

Characterizing and manipulating tight junction barriers through a claudin lens

Enrique Gamero-Estevez

Department of Human Genetics
McGill University, Montreal, Canada
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ABSTRACT

Tight junctions are structures formed between apposing cells of epithelial and endothelial barriers. Tight junctions control the diffusion of ions and molecules through the paracellular space, as well as serve as a docking platform for a multitude of cytoplasmic proteins that coordinate different cellular processes, including cell polarity. They are composed of transmembrane and cytoplasmic proteins that together determine the specific properties of a tight junction barrier. Among the 100 different proteins that are associated with tight junctions, I have focused on the claudin family, because claudins are the essential for the formation of tight junctions and their presence is directly correlated with the specific properties of a barrier.

The objectives of my thesis research were to understand the roles of individual claudins in regulating the presence of other tight junction proteins, to control and/or modify tight junction barriers by targeting specific claudins, and to understand how this could affect cell behavior. To begin to dissect how claudins can influence tight junction composition, I analyzed the interactomes of the Cldn1, -3, -4, -8 and -14 cytoplasmic domains. I discovered that each claudin preferentially interacts with specific tight junction cytoplasmic proteins, suggesting that the claudins present in a tight junction barrier have a direct effect on cytoplasmic events. I used two different approaches to modify the localization of claudins at the tight junction and study the effects on cell behavior. First I used the nutraceutical quercetin that modifies tight junction by changing the expression and localization of claudins. I found that treating a cation-selective cell line with quercetin impacted barrier tightness over time, and led to the barrier becoming tighter immediately after treatment, then leakier by six hours, and finally stabilizing after forty-eight hours such that it was tighter than in untreated control cells. This oscillation was correlated with changes in the abundance and localization of all claudins expressed in this cell line, Cldn1, -2, -3, -4 and -7. I then studied the mechanism by which the non-toxic C-terminal domain of the bacterial toxin *Clostridium perfringens* enterotoxin (C-CPE) removes claudins from tight junctions. C-CPE is able to remove six of the twenty-eight mammalian claudins from the tight junction without killing the cell. I discovered that C-CPE promotes the incorporation of claudins into extracellular vesicles, which are then released to the media. My data suggest that C-CPE-

targeted claudins may be removed and processed through different pathways, which probably are claudin-specific and through the NSF-dynamin2 pathway.

The use of C-CPE is limited by the facts that it does not remove a single claudin family member at a time, and it only targets a subset of claudins. To address this limitation, I designed and generated a series of C-CPE fusion variants that allowed me to individually target different claudins. We had previously shown that removing multiple C-CPE-responsive claudins during neural tube fusion generated open neural tubes that failed to elevate and close. My C-CPE-based protein tools allowed me to isolate the individual requirements for specific claudins during neural tube closure. I showed that Cldn8 is essential for neural fold elevation and fusion, while Cldn3 is only important for the last steps of neural tube fusion. In summary, my data have contributed to our understanding of the complexity of the tight junction and cytoplasmic plaque. It also led to the development of new tools that allow us to study individual claudins, which can be beneficial to discover the role of tight junctions in different tissues.

RÉSUMÉ

Les jonctions serrées sont des structures formées entre les cellules juxtaposées des barrières épithéliales et endothéliales. Elles contrôlent la diffusion des ions et des molécules à travers l'espace paracellulaire et servent de plateforme d'ancrage pour une multitude de protéines cytoplasmiques qui coordonnent différents processus cellulaires, incluant la polarité cellulaire. Elles sont composées de protéines transmembranaires et cytoplasmiques qui, ensemble, déterminent les propriétés spécifiques de la barrière de jonctions serrées. Parmi les centaines de protéines qui leur sont associées, je me suis concentré sur la famille des claudines parce qu'elles sont essentielles à la formation de la jonction serrée et que leur présence corrèle directement avec les propriétés spécifiques de la barrière.

Les objectifs de ma thèse étaient de comprendre les rôles individuels des claudines dans la régulation de la présence d'autres protéines de la jonction serrée, de contrôler et/ou modifier les barrières en ciblant des claudines spécifiques et de comprendre comment cela pourrait affecter le comportement cellulaire. Pour comprendre comment les claudines influencent la composition de la jonction serrée, j'ai analysé les interactomes du domaine cytoplasmique de Cldn1, -3, -4, -8 et -14. J'ai découvert que chaque claudine interagit préférentiellement avec des protéines cytoplasmiques spécifiques suggérant que les claudines présentes à la jonction ont un effet direct sur les événements cytoplasmiques. J'ai utilisé deux approches afin de modifier la localisation des claudines et étudier les effets sur le comportement cellulaire. Premièrement, j'ai utilisé le nutraceutique quercétine qui modifie la jonction serrée en changeant l'expression et la localisation des claudines. J'ai trouvé que le traitement d'une lignée cellulaire à sélectivité cationique avec la quercétine change l'étanchéité de la barrière dans le temps. Elle devient étanche immédiatement après le traitement, puis perméable après 6 heures et elle se stabilise après 48 heures à un niveau d'étanchéité plus élevé que les cellules non traitées. Cette oscillation corrèle avec des changements dans l'abondance et la localisation de toutes les claudines exprimées dans cette lignée cellulaire (Cldn1, -2, -3, -4 et -7). Ensuite, j'ai étudié comment le domaine C-terminal non-toxique de la toxine bactérienne *Clostridium perfringens* enterotoxin (C-CPE) enlève les claudines des jonctions serrées. C-CPE peut enlever 6 des 28 claudines de mammifère de la jonction sans tuer la cellule. J'ai découvert que le C-CPE promeut l'incorporation

des claudines dans les vésicules extracellulaires qui sont ensuite libérées dans le milieu. Mes résultats suggèrent que les claudines ciblées par le C-CPE sont enlevées et traitées par différents mécanismes qui sont probablement spécifiques aux claudines et par la voie NSF-dynamin2.

La limitation du C-CPE est qu'il n'enlève pas seulement un membre de la famille des claudines, mais cible un sous-groupe de claudines. Pour surmonter cette limitation, j'ai conçu et généré une série de variants fusions du C-CPE permettant de cibler individuellement différentes claudines. Nous avons précédemment montré que retirer plusieurs claudines sensibles au C-CPE durant la fusion du tube neural génère une ouverture du tube qui ne peut s'élever et se fermer. Mes protéines basées sur le C-CPE me permettent d'isoler le rôle de claudines spécifiques durant la fermeture du tube neural. J'ai montré que Cldn8 est essentielle à l'élévation du repli et de la fusion du tube neural, alors que Cldn3 est importante seulement durant les dernières étapes de la fusion. En résumé, mes résultats contribuent à une meilleure compréhension de la complexité de la jonction serrée et de la plaque cytoplasmique. Ils ont mené au développement de nouveaux outils aidant l'étude des claudines individuellement ce qui pourrait être bénéfique pour découvrir le rôle des jonctions serrées dans différent tissus.

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LIST OF ABBREVIATIONS

Å: Ångström	ILDR: Immunoglobulin-like domain-containing receptor
aa: Amino acid	IPTG: Isopropyl β -D-galactopyranoside
AF-6: Affadin	ITGA: Integrin alpha
AMPK: AMP-activated protein kinase	ITGB: Integrin beta
ALG-14: UDP-N-acetylglucosamine transferase subunit ALG14 homolog	JAM: Junctional adhesion molecule
ANXA6: Annexin A6	K⁺: Potassium ions
ARRDC1: Arrestin domain-containing protein 1	kDa: Kilodalton
ATP: Adenosine triphosphate	LLC-PK1: Lewis Lung Carcinoma Porcine Kidney Cells
Ca²⁺: Calcium ions	LN2: Ligand of Numb protein X2
Caco-2: Human Colon Carcinoma Cell Line	LSP1: Lymphocyte specific protein 1
CACNA2D3: Voltage-dependent calcium channel subunit alpha-2/delta-3	LSR: Lipolysis-stimulated lipoprotein receptor
CAR: Coxsackievirus and adenovirus receptor	MAGI: Membrane-associated guanylate kinase, WW and PDZ domain-containing protein
CARNMT1: Carnosine N-methyltransferase	MAGUK: Membrane associated guanylate kinase
C-CPE: C-terminal domain of <i>Clostridium perfringens</i> enterotoxin	MAPK: Mitogen-activated protein kinase
C-CPE₃: C-CPE variant specific of claudin-3	MARVEL: MAL related proteins for vesicle trafficking and membrane link
cdNA: Complementary deoxyribonucleic acid	MDCK: Manin-Darby canine kidney cell line
CHMP2A: Charged multivesicular body protein 2a	Mg²⁺: Magnesium ions
Cl⁻: Chloride ions	mIMCD3: Mouse inner medullary collecting duct cells
Cldn/CLDN: Claudin	miRNA: Micro-interference-RNA
CPE: <i>Clostridium perfringens</i> enterotoxin	mL: Mililiter
CPE-R: <i>Clostridium perfringens</i> enterotoxin receptor	MLCK: Myosin light chain kinase
CRMP2B: Dihydropyrimidinase-related protein 2	MPDZ: Multiple PDZ domain protein
CTTN: Cortactin	MPP5: MAGUK p55 subfamily member 5
DFNB: Deafness, Neurosensory, Autosomal Recessive	mRNA: Messenger RNA
DNA: Deoxyribonucleic acid	MUPP-1: Multi-PDZ domain protein 1
E: Embryonic day	MVB: Multi-vesicular-body
EHD4: EH domain-containing protein 4	MYH: Myosin
EL1: First extracellular loop	MYO: Myosin
EL2: Second extracellular loop	MYOF: Myoferlin
ERC-1: ELKS/Rab6-interacting/ CAST family member 1	Na⁺: Sodium ions
ERK: Extracellular Signal-Regulated Kinase	NES: Nuclear export signal
ESAM: Endothelial cell-selective adhesion molecule	NF-kβ: Nuclear factor kappa light chain enhancer of activated B cells
ESCRT: Endosomal sorting required for transport	NID2: Nidogen-2
EVP-1: Erythrocyte vesicle protein 1	NLS: Nuclear localization signal
FBN1: Fibrillin 1	nm: Nanometer
GJA-1: Gap junction alpha-1 protein	NSF: N-ethylmaleimide-sensitive factor
GLRX3: Glutaredoxin-3	Nrf2: Nuclear factor erythroid 2-related factor 2
GPCR: G-protein coupled receptor	NT: N-terminal domain of Spidroin protein
GST: Glutathione S-transferase	NT3: Specific peptide targeting Cldn3; NT::C-CPE::Cldn3EL2
GTP: Guanosine triphosphate	NT8: Specific peptide targeting Cldn8; NT::C-CPE::Cldn8EL2
HH: Hamilton and Hamburger	NTD: Neural tube defect
HEK: Human embryonic kidney	NUR2L: Chick PDZ domain-containing protein
HGS: Hepatocyte growth factor-regulated tyrosine kinase substrate	PALS1: Protein associated with Line Seven 1
His: Histidine	PAR: Partitioning defective

PATJ: PALS1-associated tight junction protein
PDZ: Post-synaptic density 95/Discs large/zonula occludens-1
PFN2: Profilin 2
PI3K: Phosphatidylinositol 3-kinase
PLK1: Polo-like kinase 1
POLDIP2: Polymerase Delta Interacting Protein 2
PPIA: Peptidylprolyl Isomerase A
PPP2R2A: serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform
Rab: Ras-associated binding protein
RALA: Ras-related protein RAL-A
RAS: Rat sarcoma virus family of proteins
RCAS: Replication competent avian retrovirus
RhoA: Ras homolog family member A
RNA: Ribonucleic acid
ROCK: Rho kinase
ROS: Reactive oxygen species
RPL: Ribosomal protein L
SH3: Src homology 3

siRNA: Short interfering ribonucleic acid
SNP: Single nucleotide polymorphism
SPTAN: Spectrin alpha chain, non-erythrocytic
SPTBN: Spectrin beta chain, non-erythrocytic
STX: Syntaxin
TAMP: Tight junction associated Marvel proteins
TER: Transepithelial electrical resistance
TGN: *Trans*-Golgi network
TJP: Tight junction protein
TPM3: Tropomyosin alpha-3 chain
TXN: Thioredoxin
UTR: Untranslated region
VCAN: Versican core protein
ZO: Zonula occludens
°C: Degrees Celsius
µg: Microgram
µL: Microliter
µm: Micrometer
µM: Micromolar

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CONTRIBUTION TO ORIGINAL KNOWLEDGE

The following are my original contributions towards understanding the individual roles of claudins in tight junctions, the use of reagents to modify claudin localization, and finally the generation of novel tools to target removal of individual claudins from tight junction barriers.

Chapter 2: In this chapter, I generated claudin tails fused to GST that were used as bait to identify the specific interactome of different claudins. This study was the first to examine the interactomes of the cytoplasmic domains of Cldn1, Cldn3, Cldn4, Cldn8 and Cldn14 in parallel. This study showed that each claudin has a unique combination of interacting proteins. I performed initial co-immunoprecipitation validation studies that confirmed that Cldn8 interacts more strongly with the tight junction adaptor protein ZO-1 than other claudins do. In this chapter, I also examined the interactomes of Cldn8 variants that were shown to differentially affect neural tube closure when overexpressed in chick embryos. My data suggest that the severe neural tube defects that result from overexpression of the Cldn8S216I variant are likely due to the decreased affinity of this variant for key tight junction cytoplasmic plaque proteins, including ZO-1, ZO-2 or MPDZ. I also showed that the interactome of Cldn8S198A, which was also associated with neural tube defects, was not dramatically different from wild-type Cldn8.

Chapter 3: In this chapter, I explored the effect of quercetin on MDCK II cells. This is the first time that the effects of quercetin on tight junction barriers were studied in a cation selective cell line. I showed that the effects of quercetin on claudin expression were claudin- and time-dependent. These changes directly affected barrier properties, which oscillated between “leakier and tighter” for the first 48h of treatment.

Chapter 4: In parallel with Chapter 3, I explored the consequence of C-CPE treatment on the removal of claudins from the tight junction. I showed that treatment of MDCK II cells with C-CPE leads to the increased secretion of extracellular vesicles. I showed that these vesicles were enriched in claudins and by using different inhibitors I showed that each claudin seem to be processed differently into extracellular vesicles. Analysis of the cargo of these extracellular

vesicles revealed that showed that the extracellular vesicles from C-CPE versus control treated cells had distinct proteomic profiles. My data suggest that an uncommon exocytosis mechanism regulated by NSF and dynamin-2 may be responsible for the changes in the extracellular vesicles that resulted from C-CPE treatment. In addition, components of more common exocytosis pathways were downregulated in the extracellular vesicles from C-CPE treated cells. These data show for the first time the mechanism used by C-CPE to remove claudins from tight junctions, and open the possibility to use this tool for the study of claudin-rich exosomes.

Chapter 5: In this chapter I designed and generated novel polypeptides tools by fusing the stabilization domain of C-CPE with the second extracellular loop of different claudins. Using these tools, I targeted the specific removal of Cldn3 or -8 from tight junctions in both MDCK II and mIMCD3 cell lines. Moreover, I used the chick embryo as a proof-of-concept and showed that my polypeptide tools were able to give rise to specific phenotypes during neural tube development distinct from C-CPE and from each other. These phenotypes were also accompanied by the specific removal of the claudin of interest. These data show for the first time a totally new idea on the generation of versatile specific claudin targeting tools. It also demonstrates that this tools will work in different tissues and species and that easy modification on the composition of the tool can allow the target of any claudin of interest. We have filed a patent for these reagents.

PREFACE TO THE THESIS

This thesis is written in accordance with the guidelines of the McGill University Graduate and Postdoctoral Studies Office. This thesis is comprised of six chapters. Four of these chapters are scientific data chapters written in manuscript format. Chapter 3 has been published and Chapter 2, 4 and 5 are manuscripts that are currently in preparation. Chapter 1 is a review of the current background literature relevant to this thesis. This chapter reviews tight junctions with an emphasis on the claudin family of tight junction proteins, and the tools that other groups have used to modify tight junctions and their barrier properties. This chapter also includes the rationale and objectives of the thesis. Chapter 2 to Chapter 5 contain experimental data in manuscript format. Chapter 2 is a manuscript in preparation describing claudin-specific interaction partners and the importance of specific amino acids in Cldn8's C-terminal cytoplasmic domain for these interactions and its function during embryonic development. Chapter 3 describes the ability of quercetin to regulate barrier properties by modifying the claudin pattern at the tight junction and has been published in the *International Journal of Molecular Science* and is reproduced in this thesis. Chapter 4 is a manuscript in preparation describing the mechanism used by *Clostridium perfringens* enterotoxin to remove claudins from tight junctions. Chapter 5 is a manuscript in preparation describing the design, generation, and proof-of concept experiments for a novel set of protein tools that I developed to specifically target removal of individual claudin family members from the tight junction. Chapter 6 provides a summary and discussion of the experimental data in Chapters 2 to 5 and describes future studies. Connecting texts between Chapter 2, 3, 4 and 5 describe the link between the manuscripts.

CONTRIBUTIONS OF AUTHORS

The experimental research presented in this thesis was developed by Enrique Gamero-Estevez under the supervision of Dr. Aimee Ryan. Enrique Gamero-Estevez carried out most experiments, participated in experimental design and troubleshooting, analyzed data, generated most of the figures and tables, wrote the first draft of each manuscript, and actively participated in the revision of manuscripts. Dr. Aimee Ryan supervised the work, participated in experimental design and troubleshooting, analyzed data, and was involved in manuscript editing. The contribution of the remaining authors to each chapter are described below.

For Chapter 2, Dr. Amanda Baumholtz performed the electroporation of Cldn8 variants and phenotypic analysis of chick embryos. Dr. Jean-François Boisclair Lachance helped to complete the biological replicates of the different experiments.

For Chapter 3, Dr. Sero Andonian, Dr. Bertrand Jean-Claude and Dr. Indra Gupta participated in conceptualization of the project and edited the manuscript.

For Chapter 4, Dr. Jenna Haverfield provided the preliminary data for the effect of C-CPE on MDCK II cells. She also performed 3 out of the 4 replicates analyzed by mass spectrometry. Dr. Jean-François Boisclair Lachance helped to complete the biological replicates of the different experiments.

For Chapter 5, Dr. Amanda Baumholtz and Elizabeth Ann Legere performed the embryo dissections and treatments with the different polypeptides. Dr. Makoto Nagano participated in project conceptualization and editing.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 OVERVIEW AND RATIONALE FOR STUDY

Epithelial and endothelial barriers separate different tissues or compartments allowing the formation of microenvironments, the specialization of structures, and the protection of specific organs. Tight junctions are essential structures that form between adjacent cells in epithelial and endothelial tissues and control paracellular transport and cell polarity. Tight junctions are composed of strands of transmembrane proteins that associate with scaffolding proteins at the cytoplasmic face of the tight junction. More than 100 proteins have been associated with tight junctions; each of them contributes specific characteristics to the junction and the epithelial or endothelial barrier (Gunzel and Fromm, 2012; Piontek et al., 2020b). Therefore, the properties and function of each biological tight junction barrier depends upon the specific proteins present.

Tight junctions play a dual role at the barrier. First, tight junctions act as a fence preventing basolateral and apical proteins and lipids from mixing, and therefore, an important structure in the regulation and maintenance of cell polarity. Second, tight junctions act as a gate by regulating the ions and molecules that can move through the paracellular space. Among the tight junction proteins, claudins are the main determinants of barrier properties. To date, more than 28 members have been described in vertebrates (Piontek et al., 2020b). Alterations in tight junction composition and particularly claudins have been linked to permeability or polarity defects that lead to different diseases in humans and animal models, including infertility, kidney stones, brain malformations, deafness, and cancer (Gamero-Estevez et al., 2018; Gunzel and Fromm, 2012). Moreover, gain- and loss-of-function studies support roles for tight junction proteins in the maintenance of tissue shape (Behr et al., 2003; Nelson et al., 2010; Wu et al., 2004), tension between cells during morphogenetic movements (Siddiqui et al., 2010), and hydrostatic pressure during lumen expansion (Bagnat et al., 2007; Moriwaki et al., 2007; Zhang et al., 2010). Therefore, understanding tight junction biology will contribute to the study, development, and identification of treatments or mechanisms to prevent the onset of different diseases.

The main purpose of my thesis research has been to develop and study tools that target claudins and to allow us to study and understand the role of individual claudins in the tight

junction. Historically, different tools have been used to study tight junctions. One tool is the *Clostridium perfringens* enterotoxin (CPE) and its derived non-toxic C-terminal domain of *Clostridium perfringens* enterotoxin (C-CPE). CPE is a protein that is able to interact with claudins and generate a pore that lead to the entry of calcium into the cell and its subsequent apoptosis. In contrast, C-CPE is able to bind to Cldn3, -4, -6, -7, -8 and -14 and remove them from the tight junction without killing the cell, and therefore, it is widely used to study the role of this subset of claudins in different models and diseases. For instance, in our lab we previously showed that C-CPE removal of Cldn3, -4 and -8 from the tight junction during neural tube closure in chick embryos leads to convergent extension defects and open neural tubes. Although C-CPE is very useful to study the tight junction barriers where these specific claudins are present, it has the limitations that it doesn't specifically target an individual claudin or other claudins different that the small subset that it naturally targets. In fact, different groups have tried to generate C-CPE variants by modifying amino acids along the molecules. However, only C-CPE variants that target individually Cldn1, -2, -3, -4 and -5 have been found. For this reason, part of my thesis research project was to generate new tools that will allow the target and removal of specific claudins from the tight junction. Derivate from bacterial toxins are not the only tools that have been used to target tight junction also nutraceuticals such as quercetin and other compounds have been shown to modify tight junction properties by changing the specific claudins members at the tight junction.

Although C-CPE and nutraceuticals have been widely used to study barriers, there is not a good understanding of the mechanisms by which these tools affect localization of claudins to the tight junction. Moreover, even though there have been numerous studies that have used knockdown of expression of single claudins or transgenic models and seen changes in barrier properties (Dube et al., 2010); we do not yet fully understand the consequences of affecting one specific claudin versus another one. In part because we do not have approaches to study individual claudins in different contexts, and we do not know what protein complexes may be formed and altered when changing localization of specific claudins. Understanding the effect and how these compounds and tools work, and generating new ones that can target specific tight

junction members, would allow us to better comprehend the tight junction and shed light on the many different diseases that are associated with tight junction defects.

1.2 TIGHT JUNCTIONS

Epithelial and endothelial barriers carry out essential functions by separating tissues and forming microenvironments. These barriers are formed by cells that interact closely with each other through their tight junctions. Tight junctions are structures formed between cells and seal the paracellular space. They are composed of proteins from apposing cells that interact tightly with each other. This interaction allows the tight junction to control the movement of small molecules or ions through the paracellular space, that is tight junctions determine which molecules can or cannot pass between cells. Tight junctions also play a role in regulating apical and basolateral polarity because they prevent the mixing of apical from basolateral protein and lipids (Figure 1.1) (Gupta and Ryan, 2010).

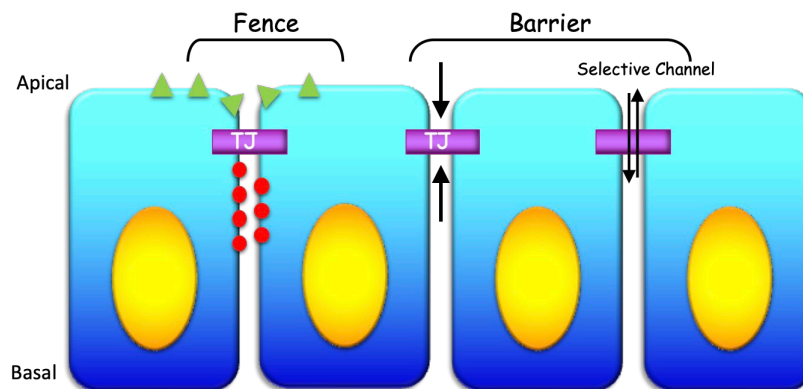


Figure 1.1: Scheme of tight junctions and its functions. Tight junctions are defined as kissing points between apposing cells that carry out two main functions: gate and fence. They act as a gate regulating the molecules and ions that can diffuse across the paracellular space; whereas they act as a fence avoiding the mixing of apical with basolateral proteins and lipids. Image taken with permission from Gupta and Ryan 2010 (Gupta and Ryan, 2010).

1.2.1 History of tight junctions

The first description of tight junctions was made by Farquhar and Palade in 1963. Using transmission electron microscopy of ultrathin sections, they identified tight junctions in the blood-testes barrier (Farquhar and Palade, 1963). Through their analyses, they observed different structures present between cells that bridge or seal the paracellular space, including tight

junctions (previously named called zonula occludens), adherens junctions (previously named zonula adherens) and desmosomes (previously named macula adherens). Tight junctions were defined as structures in which the outer leaflets of membranes from two adjacent cells merged into kissing points. Thus, they were different from other paracellular structures, such as adherens junctions and desmosomes, where there remained a 15 to 20 nm gap between apposing membranes (Farquhar and Palade, 1963; Troeger et al., 2009). Electron microscopy studies also revealed a dense cytoplasmic material associated with the tight junction that was called the cytoplasmic plaque (Farquhar and Palade, 1963). At the same time, freeze fracture electron microscopy studies of tight junction showed an apical meshwork of strands or fibrils with different complexity in different tissues that surrounded the apical domain of epithelial and endothelial cells (Staehelin et al., 1969).

The resolution of the original microscopy images led to the theory that tight junctions were made of lipids, and it was believed that the plasma membranes fused in cylindrical micelles that would leave pores where the molecules and ions could pass (Pinto da Silva and Kachar, 1982). However, the isolation of proteins from these structures rapidly disproved this theory. The first protein associated with tight junction was zonula occludens-1 (ZO-1), which was identified by Stevenson and colleagues in 1986 (Stevenson et al., 1986). It was another seven years before Furuse and colleagues discovered the first transmembrane tight junction protein, which they named occludin (Furuse et al., 1993). Subsequently in 1998 the first members of the claudin family of integral membrane proteins, Claudin1 (Cldn1) and -2, were isolated from junctional fractions (Furuse et al., 1998a). These data revealed that the tight junctions were indeed composed of proteins and of great complexity. Since 1998 more than 100 proteins have been isolated or associated with tight junctions (Gunzel and Fromm, 2012; Piontek et al., 2020b). Although lipids do not form the tight junction per se, they have important roles because tight junctions usually appear in lipid raft-like microdomains that are rich in cholesterol and sphingolipids (Lee et al., 2008; Turner, 2000).

In summary, tight junctions are apical structures between apposing cells formed by transmembrane and cytosolic proteins. The composition, regulation, formation, function and modulators of tight junction will be reviewed in the following sections.

1.2.2 Functions of the tight junction

Tight junctions are described as having a dual function. Tight junctions act as a gate by regulating the ions and small molecules that can pass through the paracellular space, and they act as a fence by preventing the mix between apical and basolateral proteins that are essential for the maintenance of apical-basal polarity (Figure 1.1).

1.2.2.1 The gate function of the tight junction

The gate function refers to the role that tight junctions have regulating which ions, molecules and cells can or cannot pass across the paracellular space (Figure 1.1). Specific aspects of the gate function are determined by the presence or absence of specific tight junction proteins. For instance, each claudin is able to form specific channels in the paracellular space that allow for the diffusion of specific ions or molecules. While junctional adhesion molecules, also present at the tight junction, coordinate the movement of immune cells across the paracellular space. Together, the specific combination of proteins present at the tight junction will dictate the barrier properties.

These barrier properties are often measured and used as indicators of the tightness of the barrier. The two most common methods used to measure “leakiness” or “tightness” of the barrier are transepithelial resistance and permeability assays. Transepithelial resistance, is a measure of the electrical resistance of a cell monolayer, that can be monitored using different electrical measurements such as impedance or ion potentials (Srinivasan et al., 2015; Van Itallie et al., 2003). Similarly, paracellular permeability assays monitor the movement of a labelled or known molecule, which can be used to detect if it can cross through a layer of cells. Different size molecules are used to study the barrier permeability including very small molecules such as mannitol or polyethylene glycol to bigger macromolecules such as dextrans (Van Itallie and Anderson, 2011). Moreover, these molecules can be charged to allow monitoring of both ion and size permeability. Both assays can be modulated by cell confluence, barrier permeability, and tight junction composition.

1.2.2.2 The fence function of the tight junction

The fence function refers to the role of tight junctions in preventing apical and basal proteins or lipids from mixing, and its ability to establish a boundary within the lateral cell membrane that maintains cell polarity (Figure 1.1). This role was discovered by cell studies that showed that when tight junctions were disturbed, there was a mixing of membrane proteins that were normally restricted to the apical or basolateral membrane (Pisam and Ripoche, 1976; Ziomek and Johnson, 1980; Ziomek et al., 1980). This is also seen when certain tight junction protein variants are overexpressed. For instance, overexpression of a C-terminally truncated occludin in MDCK cells leads to the redistribution of sphingomyelin from the apical to the basolateral membrane (Balda et al., 1996). Tight junctions also serve as docking sites for proteins involved in the maintenance of polarity, including the Par and Crumbs polarity complexes. For instance, Par-3 and aPKC were not perturbed in claudin quintuple knockout cells, but in ZO-1/ZO-2 double knockout, Par-3 and aPKC localization was fragmented and diffuse (Otani et al., 2019). In contrast, removal of claudins from the tight junctions, can lead to the mislocalization of these polarity complexes in the chicken neural tube (Baumholtz et al., 2017).

1.2.3 Formation of the tight junction

Tight junction formation has been mainly studied in the context of mesenchymal-to-epithelial transition or during blastocyst formation, while disassembly has been studied during epithelial-to-mesenchymal transition. The formation and disassembly process is usually coordinated by different transcription factors that up- or down-regulate expression of genes required for formation or disassembly of the tight junction. Tight junction formation usually starts with initiation of apical-basal polarity followed by the delivery of proteins, including cadherin, nectin, afadin or ZO-1 to the developing apical side of the cell. Next, tight junction proteins, including claudins and occludin, are trafficked to the apical domain where they are incorporated into the membrane. The recruitment of these proteins is essential for the initial establishment of polarity and the barrier formation, and maintenance. After this initial assembly, further maturation of the tight junction will occur by the exchange and redistribution of different proteins that will form the final mature tight junction.

1.2.4 Tight junction composition

Tight junctions are composed by transmembrane proteins and cytoplasmic plaque proteins (Figure 1.2). Tight junction composition is variable between different tissues, which determines the specific barrier properties. Therefore, while there are many proteins associated with tight junctions, not all of them are going to be present at the same tight junction. Moreover, some of them are going to be involved in cell-cell interactions, while others are going to be involved in linking the tight junction to the cytoskeleton or in refining the specific function of the barrier.

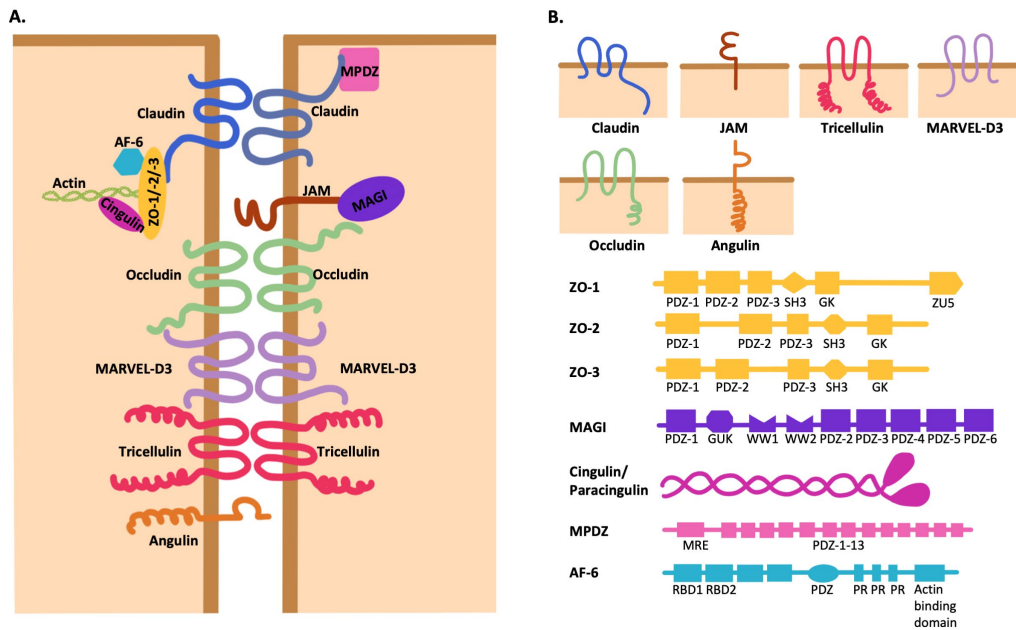


Figure 1.2: Schematic of tight junction components. Tight junctions are composed of transmembrane and cytoplasmic proteins. Among these proteins, claudins, junctional adhesion molecules and the tight junction-associated MARVEL protein family are the most relevant transmembrane proteins, while the Zona occludens family, MPDZ, cingulin and afadin are some of the most important cytoplasmic proteins that are essential to link the transmembrane proteins to the cytoskeleton and to dock other proteins to the apical side of the cell.

1.2.4.1 Transmembrane proteins

Tight junction transmembrane proteins include both single-pass (e.g. junctional adhesion molecules) and tetraspan proteins (e.g. claudins and TAMPs). The function of these

transmembrane proteins is to establish the direct interaction between apposing cell and regulate the paracellular space. In addition, their intracellular domains act as a docking platform for many other proteins that form the cytoplasmic plaque.

1.2.4.1.1 Claudins

Claudins are a family of 20-27 kDa tetraspan transmembrane proteins. To date, more than 28 members have been described in vertebrates (Piontek et al., 2020b). They are essential for the formation of tight junctions and often are the main player dictating the barrier paracellular permeability. Although occludin can form tight junction-like structures, it forms a knot and not a well defined network. In fact, claudins are the only tight junction protein that are sufficient to generate a network of tight junction-like strands when transfected in L-fibroblast cells, which lack of endogenous tight junctions (Furuse et al., 1998a). Claudins are able to differentially associate and form different intramembrane tight junction strands. For instance, cells transfected with Cldn1 and -3 form continuous and smooth intramembrane tight junction strands (Furuse et al., 1999), while cells transfected with Cldn1 and -5 form discontinuous particle chains (Morita et al., 1999b), and cells transfected with only Cldn11 generate parallel intermembrane strands that lack branching (Morita et al., 1999a). Mutations in claudin genes have been shown to be associated with a variety of diseases in humans and animal models (Table 1.1). Manipulating claudins in tight junction barriers is the primary focus of this thesis. A more detailed review of the claudin family is provided in Section 1.3.

Protein	Human associated disease	Mouse Claudin-variant phenotypes	References
Claudin-1	Neonatal ichthyosis and sclerosing cholangitis syndrome. Atypical dermatitis,. Leukoaraiosis and vessel vascular dementia	Skin leakage, water loss and neonatal death due to dehydration	(Furuse et al., 2002; Hadj-Rabia et al., 2004; Yadav and Shin, 2015)
Claudin-2	Kidney stone formation. Obstructive azoospermia. Acute pancreatitis. Malabsorptive intestine	Hypercalciuria, salt deposits in the kidney. Gallstones	(Askari et al., 2019; Curry et al., 2020; Matsumoto et al., 2014; Muto et al., 2010; Wada et al., 2013; Whitcomb et al., 2012)
Claudin-3	Spina bifida. Present in EVs from hepatobiliary cancer	Cholesterol gallstones. Atopic dermatitis	(Baumholtz et al., 2020; Ikeda et al., 2021; Tanaka et al., 2018; Yamaga et al., 2018)
Claudin-4	Present in EVs from ovarian cancer	Hydronephrosis. Lung injury	(Fujita et al., 2012; Kage et al., 2014; Li et al., 2009)
Claudin-5	Schizophrenia. Involved in 22q11.2 syndrome. Colorectal cancer with microsatellite instability	Psychosis. Seizures. Depression. Inflammation	(Greene et al., 2019; Guo et al., 2020; Omidinia et al., 2014; Son et al., 2020)
Claudin-6	Colorectal cancer with microsatellite instability	Lung conditional animals show delay in lung morphogenesis	(Jimenez et al., 2014; Son et al., 2020)
Claudin-7	Colon cancer	Neonatal death due to urinary salt wasting, dehydration and growth retardation. Intestinal loss of mucosa. Adenocarcinoma. Inflammatory bowel disease	(Hahn-Stromberg et al., 2014; Tanaka et al., 2015; Tatum et al., 2010)
Claudin-8	Neural tube defects	Kidney conditional animals develop hypertension, hypokalemia and alkalosis	(Baumholtz et al., 2020; Gong et al., 2015b)
Claudin-9	Hearing loss	Deafness	(Sineni et al., 2019)
Claudin -10a/b	Kidney and skin dysfunction	Hypermagnesemia and nephrocalcinosis	(Breiderhoff et al., 2012; Milatz, 2019)
Claudin-11	Infertility	Slowed nerve conduction. Infertility. Deafness. Reduced bone formation	(Baek et al., 2018; Chiba et al., 2012; Gow et al., 2004; Gow et al., 1999)
Claudin-14	Non-syndromic deafness DFNB29. Perrault syndrome deafness. Kidney stone formation.	Hypocalciuria and hypomagnesemia. Deafness	(Ben-Yosef et al., 2003; Faridi et al., 2017; Gong et al., 2012; Guha et al., 2015; Thorleifsson et al., 2009; Wilcox et al., 2001)
Claudin-15		Megaintestine	(Tamura et al., 2008)
Claudin-16	Familial hypomagnesemia, hypercalciuria and nephrocalcinosis (FHHNC). Amylogenesis imperfecta	Salt wasting and nephrocalcinosis	(Bardet et al., 2017; Hou et al., 2007; Konrad et al., 2008; Muller et al., 2003)
Claudin-18		Lung and stomach carcinoma. Lung defects. Increased bone resorption	(Hagen et al., 2018; LaFemina et al., 2014; Li et al., 2014; Linares et al., 2012)
Claudin-19	Familial hypomagnesemia, hypercalciuria and nephrocalcinosis (FHHNC) with severe ocular defects. Macular coloboma. Amylogenesis imperfecta	Peripheral neuropathy. Visual impairment. Renal salt wasting	(Hou et al., 2008; Khan et al., 2018; Meier et al., 1979; Miyamoto et al., 2005; Wang et al., 2019; Yamaguti et al., 2017)

Occludin	Infertility. BLC-PMG syndrome	Growth retardation. Male sterility. Chronic inflammation. Brain calcification. Gastric hyperplasia. Thinning of bone	(Ekinci et al., 2020; O'Driscoll et al., 2010; Saitou et al., 2000)
Tricellulin	Non-syndromic deafness DFNB49	Deafness	(Riazuddin et al., 2006)
JAM-A	Atherosclerosis	Decreased fertility. Increased inflammation. Angiogenesis. Cellular proliferation. Intestinal permeability	(Cavusoglu et al., 2007; Cooke et al., 2006; Shao et al., 2008)
JAM-C	Severe hemorrhagic destruction of the brain. Subependymal calcification. Congenital cataracts	Leukocytic pulmonary infiltrates. Disturbed neutrophil homeostasis. Growth retardation with increased postnatal mortality. Hydrocephalus and megaesophagus phenotype	(Imhof et al., 2007; Mochida et al., 2010)
CAR		Death due to heart and lymphatic system defects	(Pazirandeh et al., 2011)
ESAM		Vasculature leakage	(Wegmann et al., 2006)
LSR	Pediatric progressive familial intrahepatic cholestasis	Embryonic death due to liver hypoplasia and blood brain barrier leakage	(Maddirevula et al., 2019; Mesli et al., 2004)
ILDR-1	Non-syndromic deafness DFNB42	Deafness and polyuria	(Higashi et al., 2015; Higashi et al., 2013b)
ZO-1	Arrhythmogenic cardiomyopathy	Embryonic death due to severe growth defects and apoptosis of notochord, neural tube and allantois. Kidney conditionals develop proteinuria and reduce glomerular filtration	(De Bortoli et al., 2018; Itoh et al., 2014; Katsuno et al., 2008)
ZO-2	Familial hypercholaemia	Embryos arrest in early gastrulation with disturbed mesodermal differentiation. Mosaic animals were viable but infertile	(Carlton et al., 2003; Xu et al., 2009; Xu et al., 2008; Zhang et al., 2020b)
MPDZ	Autosomal recessive congenital hydrocephalus		(Al-Dosari et al., 2013)

Table 1.1: Summary of diseases associated with tight junction protein variants in humans and mouse models. This table does not include all references, it includes the 1st study where the phenotype was discovered or a recent review regarding the specific phenotypes.

1.2.4.1.2 Tight junction associated marvel proteins (TAMPs)

Members of the TAMP family are also tetraspan transmembrane proteins. Despite their structural similarity to claudins, they do not share any homology and differ in the length and structure of their different domains (Figure 1.2). The TAMP family of proteins has three principal members: occludin, tricellulin (MARVELD2) and MARVELD3. All of them have 4 transmembrane domains, 2 extracellular loops of similar size, a short intracellular loop, and long N- and C-termini oriented into the cytoplasm. The exception is occludin, which has a shorter N-terminal domain, and MARVELD3, which has a very short C-terminal domain. TAMPs also share a common MARVEL

domain that is formed by their 4 transmembrane domains and 2 extracellular loops. The MARVEL domain interacts with the lipid environment to ensure proper membrane localization. Occludin and tricellulin also have a Coiled-coil/ELL domain that is involved in their interaction with different tight junction proteins at the cytoplasmic face (Buschmann et al., 2013; Muller et al., 2005; Yaffe et al., 2012). MARVELD3 lacks the Coiled-coil/ELL domain, and its interaction with cytoplasmic proteins is thought to be through its N-terminus (Raleigh et al., 2010; Steed et al., 2009). All TAMPs are phosphorylated and the specific amino acid that is modified regulates their degradation or maintenance at the tight junction (Andreeva et al., 2001; Andreeva et al., 2006; Dorfel et al., 2013; Ikenouchi et al., 2005; Liu et al., 2016; Smales et al., 2003).

Occludin was the first tight junction transmembrane protein to be purified and characterized (Furuse et al., 1993). Expression of occludin in fibroblasts that lack tight junction, conferred adhesiveness properties to the cell (Van Itallie and Anderson, 1997). However, tight junctions can be formed in the absence of occludin; its removal from certain cell lines did not affect tight junction fibrils, cell morphology, or localization of other tight junction associated proteins, suggesting that occludin is not essential to form the tight junction (Balda et al., 1996; Wong and Gumbiner, 1997). Occludin maintains tricellulin localization at tricellular tight junctions (Ikenouchi et al., 2008; Kitajiri et al., 2014), and together with tricellulin it facilitates the formation of an anastomosing tight junction strand network (Saito et al., 2021).

Analysis of occludin knockout mice revealed that occludin has a wide range of functions in different tissues. For instance, adult occludin knockout mice exhibit growth retardation, male sterility, chronic inflammation, brain calcification, gastric hyperplasia and thinning of bones (Saitou et al., 2000). In humans, intragenic deletions in occludin that cause frameshifts or deletion of the C-terminal tail are associated with a rare autosomal recessive syndrome characterized by seizures, microcephaly, developmental delay, gray matter calcification and polymicrogyria (Abdel-Hamid et al., 2017; Aggarwal et al., 2016; Ekinici et al., 2020; Elsaid et al., 2014; Jenkinson et al., 2018; LeBlanc et al., 2013; O'Driscoll et al., 2010).

Tricellulin was first identified as a protein that was downregulated in epithelial cells undergoing epithelial-mesenchymal transition (Ikenouchi et al., 2005). Tricellulin mostly localizes to the tricellular junctions where it seems to be recruited by the angulin family of proteins

(Higashi et al., 2013b). Tricellulin can form homo- and heteromeric interactions that determine the membrane localization and tightness of the barrier. For example, in MDCK cells, tricellulin interacts heteromerically with occludin at bicellular junctions and forms homomeric interactions with other tricellulin molecules at tricellular junctions (Westphal et al., 2010). Fifteen recessive human mutations in the tricellulin gene have been associated with nonsyndromic deafness (DFNB49), suggesting an essential role of tricellulin in the tight junction barrier of the inner ear barrier (Chishti et al., 2008; Mariano et al., 2011; Riazuddin et al., 2006; Zheng et al., 2019). These findings are supported by functional studies in mice (Kamitani et al., 2015; Nayak et al., 2013; Riazuddin et al., 2006).

Of these three TAMP family members, protein interactions have been best studied for occludin, which has been shown to interact with many intracellular proteins, including ZO-1, ZO-2, ZO-3, MarvelD3 or Cav-1 (Furuse et al., 1994; Itallie and Anderson, 2012). Cording *et al.* showed that occludin can interact with itself in *cis*, with MARVELD3, and Cldn1 to -5 but not with tricellulin (Cording et al., 2013). A study by Fredriksson *et al.* further defined the occludin interactome. They performed a BioID study where biotin ligase was fused to the N- or C-terminal domains of occludin to biotinylate proteins that were in close proximity. They identified proteins involved in a variety of cell functions, including tight and adherens junctions, proteins involved in endocytosis, exocytosis and trafficking, and proteins involved in cytoskeletal regulation. Interestingly, the occludin interactomes differed based on whether the biotin ligase was fused to the N-terminus or the C-terminus domain (Fredriksson et al., 2015). Some potential interactors were common (such as ZO-1, Cldn1, Cldn2 or MAGI-3) but others were observed in close proximity to the N-terminus (such as Cldn3, -4 or -16) or to the C-terminus (such as ZO-2).

1.2.4.1.3 Junctional adhesion molecules (JAMs)

JAMs are single-span transmembrane glycoproteins of 300-400 amino acids. JAMs have three structural domains: an extracellular tail with two immunoglobulins-like domains; a single transmembrane domain; and a short cytoplasmic tail carrying a Dlg1 binding motif and a PDZ motif (Figure 1.2) (Martin-Padura et al., 1998). There are 3 classical JAMs (JAM-A, -B and -C also called JAM-1, -2 and -3) and 4 non-classical JAMs (JAM-4, JAM-L, coxsackie and adenovirus

receptor (CAR) and endothelial cell-selective adhesion molecule (ESAM)). JAMs have several functions at the tight junction, including maintenance of barrier permeability, maintenance of cell polarity, and coordination of the migration of different immune cells across the paracellular space by *trans*-interactions with integrins present in the surface of the immune cells (Aurrand-Lions et al., 2001; Aurrand-Lions et al., 2002; Corada et al., 2005; Ebnet et al., 2001; Glikli et al., 2004; Guo et al., 2009; Luissint et al., 2008; Mandicourt et al., 2007; Otani et al., 2019; Wegmann et al., 2006; Woodfin et al., 2011; Woodfin et al., 2009; Wyss et al., 2012; Yeung et al., 2008). Moreover, JAM-A regulates membrane apposition at the TJ (Otani et al., 2019).

In humans, only mutations in JAM-C have been associated with disease, where several mutations have been associated with severe hemorrhagic destruction of the brain, subependymal calcification, and congenital cataracts (Akawi et al., 2013; Mochida et al., 2010). While JAM-A has been associated with higher risk to develop atherosclerosis (Cavusoglu et al., 2007). The other JAMs although not associated with human disease have been shown to give rise to different phenotypes when mouse knockouts have been developed. For instance, JAM-A knockout mouse develop infertility and increased inflammation (Cooke et al., 2006; Laukoetter et al., 2007; Shao et al., 2008). CAR knockout mice die embryonically, and ESAM knockout mice present severe leakage along the endothelial tissue (Pazirandeh et al., 2011; Wegmann et al., 2006). JAM-C knockdown mice also develop lethal phenotypes, including hydrocephalus and megaesophagus phenotypes (Imhof et al., 2007; Wyss et al., 2012; Ye et al., 2016; Zimmerli et al., 2009). In contrast JAM-B and -4 knockout mice do not develop any significant phenotypes suggesting that their role may be compensated by other proteins (Arcangeli et al., 2011; Nagamatsu et al., 2006; Tietz et al., 2018).

1.2.4.1.4 Angulins

Angulins, are a family of single-span proteins that mark where the tricellular junction will be formed, and are essential for recruitment of proteins, such as tricellulin, to the tricellular membranes (Masuda et al., 2011). Angulins have an extracellular immunoglobulin-like domain, a single transmembrane domain and a long C-terminal cytoplasmic domain (Figure 1.2). The angulin family is composed by 3 members: lipolysis-stimulated lipoprotein receptor (LSR or

angulin-1), immunoglobulin-like domain-containing receptor-1 (ILDR-1 or angulin-2) and -2 (ILDR-2 or angulin-3).

LSR was first described as a lipoprotein receptor in the liver (Xie et al., 2018; Yen et al., 1994). It was subsequently identified in a genetic screen as a binding receptor for *Clostridium difficile* toxin (Papatheodorou et al., 2011) and shown to recruit tricellulin to tricellular tight junctions, and therefore was important for epithelial barrier function (Masuda et al., 2011). Based on sequence homology with LSR, ILDR-1 and -2 were identified as angulin family members and renamed angulin-1, -2 and -3 (Higashi et al., 2013b). The three family members are differentially expressed but at least one of three angulins is present at the tricellular tight junctions (Higashi et al., 2013b). In fact, angulin-1 is responsible for the formation of the central sealing element and for plasma membrane apposition at tricellular junctions (Sugawara et al., 2021).

ILDR-1 (angulin-2) has shown to be important in the inner ear. In humans, more than 20 ILDR-1 mutations have been linked to non-syndromic deafness DFNB42 as a consequence of outer hair cell degeneration (Higashi et al., 2013a; Higashi et al., 2013b). Moreover, ILDR-1 null mice develop deafness, polyuria and impaired urine concentration suggesting a role for ILDR-1 in the nephron barrier (Gong et al., 2017; Higashi et al., 2015; Morozko et al., 2015; Sang et al., 2015). LSR has also shown to be important at least in mice, where knockouts die during embryo development due to liver and brain phenotypes (Mesli et al., 2004; Sohet et al., 2015). In fact, LSR is associated with progressive familial intrahepatic cholestasis in humans (Maddirevula et al., 2019; Uehara et al., 2020).

1.2.4.2 Cytoplasmic plaque proteins

Cytoplasmic plaque proteins are essential to regulate and modify the properties of the barrier. They link the tight junction transmembrane proteins to the actin cytoskeleton, allowing for coordinated responses. The many different protein families found in the cytoplasmic plaque carry out different functions. Members of the multiple-PDZ-domain protein (MPDZ) and the membrane-associated guanylate kinase (MAGUK) family, including zona occludens (ZO) 1 and -2, are essential to maintain and regulate tight junction integrity (Gonzalez-Mariscal et al., 2003).

Other cytoplasmic plaque proteins, such as cingulin and afadin, are more directly involved in linking the tight junction to the actin cytoskeleton. Together the cytoplasmic plaque proteins also serve as a docking platform for many other proteins that are involved in different processes, such as cell polarity.

1.2.4.2.1 The membrane-associated guanylate kinase family (MAGUK)

The MAGUK family of proteins contain at least one PDZ (PSD-95/Dlg/ZO-1) domain, a Src homology 3 (SH3) domain, and an enzymatically inactive guanylate kinase (GUK) domain (Figure 1.2) (Anderson, 1996). They are involved in tight junction regulation, linking to the cytoskeleton and serving as a scaffold for other tight junction proteins to ensure polarity and barrier maintenance. Although there are many members of the MAGUK family, members of the zona occludens family are of the most interest for my thesis. They are often found at the tight junction, have been widely studied for their interactions with tight junction transmembrane proteins, and are often used as markers of the tight junction.

The zonula or zona occludens family (ZO) is one of the main families involved in the formation of the scaffolding complexes and the cytoplasmic plaque. They are often found at the cytoplasmic plaque of both tight and adherens junctions, and in certain conditions can also be found in the nucleus or at the leading edge of migratory cells where they interact with transcription factors or cytoskeletal proteins, and regulate cell proliferation or migration, respectively (Gonzalez-Mariscal et al., 2008; Haskins et al., 1998; Itoh et al., 1993; Jesaitis and Goodenough, 1994; Stevenson et al., 1986). There are three family members, ZO-1, ZO-2 and ZO-3, that are 1748, 1190 and 919 amino acids, respectively. They share only 15% identity, although all have three PDZ domains, one SH3 domain, and one GUK domain. In these proteins, PDZ1 is involved in binding to connexins, claudins and phosphoinositides, while the PDZ3 seems to bind to JAMs (Ebnet et al., 2000; Ernst et al., 2014; Hiroaki et al., 2018; Itoh et al., 1999; Meerschaert et al., 2009; Umetsu et al., 2011). All family members homodimerize through PDZ-2, and ZO-1 is also able to heterodimerize with ZO-2 and ZO-3 through this domain (Rueckert et al., 2012). The SH3, hinge-U5 and GUK domains, bind to occludin and afadin and are required for proper recruitment of ZO-1 to the apical junction, which is a prerequisite for normal tight junction and

cytoskeletal organization (Johnston et al., 2011; Lye et al., 2010; Ooshio et al., 2010; Rodgers et al., 2013; Tash et al., 2012). Although all family members have the same type of domains, the length of the protein and distance between the domains is different between family members, which allows them to have different functions. In addition, each zona occludens protein has different isoforms that can be differentially phosphorylated or post-translationally modified. Similarly to other proteins in the tight junction, the effect of post-translational modification depends on the specific residue and the precise modification. These modifications may increase stability at the junction, target the protein for degradation, or modulate its interactions. In the case of ZO-1 two isoforms are expressed by alternative splicing and differ by an internal 80-aminoacid domain called motif α (Willott et al., 1992). The shorter isoform (α -) is associated with leaky tight junctions, while the longer one (α +) is associated with tighter tight junctions (Willott et al., 1992). These isoforms are relevant during tight junction formation and can regulate the other proteins present at the tight junction (Willott et al., 1992).

Mouse knockouts were used to study the role of the different zona occludens proteins. ZO-1 null mice die before E11.5 due to severe growth defects and apoptosis of the notochord, neural tube, and allantois, showing the relevance of ZO-1 during development (Katsuno et al., 2008). ZO-2 null embryos die at E7.5 due to arrested gastrulation and disturbed mesodermal differentiation (Xu et al., 2008). In contrast, ZO-3 depletion does not cause any striking phenotype, suggesting that its function can be compensated by other proteins (Xu et al., 2008). In humans, relatively few variants have been associated with disease, suggesting that deleterious variants of zona occludens proteins may lead to death during embryonic development. Only some ZO-1 variants have been associated with arrhythmogenic cardiomyopathy (De Bortoli et al., 2018) and some ZO-2 variants with familial hypercholaemia with growth (Carlton et al., 2003; Zhang et al., 2020a). In contrast, in cell lines deletion of ZO-1 or ZO-2 does not cause any striking effect at the tight junction; although a small delay in tight junction formation and increased permeability to macromolecules are observed in ZO-1 knockout cell lines (Tokuda et al., 2014; Umeda et al., 2004; Van Itallie et al., 2009). However, simultaneous knockdown of ZO-1 and ZO-2 causes loss of tight junction strands, increased permeability, expansion of the apical actomyosin

contractile array, and loss of polarity (Fanning et al., 2012; Otani et al., 2019; Rodgers et al., 2013; Umeda et al., 2006; Yamazaki et al., 2008).

Conditional knockouts were used to study the role of zona occludens proteins in adult mice. Zhang *et al.* showed that mice with a conditional knockout of ZO-1 in cardiac myocytes are viable and have a normal lifespan (Dai et al., 2020; Zhang et al., 2020b). Mice with conditional knockout of ZO-1 in the liver are also viable, but the hepatic circadian rhythm is altered due to the fact, that in this tissue ZO-1 interacts with period-1, a circadian rhythm control protein, and sequesters it in the cytoplasm (Liu et al., 2020). In the intestine, ZO-1 is essential for development of unified apical surfaces (Odenwald et al., 2018), while in podocytes, ZO-1 is required for proper slit diaphragm structures. These mice had proteinuria and reduced glomerular filtration (Itoh et al., 2014). These phenotypes were even more severe if ZO-2 was also depleted in the podocytes (Itoh et al., 2018). These data suggest that ZO-1 is essential for embryonic development and that it has important functions in adult tissues that can be partially compensated by ZO-2. Finally, Xu and colleagues showed that mice that were mosaic for ZO-2 null cells were viable but infertile, due to the fact that ZO-2 is essential for proper blood-testes-barrier development (Xu et al., 2009).

Another important MAGUK protein is Pals1 (protein associated with Lin seven-1, also called MPP5). Pals1 was first described in *C. elegans* and is essential for the basolateral targeting of let-23 growth factor receptor (Kaech et al., 1998). Pals1 contains one PDZ domain and one SH3-GUK domain. Pals1 differs from other MAGUK proteins in the fact that it lacks the acidic and proline-rich domains. Instead it contains two Lin-7 binding modules and a 125 amino acid domain with no protein similarity to other MAGUK members. Pals1 forms part of the Crumbs and PATJ complex, which is restricted to the apical membrane and involved in maintaining apical-basal polarity. Knockdown of Pals1, causes severe defects in tight junctions and mislocalization of adherens junctions and exocyst complexes impairing cell polarity (Wang et al., 2007).

1.2.4.2.2 Membrane-associated guanylate kinase inverted proteins (MAGI)

MAGI proteins have a similar domain structure to MAGUK proteins but their order is inverted. The GUK domain in MAGI proteins is N-terminal to its multiple PDZ domains. MAGI

proteins also have two WW domains instead of the SH3 domain found in most MAGUK proteins (Figure 1.2). Three members of this family have been described so far: Magi-1/Bap-1, Magi-2, and Magi-3. Magi-1 has 3 isoforms that are generated from splicing variants (-1a, -1b and -1c) that localize to different cellular locations. Magi-2 acts as a scaffolding protein in synaptic junctions (Xu et al., 2001) and it is critical in the formation and maintenance of the kidney glomerular barrier (Ihara et al., 2014). In epithelial cells, Magi-2 and -3 localize to tight junctions, where they form a complex with other cytoplasmic tight junction protein (Vazquez et al., 2001).

1.2.4.2.3 Multiple PDZ domain protein-1 (MPDZ or MUPP-1)

MPDZ is a 221 kDa cytoplasmic protein that contains 13 PDZ domains (Figure 1.2) (Adachi et al., 2009; Poliak et al., 2002). MPDZ interacts with claudins via PDZ-10 and with JAMs via PDZ-9 (Hamazaki et al., 2002). Thus, it is believed that MPDZ behaves as a linker between claudins and JAMs. MPDZ may be also important for the localization and docking of other proteins, such as Pals1 (Roh et al., 2002). In humans, MPDZ variants are associated with autosomal recessive congenital hydrocephalus, suggesting that it plays an important role in the formation of the blood-brain barrier (Al-Dosari et al., 2013; Shaheen et al., 2017).

1.2.4.2.4 Cingulin and paracingulin

Cingulin and paracingulin are 149 kDa phosphoproteins that are structurally similar. They have a coiled-coil structure similar to the coiled-coil regions of myosin heavy chains, a globular tail domain, and an N-terminal globular head region (Figure 1.2) (Cordenonsi et al., 1999; Guillemot and Citi, 2006). Depletion of cingulin or paracingulin from cells revealed that they are not essential for tight junction maintenance, but both play a role in the regulation of small GTPases, such as Rac1 or RhoA, by recruiting their activators (Aijaz et al., 2005; Guillemot and Citi, 2006; Guillemot et al., 2004; Guillemot et al., 2008). Cingulin is only located at the tight junction, while paracingulin has been detected both at tight and adherens junctions (Citi and Cordenonsi, 1998; Citi et al., 1988; Ohnishi et al., 2004). Although cingulin and paracingulin interact *in vitro*, there are differences in the way that they are recruited to the tight junction, suggesting that they are controlled and regulated independently (Paschoud et al., 2011).

Cingulin's coiled-coil domain forms parallel dimers that can aggregate with other cingulin dimers; while their globular head domain interacts with ZO-2, ZO-3, afadin-6, actin or JAMs (Bazzoni et al., 2000). Interactions between cingulin and ZO-1 appears to require different domains along the cingulin molecule and is finely modulated. In fact, overexpression of cingulin causes mislocalization of ZO-1 (D'Atri et al., 2002). Cingulin's localization and its ability to interact with actomyosin and the planar apical microtubule network, suggests that it may link tight junctions to the cytoskeleton (Stevenson et al., 1989; Yano et al., 2013). In summary, cingulin and paracingulin carry out two functions: they regulate small GTPases in the cytoplasmic plaque of the tight junction, and link the tight junction proteins to the actin cytoskeleton.

1.2.4.2.5 Afadin-6 (AF-6 or MLLT4)

AF-6 is a 205 kDa polypeptide that co-localizes with ZO-1 in cultured *Xenopus* epithelial cells and is present at tight junctions and adherens junctions (Yamamoto et al., 1997). AF-6 contains a PDZ domain, two RAS-binding domains, a myosin-V domain, and one kinesin-like cargo binding domain (Figure 1.2) (Kuriyama et al., 1996). *In vitro* binding analyses showed that the RAS domain of AF-6 directly interacts with ZO-1, and inhibiting this interaction activated RAS. The same PDZ-domains of AF-6 that binds to ZO-1 also directly interact with JAMs, suggesting that these interactions are likely to be mutually exclusive and therefore involved in different functions (Ebnet et al., 2000). In fact JAMs do not localize to the tight junction in cells lacking AF-6, suggesting that AF-6 and JAMs could coordinately facilitate immune cell transmigration (Ebnet et al., 2000). AF-6 also interacts with cingulin (Cordenonsi et al., 1999). Null mutations of AF-6 disrupt epithelial interaction and cell polarity during mouse development (Zhadanov et al., 1999), thus revealing the requirement for AF-6 in cell-cell contacts at both tight and adherens junctions.

1.3 THE CLAUDIN FAMILY AND THEIR ROLE IN THE TIGHT JUNCTION

Claudins are the largest family of integral tight junction proteins and are the predominant contributors to the functional properties of tight junction barriers. This makes them the ideal target to manipulate tight junction barrier properties. Indeed, the goals of my thesis research are to understand the unique contributions of individual claudins to the tight junction cytoplasmic

plaque, and to study tools that can be used to manipulate claudin localization at the tight junction. Here, I will define their structure, classification, evolution, and interactions. I will focus particularly on the claudin family members that I have studied during my thesis; Cldn1, -2, -3, -4, -7 and -8.

As described above, occludin was the first integral protein identified that localized to the tight junction. However, the discovery that it was unable to initiate formation of tight junction strands led to the search for additional transmembrane proteins that may be involved in tight junction formation (Furuse et al., 1996; Furuse et al., 2002). Furuse and colleagues isolated Cldn1 and -2 from chicken liver tight junction membrane fractions (Furuse et al., 1998a). They showed that claudins were sufficient to generate tight junction-like strands when transfected into L-fibroblast cells that lack endogenous tight junctions. Their data suggested that claudins were essential and responsible for the formation and function of tight junctions (Furuse et al., 1998a; Furuse et al., 1998b). Subsequently, the importance of other tight junction proteins in tight junction formation and regulation has been shown, but claudins are still considered to be indispensable for the formation of tight junction strands and the main players that determine barrier function (Tsukita and Furuse, 1999; Umeda et al., 2006). Even though transfection of a single claudin is sufficient to form a tight junction, they give rise to different kinds of strands associated with different barrier properties. To date, 28 different members of the claudin family have been described in vertebrates (Piontek et al., 2020b). A more in-depth description is provided for the claudins that were studied in this thesis: Cldn1, -2, -3, -4, -7 and -8. The reason for the selection of these specific claudins is that Cldn3, -4, -7 and -8 are targets of the C-terminal domain of *Clostridium perfringens* enterotoxin (C-CPE), a commonly used tool to deplete claudins from tight junctions. We used the C-CPE peptide to demonstrate that Cldn3, -4 and -8 are required for neural tube closure (Baumholtz et al., 2017). Cldn1 and -2 are used as representatives of C-CPE-insensitive claudins. In addition, for several of my studies, I used the Madin-Darby canine kidney cells type II (MDCK II) cell line that express Cldn1, -2, -3, -4 and -7; and mouse inner medullary collecting duct cells (mIMCD) that express Cldn1, -2, -3, -4, -7 and -8.

1.3.1 Claudin structure

Claudins are members of the PMP-22/EMP/MP20/claudin superfamily (Anderson and Van Itallie, 2009). They are tetraspan transmembrane proteins that vary in length from 207 to 305 amino acids (Figure 1.3). They have a short cytoplasmic amino terminus, four transmembrane domains, two extracellular loops, a short intracellular loop, and a long intracellular C-terminal tail (Figure 1.3) (Piontek et al., 2020b).

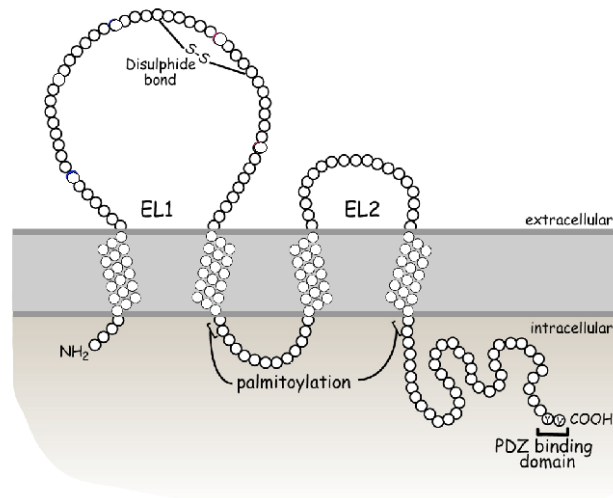


Figure 1.3: Schematic representation of a claudin molecule. Claudins are tetraspan proteins (4 transmembrane domains of 20 to 30 amino acids) that form 2 extracellular loops where the first extracellular loop is 49-59 amino acids and the second extracellular loop is 15-35 amino acids. Claudins have two cytoplasmic domains: a short ~7 amino acid N-terminal domain, except Cldn16, and a longer C-terminal domain that is 27-65 amino acids. Most post-translational modifications occur in the C-terminal domain, which interacts cytoplasmic proteins through their PDZ-binding domain.

The cytoplasmic N-terminal domain is usually only 7 amino acids, except for Cldn16, which has a long N-terminal domain of ~ 70 amino acids. Not much is known about the function of the N-terminus except that it may be processed once the claudin is inserted in the membrane (Hou and Goodenough, 2010).

The first extracellular loop is 49 to 59 amino acids. It has a conserved motif (W-GLW-C-C) where the cysteine disulfide bond acts to stabilize the loop. Crystal structures revealed that the first extracellular loops of claudins interact with each other to form a tetramer that is involved in the formation of the intercellular pore (Piontek et al., 2020b; Suzuki et al., 2014; Suzuki et al., 2017; Vecchio and Stroud, 2019). The characteristics of the pore depend on the specific amino

acid sequence within the first extracellular loop. The charged amino acids present in the first extracellular loop determines charge of ions and small molecules that can pass through the tight junction. Therefore, some claudins will form pores that are selective for cations or anions, and others create neutral pores, or can act as barriers for both cations and anions (Fuladi et al., 2020; Piontek et al., 2017; Piontek et al., 2020b; Suzuki et al., 2017; Vecchio and Stroud, 2019).

The second extracellular loop is smaller than the first loop and usually contains 15-35 amino acids. This loop is involved in *trans* interactions with claudins in the apposing cell and *cis* interactions with claudins in the same cell (Piontek et al., 2008; Suzuki et al., 2014). The size of this domain is usually related to the fibrils formed and to the tightness of the barrier. Cldn16, which has the longest second extracellular loop (35aa), forms leaky barriers that are permissive to the passage of cations (Hou and Goodenough, 2010). In contrast, Cldn11, which has the shortest second extracellular loop (15aa), creates very tight barriers, such as the blood-testes-barrier or the barriers formed in myelin sheaths (Morita et al., 1999a).

The four transmembrane domains are rich in hydrophobic amino acids, and each contains 20-30 amino acids. They participate in *cis* interactions with claudins in the same cell and are essential for the proper folding of the claudin (Rossa et al., 2014). For each claudin, the different transmembrane domains seem to participate in different interactions. For example, chimera studies with Cldn2 showed that the second transmembrane domain is necessary for *cis*-dimerization (Van Itallie et al., 2011), while mutational analysis showed that transmembrane domains 3 and 4 are responsible for the *cis*-interactions between Cldn16 and Cldn19 (Gong et al., 2015a).

Finally, the cytoplasmic C-terminal domains, which are 27 to 65 amino acids, interact with scaffolding proteins that bridge the tight junction to the actin-cytoskeleton. The cytoplasmic carboxy tail is the most heterogeneous region between claudin family members. Most claudins have a PDZ binding domain at their C-terminus, with the exception of Cldn12, -19, -21, and -24 to -27. The PDZ binding domain is thought to be the link between claudins and the PDZ domains of scaffold machinery in the cytoplasmic plaque; removal of the terminal two amino acids of the C-terminal domain (YV) prevents interactions with known cytoplasmic plaque proteins (Fredriksson et al., 2015; Hamazaki et al., 2002; Itoh et al., 1999; Jeansonne et al., 2003; Nomme

et al., 2015). This tail is also the target of many post-translational modifications that regulate claudin interactions, stability, barrier properties, and tight junction localization (D'Souza et al., 2005; Fujibe et al., 2004; Ikari et al., 2006; Nunbhakdi-Craig et al., 2002; Soma et al., 2004; Van Itallie et al., 2005).

1.3.2 Classification of claudins

Historically claudins are classified in different groups using different criteria. In terms of primary amino acid sequence, the overall identity is only 2%, and the only conserved motif is the one responsible of establishing the internal disulfide bond in the first extracellular loop. However, when compared in pairs they are 30-70% identical. In fact, 2 groups can be formed based on claudin sequence identity: “classic” claudins (Cldn1 to -10, -14, -15, -17 and 19) that have a higher identity and similarity, and “non-classic” claudins (Cldn11, -12, -13, -16, -18 and -20 to -27) that exhibit more variability and differ from the “classic” claudins. Claudins can also be classified according to whether they promote barriers that are cation- or anion-specific, or create barriers that form a tight seal that prevents the passage of any molecule. Claudins that create cation-permissive channels are Cldn2, -10b and -15, while Cldn10a and Cldn17 create anion permissive channels. Claudins that promote tighter junctions and prevent the passage of any molecule across the paracellular space are Cldn1, -3, -5, -11, -14, -18 and -19. Other claudins (Cldn4, -6, -9, -12, -13, -16 and -20 to -27) are classified as ambiguous and their effect on paracellular movement appears to depend on the tissue or conditions studied (Gunzel and Fromm, 2012; Piontek et al., 2020b). Lastly, claudins can also be classified in term of phylogeny (Figure 1.4), in this case they fall into 8 groups and 4 major clusters: Cluster 1 = Cldn3, -4, -5, -6, -8, -9 and -17; Cluster 2 = Cldn1, -2, -7, -14, -19 and -20; Cluster 3 = Cldn10, -11, -15 and -18; and Cluster 4 = Cldn12, -16, 21, -22, -23, -24, -25, -26 and -27 (Gunzel and Fromm, 2012).

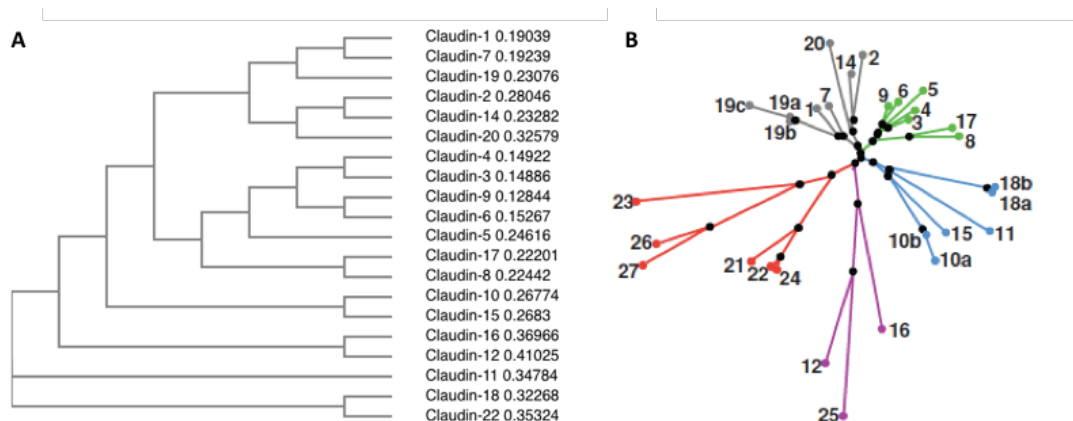


Figure 1.4: Phylogeny tree of claudins. A. Phylogenetic tree of human claudins using protein sequences from UNIPROT (except Cldn21, -23, -24, -26 and -27, which are not annotated). B. Phylogeny classification of human claudins using DNA sequences from NCBI. Figure taken with permission from Gunzel 2012 (Gunzel and Fromm, 2012).

1.3.3 Claudin evolution, genomic localization and comparison between species

Evolutionarily tight junctions are restricted to vertebrates. However tight junction-like structures, called septate junctions can be found in invertebrates with the exception of *C. elegans* that only have adherens junctions (Banerjee et al., 2006; Jonusaite et al., 2016). Moreover, claudin-like proteins can be found in these septate junctions or in the adherens junctions in many if not all invertebrate species, including sponges, invertebrates, lower chordates and *C. elegans* (Lal-Nag and Morin, 2009; Mukendi et al., 2016; Simske, 2013). For instance, *Drosophila* septate junctions have three claudin-like molecules: Sinuous, Kune-Kune, and Megatrachea that are involved in the regulation of the septate junction (Behr et al., 2003; Wu et al., 2004). These claudin-like proteins function similarly to their claudin counterparts and are able to link the cytoskeleton to the junction, and have roles in controlling barrier permeability, cell adhesion and coordinated response to stimuli (Nelson et al., 2010; Wu et al., 2004).

More than 28 claudin family members have been described in vertebrates but not all members are present in all species. The variation between species is thought to come from gene duplication events. For example, Cldn3 and Cldn4 are thought to be the result of a recent gene duplication event. They are located on the same chromosome very close to each other and share 66% identity (Loh et al., 2004). Similarly, Cldn8 and -17, and Cldn6 and -9 also appear to be a recent duplication events as they are tightly linked and these pairs share 55% and 70% identity,

respectively (Loh et al., 2004). Evolutionary this has meant that each species has different numbers of claudin genes. For example, humans have 23 claudins and 2 claudin-like proteins, while chickens have only 19 claudins, and *Xenopus* has 20 claudin family members. Cldn-13 is absent in humans but present in rodents, while chickens lack Cldn6 and -7 (Baltzegar et al., 2013; Collins et al., 2013). On the other hand, there are other species of vertebrates, such as the teleost fishes, where genomic duplications led to their having more than 64 claudin genes (Kolosov et al., 2013). In general, claudins have very few transcriptional variants. Claudin genes contain one to six exons, but many claudin proteins are encoded by a single exon. The exceptions to this are Cldn1, -7, -10, -11, -15, -16, -18, -19 and -25.

1.3.4 Claudin interactions

Mutagenesis and crystal structure analyses show that claudins interact with each other through *trans*- and *cis*-interactions. Both homo- and heterotypic interactions between different claudin family members have been identified. Interestingly, some have more affinity for themselves (homotypic interactions) while other preferentially interact with other claudin family members (Gunzel and Fromm, 2012). The interaction between claudin family members can be critical for their integration into the tight junction. For example, the interaction between Cldn4 and -8 is essential for their recruitment and integration into the tight junction (Fujita et al., 2012; Gong et al., 2015b; Hou et al., 2010). Interactions can also affect claudin function. The function of Cldn14, -16 and -19 is affected by their interaction with one another. In the kidney, Cldn16 forms a pore that mediates calcium reabsorption, however the presence of Cldn19 is required (Gong et al., 2015a; Hou and Goodenough, 2010). In contrast, Cldn19 behaves as a barrier sealing claudin when it is alone in a tight junction. Cldn14 adds a level of complexity to the Cldn16 and -19 interaction, since when Cldn14 is present it binds to Cldn16 and prevents its interaction with Cldn19, preventing formation of a calcium channel (Hou, 2012; Hou and Goodenough, 2010; Hou et al., 2009). Moreover, in lung, Cldn5 impairs the ability of Cldn18 to interact with ZO-1, destabilizing tight junctions (Lynn et al., 2020).

Claudins -1 to -5 are the best studied, mainly because they were the first claudins identified and the most common claudins studied in different tissues. Cldn16 has been studied

in depth due to its role in the calcium transport at the kidney nephrons. Moreover, Cldn11 interaction has been investigated by different groups due to the fact it is the claudin with the shortest EL2, and it is the main player in one of the tightest barriers, the blood testes barrier. A summary of all *cis*- and *trans*-interactions claudin-claudin interactions studied by different groups can be found in Figure 1.5 (Blasig et al., 2006; Coyne and Bergelson, 2005; Coyne et al., 2003; Daugherty et al., 2007; Furuse et al., 1999; Gunzel and Fromm, 2012; Hou et al., 2009; Hou et al., 2010; Inai et al., 2010; Lim et al., 2008b; Milatz et al., 2017a; Milatz et al., 2017b; Piontek et al., 2011; Van Itallie et al., 2011).

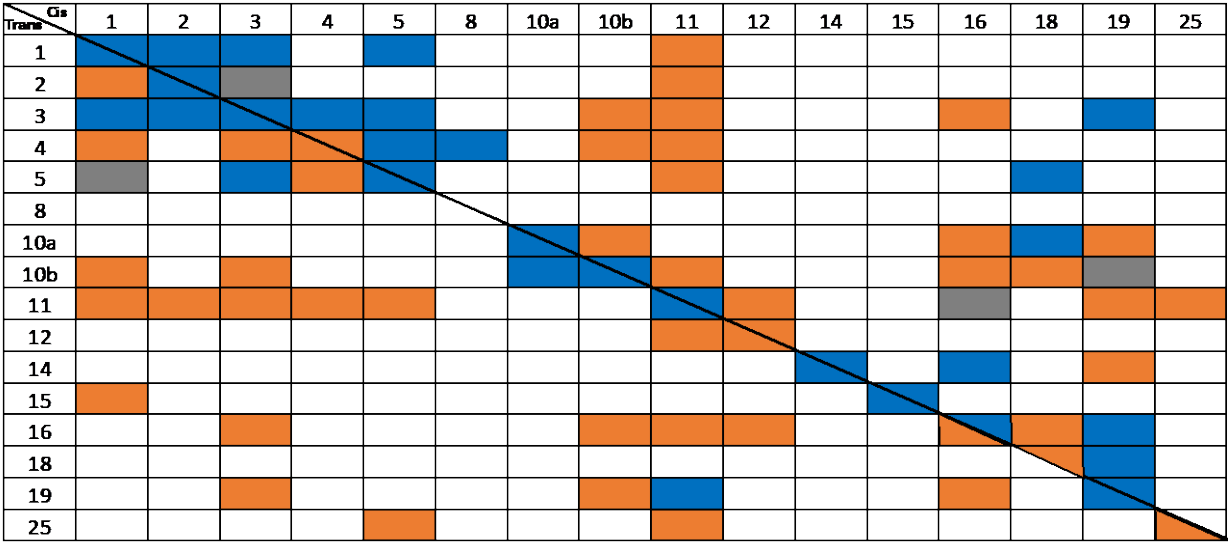


Figure 1.5: Summary of *cis*- and *trans*-interactions between claudin family members. Data for *cis*- and *trans*-interactions are shown above and below the line, respectively. Blue indicates interactions between specific claudins that have been demonstrated experimentally. Orange indicates interactions that were not able to be shown experimentally. Filled grey boxes demonstrate controversial interactions that may be context dependent. White indicates interactions that have not been studied.

Claudins also interact with other tight junction proteins either through their extracellular loops or their C-terminal domains. For instance, claudin and occludin are able to interact through their extracellular loops. Of particular interest with respect to my thesis research is the C-terminal domain and more specifically its PDZ-binding domain that interacts with cytoplasmic plaque proteins such as ZO-1, ZO-2, ZO-3 (Itoh et al., 1999), PATJ (Roh et al., 2002) and MPDZ (Hamazaki et al., 2002). In fact, the C-terminal domain interaction with zona occludens proteins is essential for claudin localization at tight junctions, and mutations of the tail can affect claudin localization.

For example, mutations in Cldn16 that affect its PDZ binding domain, prevent its interaction with ZO-1 and result in its degradation (Muller et al., 2003). In contrast, other claudins, such as Cldn1 and -5, require the cytosolic tail but not the PDZ binding domain to localize to the tight junction (Ruffer and Gerke, 2004). In addition, although interaction with ZO-1 has been reported and validated for Cldn1 to -8, not all claudins have the same affinity for cytoplasmic proteins. Claudins with tyrosine at the -6 position relative to their C-termini appear to have a higher affinity for proteins such as ZO-1. Additionally, phosphorylation of claudin or ZO-1 can affect their binding. For example, non-phosphorylated versions of Cldn2 bind with higher affinity than phosphomimetic versions (Nomme et al., 2015).

Claudins also interact with other extracellular proteins not involved in the tight junction and can act as a receptor for different viruses and bacteria allowing them to penetrate through the epithelial barrier. For instance, Cldn4 was first identified as the receptor for *Clostridium perfringens* enterotoxin (CPE) that binds to their second extracellular loop to create a pore and kill the cells (Katahira et al., 1997). Since that time CPE has been shown to interact with Cldn3, -4, -6, -7, -8 and -14 in different systems (Sonoda et al., 1999). The first extracellular loop of Cldn1 is the target receptor for Hepatitis C virus and facilitates its entry into liver cells (Liu et al., 2009).

1.3.5 Claudin-1

Claudin-1 has 211 amino acids and is encoded by 4 exons. Cldn1 protein is present in most epithelial and endothelial tissues. It is considered a barrier claudin because *in vitro* overexpression increases transepithelial resistance (TER), while removal of Cldn1 decreases TER and increases paracellular permeability (Grosse et al., 2012; Inai et al., 1999; McCarthy et al., 2000). Cldn1 has a higher affinity for other barrier forming claudins, and seems to not interact with channel forming claudins (Figure 1.5). Cldn1 was the first claudin shown to be able to interact and pull-down ZO-1, ZO-2 and ZO-3 (Itoh et al., 1999), but in fact its affinity for ZO-1 is low compared to other claudins, due to the lack of a tyrosine in position -6 (Nomme et al., 2015).

Important roles have been reported for Cldn1 in skin, liver and cholangiocytes and it has been linked to different human diseases. For instance, low levels of CLDN1 or single nucleotide polymorphisms in *CLDN1* are associated with atypical dermatitis, leukoaraiosis and vessel

vascular dementia (Asad et al., 2016; Bergmann et al., 2020; Srinivasan et al., 2017; Yadav and Shin, 2015). *CLDN1* mutations have also been linked to a higher risk of colorectal cancer or triple-negative breast cancer development (Chen et al., 2015; Hahn-Stromberg et al., 2014). Mutations in *CLDN1* cause neonatal ichthyosis and sclerosing cholangitis syndrome (NISCH), a disease mainly affecting the skin and liver (Feldmeyer et al., 2006; Hadj-Rabia et al., 2004). The skin phenotype seen in patients was confirmed with the *Cldn1* knockout mice, which die of dehydration in the neonatal period due to water loss in the epidermis (Furuse et al., 2002).

In summary, *Cldn1* is ubiquitously expressed and acts mainly as a barrier that prevents water loss across the paracellular space. This claudin has been one of the most studied due to the fact that was first identified and that it is expressed by most tissues and models.

1.3.6 Claudin-2

Claudin-2 has 230 amino acids and is encoded by a single exon. *In vitro*, *Cldn2* appears to be localized in mature tight junctions, and its localization is only observed when cells achieve optimal confluency (Amoozadeh et al., 2018). *Cldn2* functions as a cation pore that allows the transit of sodium, potassium, calcium and water across the paracellular space (Rosenthal et al., 2010; Yu, 2009; Yu et al., 2010). *Cldn2* overexpression in cell lines decreases TER and increases paracellular permeability, while silencing *CLDN2* generates the opposite effect, decreasing paracellular permeability and increasing TER, with a loss of sodium transport (Amasheh et al., 2002; Amoozadeh et al., 2015; Borovac et al., 2012; Furuse et al., 2001; Hou et al., 2006; Tokuda and Furuse, 2015). In addition to being a cation pore, *Cldn2* was shown to enhance water permeability when it was overexpressed in the kidney proximal tubule MDCK C7 cell line (Gunzel and Yu, 2013; Rosenthal et al., 2010). Moreover, although the specific interactions have not been investigated, the presence of both *Cldn4* and *Cldn8* excludes *Cldn2* from the tight junction, suggesting a mechanism of regulation of this claudin at the tight junction (Angelow et al., 2007; Capaldo et al., 2014).

Different *Cldn2* knockout mouse models have been used to understand *Cldn2* function. *Cldn2* knockout mice have decreased *trans*-epithelial reabsorption of sodium, chloride and water in the nephron. However, when these mice are challenged with a saline load, fractional excretion

of sodium and chloride are increased and the mice develop hypercalciuria (Muto et al., 2010). Analysis of a second *Cldn2* null mouse model suggested that these mice develop nephrocalcinosis as a consequence of defective reabsorption of sodium and calcium in the kidney and intestine (Curry et al., 2020). Further analyses showed that *Cldn2* is essential for bile secretion, and that its absence leads to gallstones (Matsumoto et al., 2014). In addition, *Cldn2* is essential in the intestine. *Cldn2/Cldn15* double-knockout mice die shortly after birth due to malabsorption attributed to overt hypoglycemia presumably caused by a disruption in sodium flow essential for glucose intestine absorption (Wada et al., 2013). *Cldn2* also seems to regulate proliferation and its conditional overexpression in colon cells results in an enlarged colon and increased cell proliferation (Ahmad et al., 2014).

In humans, *CLDN2* levels and mutations have been related with diseases that affect kidney, intestine, pancreas and epididymis. Single nucleotide polymorphisms in *CLDN2* introns and 3'-UTR are associated with a higher risk of nephrocalcinosis and kidney stone formation (Curry et al., 2020). A gain-of-function missense mutation in the second extracellular loop of *CLDN2* (Gly161Arg) that is predicted to cause a reduction in *cis*- and *trans*-interactions has been associated with obstructive azoospermia due to defects in the epididymal junctions (Askari et al., 2019). Meta-analysis of different polymorphisms revealed that a polymorphism in *CLDN2* is associated with a higher risk of acute pancreatitis in Caucasian populations (Aghdassi et al., 2015; Avanthi et al., 2015; Deng and Li, 2020; Derikx et al., 2015; Giri et al., 2016; Paliwal et al., 2016; Soderman et al., 2013; Whitcomb et al., 2012). In humans, *CLDN2* is upregulated in adult inflammatory bowel and celiac disease, and downregulated in child graft-versus host disease (Ishimoto et al., 2017; Ong et al., 2020; Weber et al., 2008).

In summary, *Cldn2* is one of the main claudins involved in cation and water transport. Interestingly, *Cldn2* is the only claudin observed in mature tight junctions but not usually in newly formed tight junctions.

1.3.7 Claudin3

Claudin-3, also known as erythrocyte vesicle protein 1, has 220 amino acids in humans and it is encoded by a single exon; no isoforms have been described. Cldn3 behaves as a barrier claudin; its overexpression in MDCK-I and MDCK-II cells increases TER and decreases paracellular permeability of macromolecules, anions and cations exchange without affecting water permeability (Milatz et al., 2010).

Cldn3 is found in specialized barriers, such as the blood-brain-barrier and the blood-testes-barrier and although *Cldn3* knockout mice are viable, they were shown to develop cholesterol gallstones that contain a core of calcium salt. Moreover, these mice have increased paracellular permeability of phosphate ions, which may be causative of the gallstone onset (Tanaka et al., 2018). *Cldn3* knockout mice also have leakage of sweat from the sweat gland that contributes to their atopic dermatitis (Yamaga et al., 2018). Surprisingly, they did not have any brain or fertility phenotypes, suggesting that the absence of Cldn3 in these barriers may be compensated by other claudins (Castro Dias et al., 2019; Chihara et al., 2013). Mice that were deficient for Cldn3 in the blood-testes-barrier have a delay in spermatocyte migration across the blood-testes-barrier but no defect in barrier integrity (Chihara et al., 2013). Interestingly, with the exception of the CLDN3 variant that we identified in a patient with spina bifida (Baumholtz et al., 2020), no mutations in *CLDN3* have been associated with any human diseases, although its levels are increased in patients with psoriasis and some cancers (Sikora et al., 2019; Ye et al., 2019).

To sum up, Cldn3 it is a barrier claudin that is important in different tissues to ensure barrier maintenance. Moreover, in our lab we have studied Cldn3 for its role during neural tube closure where, together with Cldn4 and -8, it is essential for the proper neural tube elevation and closure in chick embryos (Baumholtz et al., 2020; Baumholtz et al., 2017).

1.3.8 Claudin-4

Claudin-4, also known as *Clostridium perfringens* enterotoxin receptor, has 209 amino acids in humans and is encoded by one exon. To date, no variants have been described. Cldn4 function appears to be context dependent: it can act as a barrier claudin or as a channel that allows chloride ions to transit across the paracellular space (Gunzel and Fromm, 2012). In rat submandibular duct cells and MDCK II cells, Cldn4 acts as a sodium barrier preventing the passage of sodium without affecting chloride transport (Michikawa et al., 2008; Van Itallie et al., 2001; Van Itallie et al., 2003). This role can be also seen in human lungs where Cldn4 prevents sodium transport and leakage of fluid into the alveolar space (Rokkam et al., 2011; Wray et al., 2009). Unlike other claudins, Cldn4 appears preferentially in monomers (Van Itallie et al., 2011); however, when Cldn4 interacts with Cldn8, the complex forms a chloride pore, and excludes Cldn2 from the tight junction. This function can be seen in the kidney collecting duct, where it is involved in the paracellular reabsorption of chloride ions (Angelow et al., 2007; Capaldo et al., 2014; Hou et al., 2010).

Cldn4 has been relatively well studied with respect to its interactions at the cytoplasmic face of the tight junction. It interacts with MPDZ (Lanaspa et al., 2008) and zona occludens family members via its C-terminal PDZ binding domain. Cldn4's N-terminal and C-terminal interactomes were characterized using a BioID approach (Fredriksson et al., 2015). Cldn4 interacted with a similar range of candidates as those identified for occludin, including tight and adherens junction associated proteins, signaling proteins, kinases, phosphatases, proteins involved in endocytosis, exocytosis and trafficking, and proteins involved in cytoskeletal regulation. Surprisingly, interactions with ZO-1 and ZO-2 were not observed in this study.

Cldn4 null mice have increased mortality due to late onset hydronephrosis that occurs as a consequence of a maintained increased calcium and chloride fractional excretion and a progressive urinary tract obstruction due to urothelial hyperplasia (Fujita et al., 2012). *Cldn4* null nephrons also have reduced Cldn8 expression, which would be expected because in this tissue Cldn4 and -8 appear to form an heteromeric pore (Hou et al., 2010). *Cldn4* knockout mice do not have abnormal lungs, although a small increase in permeability and decreased alveolar fluid clearance was observed. However, in the context of hyperoxia *Cldn4* knockout mice exhibit a

greater degree of lung injury than wild-type littermates (Kage et al., 2014). Interestingly, mutations in *CLDN4* have not been associated with disease in humans, which suggests that they are either lethal or Cldn4 deficiency can be compensated by other claudins.

In summary, Cldn4 acts as a barrier or as an anion pore depending on which other claudins are present at the tight junction. As mentioned above together with Cldn3 and -8, Cldn4 is essential for proper neural tube closure in chick embryos (Baumholtz et al., 2017).

1.3.9 Claudin-7

There are two distinct isoforms for Cldn7. Functional differences between the isoforms are not reported. The larger isoform has 211 amino acids and is encoded by 4 exons and 2 variants with different 5'UTR. The mRNA encoding the shorter isoform lacks exon-3 and gives rise to a 158-amino acid protein in humans. Unlike other claudins, Cldn7 is localized basolaterally (Coyne et al., 2003; Dube et al., 2007; El Andalousi et al., 2020; Fujita et al., 2006; Li et al., 2004). Cldn7 has been reported to be a cation selective claudin, and as either an anion barrier or an anion selective claudin, based on the cell line or tissue being studied. In addition to its role in tight junction formation, Cldn7 has been shown to regulate cell proliferation and migration in human colorectal cancer HCT116 and in rat pancreatic cancer cell lines (Kyuno et al., 2019b; Li et al., 2019).

Cldn7 null mice die 12 days after birth with urinary sodium chloride and potassium wasting, dehydration and growth retardation, suggesting an important role for Cldn7 in the reabsorption of ions in the kidney (Fan et al., 2019; Tatum et al., 2010). Cldn7 is highly expressed in the nephric duct and the ureteric bud during branching morphogenesis of the kidney. However, Cldn7 null mice do not have any defects in *de novo* nephron formation, suggesting that the primary function of Cldn7 is to regulate paracellular transport and that if it has a function during kidney morphogenesis, it is compensated by other claudins (Khairallah et al., 2014).

Complete and intestinal-specific *Cldn7* null mice were used to study the role of Cldn7 in the intestine. Both died during the perinatal period. Analysis of the intestine revealed a disappearance of the mucosal gland structure, connective tissue hyperplasia, adenomas and inflammatory cell infiltration. The inducible conditional *Cldn7* knockout mouse was normal at

birth but following deletion of *Cldn7* it develops a similar intestinal phenotype to the other *Cldn7* knockout mice and dies (Xu et al., 2019). *Cldn7* knockout or conditional knockout mouse lines have been used as an inflammatory bowel disease-like model. In these studies, mice also developed inflammation, hyperplasia, increased colonic barrier permeability and intestinal epithelial damage including loss of crypts stem cell (Li et al., 2018; Tanaka et al., 2015; Xing et al., 2019). In humans, two *CLDN7* polymorphisms are associated with colon cancer differentiation and lymph node involvement but associations to other diseases have not been identified (Hahn-Stromberg et al., 2014).

To summarize, *Cldn7* behaves as a barrier claudin and unlike other claudins it can be localized basolaterally.

1.3.10 Claudin-8

Claudin-8 has 225 amino acids in humans and is encoded by a single exon. *Cldn8* tight junctions behave either as a sodium barrier or as a chloride channel. In MDCK II and kidney collecting duct cultured cells, *Cldn8* expression reduces paracellular permeability of cations but not of anions; while depletion of *Cldn8* causes loss of paracellular chloride permeability (Hou et al., 2010; Sassi et al., 2020; Yu et al., 2003). The different characteristics of barrier expressing *Cldn8* is probably caused by the other claudins present in the barrier. For instance, *Cldn8* interacts with *Cldn4* and -12 forming impermeable tight junction in the bladder (Acharya et al., 2004). On the other hand, in the nephron it interacts in *cis* with *Cldn4* and forms a complex that mediates chloride transport (Hou et al., 2010).

A complete *Cldn8* knockout has not been reported. Tissue specific knockout of *Cldn8* in the kidneys led to the development of hypertension, hypokalemia and metabolic alkalosis, showing the importance of *Cldn8* in ion reabsorption (Gong et al., 2015b). On the other hand, upregulation of *Cldn8* in the colon leads suggests that it has a barrier role in this tissue (Amasheh et al., 2009b). In humans, mutations of *CLDN8* have been associated with neural tube defects (Baumholtz et al., 2020).

In summary, Cldn8 can behave as a barrier or as an anion pore depending on the tissue context and the presence of other tight junction members. Together with Cldn3 and -4, it plays important roles in neural tube morphogenesis and cell shape changes (Baumholtz et al., 2017).

1.3.11 Regulation of tight junction function through post-translational modification of claudins

Tight junction proteins are regulated by many different mechanisms that affect their individual components including post-translational modification. As part of my thesis research, I investigated if the tools that I used to target claudins were acting through different signaling pathways that were involved in post-translational modification of claudins, thereby modifying the tight junction properties. Post-translational modifications can dynamically affect stability, or modify the interactions or function of components of the tight junction, ultimately affecting the tight junction barrier properties. Phosphorylation of serine, threonine and tyrosine residues has been reported for several tight junction proteins, and often affects the barrier properties. For instance, WNK kinases are known to induce phosphorylation of different claudins (Gonzalez-Mariscal et al., 2008). PKC or PKA also phosphorylate claudin, occludin or ZO-1 and affect their localization (Clarke et al., 2000a; Clarke et al., 2000b). Another kinase, MAPK can mediate both tight junction opening and tight junction sealing by targeting occludin or claudins such as Cldn6, -11 or -12. The consequence of MAPK activation will be different depending on which family member is activated or which tissue is studied (Chiba et al., 2012; Kevil et al., 2000; Thakre-Nighot and Blikslager, 2016; Yang et al., 2015). Also, PI3K, which is known to be associated with ZO-1 or occludin can modify barrier properties upon activation. These modifications are tissue and stimuli specific (Gonzalez-Mariscal et al., 2008). Three phosphatases that are important for tight junction regulation are PP2A, PP2B and PP1. PP2A and PP1 usually trigger opening of the barrier by inhibiting aPKC ζ and reducing ZO-1, occludin and Cldn1 threonine phosphorylation state (Nunbhakdi-Craig et al., 2002; Seth et al., 2007); while PP2B usually triggers barrier sealing by decreasing the phosphorylation state of cPKC α which promotes tight junction disassembly (Andreeva et al., 2006; Lum et al., 2001).

Other post-translational modifications such as palmitoylation and glycosylation have been described in tight junction proteins. For instance, Cldn14 palmitoylation controls its

localization and mutation of the cysteines involved in palmitoylation result in mislocalization and degradation of the claudin (Lynch et al., 2007; Van Itallie et al., 2005). N- and O-glycosylation has been predicted to many residues within the EL1 of different claudins, but only confirmed in Cldn26, which is N-glycosylated in the EL1 and required for Cldn26 correct membrane localization (Maher et al., 2011). Finally, polyubiquitination has been shown to target Cldn1 for degradation (Takahashi et al., 2009).

1.3.12 Trafficking, recycling and exocytosis of claudins

Some of the tools that I used for my thesis research are hypothesized to specifically remove claudins from tight junctions. Prior to describing these tools, it is important to consider our current knowledge regarding the trafficking, recycling and exocytosis of claudins at the tight junction.

1.3.12.1 Trafficking of claudins

Newly synthesized claudins are transported from the endoplasmic reticulum via Golgi to the *trans*-Golgi network, and then delivered to the apical or basolateral domains of the cell membrane. Studies in cell lines suggest that some claudins are delivered first to the basolateral domain and then internalized and re-directed to the apical domain (Polishchuk and Mironov, 2004; Slimane and Trugnan, 2003). Thus, sorting takes place at two levels – in the *trans*-Golgi network and via endosomes. Usually, apical delivery requires the association with cholesterol-glycosphingolipid-rich domains, which makes sense since tight junctions are established in lipid rafts (Kohler and Zahraoui, 2005). Specific domains are involved in trafficking. The C-terminal claudin cytoplasmic tail of Cldn1, -5, -6 and -16 is essential for proper trafficking and its truncation leads to intracellular retention in the endoplasmic reticulum due either to protein misfolding or to the inability of adaptor proteins involved in claudin trafficking to recognize the protein (Arabzadeh et al., 2006; Muller et al., 2006; Ruffer and Gerke, 2004). Moreover, post-translational modifications such as palmitoylation or phosphorylation can be essential for proper trafficking of claudins and occludin (Van Itallie et al., 2005).

Claudins can also be delivered to the membrane from endosomes derived from the *trans*-Golgi network, from recycling or endocytosis events (Stamatovic et al., 2017). Endosomes, depending on their pH, lipid content, and which Rab-GTPases or ADP ribosylation factors (ARFs) are present, can be classified as early, late or recycling endosomes. Recycled tight junction proteins are usually trafficked in early endosomes and then are further sorted for recycling, degradation or to the *trans*-Golgi network where they mix with newly synthesized tight junction proteins (Doherty and McMahon, 2009; Elkin et al., 2016). Early endosomes are rich in RAB5, EEA1, Vps34 and PIP3 (Stamatovic et al., 2017). As the early endosome matures it acquires specific proteins that will determine the point of delivery. Syntaxins, Snares and Rab-GTPases are important for both sorting and membrane fusion (Huotari and Helenius, 2011; Platta and Stenmark, 2011; van Weering et al., 2012). For example, Rab5 has 3 isoforms, Rab5A and -B target vesicles for degradation, while RabC is mostly involved in recycling (Chen et al., 2014).

1.3.12.2 Recycling of claudins

Claudins are known to have long half-life times at the membrane but as for other membrane proteins they are eventually recycled. For instance, Cldn2 and -15 have half-lives of 9 hours and 13 hours, respectively. There is also evidence suggesting that claudins can be short lived (less than two hours) in certain models (Greene et al., 2019; Krause et al., 2008; Ramirez et al., 2013; Weber et al., 2015). Internalization of tight junction complexes occurs via different endocytic pathways followed by the sorting of the endosomes either for recycling or degradation. ZO-1, occludin, JAM-A, tricellulin, and Cldn1, -2 and -4 are internalized via clathrin-dependent endocytosis (Stamatovic et al., 2017), which is mediated by clathrin-coated vesicles that coordinate with PIP2 and dynamin to bud and form the endocytic vesicle (Lampe et al., 2016; Taylor et al., 2012). Caveolae-internalization, which occurs in lipid rafts and is usually mediated by the formation of vesicles coated in caveolin-1 (Doherty and McMahon, 2009; Stamatovic et al., 2009), is considered to be the main pathway used by endothelial cells for tight junction endocytosis, although it also functions in epithelial cell layers. Occludin, JAM-A, ZO-1 and Cldn2, -3, -4, -5 and -7 are endocytosed via caveolae (Stamatovic et al., 2017). Flotilin-dependent internalization is similar to caveolae-endocytosis, where a vesicle coated in flotilin-1 and -2 buds

and gets internalized into the cell (Doherty and McMahon, 2009; Riento et al., 2009). This mechanism has been shown to recycle occludin, ZO-1 and Cldn1, -3, -5 and -8 from tight junctions (Bowie et al., 2012; Li et al., 2008a; Li et al., 2008b). Finally, macropinocytosis is a process where the membrane ruffles and then invaginates, forming a pocket-like structure supported by actin filament polymerization that forms the vesicle. Ras GTPase, PIP3, syntaxins and motor proteins are necessary for proper macropinocytosis (Lim et al., 2008a; Sharma and Henderson, 2007; Yang et al., 2005). This endocytosis pathway has been shown for internalization of occludin, JAM-A and Cldn1 internalization (Bruewer et al., 2005; Coyne et al., 2007; Stamatovic et al., 2012).

As described above, a protein can be internalized by different mechanisms depending on the stimuli or cell studied; however, the method of internalization does not necessarily relate to their final destination. Once endocytosed, the vesicles usually fuse into early endosomes whose trafficking is regulated by Rab4 and -5 (Fletcher and Rappoport, 2014; Maxfield and McGraw, 2004; van der Sluijs et al., 1992). Early endosomes can go to the lysosome, where they will be degraded, be recycled to the basal membrane or the tight junction, or be stored in the *trans*-Golgi network, where they can be re-deployed to the tight junction upon the proper stimuli (Fletcher and Rappoport, 2014; Maxfield and McGraw, 2004; Ullrich et al., 1996). The recycling speed seems to be related to which Rabs are present in the vesicle. Rab4 is associated with fast recycling, while Rab8/Rab11 and ARF6-dependent recycling is slow (Elkin et al., 2016; Grant and Donaldson, 2009). Therefore, in some cells some tight junction proteins are in constant and continuous recycling, while in other cells or upon different stimuli recycling may be very slow. For example, in MDCK cells, Cldn1 and -2 were continuously removed and replaced in the tight junction within an hour, while Cldn4 or occludin were not endocytosed in that hour. Also in MDCK cells, degradation of Cldn1 and -2 occur over several hours or days. Both sets of data suggest that each tight junction protein may have its own recycling time and that the recycling allows for a slow turnover of the proteins present at the tight junction (Chalmers and Whitley, 2012; Dukes et al., 2011a; Dukes et al., 2012). In contrast, in MTD1A cell line, occludin is continuously endocytosed, while Cldn1 remains at the tight junction, further demonstrating the controversy and the tissue specificity of these processes (Morimoto et al., 2005).

As an alternative to recycling, tight junction proteins may be degraded, which is generally characterized by the presence of Rab7, VPS34, enriched LAMP1, MLN64 and CD63 in the endocytic vesicles (Griffiths et al., 1988; Gruenberg, 2001). In MDCK II cells, Rab14 mediates the degradation of Cldn2 (Lu et al., 2014), while in brain endothelial cells, Chemokine CCL2 induces endocytosis of Cldn5 and occludin, and the 20% that was degraded was associated with Rab7 vesicles (Knowland et al., 2014). Tight junction protein degradation is also associated with ubiquitination of some proteins, such as Cldn1, or phosphorylation of Ser490 on occludin (Murakami et al., 2009; Muthusamy et al., 2014; Takahashi et al., 2009).

In summary, tight junction proteins can be removed from the membrane by different endocytic pathways. The decision of where to go and which pathway mediates their removal and recycling or degradation, is probably dependent on the tissue, the stimuli and the modification made to the specific protein.

1.3.12.3 Exocytosis of claudins

Recently there has been increased interest in understanding the role of claudins in extracellular vesicles as they have been found in extracellular vesicles in cancer patients, and have immunoregulatory functions in healthy animals (Ikeda et al., 2021; Li et al., 2009; Thuma et al., 2016). Cldn4 was the first to be reported in extracellular vesicles from the blood of ovarian cancer patients but not in healthy controls, suggesting that it may be used as a biomarker for ovarian cancer diagnosis (Li et al., 2009). Subsequently, the presence of claudins in extracellular vesicles in other cancers has been explored. The presence of Cldn3 in extracellular vesicles is used as a biomarker for hepatic cholangiocarcinoma cancer, and the presence of Cldn7 is suggestive of colorectal cancer and possible metastasis (Ikeda et al., 2021; Kyuno et al., 2019a; Kyuno et al., 2019b; Ruiz-Lopez et al., 2018; Thuma et al., 2016). These cancer-derived extracellular vesicles seem to be involved in the onset of new microenvironments that facilitate metastatic processes (Ruiz-Lopez et al., 2018). Extracellular vesicles containing claudins have also been identified in healthy tissue. For instance, Cldn5-loaded extracellular vesicles are secreted from the blood-brain-barrier endothelium and are incorporated by leukocytes to facilitate their transmigration across the barrier (Paul et al., 2016).

In my thesis, I identified extracellular vesicles secreted by kidney-derived cells. In the kidney, extracellular vesicles and mainly exosomes, have been shown to be present both in healthy and disease individuals. In fact, Stahl and colleagues proposed that exosomes play roles in cell-cell communication between nephron segments (Stahl et al., 2019). Moreover, several studies have identified kidney-derived extracellular vesicles as biomarkers for disease or as pathogenic mechanisms suggesting a role for these extracellular vesicles in the development of the disease (Thongboonkerd, 2019).

The different pathways involved in protein exocytosis can lead to the formation of different kinds of extracellular vesicles of different sizes and composition. The most common types of extracellular vesicles are exosomes and microvesicles. Exosomes range in size from 50 to 150 nm and are generated by the formation of a multivesicular body that carries several exosomes. In contrast, microvesicles range in size from 100 to 1000 nm and are formed by the budding and shedding of the plasma membrane and are usually formed by cytoskeleton polymerization. Exosomes can be formed by different pathways but the most common are the endosomal sorting complex required for transport-dependent (ESCRT-dependent) and ESCRT-independent pathways. The ESCRT complex is formed by different proteins that lead to the formation of mature multivesicular bodies that are targeted for degradation or exocytosis depending on the components of the complex. On the other hand, the most common ESCRT-independent pathway is the controlled by nSmase. This enzyme regulates ceramide conversion and through this activity the multivesicular bodies gets loaded onto ceramide promoting their delivery to the membrane where they are exocytosed (Catalano and O'Driscoll, 2020). As mentioned above, claudins have been found in different kinds of extracellular vesicles and exosomes with different functions; however, not much data is available on how claudins are incorporated nor the mechanisms involved in the formation of these extracellular vesicles.

1.3.13 Models used in this thesis to study claudin function

Tight junctions are required in all epithelial and endothelial tissues. Here I provide a brief overview of the models that I used to study claudins, including the kidney, the neural tube, chick embryos, and cell models.

1.3.13.1 Claudins in the kidney and kidney-derived cell lines

Blood filtration takes place in the kidney nephron. Each nephron segment is specialized in the reabsorption of specific ions or water, which can occur across the membrane through active transport or via the paracellular tight junction, which is tightly regulated by the claudins present. In fact, claudins are differentially expressed along the nephron and regulate the movement of different ions across the paracellular space in different segments of the nephron. For instance, in the proximal tubule water, sodium, potassium, calcium, magnesium and chloride are reabsorbed through the paracellular space, where there are high levels of Cldn2, a water and cation transporter, and Cldn10a, an anion transporter (Yu et al., 2010). The thick ascending limb is where the highest quantities of calcium and magnesium are reabsorbed; this segment is rich in Cldn14, -16 and -19, where Cldn16 and -19 form a complex that mediates the reabsorption of calcium and magnesium (Hou and Goodenough, 2010). In fact, mutations in the Cldn14, -16 or -19 have been associated with familial hypomagnesaemia with hypercalciuria and nephrocalcinosis syndrome and with the formation of kidney stones. Finally, the more distal segment of the nephron is in charge of chloride reabsorption through the paracellular space, a process that is controlled by Cldn4 and -8, which form a stable pore known to be involved in chloride reabsorption (Hou et al., 2010; Yu et al., 2010).

During my thesis research, I used two different kidney-derived cell models: Madin-Darby canine kidney cells (MDCK II) and mouse inner medullary collecting duct kidney cells (mIMCD3), which were derived from the proximal tubule and collecting duct, respectively. MDCK II cells form cation-selective barriers and express Cldn1, -2, -3, -4 and -7 (Dukes et al., 2011b; Hou et al., 2006). TER and permeability of the mIMCD3 cell line is less well characterized, in part due to the difficulty of obtaining fully confluent monolayers and tight membranes. So far, Cldn1, -2, -3, -4, -7, and -8 have been identified in mIMCD3 tight junctions.

1.3.13.2 Claudins are required for neural tube development in chick embryos

Claudin family members exhibit both overlapping and unique expression patterns throughout chick embryogenesis, including distinct expression boundaries in different tissues such as the neural tube where claudin expression boundaries are observed at the junction of non-neural and neural ectoderm (Collins et al., 2013). Different claudin expression patterns were observed in the epithelial tissues of the kidney, lung, pancreas, gonads and heart, suggesting that they carry out important functions during development in different organs (Collins et al., 2013).

Our lab has studied the role of claudin family members in neural tube development. We discovered that they are required for morphogenesis and development of the tissue. Neural tube closure involves formation of the neural plate, bending at the midline, and elevation of the neural plate to create neural folds that fuse at the midline to generate a closed neural tube. This process requires cell rearrangements and cell shape changes to allow the formation of the median hinge point that leads to the elevation of the neural folds. Studies focused on the depletion of claudins during this process showed that *Cldn3*, -4 and -8 are essential for the cell shape rearrangement, the elevation of the neural tube and proper fusion of the neural folds (Baumholtz et al., 2017).

1.3.13.3 Claudin functions in morphogenesis

Claudins have been extensively studied for their tight junction barrier function controlling the diffusion of molecules across the paracellular space. However, several studies have shown a role for claudins in maintaining cell-cell contacts during embryo morphogenesis and that they are essential for regulating changes in cell shapes during tissue movements or cell layer rearrangements. For instance, in zebrafish and *Xenopus*, claudins maintain cell-cell contact during convergent extension (Brizuela et al., 2001; Siddiqui et al., 2010). Claudins also play a role in *Drosophila* morphogenesis, where the three claudin orthologs are essential for proper tracheal tube branching and morphogenesis, and where mutant embryos cannot form the characteristic cuboidal cell shape necessary for this process (Behr et al., 2003; Nelson et al., 2010; Wu et al., 2004). As mentioned above, claudin removal during neural tube development causes defects in convergent extension and prevents apical constriction of the cells at the midline, resulting in a

failure of neural tube closure (Baumholtz et al., 2017). This study also showed that the removal of claudins from the neural tube led to mislocalization of apical proteins, including Par-3, RhoA and CDC42. Interestingly, although these proteins could localize apically, their localization close to the cell membrane was disrupted. Therefore, the role of claudins in this events is hypothesized to be as a regulator of protein localization at the apical surface where it allows for the docking of cytoplasmic plaque proteins that signal and interact with proteins involved in morphogenesis and probably with the cytoskeleton. Thus, removal of claudins would lead to the mislocalization of key proteins necessary for a coordinated response, which could explain the importance of claudins and tight junctions in their role in morphogenesis.

1.4 TOOLS TO MODIFY TIGHT JUNCTION

Many different tools have discovered that affect tight junction composition and properties. These compounds can affect the tight junction directly by recognizing specific tight junction components or indirectly by modifying one of the diverse pathways that affects gene expression, or post-translational modification or stability of tight junction proteins.

1.4.1 Compounds that indirectly affect tight junctions

The most commonly studied factors that affect tight junctions are environmental factors that deleteriously affect and alter tight junction barriers, such as toxicants or medication, factors that prevent the deleterious effects of environmental factors, such as nutraceuticals, and secreted hormones or cytokines that can modify and alter tight junction composition. We know that many of these molecules impact signaling pathways, such as mitogen activated protein kinase (MAPK), phosphatidylinositol-3-kinase (PI3K/AKT), G-coupled protein receptor (GPCR) or 5'-AMP-activated protein kinase (AMPK), that are involved in controlling cell behavior, proliferation, cell survival and cytoskeletal movements. Others directly impact pathways involved in claudin function and tight junction cytoskeletal rearrangement, such as Ras homolog gene A (RhoA). Finally, another subset, including nuclear factor-kappaB (NF- κ B) or nuclear factor erythroid derived (Nrf2), affects pathways involved in cell proliferation and inflammation that can indirectly affect the tight junction (Burkewitz et al., 2014; Campa et al., 2015; Chen et al.,

2013; Lessey et al., 2012; Magalhaes et al., 2012; Murakami and Motohashi, 2015; Sun et al., 2015; Suzuki et al., 2012). In general, increased ROS and NF- κ B, MAPK, PI3K/AKT or RhoA signaling as well as decreased AMPK and Nrf2 signaling appear to increase barrier permeability, although their exact mechanisms are not yet well understood.

As part of my thesis research I studied the effect of quercetin, a nutraceutical that is a major component of traditional medicine. Nutraceuticals are compounds present in fruits and vegetables that have been associated with a medicinal use. Some of these are common supplements, such as folic acid, while others are in our normal diet, such as polyphenols. Folic acid is a water-soluble vitamin that has been used as a mandatory supplement in many countries, including Canada, to reduce the incidence of neural tube defects during pregnancy, and has been shown to enhance the tight junction barrier (Viswanathan et al., 2017). Many different nutraceuticals such as resveratrol, epigallocatechin gallate or curcumin have been shown to have beneficial effects and enhance and protect the tight junction against different stresses (Jo et al., 2017; Wu et al., 2015; Zhang et al., 2017; Zhong et al., 2016).

Quercetin is one of the most abundant flavonoids in most vegetables. Usually it is found conjugated to sugar derivatives forming other compounds such as rutin (quercetin-3-O-rutinoside) or quercitrin (quercetin-3-O-rhamnoside). Quercetin generates “tighter” barriers through the inactivation of NF- κ B, MAPK, RhoA and PI3K/AKT signaling pathways and activation of Nrf2 and AMPK pathways in different tissues (Chuenkitiyanon et al., 2010; Tan et al., 2018; Zhu et al., 2018). Quercetin has been widely studied for its anti-inflammatory and antioxidative properties. In fact, quercetin has been shown to be able to reduce the neural tube defects in diabetic mice through the inhibition of nitric oxidase 2 protein (NOS2). The inhibition of NOS2 led to a reduction of nitric oxide and by extension of the nitrosative stress thought to be the main cause of the neural tube defect. Quercetin also increased superoxide dismutase 1 and -2 regulating DNA damage repairing factors and ameliorating the ROS stress in the embryo (Cao et al., 2016). A similar effect was observed with quercetin-3-glucoside, a quercetin derivate that is able to prevent neural tube defects at much lower concentrations (Tan et al., 2018).

Quercetin has protective roles not only during development but also later in life where it can be used to prevent the onset or treat different diseases. In a rat model, quercetin is able to

prevent kidney stone formation, and its ability to inhibit cyst formation *in vitro* has made it one of the best candidates to ameliorate autosomal dominant polycystic kidney disease (Zhu et al., 2018). In contrast, in *Xenopus* quercetin modulates the canonical WNT signaling pathway, which led to birth defects and NTD (Amado et al., 2012). This suggests that whether quercetin has beneficial effects or has teratogenic effects may depend on the species and timing of exposure.

This differential effect is also observed when looking at the tight junctions *in vitro*, where depending on the cell line or tissue, quercetin differentially affects tight junction protein expression. For instance, in the human intestine-derived Caco-2 cell line quercetin treatment increases TER and expression of Cldn2, -4 and -5 and decreases tricellulin (Amasheh et al., 2009a; Valenzano et al., 2015). Whereas in LLC-PK cells that are derived from pig kidney cells, quercetin increases TER and decreases barrier permeability by increasing the expression of Cldn5 and -7 and decreasing Cldn2 (Mercado et al., 2013). This shows that quercetin is able to tighten the tight junction by modulating different claudins, which depend on the targeted tissue or cell line. In Caco-2 cells, quercetin increases the assembly of ZO-1, occludin and Cldn1 by inhibiting PKC gamma (Suzuki and Hara, 2009). In addition, other quercetin sugar derivatives, such as rutin, have been used to prevent oxidative stress damage resulting from cisplatin treatment or hyperglycemia (Topal et al., 2018).

1.4.2 Compounds that directly affect tight junctions

Diverse bacterial toxins and compounds derived from these toxins directly target tight junctions as a way to affect the epithelium and penetrate into the body. Some of these toxins and compounds have been used to study the role of tight junctions in different barriers. For instance, *Clostridium perfringens* enterotoxin (CPE) and a truncated form derived from its C-terminus (C-CPE) has been widely used to remove claudins and assess barrier properties. Moreover, peptides, toxin variants and antibodies have been studied to be used as tools to target specific barriers. As part of my thesis research, I developed tools to specifically target individual claudins. Here, I analyze the current knowledge and advances in this context.

1.4.2.1 CPE and C-CPE

Clostridium perfringens enterotoxin (CPE) is a single chain 319 amino acid polypeptide that is associated with *C. perfringens* food poisoning in humans (Wieckowski et al., 1994). Extensive screens to identify the CPE receptor led to the cloning of CPE-Receptor (CPE-R) that was later renamed as Cldn4 (Katahira et al., 1997). A variety of studies, including dot blot assays, pull-downs, cytotoxic assays and cellular binding assays, revealed that Cldn3, -4, -6, -7, -8 and -14 also are recognized by CPE, and can mediate cytotoxicity upon binding. Therefore, this group of claudins have been termed CPE-sensitive claudins. However, not all bind CPE with equal affinity. Cldn3, -4, -6, and -7 have a high affinity for CPE, while Cldn8 and -14 bind CPE with a lower affinity (Fujita et al., 2000; Sonoda et al., 1999). More recently Cldn9 was shown to also be able to bind to CPE (Winkler et al., 2009) and Cldn1 and -2 can interact with CPE albeit with a much lower affinity than the CPE-sensitive claudins (Kimura et al., 2010). Not all claudins have been tested: the ability of Cldn17, -21, -22, and -24 to bind to CPE has not yet been determined. Mutagenesis studies revealed that CPE binds a consensus motif located in the second extracellular loop, NP(L/M)(V/L/T)(P/A) (Winkler et al., 2009). However, not all CPE-sensitive claudins have the NP(L/M)(V/L/T)(P/A) motif. More recently the electrostatic attraction model was proposed REF. It hypothesizes that the last 12 amino acids of the second extracellular loop of CPE-sensitive claudins, which are enriched in positively-charged basic amino acids, are attracted to the negatively-charged acidic amino acids located in the C-terminal domain of CPE (Kimura et al., 2010). Given that residues of the claudin first extracellular loop have been shown to bind to CPE, it is logical to think that while the motif NP(L/M)(V/L/T)(P/A) may be sufficient to bind CPE, additional residues from both the first and second extracellular loops, and an electrostatic interaction are necessary for a more stable and functional interaction.

Upon binding to claudins, CPE can form CH-1 and CH-2 complexes. CH-1 complexes are formed by six molecules of CPE bound to claudins and occludin to create a cation selective beta-barrel pore. This pore allows the entry of calcium into the cell that promotes caspase-3 mediated apoptosis (Figure 1.6) (Chakrabarti et al., 2003; Robertson et al., 2007; Shrestha et al., 2016). In contrast, CH-2 complexes are formed when interaction of CPE with claudin and occludin leads to internalization and removal of the protein from the tight junction (Shrestha et al., 2016).

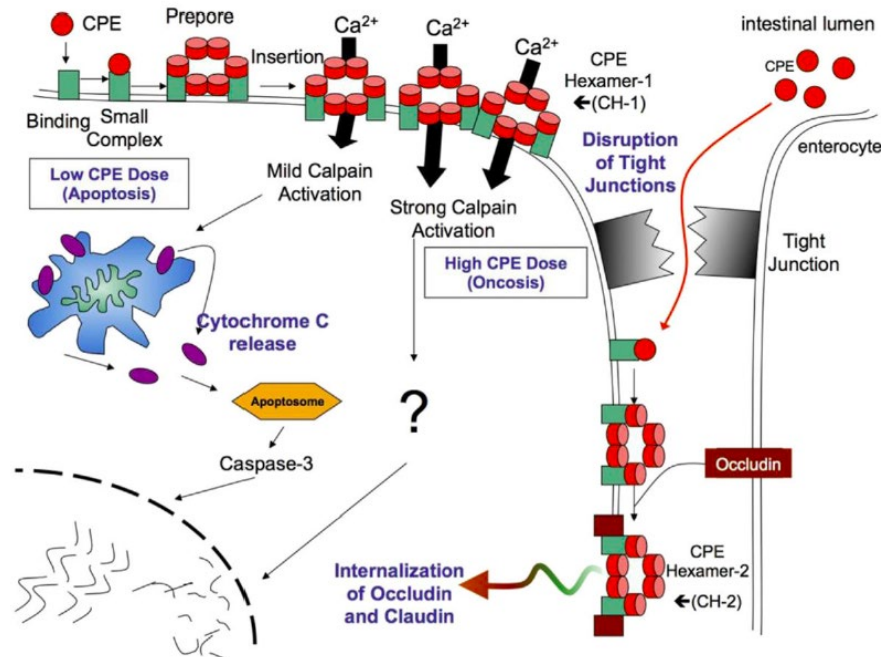


Figure 1.6: Mechanism of action of *C. perfringens* enterotoxin. *Clostridium perfringens* enterotoxin (CPE) can form 2 different complexes. CH-1, which is formed when CPE binds to claudin receptors on the surface of host cells and oligomerize into a pore. This pore will then promote the internalization of calcium and the stimulation of oncosis or apoptosis depending on the levels of CPE. On the other hand CPE can also establish CH-2 pores. These pores form in the lateral membrane and require the interaction with claudins and occludin. Instead of promoting calcium transport CH-2 complexes will promote the internalization of membrane proteins leading to the opening of the paracellular barrier. Image taken with permission from (Shrestha et al., 2016).

Structure-function analysis of CPE revealed that it is composed of different functional domains. The N-terminus is involved in the oligomerization and the cytotoxic effects, while the claudin binding domain is located in the last 30 amino acids of the C-terminal domain of CPE. In addition, a stabilization domain that is located between the cytotoxic and the claudin binding domains, is essential for the stabilization of the interaction between claudin and CPE (Hanna and McCrane, 1991; Hanna et al., 1991; Hanna et al., 1989; Kokai-Kun and McCrane, 1997; Singh et al., 2000; Smedley and McCrane, 2004). When the C-terminal 30 amino acids of CPE are removed, it becomes inactive and is unable to bind to claudins. Although the C-terminal amino acids 290-319 are sufficient for binding to claudins, longer CPE fragments (185-319) have a higher affinity for claudins suggesting the role of that sequence in the stabilization of the interaction. Moreover, this C-terminal domain (C-CPE; amino acids 185-319) does not show cytotoxic activity because it

does not contain the N-terminal cytotoxic domain. C-CPE has been widely used to study claudins and tight junctions since it can remove CPE-sensitive claudins from the tight junction without killing the cell (Saitoh et al., 2015; Shinoda et al., 2016; Takahashi et al., 2008). *In silico* modelling predicts that the absence of the N-terminal domain would prevent formation of CH-1 complexes. The presence of only CH-2 complexes could explain both the absence of cytotoxicity and the probable mechanism of action of C-CPE that should lead to the internalization of the targeted claudins (Shrestha et al., 2016; Suzuki et al., 2017).

We and others have demonstrated that C-CPE can be used to selectively and simultaneously remove a subset of claudins from tight junctions without the toxicity associated with full-length CPE (Baumholtz et al., 2017; El Andalousi et al., 2020; Sonoda et al., 1999). C-CPE is ideal to target removal of claudins: (i) it is not cytotoxic, (ii) it binds specifically to a limited subset of claudins (Cldn-3, -4, -6, -7, -8 and -14), causing their removal from tight junctions without affecting the localization of other family members, (iii) it does not affect gene expression so that upon C-CPE removal susceptible claudins can repopulate tight junctions, and (iv) it does not bind to any other cell surface proteins. Thus, phenotypes resulting from C-CPE treatments are the direct result of its interactions with claudins.

1.4.2.2 Peptidomimetics and C-CPE variants

Although C-CPE is a great tool to study claudins, it also has some limitations, including that it only targets 6 out of the 28 claudins and it does not allow selective removal of individual claudins. A number of approaches have been used to address these limitations. Site directed mutagenesis to modify the sequence of C-CPE has led to the identification of a series of C-CPE variants that predominantly target Cldn1, -2, -3, -4 or -5, and increase barrier permeability (Beier et al., 2019; Liao et al., 2016; Piontek et al., 2020a; Protze et al., 2015; Walther et al., 2012; Winkler et al., 2009). Other groups have shown that small peptides (5 amino acids) derived from the second extracellular loop of Cldn2 target Cldn2, -3 and -4, and increases barrier permeability in different cell lines (Dithmer et al., 2017; Nasako et al., 2020; Sauer et al., 2014; Staat et al., 2015; Zwanziger et al., 2012). Moreover, peptides with the exact same sequence as the C-terminal part of the first extracellular loop of Cldn1 or -5 are able to target these claudins and

increase barrier permeability to small macromolecules *in vitro*, as well as in the blood brain barrier, and the blood nerve barrier in rats (Dithmer et al., 2017; Sauer et al., 2014).

1.4.2.3 Monoclonal antibodies

Other groups have tried targeting claudin removal by using monoclonal antibodies that block the interaction with the apposing cell, thereby compromising the tight junction or the interaction with other molecules. This approach has been successfully used with monoclonal antibodies against occludin and different claudins, including Cldn1, -3, -4 and -5, which are able to bind the target claudin, promote barrier opening and increase barrier permeability *in vitro* (Fukasawa et al., 2015; Kato-Nakano et al., 2010; Okai et al., 2018; Tokunaga et al., 2007).

1.5 HYPOTHESIS AND OBJECTIVES

Tight junctions are essential in all epithelial and endothelial cell layers for proper organ and body function. Many groups have tried to develop compounds or tools that could be used to individually study tight junction components and their contribution to the barrier properties. However, few of them specifically target the proteins of interest or have been fully studied. In addition, we do not understand the underlying mechanisms of tools that are widely used to modify tight junctions, such as C-CPE and nutraceuticals. Therefore, understanding how these compounds and tools work, and generating new tools that are able to target specific tight junction proteins will allow us to gain a better understanding of the contributions of these proteins to tight junction function, in normal tissue and in diseases associated with compromised tight junction barriers.

I hypothesize that compounds that specifically target individual claudin family members can be used to modify tight junction barriers for the treatment of disease. The aims designed to address this hypothesis are centered around the understanding of current tools used to target claudins and the generation of new tools that specifically target individual claudins. First, to address the potential consequence of removing an individual claudin, I determined if claudins have unique interaction partners at the tight junction cytoplasmic plaque. In Chapter 2, I analyzed the specific cytoplasmic plaque interactors of Cldn1, -3, -4, -8 and -14, as well as, investigated the

role of different amino acids in the tail of Cldn8 in the interaction process. Then in Chapter 3, I analyzed the effect of the flavonoid quercetin that broadly targets claudin expression and localization. Quercetin, which is known to alter tight junction composition, was used to treat a kidney cation-selective cell line (MDCK II) and the data was assessed in the context of how quercetin could act to prevent kidney stones. Next in Chapter 4, I examined the mechanism used by the C-CPE peptide to remove claudins from the tight junction. C-CPE is known to bind to Cldn3, -4, -6, -7, -8 and -14 and remove them from the tight junction. The removal mechanism is thought to occur through the internalization of the Claudin-C-CPE complex. However, analysis of this process led me to discover that C-CPE treatment of MDCK II cells leads to an increase in the release of claudin-rich extracellular vesicles. Finally, in Chapter 5, I designed and generated a series of fusion proteins (NT-reagents) that specifically target removal of individual claudins from tight junctions. I used the stabilization domain of C-CPE but replaced the claudin-binding domain in C-CPE with the second extracellular loop of specific claudins. This strategy allowed me to generate specific tools that could interact and remove from specific claudins of interest from the tight junction. I validated these tools in different models through proof-of-concept experiments in cell lines and *in vivo* in chick embryos.

**CHAPTER 2: Claudin cytoplasmic domains participate in unique interactions that may affect
their role in neural tube development**

Enrique Gamero-Estevez^{1,2}, Amanda I. Baumholtz^{1,2}, Jean-François Boisclair Lachance² and
Aimee K. Ryan^{1,2,3}

¹Department of Human Genetics, McGill University, Montreal, QC H4A 3J1, Canada

²Research Institute of the McGill University Health Centre, Glen Site, Montreal, QC H4A 3J1

³Departments of Pediatrics, McGill University, Montreal, QC H4A 3J1, Canada.

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2.1 ABSTRACT

Tight junctions are essential to regulate paracellular transport as well as to coordinate morphogenesis during development. Previously we showed that claudins, the main components of tight junctions, are essential for neural tube closure. In fact, removal of Cldn3, -4 and -8 through treatment with the C-terminal domain of *Clostridium perfringens* enterotoxin (C-CPE) led to convergent extension and apical constriction defects that resulted in open neural tubes in 100% of the treated embryos. Claudins are composed of two extracellular, four transmembrane domains, and a cytoplasmic tail. They interact with other claudins in the same or apposing cell through their transmembrane or extracellular domains, respectively. In contrast, their C-terminal cytoplasmic domains interact with different scaffolding proteins and components of the actin cytoskeleton. However, a direct experiment has not been performed to identify and compare specific interactors for individual claudins in parallel. In our study, we identified claudin-specific interaction partners for Cldn1, -3, -4, -8 and -14 using the cytoplasmic C-terminal domains and embryonic protein extracts collected during neural tube closure and early organogenesis. We observed different interaction profiles for each claudin and found that each claudin preferentially interacted with specific tight junction cytoplasmic plaque proteins. Additionally, we generated a series of variants for the Cldn8 cytoplasmic C-terminal domain in which putative phosphorylation sites were modified (S198A, S216A, S216I) or the C-terminal PDZ-interaction domain deleted (Δ YV). We determined the biological effects of these variants on neural tube closure and their effect on binding to Cldn8 interaction partners. Our data suggest that each claudin preferentially binds to specific partners and that are dependent on specific residues along the tail, and that understanding these interactions can help to study and treat diseases such as neural tube defects.

2.2 INTRODUCTION

Tight junctions control the diffusion of ions and macromolecules across the paracellular space, and separate apical from basolateral proteins and lipids in polarized epithelial or endothelial cells. They are composed of strands of transmembrane proteins that associate with scaffolding and signaling proteins at the cytoplasmic face of the tight junction. Many of the

specific barrier properties of a tight junction can be attributed to the combination of claudins present at the membrane and their interaction with the cytoplasmic components (Gupta and Ryan, 2010).

Claudins are a family tetraspan proteins. The first extracellular loop is responsible for the formation of a pore that regulates the size and charge of the ions and molecules that diffuse across the paracellular space. The second extracellular loop is responsible for transdimerization with claudins in the apposing cells. The four transmembrane domains participate in cis-interactions with other claudin molecules and proteins within the same cell membrane and are responsible for the tight junction strand within a cell. Finally, the cytoplasmic C-terminal domain, is involved in direct interactions with cytoplasmic and scaffolding proteins. The interactions between the claudin C-terminal domains and scaffolding proteins bridge the tight junction to the actin-cytoskeleton, and creates a cytoplasmic plaque that serves as a docking platform where many proteins anchor or participate in transient interactions (Gonzalez-Mariscal et al., 2008; Piontek et al., 2020). Without the C-terminal domain claudins cannot interact with cytoplasmic proteins and both polarity and paracellular transport can be dysregulated (Baumholtz et al., 2017a; Ruffer and Gerke, 2004). The C-terminal two amino acids (YV in most claudin family members) seem to be critical as their removal impairs localization and interactions of different claudins with cytoplasmic proteins (Itoh et al., 1999; Ruffer and Gerke, 2004). Moreover, the C-terminal tail is a target for post-translational modifications, including phosphorylation, that can modify the affinity of claudins for their interaction partners (Nomme et al., 2015).

Roles for claudins during development of different tissues and morphogenesis have been described that may be associated with or distinct from their function in determining tight junction barrier properties. For example, gain- and loss-of-function studies support roles for tight junction proteins in the maintenance of tissue shape (Behr et al., 2003; Nelson et al., 2010; Wu et al., 2004), tension between cells during morphogenetic movements (Siddiqui et al., 2010), and hydrostatic pressure during lumen expansion (Bagnat et al., 2007; Moriwaki et al., 2007; Zhang et al., 2010). The C-terminal domain of *Clostridium perfringens* enterotoxin (C-CPE) is often used to study claudins. C-CPE is a non-toxic protein as it does not contain the N-terminal toxicity domain that is present in the full-length *Clostridium perfringens* enterotoxin, a common cause of

food poisoning that uses claudins as receptors to penetrate the intestinal barrier and promote cell apoptosis. C-CPE conserves the ability to bind and remove a subset of claudins from the tight junction but without killing the cell. C-CPE is able to target and remove Cldn3, -4, -6, -7, -8 and -14, while the localization of other claudins and tight junction proteins are not affected and remain localized at the tight junction (Shrestha et al., 2016).

As shown previously by our group, the removal of Cldn3, -4 and -8 using C-CPE during neural tube development leads to neural tube defects in the chick embryo (Baumholtz et al., 2017b). Interestingly, if only Cldn4 was removed, there was no phenotype and we observed increased expression of Cldn8 at the tight junctions depleted for Cldn4. Moreover, we showed that overexpression of human CLDN3 and CLDN8 variants in chick during neural tube development causes neural tube defects (Baumholtz et al., 2020). These data reveal the important roles for Cldn3 and Cldn8 during neural tube closure. These observations also raise the question: what is special about Cldn3 and -8 that when removed it causes more severe phenotypes than removing any other claudin?

In order to answer this question, we began by identifying proteins that interact with the claudin C-terminal domains. We performed a GST-pulldown interaction screen using the C-terminal cytoplasmic domains of five claudins that are expressed in the chick ectoderm during neural tube closure and whole extracts from two stages of chick development. We discovered that each claudin preferentially interacted with specific cytoplasmic or tight junction proteins. We then explored the ability of Cldn8 interaction partners to interact with a series of Cldn8 variants that have differential effects on neural tube closure when overexpressed in chick embryos. These variants included Cldn8S216A that has no effect on neural tube closure, Cldn8S216I whose overexpression leads to open neural tube associated with convergent extension defects, and Cldn8S198A that causes open neural tube defects in the absence of other phenotypes. Moreover, Cldn8S216I variant showed a reduced ability to interact with cytoplasmic plaque proteins, which may explain its more severe phenotype. In conclusion, our study supports that individual claudin family members participate in unique or preferential interactions at the tight junction cytoplasmic plaque that are at least in part dependent on the specific amino acid sequences in their C-terminal cytoplasmic domains.

2.3 MATERIAL AND METHODS

2.3.1 Antibodies and reagents

Primary antibodies were Cldn1 (Invitrogen, 374900, Carlsbad, CA, USA), Cldn3 (Abcam, 15102, Cambridge, UK), Cldn8 (Invitrogen, 400700Z, Carlsbad, CA, USA), GST (Santa Cruz, SC-138, Dallas, USA) and ZO-1 (Invitrogen, 339100, Carlsbad, CA, USA). In addition, secondary goat anti-rabbit (Alexa Fluor 488, Invitrogen, Carlsbad, CA, USA) and goat anti mouse (Alexa Fluor 595, Invitrogen, Carlsbad, CA, USA), were used.

2.3.2 Generation of GST-tagged claudin tails

The claudin C-terminal domains were obtained by PCR of chicken genomic DNA from HH4 mRNA using the OneStep RT-PCR kit (Qiagen) that was cloned into the pSC-A plasmid using the StrataClone OneStep RT-PCR Cloning kit (Stratagene). The sequence of each C-terminal domain is shown in Table 2.1. This plasmid was further digested to transfer the construct into the pET14b plasmid where it was fused to GST.

Cldn1	SCPRSETSYPPSRGYPKNAPSTGKDYV
Cldn3	SCPPKDERYAPSKVAYSAPRSAVTSYDKRNYV
Cldn4	CCSCPPRGEKPYSAKYSAARSLPASNYV
Cldn8	RCCEKTRSYNYSMPQHLPLNQHLHGATESAYSQSQYV
Cldn14	SGQCGTDQGPRQPRPCSTRTAPSCRPTAYKGNHASSLTSASHSGYRLNDYV

Table 2.1: Amino acid sequence of the claudin tails used to generate GST-Claudin tail fusion proteins.

2.3.3 Generation of claudin wild-type and mutant constructs

Full-length Claudin-8 cDNA (Accession #XM_004938379.2) was cloned from HH4 mRNA using the OneStep RT-PCR kit (Qiagen) into the pSC-A plasmid using the StrataClone OneStep RT-PCR Cloning kit (Stratagene). Predicted phosphorylation sites conserved in the C-terminal tail of chicken, mouse and human Claudin-8 were identified using NetPhos 2.0 and mutated to phosphorylation-null alanine residues in chicken Claudin-8 at positions S198 and S216. S216 was also mutated to isoleucine. The PDZ-binding domain was removed by inserting a premature stop codon immediately upstream of the YV, the terminal two amino acids. Site-directed mutagenesis

was performed using the Stratagene QuikChange II Site-Directed Mutagenesis kit according to manufacturer's directions (Agilent Technologies, Santa Clara, CA). Sequence identity of cDNA clones was confirmed by sequencing at the Genome Quebec Innovation Centre (Montreal, Quebec, Canada). Claudin-8 wild-type and variant constructs were then cloned into the pCanHA3 and pMES expression plasmids and the tail sequences were cloned into pET14b plasmids as described above.

2.3.4 Preparation of chick embryo extract

Chick embryos were collected at either Hamburger-Hamilton (HH) stage 8 or at day 4, rinsed in PBS and flash frozen in liquid nitrogen. The day of the pull-down experiment, the chick embryos were defrosted and lysed in lysis buffer (20mM Tris, pH7.5, 10mM NaCl, 50mM NaF, 1% NP40 and 0.5% sodium deoxycholate) with protease inhibitors (1.45nM Pepsatin A, 2.1nM Leupeptin, 0.15nM Aprotinin and 0.57mM PMSF) at a ratio of 1mL per mg of tissue. The lysate was then passed through a 24-gauge syringe needle to ensure its complete dissociation and incubated on ice for 15 minutes. The lysate was then centrifuged for 30 minutes at 13000 rpm and the supernatant was collected and added to beads for the pull-down experiments as described below.

2.3.5 GST pull-down experiment

Plasmids encoding GST fusion proteins with the wild-type or variant claudin cytoplasmic C-terminal domains were transformed into *E. coli* BL21 cells and plated on ampicillin lysogeny broth (LB) plates. Individual colonies were used to inoculate 4 mL of LB media that was culture overnight and then 1 mL was used to inoculate 100 mL of LB. When an absorbance 600 of 0.3-0.6 OD was reached, 1M IPTG stock solution was added to a final concentration of 0.2mM, and the culture was incubated overnight at 22°C with constant agitation at 250rpm. Bacterial cell pellets were lysed in 5 volumes of resuspension buffer (50mM Tris pH7.5, 150mM NaCl, 0.1mM EDTA) with protease inhibitors (1.45nM Pepsatin A, 2.1nM Leupeptin, 0.15nM Aprotinin and 0.57mM PMSF) and incubated on ice for 20 minutes in presence of Lysozyme to a final concentration of 20 µg/mL and sodium deoxycholate was added to a final concentration of 0.2%.

The lysate was sonicated (6 times for 30 seconds at 30° amplitude) and then centrifuged at 15,000rpm for 30 minutes. The supernatant containing soluble GST-tagged fusion proteins was incubated at room temperature for 2h with 100 μ L of 50% slurry of glutathione agarose beads. Agarose beads were collected by low speed centrifugation and washed 4 times in 10-bed volumes of washing buffer (50mM Tris pH7.5, 0.15M NaCl). A 5ml aliquot of the washed beads was separated on a denaturing polyacrylamide gel, which was then stained with Coomassie brilliant blue. Equal amounts of fusion proteins bound to glutathione agarose beads (40-80 μ l depending on the Coomassie brilliant blue) were transferred to a new microcentrifuge tube and approximately 1000mg of chick extract from HH stage 8 or day 4 embryos were added and incubated at room temperature overnight with rocking. Twenty-two dozen HH8 embryos were used per tail, while for 2 dozen day 4 embryos were used per pulldown. Beads were washed 4 times in 10-bed volumes with washing buffer (50mM Tris pH7.5, 0.15M NaCl). Finally, washed beads were sent to mass spectrometry or used for western blot analysis in order to identify or validate specific interactors.

2.3.6 Mass spectrometry

For each sample, a single stacking gel bandwidth of all proteins was reduced with DTT, alkylated with iodoacetic acid and digested with trypsin. Extracted peptides were re-solubilized in 0.1% aqueous formic acid and loaded onto a Thermo Acclaim Pepmap (Thermo, 75 μ M ID X 2cm C18 3 μ M beads) precolumn and then onto an Acclaim Pepmap Easyspray (Thermo, 75 μ M X 15cm with 2 μ M C18 beads) analytical column separation using a Dionex Ultimate 3000 uHPLC at 250 nl/min with a gradient of 2-35% organic (0.1% formic acid in acetonitrile) over 3 hours. Peptides were analyzed using a Thermo Orbitrap Fusion mass spectrometer operating at 120,000 resolution for MS1 with HCD sequencing at top speed (15,000 resolution) for all peptides with a charge of 2+ or greater. The raw data were converted into Mascot generic format (.mgf) for comparison to *Gallus* protein sequences (Uniprot 2020) using the Mascot 2.5.1 search engine (Matrix Science). The data was also compared to *E. coli* database to remove false positive hits. The database search results were loaded onto Scaffold Q+ Scaffold_4.4.8 (Proteome Sciences) for statistical analysis and data visualization. Pinnacle (Optys Tech) was used to

quantify all detected peptides using a MS1 quantification workflow (Targeted Quantification: Label Free DDA) wherein the peptide specific XICs from the raw mass spec data (.raw) were used to directly compare all identified peptide (*.dat) amounts across all experiments using precursor ion integrals (in counts).

Proteins with less than 2 peptides per protein, high ambiguity or confidence less than 95% were excluded from the analysis. The remaining peptides were analyzed by comparing their abundance using total spectral counts and performing a Fisher's F statistical test and corrected for multiple comparison using Bonferroni correction. Candidate interaction proteins were determined to be peptides that were significantly different ($p < 0.0001$) or at least appeared with a 2-fold difference in reference to the control samples. Samples that only appeared in only one of the three replicates were excluded from the analysis unless more than 5 peptides were present. For claudin tail pulled-downs, GST was used as control reference, while for Cldn8 variants, Cldn8 wild-type tail was used as control reference.

2.3.7 Immunoprecipitation

500 µg to 1 mg of HH 8 or Day 4 chick embryos were collected and lysed with 1 mL/mg tissue lysis buffer (20mM Tris pH7.5, 10mM NaCl, 50mM NaF, 1%NP40 and 0.2% SDS) with protease inhibitors (1.45nM Pepsatin A, 2.1nM Leupeptin, 0.15nM Aprotinin and 0.57mM PMSF). The lysed embryos were forced through a 24-gauge syringe needle to ensure complete dissociation and then incubated on ice for 15 minutes. The lysates were centrifuged for 30 minutes at 13000 rpm and the supernatant was collected. Samples were incubated with 20 µL of Protein G beads (Roche, Basel, Switzerland) for 30 minutes at room temperature in order to remove non-specific interactors. The supernatant was transferred to a new tube and then incubated overnight at 4°C with primary antibody (1.5 µg of antibody per 0.5 mg of protein). The sample was then incubated for 2 hours at room temperature with 40 µL of Protein G beads and subsequently washed in washing buffer (50mM Tris pH7.5, 0.15M NaCl). The beads were then resuspended in loading dye, separated on denaturing polyacrylamide gels and subjected to western blot analysis.

2.3.8 Western blot analysis

For western blot analysis, beads from pulldown or immunoprecipitation experiments were separated by 12.5% SDS-PAGE gels and transferred onto PVDF membranes. PVDF membranes were blocked for 1h with 5% BSA in TBST (Tris-NaCl, pH X, 0.3% Tween-20). Membranes were incubated overnight at 4°C with primary antibodies in TBST: Cldn1, -3, -8, ZO-1 (1:1000) or GST (1:10000). Membranes were then washed with TBST and incubated with goat-anti-rabbit-HRT conjugated or goat-anti-mouse-peroxidase conjugated (1:5000) secondary antibodies for 1h at RT. Membranes were revealed using Clarity Western ECL substrate (Biorad Laboratories, 1705061, Hercules, CA, USA), and imaged using Amersham imager 600 (GE Healthcare, Little Chalfont, United Kingdom).

2.3.9 Cell culture

Human embryonic kidney cells (HEK293 cells) were obtained from ATCC. They were incubated at 37°C and 5% CO₂ in Dulbecco's Modified Eagle's medium (DMEM), supplemented with 10% FBS, 51 IU penicillin, 50 µg/mL streptomycin and 16 µg/mL of gentamicin (Wisent BioProducts, Quebec, QC, Canada).

2.3.10 Immunolocalization of ectopically expressed claudins

HEK293 cells grown on coverslips in a 24-well plate were transfected at 70-90% confluence with pMES or pCanHA3 expression vectors encoding wild-type or variant claudins using Lipofectamine LTX (Invitrogen) in a 1:1 DNA:reagent ratio. Transfected cells were incubated for 24-48 hours at 37°C. After rinsing with PBS, cells were fixed with 10% trichloroacetic acid for 15 minutes at 4°C. Cells were then washed with PBS and blocked with 10% normal goat serum in PBS with 0.3% Triton-100 (PBST) and then incubated overnight at 4°C with primary antibodies in 5% normal goat serum in PBST at 1:100 concentration. After the overnight incubation, primary antibody was removed and Alexa Fluor-conjugated secondary antibodies (1:500) in PBST were added for 1h at RT. Coverslips were washed three times for 5 minutes with PBS and then placed on slides with SlowFade Gold Antifade kit (Molecular Probes), which contained DAPI to enable

visualization of the nuclei and imaged using a laser scanning confocal microscope (LSM780, Leica, Germany).

2.3.11 Overexpression assay in the chick embryo

pMES-claudin constructs (5 $\mu\text{g}/\mu\text{L}$) were injected into the space between the vitelline membrane and the epiblast of HH4 embryos cultured on agar-albumen plates using a Narishige IM 300 microinjector and electroporated using a Protech CUY21SC square electroporator (five 3 V/50 ms pulses at 500 ms intervals). Manipulated embryos were cultured until the indicated stage. HH11-12 embryos were scored for defects in neural tube closure and convergent extension. Moreover, statistical significance of the phenotypes that appeared upon Cldn8 variant electroporation was analyzed by Chi-square analysis where significance was considered when $p < 0.005$.

2.4 RESULTS

2.4.1 Each claudin has specific sets of interaction partners

To determine if all claudins interact with the same cytoplasmic plaque proteins, we performed GST-pulldown assays. We generated GST-fusion proteins for the cytoplasmic C-terminal domain of five claudins that are expressed in the chicken neural and non-neural ectoderm during neural tube closure (Cldn1, -3, -4, -8 and -14). These GST-Claudin tail fusion proteins were then used to isolate interacting proteins from a Day 4 chick embryo extract. Three biological replicates were performed and interacting proteins were analyzed by mass spectrometry. For analysis, the three samples for each claudin were compared to their corresponding GST-control. The complete list of proteins isolated with each claudin C-terminal cytoplasmic tail is provided in Appendix 1.

The pipeline used for the analysis of our dataset and the number of proteins remaining after each filtering step is outlined in Figure 2.1. We focused initially on proteins that were identified with a confidence level $>95\%$ and that were present in the *Gallus* database. This step excluded false positives and proteins that could come from the *E. coli* background where the claudin tail fusion proteins were generated. For further analysis, we first excluded proteins with

less than 2 peptides per protein, retaining those proteins where two different peptides were identified one or more times and or a single peptide was identified multiple times. The abundance of the remaining peptides was compared using total spectral counts, performing a Fisher's F statistical test, and corrected for multiple comparison using Bonferroni. Candidate interaction proteins were determined to be peptides that were significantly different ($p < 0.0008$) or at least appeared with a 2-fold difference with respect to the GST control samples, which would indicate preferential binding to claudin tails but not to the GST control. Finally, each candidate was examined manually and samples that appeared in only one of the three replicates or those that showed great inconsistency between replicates were excluded from further analysis, with the exception for those proteins where more than 5 peptides were detected.

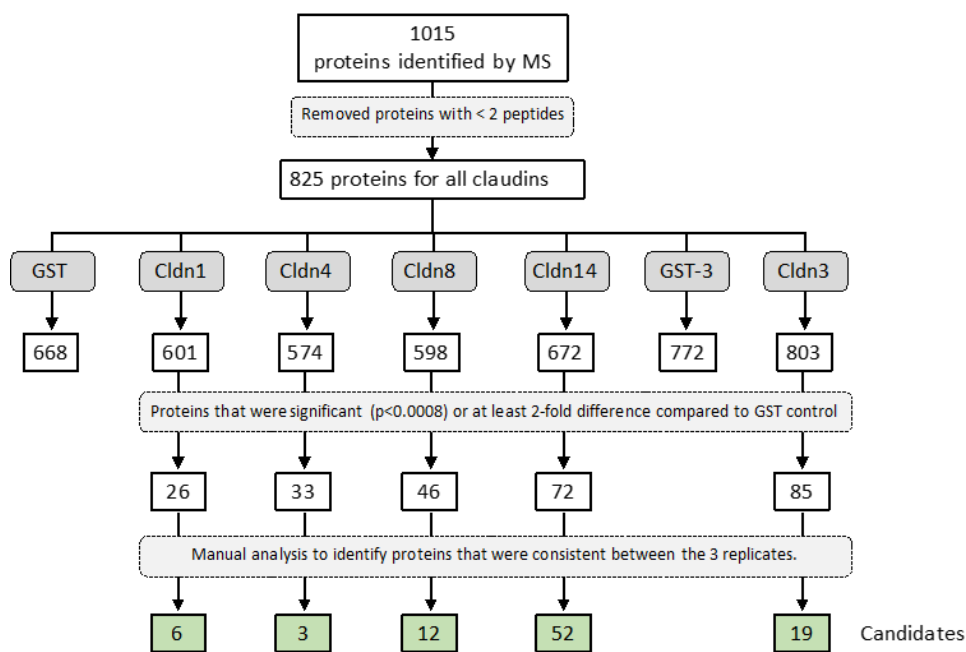


Figure 2.1: Pipeline used for the analysis of the mass spectrometry dataset. Numbers inside the boxes correspond to number of proteins carried forward between steps.

After following these criteria we were able to prioritize a limited subset of proteins from the initial 1015 proteins for more in-depth analysis. We found that each claudin preferentially interacted with distinct subsets of proteins. GST-Cldn1 preferentially pulled down six proteins when compared to GST-control, including ZO-2, SEC24C, CRMP2B, PPIA, RPL23 and ALG14 (Table 2.2). GST-Cldn3 was able pull-down 19 proteins compared to its GST-control, including ZO-1, ZO-2, SEC23A, PALS1, PFN2, GJA1, CARNNMT1, POLDIP2 and PLK1 (Table 2.2). Cldn4 was the least

interactive claudin as it preferentially interacted with only CACNA2D3, PPP2R2A and ALG14 (Table 2.2). In contrast, GST-Cldn8 interacted with 12 proteins when compared to GST-control, including MPDZ, ZO-1, ZO-2, MLLT4, GLRX3, LNX2, TXN and PPP2R2A (Table 2.2). Finally, GST-Cldn14 appeared to be the most promiscuous claudin and it preferentially pulled down 52 proteins when compared to GST-control, including cRhoA, PPIA, SSH2, NUR2L, LSP1, CTTN, Collagen XVIII, VCAN, FBN1, NID2, SEC61B or ANXA6 (Table 2.2). As an internal control, we were able to confirm the identify of each pull-down fraction by the presence of peptides corresponding to the claudin bait, with the exception of Cldn4, whose sequence is not yet annotated in the Uniprot *Gallus* database (Table 2.2). Cldn4 was inserted manually in our database and we were successfully able to identify it in our dataset.

List of proteins preferentially pulled down by GST-Cldn tails when compared to GST-control						
	Protein name	Accession number	F test	Folds	Total spectral counts Claudin	Total spectral counts GST
Cldn1	Cldn1	Q57MG2_CHICK	<0.0001	inf	101	0
	SEC24C	E1BUD8_CHICK	0.0014	inf	9	0
	CRMP2B	Q71SG1_CHICK	0.0042	10	10	1
	PPIA	D0EKR3_CHICK	0.12	3	6	1
	ALG14	E1BSX2_CHICK	0.013	2.3	21	9
	RPL23	E1BY89_CHICK	0.035	3.3	10	2
	ZO-2/ TJP2	A0A1D5PA43_CHICK	0.00082	2.7	32	11
Cldn3	Cldn3	Q98SR2_CHICK	<0.0001	92	92	1
	ZO-1/TJP1	A0A1D5NWX9_CHICK	<0.0001	inf	71	0
	PALS1/MPP5	E1C3F8_CHICK	<0.0001	inf	63	0
	ZO-2/ TJP2	A0A1D5PA43_CHICK	<0.0001	inf	61	0
	SEC23A	Q5ZK03	<0.0001	inf	36	0
	PFN2	Q5ZL50_CHICK	<0.0001	inf	34	0
	GJA1	CXA1_CHICK	<0.0001	inf	30	0
	SEC24C	E1BUD8_CHICK	<0.0001	inf	29	0
	CARNMT1	CARME_CHICK	<0.0001	inf	17	0
	POLDIP2	A0A1D5PSI7_CHICK	0.0001	inf	9	0

	THOP1	A0A1D5PIZ1	0.00044	15	15	1
	FBXO21	A0A1D5PHM5	0.01	inf	9	0
	PLK1	F1NCX7_CHICK	0.044	7	7	1
	UTP15	Q5F3D7	0.11	3.5	7	2
	KRT14	A0A1D5PXW6_CHICK	0.0032	3.4	24	7
	KRT18	A0A1D5PHS2_CHICK	<0.0001	2.2	177	82
	H1.11R	P08288	0.00021	2	92	35
	APOB	F1NV02_CHICK	0.13	2	14	7
	RPS2	E1C4M0_CHICK	0.29	2	6	3
Cldn4	CACNA2D3	F1NX89_CHICK	0.0087	inf	8	0
	PPP2R2A	F1NBJ2_CHICK	0.079	2	6	2
	ALG14	E1BSX2_CHICK	0.0075	2	14	7
Cldn8	Cldn8	E1BS22_CHICK	<0.0001	23	205	9
	MPDZ/MUPP-1	F1N9G0_CHICK	<0.0001	inf	25	0
	Cldn1	Q5ZMG2_CHICK	0.00051	inf	8	0
	ZO-2/ TJP2	A0A1D5PA43_CHICK	<0.0001	13	150	11
	GLRX3	F1NNP6_CHICK	0.0087	inf	5	0
	LNK2	A0A1D5PZR2_CHICK	0.0034	inf	5	0
	TXN	THIO_CHICK	0.00054	inf	9	0
	ZO-1/ TJP1	A0A1D5NWX9_CHICK	<0.0001	7.6	356	46
	MLLT4/ afadin-6	E1BYF5_CHICK	<0.0001	6.8	34	5
	PPP2R2A	F1NBJ2_CHICK	0.012	3	9	2
	KPNB1	A0A1D5P1W7_CHICK	0.0046	2.3	14	6
	PRDX1	PRDX1_CHICK	0.0001	1.8	40	24
Cldn14	Cldn14	E1C5Q7_CHICK	<0.0001	inf	27	0
	ATAD3A	A0A1D5PVA4_CHICK	0.0079	inf	7	0
	HNRNPU	F1NSP8_CHICK	0.02	8	8	1
	LBR	F6UZR6_CHICK	0.02	8	8	1
	RPS27	A0A1D5NZ06_CHICK	0.063	6	4	1
	RPS12	A0A1I7Q419_CHICK	0.063	6	6	1
	SEC61B	F1NWX7_CHICK	0.063	6	6	1
	HNRNPA3	A0A1D5PXH1_CHICK	0.11	5	5	1

cRhoA	O93467_CHICK	0.19	4	4	1
Caprin 1	Q5XNV3_CHICK	0.19	4	4	1
MTX2	A0A1L1RNR7_CHICK	0.19	4	4	1
PPIA	D0EKR3_CHICK	0.091	3.5	7	1
ATP1A1	A0A1D5P0G4_CHICK	0.0048	3.3	20	6
RPS14	A0A1D5PK33_CHICK	0.025	3.2	13	4
COX5A	E1C043_CHICK	0.039	3	12	4
RPL6	Q8UWG7_CHICK	0.01	2.6	22	9
NUR2L	A0A1D5PF01_CHICK	0.091	2.5	10	4
NID2	F1NDL4_CHICK	0.23	2.5	5	2
LRPPRC	F1NGU3_CHICK	0.23	2.5	5	2
NDUFA5	E1BRT9_CHICK	0.23	2.5	5	2
ACAT1	E1C0Q5_CHICK	0.23	2.5	5	2
LSP1	Q5F3Y0_CHICK	0.0017	2.4	40	17
SAMM50	E1C8A2_CHICK	0.032	2.4	17	7
RPL13	RL13_CHICK	0.073	2.4	12	5
RPL4	A0A1D5NUQ4_CHICK	0.018	2.3	23	10
RPL23	E1BY89_CHICK	0.17	2.3	7	2
HNRNPM	F7B5K7_CHICK	0.018	2.3	23	9
ANXA6	A0A1D5PY67_CHICK	0.17	2.3	7	3
AIF3A	A0A1D5PGY9_CHICK	0.17	2.3	6	3
NPM1	F1NVA4_CHICK	0.031	2.2	20	9
RPL10A	F6SU35_CHICK	0.085	2.2	13	6
RPS19	A0A1D5PDV6_CHICK	0.13	2.2	9	4
Collagen XVIII	O93419_CHICK	0.085	2.2	13	6
CTTN	F1NU55_CHICK	0.0096	2.1	32	15
VCAN	E1BZM6_CHICK	0.055	2.1	17	8
PAWR	F1NA74_CHICK	0.055	2.1	17	8
TPM3	H9L3K0_CHICK	<0.0001	2	360	181
FBN1	A0A1D5NW86_CHICK	0.00046	2	67	33
cRPS11	Q98TH5_CHICK	0.15	2	10	4
EIF4A2	A0A1L1RUX2_CHICK	0.077	2	16	8

AHNAK2	E1BUP8_CHICK	0.096	2	14	7
COX6C	A0A1D5PN05_CHICK	0.2	2	8	4
LRRFIP1	F1NCG5_CHICK	0.2	2	8	4
ZP3	A0A1I7Q3Y5_CHICK	0.2	2	8	4
LOC107049545	A0A1D5PUI6_CHICK	0.26	2	6	3
RPL19	A0A1L1S086_CHICK	0.26	2	6	3
CHCHD3	A0A1L1RNV8_CHICK	0.15	2	10	5
HAPLN3	E1BUN0_CHICK	0.35	2	4	0
SSH2	A0A1D5PGJ7_CHICK	0.35	2	4	2

Table 2.2: List of proteins preferentially pulled down by each claudin tail. Proteins (and accession numbers) that were preferentially pulled down by each claudin tail. Total spectral counts and folds are annotated for each protein. Folds were calculated by dividing the total spectral counts for a protein in GST-Cldn tails by the number found in GST control. If no protein was found in the control sample, the folds were impossible to calculate accurately and therefore, they were indicated as infinity (inf). Internal controls are also added to the list of candidates. Fisher exact test and Bonferroni correction was performed to determine significance, which was achieved when $p < 0.0008$ (in bold).

The cytoplasmic C-termini of claudins are known to interact with proteins in the tight junction cytoplasmic plaque, including the scaffolding protein ZO-1 (Nomme et al., 2015). Therefore, we were particularly interested in examining proteins that are known to be associated with tight junctions and/or their regulation (Table 2.3). A comparison of the proteins pulled down by the different claudins showed that each claudin C-terminal tail interacted with a distinct subset of proteins. We observed that one of the main tight junction scaffolding proteins, Zona Occludens-1 (ZO-1), was preferentially pulled down by Cldn8 and -14: 356 and 67 spectral counts versus only 46 in the control. In contrast, ZO-1 was not enriched in Cldn1 and -4 pull-downs. Zona Occludens-2 (ZO-2), another tight junction scaffolding protein, was also preferentially pulled down by Cldn8 and to a lower degree by Cldn1: 150 and 32 spectral counts compared to 11 in the control. In contrast ZO-2 was not enriched in the Cldn4 and -14 pools over the GST control. MPDZ, also called MUPP-1, a tight junction scaffolding protein, was present in one replicate of the proteins pulled down by Cldn1 and two of the replicates for Cldn8 but was absent in the GST control and Cldn4 and Cldn14 tails interaction fractions. MLLT4, also called afadin-6, was pulled down preferentially by Cldn8 (34 spectral counts compared to 5 in the control), but not by the

other claudins. RhoA, which is known to regulate tight junctions, was preferentially pulled down by Cldn14 and absent in the other claudin pull-downs. Cldn3, which was assayed in a separate set of experiments, preferentially pulled down ZO-1 and ZO-2: 71 and 61 counts, respectively, which were both absent in the GST control. Similarly to Cldn1, MPDZ was pulled down by Cldn3 versus GST but only in one of the 3 replicates.

	GST (Cldn1, -4, -8, -14)	Cldn1	Cldn4	Cldn8	Cldn14	GST (Cldn3)	Cldn3
Zona Occludens-1	46	39	22	356	67	0	71
Zona Occludens-2	11	32	13	150	18	0	61
MPDZ/ MUPP-1	0	8	0	25	0	0	12
MLLT4/afadin-6	5	2	3	34	5	0	0
PALS1/MPP5	0	0	17	4	0	0	63
cRhoA	1	2	0	1	4	4	0

Table 2.3: List of tight junction related proteins found in the pull-down analysis and preferentially pulled-down by a specific claudin. Tight junction proteins identified in the pulldown fractions. Numbers correspond to spectral counts.

The cytoplasmic domains of claudins are subject to significant post-translational modification and we were interested to determine if any of these interactions would be observed in our pull-down assay. ALG14, a N-glycosylation enzyme, was pulled down by Cldn1, -4 and -8; PPIA, a prolyl *cis/trans* isomerase, by Cldn1, -4 and -14; PPP2R2A, a Serine/Threonine phosphatase by Cldn3, -4 and -8; SSH2, a Tyrosine phosphatase, by Cldn14; or PLK1, a Serine/Threonine kinase, by Cldn3 (Table 2.4). We also observed that several cytoskeleton-associated proteins preferentially interacted with specific claudins. For example, Cldn1 pulled down CRMP2B that is a microtubule binding protein, Cldn3 pulled down PFN2 that is involved in actin binding and polymerization, while Cldn14 pulled down NUR2L, LSP1P1, CTTN or TPM3, all of which are involved in actin binding and cytoskeleton organization. In contrast, Cldn4 and -8 did appear pulldown any cytoskeleton-associated proteins (Table 2.5).

	GST (Cldn1, -4, -8, -14)	Cldn1	Cldn4	Cldn8	Cldn14	GST (Cldn3)	Cldn3
ALG-14	9	21	14	15	7	4	8
PPIA	1	6	15	4	7	6	0
PLK1	0	1	0	0	2	0	7
PPP2R2A	2	0	6	9	2	0	8
SSH2	2	1	0	0	4	0	0

Table 2.4: List of proteins involved in post-translational modifications found in the pull-down analysis and preferentially pulled down by a specific claudin C-terminal domain. List of proteins involved in post-translational modifications that were preferentially pulled down by claudins and its comparison with other claudin tails. Numbers indicate spectral counts.

	GST (Cldn1, -4, -8, -14)	Cldn1	Cldn4	Cldn8	Cldn14	GST (Cldn3)	Cldn3
CRMP2B	1	10	3	0	0	3	2
PFN2	1	0	4	2	1	0	34
NUR2L	4	0	1	0	10	0	0
LSP1P1	17	22	7	14	40	0	1
CTTN	15	13	6	8	32	0	2
TPM3	181	184	97	187	360	60	75

Table 2.5: List of cytoskeleton-associated proteins found in the pull-down analysis and preferentially pulled-down by a specific claudin. List of cytoskeleton proteins that were preferentially pulled down by claudins and its comparison with other claudin tails. Numbers indicate spectral counts.

Since our pulldowns occur in an *in vitro* setting we validated these interactions *in vivo*. To validate the interactions between the claudin cytoplasmic tail and the proteins identified in the pull-down fraction, we performed co-immunoprecipitation assays. To date I have confirmed the interaction with ZO-1; assays with other candidates are ongoing. To confirm this interaction the pulldowns were repeated using fresh extracts and analyzed by western blots. Western blot analysis confirmed that ZO-1 was preferentially pulled down by Cldn8. Cldn1 and -14 were not able to pulldown ZO-1 even though that the amount of GST-fusion protein similar for all claudins (Figure 2.2A). Co-immunoprecipitation with ZO-1 antibody was used analyze if claudins were co-

immunoprecipitated. ZO-1 antibody successfully immunoprecipitated ZO-1 protein (Figure 2.2B) and while Cldn3 and -8 were co-immunoprecipitated, interaction with Cldn1 was not observable (Figure 2.2C).

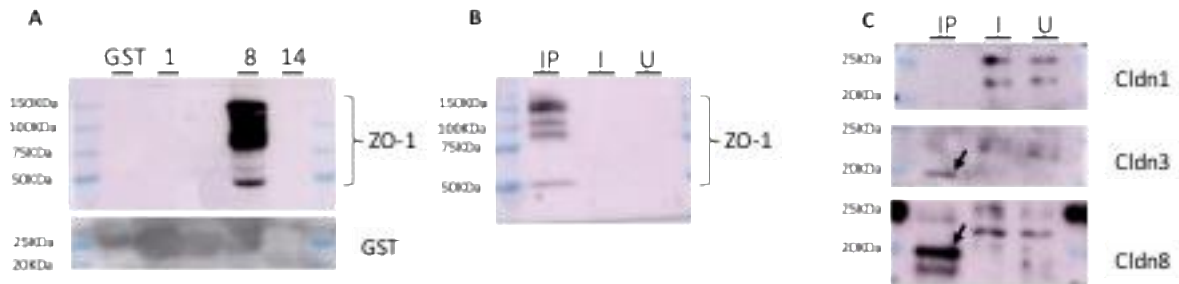


Figure 2.2: Validation that ZO-1 is preferentially pulled down by Cldn3 and -8. **A.** Western blot against ZO-1 and GST of pulled-downs using GST-Claudin tails as bait of D3 chick embryo extract (n=2). **B.** Western blot against ZO-1 of D3 chick embryo immunoprecipitated with ZO-1 antibody. **C.** Western blot against Cldn1, -3 and -8 of D3 chick embryo immunoprecipitated with ZO-1 antibody.

2.4.2 Variants in the Cldn8 cytoplasmic C-terminus disrupt neural tube closure

As described above Cldn8 appeared to interact with several proteins associated with the tight junction cytoplasmic plaque. Our previous studies show that Cldn8 is required for the cell shape and morphogenetic changes in the neural ectoderm during the early stages of neural tube closure (Baumholtz et al., 2017b). We observed that removal of Cldn4 and Cldn8 from the neural ectoderm causes a failure in convergent extension movements in the neural ectoderm and of apical constriction in cells at the midline of the neural plate. In contrast, removal of only Cldn4 has no effect on neural tube closure (Baumholtz et al., 2017b). We also discovered that a human CLDN8 variant P216L causes neural tube defects when it is overexpressed in chick embryos. These data suggest that proteins that interact with the Cldn8 cytoplasmic C-terminal tail are critical for its role in neural tube closure. To further investigate the role of amino acid residues in the Cldn8 cytoplasmic domain in regulating embryogenesis and their effect on protein interactions, we generated three variants at putative serine phosphorylation sites in chick Cldn8 (Cldn8S198A, Cldn8S216A, Cldn8S216I) and one that deleted the PDZ binding domain at its C-terminus (Cldn8ΔYV) (Figure 2.3A). All variants localized to tight junctions in transfected HEK293 cells (Figure 2.3B). Electroporation of neural plate stage embryos with wild-type Cldn8 expression vector did not significantly affect neural tube closure and convergent extension (Figure 2.3C):

92% had closed neural tubes (n=45/49) and 96% were normal length (n=47/49). Similarly, $\geq 90\%$ of the embryos electroporated with Cldn8 Δ YV (n=39/42) and Cldn8S216A (n=45/50) had closed neural tubes. A significant increase in NTDs was observed in embryos electroporated with the Cldn8S216I variant (n=21/65; $c^2=9.5$, $p<0.002$) and more than two-thirds of these embryos also had convergent extension defects (n=15/21) (Figure 2.3E, F). Histological analysis of transverse sections through the neural tube of embryos overexpressing the Cldn8S216I variant revealed a flat and broad midline, indicating that apical constriction of midline neural ectoderm cells did not occur (Figure 2.3D). Interestingly, the Cldn8S198A variant also caused a significant increase in open NTDs (n=7/17; $c^2=9.9$, $p<0.002$) but did not affect convergent extension (n=2/17; $c^2=1.31$, $p=0.253$) (Figure 2.3E, F). These data suggest that residues within the Cldn8 C-terminus may have distinct interaction partners that regulate different morphogenetic events during neural tube closure.

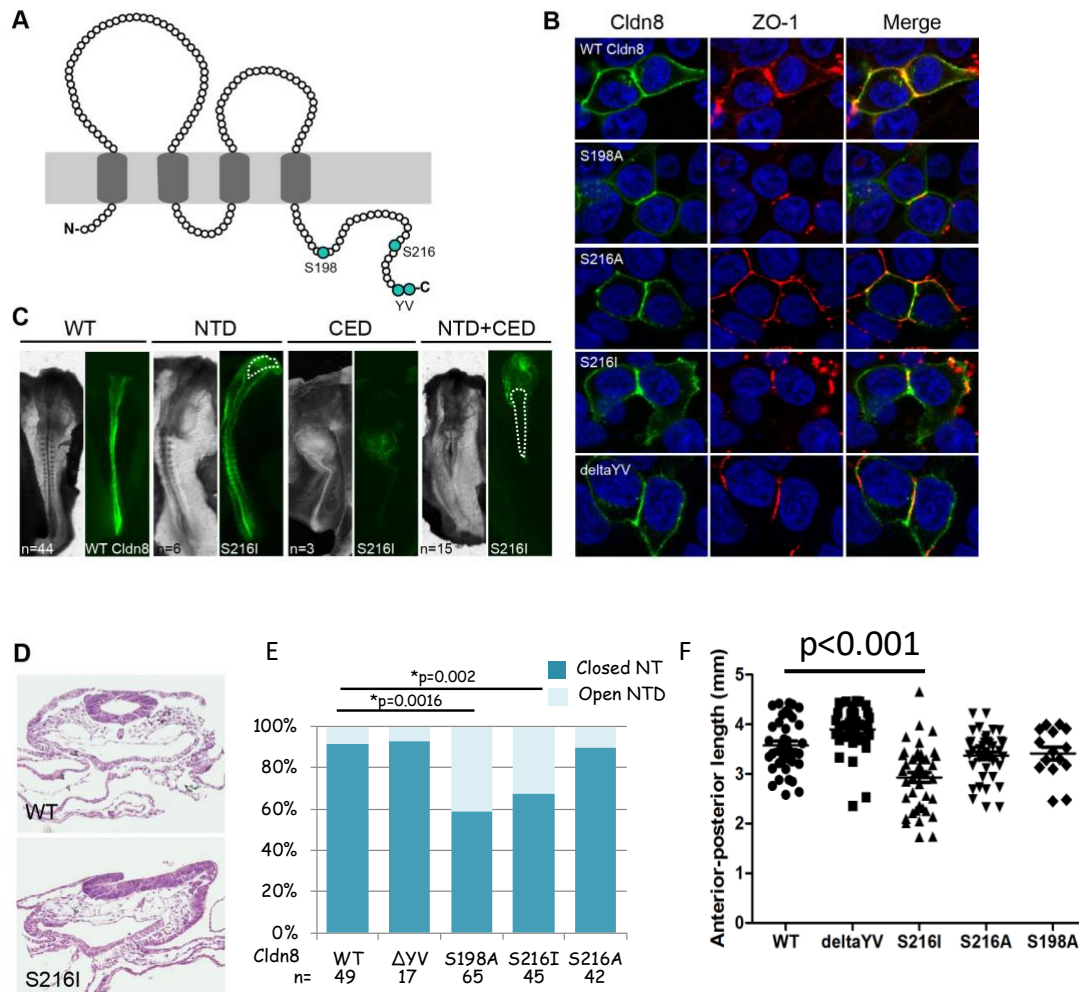


Figure 2.3: Study and profiling of Cldn8 variants. **A.** Scheme of a Cldn8 molecule. In light blue are the residues mutated or deleted in the case of Δ YV to create the Cldn8 variants. **B.** Immunofluorescence of HEK293 cells transfected with Cldn8 variants. In blue is DAPI, in red ZO-1 a marker of tight junction, and in green is Cldn8. **C.** Pictures of chick embryos electroporated with the different Cldn8 variants. The area of electroporation is labelled with GFP. **D.** Hematoxylin and eosin stained transverse sections through the neural tube of chick embryos electroporated with wild-type Cldn8 or Cldn8S216I. **E.** Summary of neural tube (NT) closure and open neural tube defects (NTD) in electroporated chick embryos. The total number of embryos per group are shown below each bar. Statistical significance following a Chi-square statistical test is shown. **F.** Graph showing the lengths of the chick embryos electroporated with either wild-type Cldn8 or Cldn8 variants. Statistical significance following a Chi-square statistical test is shown. $p < 0.005$ denotes significance.

These data led us to determine if these Cldn8 tail variants affected the ability of Cldn8 to interact with cytoplasmic partners and if this could contribute to the differences in phenotype and penetrance. GST-fusion proteins for each of these variants were prepared and used in pull-down assays. For these assays, we used whole cell extracts from HH stage 6-8 embryos because this is the developmental stage at which Cldn8 is required for neural tube morphogenesis. Proteins that were isolated from these pull-down assays were characterized by mass spectrometry and analyzed following the criteria described above. The full data set is shown in Appendix 2. The pipeline to identify candidates for further analysis was the same as that used for the claudin tails. The pipeline and protein counts are shown in Figure 2.4.

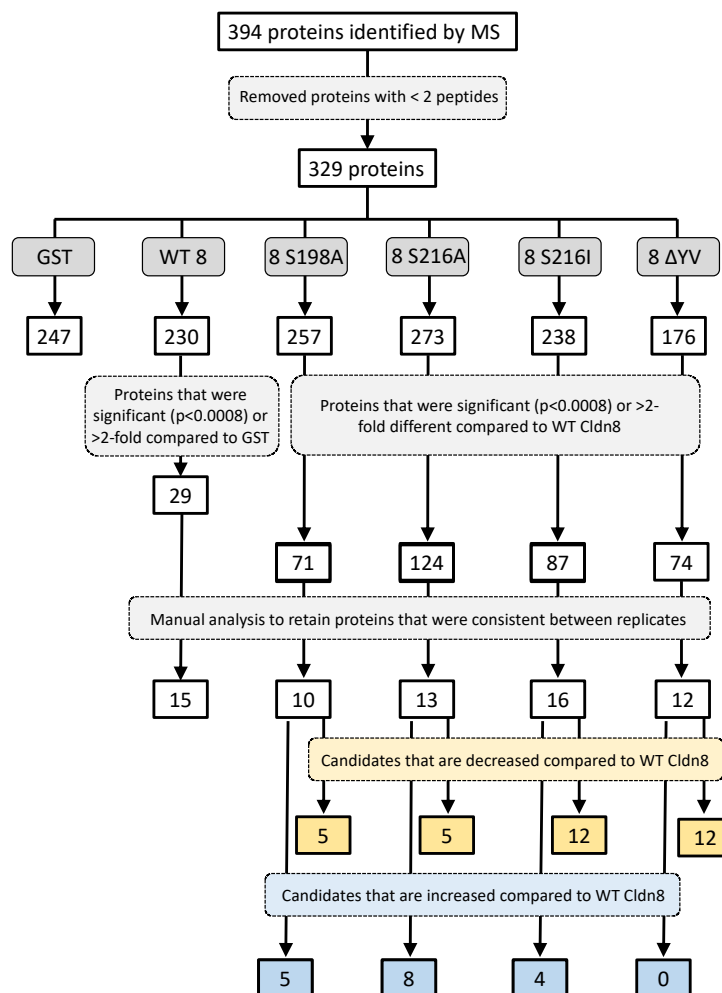


Figure 2.4: Pipeline used to analyze wild-type Cldn8 and Cldn8 variants interactors. For this analysis wild-type Cldn8 was compared to GST Control (Table 2.6), while Cldn8 variants were compared to wild-type Cldn8 (Table 2.7 and 2.8). Numbers inside the boxes correspond to number of proteins carried forward to the next step.

The pull-downs with the Cldn8 variants were done using protein extracts from HH6-8 embryos. Therefore, we first compared the profile of proteins that interacted with wild-type Cldn8 from HH6-8 embryo extracts to that obtained with the extracts from day 4 chick embryos (Table 2.2 and 2.6). A direct statistical comparison is not yet possible because the HH6-8 pull-downs have only been done in duplicate, while day 4 chick embryo pull-downs were done in triplicate. However, we found that most of the interactors identified from the Day 4 chick embryo protein extracts were also present in the pull-downs from the HH6-8 embryo protein extracts, including tight junction proteins such as ZO-1, ZO-2, MPDZ or MLLT4. In addition, we found other tight junction related proteins in the HH6-8 pull-down that were absent in the Day 4 chick embryo pull-downs, including ZO-3, PALS1 and MAGI-1 (Table 2.6).

Protein name	Accession number	F test	Fold	Total spectral counts	
				WT Cldn8	GST control
ZO-2/ TJP2	A0A1D5P943_CHICK	inf	<0.0001	162	0
ZO-1/ TJP1	A0A1D5NWX9_CHICK	inf	<0.0001	339	0
MPDZ/ MUPP-1	F1N9G0_CHICK	inf	<0.0001	63	0
PALS1/MPP5	E1C3F8_CHICK	inf	<0.0001	17	0
PPP2R2A	A0A1D5PED5	inf	0.0035	13	0
GJA1	CXA1_CHICK	inf	<0.0001	11	0
MLLT4/ Afadin-6	A0A1D5P8Z8_CHICK	inf	0.01	7	0
MIF	MIF_CHICK	inf	0.18	4	0
MAGI-1	F1NMZ1_CHICK	inf	0.00045	13	0
SEC24C	E1BUD8	inf	0.00024	15	0
ALDH9A1	A0A1L1RQM3_CHICK	inf	<0.0001	11	0
SEC24B	A0A1D5PCR2	inf	0.11	5	0
HRNR	Q86YZ3	inf	0.31	3	0
ZO-3/ TJP-3	F1N8X8_CHICK	inf	0.019	7	0
YWHAQ	A0A1D5NW39	3	0.25	6	2

Table 2.6: List of proteins preferentially pulled down by wild-type Cldn8 when compared to GST control using HH6-8 embryos. List of proteins and their accession numbers that were preferentially pulled down wild-type Cldn8. Total spectral counts and folds are annotated for each protein. Folds were calculated by dividing the total spectral counts of proteins in GST-Cldn tails by the amount of that protein found in GST control. If no protein was found in the control the folds were impossible to calculate accurately and therefore they were indicated as infinite (inf). Internal controls are also added to the list of candidates. Proteins that were also identified on the day 4 pulled-downs are marked in blue. Fisher exact test and Bonferroni correction was performed to determine significance, which was achieved when $p < 0.0008$ (in bold).

We then explored the difference in interactors between wild-type Cldn8 and Cldn8 variants. Cldn8 variants had proteins to which they preferentially interacted and some where they did not interact to the same degree as wild-type Cldn8. Proteins that did not differ in their degree of interaction between wild-type Cldn8 and the variants were not included in these tables. Proteins of interest or related to tight junctions will be further analyzed in the following section. A list of increased and decreased interaction data is presented in Tables 2.8 and 2.9. Briefly, Cldn8S198A interactions with PALS1, MAGI-1, MPDZ and ZO-3 known to be involved in tight junctions were lower than wild-type Cldn8. Cldn8S216A preferentially pulled down cytoskeleton associated proteins such as SPTAN1, SPTANB1, MYH15, MYO18A, MYO5A or MYO6 when compared to wild-type Cldn8; however, interaction was lower for other proteins involved in tight junction formation or maintenance such as MPDZ, MAGI-1, ZO-3 or PALS1. Cldn8S216I pulled down higher amounts of proteins than wild-type Cldn8 mostly related to the cytoskeleton such as SPTAN1, SPTANB1, MYO5A or MYH15; while it decreased the interaction with other subset of proteins, which were preferentially pulled down by wild-type Cldn8, including proteins involved in tight junction formation such as ZO-1, ZO-2, ZO-3, MPDZ, MLLT4, MAGI-1 or PALS1. Finally, when Cldn8ΔYV was compared to wild-type Cldn8 no new or increased interaction was detected. On the other hand, its interaction with many other proteins was decreased to the point that they were not identified in the Cldn8ΔYV interactors, including junction related proteins such as ZO-1, ZO-2, ZO-3, MPDZ, MLLT4, MAGI-1, GJA1, SEC24A, SEC24C or PALS1 (Tables 2.8 and 2.9).

				Total spectral counts			
	Protein name	Accession number	F test	Fold	Cldn8 variant	WT Cldn8	GST
Cldn8 S198A	PALS1/MPP5	E1C3F8_CHICK	<0.0001	0.1	2	17	0
	MPDZ/ MUPP-1	F1N9G0_CHICK	<0.0001	0.1	7	63	0
	MAGI-1	F1NMZ1_CHICK	0.0085	0.3	3	13	0
	ZO-3/ TJP3	F1N8X8_CHICK	0.0037	0	0	7	0
	Ovocleidin-116	OC116_CHICK	0.036	0	0	5	0
Cldn8 S216A	MPDZ/ MUPP-1	F1N9G0_CHICK	<0.0001	0.3	21	63	0
	PALS1/MPP5	E1C3F8_CHICK	<0.0001	0.1	2	17	0
	MAGI-1	F1NMZ1_CHICK	0.00027	0.08	1	13	0
	ZO-3/ TJP3	F1N8X8_CHICK	0.0094	0	0	7	0
	Ovocleidin-116	OC116_CHICK	0.037	0	0	5	0
Cldn8 S216I	ZO-1/ TJP1	A0A1D5NWX9_CHICK	<0.0001	0.41	142	339	0
	ZO-2/ TJP2	A0A1D5P943_CHICK	<0.0001	0.17	29	162	0
	SEC24C	E1BUD8	0.0023	0	0	15	0
	MPDZ/ MUPP-1	F1N9G0_CHICK	<0.0001	0	0	63	0
	PALS1/MPP5	E1C3F8_CHICK	<0.0001	0	0	17	0
	MLLT4/ Afadin-6	A0A1D5P8Z8_CHICK	0.0078	0	0	7	0
	MAGI-1	F1NMZ1_CHICK	0.00029	0	0	13	0
	SEC23B	A0A1I7Q406	0.051	0	0	5	0
	Sec24B	A0A1D5PCR2	0.092	0	0	5	0
	GJA1	CXA1_CHICK	0.011	0.45	5	11	0
	Ovocleidin-116	OC116_CHICK	0.051	0	0	5	0
	ZO-3/ TJP3	F1N8X8_CHICK	0.015	0	0	7	0
	ZO-2/ TJP2	A0A1D5P943_CHICK	<0.0001	0	0	162	0
	ZO-1/ TJP1	A0A1D5NWX9_CHICK	<0.0001	0	0	339	0
	MPDZ/ MUPP-1	F1N9G0_CHICK	<0.0001	0	0	63	0
Cldn8 ΔYV	PALS1/MPP5	E1C3F8_CHICK	<0.0001	0	0	17	0
	GJA1	CXA1_CHICK	<0.0001	0	0	11	0
	MLLT4/ Afadin-6	A0A1D5P8Z8_CHICK	0.011	0	0	7	0
	MAGI-1	F1NMZ1_CHICK	0.00054	0	0	13	0
	SEC24C	E1BUD8	0.00029	0	0	15	0
	SEC24B	A0A1D5PCR2	0.11	0	0	5	0
	SEC23B	A0A1I7Q406	0.065	0	0	5	0
	ZO-3/ TJP3	F1N8X8_CHICK	0.021	0	0	7	0
	Ovocleidin-116	OC116_CHICK	0.065	0	0	5	0

Table 2.7: Proteins preferentially pulled down by wild-type Cldn8 compared to Cldn8 tail variants from HH6-8 chick embryo protein extracts. Total spectral counts and folds were annotated for each protein that was preferentially pulled down by wild-type Cldn8 as compared to Cldn8 tail variants. Folds were calculated by dividing the total spectral counts proteins in GST-Cldn8 variant tails by the counts for that protein in the GST-wild-type Cldn8 tail pull-down fraction. Internal controls are also added to the list of candidates. Even though Control sample is

wild-type Cldn8, we also add information about the presence or absence on the GST only pulled-down. Fisher exact test and Bonferroni correction was performed to determine significance, which was achieved when $p < 0.0008$ (in bold).

	Protein name	Accession number	F test	Fold	Total spectral counts		
					Cldn8 variant	WT Cldn8	GST
Cldn8 S198A	RPS19	A0A1D5PDV6	0.26	inf	4	0	0
	FBXO21	F1NDZ7_CHICK	0.36	2.6	6	2	0
	Apovitellenin-1	APOV1_CHICK	0.1	2.5	18	7	8
	HSPD1	Q5ZL72	0.17	2.4	13	5	4
	TXNRD3	A0A1D5NVZ3_CHICK	0.26	2.3	10	4	2
Cldn8 S216A	SPTAN1/ Fodrin	A0A1D5PVG1	<0.0001	inf	41	0	16
	MYH15	F1NM49	<0.0001	73	73	1	15
	SPTBN1	A0A1D5PJY1	<0.0001	inf	26	0	4
	MYO18A	E1BY27	<0.0001	10	50	5	20
	MYO5A	A0A1D5PRP3	<0.0001	11	34	3	9
	MYO6	F1NWI9	<0.0001	inf	20	0	9
	HK1	F1NZJ2	0.0036	5	22	4	10
	Gelsolin	O93510	0.0022	2.4	53	22	38
Cldn8 S216I	SPTAN1/ Fodrin	A0A1D5PVG1	<0.0001	inf	32	0	16
	SPTBN1	A0A1D5PJY1	0.00074	inf	11	0	4
	MYH15	F1NM49	<0.0001	40	40	1	15
	MYO5A	A0A1D5PRP3	0.0044	6.7	20	3	9

Table 2.8: Proteins preferentially pulled down by Cldn8tail variants compared to wild-type Cldn8 from HH6-8 chick embryo protein extracts. List of proteins and accession numbers of proteins identified to be preferentially pulled down by each Cldn8tail variant. Folds were calculated by dividing the total spectral counts proteins in GST-Cldn8 variant tails by the amount of that protein found in wild-type Cldn8 tail pull-down fractions. If no protein was found in the control, the folds were impossible to calculate accurately and therefore they were indicated infinity (inf). Internal controls are also added to the list of candidates. Fisher exact test and Bonferroni correction was performed to determine significance, which was achieved when $p < 0.0008$ (in bold).

In summary, all variants appeared to lose their ability to interact with MAGI-1, ZO-3 and MPDZ. The S216I variant appears to have reduced interaction with all of these proteins, while Cldn8 Δ YV did not interact with any tight junction protein. Cldn8S198A and S216A retain the ability to interact with ZO-1, ZO-2 and Afadin-6, while this interaction appears weaker for Cldn8S216I. *De novo* interactions were observed for some cytoskeleton associated proteins, such as SPTBN1 and SPTAN1, also known as fodrin, with Cldn8S216 variants. As an internal control we

manually annotated the sequence of the GST-Cldn8 and showed that it or Cldn8 variants appeared in all of the pull-downs in a relative similar abundance (< 1.2 folds difference). A comparison of interaction of tight junction related proteins and fodrin with wild-type Cldn8 and Cldn8 variants can be found on Table 2.9

	Total Spectral Counts					
	GST	WT Cldn8	Cldn8S198A	Cldn8S216A	Cldn8S216I	Cldn8ΔYV
ZO-1	0	339	341	310	140	0
ZO-2	0	162	170	138	29	0
ZO-3	0	7	0	0	0	0
MPDZ/MUPP-1	0	63	7	21	0	0
MLLT4/ Afadin-6	0	7	8	11	0	0
MAGI-1	0	13	3	1	0	0
PALS1/MPP5	0	17	2	2	0	0
SPTAN-1/fodrin	16	0	9	41	32	1
SPTBN-1	4	0	0	26	11	0
GST-peptide (internal control)	4082	4254	5141	4651	4571	5178

Table 2.9: Comparison of tight junction proteins pulled down by wild-type and variant Cldn8 from HH6-8 chick embryo protein extracts. List of tight junction proteins that were preferentially pulled down by wild-type Cldn8 or Cldn8 variants and the comparison with each other. Numbers indicate spectral counts.

The protein interactions identified by mass spectrometric analysis of the pull-down experiments are not quantitative and it is necessary to validate these data. To confirm Cldn8's interaction with ZO-2, the pull-downs were repeated with the different variants and confirmed by western blot. Although Cldn8ΔYV did not pull-down ZO-1, all other variants and wild-type Cldn8 successfully pulled ZO-1 down (Figure 2.5). Repetition and in vivo validation are necessary to confirm if the decreased interaction seen by mass spectrometry is real. In this western blot, we also confirmed the relative amount of GST fusion protein bait was similar between the different variants and wild-type (Figure 2.5). The different candidates will be further validated in ongoing experiments by measuring their expression and localization in vivo in the neural ectoderm of chick embryos electroporated with the different variants.

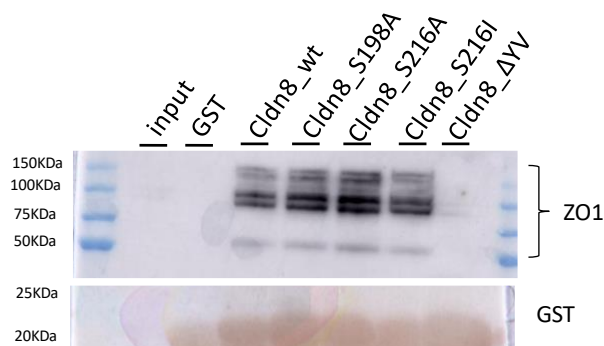


Figure 2.5: Validation that ZO-1 is pulled down by some Cldn8 variants. Western blot against ZO-1 and GST of pulled-downs using GST-Claudin8 variant tails as bait of HH8 chick embryo.

2.5 DISCUSSION

Characterizing the specific interactions of individual claudin family members is essential to understand the consequences of removing a specific claudin on interactions at the tight junction cytoplasmic plaque and how these interactions are important for function. In this study, we described the interactomes for Cldn1, -3, -4, -8 and -14 cytoplasmic C-terminal tails in whole cell extracts from day 4 chick embryos. For Cldn8, we explored the impact of Cldn8 cytoplasmic tail variants on these interactions and its role in neural tube closure. Our study revealed that each claudin preferentially interacts with a subset of proteins

The ability of claudins to interact with different cytoplasmic plaque proteins has been previously studied. Jeansonne *et al.* used Cldn8 as bait in a yeast two-hybrid system to show that it could interact with MPDZ. They confirmed this interaction in MDCK cells, where they showed that Cldn8 and MPDZ were able to co-immunoprecipitate and colocalize when transfected in MDCK cells, and consequently change the TER barrier properties (Jeansonne et al., 2003). In addition, Itoh et al., showed that the PDZ1 domain in ZO-1 interacts is able to interact with claudin 1-8 (Itoh et al., 1999). But the question remained as to whether this interaction happens in vivo or if ZO-1 has a preference for individual claudin family members.

From our experiment, it is clear that each claudin preferentially interacts with specific proteins. Moreover, Cldn3, -8 and -14 appear to participate in a broader spectrum of interactions than Cldn 1 and -4. Interestingly, although all claudins are supposed to interact with ZO-1, in our dataset Cldn3, -8 and -14 were able to bind to ZO-1 while Cldn1 and -4 appear not to bind to ZO-1. Similar differences were described by Nomme et al. who described that Cldn8 bound to ZO-1

with a higher affinity than other claudins (Nomme et al., 2015). These differences in affinity and interaction may be due to the amino acid sequence or charge, or post-translational modifications that could increase their interaction. Proteomic screening approaches to identify proteins that are in the proximity of Cldn4, occludin and ZO-1 were done using biotin ligase (Fredriksson et al., 2015; Van Itallie et al., 2013). These studies revealed that the Cldn4 C-terminal domain is in the proximity of many other tight junction proteins. While BioID is useful to identify proteins that are in close proximity to a protein of interest, they do not provide concrete evidence that the interaction is direct. In fact, the BioID experiments of Van Itallie and colleagues showed that ZO-1 is surrounded by all the claudins present in the cell, including Cldn1, -2, -3, -4, or -7, even though we know their tails do not directly interact (Van Itallie et al., 2013). A more recent BioID study by the same group using Cldn4 fused to biotin ligase suggests that ZO-1 is not in close proximity with Cldn4 (Fredriksson et al., 2015). Indeed, analysis of our GST-pulldown data suggests that not all claudins bind to ZO-1 with the same affinity. We showed that the ability of each claudin to interact with other tight junction proteins is different and dependent on the specific claudin, not only in the case of ZO-1 but also for ZO-2, MPDZ, PALS1 or MLLT4.

Our proteomic study revealed that each claudin interacted with a different profile of proteins. For instance, Cldn3 and -8 interacted with many known cytoplasmic tight junction proteins that are known to maintain and regulate tight junction architecture, such as ZO-1, ZO-2, MPDZ or MLLT4. For Cldn8, we observed these interactions in protein extracts from day 4 and HH6-8 (~1.25-1.5) embryos. In contrast, Cldn14 barely interacted with tight junction proteins, rather more of its interaction were with cytoskeleton associated proteins or extracellular matrix proteins. Cldn4 had few interaction partners; only CACNA2D3, a calcium channel protein and two enzymes involved in post-translational modification (PPP2R2A and ALG14) were identified. Finally, Cldn1 was observed to interact with a wide range of proteins but the only tight junction protein seen to bind to Cldn1 was ZO-2. These different patterns suggest that claudins may have different roles in the tight junction cytoplasmic plaque. This also correlates with our unpublished data that depleting Cldn3 or Cldn8 leads to more severe defects in neural tube closure than removal of Cldn4 from tight junctions (Baumholtz et al., 2017b).

In our experimental paradigm, we used the claudin tail as bait; however, we did not remove endogenous claudins. This could mean that proteins present in the embryo extract may bind with higher affinity to claudins present in the extract rather than the claudin tail used as bait. Validation of our observation *in vivo* by co-immunoprecipitation assays is crucial to understand the full interactome and affinity assays might be of interest in the future to measure relative binding capacity of each claudin to the different cytoplasmic proteins.

The amino acid sequence and the post-translational modifications of amino acid residues often control the ability and affinity of proteins interactions. In fact, when claudins are phosphorylated at the tyrosine 6 amino acids from their C-terminus (chicken Cldn8Y219), their ability to interact with ZO-1 decreases (Nomme et al., 2015). In other instances, phosphorylation of claudin tails increases their stability at the membrane or targets them for degradation. These effects are tissue and stimuli dependent and probably related to the amino acid that is modified and the other proteins expressed in the tissue that interact with the claudin (Gonzalez-Mariscal et al., 2008). In our study, we did not modify tyrosine 219 but did change the nearby amino acid Serine-216 to isoleucine or alanine. We also modified the putative phosphorylation site at Serine-198 to alanine and created a variant that was missing the two C-terminal amino acids of claudins known to be essential for claudin's ability to interact with PDZ-domains of other proteins within the tight junction cytoplasmic plaque. The effects of these variants on neural tube closure did not correlate with the effects on their ability to interact with tight junction proteins as compared to wild-type Cldn8. The Cldn8S198A and S216A variants had similar effects on protein interactions: both interacted similarly with ZO-1, ZO-2 and afadin-6, but did not interact with MPDZ, ZO-3 or MAGI-1. Yet, while Cldn8S216A did not affect neural tube closure, the Cldn8S198A variant increased the incidence of open neural tubes. In contrast, Cldn8S216I and ΔYV variants showed reduced binding to all Cldn8-enriched interaction partners, but while embryos overexpressing Cldn8S216I had open NTDs as well as defects in convergent extension, embryos overexpressing the Cldn8 ΔYV variant looked like wild-type embryos. This suggests that the phenotypes may be due to *de novo* interactions or improper interactions with cytoplasmic proteins, rather than the loss of interactions with tight junction proteins. Interestingly, both Cldn8S216A and S216I pulled down SPTAN1, also known as fodrin, which interacts with calmodulin in a calcium-dependent

manner and may be a candidate for calcium-dependent movement of the cytoskeleton at the membrane. This interaction was not observed for wild-type Cldn8 nor for the Cldn8S198A or ΔYV variants. A role for alpha II spectrin/fodrin in neural tube closure was proposed several decades ago based on its expression pattern during neural tube closure (Sadler et al., 1986) and null mice have NTDs (Stankewich et al., 2011). Obviously, this interaction needs to be confirmed and its relevance to neural tube closure explored. Given that Cldn8 ΔYV variant had very few interactions, it is possible that the absence of a phenotype is due to its inability to compete with other claudins present at the tight junction.

With this study, we aimed to understand better the role of each claudin during neural tube closure and to investigate if there were differences in the specific cytoplasmic proteins that could interact with each of the claudin tails. We successfully showed that each claudin can interact preferentially with specific cytoplasmic proteins. We also showed that the maintenance of this interactions may be essential for the proper neural tube closure. Altogether our work contributes to increasing our knowledge about claudin interactomes, which is essential to better understand tight junctions' biology and regulation.

2.6 AUTHOR CONTRIBUTIONS

Conceptualization, Enrique Gamero-Estevez, Amanda Baumholtz and Aimee K. Ryan; Formal analysis, Enrique Gamero-Estevez, Amanda Baumholtz, Jean-Francois Boisclair Lachance; Funding acquisition, Aimee K. Ryan; Investigation, Enrique Gamero-Estevez, Amanda Baumholtz and Aimee K. Ryan; Methodology, Enrique Gamero-Estevez, Amanda Baumholtz and Aimee K. Ryan; Writing – original draft, Enrique Gamero-Estevez; Writing – review & editing, Aimee K. Ryan.

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2.9 CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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2.11 CONNECTING TEXT BETWEEN CHAPTER 2 AND 3

In Chapter 2 I studied the interactome of the C-terminal domain of five different claudins with the intention of understanding their complexity. Characterizing the specific interactions of individual claudin family members is essential to understand the consequences of removing a specific claudin from the tight junction. I showed that each claudin appears to interact preferentially with a different subset of proteins. I extended these analyses for Cldn8 and evaluated the requirement for specific amino acids in its cytoplasmic tail for these interactions. In Chapter 3, I shift the focus to examine the effects of quercetin, a compound that have been shown to modify tight junctions in different models. I explore the effect of quercetin on claudin expression and localization in a cation-selective cell line. These data allowed me to correlate effects on the tight junction barrier with changes in localization or modification of specific claudins. This work was published in the *International Journal of Molecular Science* in 2019 and is reproduced in this Chapter.

CHAPTER 3: Temporal effects of quercetin on tight junction barrier properties and claudin expression and localization in MDCK II cells

Enrique Gamero-Estevez^{1,2}, Sero Andonian³, Bertrand Jean-Claude^{2,4}, Indra Gupta^{1,2,5} and Aimee K. Ryan^{1,2,5}

¹Department of Human Genetics, McGill University, Montreal, QC H4A 3J1, Canada

²Research Institute of the McGill University Health Centre, Glen Site, Montreal, QC H4A 3J1

³Division of Urology, McGill University, Montreal, QC H4A 3J1, Canada

⁴Department of Medicine, McGill University, Montreal, QC H4A 3J1, Canada

⁵Departments of Pediatrics, McGill University, Montreal, QC H4A 3J1, Canada.

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3.1. ABSTRACT

Kidney stones affect 10% of the population. Yet, there is relatively little known about how they form or how to prevent and treat them. The claudin family of tight junction proteins has been linked to the formation of kidney stones. The flavonoid quercetin has been shown to prevent kidney stone formation and to modify claudin expression in different models. Here we investigate the effect of quercetin on claudin expression and localization in MDCK II cells, a cation-selective cell line, derived from the proximal tubule. For this study, we focused our analyses on claudin family members that confer different tight junction properties: barrier-sealing (Cldn1, -3 and -7), cation-selective (Cldn2) or anion-selective (Cldn4). Our data revealed that quercetin's effects on the expression and localization of different claudins over time corresponded with changes in transepithelial resistance, which was measured continuously throughout the treatment. In addition, these effects appear to be independent of PI3K/AKT signaling, one of the pathways that is known to act downstream of quercetin. In conclusion, our data suggest that quercetin's effects on claudins result in a tighter epithelial barrier, which may reduce the reabsorption of sodium, calcium and water, thereby preventing the formation of a kidney stone.

3.2 INTRODUCTION

Kidney stones affect 10% of the population and recur in 50% of adults. They are associated with renal failure in children and adults. They cause extreme pain and have a significant financial burden to society (Evan, 2010; Khan et al., 2016; Walker, 2019). Given these morbidities, surprisingly little is known about why stones recur and how they can be prevented. Most stones are calcium-based with calcium oxalate more frequently observed than calcium phosphate stones (Walker, 2019). Kidney stones result from the accumulation of salts along the kidney nephron, where an important factor in salt reabsorption is the epithelial tight junction barrier. Tight junctions are the most apical junction between apposing cells, where they compartmentalize the apical and the basolateral intercellular space and regulate the passive paracellular movement of water and solutes (Anderson and Van Itallie, 2009). The ion specificity of the tight junction barrier is determined by the claudin family of tetraspanin proteins, which

contains close to 30 members (Gupta and Ryan, 2010; Tsukita et al., 2019; Van Itallie and Anderson, 2006). Claudins can bind hetero- and homotypically with claudins in the same cell through their transmembrane domains or to claudins in the apposing cell through their extracellular loops (Gupta and Ryan, 2010). The combination of claudins expressed within an epithelium determines the tightness and selectivity of the tight junction barriers (Yu, 2015).

The claudin composition of tight junctions varies along the different segments of the nephron and, consequently, ions and salts are differentially reabsorbed in the different nephron segments (Yu, 2015). Reabsorption of calcium from the urine filtrate predominantly occurs in two segments: the proximal tubule reabsorbs 70% and the thick ascending limb reabsorbs 25% of the calcium. Several claudin family members are implicated in calcium reabsorption. A common sequence variant in CLDN14 and rare mutations in CLDN16 and -19, all of which are expressed in the thick ascending limb, are risk factors for the formation of calcium-based kidney stones in humans (Konrad et al., 2006; Simon et al., 1999; Thorleifsson et al., 2009). Both Cldn2 and -10 null mice develop nephrocalcinosis (Breiderhoff et al., 2012; Muto et al., 2010), while Cldn16 and -19 knockdown mice develop hypercalciuria (Himmerkus et al., 2008; Hou et al., 2009). Cldn7 is also essential for salt reabsorption: Cldn7 null mice die shortly after birth due to salt loss and dehydration (Tatum et al., 2010). Although not known to participate in ion exchange, Cldn3 has been shown to form a complex with Cldn16 and -19 in the thick ascending limb, which is essential for calcium and magnesium reabsorption in this segment (Plain and Alexander, 2018). Cldn4 has a critical role in chloride reabsorption in the kidney collecting duct where it forms a pore with Cldn8 (Gong and Hou, 2017). The role of Cldn1 in ion reabsorption is less clear; however, it is present in different kidney segments and may be important in diabetic nephropathy (Wakino et al., 2015). Because claudins are essential for sodium, calcium and water reabsorption in the nephron, targeting this claudin-based epithelial barrier may be a successful approach to decrease salt reabsorption and prevent kidney stone formation

The flavonoid quercetin prevents kidney stone formation in a rat model (Zhu et al., 2014). Quercetin alters claudin expression in the Caco-2 intestine-derived cell line and in LLC-PK1 cells, an anion-selective cell line derived from the renal proximal tubule (Mercado et al., 2013; Suzuki and Hara, 2009; Suzuki et al., 2011; Valenzano et al., 2015). To date, no one has studied the effect

of quercetin in a cation-selective cell line that models an epithelial barrier which is permeable to calcium and sodium. We hypothesize that quercetin may prevent kidney stones through its effects on claudins at tight junctions in the epithelial barrier of the nephron. For our study, we used Madin-Darby Canine Kidney cells (MDCK II), which are cation-selective and derived from the proximal tubule where the majority of sodium and calcium reabsorption occurs. MDCK II cells express Cldn1, -2, -3, -4 and -7 (Dukes et al., 2011; Hou et al., 2006), which allows us to investigate the effect of quercetin on claudins with different properties: barrier-sealing (Cldn1, -3 and -7), cation-selective (Cldn2) or anion-selective (Cldn4) within a cation-selective cell line (Borovac et al., 2012; Gunzel and Fromm, 2012; Rosenthal et al., 2017; Van Itallie et al., 2003). The MDCK II cell line has been widely studied and its TER and cation permeability properties are well characterized. Cldn2 confers the leaky barrier properties of MDCK II cells. If Cldn2 is depleted, TER increases and sodium and chloride potentials are significantly reduced (Tokuda and Furuse, 2015).

We found that quercetin differentially affected both the expression and the localization of some claudins over time. Quercetin also significantly increased transepithelial resistance. Our data suggest that after treatment with quercetin, the barrier becomes tighter, which could lead to a decrease in cation and water reabsorption. This may result in a more favorable urinary filtrate that is less prone to crystal supersaturation and the formation of a stone.

3.3 RESULTS

3.3.1 Quercetin increased transepithelial resistance of MDCK II cells

Transepithelial resistance (TER) is a measure of the electrical resistance of a cell monolayer and is modulated by cell confluence, barrier permeability and tight junction composition. For these studies, we used the cellZscope (NanoAnalytics) to continuously measure TER in MDCK II cells from the time of plating until several days after confluence when stable, mature tight junctions are formed. In cation-selective cell lines, TER increases as cells become closer or when paracellular movement of cations is reduced (Van Itallie et al., 2003). As predicted, there was an increase in TER immediately after seeding, when cells are dividing and confluence is increasing (Figure 3.S1A). Under control conditions, MDCK II cells reached a peak resistance of 130 $\Omega \cdot \text{cm}^2$

~40h after seeding. Once maximum confluence is achieved, contact inhibition and tight junction remodeling takes place, which leads to a decrease in the TER that eventually stabilizes and becomes constant as the cells form stable mature tight junctions (Dembla et al., 2016; Srinivasan et al., 2015). In MDCK II cells, this translated into a decrease in TER that then remained at 50-80 $\Omega\cdot\text{cm}^2$, which is characteristic for mature tight junctions in this model (Dembla et al., 2016). Therefore, this was the time point selected for treatment with quercetin since it best represents the mature renal proximal tubule epithelium.

We confirmed that 400 μM quercetin, the concentration used in previous studies (Mercado et al., 2013; Valenzano et al., 2015), was also the most optimal for our experiments through a dose response curve (Figure 3.S1). We monitored the effects of quercetin on TER for ~96h after treatment. Figure 3.1A shows the TER profile for one of the three biological replicates, which were each done in triplicate. Analysis of all data showed a small expected increase in TER immediately after treatment, due to the removal of the cells from the incubator and change of media. In control cells, the TER stabilized within 4-5h and then remained at a resistance of ~50 $\Omega\cdot\text{cm}^2$ (Figure 1A). Two-way ANOVA analysis showed that TER changed significantly over time and in a treatment-dependent manner ($P_{\text{int}}=0.0405$; $P_{\text{time}}<0.0001$; $P_{\text{treat}}<0.0001$). Cells treated with 400 μM quercetin exhibited a progressive increase in TER, reaching significance at 3h when the TER was ~15 $\Omega\cdot\text{cm}^2$ higher than the control cells ($P=0.049$; quercetin-treated = 90.04 ± 4.01 $\Omega\cdot\text{cm}^2$ versus control cells = 70.7 ± 1.62 $\Omega\cdot\text{cm}^2$). The TER remained significantly increased until 5h post-treatment ($P=0.046$; quercetin-treated = 86.33 ± 2.94 $\Omega\cdot\text{cm}^2$ versus control cells = 66.86 ± 3.59 $\Omega\cdot\text{cm}^2$) and then progressively decreased to ~5 $\Omega\cdot\text{cm}^2$ below control levels 15h after treatment, which was not significant (15h: $P>0.99$; control 60.08 ± 3.61 , quercetin 62.21 ± 2.37). Following the decrease, TER increased again, reaching a steady state level of 10 $\Omega\cdot\text{cm}^2$ above control, 36h after treatment, which was statistically significant and remained significantly increased for the duration of the experiment (36h: $P=0.0071$; control 54.7 ± 2.31 , quercetin 78.05 ± 5.19) (48h: $P<0.0001$; control 53.35 ± 1.8 , quercetin 85.68 ± 2.55) (Figure 3.1).

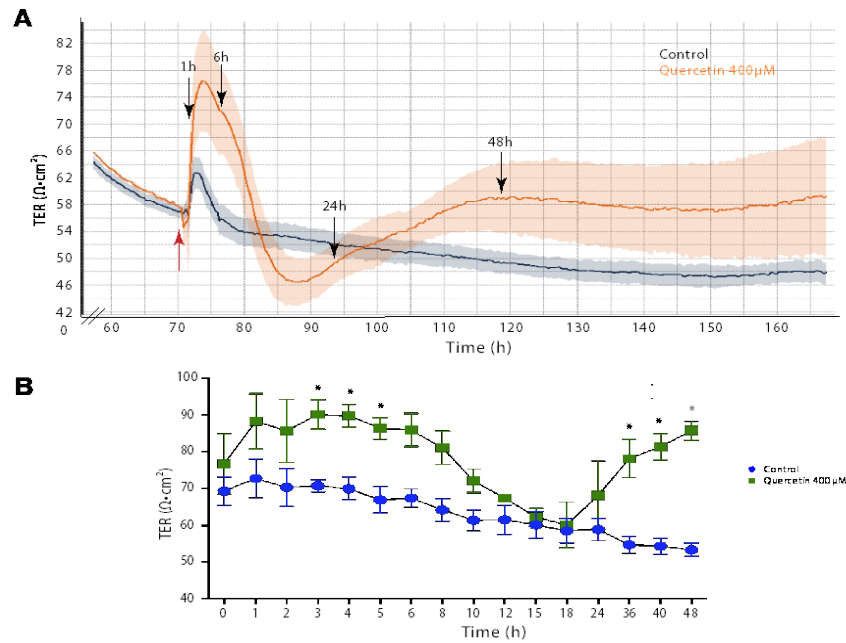


Figure 3.1: Quercetin caused oscillations in transepithelial resistance (TER) of MDCK II cells. (A) Representative plot of TER in control cells (black) and cells treated with 400 μM quercetin (orange) from one biological replicate performed in triplicate. Red arrow indicates when quercetin was added to the culture medium. Black arrows indicate the time points taken for western blot and immunofluorescence analysis. (B) TER of control and quercetin-treated cells at different time points after treatment from three independent experiments performed in triplicate. Two-way ANOVA was performed ($P_{\text{int}}=0.04$; $P_{\text{time}}<0.0001$; $P_{\text{treat}}<0.0001$). Mean and SEM are plotted. *Denotes significance, $P<0.05$.

3.3.2 Quercetin treatment caused claudin-specific changes in expression and membrane localization

To determine if the changes in TER caused by quercetin treatment corresponded with different claudin profiles, cells were collected 1, 6, 24 and 48 hours after treatment. Claudin expression was assessed by western blot analysis and localization to the tight junction barrier was assessed by immunofluorescence. Immunofluorescence also provided a qualitative assessment of claudin expression. Five claudins expressed in MDCK II cells were studied: Cldn1, -3 and -7 that have barrier-sealing functions, Cldn2 that is involved in cation pore formation, and Cldn4 that is involved in anion pore formation. For all experiments, cells were cultured for 72h before treatment with 400 μM quercetin to ensure that the cells had established mature tight junctions.

3.3.2.1. Cldn1

Western blot analysis revealed a significant decrease in Cldn1 expression over time in both controls and quercetin-treated cells ($P_{\text{time}}=0.021$). Quercetin treatment significantly lowered Cldn1 levels at 48h compared to controls ($P=0.038$; control 1.47 ± 0.55 ; quercetin 0.44 ± 0.18). A change in the relative abundance in the two migratory bands was observed at 24h, although the total amount of Cldn1 was not affected (Figure 3.2A-B). Immunofluorescence analysis revealed decreased levels of Cldn1 at 1, 6 and 48h (Figure 3.2C). A reduction in Cldn1 co-localization with ZO-1 can be seen at 1 and 48h, although it was not significant ($P=0.3$ and $P=0.2$, respectively) (Figure 3.2D). These data suggest that even though general levels of Cldn1 were decreased, the remaining Cldn1 still co-localized with ZO-1.

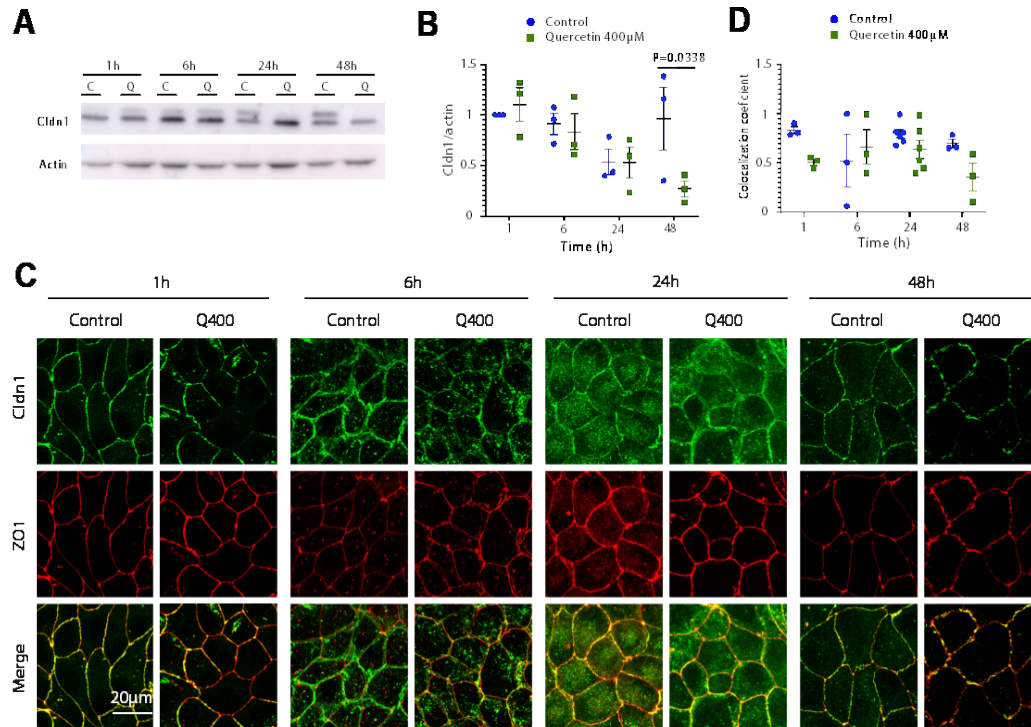


Figure 3.2: Analysis of Cldn1 expression and localization in MDCK II cells following quercetin treatment. (A) Western blot analysis of Cldn1 expression in cell lysates from control and 400μM quercetin-treated MDCK II cells. Actin was used as a loading control. **(B)** Densitometry measurements of Cldn1 and actin intensity on western blot were normalized to expression at 1h. Normalized Cldn1 expression relative to normalized actin expression is plotted ($P_{\text{int}}=0.11$; $P_{\text{time}}=0.022$; $P_{\text{treat}}=0.16$). **(C)** Immunofluorescence of control and 400μM of quercetin-treated MDCK II cells at 1h, 6h, 24h and 48h. Cldn1 is shown in green and ZO-1 is shown in red. **(D)** Localization of claudin at the tight junction was assessed by determining the amount of ZO-1 that

was co-localized with claudin expression ($P_{\text{int}}=0.24$; $P_{\text{time}}=0.35$; $P_{\text{treat}}=0.055$). For all graphs, each point corresponds to an independent experiment; mean and SEM are shown. C=Control and Q=Quercetin. Scale bar, 20 μm .

3.3.2.2. Cldn2

Two-way ANOVA analysis on Cldn2 expression showed that Cldn2 decreased significantly with quercetin treatment ($P_{\text{treat}}=0.0021$), while changes in time and interaction only suggested significance ($P_{\text{int}}=0.0525$; $P_{\text{time}}=0.096$). Sidak's multiple comparison test showed that the decrease observed at 48h after treatment with quercetin was significant ($P=0.0011$; control 2.0 ± 0.79 ; quercetin 0.06 ± 0.057). At 24h, no Cldn2 was observed by western blot analysis in quercetin-treated cells (Figure 3.3A-B). As previously reported, Cldn2 detection by immunofluorescence was patchy within the epithelial monolayer of control MDCK II cells (Amoozadeh et al., 2018), with clusters of cells showing high levels of Cldn2 expression and adjacent groups of cells showing virtually no expression (Figure 3.3C). Localization of Cldn2 was significantly changed after treatment with quercetin ($P_{\text{treat}}=0.0055$). At 1h and 6h after quercetin treatment, no changes in localization of Cldn2 were observed. However, at 24h and 48h after treatment, quercetin-treated cells showed a significant reduction both in the amount of Cldn2 and in the portion of Cldn2 that co-localized with ZO-1 (24h: $P=0.036$; control 0.54 ± 0.11 , quercetin 0.14 ± 0.08 ; 48h: $P=0.034$; control 0.52 ± 0.12 , quercetin 0.12 ± 0.05) (Figure 3.3C-D).

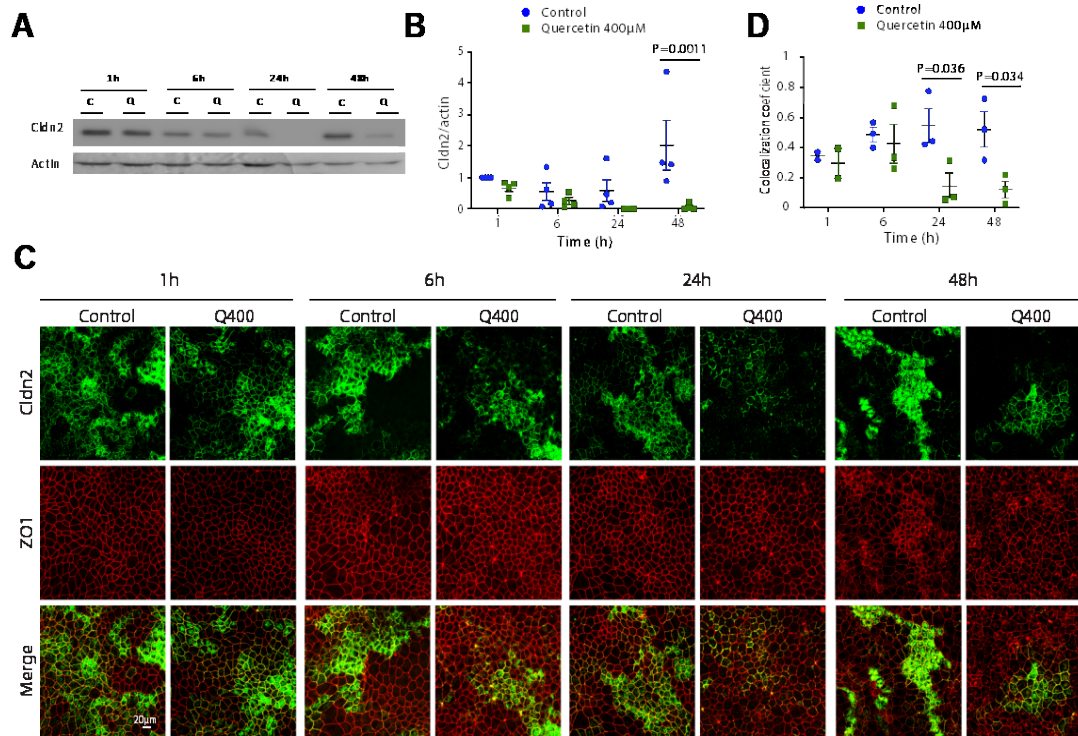


Figure 3.3: Analysis of Cldn2 expression and localization in MDCK II cells following quercetin treatment. (A) Western blot analysis of Cldn2 expression in cell lysates from control and 400 μ M quercetin-treated MDCK II cells. Actin was used as a loading control. (B) Densitometry measurements of Cldn2 and actin intensity on western blot were normalized to control at 1h. Normalized Cldn2 expression relative to normalized actin is plotted ($P_{\text{int}}=0.052$; $P_{\text{time}}=0.096$; $P_{\text{treat}}=0.0021$) (C) Immunofluorescence of control and 400 μ M of quercetin-treated MDCK II cells at 1h, 6h, 24h and 48h. Cldn2 is shown in green and the ZO-1 is shown in red. (D) Localization of claudin at the tight junction was assessed by determining the amount of ZO-1 that co-localized with claudin expression ($P_{\text{int}}=0.15$; $P_{\text{time}}=0.46$; $P_{\text{treat}}=0.0055$). For all graphs, each point corresponds to an independent experiment; mean and SEM are shown. C=Control and Q=Quercetin. Scale bar, 20 μ m.

3.3.2.3. Cldn3

Western blot analysis showed that quercetin caused a significant treatment-dependent increase in Cldn3 expression ($P_{\text{treat}}=0.0395$). In marked contrast to the effects on Cldn1 and -2, Cldn3 expression was increased at 24h and 48h after treatment, although it was only statistically significant at 48h ($P=0.04$; control 0.29 ± 0.1 ; quercetin 2.4 ± 0.98) (Figure 3.4A-B). Although there appeared to be a general decrease in Cldn3 detection by immunofluorescence after 1h and 6h of treatment (Figure 3.4C), co-localization analysis showed that there was no effect on Cldn3 co-

localization with ZO-1 at 1h and 6h ($P=0.79$ and $P=0.99$, respectively) (Figure 3.4D). Cldn3 also co-localized with ZO-1 at later time points.

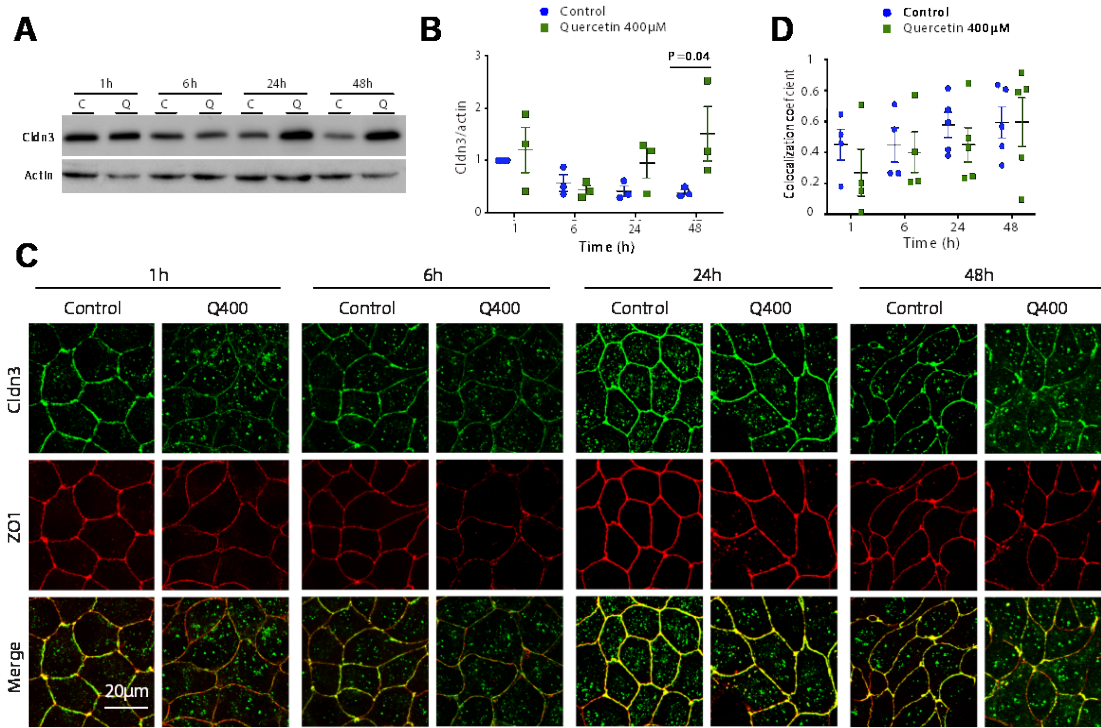


Figure 3.4: Analysis of Cldn3 expression and localization in MDCK II cells following quercetin treatment. (A) Western blot analysis of Cldn3 expression in cell lysates from control and 400μM quercetin-treated MDCK II cells. Actin was used as a loading control. (B) Densitometry measurements of Cldn3 and actin were normalized to control at 1h. Normalized Cldn3 expression relative to normalized actin is plotted ($P_{int}=0.16$; $P_{time}=0.16$; $P_{treat}=0.039$). (C) Immunofluorescence of control and 400μM of quercetin-treated MDCK II cells at 1h, 6h, 24h and 48h. Cldn3 is shown in green and ZO-1 is shown in red. (D) Localization of claudin at the tight junction was assessed by determining the amount of ZO-1 that co-localized with claudin expression ($P_{int}=0.87$; $P_{time}=0.24$; $P_{treat}=0.31$). For all graphs, each point corresponds to an independent experiment; mean and SEM are shown. C=Control and Q=Quercetin. Scale bar, 20 μm.

3.3.2.4. Cldn4

Cldn4 expression changed over time following quercetin treatment ($P_{int}<0.0001$; $P_{time}=0.0017$; $P_{treat}<0.0001$). At 24h and 48h, Cldn4 expression was significantly increased by western blot analysis (control = 0.67 ± 0.37 ; quercetin = 2.74 ± 0.36 , $P<0.0001$ and control = 0.79 ± 0.36 ; quercetin = 2.93 ± 0.37 , $P<0.0001$, respectively) (Figure 3.5A-B). In contrast, by immunofluorescence, qualitatively, Cldn4 was downregulated at 48h (Figure 3.5C), but co-

localized with ZO-1 (Figure 3.5D). This could be a consequence of possible claudin modifications that may avoid correct recognition of the epitope by the antibody. At this point, we cannot explain the discrepancy in Cldn4 expression as assessed by western blot and immunofluorescence.

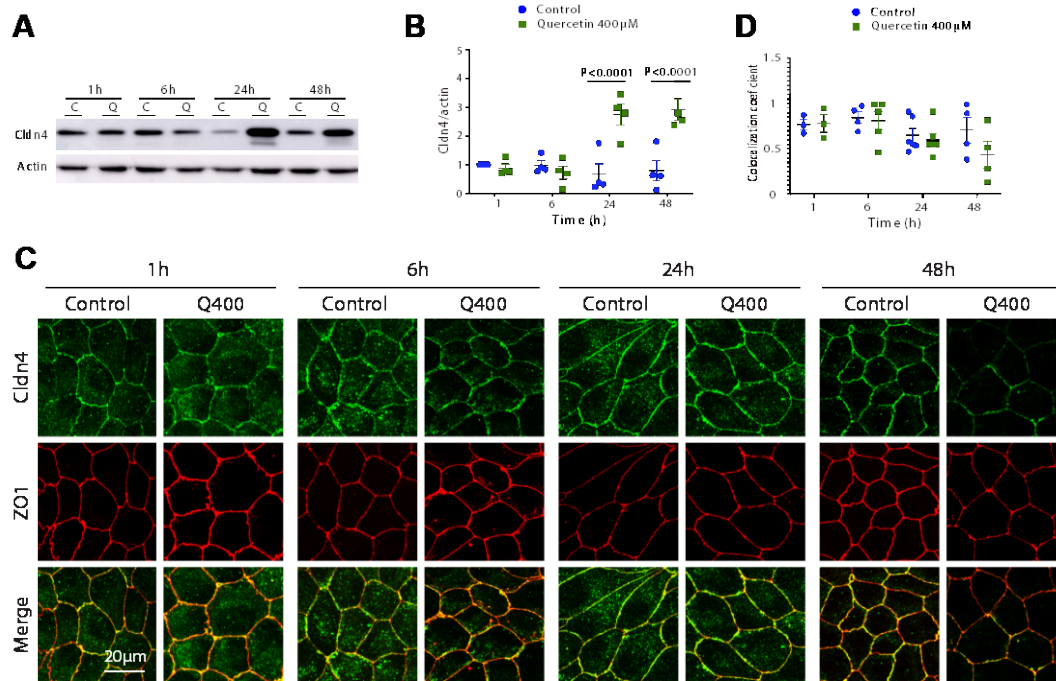


Figure 3.5: Analysis of Cldn4 expression and localization in MDCK II cells following quercetin treatment. (A) Western blot analysis of Cldn4 expression in cell lysates from control and 400μM quercetin-treated MDCK II cells. Actin was used as a loading control. (B) Densitometry measurements of Cldn4 and actin intensity on western blot were normalized to control levels at 1h. Normalized Cldn4 expression relative to normalized actin is plotted ($P_{\text{int}} < 0.0001$; $P_{\text{time}} = 0.0017$; $P_{\text{treat}} < 0.0001$). (C) Immunofluorescence of control and 400μM of quercetin-treated MDCK II cells at 1h, 6h, 24h and 48h. Cldn4 is shown in green and ZO-1 is shown in red. (D) Localization of claudin at the tight junction was assessed by determining the amount of ZO-1 that co-localized with claudin expression ($P_{\text{int}} = 0.54$; $P_{\text{time}} = 0.051$; $P_{\text{treat}} = 0.24$). For all graphs, each point corresponds to an independent experiment; mean and SEM are shown. C=Control and Q=Quercetin. Scale bar represent 20 μm.

3.3.2.5. Cldn7

In contrast to the other claudin family members examined, Cldn7 expression and localization were not affected by quercetin treatment (Figure 3.6). At 24h, there was an increased level of Cldn7 immunofluorescence in the cytoplasm (Figure 3.6C) but this was not associated

with increased co-localization with ZO-1 at the tight junction (Figure 3.6D). This may suggest that Cldn7 incorporation into the tight junction complex is saturated at this time point.

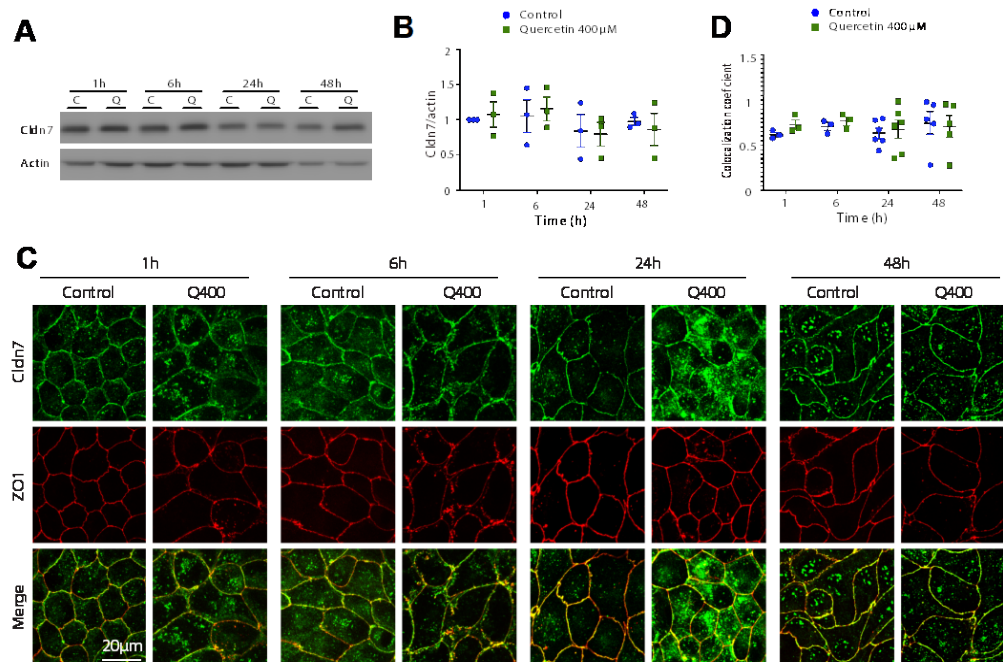


Figure 3.6: Analysis of Cldn7 expression and localization in MDCK II cells following quercetin treatment. (A) Western blot analysis of Cldn7 expression in cell lysates from control and 400μM quercetin-treated MDCK II cells. Actin was used as a loading control. (B) Densitometry measurements of Cldn7 and actin intensity on western blot were normalized to control at 1h. Normalized Cldn7 expression relative to normalized actin is plotted (P_{int}=0.916; P_{time}=0.41; P_{treat}=0.98). (C) Immunofluorescence of control and 400μM of quercetin-treated MDCK II cells at 1h, 6h, 24h and 48h. Cldn7 is shown in green and ZO-1 is shown in red. (D) Localization of claudin at the tight junction was assessed by determining the amount of ZO-1 that co-localized with claudin expression (P_{int}=0.89; P_{time}=0.8; P_{treat}=0.55). For all graphs each point corresponds to an independent experiment; mean and SEM are shown. C=Control and Q=Quercetin. Scale bar, 20 μm

3.3.3 Quercetin's effects on claudin expression and localization is independent of the PI3K/AKT pathway

Quercetin has been identified as a strong inhibitor of the PI3K/AKT pathway (Jiang et al., 2016; Welker and Kulik, 2013). Given that receptor tyrosine kinases and pAKT phosphorylate and regulate claudins both directly and indirectly (Khan et al., 2017; Lin et al., 2013), we hypothesized that quercetin affects claudin expression through the PI3K/AKT pathway. Western blot analysis

of pPI3K, AKT and pAKT was performed following quercetin treatment. The levels of pPI3K and AKT in MDCK II cells did not change significantly after quercetin treatment. pAKT was reduced in quercetin-treated cells at 1h and 6h after treatment. pAKT levels could not be detected at 1h and 6h after quercetin treatment limiting the statistical analysis at these time points. At 24h and 48h, pAKT was decreased, but this was not statistically significant ($P=0.99$ and $P=0.7$, respectively). These data suggest that quercetin inhibits the AKT/pAKT pathway in MDCK type II cells (Figure 3.7A-B).

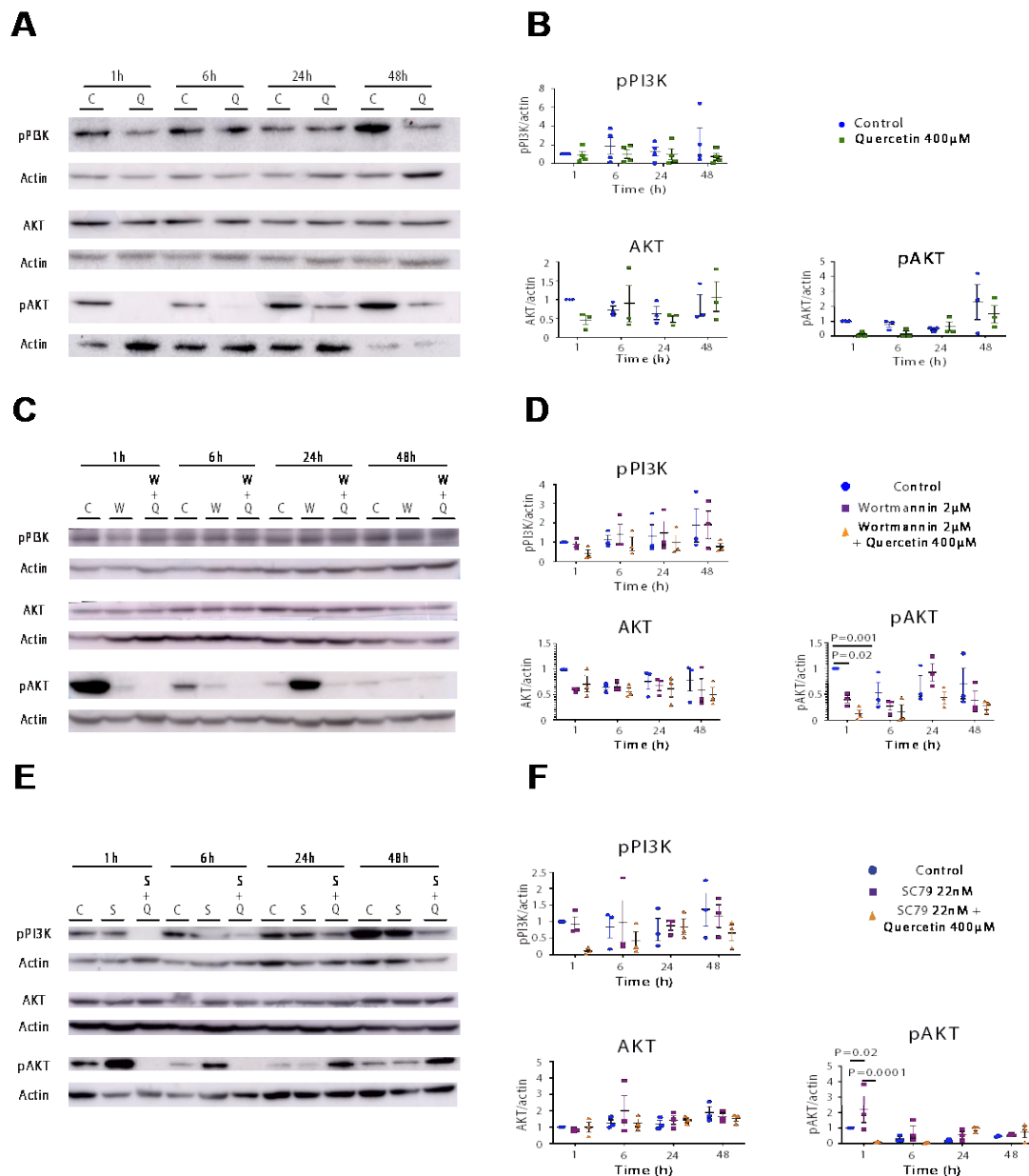


Figure 3.7: Analysis of pPI3K, pAKT and AKT expression in MDCK II cells following quercetin treatment. (A) Western blot analysis of claudin expression in cell lysates from control and 400 μ M quercetin-treated MDCK II cells. Actin was used as a loading control. **(B)** Densitometry measurements of band intensity on western blot were normalized to control at 1h and protein expression was plotted relative to normalized actin expression. pPI3K ($P_{\text{int}}=0.67$; $P_{\text{time}}=0.79$; $P_{\text{treat}}=0.14$), AKT ($P_{\text{int}}=0.44$; $P_{\text{time}}=0.49$; $P_{\text{treat}}=0.68$), and pAKT ($P_{\text{int}}=0.68$; $P_{\text{time}}=0.027$; $P_{\text{treat}}=0.142$) measurements were plotted. **(C)** Western blot analysis of pPI3K, pAKT and AKT expression in cell lysates from control, 2 μ M wortmannin-treated and 2 μ M wortmannin plus 400 μ M quercetin-treated MDCK II cells. Actin was used as a loading control. **(D)** Densitometry measurements of band intensity on western blot were normalized to control at 1h and protein expression was plotted relative to normalized actin expression. pPI3K ($P_{\text{int}}=0.97$; $P_{\text{time}}=0.03$; $P_{\text{treat}}=0.13$), AKT ($P_{\text{int}}=0.79$; $P_{\text{time}}=0.53$; $P_{\text{treat}}=0.099$), and pAKT ($P_{\text{int}}=0.15$; $P_{\text{time}}=0.064$; $P_{\text{treat}}=0.0008$) measurements were plotted. **(E)** Western blot analysis of pPI3K, pAKT and AKT expression in cell lysates from control 22nM SC79-treated and 22nM SC79 plus 400 μ M quercetin-treated MDCK II cells. Actin was used as a loading control. **(F)** Densitometry measurements of band intensity on western blot were normalized to control at 1h and protein expression was plotted relative to normalized actin expression. pPI3K ($P_{\text{int}}=0.81$; $P_{\text{time}}=0.51$; $P_{\text{treat}}=0.078$), AKT ($P_{\text{int}}=0.79$; $P_{\text{time}}=0.52$; $P_{\text{treat}}=0.099$), and pAKT ($P_{\text{int}}=0.16$; $P_{\text{time}}=0.063$; $P_{\text{treat}}=0.0008$) measurements were plotted. Each point corresponds to an independent experiment; mean and SEM are shown. W=Wortmannin; S=SC79; C=Control and Q=Quercetin.

To query if quercetin's effects on claudin expression were due to impairment of the PI3K/AKT pathway, we inhibited or activated the PI3K/AKT pathways using wortmannin or SC79, respectively, alone or in combination with quercetin. As predicted, treatment with wortmannin caused a transient decrease in pAKT in MDCK II cells at 1h and 6h and SC79 increased pAKT at 1h and 6h (Figure 3.7C-F). In both cases, effects were greatly attenuated by 24h. However, neither activation nor inhibition of the pAKT pathway recapitulated the effects on claudins observed following quercetin treatment (Figure 3.8), and neither wortmannin nor SC79 rescued the effects of quercetin. Therefore, our data suggest that quercetin is not acting through the PI3K/AKT pathway to effect changes in claudin protein expression.

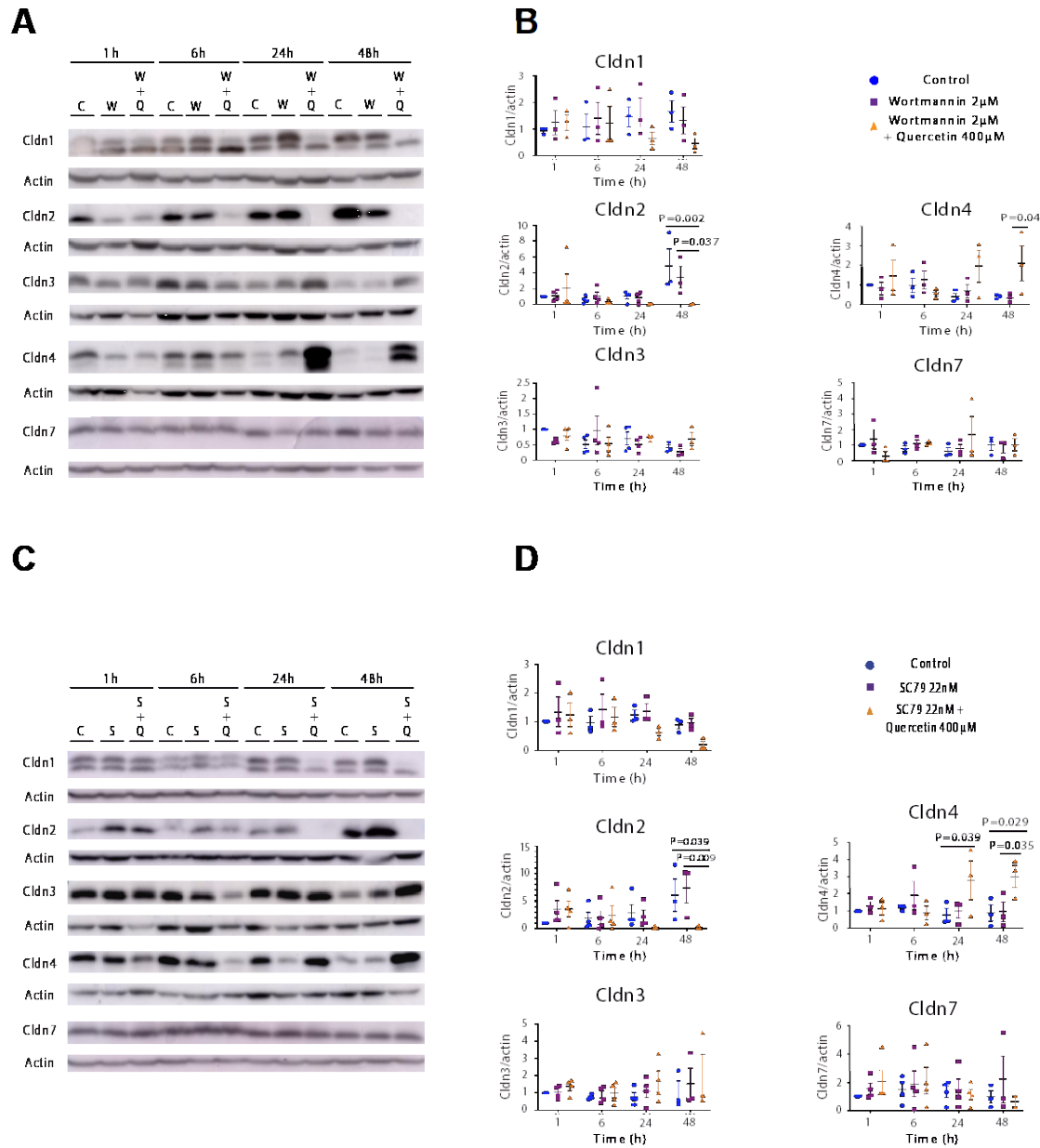


Figure 3.8: Analysis of Cldn1, -2, -3, -4 and -7 expression in MDCK II cells following wortmannin or SC79 treatment. (A) Western blot analysis of claudin expression in cell lysates from control, 2 μ M wortmannin-treated and 2 μ M wortmannin plus 400 μ M quercetin-treated MDCK II cells. Actin was used as a loading control. (B) Densitometry measurements of band intensity on western blot were normalized to control at 1h and protein expression was plotted relative to normalized actin expression. Cldn1 ($P_{\text{int}}=0.6$; $P_{\text{time}}=0.98$; $P_{\text{treat}}=0.24$), Cldn2 ($P_{\text{int}}=0.059$; $P_{\text{time}}=0.016$; $P_{\text{treat}}=0.097$), Cldn3 ($P_{\text{int}}=0.29$; $P_{\text{time}}=0.26$; $P_{\text{treat}}=0.78$), Cldn4 ($P_{\text{int}}=0.21$; $P_{\text{time}}=0.97$; $P_{\text{treat}}=0.041$) and Cldn7 ($P_{\text{int}}=0.41$; $P_{\text{time}}=0.98$; $P_{\text{treat}}=0.83$) measurements were plotted. (C) Western blot analysis of claudin expression in cell lysates from control 22nM SC79-treated and 22nM SC79 plus 400 μ M quercetin-treated MDCK II cells. Actin was used as a loading control. (D) Densitometry measurements of band intensity on western blot were normalized to control at 1h

and protein expression was plotted relative to normalized actin expression. Cldn1 ($P_{\text{int}}=0.6$; $P_{\text{time}}=0.15$; $P_{\text{treat}}=0.11$), Cldn2 ($P_{\text{int}}=0.09$; $P_{\text{time}}=0.14$; $P_{\text{treat}}=0.11$), Cldn3 ($P_{\text{int}}=0.99$; $P_{\text{time}}=0.46$; $P_{\text{treat}}=0.21$), Cldn4 ($P_{\text{int}}=0.082$; $P_{\text{time}}=0.73$; $P_{\text{treat}}=0.047$) and Cldn7 ($P_{\text{int}}=0.87$; $P_{\text{time}}=0.78$; $P_{\text{treat}}=0.5$) measurements were plotted. Each point corresponds to an independent experiment; mean and SEM are shown. W=Wortmannin; S=SC79; C=Control and Q=Quercetin.

3.4 DISCUSSION

The purpose of this study was to determine the effect of quercetin on the cation-selective tight junction barrier in MDCK II cells, which models the proximal tubule of the kidney. We showed that the effects of quercetin on the MDCK barrier were stabilized 48h after treatment, resulting in an increased TER of at least 20% over the TER observed in untreated cells. The quercetin-dependent increase in TER correlates with decreased expression of Cldn1 and decreased expression and localization of Cldn2 to the tight junction. Although a significant increase in Cldn3 and -4 was observed by western blot analysis, tight junction localization of Cldn3, -4 and -7 were not affected at this timepoint.

In the mature kidney, transepithelial resistance in the proximal tubule is $<10 \Omega \cdot \text{cm}^2$, indicating that this nephron segment has a leaky barrier, which correlates with its important role in salt and water reabsorption (Van Itallie et al., 2003). Therefore, the increase of $10\text{-}20 \Omega \cdot \text{cm}^2$ observed following quercetin treatment of MDCK II cells would be very relevant in the context of the proximal tubule, where similar oscillations could completely change its barrier properties.

The TER oscillation observed during the first 48h after quercetin treatment reflects dynamic tight junction remodeling and correlates with the changes in claudin expression that we observed. The decrease in TER observed at 6h coincided with a decrease in the immunofluorescent levels of barrier-sealing claudins, Cldn1 and -3. This is predicted to translate into a 'leakier' barrier, and consequently, a reduced TER. The increased TER at 24h and 48h coincided primarily with a decrease in the expression and tight junction localization of Cldn2, a cation-selective pore-forming claudin. Decreased Cldn2 at the tight junction is predicted to reduce paracellular movement of cations (Tokuda and Furuse, 2015), including sodium and calcium, and water, and may contribute to the increase in TER, as previously described for Cldn4 in MDCK (Van Itallie et al., 2003).

Our data and those obtained by other groups, suggest that the effects of quercetin are cell-line dependent (Mercado et al., 2013; Piegholdt et al., 2014; Suzuki and Hara, 2009; Suzuki et al., 2011; Tokuda and Furuse, 2015; Valenzano et al., 2015). When Tokuda and Furuse depleted Cldn2 from MDCK II cells, they observed increased TER and decreased sodium and chloride potential (Tokuda and Furuse, 2015). The increase in TER was significantly higher than what we observed ($>1000 \Omega \cdot \text{cm}^2$), perhaps due to compensation by other claudins in response to removal of Cldn2. In their experiment, depletion of Cldn2 led to increased tight junction localization of Cldn1, -3, -4 and -7, which would effectively tighten the barrier and increase TER. In our quercetin-treated cells, the 90% reduction of Cldn2, was not accompanied by an increased localization of other claudins to the tight junction: Cldn1 expression was decreased, Cldn3 and -4 expression was increased but localization to the tight junction was unchanged, and Cldn7 was unchanged. Thus, quercetin causes a different net effect on claudin expression/localization compared to depletion of Cldn2 alone and, as a result, there is a more modest increase in TER.

Quercetin also increased TER of the anion-selective barrier in LLC-PK1 cells, here, expression of Cldn2 and -3 were downregulated while Cldn4, -5 and -7 were upregulated (Mercado et al., 2013). However, claudin localization was not assessed. An oscillation and final increase in TER following quercetin treatment, has been observed in Caco-2 cells (Suzuki and Hara, 2009; Suzuki et al., 2011), where TER oscillated similarly to what we observed in MDCK II cells during the first 48h after treatment. In this case Cldn1 was displaced to the cytoskeletal fraction, Cldn4 expression was increased, while Cldn3 was unperturbed. In contrast, Valenzano et al., did not report any effect on TER after 17h of quercetin treatment on Caco-2 cells, although Cldn2, -4 and -5 were increased (Valenzano et al., 2015). These data together with our findings indicate that quercetin is able to tighten tight junction barriers, but does so by differentially affecting different claudin family members, in a cell-type specific manner.

We observed two migratory bands for Cldn1 and -4 in our western blot analyses. Previous studies have linked these different migratory bands to either claudin degradation (Horng et al., 2017) or claudin phosphorylation (Aono and Hirai, 2008; Fujibe et al., 2004). Claudins are known to undergo post-translational modifications, including phosphorylation, glycosylation, palmitoylation and ubiquitination (Shigetomi and Ikenouchi, 2018). These modifications are

thought to rapidly change claudin stability at the tight junction. For instance, dephosphorylation of Cldn1 and -2 is a signal for degradation and reduces their presence at tight junctions (Fujii et al., 2016; Van Itallie et al., 2012). In the case of Cldn4, studies have shown that the effects depend on the site of phosphorylation. In some cases, phosphorylation leads to the disruption of the tight junction (D'Souza et al., 2007; Tanaka et al., 2005), while in others, phosphorylation increases the stability of Cldn4 at the tight junction (Aono and Hirai, 2008). Further experiments are required to elucidate if the two migratory bands are a consequence of different post-translational modifications or if, they correspond to degradation products (Horng et al., 2017).

Other studies that have looked at quercetin's effects on tight junction barrier properties and claudin expression have been based only on western blot analyses. The lack of congruence between our western blot and immunofluorescence expression data for some of the claudins may reflect the availability of claudin epitopes for detection using these two methods. It could also reflect disparities in how post-translational modifications or protein degradation can be assessed by western blot versus immunofluorescence. For instance, in the case of Cldn1, at 24 and 48 hours, the western blot data shows a loss of the slower migrating band and decreased Cldn1 expression in the quercetin-treated cells. In contrast, the levels by immunofluorescence are equivalent to the control cells. This could suggest that the immunofluorescence signal is primarily due to recognition of the faster migrating band.

Cldn2 immunofluorescence exhibited quite a different pattern compared to other claudin family members. In contrast to most claudins, Cldn2 was highly expressed in some regions and almost absent in other regions within confluent cell layer. This has been previously described and high Cldn2 levels appear to correlate with increased cell confluence (Amoozadeh et al., 2018). The increased Cldn2 during tight junction maturation, can be seen clearly seen in the control cells by both western blot analysis and by colocalization with ZO-1 at tight junctions.

The mechanism(s) by which quercetin impacts the tight junction barrier through its effects on claudin expression and localization remains unresolved. Quercetin is known to bind to several membrane-bound nutraceutical receptors that signal through different pathways to reduce oxidative stress. Quercetin also interacts directly with several kinases (Chuenkitiyanon et al., 2010; Gamero-Estevez et al., 2018; Jiang et al., 2019; Tan et al., 2018; Zhu et al., 2018). Some

studies show that it is a potent inhibitor of PI3K/AKT pathway (Jiang et al., 2016; Maurya and Vinayak, 2016); although there is some controversy (Du et al., 2016; Jiang et al., 2019; Wang et al., 2011), while others show that it inhibits the ERK and NF- κ B pathways (Wu et al., 2017) and activates the AMPK pathway (Jiang et al., 2019; Qiu et al., 2018). However, these studies have been done on different cell lines, using different conditions and measuring different outcomes. Therefore, it remains unclear if quercetin acts upstream of all of these pathways, or if its effects are cell-type specific. We showed that quercetin inhibits PI3K/AKT pathway in MDCK II cells. However, inhibition the PI3K/AKT pathway was not sufficient to recapitulate quercetin's effects on claudin expression.

So, how does this translate to the potential therapeutic effects of quercetin to prevent kidney stone formation in the context of the proximal tubule? The site of kidney stone formation is not well understood. Some studies suggest that kidney stones are formed in the interstitium, while others suggest that they form in the lumen of the nephron (Bird and Khan, 2017; Coe et al., 2010; Khan et al., 2016). Here, we showed that quercetin led to a relative increase in barrier claudins at the tight junction relative to the pore-forming claudins in MDCK II cells. Although direct measurements of cation potential and water permeability were not performed, our data suggest that quercetin may be beneficial by preventing sodium, calcium, and water within the urine from crossing the epithelial barrier to enter the interstitium. We believe that the effects seen for Cldn2 are essential to explain what may be happening in the nephron. Cldn2 is a cation-selective pore forming claudin that promotes the paracellular movement of sodium, calcium and water (Rosenthal et al., 2017). A decrease in Cldn2 is predicted to lead to a reduction in reabsorption of these substances. This would then prevent the transport of sodium and calcium in the interstitium which can drive stone formation. Alternatively, quercetin may effectively tighten the proximal tubular epithelium to maintain the water content within the urinary lumen of this segment to keep calcium and sodium solubilized (Tokuda et al., 2016). This mechanism could correlate with the beneficial effects that high-water diets or diuretic drugs have in the prevention of kidney stones. In vivo experiments are necessary to discern which specific scenario is taking place and whether this effect could be beneficial for the prevention of kidney stones.

3.5 MATERIAL AND METHODS

3.5.1 Cell culture

Madin-Darby Canine Kidney cells II (MDCK II) cells were obtained from ATCC. They were incubated at 37°C and 5% CO₂ in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 51 IU penicillin, 50 µg/mL streptomycin and 16 µg/mL of gentamicin (Wisent BioProducts, Quebec, Canada).

3.5.2 Chemicals and antibodies

Quercetin (Sigma-Aldrich, Darmstadt, Germany) was added to the culture media, and heated at 37°C with continuous stirring for at least 30 minutes in order to ensure that it was dissolved. It was used at the working concentration of 400 µM. PI3K/AKT pathway modulators Wortmannin (Abcam, 120148, Cambridge, UK) and SC79 (Abcam, 146428, Cambridge, UK) were used at working concentrations of 2 µM and 22 nM respectively.

Primary antibodies used for immunofluorescence and western blotting were: Cldn1 (Invitrogen, 374900, Carlsbad, California, USA), Cldn2 (Invitrogen, 516100, Carlsbad, California, USA), Cldn3 (Abcam, 15102, Cambridge, UK), Cldn4 (Invitrogen, 364800, Carlsbad, California, USA), Cldn7 (Spring Bioscience, E10594, Pleasanton, California, USA), ZO-1 (Invitrogen, 339100, Carlsbad, California, USA), pPI3K (Cell signaling, 4228, Danvers, Massachusetts, USA), pAKT (Cell signaling, 9271, Danvers, Massachusetts, USA), AKT (Cell signaling, 9272, Danvers, Massachusetts, USA) and pan-actin (Cell signaling, 4968, Danvers, Massachusetts, USA,). In addition, secondary goat anti-rabbit (Alexa Fluor 595 and 488, Invitrogen, Carlsbad, California, USA), goat anti mouse (Alexa Fluor 595 and 488, Invitrogen, Carlsbad, California, USA), goat anti-rabbit-HRT conjugated (Cell Signaling, 70748, Danvers, Massachusetts, USA) and goat anti mouse peroxidase conjugated (Jackson ImmunoResearch, 115-035-146, West Grove, Pennsylvania, USA) were used.

3.5.3 Transepithelial resistance (TER)

Cells were seeded on sterile 0.4 µm pore size, 12-well transparent polyethylene terephthalate inserts (Corning, New York, USA) at a cell density of 0.15×10^6 cells/insert and

placed in the automated cell monitoring CellZscope system (NanoAnalytics, Münster, Germany). The CellZscope system provides noninvasive, continuous monitoring of cell monolayers impedance, capacitance and resistance.

The tissue culture inserts were placed into a 12-well cell module, and the system was incubated at 37°C and 5% CO₂. TER measurements, expressed in ohm square centimeters, were recorded in real time every 20 minutes. DMEM in both the apical and basal compartments was replaced with fresh DMEM (control) or DMEM plus 400µM quercetin at 72h, at which point mature tight junctions were established and stabilized (Resistance $\cong 70 \Omega \cdot \text{cm}^2$). Measurements were recorded in real time every 20 minutes for at least 90h following treatment. Each time the machine was removed from the incubator or the media was changed, a small increase in TER is expected which then stabilizes.

3.5.4 Immunofluorescence staining

Cells were seeded on coverslips in 12-well plates at a cell density of 0.1×10^6 cells/well. Once confluence and tight junction maturity were achieved ($\approx 72\text{h}$), cells were treated with DMEM (control) or DMEM plus 400µM quercetin and collected at 1, 6, 24 or 48 hours. Cell layers were rinsed with phosphate buffer saline (PBS) and fixed in 10% trichloroacetic acid for 15 minutes at 4°C. Cells were then blocked with 10% normal goat serum in phosphate buffer saline and 0.3% Triton-100 (PBST) and incubated overnight at 4°C with 5% normal goat serum in PBST and primary antibodies: Cldn1, -2, -3 and -4 (1:100), Cldn7 (1:50), and ZO-1 (1:150). Cells were washed with PBS. Alexa Fluor-conjugated secondary antibodies (1:500) in PBST were added for 1h at RT. Coverslips were washed with PBS and then placed on slides with Slowfade Gold with DAPI (Invitrogen, Carlsbad, California, USA). Z-stacks were imaged using a Zeiss LSM780 laser scanning confocal microscope. To determine the amount of claudin localized in the membrane, maximum intensity projections were obtained for each stack of images and then ZEN software colocalization analysis was performed. Only M2 results are presented; M2 makes reference to how many of the red pixels (ZO-1) coincide with green pixels (claudin). A high M2 indicates high claudin at the membrane while a low M2 indicates high ZO-1 alone, which relates to a low claudin

at the membrane. M2 is not a reading of abundance or intensity levels and therefore it only provides information about localization.

3.5.5 Western blot analysis

Cells were seeded in 10cm plates at a cell density of 2×10^6 cells/plate. Once confluence and tight junction maturity was reached (≈ 72 h), cells were treated with DMEM (control) or DMEM plus 400 μ M quercetin for 1, 6, 24 or 48 hours, and then collected by physical scraping into lysis buffer (25mM Tris-HCl Ph7.4; 10mM sodium pyrophosphate; 25mM NaCl; 10mM sodium fluoride; 2mM EGTA; 2mM EDTA; 1% NP40; 0.1% SDS; 1.45nM Pepsatin A; 2.1nM Leupeptin; 0.15nM Aprotinin and 0.57 μ M PMSF). Protein concentration was determined by Bradford assay and 50 μ g per sample were separated by 12.5% SDS-PAGE and transferred onto PVDF membranes. Membranes were blocked for 1h with 5% non-fat milk in PBST or with 5% BSA in Tris-NaCl buffer and 0.3% of Tween-20 (TBST). Membranes were incubated overnight at 4°C with primary antibodies in PBST or TBST: Cldn1, -2, -3, -4, -7, pPI3K, pAKT and AKT (1:1000) or pan-actin (1:2000). Membranes were then washed with PBST or TBST and then incubated with goat-anti-rabbit-HRT conjugated or goat-anti-mouse-peroxidase conjugated (1:5000) secondary antibodies for 1h at RT. Membranes were revealed using Clarity Western ECL substrate (Biorad laboratories, 1705061, Hercules, California, USA), and imaged using Amersham imager 600 (GE Healthcare, Little Chalfont, United Kingdom). Band densities were quantified by densitometry using ImageJ, normalized against the 1h control sample on each blot and then to normalized actin. Because some variability was observed in actin, we performed a statistical analysis of actin expression from all experiments and determined that there was no significant change over time or in response to quercetin treatment.

3.5.6 Statistics

All plotted results are mean $\pm$ SEM. Statistical analyses were performed using Graph-Pad PRISM (version 8.02, GraphPad Software, Inc.). Two-way ANOVA analysis was used for effects of Treatment, Time and Interaction. Sidak's multiple comparison test was used to compare the

effect of treatment at the different time points. For TER Figure 3.S1B, unpaired T-test were performed at the specific time points. Significance is considered if $P < 0.05$.

3.6 SUPPLEMENTARY MATERIALS

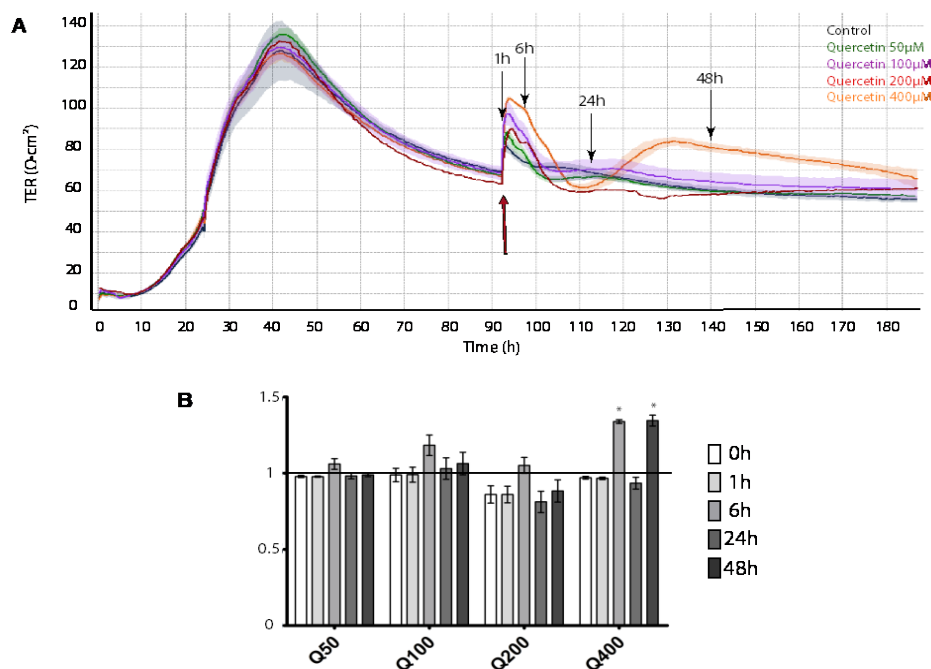


Figure 3.S1: TER of dose response curve of quercetin. (A) Comparison of TER of control (black) versus cells treated with 50 μM (green), 100 μM (purple), 200 μM (red) or 400 μM of quercetin (orange). **(B)** Measurement of quercetin treated TER values relative to control cells at different time points and different treatment concentrations. The horizontal line delineates control level. * $P < 0.05$.

3.7 AUTHOR CONTRIBUTIONS

Conceptualization, Enrique Gamero-Estevez, Sero Andonian, Bertrand Jean-Claude, Indra Gupta and Aimee K. Ryan; Formal analysis, Enrique Gamero-Estevez; Funding acquisition, Sero Andonian, Indra Gupta and Aimee K. Ryan; Investigation, Enrique Gamero-Estevez, Indra Gupta and Aimee K. Ryan; Methodology, Enrique Gamero-Estevez, Indra Gupta and Aimee K. Ryan; Writing – original draft, Enrique Gamero-Estevez; Writing – review & editing, Indra Gupta and Aimee K. Ryan.

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3.10 CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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3.11 CONNECTING TEXT BETWEEN CHAPTER 3 AND 4

In Chapter 3, I showed that quercetin is able to alter claudin localization and expression and that this effect is claudin dependent. These changes impacted barrier properties over time. Initially, quercetin led to the barrier becoming tighter. By six hours the barrier became leakier and after 24h of treatment the barrier became tighter again, stabilizing after 48h in a barrier that was tighter than its control counterpart. This oscillation over time could in fact be matched to the change in individual claudin patterns, suggesting that each claudin has a specific role in the cell, and that modifying or changing the claudin composition of the tight junction directly affects barrier properties. In Chapter 4, I explore a different tool, called C-CPE. C-CPE stands for C-terminal domain of *Clostridium perfringens* enterotoxin. C-CPE has been widely used for the study of tight junctions since it is able to bind and remove a subset of claudins from the tight junction without killing the cell. However, no one has really answered the question of how C-CPE works. In Chapter 4, I study the mechanism of action of C-CPE and what happens to the specific claudins it targets for removal from tight junctions after they are internalized. This will help us understand tight junction dynamics and how to target specific barriers. Further analysis to validate the effects of claudin removal on extracellular vesicle cargo as well as individual claudin-rich vesicles is ongoing. Western blot and density gradients will be used to validate the mass spectrometry data and to identify the size of the vesicles that contain the cargo of interest.

**CHAPTER 4: Treatment with the C-terminal domain of *Clostridium perfringens* enterotoxin
leads to release of claudin-rich extracellular vesicles in MDCK II cells**

Enrique Gamero-Estevez^{1,2}, Jenna Haverfield^{1,2}, Jean-François Boisclair Lachance^{1,2}, Indra
Gupta^{1,2,3} and Aimee K. Ryan^{1,2,3}

¹Department of Human Genetics, McGill University, Montreal, QC H4A 3J1, Canada

²Research Institute of the McGill University Health Centre, Glen Site, Montreal, QC H4A 3J1

³Departments of Pediatrics, McGill University, Montreal, QC H4A 3J1, Canada.

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4.1 ABSTRACT

Clostridium perfringens enterotoxin (CPE) interacts with a subset of the claudins present in the intestinal epithelium and promotes cell apoptosis and barrier breaks, which give rise to the symptoms associated with food poisoning. The C-terminal domain of *Clostridium perfringens* enterotoxin (C-CPE) is often used as a non-toxic reagent to remove a subset of claudin family members from tight junctions and promote changes in barrier properties. This reagent is able to bind and remove a subset of claudins from the tight junction without damaging the cell. It has been suggested that C-CPE binding to claudins results in formation of a complex that leads to the internalization of claudins. However, we do not know what happens to the claudins once they are removed from the tight junction. In addition, evidence from immunofluorescence studies suggests that C-CPE removal of claudins from tight junctions does not result in cytoplasmic accumulation, while western blot analysis shows that levels of claudins are not altered. Therefore, we asked the question: Are claudins being secreted as a consequence of C-CPE treatment? Here, we show that following C-CPE treatment there is an increase in the number of extracellular vesicles and that these vesicles are enriched with all claudins expressed in the MDCK II cell line, including Cldn1, -2, -3, -4, -6 and -7. We were able to determine that C-CPE specially increased the abundance of 100-300nm vesicles. Moreover, use of reagents that inhibit exosome and microvesicle formation did not permit identification of a specific secretion pathway. However, the observation that the inhibitor effects were in part claudin specific suggests that each claudin may be regulated and removed following a different, specific mechanism. Mass spectrometry analysis of the cargo from C-CPE-derived vesicles showed that these vesicles were enriched in claudins and showed evidence that the NSF-dynamin2 pathway was upregulated at the expense of other more commonly studied exocytic pathways.

4.2 INTRODUCTION

Clostridium perfringens enterotoxin (CPE) is a single chain 319 amino acid polypeptide that causes the gastrointestinal symptoms associated with *C. perfringens* type A food poisoning in humans (Wieckowski et al., 1994). CPE binds to a subset of the claudins present in the intestinal epithelium, which results in the formation of a pore that allows calcium to enter the cell and

subsequently leads to apoptosis (Chakrabarti et al., 2003; Shrestha et al., 2016). However, if the N-terminal cytotoxic domain of CPE is removed, the resultant C-terminal domain of CPE (C-CPE; aa.186-319) is able to bind to Claudin-3, -4, -6, -7, -8 and -14 and remove them from tight junctions without killing the cell (Shinoda et al., 2016; Takahashi et al., 2008). Claudins are a family of 20-27 kDa tetraspan transmembrane proteins that have at least 28 family members in mammals (Piontek et al., 2020). They are essential to form tight junctions and determine the barrier properties of the epithelium with respect to its ability to promote or inhibit the paracellular passage of ions and small molecules. Therefore, C-CPE has been used by us and others to study claudins and their role within tight junctions, as well as to study different epithelial barriers (Baumholtz et al., 2017; Saitoh et al., 2015).

Several studies have addressed the mechanism by which CPE and C-CPE function. When CPE binds to its receptor claudin, it can form two different hexamer complexes: CH-1 and CH-2. The CH-1 complex leads to the formation of a calcium pore that allows an influx of calcium that ultimately causes cell lysis. On the other hand, the CH-2 complex leads to the internalization of claudins and occludin from the tight junction (Robertson et al., 2007; Shrestha et al., 2016; Singh et al., 2000). Removal of amino acids within the cytotoxic domain of CPE (aa. 80-106) completely blocks the cytotoxicity of CPE and the formation of CH-1 complexes. This suggests that C-CPE can only form CH-2 complexes that remove claudins from the tight junction when the complex is internalized by the cell (Shrestha et al., 2016; Smedley and McClane, 2004). Interestingly, although C-CPE is able to remove claudins from tight junctions, they do not accumulate in the cytoplasm, yet the level of claudins do not change by western blot. Therefore, this raises the question, where are claudins going?

There is a growing interest in the study of claudins in extracellular vesicles where they seem to appear in different diseases, including their use as biomarkers for ovarian cancer and other tumors (Ikeda et al., 2021; Kyuno et al., 2019a; Kyuno et al., 2019b; Li et al., 2009). The most common types of extracellular vesicles are exosomes and microvesicles. Exosomes range in size from 50 to 150 nm and they are generated by the formation of a multivesicular body that holds several exosomes and releases them to the extracellular space upon fusion of the multivesicular body with the cell membrane. In contrast, larger microvesicles, ranging in size

from 100 to 1000 nm, are formed by budding and shedding at the plasma membrane (van Niel et al., 2018).

We hypothesized that C-CPE removes claudins from the tight junction by internalization and then processes these complexes and releases claudins from the cell in extracellular vesicles. We discovered that C-CPE treatment increases the number of extracellular vesicles secreted, and that these vesicles are enriched in claudins. We also studied the effects of reagents that inhibit exosomal and microvesicle formation on extracellular vesicle secretion. Our data suggest that each claudin may be independently incorporated into tight junctions following treatment with C-CPE. Understanding the generation of claudin-rich extracellular vesicles and the profile of other cargo contained in the same populations is important to understand the potential functions of these vesicles.

4.3 MATERIAL AND METHODS

4.3.1 C-CPE and GST purification

Plasmid pGEX6P1 carrying GST-tagged C-CPE was obtained from Dr. Furuse. GST was used as a negative control. GST and GST::C-CPE were purified following a standard GST purification protocol. Briefly, plasmids encoding GST or C-CPE were transformed into *E. coli* BL21 cells and plated on ampicillin lysogeny broth (LB) plates. Individual colonies were used to inoculate overnight cultures in LB-amp media, which were used to inoculate fresh cultures for protein production. When an absorbance 600 of 0.3-0.6 OD was reached, IPTG was added to a final concentration of 0.2mM, and the culture was incubated overnight at 22°C with constant agitation at 250rpm. Bacterial cell pellets were lysed in 5 volumes of resuspension buffer (50mM Tris pH7.5, 150mM NaCl, 0.1mM EDTA) with protease inhibitors (1.45nM Pepsatin A, 2.1nM Leupeptin, 0.15nM Aprotinin and 0.57mM PMSF). Lysozyme was added to a final concentration of 20 µg/mL and mixtures was incubated on ice for 20 minutes. Sodium deoxycholate and NaCl were added to a final concentration of 0.2% and 0.5M, respectively. The lysate was sonicated (9 times for 30 seconds at 30° amplitude) and then centrifuged at 15,000rpm for 30 minutes. The supernatant containing soluble GST or GST-tagged C-CPE was incubated at 4 °C overnight with 100 µL of a 50% slurry of glutathione agarose beads. Agarose beads were collected by low-speed

centrifugation and washed 4 times in 10-bed volumes of washing buffer (50mM Tris pH7.5, 0.5M NaCl). Washed beads were transferred to a column and washed further until eluant was free of protein as assessed by standard Bradford assay. Protein was eluted from the beads by adding 1 bed volume of elution buffer (50mM Tris pH7.5, 150mM NaCl, 20mM reduced Glutathione). This was repeated until ~10 fractions were collected. A Bradford protein assay was used to identify the eluted fractions containing purified recombinant protein, which were pooled and dialyzed overnight against phosphate-buffered saline (PBS). Recombinant protein was aliquoted and stored at -80°C in PBS.

4.3.2 Chemicals and antibodies

Primary antibodies used were: Cldn1 (Invitrogen, 374900, Carlsbad, CA, USA), Cldn2 (Invitrogen, 516100, Carlsbad, CA, USA), Cldn3 (Abcam, 15102, Cambridge, UK), Cldn4 (Invitrogen, 364800, Carlsbad, CA, USA), Cldn7 (Spring Bioscience, E10594, Pleasanton, CA, USA), ZO-1 (Invitrogen, 339100, Carlsbad, CA, USA), and pan-actin (Cell signaling, 4968, Danvers, MA, USA). In addition, secondary goat anti-rabbit (Alexa Fluor 488, Invitrogen, Carlsbad, CA, USA) and goat anti mouse (Alexa Fluor 595, Invitrogen, Carlsbad, CA, USA), were used.

Extracellular pathway inhibitors used were: Manumycin A (Millipore-Sigma, M6418, Massachusetts, USA), GW4869 (Millipore-Sigma, D1692, Massachusetts, USA), Y27632 (Millipore-Sigma, Y0503, Massachusetts, USA) and (-)Blebbistatin (Millipore-Sigma, B0560, Massachusetts, USA).

4.3.3 Purification of extracellular vesicle from MDCK II cells

Madin–Darby Canine Kidney II cells (MDCK II) were obtained from ATCC. They were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FBS depleted of extracellular vesicles, 51 IU penicillin, 50 µg/mL streptomycin and 16 µg/mL of gentamicin (Wisent BioProducts, Quebec, QC, Canada) and grown at 37°C with 5% CO₂.

In order to gather enough extracellular vesicles for the different experiments, 2.2x10⁶ MDCK II cells were seeded in 150mm plates, with a total of 5 plates per treatment group. Cells were cultured in DMEM for 72h to ensure confluency. GST (control) or C-CPE was added at a final

concentration of 50 µg/mL and incubation was continued for 48h to accumulate extracellular vesicles. The media was collected and analyzed as previously described (Choi et al., 2021). Briefly, the collected media was centrifuged at 3000xg for 10 minutes to remove cell debris. The supernatant was filtered through a 0.8 µm filter and then concentrated to 0.5 mL using Amicon Ultra 100 kDa filter unit (UFC910008, Millipore-Sigma, Massachusetts, USA) by continuous centrifugation at 4000 x g. Sample volume was increased to 15 mL with phosphate buffer saline (PBS) and re-concentrated to 0.5 mL twice. The concentrated supernatant was combined with 2.5 mL of PBS and centrifuged at 100,000 x g for 1h at 4°C. The extracellular vesicle pellet was resuspended in PBS and centrifuged as previously. The pellet was resuspended in RIPA buffer (50 mM Tris pH7.5, 150 mM NaCl, 100 mM EDTA, 1%NP-40, 0.5% sodium deoxycholate, 0.1% SDS) with protease inhibitors (1.45 nM Pepsatin A, 2.1 nM Leupeptin, 0.15 nM Aprotinin and 0.57 mM PMSF) for western blots and without the protease inhibitors for mass spectrometry analysis.

4.3.4 Extracellular vesicle inhibitor treatment

For the inhibitor treatments, 2.2x10⁶ MDCK II cells were seeded in 150mm plates with a total of 5 plates per treatment group. Cells were cultured for 72h to ensure confluency prior to treatment with Manumycin A (10µM), GW4869 (10µM), Y27632 (10µM) and (-)Blebbistatin (50µM). At 3h after addition of inhibitors, GST or C-CPE was added to the media at a final concentration of 50 µg/mL and cells were incubated for 48h. Cells and extracellular vesicles were collected and purified as previously described.

4.3.5 NanoSight analysis

To count the number of extracellular vesicles in the different treatments, 0.5 mL of cell culture media 24h after each treatment were analyzed using NanoSight NS300 containing a 532 nm laser (NanoSight Ltd., UK). Three recordings of 30 seconds at 37°C were captured with camera level 15 and processed with detection threshold of 5 and blur size on auto using NTA software (version 3.0).

4.3.6 Western blot analysis

Cells were cultured as described above and collected by physical scraping into lysis buffer (25 mM Tris-HCl pH7.4; 10 mM sodium pyrophosphate; 25 mM NaCl; 10 mM sodium fluoride; 2 mM EGTA; 2 mM EDTA; 1% NP40; 0.1% SDS; 1.45 nM Pepstatin A; 2.1 nM Leupeptin; 0.15 nM Aprotinin, and 0.57 μ M PMSF). Protein concentration was determined by Bradford assay and 20 μ g per sample were separated by 12.5% SDS-PAGE and transferred onto PVDF membranes. Extracellular vesicles were collected in RIPA buffer as described above. Protein concentration was determined by BCA assay (ThermoFisher Scientific, MA, USA) and 1 μ g of sample was separated by 12.5% SDS-PAGE and transferred onto PVDF membranes.

PVDF membranes were blocked with 5% BSA in TBST (19mM Tris-HCl, pH7.4, 137mM NaCl, 0.3% Tween-20) for 1h at room temperature. Membranes were incubated overnight at 4°C with primary antibodies in TBST: Cldn1, -2, -3, -4, -7, CD9 (1:1000) or pan-actin (1:2000). Membranes were then washed with TBST and incubated with goat-anti-rabbit-HRT or goat-anti-mouse-peroxidase conjugated secondary antibodies (1:5000) for 1h at RT. Membranes were revealed using Clarity Western ECL substrate (Biorad laboratories, 1705061, Hercules, CA, USA), and imaged using the Amersham imager 600 (GE Healthcare, Little Chalfont, United Kingdom). Band densities were quantified by densitometry using ImageJ and normalized against actin.

4.3.7 Immunofluorescence

Cells were seeded on coverslips in 12-well plates at a cell density of 0.1×10^6 cells/well. Once confluence and tight junction maturity were achieved (~72 h), cells were treated with DMEM plus 50 μ g/mL GST (control) or C-CPE and collected 24h after treatment. Cells were rinsed with PBS and fixed in 10% trichloroacetic acid for 15 min at 4°C. Cells were then blocked with 10% normal goat serum in phosphate buffered saline and 0.3% Triton-100 (PBST) and incubated overnight at 4°C with 5% normal goat serum in PBST and primary antibodies: Cldn3 (1:100) and ZO-1 (1:150). Cells were washed with PBS and then incubated with Alexa Fluor-conjugated secondary antibodies (1:500) in PBST for 1h at room temperature. Cells were washed with PBS and then placed on slides with FluorSave reagent (EMD Millipore, 34789, Massachusetts, USA). Z-stacks

were imaged using a Zeiss LSM780 laser scanning confocal microscope and maximum intensity projections were obtained for each stack of images.

4.3.8 Mass spectrometry

Protein concentration of the extracellular vesicles was determined by BCA assay and 10µg of protein from each treatment group was used for mass spectrometry. For each sample, a single stacking gel bandwidth of all proteins was reduced with DTT, alkylated with iodoacetic acid and digested with trypsin. Extracted peptides were re-solubilized in 0.1% aqueous formic acid and loaded onto a Thermo Acclaim Pepmap (Thermo, 75µM ID X 2cm C18 3µM beads) precolumn and then onto an Acclaim Pepmap Easyspray (Thermo, 75µM X 15cm with 2µM C18 beads) analytical column separation using a Dionex Ultimate 3000 uHPLC at 250 nl/min with a gradient of 2-35% organic (0.1% formic acid in acetonitrile) over 3h. Peptides were analyzed using a Thermo Orbitrap Fusion mass spectrometer operating at 120,000 resolution for MS1 with HCD sequencing at top speed (15,000 resolution) for all peptides with a charge of 2+ or greater. The raw data were converted into .mgf format (Mascot generic format) for searching using the Mascot 2.5.1 search engine (Matrix Science) against *Canis familiaris* protein sequences (Uniprot 2020). The database search results were loaded onto Scaffold Q+ Scaffold_4.4.8 (Proteome Sciences) for statistical treatment and data visualization. Pinnacle (Optys Tech) was used to quantify all detected peptides using a MS1 quantification workflow (Targeted Quantification: Label Free DDA) wherein the peptide specific XICs from the raw mass spec data (.raw) were used to directly compare all identified peptide (*.dat) amounts across all experiments using precursor ion integrals (in counts).

Proteins with less than 2 peptides per protein (same or different peptides for the same proteins), high ambiguity or confidence less than 95% were excluded from the analysis. The remaining peptides were analyzed by Fisher's exact test and corrected for multiple comparison using Bonferroni. Candidates were determined to be those with peptides that were significantly different ($p < 0.05$) and that had at least a 2-fold difference between the GST and C-CPE samples.

4.3.9 Statistics

All plotted results are mean $\pm$ SEM. Statistical analyses were performed using Graph-Pad PRISM (version 8.02, GraphPad Software, Inc., California, USA). t-test and two-way ANOVA analysis were used for effects of treatment. Tukey's or Sidak's multiple comparison test was used to compare the effect of the different treatments. Significance is considered if $p < 0.05$.

4.4 RESULTS

4.4.1 Extracellular vesicles from C-CPE treated cells are enriched in claudins

As shown by us and others, C-CPE specifically removes a subset of claudins from tight junctions, including Cldn3, -4, -6, -7, -8 and -14. The proposed mechanism of removal has been extrapolated from studies with CPE and suggests that claudins are internalized upon C-CPE treatment. Interestingly, C-CPE does not affect transcription or translation (Data not shown) and we observed that claudins did not accumulate in the cytoplasm (Figure 4.S1). Given the increasing reports describing claudins in extracellular vesicles in different models (Ikeda et al., 2021; Kyuno et al., 2019a; Kyuno et al., 2019b; Li et al., 2009), we decided to examine whether C-CPE affected the inclusion of claudins in extracellular vesicles secreted from MDCK II cells. Extracellular vesicles from MDCK II cells treated with 50 $\mu\text{g}/\text{mL}$ of GST or 50 $\mu\text{g}/\text{mL}$ of C-CPE were purified as described in material and methods and 1 μg of protein was loaded per lane. Western blots against different claudins showed that all claudins expressed in MDCK II were present in extracellular vesicles including Cldn1, -2, -3, -4 and -7 (Figure 4.1). Moreover, we observed that the presence of claudins was significantly enriched in the extracellular vesicles derived from C-CPE-treated cells, while other extracellular vesicle control markers, such as actin, remained unchanged (Figure 4.1). In fact, overall claudin content on extracellular vesicles from cells treated with GST or C-CPE samples were significantly different (Two-way ANOVA: $P_{\text{treat}}=0.0006$). In addition, Cldn3 was significantly increased in the C-CPE extracellular vesicles compared to control vesicles ($p=0.038$); and Cldn1 trended towards significance ($p=0.13$). Although the rest of claudins were clearly increased in the C-CPE derived extracellular vesicles when compared to control, the increment was variable between experiments, which may be the reason why statistical significance was not achieved. Western blot analysis of the cell extracts showed that claudin levels were not affected

by C-CPE treatment, with exception of Cldn4 that it is significantly increased in cells treated with C-CPE (Supplemental figure 4.1).

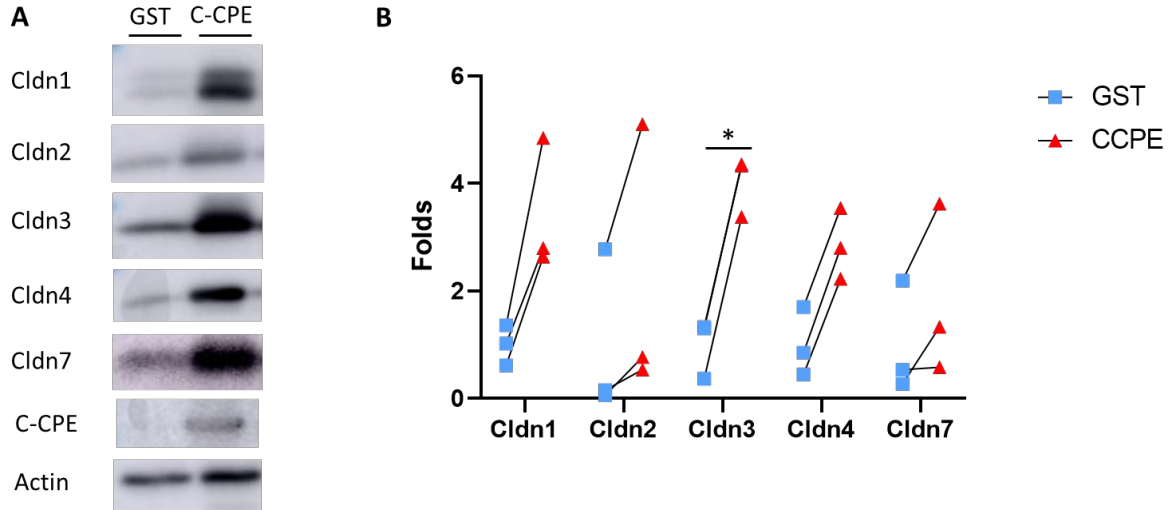


Figure 4.1: Claudins are increased in extracellular vesicles derived from C-CPE-treated MDCK II cells. **A.** Western blots against claudins and actin loading control of extracellular vesicles from cells treated with 50 μ g/mL of GST (control) or C-CPE. **B.** Western blot quantification normalized against actin. Each square corresponds to an independent experiment. Strings are added to show pairing. Two-way ANOVA was performed ($P_{\text{int}}=0.55$; $P_{\text{V.size}}=0.55$; $P_{\text{Treat}}=0.0006$). *Denotes significance, $p<0.05$.

4.4.2 C-CPE increases the number of extracellular vesicles secreted

To determine if the increased levels of claudins in the extracellular vesicles derived from C-CPE-treated cells treated was associated with an overall increase in the number or type of extracellular vesicles, we first determined the concentration of extracellular vesicles. In order to measure this, we collected media from MDCK II cells treated with 50 μ g/mL of GST (control) or C-CPE and calculated the total number of vesicles using a nanoparticle tracker analysis system. We observed that C-CPE treatment caused a significant increase in the total number of extracellular vesicles secreted by cells ($p<0.05$) (Figure 4.2A). We then analyzed the abundance of different sized vesicles using a nanoparticle tracking analysis system. We observed significant increases in the number of extracellular vesicles (Two-way ANOVA $p_{\text{treat}}<0.0001$), particularly in the 100-300 nm range: 100-150 ($p=0.035$), 150-200 ($p<0.0001$), 200-250 ($p=0.0002$) and 250-300 ($p=0.012$) (Figure 4.2B). Comparison of the relative proportion of each size class using a Two-way

ANOVA test showed that there was no difference between the GST- and C-CPE-treated cells, indicating that the increase of vesicle secretion was proportional along the different vesicle size range ($p_{\text{treat}}=0.99$) (Figure 4.2C).

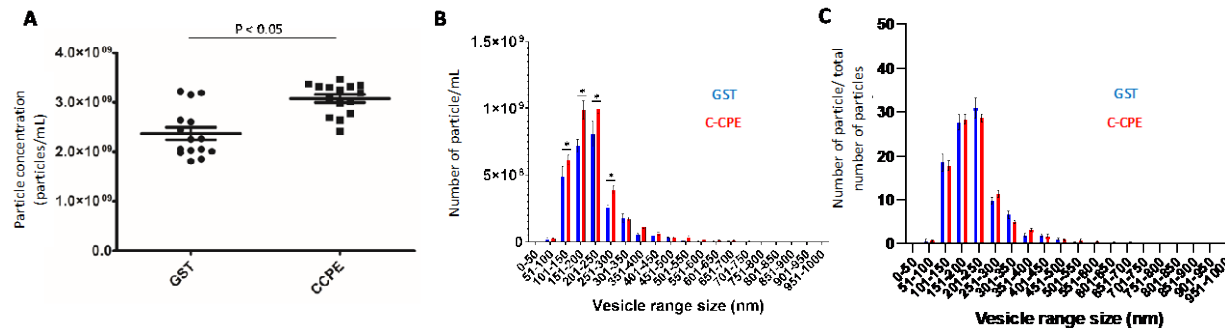


Figure 4.2: C-CPE treatment increases the number and average size of extracellular vesicles secreted from MDCK II cells. **A.** The total number of extracellular vesicles secreted by MDCK II cells treated with 50 $\mu\text{g/mL}$ of GST or C-CPE. Each dot represents a separate measurement from a total of 5 biological and technical replicates. T-Test revealed statistical difference between treatments ($p < 0.05$). **B.** Analysis of the size of extracellular vesicles secreted by cells treated with 50 $\mu\text{g/mL}$ of GST or C-CPE. Two-way ANOVA was performed ($P_{\text{int}} < 0.0001$; $P_{\text{V.size}} < 0.0001$; $P_{\text{Treat}} < 0.0001$). Mean and SEM are plotted. *Denotes significance, $p < 0.05$. **C.** Analysis of the proportion of extracellular vesicles of each size secreted by cells treated with 50 $\mu\text{g/mL}$ of GST or C-CPE compared to the total number of extracellular vesicles secreted. Two-way ANOVA was performed ($P_{\text{int}} = 0.93$; $P_{\text{V.size}} < 0.0001$; $P_{\text{Treat}} = 0.99$). Mean and SEM are plotted.

4.4.3 Inhibitor treatments show that several pathways are involved in claudin extracellular vesicle secretion

Nanoparticle tracker analysis showed that the population of extracellular vesicles between 100-300 nm are the main fractions increased by C-CPE. This size-fraction corresponds to reported sizes of both exosomes and microvesicles. In order to investigate the category of extracellular vesicles and the mechanism underlying claudin enrichment following C-CPE-treatment, we pretreated MDCK II cells with different inhibitors that block either the exosomal pathway (Manumycin A and GW4869) or microvesicle release (Y27632 and Blebbistatin), prior to adding GST or C-CPE (Figure 4.3) (Catalano and O'Driscoll, 2020; Lv et al., 2020). Western blot analysis of these extracellular vesicles reinforced our initial observation that all claudins were significantly increased in extracellular vesicles obtained from C-CPE-treated MDCK II cells (Two-way ANOVA $P_{\text{treat}} < 0.02$ for all claudins; Figure 4.3A). Although none of the inhibitors were able to

fully block the release of all claudin-rich extracellular vesicles upon C-CPE treatment, some of the inhibitors appear to be able to reduce the amount of some claudins in the extracellular vesicles. Cldn1 was increased in C-CPE-treated cells and cells pre-treated with Y27632 or Manumycin-A; however, GW4869 and Blebbistatin hindered the increased release of Cldn1 enriched extracellular vesicles upon C-CPE treatment. C-CPE's effect on the secretion of Cldn3-rich extracellular vesicles was also reduced; cells pre-treated with any of the inhibitors reduced the amount of Cldn3 in the secreted extracellular vesicles, although there was still a great increase on Cldn3 in extracellular vesicles from C-CPE-treated cells when compared with GST-treated cells. In contrast, the presence of Cldn2 and -7 in extracellular vesicles from C-CPE-treated cells did not appear to be altered when cells were pretreated with any of the inhibitors. Finally, the presence of Cldn4 in extracellular vesicles was not affected by inhibitors, with the exception of Manumycin-A which increased Cldn4 in extracellular vesicles when combined with C-CPE treatment.

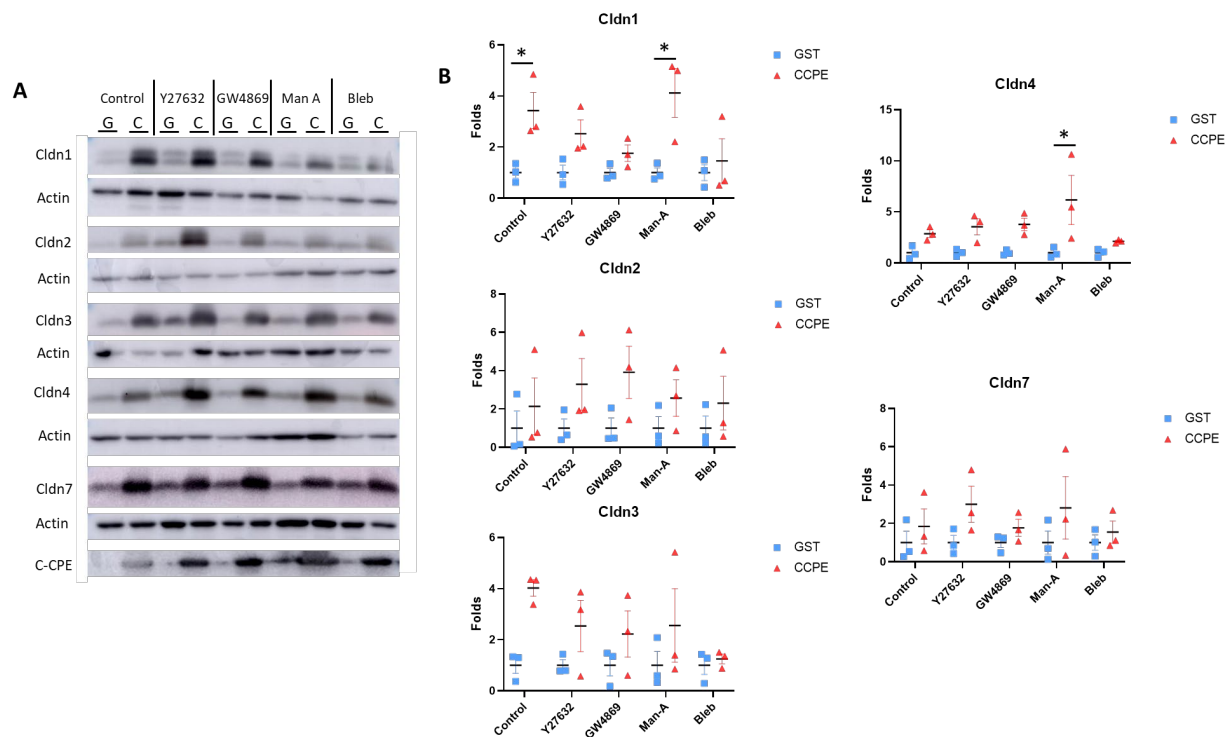


Figure 4.3: Inhibition of extracellular vesicle release partially impair C-CPE effect. **A.** Western blots of 1 μ g of extracellular vesicles obtained from MDCK II cells treated pretreated with exosome or microvesicle release inhibitors and with 50 μ g/mL GST or C-CPE. Actin was used as a loading control. **B.** Western blot quantification using actin as a loading control. Folds with respect

GST-treated control are plotted. Each dot corresponds to an independent experiment. Two-way ANOVA was performed on each claudin by separate: Cldn1 ($P_{\text{int}}=0.1$; $P_{\text{inhibitor}}=0.1$; $P_{\text{Treat}} < 0.0001$); Cldn2 ($P_{\text{int}}=0.9$; $P_{\text{inhibitor}}=0.9$; $P_{\text{Treat}}=0.01$); Cldn3 ($P_{\text{int}}=0.41$; $P_{\text{inhibitor}}=0.41$; $P_{\text{Treat}}=0.$); Cldn4 ($P_{\text{int}}=0.21$; $P_{\text{inhibitor}}=0.21$; $P_{\text{Treat}} < 0.0001$); Cldn7 ($P_{\text{int}}=0.83$; $P_{\text{inhibitor}}=0.83$; $P_{\text{Treat}}=0.02$). Mean and SEM are plotted. *Denotes significance, $p < 0.05$.

4.4.4 Effects of C-CPE treatment on extracellular vesicle cargo

Several extracellular vesicles markers were assayed in order to adhere to MISEV2018 guidelines (Thery et al., 2018). However, none appeared to be consistent with the increases in claudins. For this reason we subjected the extracellular vesicles from C-CPE-treated cells and control cells to mass spectrometry analysis to determine the composition of the extracellular vesicles. Extracellular vesicles from C-CPE- and GST-treated cells were purified and 10 μg of protein from each sample was analyzed by mass spectrometry. Proteins with less than 2 peptides per protein, high ambiguity or confidence less than 95% were excluded from the analysis. The remaining peptides were analyzed by Fisher's exact test and corrected for multiple comparison using Bonferroni. Proteins that were significantly different ($p < 0.05$) or had a 2-fold difference between the GST and C-CPE samples were categorized with respect to their predicted function. Full data can be found in Appendix 3.

In total 2335 proteins were identified in our mass spectrometry analysis (Figure 4.4). After applying the criteria explained above, a total of 903 proteins were found to be differently expressed in the extracellular vesicles derived from C-CPE-treated cells. Specifically, 368 protein were upregulated in the extracellular vesicles from C-CPE-treated cells, while 535 were downregulated. We then applied a manual analysis where inconsistent data were eliminated. Finally, 140 proteins were found to be upregulated and 223 were found to be downregulated in the C-CPE extracellular vesicles compared to GST. These proteins were associated with a number of different pathways (Figure 4.5). Proteins involved in tight junctions were upregulated in the extracellular vesicles from C-CPE-treated cells, including all of the claudins present in MDCK II cells which were increased by 1.4- to 2.4-fold (Table 4.1). Other proteins involved in cell adhesion were downregulated in the C-CPE-associated extracellular vesicles, including integrins ITGB4 and ITGA2 (Table 4.2). Components of extracellular vesicle biogenesis pathways were different between C-CPE and control samples. The only biogenesis pathway that was upregulated in the

extracellular vesicles obtained from C-CPE-treated cells was the NSF and dynamin-2 pathways that are involved in unconventional exocytosis (NSF, 5-fold; dynamin-2, 4.2-fold) (Table 4.1). Pathways involved in exosome formation and exocytosis were all downregulated in the C-CPE population, including proteins involved in microvesicle formation (e.g. MYOF, 0.1-fold; EHD4, 0.3-fold), ESCRT-dependent exosome formation (e.g. HGS, 0.2-fold; CHMP2A, 0.2-fold; IST1, 0.2-fold), ectosome formation (e.g. ARRDC1, 0.5-fold) and some other less common exocytic pathways (e.g. RALA, 0.4-fold; ERC1, 0.4-fold; STX4, 0.4-fold) (Table 4.2).

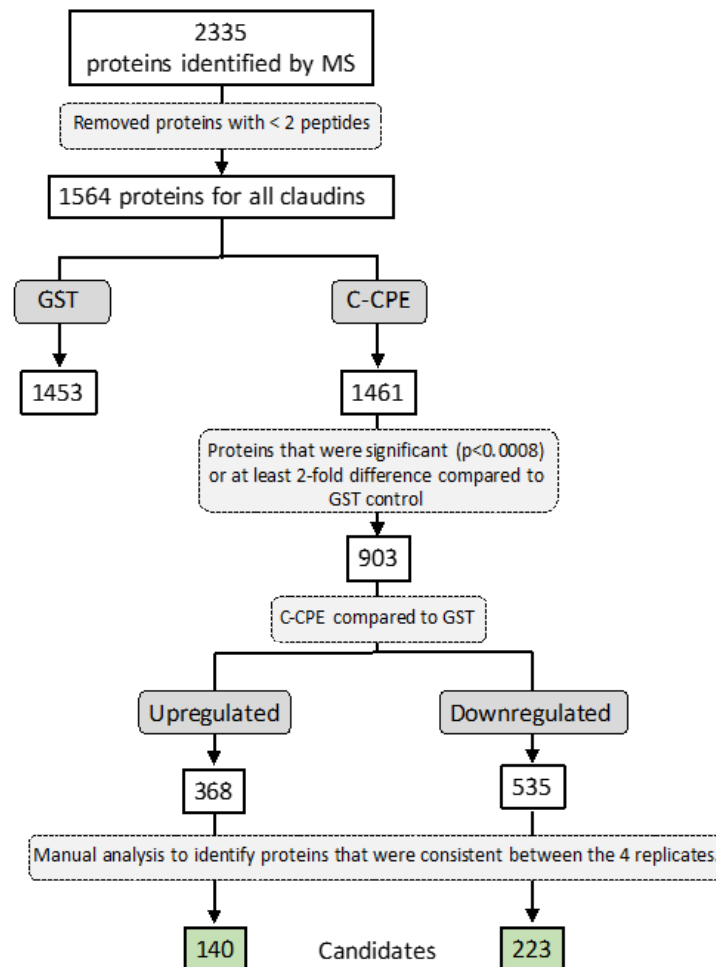


Figure 4.4: Pipeline used for the analysis of the mass spectrometry dataset. Numbers inside the boxes correspond to number of proteins carried forward between steps

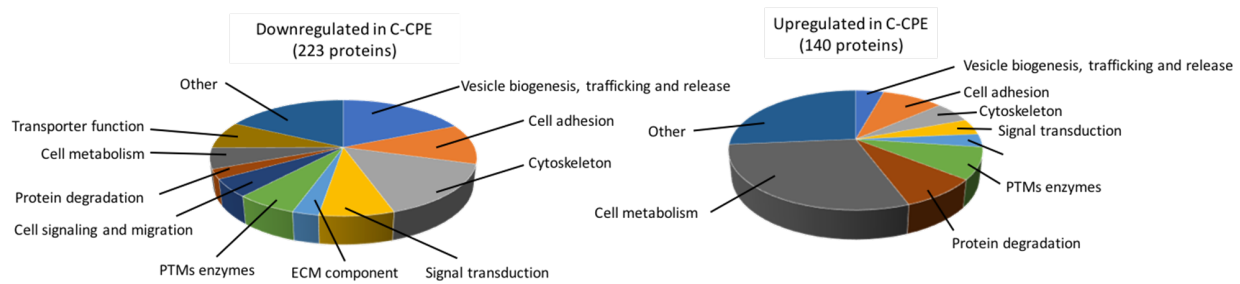


Figure 4.5: Pathway components that are up- or downregulated in the extracellular vesicles from C-CPE-treated MDCK II cells. Pie charts illustrating a general overview of the pathways up- or down regulated in extracellular vesicles from C-CPE-treated MDCK II cells when compared to extracellular vesicles from GST-treated MDCK II cells.

Pathway	Protein name	Fold change
Vesicle biogenesis trafficking and release	EXT1	9.4
	EXT2	6
	NSF	5
	PGAP1	4.9
	VPS29	4.2
	ATP6V1A	2.6
Cell adhesion	FERMT3	19
	EFEMP1	13
	TGFB1	3.9
	LAMC2	3.4
	LAMB3	3
	FBLN1	2.7
	CLDN7	2.4
	LAMA3	2.3
	CLDN3	2
	CLDN1	1.8
	CLDN6	1.8
	CLDN4	1.6
	CLDN2	1.4
Cytoskeleton related	TUBA8	210
	FSCN1	16
	MAPRE2	7.8
	EML2	6.2

	TUBB1	4.2
	DNM2	4.2
	ACTR2	2.8
	TTLL12	2.4
Signal transduction	LTBP1	10
	BMP1	8.8
	MAP2K1	8
	PA2G4	7.2
	MAPK1	5.2
	ARHGEF17	4.7
	TBC1D32	3.2
Extracellular matrix component	OLFML3	12
	BGN	7.1
	PRG4	4.5
	LOXL1	2.8
	PODN	2.7
Enzymes involved in post-translational modifications	B3GAT3	13
	ST3GAL4	10
	HS3ST1	7.5
	GAL3ST2	6.2
	FAM20C	6
	P4HA1	5.1
	QSOX1	4.8
	LOC482182	4.8
	PPP1CC	4.7
	PPP1R7	3.3
	CHST11	3
Protein degradation and maturation	PRMT1	2.9
	PCSK5	15
	CPN1	11
	ADAMDEC1	9.6
	PCSK6	7
	LOC484960	6.8
	ADAMTS13	5.5

Enzymes involved in cell metabolism	SERPINE1	5.5
	TIMP3	5.4
	CTSH	4.5
	PSMC5	4.2
	SERPINE2	3.1
	PYGL	25
	PYGB	22
	ASS1	18
	LIPG	15
	MAT1A	15
	IDH1	14
	ADH5	12
	PLAT	11
	ASL	11
	PGM1	10
	UGDH	10
	MPST	9.5
	QARS	9.2
	UXS1	8.4
	PGLYRP2	8
	ADK	7.8
	GSTM3	7.3
	CNDP2	7
	AKR1C3	6.8
	TKFC	6.8
	GALK1	6.5
	UAP1	6.5
	GGH	6.4
	FBP1	6
	RGN	5.7
	SHMT1	5.5
	PCYOX1L	5
	PLA2G3	5
	GMPPB	4.8

	DHFR2	4.8
	AHCY	4.6
	CMBL	4.5
	GMPS	4.4
	XPNPEP1	4.2
	QDPR	4
	BLVRA	3.8
	PFAS	3.8
	PFKL	3.4
	GLYAT	3
	PKM	2.1
	MTHFD1	1.9
DNA and RNA repair and regulation	TACC1	60
	ENO3	21
	RRM1	12
	APEX1	10
	EIF4A3	9.1
	MCM2	8.5
	PRPF19	7
	DHX9	6
	DHX15	5
	STRAP	4.8
	NONO	4.2
	ACO1	4.1
	DARS	4.1
	DDX1	4
	ACTL6A	3.5
	UPF1	3.5
	TARS	2.5
Ribosome related	NAA15	4.8
	EIF3I	4.8
	RPL10A	4.6
	RPLP0	4.5
	RACK1	4.2

Other	RPL30	4.2
	NOP58	3.8
	F9	13
	OGN	12
	DNM1L	9.2
	CLEC11A	8
	BPGM	6.5
	TOR3A	6.5
	F2	5.5
	PLTP	5.3
	NUTF2	4
	CLU	3.4
	HSPB1	3.3
	RAN	2.6
	F5	2.4

Table 4.1: List of proteins upregulated in extracellular vesicles from C-CPE treated MDCK II cells. Protein name and folds are annotated. Folds were calculated by dividing the total spectral counts found in extracellular vesicles from C-CPE treated MDCK II cells by the spectral counts found in extracellular vesicles from GST treated MDCK II cells for each of the proteins.

Pathway	Protein name	Fold
Vesicle biogenesis trafficking and release	FLOT1	0.1
	MYOF	0.1
	RAB21	0.2
	IGF2R	0.2
	HGS	0.2
	CHMP2A	0.2
	IST1	0.2
	CHMP1A	0.2
	GOLT1B	0.2
	CAVIN1	0.2
	TM9SF2	0.2
	AP1B1	0.3
	PDCD6	0.3
	STXBP2	0.3

	RAB35	0.3
	CLSTN1	0.3
	SPTBN1	0.3
	SPTAN1	0.3
	VPS4B	0.3
	ANXA4	0.3
	ANXA8L1	0.3
	EHD4	0.3
	CAV1	0.3
	AP2A2	0.4
	STX4	0.4
	RAB7A	0.4
	ERC1	0.4
	RAB11B	0.4
	RAB5A	0.4
	TMEM30A	0.4
	STAM	0.4
	RALA	0.4
	ANXA2	0.4
	EHD2	0.4
	SH3GL1	0.4
	CYFIP2	0.4
	EHD1	0.5
	ANXA13	0.5
	ARRDC1	0.5
	CLTC	0.6
Cell adhesion	COL17A1	0.2
	EPB41L5	0.2
	PKP3	0.2
	L1CAM	0.2
	ITGB4	0.3
	DSG2	0.3
	CCDC80	0.3
	PODXL	0.3

Cytoskeleton related	EDIL3	0.3
	CDH1	0.3
	ITGA2	0.3
	NECTIN2	0.3
	AFDN	0.3
	SIRPA	0.4
	ITGAV	0.4
	VCL	0.4
	ITGA6	0.4
	ITGA3	0.4
	CDCP1	0.4
	CEACAM1	0.4
	LAMB1	0.4
	CXADR	0.5
	ITGB1	0.5
	LAMC1	0.5
	EPCAM	0.5
	CEP55	0.1
	EPS8L1	0.2
	BAIAP2L1	0.2
	CAPG	0.2
	FLNB	0.3
	SLC9A3R1	0.3
	EPS8	0.3
	EPS8L2	0.3
	RAP1A	0.3
	FAM49B	0.3
	TPM4	0.3
	KIF23	0.3
	IQGAP1	0.3
	CFL2	0.3
	PLXNA2	0.3
	UTRN	0.3
	TNKS1BP1	0.3

Signal transduction	RDX	0.3
	MYO1C	0.3
	ACTN1	0.3
	ACTN4	0.3
	CYFIP1	0.4
	RAP2C	0.4
	ANXA1	0.4
	PLXNA1	0.4
	TNS3	0.4
	BAIAP2	0.4
	EZR	0.4
	MSN	0.4
	PLEC	0.4
	RRAS	0.5
	JUP	0.5
	CFL1	0.5
	CDC42	0.5
	GLG1	0.1
	PLCB3	0.2
	MVP	0.2
	MAP4K4	0.2
	GPRC5A	0.3
	GPRC5C	0.3
	GNA11	0.3
	GNA13	0.3
	EGFR	0.3
	CD109	0.3
	ARHGEF7	0.3
	GNAS	0.4
	CTNNB1	0.4
	GNAI3	0.4
	SFN	0.4
	BSG	0.5
	GNB1	0.5

Extracellular matrix component	NID1	0.2
	SPP1	0.2
	GPC1	0.3
	COL18A1	0.4
	HSPG2	0.4
	FN1	0.5
Enzymes involved in post-translational modifications	ZDHHC20	0.1
	RPN2	0.1
	NEDD4L	0.2
	P4HB	0.2
	CSNK2A1	0.2
	PDIA3	0.2
	PDIA4	0.2
	DDOST	0.2
	TKT	0.3
	UBE2L3	0.3
	ITCH	0.3
	ZDHHC5	0.3
	CSNK1A1	0.3
	PPIB	0.3
Cell signaling and migration	PHB2	0.09
	PHB	0.1
	CPNE3	0.2
	SLIT2	0.2
	PLXNB2	0.3
	SRGAP2	0.3
	MARCKSL1	0.4
	SLIT3	0.4
	LGALS3	0.5
Planar-cell-polarity regulation	VANG1	0.3
	PTK7	0.4
Protein degradation and maturation	RPN1	0.2
	PSMB3	0.3
	LAMP1	0.3

	ENPEP	0.4
	CAPN5	0.4
	MMP2	0.5
Enzymes involved in cell metabolism	NME2	0.1
	CYBRD1	0.3
	TMX1	0.3
	NME1	0.3
	OAT	0.3
	CPD	0.3
	ACSL4	0.3
	DNASE1L1	0.3
	PLCD1	0.4
Calcium regulation	ANXA6	0.2
	CPNE1	0.3
	CPNE8	0.4
	ATP2B4	0.4
Transporter	ABCC4	0.2
	SLC4A7	0.2
	CLIC2	0.2
	LRRC8A	0.2
	SLC7A5	0.3
	SLC7A1	0.3
	SLC2A1	0.3
	STOM	0.4
	SLC5A3	0.4
	ATP1A1	0.4
	ATP1A4	0.4
	SLC16A1	0.4
	SLC3A2	0.4
	SLC44A1	0.4
	SLC1A4	0.4
	SLC1A5	0.4
	SLC12A2	0.4
	YBX1	0.2

DNA and RNA repair and regulation	H2AFY	0.3
	HIST2H2AC	0.3
	TOP1	0.3
	EIF5A	0.4
	PIR	0.4
Mitochondria	VDAC1	0.09
	SLC25A5	0.1
	ATP5F1A	0.1
	VDAC2	0.1
	SLC25A3	0.2
	FLVCR1	0.3
Chaperone	PDIA6	0.1
	CANX	0.2
	LAMP2	0.3
	BCAP31	0.3
	CALR	0.3
	HSPD1	0.3
	DNAJA1	0.4
	HSPA5	0.5
Other	AHNAK	0.05
	ANLN	0.1
	PPL	0.1
	SYPL1	0.2
	TMEM43	0.2
	ANXA5	0.2
	ATP1B3	0.2
	RPL3	0.3
	CASK	0.3
	DIP2B	0.3
	LSR	0.4
	HEBP1	0.4
	MFGE8	0.4
	RNH1	0.4
	ANXA11	0.4

CTNNA1	0.4
TAGLN2	0.4
NDRG1	0.5
PVR	0.5
PTGFRN	0.5

Table 4.2: List of proteins downregulated in extracellular vesicles from C-CPE treated MDCK II cells. Protein name and folds are annotated. Folds were calculated by dividing the total spectral counts found in extracellular vesicles from C-CPE treated MDCK II cells by the spectral counts found in extracellular vesicles from GST treated MDCK II cells for each of the proteins.

4.5 DISCUSSION

C-CPE is a widely used tool to study tight junctions and claudin biology. However, until now there was no evidence of what happened to claudins once C-CPE removes them from tight junctions. Our study showed that C-CPE led to increased release of extracellular vesicles that are enriched in claudins. We also showed that the claudin enrichment of extracellular vesicles can be partially blocked by pre-treatment with inhibitors of different exosome or microvesicle release pathways. This effect seems to be specific to certain inhibitor-claudin combinations. Moreover, characterization of the extracellular vesicle cargo showed that C-CPE treatment promotes an unconventional exocytosis pathway that involves NSF and dynamin-2.

Our data revealed that claudins are normally incorporated into extracellular vesicles in MDCK II cells. Upon C-CPE treatment there was an increase in the number of extracellular vesicles and the abundance of claudins within the extracellular vesicles, although only the increase in Cldn3 reached significance. Our mass spectrometry analysis of the extracellular vesicle cargo confirmed the increase of claudins in the vesicles. These data suggest that upon C-CPE treatment claudins are enriched in extracellular vesicles and present in a higher abundance than in extracellular vesicles from untreated cells.

In order to confirm that the particles seen in our analysis are in fact extracellular vesicles, it is necessary to adhere to the MISEV2018 guidelines (Thery et al., 2018). None of the standard extracellular vesicle markers seem to be specific to our population. Using the size of the extracellular vesicles is not conclusive as well since our preliminary analysis of the vesicle size revealed that C-CPE causes an increase in all sizes of vesicles, particularly those in the 100-300nm,

range that is shared by exosomes (50-150nm) and microvesicles (100-1000nm). Electron-microscopy analysis will be critical to confirm the exact population, distribution and biogenesis of the extracellular vesicles identified in the media from C-CPE-treated cells.

To begin to unravel the underlying biogenesis mechanism of the additional extracellular vesicles in the C-CPE-treated cells, we used different inhibitors to try to understand the pathways responsible for generating claudin-rich extracellular vesicles (Catalano and O'Driscoll, 2020; Lv et al., 2020). Two of the inhibitors that we used are known to inhibit the most common exosome formation pathways: GW4869 and ManumycinA. GW4869 inhibits neutral sphingomyelinase (nSMase) which is involved in the ceramide pathway and essential for the biogenesis of lipid-rich exosomes. Manumycin A inhibits the small GTPase RAS that is involved in the formation of ESCRT-dependent exosomes. We also used Y27632, which inhibits ROCK activity, and (-)Blebbistatin, which inhibits myosin II and impairs actin polymerization, vesicle motility and budding of the membrane, thereby blocking vesicle release from the plasma membrane. Interestingly, although some of these inhibitors were able to attenuate the increase of some claudins in the extracellular vesicles purified from the MDCK II cells treated with C-CPE, none were able to completely eliminate the effect of C-CPE on the extracellular vesicle population. However, these data are not sufficient to conclude which pathway is participating in increasing the release of claudin-enriched vesicles. For instance, the increase in Cldn1 in extracellular vesicles following C-CPE treatment was diminished when cells were treated with GW4869 or Blebbistatin. The increase in Cldn3 was blocked by most inhibitors, while Cldn2 and 7 enrichment was not affected by any of the inhibitors. Interestingly, pretreatment with Manumycin-A led to an additional increase in Cldn4 in the extracellular vesicles, suggesting that inhibiting RAS proteins by farnesylation and attachment to the plasma membrane somehow promotes inclusion of Cldn4 into extracellular vesicles. The observation that each inhibitor differentially affected the claudin profile in the extracellular vesicles, suggests that each claudin may in fact be processed differently. It still needs to be determined if claudins are in the same vesicle or if each is being packaged into specific vesicles via different pathways. This will make sense with other processes where claudins are specifically modified and regulated even though they are usually treated as a whole when studying tight junctions.

Mass spectrometry analysis of the extracellular vesicle population identified 223 downregulated and 140 upregulated proteins in extracellular vesicles from C-CPE-treated cells. Pathway analysis revealed that many downregulated proteins were associated with the cytoskeleton, signal transduction, cell signaling, transporter function or vesicle biogenesis, trafficking and release, while upregulated proteins were involved in protein degradation or cell metabolism.

In other cases, distinct protein families with similar functions appear to be differentially expressed. For example, the increase of claudins in extracellular vesicles was accompanied by a decrease in integrins, another family of transmembrane proteins that are commonly found in extracellular vesicles where they function to anchor and interact with specific receptors in the target cell (Hurwitz and Meckes, 2019). It is tempting to speculate that claudins might play a similar role.

The mass spectrometry study also allowed us to ask about biogenesis mechanism. We observed that in the vesicles secreted from C-CPE-treated cells only NSF-Dynamin2 pathway was upregulated, whereas many other exocytic pathways were downregulated. The ATPase N-ethylmaleimide-sensitive fusion protein (NSF) and Dynamin-2 function in an unconventional exocytosis pathway that has only been reported in neurotransmitter recycling (Hussain and Davanger, 2011). VPS29, a protein involved in the cargo selection of the retromere complex was also upregulated in the C-CPE extracellular vesicles (4.2 folds). The retromere complex is involved in recycling of different membrane proteins where VPS29 plays an important role selecting the cargo of the recycling vesicles (Seaman, 2012). In contrast, proteins involved in microvesicle formation (myoferlin, EDH family, etc.), ESCRT dependent exosome formation (HGS, CHMP2A, IST1, etc.), ectosome formation (ARRDC1) or other exocytic pathways (RALA, ERC1, STX4, etc.) were downregulated in the extracellular vesicles from C-CPE treated cells. It is tempting to speculate that C-CPE may activate the retromere complex that leads to the recycling of membrane proteins and their processing through the unconventional exocytic NSF-Dynamin 2 pathway in detriment of other exocytosis pathways (Figure 4.6). However, it remains to be determined if claudins are being incorporated into the extracellular vesicles through the NSF-dynamin2 pathways or if the process is controlled by another exocytic pathway. Also, we should

keep in mind that each claudin may be processed via a different pathway so the exocytic pathway must be validated for each individual claudin.

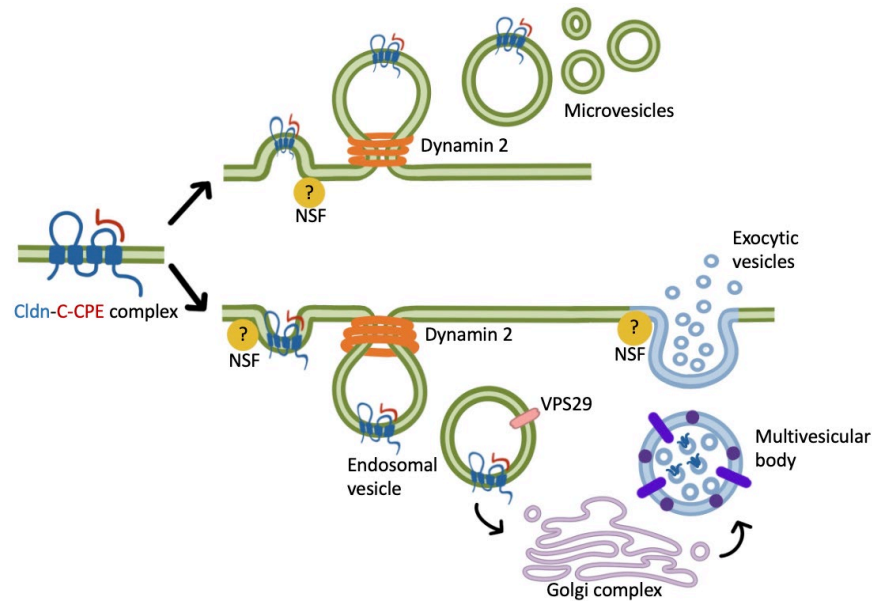


Figure 4.6: Schematic representation of the possible mechanisms used by C-CPE to remove claudins

Another possibility could be that C-CPE increases the release of supermeres. Our mass spectrometry data revealed an increase in TGFB1 in the extracellular vesicles from C-CPE treated cells. TGFB1 has been recently proposed as a marker for supermeres, which are small extracellular vesicles that are morphologically distinct from exosomes and exomeres. Supermeres are of great interest due to the fact that they harbor specific biomarkers (Zhang et al., 2021).

In this study, we showed that claudins accumulate in extracellular vesicles upon C-CPE treatment. However, the significance of the claudins in these vesicles remains unknown. We believe that the inclusion of claudins in extracellular vesicles could be due to one of the following reasons: (1) The extracellular vesicles containing claudins could carry information about C-CPE and be used as a way to communicate and alert surrounding cells about C-CPE or the barrier dysfunction. (2) The secretion of claudin into extracellular vesicles could be a way to alert the immune system of a probable infection. (3) The accumulation of claudins due to their removal from the membrane may lead the cell to secrete the extracellular vesicle without a clearly defined biological function.

In summary, for the first time we showed that C-CPE treatment leads to increased incorporation of claudins into extracellular vesicles, potentially via distinct pathways for each claudin. We also showed that not only do claudins get incorporated into extracellular vesicles, but that the number of extracellular vesicles is significantly increased upon C-CPE treatment and that the cargo incorporated into these extracellular vesicles is different from the proteins present in control extracellular vesicles. Moreover, our data opens the possibility of using C-CPE as a tool to generate claudin-rich extracellular vesicles useful for the study of different processes and diseases.

4.6 SUPPLEMENTARY MATERIALS

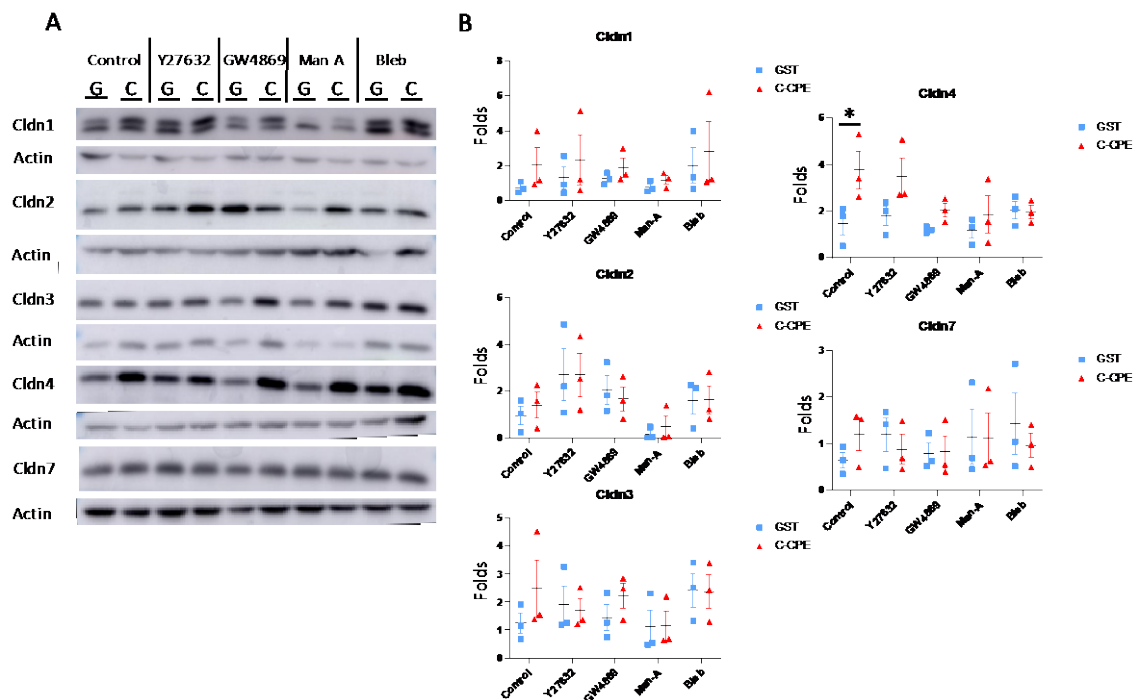


Figure 4.S1: Western blot of cells treated with GST or C-CPE in presence of exosome or microvesicle release inhibitors. A. Western blots of 20 μ g of protein from MDCK II cells treated with exosome or microvesicle release inhibitors and with 50 μ g/mL GST or C-CPE and their corresponding actin loading control. **B.** Western blot quantification using actin as a loading control. Folds respect GST-treated control is plotted. Each dot corresponds to an independent experiment. Two-way ANOVA was performed on each claudin by separate: Cldn1 ($P_{\text{int}}=0.98$; $P_{\text{Inhibitor}}=0.57$; $P_{\text{Treat}}=0.15$); Cldn2 ($P_{\text{int}}=0.96$; $P_{\text{Inhibitor}}=0.02$; $P_{\text{Treat}}=0.85$); Cldn3 ($P_{\text{int}}=0.71$; $P_{\text{Inhibitor}}=0.37$; $P_{\text{Treat}}=0.35$); Cldn4 ($P_{\text{int}}=0.21$; $P_{\text{Inhibitor}}=0.12$; $P_{\text{Treat}}=0.0035$); Cldn7 ($P_{\text{int}}=0.76$; $P_{\text{Inhibitor}}=0.88$; $P_{\text{Treat}}=0.86$). Mean and SEM are plotted. *Denotes significance, $p<0.05$

4.7 AUTHOR CONTRIBUTIONS

Conceptualization, Enrique Gamero-Estevez, Jenna Haverfield and Aimee K. Ryan;
 Formal analysis, Enrique Gamero-Estevez, Jenna Haverfield; Funding acquisition, Aimee K. Ryan;
 Investigation, Enrique Gamero-Estevez, Jenna Haverfield and Aimee K. Ryan; Methodology,
 Enrique Gamero-Estevez, Jenna Haverfield and Aimee K. Ryan; Writing – original draft, Enrique
 Gamero-Estevez; Writing – review & editing, Aimee K. Ryan.

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4.10 CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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4.11 CONNECTING TEXT BETWEEN CHAPTER 4 AND 5

In Chapter 4, I showed that C-CPE treatment of MDCK II cells led to the increased release of extracellular vesicles into the media. Moreover, these extracellular vesicles were enriched in claudins, suggesting that upon C-CPE treatment, claudins are incorporated in extracellular vesicles and secreted. This shed light on how C-CPE may work to remove claudins from the tight junctions; however, C-CPE still has limitations for its use as a tool to study claudins and tight junctions. One challenge is that C-CPE does not remove a single claudin family member at a time. Another is that C-CPE targets removal of only a limited subset of claudins, only 6 out of the 28 claudins present in mammals. Although a number of C-CPE variants have been generated that target individual claudin family members, including Cldn1, Cldn2, Cldn3, Cldn4 and Cldn5, most claudins cannot be uniquely targeted and removed. In Chapter 5 I describe my research to design and generate a new set of tools based on the C-CPE backbone that can be used to individually target any claudin family member. This work is presented as a draft of a manuscript that will be submitted shortly. In addition, we (E. Gamero, M. Nagano and A. Ryan) have filed a patent for these reagents.

CHAPTER 5:

Claudin-specific peptides target removal of individual claudins from the tight junction

Enrique Gamero-Estevez^{1,2}, Amanda I. Baumholtz^{1,2}, Elisabeth-Ann Legere^{1,2}, Makoto Nagano³
and Aimee K. Ryan^{1,2,4}

¹Department of Human Genetics, McGill University, Montreal, QC, H3A 0C7, Canada

²Research Institute of the McGill University Health Centre, Glen Site, Montreal, QC, H4A 3J1,
Canada

³Department of Obstetrics and Gynecology, McGill University, Montreal, QC, H4A 3J1, Canada

⁴Departments of Pediatrics, McGill University, Montreal, QC, H4A 3J1, Canada.

Manuscript in preparation.

5.1 ABSTRACT

Epithelial and endothelial tight junction barriers are composed of transmembrane proteins that interact between apposing cells. One of the main components of tight junctions are claudins, a family of tetraspan membrane proteins. Our goal is to modify epithelial or endothelial barriers by specifically targeting the removal of individual claudin family members. One available tool to target a subset of claudins is the C-terminal domain of *Clostridium perfringens* enterotoxin (C-CPE), which is able to bind Cldn3, -4, -6, -7, -8 and -14 with different affinities and remove them from the tight junction without damaging the cell. Small peptides and C-CPE variants that target individual claudins have also been developed. However, most claudin family members are not individually targeted by these tools. To address this technology gap, we generated a set of peptide tools that target removal of specific claudin family members. In our design, the claudin binding domain of C-CPE was replaced with the claudin second extracellular loop that is responsible for the dimerization of claudins across the paracellular space of apposing cells. Specifically, the claudin-targeting peptides were generated by fusing in series cDNAs that encode a histidine tag, a solubility tag (NT), and the stabilization domain of *Clostridium perfringens* enterotoxin (CPE) to the cDNA sequence encoding the claudin second extracellular loop. As a proof-of-concept we demonstrated that Cldn3- and Cldn8-targeting proteins (NT3 and NT8) specifically removed Cldn3 and -8, respectively, in cultured MDCK II and mIMCD3 cells, and in *ex ovo* cultured chick embryos. The neural tube phenotypes generated following tight junction depletion of Cldn3 or Cldn8 overlapped with the phenotypes observed when Cldn3, -4 and -8 were removed using C-CPE. Our approach offers the possibility of creating a fusion peptide that can target the specific removal of each member of the claudin family. These tools will advance our ability to study any epithelial barrier and dissect the role of individual claudin family members in the barrier.

5.2 INTRODUCTION

Tight junctions are essential structures between apposing cells in epithelial and endothelial tissues that control the movement of ions and molecules across the paracellular space, and separate apical from basolateral proteins and lipids to allow the correct polarization

of the cell. Tight junctions are composed of anastomosing strands of transmembrane proteins that associate with scaffolding proteins at the cytoplasmic face of the tight junction. Among the more than 100 proteins associated with tight junctions, it is the combination of claudin family members and their interactions with cytoplasmic components that ultimately determines the properties of a tight junction (Gunzel and Yu, 2013).

Claudins are a family of 20-27 kDa tetraspan transmembrane proteins. To date, 28 members have been described in mammals (Piontek et al., 2020b). They have four main functional domains. The first extracellular loop is involved in the formation of a pore that determines the size and charge of ions and small molecules that can pass through the tight junction. The second extracellular loop participates in *trans*-dimerization with claudins in apposing cells. The four transmembrane domains participate in *cis*-interactions with other claudin molecules within the membrane. Finally, the cytoplasmic C-terminal domain interacts with scaffolding proteins that bridge the tight junction to the actin-cytoskeleton (Itoh et al., 1999).

Claudins have been studied to understand developmental processes, as well as their role as a target for drug delivery to specific barriers (Hashimoto et al., 2019). The specific properties of the tight junction paracellular barrier are determined by the combination of claudins present, as each claudin is distinct with respect to its ability to promote or inhibit the paracellular passage of ions and small molecules. Gain- and loss-of-function studies demonstrate additional roles for claudins, including the maintenance of tissue shape (Behr et al., 2003; Nelson et al., 2010; Wu et al., 2004), tension between cells during morphogenetic movements (Siddiqui et al., 2010), and hydrostatic pressure during lumen expansion (Bagnat et al., 2007; Moriwaki et al., 2007; Zhang et al., 2010). Claudin variants have been associated with several human diseases, including deafness, kidney stones, cancer, infertility, and brain defects, supporting their roles in maintaining the tight junction paracellular barrier and in tissue morphogenesis (Baumholtz et al., 2020; Gunzel and Yu, 2013; Yu, 2015).

One of the tools that has been used to study claudin function is derived from the *Clostridium perfringens* enterotoxin (CPE), a single chain 319 amino acid polypeptide that leads to the gastrointestinal symptoms associated with *C. perfringens* type A food poisoning in humans

(Wieckowski et al., 1994). CPE binds to a subset of the claudins present in the intestinal epithelium, which results in the formation of a pore that allows calcium to enter the cell and subsequently leads to apoptosis (Chakrabarti et al., 2003; Shrestha et al., 2016). Consequently, there is a break in the intestinal barrier that leads to the sequelae of events associated with food poisoning. CPE is comprised of 3 functional domains: an N-terminal cytotoxic domain (amino acids 45-116) that mediates the calcium-pore formation, a C-terminal claudin binding domain (amino acids 290-319) that interacts with the second extracellular loop of Cldn3, -4, -6, -7, -8 and -14, and a stabilization domain (amino acids 184-290) that is essential to stabilize the interaction (Hashimoto et al., 2019).

The C-terminal half of *Clostridium perfringens* enterotoxin (C-CPE) contains the stabilization and claudin binding domains of CPE (aa.186-319). C-CPE is ideal to target removal of claudins because (i) it is not cytotoxic, (ii) it binds specifically to a limited subset of claudins (Cldn-3, -4, -6, -7, -8 and -14) and removes them from tight junctions without affecting the localization of other family members, (iii) it does not affect gene expression so that upon C-CPE removal susceptible claudins can repopulate tight junctions, and (iv) it does not bind to any other cell surface proteins. Previously we and others demonstrated that C-CPE can be used to selectively and simultaneously remove a subset of claudins from tight junctions without the toxicity associated with full-length C-CPE (Baumholtz et al., 2017; El Andalousi et al., 2020; Takahashi et al., 2008). Thus, phenotypes resulting from C-CPE treatments are the direct result of its interactions with specific claudins.

However, C-CPE also has some limitations, including that it only targets 6 out of the 28 claudins and it does not allow selective removal of individual claudin family members. A number of approaches have been used to address these limitations. Site-directed mutagenesis to modify the sequence of C-CPE led to the identification of C-CPE variants that predominantly target Cldn1, -2, -3, -4 and/or -5, and that increase barrier permeability (Beier et al., 2019; Liao et al., 2016; Piontek et al., 2020a; Protze et al., 2015; Walther et al., 2012; Winkler et al., 2009). Other groups have shown that small claudin-specific peptides (5 amino acids) corresponding to the EL2 domain of Cldn2 can be used to block its *trans*-interactions with Cldn2, -3 and -4, and increase barrier permeability (Dithmer et al., 2017; Nasako et al., 2020; Sauer et al., 2014; Staat et al., 2015;

Zwanziger et al., 2012). Peptides with the exact same sequence as the C-terminal part of the EL1 of Cldn1 or Cldn5 are able to target these claudins and increase barrier permeability to small macromolecules *in vitro*, including in the blood brain barrier and the blood nerve barrier in rats (Dithmer et al., 2017; Sauer et al., 2014). Finally, monoclonal antibodies against different claudins, such Cldn3 or -5, are able to bind the target claudin, promote barrier opening, and increase barrier permeability *in vitro* (Fukasawa et al., 2015; Kato-Nakano et al., 2010; Okai et al., 2018; Tokunaga et al., 2007).

Here, we report our *in vitro* and *in vivo* proof-of-concept data for our novel approach to specifically target Cldn3 and -8, which were chosen because of their importance during neural tube closure and due to the fact that we have a direct biological assay to confirm the efficacy of these tools. Specifically, we substituted the claudin binding domain of C-CPE with the EL2 of a specific claudin. This tool can be adapted for all claudin family members and will have direct applications for the study of tight junction barriers.

5.3 RESULTS

5.3.1 Design and purification of fusion peptides that target removal of individual claudins from tight junctions

Our goal was to develop a series of tools that transiently remove individual claudin family members from tight junctions that can be used to facilitate the controlled opening of these barriers for short periods of time. Others have previously used short peptides corresponding the first or second extracellular loop to open barriers (Dithmer et al., 2017; Nasako et al., 2020; Sauer et al., 2014; Staat et al., 2015; Zwanziger et al., 2012). However, in our hands, the effect of these peptides was very rapid and had no significant effect on the localization of the claudin of interest. (Data not shown.) We and others have used the C-CPE reagent but its limitation was its specificity: it removes multiple claudins simultaneously. Therefore, we designed a tool that combined the properties of both of these reagents such that it would specifically target individual claudin family members, while allowing the barriers to be opened for controlled time periods. For this peptide reagent we replaced the claudin binding domain of C-CPE (amino acids 290-319) with the sequence of the second extracellular loop (EL2) of a specific claudin. For these proof-of-principle

experiments we used mouse Cldn3 (amino acids 142-161) or Cldn8 (amino acids 142-166) (Table 5.1). Given that the EL2 is responsible for claudin homodimerization, we predicted that this domain would provide target specificity. Therefore, we predict that these new fusion proteins will bind and remove the claudin of interest similar to what C-CPE does but being specific of whichever claudin EL2 we include in our tools. We chose to use the sequence of mouse EL2 for these studies because it is expected to have the broadest applications across the different species that we use. This truncated C-CPE::EL2 cDNA was then transferred to different plasmids with different purification tags.

Construct	Amino acid sequence
C-CPE	RCVLTVPSTDIEKEILDAAATERLNLTDALNSNPAGNLYDWRSSNSYPWTQKLNHLTITATGQKYRILASKIV DFNIYSNNFNFLVKLEQSLGDGVKDHVYDISLDAGQYVLMKANSSYSGNYPYSILFQKF
mCldn3EL2	WSANTIIRDFYNPLVPEAQKREMG
mCldn8EL2	WVANSIIRDFYNPLVDVALKRELGEALY
C-CPE::Cldn3EL2	RCVLTVPSTDIEKEILDAAATERLNLTDALNSNPAGNLYDWRSSNSYPWTQKLNHLTITATGQKYRILASKIV DFNIYSNNFNFLVKLEQSLGDGVKDHVYDISSSIIRDFYNPLVPEAQKREMG
C-CPE::Cldn8EL2	RCVLTVPSTDIEKEILDAAATERLNLTDALNSNPAGNLYDWRSSNSYPWTQKLNHLTITATGQKYRILASKIV DFNIYSNNFNFLVKLEQSLGDGVKDHVYDISSSIIRDFYNPLVDVALKRELGEALY

Table 5.1: Sequences of the different constructs and domains. Amino acid sequences of the initial and final constructs used to generate the different tools.

Initial attempts to purify these fusion proteins using the GST tag, resulted in extremely low yields due to the fact that the protein was highly insoluble, and we were unable to obtain sufficient quantities of soluble protein. To increase the yield and solubility of the C-CPE::EL2 peptides we made several modifications to the bacterial culture and protein induction conditions (Table 5.2). These modifications included inducing protein production at different bacterial culture densities, the amount of IPTG used for protein induction, and the incubation temperature and duration of fusion protein production. However, none of these modifications resulted in higher solubility of the GST fusion proteins.

Absorbance A ₆₀₀ before induction	0.3	0.4	0.6	1.6		
IPTG concentration	0.05mM	0.1mM	0.2mM	0.4mM	0.8mM	
Temperature of growth after induction	Room Temp. overnight	37°C overnight	22°C overnight	Room Temp. 4h	37°C 4h	22°C 4h
Buffer used for resuspension	Hepes buffer	Tris buffer (pH 6.9)	Tris buffer +Urea	Tris buffer +Sarkosyl (SLS) 0.1-10%		
Sonication	Small probe 3 times	Small probe 6 times	Small probe 9 times	Small probe 12 times	Sonicator bath	
Detergent treatment before beads	+triton X100	Octyl- glucoside	CHAPS	No detergent		
Beads treatment (GST/His/TRX)	Soluble protocol	Insoluble protocol				
Dialysis buffer	PBS	Tris-Glycerol				

Table 5.2: Summary of the strategies used to troubleshoot and improve purification solubility. Overview of the different steps that were troubleshoot to improve solubility and yield of the different tools. In bold are the strategies that allow better purification.

Next we modified our bacterial cell lysis and purification protocols in an attempt to increase the yield of bacterial fusion proteins. We compared several different lysis buffers and a range of Sarkosyl concentrations (0.1-10%) in Tris-buffered solutions. Sarkosyl concentrations of 2% or higher improved the yield of soluble protein in the lysate. However, under these conditions the interaction of the fusion protein with the purification beads was also enhanced and we were not able to elute the proteins from the beads. Attempts to elute the protein using other detergents, including TritonX-100, Octyl-glucoside or CHAPS, to weaken the interaction between protein and beads were unsuccessful (Table 5.2). Increased sonication was also not able to improve the yield of soluble protein.

Replacing the GST-tag with a His-tag was also unable to increase the solubility of the fusion protein. Therefore, we used a denaturation purification protocol to extract more protein from the insoluble protein pellet. After sonication, the pellet was resuspended in 8M urea to solubilize the protein for purification on Ni-beads and elution. The protein was renatured using sequential dialysis in decreasing concentrations of urea. Unfortunately, the protein precipitated

upon dialysis and changing the dialysis buffer did not prevent precipitation nor increase the recovery of folded soluble protein.

Due to the challenges that we faced in obtaining soluble protein, we decided to include a solubility tag. For this we tested both adding Thioredoxin (TRX) to the C-terminal domain of our constructs or adding the N-terminal domain of the Spidroin protein (NT) to the N-terminal domain of our construct. Both of these tags have been shown to increase the solubility of membrane and highly insoluble proteins (Correddu et al., 2019; Kronqvist et al., 2017). Indeed, in our hands, both tags increased the yield of soluble protein. Given that the NT-tag resulted in a 4-fold higher yield and a 4-fold higher concentration of soluble protein relative to what we obtained for TRX-tagged proteins (0.75 mg per liter of bacterial culture at 6.8 mg/mL versus 0.2 mg per liter bacterial culture at 1.5 mg/mL; Table 5.3), we incorporated the NT-tag into all of our fusion protein constructs. We then re-optimized the purification strategy testing the parameters described for GST-tag in Table 5.2 and determined that induction of fusion protein production with 0.2mM IPTG followed by culturing at 22°C overnight produced the highest yield of protein (0.75 mg/L) that was soluble at concentrations of >6 mg/mL. Addition of detergents or urea was not required. A summary of the different peptide structure and their yield and concentration can be found in Figure 5.1 and Table 5.3. We generated His::NT::C-CPE::EL2 polypeptides that could target Cldn3 and Cldn8 (referred to as NT3 and NT8, respectively). These peptides were used in proof-of-concept experiments to validate our strategy.

Construct	Maximum concentration (µg/µL)	Yield (mg/L)
GST	20	4
GST::C-CPE	20	2
GST::C-CPE::Cldn3EL2	Not soluble	-
GST::C-CPE::Cldn8EL2	Not soluble	-
His::C-CPE::Cldn3EL2	Not soluble	-
His::C-CPE::Cldn8EL2	Not soluble	-
C-CPE::Cldn3EL2::TRX::His	1.5	0.2
C-CPE::Cldn8EL2::TRX::His	Not soluble	-
His::NT::C-CPE::Cldn3EL2	6	0.75
His::NT::C-CPE::Cldn8EL2	6.8	0.75

Table 5.3: Summary of solubility and yield of protein obtained using different purification tags. Summary of the yields and maximum concentration from different constructs obtained using the optimized purification protocol for each.

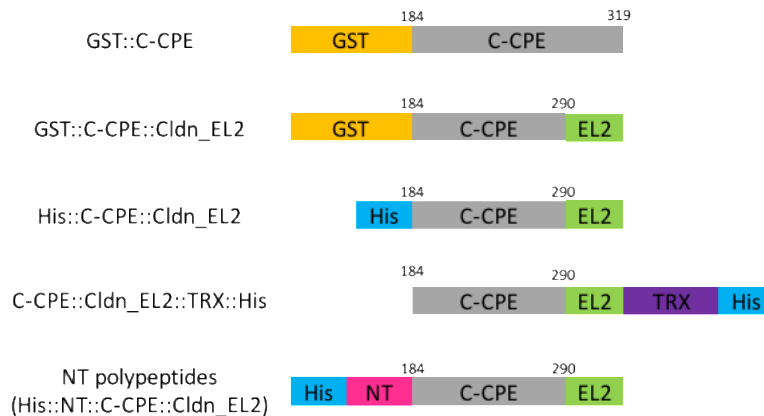


Figure 5.1: Scheme of the different C-CPE::Claudin fusion proteins. Schematic showing the peptides generated using GST, His and/or TRX or NT solubility tags fused. In all peptides the claudin binding domain of C-CPE (290-319) was replaced by the EL2 of Cldn3 or Cldn8.

5.3.2 NT peptides specifically remove claudins from tight junctions in cultured cells

We tested the ability of NT3 and NT8 to remove the targeted claudin family member from tight junctions in cell lines. First, we used the mouse inner medullary collecting duct-derived cell line (mIMCD3), which expresses several claudins, including Cldn3, -4, -7 and -8 that are targeted by C-CPE, and Cldn1 that is C-CPE insensitive. mIMCD3 cells were grown to confluence and then

treated with 50, 100 or 200 $\mu\text{g}/\text{mL}$ of NT3 or NT8 protein. Cells were collected 3h after treatment and immunofluorescence was used to examine the effects on Cldn3 and -8 localization (Figure 5.2). Neither NT3 nor NT8 had any effect on claudin localization at the lowest dose of 50 $\mu\text{g}/\text{mL}$. However, 100 $\mu\text{g}/\text{mL}$ of NT3 or NT8 decreased the colocalization of Cldn3 or Cldn8, respectively, with ZO-1 at the tight junction. There did not appear to any additional effect on Cldn8 localization when the dose of NT8 was increased to 200 $\mu\text{g}/\text{mL}$; while an even higher decrease on Cldn3 was observed when the dose of NT3 was increase to 200 $\mu\text{g}/\text{mL}$. We decided to use for further experiments a final concentration of NT peptide of 100 $\mu\text{g}/\text{mL}$.

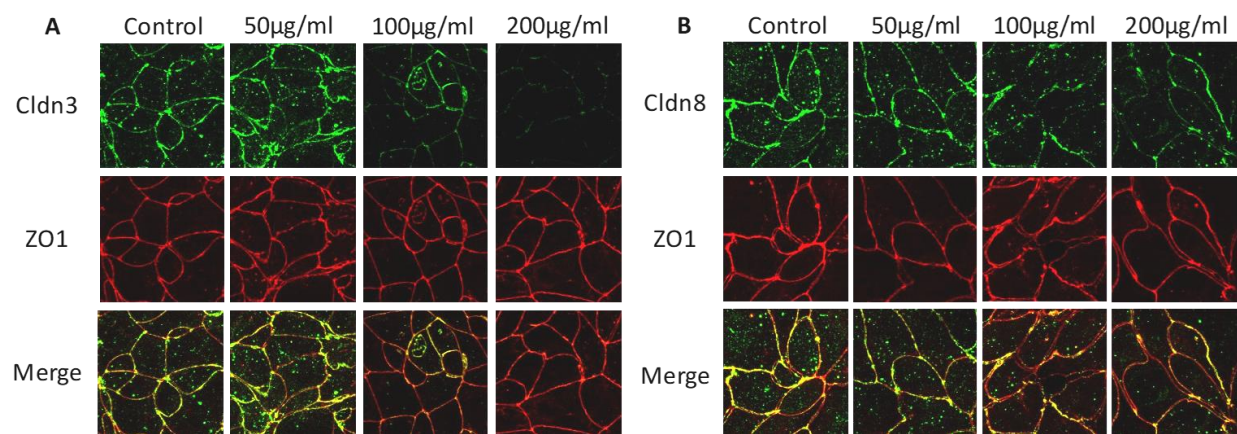


Figure 5.2: Analysis of Cldn3 and -8 localization after treatment with different doses of NT3 or NT8. **A.** mIMCD3 cells were treated with 50, 100 or 200 $\mu\text{g}/\text{mL}$ of NT3. Cldn3 (green) and ZO-1 (red) localization was monitored by immunofluorescence 3h after treatment. **B.** mIMCD3 cells were treated with 50, 100 or 200 $\mu\text{g}/\text{mL}$ of NT8. Cldn8 (green) and ZO-1 (red) localization was monitored by immunofluorescence 3h after treatment.

We also tested whether NT3 was able to remove Cldn3 from tight junctions in Madin Darby Canine Kidney (MDCK II) cells, which have been used extensively to analyze claudins and tight junction function. MDCK II cells have a different claudin expression profile than mIMCD3 cells and so allow us to determine if the NT reagents work in the context of different species and claudin profiles. They do not express Cldn8 and therefore only the NT3 reagent could be tested. Similarly to what was observed in mIMCD3 cells, Cldn3 was removed from the tight junctions of NT3-treated MDCKII at both 3h and 24h (Figure 5.3). These data demonstrate that NT3 is able to target removal of both mouse and canine Cldn3.

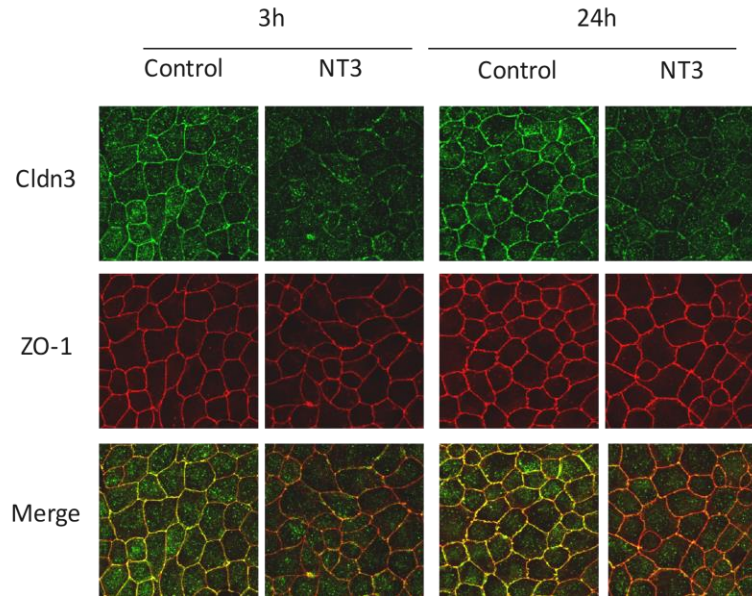


Figure 5.3: Analysis of Cldn3 localization in MDCK II cells after 3 and 24 hours of treatment with 100 $\mu\text{g}/\text{mL}$ of NT3. MDCK II cells were treated with 100 $\mu\text{g}/\text{mL}$ of NT3. Immunofluorescence against Cldn3 (green) and ZO-1 (red) was performed at 3h and 24h after treatment.

Next we assessed the effect on claudin localization over time. mIMCD3 cells were grown to confluence, treated with 100 $\mu\text{g}/\text{mL}$ of NT3 or NT8, and collected at 3h, 6h, 12h or 24h. Cldn3 or Cldn8 localization was assessed by immunofluorescence (Figure 5.2 and 5.4). Both NT3 and NT8 removed their respective targets from the tight junction by 3h after treatment (Figure 5.2). In cells treated with NT3, Cldn3 remained significantly reduced at the tight junction at 24h after the start of treatment (Figure 5.4A). In contrast, in NT8-treated cells, Cldn8 was reduced at tight junctions at 12h, but by 24h it had relocated to tight junctions (Figure 5.4B).

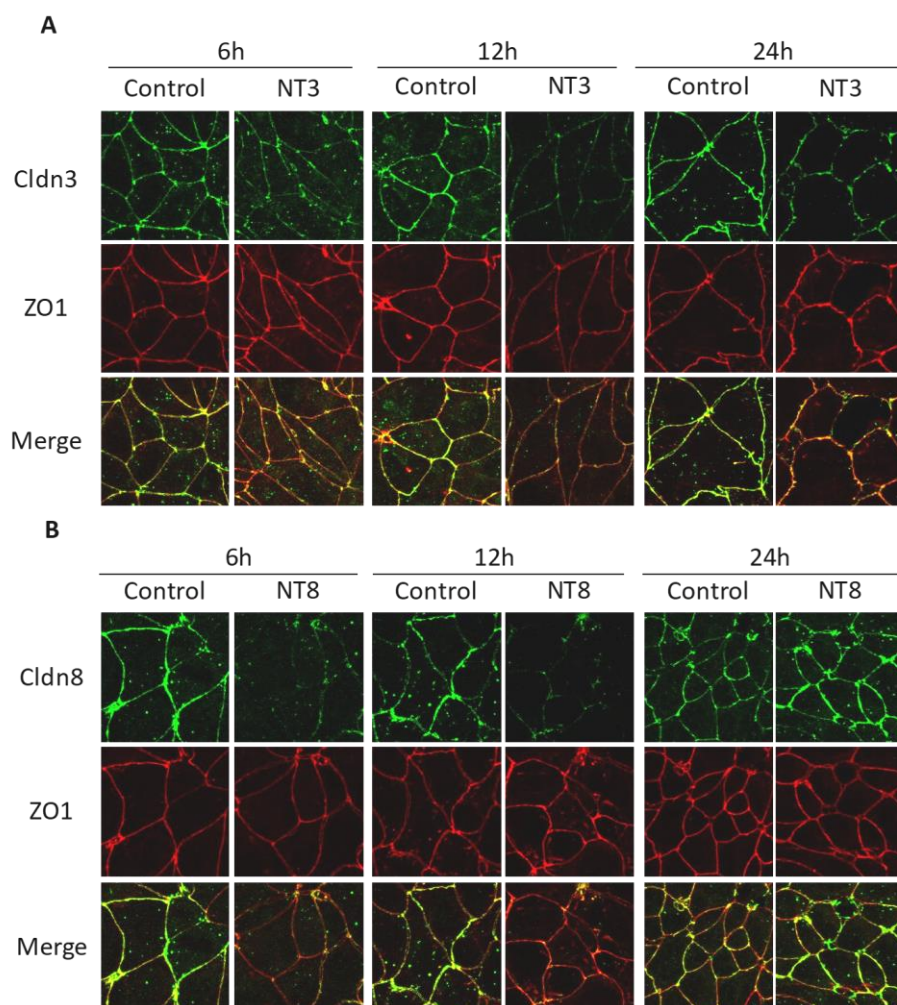


Figure 5.4: Analysis of Cldn3 and 8 localization in mIMCD3 cells after 6, 12 or 24 hours of treatments with 100 µg/mL of NT3 or NT8. A. Immunofluorescence on control and NT3-treated mIMCD3 cells with antibodies against Cldn3 (green) and ZO-1 (red) performed 6, 12 or 24h after treatment with 100 µg/mL of NT3. **B.** Immunofluorescence on control and NT8-treated mIMCD3 cells with antibodies against Cldn8 (green) and ZO-1 (red) performed 6, 12 or 24h after treatment with 100 µg/mL of NT8.

We then tested the specificity of our peptide. mIMCD3 cells were grown to confluence and treated with 100 µg/mL of NT3 or NT8 for 3h or 24h and the effects on tight junction localization of Cldn1, -3, -4, -7 and -8 were assessed by immunofluorescence (Figures 5.5). At 3h of NT3 treatment, localization of Cldn1 and -4 to the tight junction was not affected, while Cldn7 was slightly reduced and Cldn8 was increased. At 24h Cldn1, -4, -7 and -8 were unaffected by the NT3 treatment, while Cldn3 remained reduced as described above. In contrast to NT3, NT8 had more variable effects on other claudins. At 3h, Cldn1 was increased while Cldn3 was decreased

at the tight junction. However, by 24h no effect was observed in any of the claudins when compared to control (Figures 2B, 4B and 5).

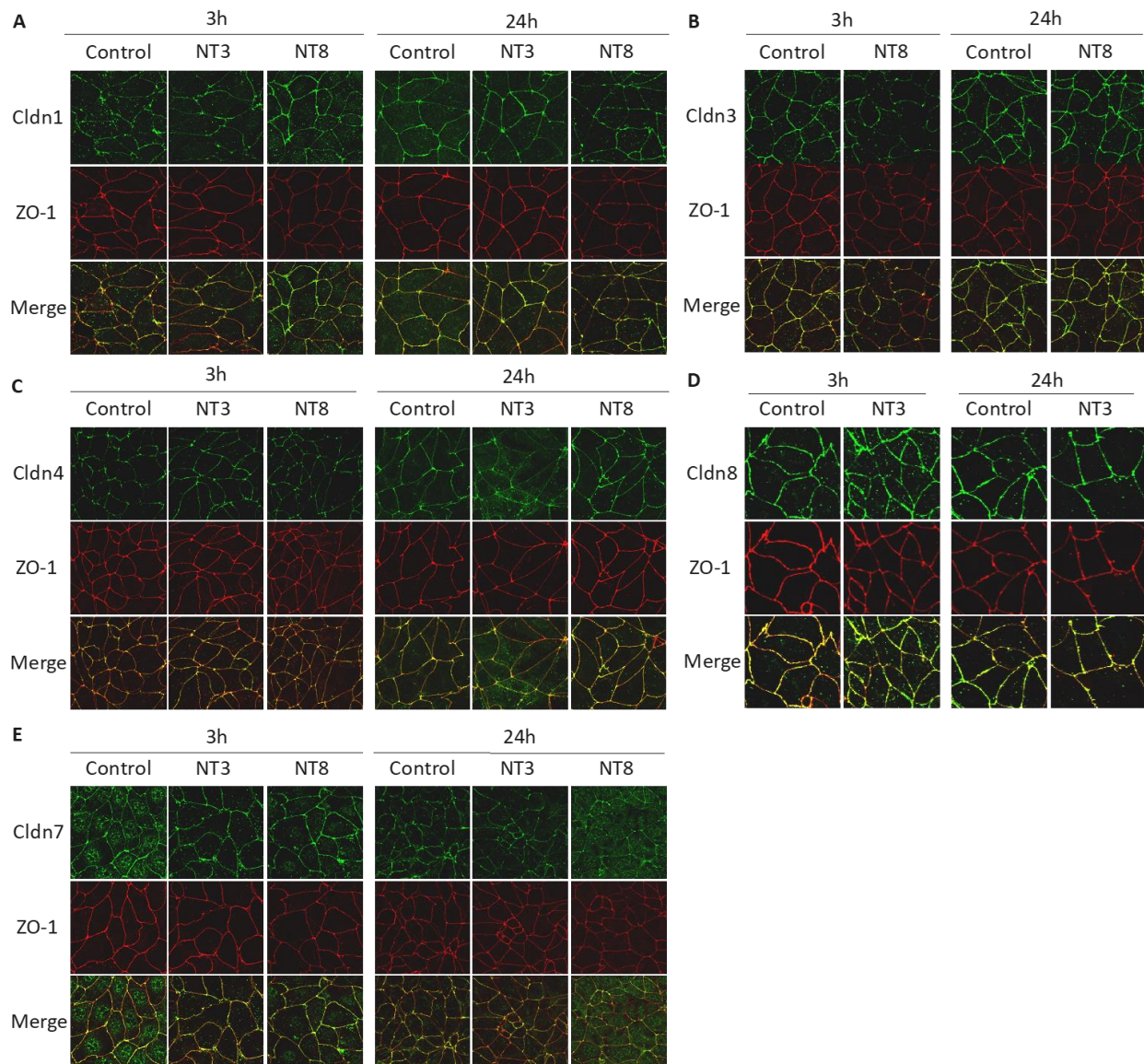


Figure 5.5: Analysis of Cldn1, -3, -4, -7 and 8 localization in mIMCD3 cells after 3 or 24 hours of treatments with NT3 or NT8. **A.** Immunofluorescence on mIMCD3 against Cldn1 (green) and ZO-1 (red) performed 3 or 24h after treatment with 100 $\mu\text{g}/\text{mL}$ of NT3 or NT8. **B.** Immunofluorescence on mIMCD3 against Cldn3 (green) and ZO-1 (red) performed 3 or 24h after treatment with 100 $\mu\text{g}/\text{mL}$ of NT8. **C.** Immunofluorescence on mIMCD3 against Cldn4 (green) and ZO-1 (red) performed 3 or 24h after treatment with 100 $\mu\text{g}/\text{mL}$ of NT3 or NT8. **D.** Immunofluorescence on mIMCD3 against Cldn7 (green) and ZO-1 (red) performed 3 or 24h after treatment with 100 $\mu\text{g}/\text{mL}$ of NT3 or NT8. **E.** Immunofluorescence on mIMCD3 against Cldn8 (green) and ZO-1 (red) performed 3 or 24h after treatment with 100 $\mu\text{g}/\text{mL}$ of NT3.

5.3.3 NT3 and NT8 give rise to distinct neural tube defects in the chick embryo

We previously showed that using wild-type C-CPE to remove Cldn3, -4 and -8 from neural plate stage chick embryos causes severe neural tube defects (Baumholtz et al., 2017). In the C-CPE-treated embryos the neural folds fail to elevate due to defects in apical constriction at the midline of the neural ectoderm, which expresses Cldn4 and Cldn8 but not Cldn3. Treatment with a C-CPE variant that is specific for Cldn4 (C-CPE₄) had no effect on apical constriction or neural tube closure, suggesting that Cldn8 is the critical claudin in the neural ectoderm. In contrast, a C-CPE variant that only targets Cldn3 (C-CPE₃) caused a milder phenotype due to a failure in the final phase of neural tube closure in 50% of embryos (unpublished observation). Therefore, we used the chick embryo model to test the biological activity of our NT peptides and confirm their specificity *in vivo*. Based on our previous studies we predicted that NT8 would cause more severe defects in neural tube closure since its expression pattern and our studies with C-CPE, suggest that Cldn8 is required in the neural ectoderm for the cell shape changes at the midline of the neural plate that are essential for neural fold elevation. In contrast, Cldn3 is expressed only in the non-neural ectoderm and appears to be required for the final steps in neural tube closure.

Chick embryos were cultured *ex ovo* using the Cornish pasty method with 200 µg/mL of NT3 or NT8 added to the culture media (Nagai et al., 2011). One-third (n=10/31) of NT3-treated embryos developed normally and had closed neural tubes. In contrast two-thirds (n=21/31) of NT3-treated embryos had neural tube defects. Approximately 40% of these embryos (n=9/21) had openings in the cranial region while the remaining 60% of the embryos (n=14/21) had mild defects that appeared to be due to a failure of neural fold fusion (Figure 5.6B). These defects were similar to those observed with C-CPE₃ (also known as C-CPE-LDR (Piontek et al., 2020a; Winkler et al., 2009), where the initial steps of neurulation proceed normally but the final step of neural tube closure and neural fold fusion fail (Figure 5.6A). Immunofluorescence confirmed that NT3 removed Cldn3 from the tight junction in the non-neural ectoderm as we previously observed with C-CPE and C-CPE₃. In contrast, levels of Cldn4 and -8 were somewhat elevated in NT3-treated embryos (Figure 5.7).

Embryos treated with NT8 exhibited a broader range of phenotypes. Completely open neural tubes were observed in 40% of the embryos (n=11/27). This phenotype was more severe

than the neural tube defects observed in embryos treated with NT3 but less severe than the ones observed in C-CPE-treated embryos. The remaining embryos had mild openings along the neural tube (n=5/27), small cranial openings (n=5/27) or closed neural tubes that were indistinguishable from control embryos (n=6/27) (Figure 5.6C). Immunofluorescent studies showed that Cldn3 was not affected by NT8 in non-neural ectoderm. In contrast, both Cldn4 and -8 were removed tight junctions in the neural ectoderm in NT8-treated embryos. The loss of Cldn4 in the neural ectoderm is not surprising as Cldn8 is required in the kidney to recruit Cldn4 to tight junctions (Hou et al., 2010) and we observed increased Cldn8 when we removed Cldn4 using C-CPE₄ (Baumholtz et al., 2017). An increased level of Cldn4 was observed in the non-neural ectoderm (Figure 5.7).

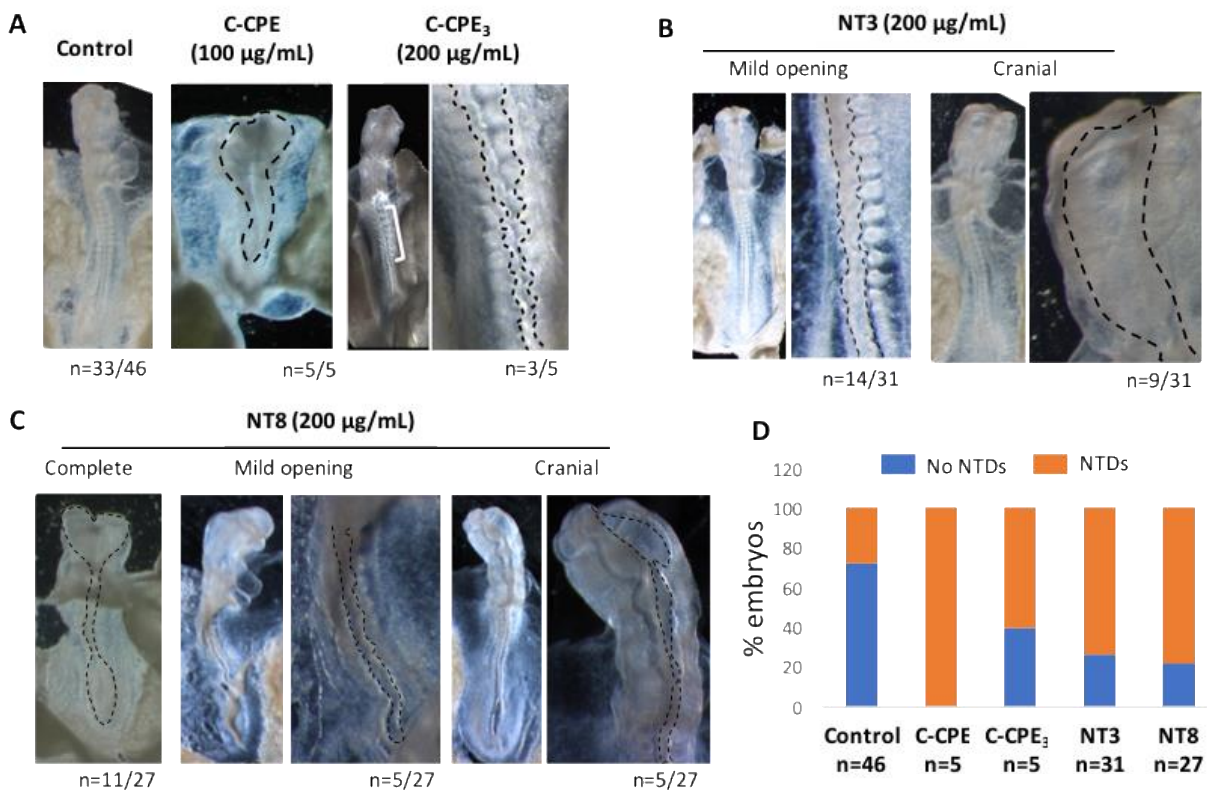


Figure 5.6: Embryos treated with NT3 or NT8 have neural tube defects. **A.** Representative images of control embryo treated with GST, an embryo treated with C-CPE, and an embryo treated with C-CPE₃. **B.** Representative images of phenotypes in embryos treated with 200 µg/mL of NT3. NTDs are outlined by the dotted line. **C.** Representative images of NTDs phenotype observed in chick embryos 24h after treatment with 200 µg/mL of NT8. **D.** Incidence of NTDs in the different treatment groups.

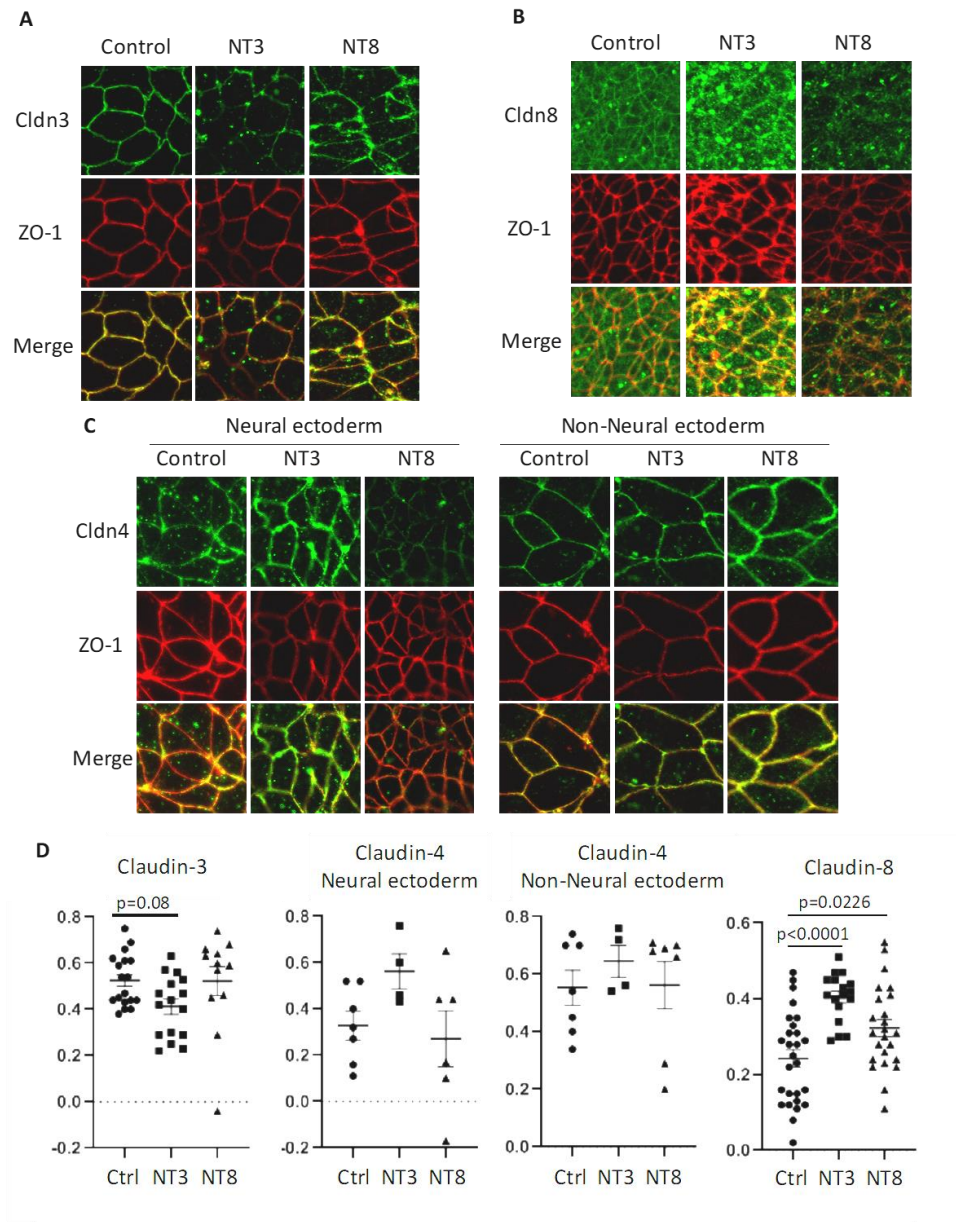


Figure 5.7: NT3 and NT8 affect tight junction localization of Cldn3 and Cldn8 in chick embryos.

A. Representative images of Cldn3 (green) and ZO-1 (red) immunofluorescence in the non-neural ectoderm of chick embryos after 5h of treatment with 200 $\mu\text{g}/\text{mL}$ of NT3 or NT8. **B.** Representative images of Cldn8 (green) and ZO-1 (red) immunofluorescence in the neural ectoderm of chick embryos after 5h of treatment with 200 $\mu\text{g}/\text{mL}$ of NT3 or NT8. **C.** Representative images of Cldn4 (green) and ZO-1 (red) immunofluorescence in the non-neural or neural ectoderm of chick embryos after 5h of treatment with 200 $\mu\text{g}/\text{mL}$ of NT3 or NT8. **D.** Pearson colocalization coefficients illustrating the amount of claudin that colocalize with ZO-1. Each dot corresponds to a different field measured, an average of 3 fields for each of 2-6 embryos per condition. ANOVA statistical test was performed and significance is considered if $P < 0.05$ while trend is considered if $P < 0.01$.

5.4 DISCUSSION

Defining how tight junctions behave and the role of specific claudins within this structure are essential to our understanding of the impact of tight junction and epithelial barrier changes in normal tissue and disease. We designed a novel modular peptide strategy to permit the targeting of individual claudins and specifically remove them from tight junctions. For these peptides, we replaced the claudin binding domain in C-CPE with the EL2 domain of a claudin to permit targeting removal of that claudin from the tight junction. Our proof-of-concept data for the NT3 and NT8 peptides reveal that these tools are effective and specific *in vitro* and *in vivo*.

Several groups have developed tools to target specific claudins. The most common strategy has been the use of peptides, which have been able to target Cldn1, -2 and -5 (Dithmer et al., 2017; Nasako et al., 2020; Sauer et al., 2014; Staat et al., 2015; Zwanziger et al., 2012). For these peptides the C-terminal part of EL1 of the specific claudin has been used successfully to increase the permeability of different barriers. Another approach has been the development of C-CPE variants that recognize fewer or individual claudins. This strategy has been successful in generating C-CPE variants that predominantly target Cldn1, -2, -3, -4 and/or -5 (Beier et al., 2019; Liao et al., 2016; Piontek et al., 2020a; Protze et al., 2015; Walther et al., 2012; Winkler et al., 2009) but it still remains to be proven that modifications on the sequence can expand the specificity of C-CPE to any claudin. Monoclonal antibodies have also been used to target specific claudins such as Cldn3 or -5 successfully (Fukasawa et al., 2015; Kato-Nakano et al., 2010; Okai et al., 2018; Tokunaga et al., 2007).

Our tool has major advantages when compared to the existing techniques: it is easy to generate and can be adapted to generate fusion peptides that can target any claudin family member. The only limitation that we have encountered is the solubility of the fusion peptide. As with previous studies we found that removing the terminal amino acids from C-CPE drastically reduced its solubility (Takahashi et al., 2008). Fortunately, we were able to solve this issue with the inclusion of the NT solubility tag that permits purification of sufficient quantities of soluble and biologically active fusion protein.

Our peptide tool was generated using cDNA sequences that encode the mouse EL2 domain due to its identity with other species of interest such as human (Cldn3: 96%; Cldn8: 85%),

chicken (Cldn3: 84%; Cldn8: 85%) and dog (Cldn3: 100%; Cldn8: 75%). However, we were able to demonstrate through our *in vitro* and *in vivo* proof-of-concept experiments that the peptide could remove mouse (mIMCD3 cells), dog (MDCK II cells) and chick claudins. In addition, we showed that the tool could be used to target removal of a specific individual claudin family member. Moreover, removal of claudins was observed within 3 hours of treatment. We predict that these findings will be extended for any claudin family member.

We used the chick embryo model to assess function and specificity, since we can compare the effect of our peptides to the effects of C-CPE, C-CPE₃ and C-CPE₄ on neural tube closure that we have previously described (Baumholtz et al., 2017). Chick embryos treated with concentrations of NT3 >200 µg/mL had phenotypes that were indistinguishable from those caused by C-CPE₃ treatment. Both C-CPE₃ and NT3 depleted Cldn3 from the tight junction in the non-neural ectoderm. Interestingly, both Cldn4 in the neural and non-neural ectoderm and -8 in the neural ectoderm were increased in embryos treated with NT3. The fact that Cldn4 is affected in the non-neural ectoderm, suggest that maybe the loss of Cldn3 could be partially compensated by increasing Cldn4 at the tight junction; however, its presence is unable to allow the proper embryo development. This was not surprising given our previous finding that removing Cldn4 from tight junctions does not affect neural tube closure. On the other hand the effect on neural ectoderm claudins we do not fully understand, since Cldn3 is not expressed in this tissue. NT8-treated embryos exhibited more severe neural tube defects than NT3-treated embryos but less severe than C-CPE-treated embryos. This could be a consequence of the claudins present since the localization of Cldn4 and -8 was altered in the NT8 treated embryos, but not the localization of Cldn3.

In mIMCD3 cells, NT8 was able to reduce Cldn8 at the tight junction, although it seemed to be more promiscuous than NT3 at early time points. The fact that NT3 had an effect on Cldn8 at 3h and that NT8 targeted Cldn3 at 3h could also mean that these claudins may act as a partner in this cell line and therefore be affected by our tools. On the other hand, the Cldn3 decrease and Cldn1 increase observed upon NT8 treatment could be an indirect effect of removing Cldn8 from the barrier and the change on localization of Cldn1 and -3 could be a compensation mechanism by the cell rather than a NT8 direct effect. At the same time, the increased Cldn8 expression

observed following NT3 treatment in chick embryos or the increase in Cldn1 following treatment of mIMCD3 cells might also reflect the cell's compensatory response. This kind of responses have been observed previously upon removal of specific claudins in different cell models (Baumholtz et al., 2017; Kage et al., 2014; Meoli and Gunzel, 2020).

In the past, we showed that the removal of Cldn3, -4 and -8 using C-CPE generates severe neural tube defects (NTDs)(Baumholtz et al., 2017). These NTDs, are characterized by an open neural tube where apical constriction, neural fold elevation and neural fold fusion are defective. On the other hand, removing only Cldn3 by using a C-CPE variant that only targets Cldn3 (C-CPE₃) causes a milder phenotype where the neural tube is open due to a failure in neural fold fusion, whereas apical constriction and neural fold elevation occur normally. Cldn3 is expressed early in the non-neural ectoderm and Cldn8 in the neural ectoderm. In contrast, Cldn4 is expressed in both the non-neural and the neural ectoderm. We previously showed that removing Cldn4 using a C-CPE variant that only targets Cldn4 (C-CPE₄) does not cause neural tube defects. Although Cldn4 is removed from both tissues when treated with C-CPE₄, the level of Cldn8 localization to the tight junction was increased, which suggests that the loss of Cldn4 can be compensated by increasing the amount of Cldn8 at the tight junction. Because of this data, we expected Cldn4 to be affected upon NT8 treatment. In fact, Cldn4 was affected in the chick embryo. In the neural ectoderm it was decreased while in the non-neural ectoderm it was increased upon NT8 treatment, which suggest that Cldn4 cannot compensate for the loss of Cldn8. This would make sense since in other tissues such kidney, Cldn8 has shown to be essential for the proper recruitment of Cldn4 to the tight junction (Hou et al., 2010). Another possibility could be that removing a claudin from the tight junction using C-CPE can remove by extension claudins that it interacts with at tight junctions. Similar situation could be happening here, where Cldn4 and -8 are partners in the neural ectoderm and removal of Cldn8 may remove Cldn4, whereas in the non-neural ectoderm where Cldn8 is absent, Cldn4 maybe try to compensate for the loss of claudin in the neural ectoderm. In contrast, when studying NT8 effect on mIMCD3 cells, Cldn4 was not changed when cells were treated with NT8, which could mean that in mIMCD3 Cldn4 and -8 are not interacting partners.

Comparison of the biological activity of NT3 and NT8 suggests that NT3 is more effective and for longer period of time at removing Cldn3 than NT8 was for removing Cldn8. This may be due to the levels of expression of the different claudins and the observation that NT8 appears to be more promiscuous at early time points.

Also important is the fact that our peptides showed a different activity than C-CPE itself. In previous studies treatment with C-CPE on chick embryos caused neural tube defects in 100% of the embryos and affected the localization of Cldn3, -4, -8. This wide effect in claudin localization is not seen in the chick embryos treated with NT3 or NT8 suggesting that with our approach we can modify the specificity of C-CPE and target individual claudins *in vivo*.

In summary, we have developed a new approach that allow the easy generation of tools that can target and remove specific claudins *in vitro* and *in vivo*, which can be of high interest to understand how barrier works and the role of specific claudins. It also facilitates the generation of new tools to target specific barrier and potentially help with drug or cell delivery to specific tissues.

5.5 MATERIAL AND METHODS

5.5.1 Chemicals and antibodies

Primary antibodies used for immunofluorescence were: Cldn1 (Invitrogen, 374900, Carlsbad, CA, USA), Cldn3 (Abcam, 15102, Cambridge, UK), Cldn4 (Invitrogen, 364800, Carlsbad, CA, USA), Cldn7 (Spring Bioscience, E10594, Pleasanton, CA, USA), Cldn8 (Invitrogen, 400700Z, Carlsbad, CA, USA) and ZO-1 (Invitrogen, 339100, Carlsbad, CA, USA). In addition, secondary goat anti-rabbit (Alexa Fluor 488, Invitrogen, Carlsbad, CA, USA) and goat anti mouse (Alexa Fluor 595, Invitrogen, Carlsbad, CA, USA), were used.

5.5.2 Plasmid generation

A pGEX6P1 plasmid encoding GST-tagged C-CPE was kindly provided by Dr. M. Furuse (Moriwaki et al., 2007). The cDNA clones encoding C-CPE::EL2 fusion proteins were generated by replacing the nucleotide sequences encoding the C-CPE claudin binding domain (amino acids 290-319) with nucleotide sequences encoding the EL2 domain of the targeted claudin. Briefly, an

internal XhoI site was generated on amino acid 290 of C-CPE by site-directed mutagenesis and the sequence encoding amino acids 290-319 were removed by a XhoI/BamHI restriction digest. The cDNA sequence encoding the claudin EL2 domain was PCR amplified with appropriate restriction sites in the 5' and 3' amplification primers. All cDNA sequences were confirmed by Sanger sequencing.

To create His-tagged proteins, the C-CPE::EL2 fusion gene was PCR amplified and cloned into pET-14b plasmid carrying a His-tag (obtained from Addgene). A similar strategy was used to incorporate create a series of C-terminal TRX::His tagged C-CPE::EL2 fusion genes or the series of N-terminal His::NT tagged fusions. The plasmid carrying TRX was obtained from Addgene (Massachusetts, USA) and the NT parent vector was generously provided by Dr. Jan Johansson. C-CPE. Finally, for positive control and comparison we used a variant of C-CPE that only target Cldn3, C-CPE₃. C-CPE₃ was given by Dr. Piontek

5.5.3 Protein purification

GST and His-tagged proteins were purified using an optimized protocol that overcame initial problems with obtaining sufficient quantities of soluble protein. cDNA expression vectors were transformed into E. Coli BL21 cells, plated and an individual colony was used to inoculate 5 mL of lysogeny broth (LB) media. 4 mL of the overnight culture was used to inoculate 400 mL of LB. Bacterial cultures were grown to an absorbance 600 of 0.3-0.6 OD at which point protein expression was induced by the addition of IPTG to a final concentration of 0.2mM. The culture was incubated overnight at 22°C with constant agitation (250rpm). Bacterial cell pellets were resuspended in 5 volumes of resuspension buffer (GST-tagged proteins: 50mM Tris pH7.5, 150mM NaCl, 0.1mM EDTA; His-tagged proteins: 50mM Tris pH7.5, 150mM NaCl, 10mM imidazole) with protease inhibitors (1.45nM Pepsatin A, 2.1nM Leupeptin, 0.15nM Aprotinin and 0.57mM PMSF) and incubated on ice for 20 minutes to lyse the cells. Sodium deoxycholate and NaCl were added to a final concentration of 0.2% and 0.5M, respectively. The lysate was sonicated (9 times for 30 seconds at 30° amplitude) and then centrifuged at 15,000rpm for 30 minutes. The resulting supernatants containing GST- or His-tagged fusion proteins were incubated at 4°C overnight with glutathione or Ni-NTA agarose beads, respectively. Agarose

beads were collected by low speed centrifugation and washed 4 times in 10-bed volumes with washing buffer (GST-tagged proteins: 50mM Tris pH7.5, 0.5M NaCl; His-tagged proteins: 50mM Tris pH7.5, 0.5M NaCl and 15mM imidazole). Washed beads were transferred to a column and washed further until eluant was free of protein as assessed by standard Bradford assay. Protein was eluted from the beads by adding 1 bed volume of elution buffer (GST-tagged proteins: 50mM Tris pH7.5, 150mM NaCl, 20mM reduced Glutathione; His-tagged proteins: 50mM Tris pH7.5, 150mM NaCl and 300mM imidazole). This was repeated until ~10 fractions were collected. A Bradford protein assay was used to identify the eluted fractions containing purified recombinant protein, which were pooled and were dialyzed overnight against phosphate-buffered saline (PBS). Recombinant protein was aliquoted and stored at -80°C in PBS

5.5.4 Cell culture

Madin–Darby Canine Kidney Type II cells (MDCK II) and mouse inner medullary collecting duct cells (mIMCD3) were obtained from ATCC. They were incubated at 37°C and 5% CO₂ in Dulbecco's modified Eagle's medium (DMEM) or Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) respectively, supplemented with 10% FBS, 51 IU penicillin, 50 µg/mL streptomycin and 16 µg/mL of gentamicin (Wisent BioProducts, Quebec, QC, Canada).

MDCK II and mIMCD3 cells were seeded on coverslips in 12-well plates at a cell density of 0.1×10^6 cells/well and cultured for 48h at which point cells were confluent and tight junctions were well-established. Cells were treated with culture media (Control), NT3 (100 µg/mL) or NT8 (100 µg/mL) and collected at 3 or 24h for analysis.

5.5.5 Immunofluorescence staining

Cell layers were rinsed with PBS and fixed in 10% trichloroacetic acid for 15 min at 4°C. Cells were washed with PBS and blocked with 10% normal goat serum in PBS with 0.3% Triton-100 (PBST) and then incubated overnight at 4°C with primary antibodies for Cldn1, -3, -4, or -7 (1:100) and ZO-1 (1:150), or for 48h at 4°C with antibodies for Cldn8 (1:100) and ZO-1 in PBST with 5% normal goat serum (Cldn3, and -8 (1:100) and ZO-1 (1:150). Alexa Fluor-conjugated secondary antibodies (1:500) in PBST were added for 1h at RT. Coverslips were washed with PBS

and then placed on slides with FluorSave reagent (EMD Millipore, 34789, Massachusetts, USA). Z-stacks were imaged using a Zeiss LSM780 laser scanning confocal microscope. All experiments were performed in triplicate and 2-3 different fields were imaged on each coverslip.

5.5.6 Chick embryo culture and immunostaining

Fertilized eggs (Couvoir Simetin, Mirabel, Canada) were incubated at 38.5°C. Embryos were collected at stage HH6, according to Hamilton and Hamburger (HH) criteria (Hamburger and Hamilton, 1951) and cultured using the modified Cornish pasty method (Nagai et al., 2011). Purified GST (100 µg/mL), GST-C-CPE (100 µg/mL), GST-C-CPE₃ (200 µg/mL), NT::C-CPE::Cldn3EL2 (NT3; 200 µg/mL) and NT::C-CPE::Cldn8EL2 (NT8; 200 µg/mL) were added directly to the Cornish pasty culture medium. Embryos were collected at 5h and 24h and subjected to whole mount immunofluorescence and morphological analysis, respectively, as previously described (Baumholtz et al., 2017). In brief, treated embryos were fixed in 10% TCA for 30 minutes at 4°C. Embryos were washed with PBS and blocked with 10% normal goat serum in PBS with 0.3% Triton-100 (PBST) and then incubated overnight at 4°C with primary antibodies for Cldn3 or -4 (1:100) and ZO-1 (1:150), or for 48h at 4°C with antibodies for Cldn8 (1:100) and ZO-1 in PBST with 5% normal goat serum (Cldn3, and -8 (1:100) and ZO-1 (1:150). Alexa Fluor-conjugated secondary antibodies (1:500) in PBST were added for 1h at RT. Coverslips were washed with PBS and then placed on slides with FluorSave reagent (EMD Millipore, 34789, Massachusetts, USA). Z-stacks were imaged using a Zeiss LSM780 laser scanning confocal microscope. Pictures were taken from both non-neural and neural ectoderm.

5.5.7 Image analysis

Z-stacks were taken for both cells and chick embryos. To account for the plane variation in cells, maximum intensity projections were obtained for each stack of images. The single stack from chick embryos and the maximum intensity projection from cells immunofluorescence were then analyzed using the ZEN software to determine the amount of claudin localized in the membrane. The amount of claudin colocalizing with ZO-1 at the membrane was measured and plotted as Pearson coefficients, which takes into account both localization and intensity, to measure the amount of claudin localized at the membrane.

5.5.8 Statistics

Data are graphed as mean $\pm$ SEM. Statistical analyses were performed using Graph-Pad PRISM (version 8.02, GraphPad Software, Inc., California, USA). t-test and one-way ANOVA analysis were used to analyze the effects of treatment. Tukey's multiple comparison test was used to compare the effect of the different treatments. Differences were considered significant when $p < 0.05$.

5.6 AUTHOR CONTRIBUTIONS

Conceptualization, Enrique Gamero-Estevez, Amanda Baumholtz, Indra Gupta, Makoto Nagano and Aimee K. Ryan; Formal analysis, Enrique Gamero-Estevez, Amanda Baumholtz, Elisabeth Legere; Funding acquisition, Aimee K. Ryan and Makoto Nagano; Investigation, Enrique Gamero-Estevez, Amanda Baumholtz, Elisabeth Legere, Makoto Nagano and Aimee K. Ryan; Methodology, Enrique Gamero-Estevez, Makoto Nagano and Aimee K. Ryan; Writing – original draft, Enrique Gamero-Estevez; Writing – review & editing, Makoto Nagano and Aimee K. Ryan.

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5.9 CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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CHAPTER 6: DISCUSSION

6.1 OVERVIEW AND MAJOR FINDINGS

Tight junctions and claudins have often been studied to understand paracellular transport and barriers in epithelial and endothelial cell layers. However, gain- and loss-of-function studies have demonstrated additional roles for claudins, including the maintenance of tissue shape (Behr et al., 2003; Nelson et al., 2010; Wu et al., 2004), targets for drug delivery (Hashimoto et al., 2019), involvement in developmental processes (Baumholtz et al., 2017a; El Andalousi et al., 2020), tension between cells during morphogenetic movements (Siddiqui et al., 2010), and hydrostatic pressure during lumen expansion (Bagnat et al., 2007; Moriwaki et al., 2007; Zhang et al., 2010). Moreover, several claudin variants are associated with human diseases, including deafness, kidney stones, cancer, infertility, and brain defects (Baumholtz et al., 2020; Gunzel and Yu, 2013; Yu, 2015). In order to more fully understand the specific role of each member of the claudin family, I began by identifying the cytoplasmic interaction partners of a subset of claudin family members, then investigated the effects and mechanisms of two reagents that alter the claudin composition of tight junctions, and finally I generated novel tools that can be used to remove individual claudin family members from tight junctions. These tools allowed us to also study what happens to the barrier when a claudin is removed or exchanged, and the downstream implications of removing a specific claudin on other cellular processes.

In Chapter 2, I describe how claudins may influence tight junction composition by analyzing the interaction partners of individual claudins. There are a few studies that suggest that each claudin has a different affinity for the common cytoplasmic plaque tight junction molecules such as ZO-1 (Nomme et al., 2015). In this study I showed that each of the claudins preferentially interacted with a different profile of proteins. For instance, Cldn14 mainly interacted with cytoskeletal and extracellular matrix proteins while Cldn3 and Cldn8 preferentially pulled down ZO-1 and other cytoplasmic plaque proteins such as ZO-2, MPDZ or MLLT4, which were not pulled down by Cldn4 or Cldn14. In addition, I studied the effect of mutating specific residues within the Cldn8 cytoplasmic C-terminal tail on the Cldn8's protein interactions. I used a series of Cldn8 variants where amino acids predicted to be phosphorylated were replaced by alanine or isoleucine (S198A, S216A and S216I) as well as a variant where the two terminal amino acids known to interact with PDZ domains in cytoplasmic plaque proteins were deleted (Δ YV).

Interestingly, these variants had different effects on neural tube closure when electroporated into the epiblast of chick embryos. Cldn8S216I generated the most severe phenotype: chick embryos exhibited both convergent extension defects and a failure of neural tube closure. In contrast, Cldn8S198A affected neural tube closure but not convergent extension. The other variants did not affect either neural tube closure or convergent extension. To understand if the differences in phenotypes were due to effects on the variant Cldn8 C-terminus's ability to interact with cytoplasmic interaction partners, I compared the profile of proteins that interacted with the variants to those that interacted with wild-type Cldn8. While most variants bound to the same cytoplasmic proteins, Cldn8S216I appeared to have a lower affinity for ubiquitously expressed tight junction proteins, such as ZO-1, ZO-2 or MUPP-1. This may explain why this variant gave rise to the most severe phenotypes. In comparison, Cldn8 Δ YV did not interact with common tight junction proteins and it did not generate any phenotype. The absence of phenotype is likely due to its inability to compete with the wild-type Cldn8 present in the neural ectoderm. Therefore, while Cldn8 Δ YV is unable to bind cytoplasmic proteins, Cldn8S216I may interfere and bind inappropriately to these proteins leading to a more severe phenotype.

In Chapter 3, I describe my data using the nutraceutical quercetin that had previously been shown to modify the abundance of claudins present at the tight junction leading to a tighter barrier in anion-selective cell models. However, no information on cation-selective cell lines was available. Therefore, I asked if quercetin also had a tightening effect in the cation-selective cell line MDCK II. Interestingly, my research showed that the claudins affected by quercetin were different depending on the cell line studied. I showed that both expression and localization of claudins change over time and that these effects were claudin specific. These changes in claudin composition at tight junctions could be related to oscillations in barrier properties that made the barrier tighter or leakier overtime. Ultimately the changes in claudin expression caused by quercetin led to the establishment of a stable and tighter barrier than the control cells after 48h of culture.

In Chapter 4, I examined the mechanism that C-CPE uses to remove claudins from the tight junction. It was known that claudins are internalized upon treatment with CPE, however, the levels of claudins in the cytoplasm nor their levels of expression changed when cells were

treated with C-CPE. In collaboration with a postdoctoral fellow we discovered that C-CPE treatment promotes claudin incorporation into extracellular vesicles. Moreover, I showed that inhibiting extracellular vesicle formation hindered the ability of C-CPE to generate claudin-rich extracellular vesicles. Interestingly, the incorporation of each specific claudin into the extracellular vesicles following C-CPE treatment was differentially affected by the different inhibitors. This led me to hypothesize that each claudin is independently processed by different pathways and potentially incorporated into specific extracellular vesicles. I also studied the other cargo in the extracellular vesicles derived from C-CPE-treated cells. Preliminary analysis of these cargo proteins revealed that following C-CPE treatment there was an upregulation in components of the NSF-Dynamin2 secretion pathway, which is an unconventional exocytosis pathway often used during neurotransmitter recycling. These data, combined with the observation that other extracellular vesicle generation pathways were downregulated following C-CPE treatment, led me to hypothesize that the NSF-Dynamin2 pathway is involved in the biogenesis of claudin-rich extracellular vesicles. Further studies are needed to explore this interesting finding.

Finally in Chapter 5, I describe my approach to address the main limitations of C-CPE through the generation of polypeptide tools that target and remove specific claudins. To achieve this goal, I generated polypeptides by fusing the C-CPE stabilization domain to the second extracellular loop of the claudin of interest. The tools successfully removed the target claudin from the tight junction and the peptides that targeted removal of Cldn3 and Cldn8 were validated by *in vivo* proof-of-principle. These experiments supported our previous findings that Cldn8 is essential for neural plate elevation and closure, while Cldn3 is important for the fusion of the non-neural ectoderm at the midline, the last step of neural tube closure.

In summary, my work increased our knowledge about claudins. It is clear that each claudin is different from other claudin family members and has a unique interactome. I also studied and generated different tools that can be used to study the role of specific claudins, and open new possibilities to study the individual contributions of claudins at the tight junction and their impact on barrier properties.

6.2 GENERAL DISCUSSION

6.2.1 Removing and modifying claudins present at the tight junction alters barrier properties

As shown by others, tight junction barrier properties are directly linked to the claudins present at the tight junction. In Chapter 3, I showed that modifying the pattern and abundance of specific claudins at the tight junction correlated with changes in barrier properties, generating “leakier or tighter” barriers depending on the claudins present at the membrane. Moreover, removal of specific members from the tight junction can lead to the tissue impairment and defects on developmental processes as shown in Chapter 5. It is true that depending on the claudins present at the tight junctions, their extracellular interactions may affect paracellular permeability. However, barrier properties are not only dependent on this. In fact, in Chapter 2, I showed that each claudin is able to bind to specific proteins, and therefore, altering the levels of claudins in the tight junction could change the pattern and interaction of cytoplasmic plaque proteins, and consequently affect barrier properties and cell functions. For instance, Cldn3 and Cldn8 preferentially interacted with cytoplasmic proteins, including ZO-1, ZO-2, MPDZ or MLLT4. Therefore, if Cldn3 or Cldn8 are removed from a barrier it is logical to hypothesize that the cytoplasmic proteins that they interact with may not remain localized at the tight junction. This could affect cell behavior and function. It may also limit the ability of other claudins, whose affinity for these interaction partners is lower, to compensate for the loss of Cldn3 or Cldn8. This could in fact be the reason why when Cldn8S216I is electroporated in the chick embryo, it gave rise to severe phenotypes. Moreover, the removal of a specific claudin from a barrier may promote the interaction of cytoplasmic proteins with other proteins present at the tight junction, which might be sufficient to affect the tight junction barrier. Therefore, the effect on the tight junction may be affected by these specific cytoplasmic interactions, in addition to the effect of claudins present at the tight junction and their extracellular interactions.

6.2.2 Why do we need new reagents to target individual claudin removal?

Several groups have tried to obtain peptides, C-CPE variants or antibodies that are capable of removing individual claudins. However, each of the tools that have been reported have some disadvantages. For instance, although peptides that are usually 10-15 amino acids work *in vitro*,

their potential non-specific binding may limit their use *in vivo*. Their effects are also very transient. C-CPE variants have the advantage of being more stable and present an interesting approach could work well. However, mutagenesis of amino acids in the claudin binding domain has only led to a small number of C-CPE variants that target specific claudins, Cldn1, -3, -4 and -5. Finally, antibodies are robust tools that have been shown to inhibit the interaction of several tight junction proteins, including claudins, occludin and JAMs. However, their size presents a problem for accessibility to certain barriers, as well they require extensive labor to identify, select and manufacture each individual antibody. Moreover, antibodies do not remove the claudin but instead block the interaction of the claudin with the claudin in the apposing cell, and/or other proteins based on the epitope that is being recognized.

For this reason, in Chapter 5 I developed a new strategy, whereby the claudin binding domain in C-CPE was replaced with the second extracellular loop of the targeted claudin. The second extracellular loop is known to be important for homodimerization of claudins and so this domain was predicted to target the remainder of the C-CPE protein to a specific claudin. Indeed, this tool appeared to solve the problems of existing tools. They are small enough to access different barriers, but very specific for the claudin of interest. Moreover, my strategy allows for substitution of any claudin EL2, which make this tool adaptable to potentially target any of the 28 claudin family members.

Although this tool shows to be promising to target specific proteins; it is still to be shown that this strategy work for other claudins. In our proof of concept experiments, we observed that NT8 was able to affect not only Cldn8 but also other claudins, while the effect of NT3 was more restricted to Cldn3. It still needs to be investigated if this is as a consequence of the specificity of our tool, or if it is due to the fact that our peptide could also bind to the natural partners of the claudin of interest.

Moreover, the duration of the effect is longer for NT3 than NT8. This fact could be due to different affinity between our tools and the claudin of interest; to the importance of the claudin in the tight junction; or to the relative amount that may reduce the bioavailability of our tool.

6.2.3 Claudins are enriched in extracellular vesicles that are secreted upon C-CPE treatment

Different groups have studied the mechanism of CPE and shown that upon treatment claudins get internalized. However, no one had asked the question “what happens next?”. In Chapter 4, I showed that claudins get packaged into extracellular vesicles that are then secreted. There is an increasing interest in the study of claudins in extracellular vesicles, including their use as biomarkers for ovarian cancer, and other tumors (Ikeda et al., 2021; Li et al., 2009; Thuma et al., 2016). The fact that C-CPE promotes the incorporation of claudins into extracellular vesicles, which may be different for individual claudins, opens the possibility to use this tool or the peptides designed in Chapter 5 to generate and obtain claudin-specific extracellular vesicles. These vesicles would enable the production and functional analysis of the role of specific claudins in an extracellular vesicle. For instance, in the cancer field it is currently believed that claudin-rich extracellular vesicles serve to guide the vesicles to the tissue of interest (Kyuno et al., 2019b). However, it is impossible to obtain sufficient quantities of these vesicles to study the role of claudins. Therefore, the peptides described in Chapter 5 may allow us to generate claudin-specific vesicles that allow the interrogation of their role and what tissue they target.

In the case of C-CPE the incorporation of claudins into extracellular vesicles is intriguing since we do not understand why treatment with C-CPE leads to the generation of claudin-rich extracellular vesicles. Other groups showed that CPE is able to remove the claudins by internalization. This occurs without increased cytoplasmic accumulation or changes in claudin expression within the first 24h of treatment. At first we thought that the claudins that were targeted for removal from tight junctions may be degraded or shuttled into multivesicular bodies and secreted as exosomes. Unfortunately, I could not prove this by measuring the size of the extracellular vesicles secreted or by using specific exosomal inhibitors. The logical explanation would be that since inhibitors of vesicle formation hinder C-CPE activity that each claudin may follow a different mechanism to be incorporated into specific extracellular vesicles.

In addition, we believe that the incorporation of claudins into extracellular vesicles may affect the functions of the vesicles. For instance, it could be that these vesicles carry information to surrounding cells and alert them of the barrier integrity dysfunction caused by C-CPE treatment. Similarly, it could be a mechanism to alert and inform the immune system of the

presence of C-CPE, this could make sense because C-CPE is a bacterially-derived protein and this mechanism has already been described for enteric bacteria (Ayyar and Moss, 2021). In contrast, it could also be that the massive removal of claudin from the membrane just triggers the exocytosis of the excess of protein without a clearly defined biological function. These topics and vesicle functions will be definitely interesting to explore in the future.

6.2.4 Role of claudins during neural tube fusion

In the developing neural tube the expression pattern of specific claudins is totally different; for instance, Cldn4 appears in both neural and non-neural ectoderm, Cldn3 is only present in the non-neural ectoderm, and Cldn8 is enriched in the neural ectoderm. In our lab, we previously showed that Cldn3, -4 and -8 are essential for neural tube formation (Baumholtz et al., 2017b). While we were able to discern the roles of Cldn3 and -4 by using existing C-CPE specific variants; we were only able to discern the role of Cldn8 by default due to the lack of a Cldn8-specific C-CPE variant. I generated reagents that were able to target individual claudins and I was able to confirm the previous unpublished data that Cldn3 is required in the non-neural ectoderm for neural fold fusion, the final step of neural tube closure. While NT8 allowed me to confirm that the phenotype seen by C-CPE in the neural ectoderm was mainly due to effects on claudin-8. Moreover, these data combined with the data that Cldn8 variants generated neural tube opening and convergent extension defects, suggest that Cldn8 is essential to maintain tight junction function and the cytoplasmic plaque proteins properly localized. In fact, previous work from our lab showed that removal of claudins from the tight junction during neural tube development led to mislocalization of polarity proteins such as PAR-3, Rho-A or Cdc42. Therefore, the phenotype seen in the chick embryos when claudins are removed or variants are overexpressed may be caused by important cytoplasmic proteins being mislocalized. I showed that Cldn8 interacts with ZO-1, ZO-2, MPDZ and MLLT4. All of these proteins are essential for linking the tight junction to the cytoskeleton and often act as a docking platform for other cytoplasmic proteins involved in morphogenetic processes or cell polarity, as observed for PAR-3, Rho-A or Cdc42.

6.2.5 The individual claudin family members should be studied independently

By exploring the mechanism used by C-CPE to remove claudins, I observed that claudins are incorporated into vesicles and released to the media. Moreover, inhibition studies showed that the presence of claudin in secreted extracellular vesicles can be hindered by different inhibitors but that these effects are claudin-dependent, suggesting that each claudin may be removed from the tight junction differently or incorporated in specific and separate vesicles. These data in combination with my discovery that each claudin preferentially interacts with different cytoplasmic plaque proteins, suggest that each claudin could behave independently.

Most groups consider the claudin family as one unit, and try to extrapolate their data between claudins or different barriers. However, the data that I generated support that each claudin is different. Even though they belong to the same family, their interactions and recycling mechanisms may be distinct and they should be considered independently. To reinforce this idea, I showed that quercetin had the same effect on the barrier properties of MDCK II cells as previously reported by other groups for different cell lines. However, in each cell line the effect seem to occur by the changes of expression of different claudins with the ultimate effect of tightening the barrier suggesting that either the process in place are different in each cell line or that the complexes formed at the tight junction are different and cell line specific. In summary, the data collected in my thesis suggest that tight junctions and the claudin roles are complex and need to be interrogated one-by-one.

6.3 FUTURE DIRECTIONS

Each claudin is different with respect to their roles in dictating barrier properties, their specific interactors, and their regulation, trafficking and recycling mechanism. In my studies, examined the role of individual claudins in the barrier and determined how their interactomes differ. These findings still need to be validated *in vivo*. In addition, I generated peptides that are able to target individual claudins and provide proof-of-concept data. These experiments need to be extended to show that the peptide constructs will work with other claudins. I have been collaborating with Dr. Makoto Nagano to target specific claudins in the blood testes barrier and transiently open it for the delivery of cells and drugs. In addition, validation of the mechanisms

used by each claudin to get incorporated into extracellular vesicles upon C-CPE treatment or validation of interactions of claudins with the cytoplasmic proteins in different models have not been studied yet. For these outstanding questions, I propose the following future experiments.

6.3.1 Use of NT5 and NT11 to target blood brain and blood testes barriers

In my proof-of-concept experiments with the claudin-specific peptides, I focused on reagents that target claudins that are targeted by C-CPE. For subsequent studies it will be of interest to generate protein tools that target non-C-CPE sensitive claudins. For instance, targeting the blood brain barrier by targeting Cldn5 or the blood testes barrier by targeting Cldn11 seem very interesting options to prove that our strategy can be used against any claudin of interest, including non-C-CPE sensitive claudins. In fact, in collaboration with Dr. Nagano's lab we are exploring the possibility of transiently opening the blood testes barrier to deliver cells or drugs by targeting and removing Cldn11.

6.3.2 Validate mechanism that leads to incorporation of claudins into extracellular vesicles

From our mass spectrometry data, the only exocytic pathway that was upregulated in extracellular vesicles C-CPE treated cells was the NSF-Dynamin2 pathway. However, we have not proven that this pathway is responsible for the incorporation of claudins into the extracellular vesicles. For future experiments, it will be of interest to study if all or some of the claudins are being processed via this mechanism or if instead they use any other mechanism. If indeed claudins are processed by NSF-Dynamin 2 pathways, we will need to discern between exosomal or microvesicles. One possibility is to follow what occurs to the claudin of interest upon treatment in real time. In order to study this, we have generated claudins fused to GFP in their amino terminal domain, which are able to localize to the membrane and it could help us follow in real time what occurs to the claudin once they interact with C-CPE.

6.3.3 Characterization of claudin-rich extracellular vesicles generated by C-CPE and study the function of claudins in these extracellular vesicles

Although my data suggests that claudins are packaged into extracellular vesicles following C-CPE treatment, we need to prove that these are actually extracellular vesicles and characterize them according to the MISEV2018 guidelines (Thery et al., 2018). Firstly, electron-microscopy data is necessary to reveal the ultrastructure extracellular vesicles. This may also inform us of a probable biogenesis pathway. Second, identification of specific kinds of extracellular vesicles using protein markers should be explored in depth to determine which kind of extracellular vesicles we are working with or if C-CPE treatment generates a new kind of extracellular vesicle that has not been reported previously.

In the context of cancer, where claudins in extracellular vesicles are used as biomarkers and have been hypothesized to be involved in the metastatic process, understanding the role of each claudin in these vesicles is key (Kyuno et al., 2019a; Kyuno et al., 2019b; Tabaries et al., 2015). Thus, the first thing to consider is if the extracellular vesicles secreted by C-CPE-treated cells have a specific function. Experiments are currently ongoing using the presumed extracellular vesicles purified from C-CPE-treated MDCKII cells and adding them to untreated MDCK II cells. Analysis of effects on TER and barrier properties will be prioritized since these data will inform us if the vesicles have an effect in modulating the overall barrier properties and/or can cross the tight junction barrier. An important caveat to consider lays in the difficulty to separate the effects coming from the claudin vesicle versus the remaining C-CPE in the media or in the vesicles themselves.

In addition to their role in cancer and the fact that each claudin is different, the study of the entire population as a whole will be further refined. Therefore, we should sort out claudin specific extracellular vesicles or differentiate between different size vesicles using density gradients in order to fully understand the differences between populations of extracellular vesicles. In addition, pull-downs using antibodies or the NT peptides could help identify specific extracellular vesicle populations and understand if claudins are incorporated in the same or in different vesicles. Moreover, density gradients are underway to identify the size of vesicles containing claudins. Preliminary data suggested that C-CPE treatment changes the size of vesicles

where claudins are incorporated. However, the shift in size of claudin-containing vesicle was not the same for all claudins. If validated this would support our hypothesis that each claudin is being incorporated into different kind of vesicles.

Furthermore, there is the possibility that using the NT reagents we could remove a single claudin and therefore generate extracellular vesicles carrying only this claudin. Generating extracellular vesicles using the C-CPE::EL2 peptides or isolation of extracellular vesicles carrying an individual claudin can facilitate examination of the function of each claudin within these vesicles. For instance, treatment of cancer cell lines with the specific polypeptides, could lead to the generation of extracellular vesicles rich in the claudin of interest. Finally, injecting labeled bulk extracellular vesicles or extracellular vesicles carrying single claudins could be tracked and analyzed *in vivo* to understand where do they go and what do they do, which may help both to improve their biomarker detection and to understand their function.

6.3.5 Validate the preferential interaction seen in our pull-down analysis

My study to identify proteins that interact with the claudin C-terminal cytoplasmic tail, showed that each claudin specifically interacts with cytoplasmic plaque proteins. I validated this observation for ZO-1. However, the interactions with other proteins remain to be validated, including tight junction proteins such as ZO-2 or MUPP-1. Finally, Cldn8 variants gave rise to specific phenotypes and in the case of Cldn8S216I, a reduction in interaction was seen between this claudin variant and known cytoplasmic plaque proteins. The next steps will be to determine if the phenotypes are a consequence of the changes in interaction or if there may be an alternate explanation. Therefore, imaging the localization of interactors *in vivo* upon electroporation of Cldn8 variants will clarify if the phenotypes are correlated to tight junction abundance of the specific cytoplasmic proteins.

6.4 CONCLUDING SUMMARY

My thesis research focused on the development and assessment of different tools to study individual claudins and the impact of removing or modifying specific claudin members on tight junctions and epithelial barriers. My work pivoted around three different tools to modify

claudin localization: Quercetin, C-CPE and NT polypeptides. My data showed that each claudin is different from other claudin family members, including their roles at the tight junction, their ability to interact with cytoplasmic plaque proteins, and the manner in which they are removed from tight junctions in response to C-CPE treatment. Moreover, I was able to generate a very useful tool following a totally novel approach, that will allow to target specifically any claudin of interest and temporally remove it from the tight junction.

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APPENDIX 1

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#	Visible?	Starred?	Bio View: 2120 Proteins in 1487 Clusters With 685 Hidden	Accession Number	Molecular Weight	Protein Grouping Ambiguity	Probability Legend:																											
							over 95% 80% to 94% 50% to 79% 20% to 49% 0% to 19%																											
														Cld3_B			Cldn1			Cldn4			Cldn8			Cldn14			Control			Control3		
							CLD3_5	Cld3_6	Cld3_4	L_2	L_3	L_1	Cld4_4	4_2	4_3	8_2	8_3	8_1	14_2	14_3	14_1	GST_control_2	GST_control_3	GST_control_1	GST_Control_5	GST_control_6	GST_control_4							
743			Uncharacterized protein OS=Gallus gallus GN=IPO8 PE=4 SV=2	A0A1D5P...	120 k...	★																												
744			Transcription factor BTF3 OS=Gallus gallus GN=BTF3 PE=3 SV=1	A0A11LR...	18 kDa																													
745			Uncharacterized protein OS=Gallus gallus GN=CNOT2 PE=4 SV=1	A0A1D5N...	59 kDa																													
746			Single-stranded DNA-binding protein 3 OS=Gallus gallus GN=SSBP3 PE=4 SV...	A0A1D5P...	37 kDa		2																											
747			Uncharacterized protein OS=Gallus gallus GN=ILF2 PE=4 SV=1	A0A11LR...	23 kDa		1																											
748			High mobility group protein 81 OS=Gallus gallus GN=HMG81 PE=4 SV=1	A0A11LR...	12 kDa		1																											
749			Uncharacterized protein OS=Gallus gallus GN=RCOR1 PE=4 SV=3	E1BWG6...	51 kDa		1																											
750			Coronin OS=Gallus gallus GN=ORO2A PE=3 SV=2	E1BWN6...	61 kDa																													
751			Uncharacterized protein OS=Gallus gallus GN=NOL10 PE=4 SV=1	E1BXP3_C...	80 kDa		2																											
752			Uncharacterized protein OS=Gallus gallus GN=HMBOX1 PE=4 SV=3	E1BZ53_C...	44 kDa		2																											
753			Uncharacterized protein OS=Gallus gallus PE=4 SV=3	E1C8T8_C...	69 kDa		2																											
754			Nucleolar complex protein 4 homolog OS=Gallus gallus OX=9031 GN=NOC4L...	Q5ZJC7	58 kDa		2																											
755			Ras-related protein Rab-2A OS=Gallus gallus OX=9031 GN=RAB2A PE=2 SV=1	Q90965	24 kDa		1																											
756			Ras-related protein Rab-5C OS=Gallus gallus GN=RAB5C PE=1 SV=1	RAB5C_C...	24 kDa																													
757			Citrate synthase, mitochondrial OS=Gallus gallus OX=9031 GN=CS PE=1 SV=1	P23007	47 kDa																													
758			Eukaryotic initiation factor 4A-III OS=Gallus gallus GN=EIF4A3 PE=3 SV=1	A0A1D5N...	47 kDa	★	4				3																							
759			Uncharacterized protein OS=Gallus gallus GN=KDM1A PE=4 SV=1	A0A1D5P...	91 kDa	★	1																											
760			Serum albumin OS=Gallus gallus GN=ALB PE=3 SV=1	A0A1D5N...	70 kDa																													
761			Uncharacterized protein OS=Gallus gallus GN=WDR75 PE=4 SV=1	A0A11LR...	42 kDa		0																											
762			Uncharacterized protein OS=Gallus gallus GN=ZNF618 PE=4 SV=1	A0A1D5N...	103 k...		1																											
763			Erythroid protein 4.1 OS=Gallus gallus GN=EPB41 PE=4 SV=1	R4GHW9...	97 kDa																													
764			Uncharacterized protein OS=Gallus gallus GN=ATXN2 PE=4 SV=1	A0A1D5P...	120 k...																													
765			Uncharacterized protein OS=Gallus gallus GN=MYEF2 PE=4 SV=2	A0A1D5P...	66 kDa																													
766			Casein kinase II subunit alpha OS=Gallus gallus GN=CSNK2A1 PE=2 SV=1	CSK21_C...	45 kDa																													
767			Uncharacterized protein OS=Gallus gallus PE=4 SV=1	A0A1D5P...	66 kDa		1																											
768			Uncharacterized protein OS=Gallus gallus GN=UBE2L3 PE=3 SV=1	A0A1D5N...	18 kDa	★	2																											
769			Acetyltransferase component of pyruvate dehydrogenase complex OS=Gallus ...	E1CG65_C...	67 kDa	★	4																											
770			Isocitrate dehydrogenase (NAD) subunit, mitochondrial OS=Gallus gallus GN=...	A0A1D5P...	48 kDa																													
771			Uncharacterized protein OS=Gallus gallus GN=HNRNPD PE=4 SV=1	F6QGM9...	35 kDa		1																											
772			Cluster of Uncharacterized protein OS=Gallus gallus GN=IMP4 PE=4 SV=1 (R4...	R4GJAS [3]	33 kDa	★	2	4																										
773			Cluster of Uncharacterized protein OS=Gallus gallus PE=4 SV=1 (A0A1D5P1M...	A0A1D5P...	21 kDa	★	0			1	1																							
774			Cluster of Uncharacterized protein OS=Gallus gallus GN=POLDIP2 PE=4 SV=1 ...	tr A0A1D...	43 kDa	★	5	4	5																									
775			Cluster of Uncharacterized protein OS=Gallus gallus GN=SLRP PE=4 SV=1 (A...	A0A1D5P...	12 kDa	★			1	0																								
776			Cluster of Uncharacterized protein OS=Gallus gallus GN=NOP14 PE=4 SV=3 (E...	E1C936 [2]	97 kDa	★	2	1																										
777			Cluster of Uncharacterized protein OS=Gallus gallus GN=ERP44 PE=4 SV=3 (tr...	tr E1BSL7...	47 kDa	★			1																									
778			Cluster of Collapsin response mediator protein-18 OS=Gallus gallus GN=CR...	Q715G3_C...	62 kDa	★			2																									
779			Cluster of Uncharacterized protein OS=Gallus gallus GN=MDK PE=4 SV=1 (A0...	A0A11LR...	13 kDa	★	2																											
780			Cluster of Uncharacterized protein OS=Gallus gallus GN=SRSF10 PE=4 SV=1 (...)	A0A1D5P...	21 kDa	★	2																											
781			Cluster of Poly (ADP-ribose) polymerase OS=Gallus gallus GN=PARP3 PE=4 S...	E1BS10_C...	59 kDa	★	0	1																										
782			Cluster of Uncharacterized protein OS=Gallus gallus GN=SPTBN5 PE=4 SV=4 (...)	F1NV58_C...	452 k...	★																												
783			Cluster of Uncharacterized protein OS=Gallus gallus GN=ORC1 PE=2 SV=1 (Q...	Q5ZMC5_C...	97 kDa	★																												
784			Cluster of Uncharacterized protein OS=Gallus gallus GN=HSPA4 PE=3 SV=1 (A...	A0A1D5P...	94 kDa	★	2																											
785			Glutathione peroxidase OS=Gallus gallus GN=GPX7 PE=3 SV=1	A0A1D5N...	16 kDa																													
786			Uncharacterized protein OS=Gallus gallus GN=ABC86 PE=4 SV=1	A0A1D5N...	102 k...																													
787			Uncharacterized protein OS=Gallus gallus GN=MAU2 PE=4 SV=1	A0A1D5P...	80 kDa		2																											
788			Uncharacterized protein OS=Gallus gallus GN=UHRF1 PE=4 SV=1	A0A1D5P...	98 kDa		2																											
789			Uncharacterized protein OS=Gallus gallus GN=ATP6V1B2 PE=3 SV=1	A0A1D5P...	56 kDa																													
790			Uncharacterized protein OS=Gallus gallus GN=BYSL PE=4 SV=1	A0A11LR...	35 kDa																													
791			Uncharacterized protein OS=Gallus gallus GN=BCL7A PE=4 SV=4	E1BWB1_C...	24 kDa		2																											
792			Uncharacterized protein OS=Gallus gallus GN=CENPJ PE=4 SV=4	E1COR8_C...	143 k...																													
793			Uncharacterized protein OS=Gallus gallus GN=ORC3 PE=4 SV=2	F1P1T6_C...	58 kDa																													
794			Gag and reverse transcriptase polyprotein OS=Gallus gallus PE=4 SV=1	R4QXY1_C...	174 k...																													
795			Uncharacterized protein OS=Gallus gallus GN=NUDT21 PE=4 SV=3	E1C538_C...	26 kDa																													
796			Uncharacterized protein OS=Gallus gallus PE=4 SV=1	A0A11LR...	24 kDa																													
797			Uncharacterized protein OS=Gallus gallus PE=4 SV=2	R4GI80_C...	99 kDa																													
798			MICOS complex subunit OS=Gallus gallus PE=3 SV=2	E1C4D9_C...	28 kDa																													
799			Carbonic anhydrase 2 OS=Gallus gallus GN=CA2 PE=4 SV=1	A0A11LR...	21 kDa																													
800			Uncharacterized protein OS=Gallus gallus GN=RECQL PE=4 SV=1	A0A1D5P...	74 kDa	★		1																										
801			Uncharacterized protein OS=Gallus gallus GN=YY1 PE=4 SV=2	F1NHV6_C...	45 kDa		2																											
802			Serine/threonine-protein kinase Chk1 OS=Gallus gallus GN=CHK1 PE=1 SV...	Q8ACY9	54 kDa		2																											
803			Uncharacterized protein OS=Gallus gallus GN=C1H11ORF54 PE=4 SV=2	tr F1NY09...	35 kDa																													
804			Uncharacterized protein OS=Gallus gallus GN=MARCKSL1 PE=2 SV=1	Q5ZKS0_C...	21 kDa																													
805			Uncharacterized protein OS=Gallus gallus GN=HMGN1 PE=4 SV=1	F1P1																														

APPENDIX 2

233

234

235

#	Visible? Starred?	Bio View: 590 Proteins in 329 Clusters	Accession Number	Molecular Weight	Protein Grouping Ambiguity	Probability Legend:										8wt	GST	S198A	S216A	S216I	delYV
						over 95%	80% to 94%	50% to 79%	20% to 49%	0% to 19%											
307		⚡ Claustrin OS=Gallus gallus PE=2 SV=1	Q90784 (+1)	117 k...																	
308		⚡ Uncharacterized protein OS=Gallus gallus GN=TFAP2A PE=4 SV=1	tr F1P4X0 F1P4X0...	48 kDa																	
309		⚡ Clathrin heavy chain OS=Gallus gallus GN=CLTC PE=3 SV=2	F1NW23	192 k...																	
310		⚡ Uncharacterized protein OS=Gallus gallus GN=RCJMB04_19g16 PE=2 SV=1	Q5F3E6	57 kDa																	
311		⚡ Uncharacterized protein OS=Gallus gallus GN=RCJMB04_1b10 PE=2 SV=1	Q5F4C6	109 k...																	
312		⚡ Uncharacterized protein OS=Gallus gallus GN=PHF14 PE=4 SV=2	tr A0A1D5P3P0 A...	104 k...																	
313		⚡ Histone-lysine N-methyltransferase SUV39H2 OS=Gallus gallus GN=SUV39H2 PE=2 SV=1	sp Q5F3W5 SUV9...	47 kDa																	
314		⚡ Core histone macro-H2A.1 OS=Gallus gallus GN=H2AFY PE=1 SV=3	sp O93327 H2AY...	40 kDa																	
315		⚡ T-complex protein 1 subunit delta OS=Gallus gallus GN=CCT4 PE=3 SV=1	A0A1L1RM13 (+2)	52 kDa																	
316		⚡ Uncharacterized protein OS=Gallus gallus GN=NME2 PE=3 SV=1	A0A1D5PY99	14 kDa																	
317		⚡ Uncharacterized protein OS=Gallus gallus GN=TFAM PE=4 SV=1	A0A1L1RW12	16 kDa																	
3...		⚡ Cluster of Calponin OS=Gallus gallus GN=ALG14 PE=3 SV=3 (E1BSX2)	E1BSX2 [2]	37 kDa																	
3...		⚡ Cluster of Uncharacterized protein OS=Gallus gallus GN=RP519 PE=4 SV=1 (A0A1D5PDV6)	A0A1D5PDV6 [2]	15 kDa																	
3...		⚡ Cluster of Uncharacterized protein OS=Gallus gallus PE=3 SV=1 (tr A0A1D5P5F5 A0A1D5P5F5_CHICK)	tr A0A1D5P5F5 A...	30 kDa																	
3...		⚡ Cluster of Uncharacterized protein OS=Gallus gallus GN=RBMX PE=4 SV=1 (tr F1NZD3 F1NZD3_CHICK)	tr F1NZD3 F1NZD...	42 kDa																	
3...		⚡ Cluster of Histone deacetylase 1 OS=Gallus gallus GN=HDAC1 PE=2 SV=1 (sp P56517 HDAC1_CHICK)	sp P56517 HDAC...	55 kDa																	
3...		⚡ Cluster of Uncharacterized protein OS=Gallus gallus GN=HNRNPA3 PE=4 SV=1 (tr A0A1D5P5E1 A0A1D5P5E1_CHICK)	tr A0A1D5P5E1 A...	32 kDa																	
324		⚡ Histone-binding protein RBBP4 OS=Gallus gallus GN=RBBP4 PE=4 SV=1	F2Z4M0 (+1)	48 kDa																	
325		⚡ Histone-binding protein RBBP4 OS=Gallus gallus GN=RBBP4 PE=1 SV=3	sp Q9W715 RBBP4...	48 kDa																	
326		⚡ Uncharacterized protein OS=Gallus gallus GN=SALL3 PE=4 SV=1	tr A0A1D5NTS5 A...	134 k...																	
327		⚡ Nucleolin OS=Gallus gallus GN=NCL PE=4 SV=1	A0A1D5NZ30 (+1)	75 kDa																	
3...		⚡ Cluster of Core histone macro-H2A OS=Gallus gallus GN=H2AFY2 PE=4 SV=1 (A0A1L1RSN9)	A0A1L1RSN9 [4]	40 kDa																	
3...		⚡ Cluster of Lens epithelium-derived growth factor OS=Gallus gallus GN=PSIP1 PE=2 SV=1 (sp Q5XXA9 PSIP1_CHICK)	sp Q5XXA9 PSIP1...	65 kDa																	

APPENDIX 3

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
								Probability Legend:							
								over 95%							
								80% to 94%							
								50% to 79%							
								20% to 49%							
								0% to 19%							
1			Fibronectin OS=Canis lupus familiaris OX=9615 GN=FN1 PE=4 SV=2	F1P6H7_CANLF	FN1	267 ...		889	412	478	464	475	200	129	313
2			Heparan sulfate proteoglycan 2 OS=Canis lupus familiaris OX=9615 GN=HSPG2 PE=4 SV=1	J9NRJ0_CANLF	HSPG2	468 ...		766	479	309	543	360	108	104	281
3			Rho guanine nucleotide exchange factor 17 OS=Canis lupus familiaris OX=9615 GN=ARH...	E2RE02_CANLF	ARHGEF17	222 ...		7	7	4	0	41	43	2	0
4			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=HSPA8 PE=3 SV=1	E2ROT6_CANLF	HSPA8	71 k...	★	203	88	123	182	162	54	105	134
5			Plectin OS=Canis lupus familiaris OX=9615 GN=PLEC PE=4 SV=2	F1PH55_CANLF	PLEC	534 ...	★	227	126	114	198	153	17	37	40
6			Actin, cytoplasmic 1 OS=Canis lupus familiaris OX=9615 GN=ACTB PE=2 SV=3	ACTB_CANLF	ACTB	42 k...	★	194	91	55	164	145	49	67	107
7			Clusterin OS=Canis lupus familiaris OX=9615 GN=CLU PE=2 SV=1	CLUS_CANLF	CLU	52 k...		62	50	19	62	204	64	145	241
8			Thrombospondin 1 OS=Canis lupus familiaris OX=9615 GN=THBS1 PE=4 SV=2	F1PB16_CANLF	THBS1	130 ...	★	36	148	46	205	85	78	53	160
9			Clathrin heavy chain OS=Canis lupus familiaris OX=9615 GN=CLTC PE=3 SV=2	F1PHQ0_CANLF	CLTC	192 ...	★	203	30	63	187	126	13	35	110
10			NCK associated protein 5 OS=Canis lupus familiaris OX=9615 GN=NCKAP5 PE=4 SV=2	F1P198_CANLF	NCKAP5	212 ...		1				166	47	4	20
11			Annexin A2 OS=Canis lupus familiaris OX=9615 GN=ANXA2 PE=1 SV=1	ANXA2_CANLF	ANXA2	39 k...		217	78	89	143	106	28	29	55
12			Programmed cell death 6 interacting protein OS=Canis lupus familiaris OX=9615 GN=PDC...	F1PFG6_CANLF	PDCD6IP	96 k...		142	43	94	123	126	30	63	101
13			Milk fat globule-EGF factor 8 protein OS=Canis lupus familiaris OX=9615 GN=MFGE8 PE=...	F1PF25_CANLF	MFGE8	48 k...		137	69	106	174	86	29	25	59
14			Prostaglandin F2 receptor inhibitor OS=Canis lupus familiaris OX=9615 GN=PTGFRN PE=4...	F1PR26_CANLF	PTGFRN	97 k...		230	61	84	98	133	36	34	34
15			Serum albumin OS=Canis lupus familiaris OX=9615 GN=ALB PE=1 SV=3	ALBU_CANLF	ALB	69 k...		71	121	161	44	62	75	50	66
16			Sodium/potassium-transporting ATPase subunit alpha OS=Canis lupus familiaris OX=96...	F1PBA1_CANLF	ATP1A1	113 ...	★	162	81	90	149	113	22	20	39
17			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=RP527A PE=4 SV=2	F1PE24_CANLF	RP527A	18 k...		113	16	41	129	106	33	28	86
18			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=SDCBP PE=4 SV=2	F1PKR0_CANLF	SDCBP	32 k...		180	51	79	86	141	21	40	48
19			Talin 1 OS=Canis lupus familiaris OX=9615 GN=TLN1 PE=4 SV=2	F1P5C2_CANLF	TLN1	270 ...	★	117	44	75	58	87	13	89	116
20			Tubulin beta chain OS=Canis lupus familiaris OX=9615 GN=TUBB PE=3 SV=1	E2QSF4_CANLF	TUBB	50 k...	★	101	36	24	81	89	42	115	123
21			Glyceraldehyde-3-phosphate dehydrogenase OS=Canis lupus familiaris OX=9615 GN=GA...	F1PT29_CANLF	GAPDH	35 k...	★	61	18	28	74	88	33	47	155
22			Equilibrative nucleoside transporter 1 OS=Canis lupus familiaris OX=9615 GN=HSP90AB1...	E2RL53_CANLF	HSP90AB1	83 k...	★	69	33	26	98	64	33	41	94
23			Eukaryotic translation elongation factor 2 OS=Canis lupus familiaris OX=9615 GN=EEF2 P...	F6XRY2_CANLF	EEF2	95 k...	★	53	36	24	61	67	65	48	128
24			Filamin A OS=Canis lupus familiaris OX=9615 GN=FLNA PE=4 SV=2	F1PWW0_CANLF	FLNA	281 ...	★	99	44	45	103	54	16	47	56
25			Transforming growth factor beta induced OS=Canis lupus familiaris OX=9615 GN=TGFB1 ...	J9P2W2_CANLF	TGFB1	72 k...		26	30	13	23	123	111	27	97
26			Moesin OS=Canis lupus familiaris OX=9615 GN=MSN PE=4 SV=2	E2R7F1_CANLF	MSN	68 k...	★	107	49	51	79	70	14	7	14
27			Tubulin alpha chain OS=Canis lupus familiaris OX=9615 GN=TUBA8 PE=3 SV=1	F6X9E9_CANLF	TUBA8	50 k...	★					231	120	29	42
28			Tubulin alpha chain OS=Canis lupus familiaris OX=9615 GN=TUBA1B PE=3 SV=1	L7N0B2_CANLF	TUBA1B	50 k...	★	54	35	37	69	78	48	64	80
29			Actinin alpha 4 OS=Canis lupus familiaris OX=9615 GN=ACTN4 PE=4 SV=1	E2R5T9_CANLF	ACTN4	105 ...	★	118	65	28	134	72	20	3	14
30			Agrin OS=Canis lupus familiaris OX=9615 GN=AGRN PE=4 SV=2	F1Q2Z6_CANLF	AGRN	217 ...		70	93	38	73	31	41	18	81
31			Catenin alpha 1 OS=Canis lupus familiaris OX=9615 GN=CTNNA1 PE=4 SV=1	E2R9S7_CANLF	CTNNA1	100 ...	★	139	57	40	58	84	18	8	8
32			Valosin containing protein OS=Canis lupus familiaris OX=9615 GN=VCP PE=3 SV=2	E2RLQ9_CANLF	VCP	91 k...		122	33	19	79	83	22	16	28
33			Annexin A13 OS=Canis lupus familiaris OX=9615 GN=ANXA13 PE=1 SV=2	ANX13_CANLF	ANXA13	35 k...		118	37	49	56	87	6	16	16
34			Serpin family C member 1 OS=Canis lupus familiaris OX=9615 GN=SERPINC1 PE=3 SV=2	E2RES2_CANLF	SERPINC1	52 k...		22	26	21	49	40	35	60	81
35			Galectin 3 binding protein OS=Canis lupus familiaris OX=9615 GN=LGALS3BP PE=4 SV=1	E2RKQ6_CANLF	LGALS3BP	62 k...		148	67	33	34	99	42	6	12
36			Transforming acidic coiled-coil containing protein 1 OS=Canis lupus familiaris OX=9615 ...	E2R4V5_CANLF	TACC1	84 k...		1	2			118	106	15	0
37			Myosin-9 OS=Canis lupus familiaris OX=9615 GN=MYH9 PE=3 SV=2	F1P9J3_CANLF	MYH9	226 ...	★	70	31	23	67	69	13	40	42
38			Integrin beta OS=Canis lupus familiaris OX=9615 GN=ITGB1 PE=3 SV=2	E2RT60_CANLF	ITGB1	88 k...		101	44	44	56	74	18	15	14
39			Pyruvate kinase OS=Canis lupus familiaris OX=9615 GN=PKM PE=3 SV=2	F1PHR2_CANLF	PKM	60 k...	★	37	14	23	57	54	30	63	127
40			Tubulointerstitial nephritis antigen like 1 OS=Canis lupus familiaris OX=9615 GN=TINAG...	E2QXH3_CANLF	TINAGL1	52 k...		82	51	45	54	74	33	31	24
41			Ezrin OS=Canis lupus familiaris OX=9615 GN=EZR PE=4 SV=1	E2RSI6_CANLF	EZR	69 k...	★	121	63	51	82	88	16	15	19
42			Integrin subunit alpha 6 OS=Canis lupus familiaris OX=9615 GN=ITGA6 PE=3 SV=2	E2RL88_CANLF	ITGA6	122 ...	★	94	48	41	50	61	8	7	9
43			Junction plakoglobin OS=Canis lupus familiaris OX=9615 GN=JUP PE=4 SV=1	A0A140T8E6_C...	JUP	82 k...	★	80	33	40	61	55	9	13	22
44			Annexin OS=Canis lupus familiaris OX=9615 GN=ANXA1 PE=3 SV=1	F1P6B7_CANLF	ANXA1	39 k...		121	43	21	67	59	13	9	18
45			Fatty acid synthase OS=Canis lupus familiaris OX=9615 GN=FASN PE=4 SV=2	F1Q2F6_CANLF	FASN	273 ...		62	11	17	36	61	13	33	54
46			Prothrombin OS=Canis lupus familiaris OX=9615 GN=F2 PE=3 SV=1	J9NSF9_CANLF	F2	70 k...		11	13	16	14	55	42	105	95
47			Cofilin 1 OS=Canis lupus familiaris OX=9615 GN=CFL1 PE=3 SV=2	F1PQN5_CANLF	CFL1	19 k...	★	69	38	41	62	45	15	13	24
48			Lysyl oxidase like 2 OS=Canis lupus familiaris OX=9615 GN=LOXL2 PE=4 SV=2	E2RCV4_CANLF	LOXL2	86 k...		49	49	15	51	52	18	12	43
49			Epidermal growth factor receptor pathway substrate 8 OS=Canis lupus familiaris OX=961...	F1PLS1_CANLF	EP58	92 k...		102	22	49	58	49	3	5	4
50			Laminin subunit alpha 3 OS=Canis lupus familiaris OX=9615 GN=LAMA3 PE=4 SV=2	E2RPP1_CANLF	LAMA3	368 ...	★	17	20	5	33	54	35	9	78
51			Fibulin-1 OS=Canis lupus familiaris OX=9615 GN=FBLN1 PE=3 SV=2	F1PLV6_CANLF	FBLN1	78 k...		8	16	10	36	20	24	47	98
52			IKBK interacting protein OS=Canis lupus familiaris OX=9615 GN=IKBIP PE=4 SV=2	F1PXI6_CANLF	IKBIP	43 k...		1	3			49	74	1	2
53			EH domain containing 1 OS=Canis lupus familiaris OX=9615 GN=EHD1 PE=3 SV=2	F1PSK6_CANLF	EHD1	53 k...	★	96	24	27	43	60	7	16	17
54			Major vault protein OS=Canis lupus familiaris OX=9615 GN=MVP PE=4 SV=2	F1P914_CANLF	MVP	99 k...		74	25	38	79	35			11
55			L-lactate dehydrogenase OS=Canis lupus familiaris OX=9615 GN=LDHA PE=3 SV=2	F1PVW0_CANLF	LDHA	40 k...	★	47	24	18	46	57	25	21	38
56			Heat shock protein 90 alpha family class A member 1 OS=Canis lupus familiaris OX=9615...	F1PGY1_CANLF	HSP90AA1	73 k...	★	54	23	15	86	45	25	48	84
57			Laminin subunit beta 3 OS=Canis lupus familiaris OX=9615 GN=LAMB3 PE=4 SV=2	F1PFM5_CANLF	LAMB3	129 ...		16	17	7	26	71	34	22	71
58			Histone H4 OS=Canis lupus familiaris OX=9615 PE=3 SV=1	L7N0L3_CANLF		11 k...		84	30	18	54	39	8	17	35
59			Laminin subunit gamma 2 OS=Canis lupus familiaris OX=9615 GN=LAMC2 PE=4 SV=2	F1PG66_CANLF	LAMC2	131 ...		16	16	5	24	81	37	21	67
60			Alpha-fetoprotein OS=Canis lupus familiaris OX=9615 GN=AFP PE=4 SV=1	F1PXN2_CANLF ...	AFP	69 k...		20	30	25	9	15	16	7	9
61			Histone H2B OS=Canis lupus familiaris OX=9615 GN=LOC608682 PE=3 SV=1	H9GWB1_CANLF...	LOC608682	14 k...	★	80	16	19	36	47	3	13	22
62			EGF like repeats and discoidin domains 3 OS=Canis lupus familiaris OX=9615 GN=EDIL3 ...	F1P8D5_CANLF	EDIL3	54 k...		54	26	41	62	24	10	9	12
63			Neuron derived neurotrophic factor OS=Canis lupus familiaris OX=9615 GN=NDNF PE=4 S...	E2RTM5_CANLF	NDNF	65 k...		52	55	11	35	43	19	7	34
64			Phosphoglycerate kinase OS=Canis lupus familiaris OX=9615 GN=PGK1 PE=3 SV=1	E2RRC9_CANLF	PGK1	44 k...		3							

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
				Probability Legend:											
				over 95%											
				80% to 94%											
				50% to 79%											
				20% to 49%											
				0% to 19%											
86			EH domain containing 2 OS=Canis lupus familiaris OX=9615 GN=EHD2 PE=3 SV=2	F1PNP7_CANLF	EHD2	61 k...	★	61	20	16	23	33	4	7	3
87			Histone H2A OS=Canis lupus familiaris OX=9615 GN=LOC611231 PE=3 SV=1	J9P2B7_CANLF (...LOC611231		14 k...	★	48	17	14	26	22	6	2	16
88			HtrA serine peptidase 1 OS=Canis lupus familiaris OX=9615 GN=HTRA1 PE=4 SV=1	J9P2L4_CANLF	HTRA1	43 k...		26	15	11	26	28	26	15	63
89			Alpha 2-HS glycoprotein OS=Canis lupus familiaris OX=9615 GN=AHSG PE=4 SV=2	E2QUV3_CANLF	AHSG	39 k...		28	39	23	23	17	23	9	24
90			Catenin beta-1 OS=Canis lupus familiaris OX=9615 GN=CTNNB1 PE=1 SV=1	CTNB1_CANLF	CTNNB1	86 k...	★	81	23	23	40	48	5	4	13
91			Na(+)/H(+) exchange regulatory cofactor NHE-RF OS=Canis lupus familiaris OX=9615 GN...	E2RGF3_CANLF	SLC9A3R1	40 k...		56	25	14	58	30	3	3	8
92			Spectrin alpha, non-erythrocytic 1 OS=Canis lupus familiaris OX=9615 GN=SPTAN1 PE=4 ...	F1PAP5_CANLF	SPTAN1	285 ...		70	30	4	32	32	3	1	2
93			Triosephosphate isomerase OS=Canis lupus familiaris OX=9615 GN=TP1 PE=3 SV=1	A0A0A0MPD0_...	TP1	32 k...		43	17	14	48	39	11	14	15
94			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=1	G1K268_CANLF...		71 k...	★	36	17	34	57	33	15	50	51
95			T-complex protein 1 subunit delta OS=Canis lupus familiaris OX=9615 GN=CCT4 PE=3 S...	F1Q331_CANLF	CCT4	58 k...		44	19	8	26	32	10	20	27
96			WD repeat domain 1 OS=Canis lupus familiaris OX=9615 GN=WDR1 PE=2 SV=2	F1PR93_CANLF	WDR1	66 k...		23	12	10	35	20	16	23	37
97			Vinculin OS=Canis lupus familiaris OX=9615 GN=VCL PE=4 SV=1	J9P4F3_CANLF	VCL	117 ...		51	25	20	32	38	2	3	7
98			Arrestin domain containing 1 OS=Canis lupus familiaris OX=9615 GN=ARRDC1 PE=4 SV=2	F1Q1C9_CANLF	ARRDC1	46 k...		51	16	30	35	37	8	9	14
99			Eukaryotic initiation factor 4A-I OS=Canis lupus familiaris OX=9615 GN=EIF4A1 PE=3 SV...	F1P895_CANLF	EIF4A1	46 k...	★	24	10	9	22	35	20	24	33
100			Integrin subunit alpha 3 OS=Canis lupus familiaris OX=9615 GN=ITGA3 PE=3 SV=2	F1Q439_CANLF	ITGA3	117 ...		71	22	17	28	39	7	3	2
101			L-lactate dehydrogenase OS=Canis lupus familiaris OX=9615 GN=LDHB PE=3 SV=2	F1PIB3_CANLF	LDHB	39 k...	★	26	13	14	29	46	25	36	34
102			Ubiquitin like modifier activating enzyme 1 OS=Canis lupus familiaris OX=9615 GN=UBA...	E2RGH5_CANLF	UBA1	117 ...		29	5	5	25	25	10	31	37
103			Laminin subunit gamma 1 OS=Canis lupus familiaris OX=9615 GN=LAMC1 PE=4 SV=2	F1PHK9_CANLF	LAMC1	165 ...		41	34	14	26	26	7	6	16
104			Amino acid transporter OS=Canis lupus familiaris OX=9615 GN=SLC1A5 PE=3 SV=1	F1PPI5_CANLF	SLC1A5	58 k...		43	33	29	35	34	15	5	5
105			Integrin subunit alpha 2 OS=Canis lupus familiaris OX=9615 GN=ITGA2 PE=3 SV=2	E2REA9_CANLF	ITGA2	130 ...		57	19	28	37	31	6	1	7
106			Fructose-bisphosphate aldolase OS=Canis lupus familiaris OX=9615 GN=ALDOA PE=3 SV...	F1PBT3_CANLF	ALDOA	75 k...	★	29	6	9	28	24	12	26	17
107			Plasminogen activator OS=Canis lupus familiaris OX=9615 GN=PLAT PE=3 SV=2	F1PWE1_CANLF	PLAT	64 k...		9	3			68	28	16	35
108			ADAM metallopeptidase domain 10 OS=Canis lupus familiaris OX=9615 GN=ADAM10 PE...	F1PGR0_CANLF	ADAM10	85 k...		43	7	17	20	50	8	3	9
109			Rab GDP dissociation inhibitor OS=Canis lupus familiaris OX=9615 GN=GD12 PE=3 SV=1	F1PBL7_CANLF	GD12	49 k...	★	33	15	11	20	33	8	6	13
110			Plasminogen OS=Canis lupus familiaris OX=9615 GN=PLG PE=3 SV=2	F1Q421_CANLF	PLG	91 k...		9	5	8	18	63	48	12	23
111			Aldehyde dehydrogenase 1 family member A1 OS=Canis lupus familiaris OX=9615 GN=A...	J9NS92_CANLF	ALDH1A1	55 k...	★	18	2	6	31	30	6	26	47
112			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=BSG PE=4 SV=1	E2QZT4_CANLF	BSG	29 k...		38	27	30	35	29	17	10	6
113			Chaperonin containing TCP1 subunit 8 OS=Canis lupus familiaris OX=9615 GN=CCT8 PE...	J9NRN0_CANLF	CCT8	78 k...		43	9	7	25	28	5	32	18
114			Chaperonin containing TCP1 subunit 2 OS=Canis lupus familiaris OX=9615 GN=CCT2 PE...	F6V0D8_CANLF	CCT2	57 k...		52	13	7	24	28	6	20	16
115			Apolipoprotein B OS=Canis lupus familiaris OX=9615 GN=APOB PE=4 SV=1	F1P8Z5_CANLF	APOB	502 ...		26	21	22	5	22	13	14	20
116			5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase ...	F1P797_CANLF	ATIC	65 k...		18	4	1	19	20	12	21	47
117			Transgelin OS=Canis lupus familiaris OX=9615 GN=TAGLN2 PE=3 SV=2	F1P6P2_CANLF	TAGLN2	22 k...		59	17	12	33	32	5	0	8
118			Heat shock protein family A (Hsp70) member 5 OS=Canis lupus familiaris OX=9615 GN=H...	F1PIC7_CANLF	HSPA5	72 k...	★	45	24	63	36	24	11	19	22
119			40S ribosomal protein S4 OS=Canis lupus familiaris OX=9615 GN=RP54X PE=3 SV=1	F2Z4Q1_CANLF	RP54X	30 k...		17	9	8	13	26	9	11	36
120			CD44 antigen OS=Canis lupus familiaris OX=9615 GN=CD44 PE=4 SV=2	F1PTZ7_CANLF	CD44	77 k...		45	20	22	34	40	5	11	1
121			T-complex 1 OS=Canis lupus familiaris OX=9615 GN=TCP1 PE=3 SV=1	E2R0L9_CANLF	TCP1	60 k...		40	7	3	24	32	7	16	31
122			Laminin subunit beta 1 OS=Canis lupus familiaris OX=9615 GN=LAMB1 PE=4 SV=2	F1PCD8_CANLF	LAMB1	182 ...		39	39	18	13	21	17	2	7
123			Galectin OS=Canis lupus familiaris OX=9615 GN=LGALS3 PE=4 SV=1	F6PME1_CANLF ...LGALS3		31 k...		30	12	11	36	18	3	4	21
124			Radixin OS=Canis lupus familiaris OX=9615 GN=RDYX PE=4 SV=2	E2RR6C_CANLF	RDX	71 k...	★	100	46	29	54	54	10		
125			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9NXR3_CANLF		89 k...		42	19	19	28	38	6	2	3
126			Dynein cytoplasmic 1 heavy chain 1 OS=Canis lupus familiaris OX=9615 GN=DYNC1H1 PE...	F1PGV9_CANLF	DYNC1H1	532 ...		22	1	9	5	32	1	23	14
127			Stomatins OS=Canis lupus familiaris OX=9615 GN=STOM PE=4 SV=2	F1PFS1_CANLF	STOM	26 k...		51	16	15	21	34	4	2	3
128			GPI inositol-deacylase OS=Canis lupus familiaris OX=9615 GN=PGAP1 PE=3 SV=1	J9NRJ8_CANLF	PGAP1	106 ...		2	4	2	1	8	11	20	5
129			Annexin OS=Canis lupus familiaris OX=9615 GN=ANXA11 PE=3 SV=1	E2QXN8_CANLF	ANXA11	54 k...	★	30	17	23	34	23	9	6	2
130			Actinin alpha 1 OS=Canis lupus familiaris OX=9615 GN=ACTN1 PE=4 SV=2	E2QY08_CANLF	ACTN1	105 ...	★	83	48	19	101	46	14	3	14
131			T-complex protein 1 subunit gamma OS=Canis lupus familiaris OX=9615 GN=CCT3 PE=3...	E2R879_CANLF	CCT3	61 k...		32	4	4	17	23	6	30	16
132			Cell division control protein 42 homolog OS=Canis lupus familiaris OX=9615 GN=CDC42...	CDC42_CANLF	CDC42	21 k...	★	43	16	14	25	24	8	1	14
133			Collagen type V alpha 2 chain OS=Canis lupus familiaris OX=9615 GN=COL5A2 PE=4 SV=2	F1PG08_CANLF	COL5A2	145 ...	★	19	20	20	14	19	2	8	10
134			Sodium/potassium-transporting ATPase subunit beta-1 OS=Canis lupus familiaris OX=9...	ATP1B1_CANLF	ATP1B1	35 k...		19	15	17	25	21	7	8	10
135			BAI1 associated protein 2 like 1 OS=Canis lupus familiaris OX=9615 GN=BAIAP2L1 PE=4 ...	F1PWE6_CANLF	BAIAP2L1	55 k...	★	41	4	18	15	15		1	1
136			2',3'-cyclic-nucleotide 3'-phosphodiesterase OS=Canis lupus familiaris OX=9615 GN=CN...	F1PY13_CANLF	CNP	47 k...		35	17	17	25	30	5	0	
137			Chaperonin containing TCP1 subunit 6A OS=Canis lupus familiaris OX=9615 GN=CCT6A ...	E2RLL6_CANLF	CCT6A	58 k...		49	6	4	12	23	4	10	9
138			Guanine nucleotide-binding protein G(i)/G(s)/G(t) subunit beta-1 OS=Canis lupus familia...	G8B1_CANLF	GNB1	37 k...	★	33	16	13	27	25	7	5	9
139			Semaphorin 3C OS=Canis lupus familiaris OX=9615 GN=SEMA3C PE=3 SV=2	E2R0R3_CANLF	SEMA3C	85 k...		9	21	4	10	27	18	15	36
140			Tetraspanin OS=Canis lupus familiaris OX=9615 GN=CD81 PE=3 SV=2	F1PB66_CANLF	CD81	25 k...		37	9	11	15	38	13	12	12
141			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=DNAJA1 PE=3 SV=1	E2RM09_CANLF	DNAJA1	45 k...		48	5	14	21	25	2	8	2
142			Filamin B OS=Canis lupus familiaris OX=9615 GN=FLNB PE=4 SV=2	E2R0F2_CANLF	FLNB	281 ...	★	38	20	8	34	18	4	6	5
143			Histone H3 OS=Canis lupus familiaris OX=9615 GN=LOC611519 PE=3 SV=1	J9P2B2_CANLF	LOC611519	15 k...	★	32	2	1	7	10	0	2	9
144			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC100856782 PE=3 SV...	J9NS87_CANLF	LOC100856782	59 k...		32	9	5	19	23	8	8	17
145			Chloride intracellular channel protein OS=Canis lupus familiaris OX=9615 GN=CLIC4 PE=...	E2RG14_CANLF	CLIC4	29 k...	★	35	9	3	30	27	5	9	14
146			Cytoplasmic FMRI-interacting protein OS=Canis lupus familiaris OX=9615 GN=CYFIP1 PE...	F1PDQ4_CANLF	CYFIP1	146 ...	★	33	15	17	20	24	2	4	4
147			Tripeptidyl peptidase 2 OS=Canis lupus familiaris OX=9615 GN=TPP2 PE=4 SV=2	E2R9J9_CANLF	TPP2	140 ...		51	3	2	31	22		2	6
148			Spectrin beta chain OS=Canis lupus familiaris OX=9615 GN=SPTBN1 PE=3 SV=2	F1PXU1_CANLF	SPTBN1	274 ...	★	34	17	5	20	17	2		
149			Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein beta OS=Ca...	F1PKW7_CANLF	YWHA8	28 k...	★	56	25	27	26	50	7	10	10
150			Endoplasmic reticulum protein OS=Canis lupus familiaris OX=9615 GN=HSP90B1 PE=1 SV=1	ENPL_CANLF (+1)	HSP90B1	93 k...	★	23	13	36	21	12	5	14	24
151			Solute carrier family 16 member 1 OS=Canis lupus familiaris OX=9615 GN=SLC16A1 PE=...	F1PCQ3_CANLF	SLC16A1	54 k...		36	8	28	24	27	2	5	7
152			Complement C3 OS=Canis lupus familiaris OX=9615 GN=C3 PE=4 SV=2	F1PIX8_CANLF	C3	187 ...		10	13	15	8	10	10	9	18
153			Karyopherin subunit beta 1 OS=Canis lupus familiaris OX=9615 GN=KPNB1 PE=4 SV=1	F6X637_CANLF	KPNB1	97 k...									

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
								Probability Legend:							
								over 95%							
								80% to 94%							
								50% to 79%							
								20% to 49%							
								0% to 19%							
171			ARP3 actin related protein 3 homolog OS=Canis lupus familiaris OX=9615 GN=ACTR3 PE=...	F1P679_CANLF	ACTR3	48 k...		24	8	7	15	15	9	15	23
172			Plexin B2 OS=Canis lupus familiaris OX=9615 GN=PLXNB2 PE=4 SV=2	F1P955_CANLF	PLXNB2	205 ...	★	42	6	16	11	12	1	1	5
173			Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 OS=Canis lup...	RPN1_CANLF	RPN1	69 k...		30	12	31	16	11	2	4	1
174			Chaperonin containing TCP1 subunit 5 OS=Canis lupus familiaris OX=9615 GN=CCT5 PE=...	E2R881_CANLF	CCT5	60 k...		31	6	2	17	19	6	18	21
175			Family with sequence similarity 129 member B OS=Canis lupus familiaris OX=9615 GN=F...	E2RR68_CANLF	FAM129B	84 k...		25	8	4	21	18	5	2	10
176			Calnexin OS=Canis lupus familiaris OX=9615 GN=CANX PE=1 SV=3	CALX_CANLF	CANX	68 k...		33	8	35	15	10	1	7	
177			6-phosphogluconate dehydrogenase, decarboxylating OS=Canis lupus familiaris OX=961...	F1PE09_CANLF	PGD	53 k...		17	5	4	23	15	6	13	15
178			Peptidylprolyl isomerase OS=Canis lupus familiaris OX=9615 GN=FKBP4 PE=4 SV=2	E2QW55_CANLF	FKBP4	52 k...		15	6	6	17	13	6	21	14
179			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC477072 PE=4 SV=1	F6V1W9_CANLF	LOC477072	111 ...		12	15	23	16	13	12	11	22
180			Alpha-1,4 glucan phosphorylase OS=Canis lupus familiaris OX=9615 GN=PYGL PE=3 SV=2	F1PB77_CANLF	PYGL	100 ...	★	1	2	0		14	17	20	50
181			Collagen type V alpha 1 chain OS=Canis lupus familiaris OX=9615 GN=COL5A1 PE=4 SV=2	F1PHX8_CANLF	COL5A1	180 ...		14	35	1	14	33	7		
182			Calpain 5 OS=Canis lupus familiaris OX=9615 GN=CAPN5 PE=3 SV=2	F1PB20_CANLF	CAPN5	73 k...		32	4	9	23	20	0		3
183			Carcinoembryonic antigen-related cell adhesion molecule 1 isoform 4L OS=Canis lupus fa...	Q00482_CANLF	CEACAM1	56 k...	★	32	16	18	18	23	6	3	2
184			Ribosomal protein S2 OS=Canis lupus familiaris OX=9615 GN=RP52 PE=3 SV=2	E2QWY6_CANLF	RP52	31 k...		12	10	8	9	15	6	6	18
185			NCK associated protein 1 OS=Canis lupus familiaris OX=9615 GN=NCKAP1 PE=4 SV=2	F1PH57_CANLF	NCKAP1	126 ...		30	5	10	12	23	1	2	2
186			Integrin beta OS=Canis lupus familiaris OX=9615 GN=ITGB3 PE=3 SV=1	F1PPG5_CANLF	ITGB3	87 k...		28	7	19	12	23	6	2	4
187			Transforming protein RhoA OS=Canis lupus familiaris OX=9615 GN=RHOA PE=4 SV=1	F6XY66_CANLF	RHOA	22 k...	★	28	14	12	17	25	5	3	5
188			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=CXADR PE=4 SV=2	F1P992_CANLF	CXADR	39 k...		38	13	4	18	23	6	3	2
189			Acyl-CoA synthetase long chain family member 4 OS=Canis lupus familiaris OX=9615 GN...	F1PEH9_CANLF	ACSL4	74 k...	★	25	9	17	21	15	2	2	4
190			Cysteine rich angiogenic inducer 61 OS=Canis lupus familiaris OX=9615 GN=CYR61 PE=4...	E2RAA0_CANLF	CYR61	42 k...		26	15	6	13	20	9	2	3
191			Stratifin OS=Canis lupus familiaris OX=9615 GN=SFN PE=3 SV=2	F1PQ93_CANLF	SFN	28 k...	★	46	21	22	34	29	3	9	11
192			RAS related OS=Canis lupus familiaris OX=9615 GN=RRAS PE=4 SV=1	E2R5G6_CANLF	RRAS	24 k...	★	32	10	4	14	22	3	2	1
193			AP complex subunit beta OS=Canis lupus familiaris OX=9615 GN=AP2B1 PE=3 SV=2	E2RIV1_CANLF	AP2B1	106 ...	★	22	8	4	22	24	1	3	6
194			Glucose-6-phosphate 1-dehydrogenase OS=Canis lupus familiaris OX=9615 GN=G6PD P...	J9P9E9_CANLF	G6PD	67 k...		6	7	4	22	10	5	1	11
195			40S ribosomal protein S3 OS=Canis lupus familiaris OX=9615 GN=RP53 PE=1 SV=1	RS3_CANLF	RP53	27 k...		12	5	3	13	23	8	9	33
196			NAD(PH) quinone dehydrogenase 1 OS=Canis lupus familiaris OX=9615 GN=NQO1 PE=4 S...	F1PBZ4_CANLF	NQO1	31 k...		18	6	9	28	20	4	15	17
197			Tetraspanin OS=Canis lupus familiaris OX=9615 GN=TSPAN8 PE=3 SV=1	E2QSC8_CANLF	TSPAN8	26 k...		27	10	10	17	23	12	8	6
198			Aconitase 1 OS=Canis lupus familiaris OX=9615 GN=ACO1 PE=3 SV=2	E2RMX9_CANLF	ACO1	100 ...		5	2	4	6	13	7	10	39
199			Glucosidase II alpha subunit OS=Canis lupus familiaris OX=9615 GN=GANAB PE=3 SV=1	E2R729_CANLF	GANAB	109 ...		10	1	7	1	23	7	7	35
200			Stannocalcin 1 OS=Canis lupus familiaris OX=9615 GN=STC1 PE=4 SV=1	E2RST6_CANLF	STC1	27 k...		8	7	8	19	18	15	9	23
201			Ras-related C3 botulinum toxin substrate 1 OS=Canis lupus familiaris OX=9615 GN=RAC...	F1PYQ1_CANLF	RAC1	24 k...	★	19	7	11	16	20	4	9	10
202			FAT atypical cadherin 1 OS=Canis lupus familiaris OX=9615 GN=FAT1 PE=3 SV=1	F6XXM1_CANLF	FAT1	556 ...	★	19	10	14	1	15	0	1	3
203			Slit guidance ligand 3 OS=Canis lupus familiaris OX=9615 GN=SLIT3 PE=4 SV=2	F1Q3G2_CANLF	SLIT3	145 ...	★	46	3	0	13	22		2	1
204			Myoferlin OS=Canis lupus familiaris OX=9615 GN=MYOF PE=4 SV=1	J9P3K0_CANLF	MYOF	235 ...		32	9	10	22	12		0	
205			Tyrosine-protein kinase OS=Canis lupus familiaris OX=9615 GN=YES1 PE=3 SV=2	F1PSB7_CANLF	YES1	61 k...	★	18	7	9	14	19	3	3	8
206			Vitronectin OS=Canis lupus familiaris OX=9615 GN=VTN PE=4 SV=1	F1PUM6_CANLF	VTN	54 k...		6	6	6	11	21	25	4	25
207			Protein disulfide-isomerase OS=Canis lupus familiaris OX=9615 GN=PDIA3 PE=3 SV=1	E2RD86_CANLF	PDIA3	57 k...		22	11	27	8	7	1	2	
208			Ras-related protein Rab-7a OS=Canis lupus familiaris OX=9615 GN=RAB7A PE=1 SV=1	RAB7A_CANLF	RAB7A	24 k...		27	11	9	23	13	3	5	6
209			Tubulin tyrosine ligase like 12 OS=Canis lupus familiaris OX=9615 GN=TTLL12 PE=4 SV=2	F1PQD3_CANLF	TTLL12	77 k...		8	0	2	18	13	3	23	30
210			Peptidyl-prolyl cis-trans isomerase OS=Canis lupus familiaris OX=9615 PE=3 SV=1	F1PK62_CANLF		18 k...	★	16	12	8	15	20	10	8	1
211			Vacuolar protein sorting-associated protein 35 OS=Canis lupus familiaris OX=9615 GN=V...	E2QRX1_CANLF	VPS35	92 k...		18	7	7	14	15	8	17	17
212			Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein gamma OS=...	E2QVP6_CANLF	YWHAQ	28 k...	★	33	18	17	22	31	6	11	5
213			DLA class II histocompatibility antigen, DR-1 beta chain precursor OS=Canis lupus familia...	F6V690_CANLF	HLA-DRB1	31 k...	★	23	11	11	26	25	6	7	6
214			Keratin 19 OS=Canis lupus familiaris OX=9615 GN=KRT19 PE=3 SV=1	F1Q0N9_CANLF	KRT19	44 k...	★	22	10	6	24	21	7	0	0
215			Guanine nucleotide-binding protein (G)s subunit alpha OS=Canis lupus familiaris OX=96...	GNAS_CANLF	GNAS	46 k...	★	35	10	12	19	22	2	3	2
216			Annexin OS=Canis lupus familiaris OX=9615 GN=ANXA5 PE=2 SV=2	E2RQ14_CANLF	ANXA5	36 k...		28	13	15	29	13			
217			Plastin 3 OS=Canis lupus familiaris OX=9615 GN=PLS3 PE=4 SV=1	F6V234_CANLF	PLS3	71 k...	★	27	2	3	29	18	4	7	8
218			Tumor susceptibility 101 OS=Canis lupus familiaris OX=9615 GN=TSG101 PE=4 SV=2	E2RGP6_CANLF	TSG101	44 k...		31	6	15	14	21	2	8	6
219			Lamin A/C OS=Canis lupus familiaris OX=9615 GN=LMNA PE=3 SV=1	J9NSW5_CANLF	LMNA	84 k...	★	20	6	4	36	17	0	0	2
220			Keratin, type I cytoskeletal 10 OS=Canis lupus familiaris OX=9615 GN=KRT10 PE=3 SV=2	F1PYU9_CANLF	KRT10	58 k...	★	20	11	25	8	13	6	5	
221			Transketolase OS=Canis lupus familiaris OX=9615 GN=TKT PE=4 SV=2	F1PE28_CANLF	TKT	63 k...		26	9	4	38	13	2		4
222			Carboxypeptidase D OS=Canis lupus familiaris OX=9615 GN=CPD PE=4 SV=1	E2R830_CANLF	CPD	153 ...		31	4	9	17	14		1	
223			MHC class II DR alpha chain OS=Canis lupus familiaris OX=9615 GN=DLA DRA PE=3 SV=1	Q30437_CANLF	DLA	29 k...	★	26	13	15	18	20	6	5	3
224			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=DNAJB11 PE=4 SV=2	F1PXX5_CANLF	DNAJB11	41 k...		5	21	3	8	15	12	15	17
225			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC609365 PE=4 SV=2	F1PWC7_CANLF	LOC609365	43 k...		27	6	5	26	21	2	1	3
226			Myosin ID OS=Canis lupus familiaris OX=9615 GN=MYO1D PE=3 SV=2	F1PRN2_CANLF	MYO1D	116 ...		26	9	8	22	14	0		
227			Tissue factor OS=Canis lupus familiaris OX=9615 GN=F3 PE=2 SV=1	Q4W6L5_CANLF	F3	33 k...		23	13	14	13	22	7	5	2
228			Periplakin OS=Canis lupus familiaris OX=9615 GN=PPL PE=4 SV=1	J9NU37_CANLF	PPL	204 ...		43	0	3	18	7	0	0	
229			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9P9V0_CANLF		14 k...	★	17	11	11	26	17	5	3	14
230			EH domain containing 4 OS=Canis lupus familiaris OX=9615 GN=EHD4 PE=3 SV=2	F1P719_CANLF	EHD4	61 k...	★	49	11	14	11	19	1	4	4
231			GC, vitamin D binding protein OS=Canis lupus familiaris OX=9615 GN=GC PE=4 SV=2	F1P841_CANLF	GC	54 k...		13	11	11	9	8	12	5	16
232			Septin 2 OS=Canis lupus familiaris OX=9615 GN=SEPT2 PE=3 SV=2	F1PEK5_CANLF	SEPT2	41 k...		11	7	5	22	9	4	5	18
233			Adenyllyl cyclase-associated protein OS=Canis lupus familiaris OX=9615 GN=CAP1 PE=3 ...	E2QZ50_CANLF	CAP1	51 k...		23	6	4	17	16	2	9	6
234			G protein subunit alpha i3 OS=Canis lupus familiaris OX=9615 GN=GNAI3 PE=4 SV=2	F1PQ60_CANLF	GNAI3	41 k...	★	33	14	15	39	23	6	8	6
235			Ribosomal protein S16 OS=Canis lupus familiaris OX=9615 GN=RP516 PE=3 SV=2	F1PKR6_CANLF	RP516	18 k...		8	6	6	5	12	8	11	16
236			26S proteasome non-ATPase regulatory subunit 2 OS=Canis lupus familiaris OX=9615 G...	E2RCP9_CANLF	PSMD2	100 ...		14	6	4	7	12	4	18	9
237			Apolipoprotein A-I OS=Canis lupus familiaris OX=9615 GN=APOA1 PE=3 SV=1	F1PDJ5_CANLF	APOA1	30 k...		9	15	13	8	13	14	17	11
238			Phosphoglycerate mutase OS=Canis lupus familiaris OX=9615 GN=PGAM1 PE=3 SV=2	E2RT65_CANLF	PGAM1	29 k...		13	8	3	12	18	10	9	15
239			Importin 5 OS=Canis lupus familiaris OX=9615 GN=IPO5 PE=4 SV=2	E2RF06_CANLF	IPO5	123 ...		26	11	3	12	14	5	4	4

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
								Probability Legend:							
								over 95%							
								80% to 94%							
								50% to 79%							
								20% to 49%							
								0% to 19%							
256			Protein disulfide-isomerase OS=Canis lupus familiaris OX=9615 GN=P4HB PE=3 SV=2	F1PL97_CANLF	P4HB	57 k...		21	8	21	3	6	1	0	
257			40S ribosomal protein SA OS=Canis lupus familiaris OX=9615 GN=RPSA PE=3 SV=2	E2RJ06_CANLF	RPSA	35 k...		7	4	2	9	16	7	18	27
258			Plexin A1 OS=Canis lupus familiaris OX=9615 GN=PLXNA1 PE=4 SV=2	F1P6G0_CANLF	PLXNA1	212 ...	★	23	6	7	5	13		1	
259			Ras-related protein Rab-1A OS=Canis lupus familiaris OX=9615 GN=RAB1A PE=1 SV=3	RAB1A_CANLF	RAB1A	23 k...	★	20	8	9	25	12	7	4	9
260			Syntaxin-binding protein 2 OS=Canis lupus familiaris OX=9615 GN=STXB2 PE=2 SV=1	STXB2_CANLF	STXB2	67 k...	★	20	9	7	20	11	2	3	3
261			Intercellular adhesion molecule 1 (Fragment) OS=Canis lupus familiaris OX=9615 GN=ICAM1 PE=4 SV=1	ICAM1_CANLF	ICAM1	58 k...	★	20	9	5	21	14	3		
262			L1 cell adhesion molecule OS=Canis lupus familiaris OX=9615 GN=L1CAM PE=4 SV=1	E2R2V6_CANLF	L1CAM	140 ...	★	24	12	9	14	10	1		
263			Serpin family H member 1 OS=Canis lupus familiaris OX=9615 GN=SERPINH1 PE=1 SV=1	E2RHY7_CANLF	SERPINH1	47 k...		6	3	14	2	11	7	19	17
264			Hepatocyte growth factor receptor OS=Canis lupus familiaris OX=9615 GN=MET PE=3 SV=1	J9P743_CANLF	MET	159 ...	★	20	8	5	10	19	2	2	3
265			ATP binding cassette subfamily B member 9 OS=Canis lupus familiaris OX=9615 GN=ABCB9 PE=4 SV=1	E2R807_CANLF	ABCB9	103 ...		27	9	10	3	26	0	4	2
266			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=2	F1PW98_CANLF	KRT8	55 k...	★	25	9	11	28	12	1		
267			Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein eta OS=Canis lupus familiaris OX=9615 GN=YWHAH PE=4 SV=1	J9P6N4_CANLF	YWHAH	28 k...	★	32	22	15	27	29	5	8	8
268			Ribonucleoside-diphosphate reductase OS=Canis lupus familiaris OX=9615 GN=RRM1 PE=4 SV=1	E2R8P1_CANLF	RRM1	90 k...		3	1	0		12	10	18	19
269			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=1	F6Y478_CANLF		19 k...	★	21	13	14	27	19	5	12	12
270			60S ribosomal protein L6 OS=Canis lupus familiaris OX=9615 GN=RPL6 PE=3 SV=1	F1Q424_CANLF	RPL6	33 k...		15	13	6	14	6	5	2	10
271			Septin 7 OS=Canis lupus familiaris OX=9615 GN=SEPT7 PE=3 SV=2	F1Q067_CANLF	SEPT7	45 k...		13	8	6	20	8	2	8	9
272			Tetraspanin OS=Canis lupus familiaris OX=9615 GN=CD82 PE=3 SV=2	F1PLP3_CANLF	CD82	33 k...		18	8	13	12	15	8	10	5
273			Capping actin protein of muscle Z-line alpha subunit 1 OS=Canis lupus familiaris OX=9615 GN=CAPZA1 PE=4 SV=1	F1P7P3_CANLF	CAPZA1	34 k...	★	13	4	4	13	11	1	9	4
274			Poliovirus receptor OS=Canis lupus familiaris OX=9615 GN=PVR PE=4 SV=2	F1PV57_CANLF	PVR	44 k...		19	7	7	10	17	3	0	1
275			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=2	E2R7F3_CANLF		46 k...		21	3	9	10	18		1	2
276			Basal cell adhesion molecule OS=Canis lupus familiaris OX=9615 GN=BCAM PE=4 SV=2	F1PWJ7_CANLF	BCAM	68 k...		23	3	9	16	19	4	3	4
277			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC611458 PE=4 SV=1	F6UMP8_CANLF	LOC611458	166 ...		4	1	20	3	1	1	16	16
278			Dihydropyrimidinase like 2 OS=Canis lupus familiaris OX=9615 GN=DPYSL2 PE=4 SV=2	F1P9U4_CANLF	DPYSL2	74 k...	★	11	0	0	10	10	2	14	16
279			Cadherin-1 OS=Canis lupus familiaris OX=9615 GN=CDH1 PE=1 SV=2	CADH1_CANLF	CDH1	98 k...		20	14	6	19	11	3		4
280			Slit guidance ligand 2 OS=Canis lupus familiaris OX=9615 GN=SLIT2 PE=4 SV=2	F1P8A1_CANLF	SLIT2	170 ...	★	35	17	3	1	12	1	0	
281			Kinesin-like protein OS=Canis lupus familiaris OX=9615 GN=KIF23 PE=3 SV=2	F1PML0_CANLF	KIF23	110 ...		14	6	5	20	8	0	3	1
282			RuvB-like helicase OS=Canis lupus familiaris OX=9615 GN=RUVBL1 PE=3 SV=2	E2RQC9_CANLF	RUVBL1	50 k...		29	4	2	14	19	2	6	5
283			Actin, alpha, cardiac muscle 1 OS=Canis lupus familiaris OX=9615 GN=ACTC1 PE=3 SV=1	F2Z4N7_CANLF	ACTC1	42 k...	★	85	47	35	106	74	30	46	66
284			Copine 1 OS=Canis lupus familiaris OX=9615 GN=CPNE1 PE=4 SV=1	E2R9N5_CANLF	CPNE1	59 k...	★	27	7	9	18	14		1	2
285			60S acidic ribosomal protein P0 OS=Canis lupus familiaris OX=9615 GN=RPLP0 PE=3 SV=1	F1PUX4_CANLF	RPLP0	34 k...		5	5	1	2	17	10	20	11
286			Fermitin family member 3 OS=Canis lupus familiaris OX=9615 GN=FERMT3 PE=4 SV=1	E2RK64_CANLF	FERMT3	76 k...				2	1	10	8	23	36
287			Phosphoinositide phospholipase C OS=Canis lupus familiaris OX=9615 GN=PLCB3 PE=4 SV=1	F1JHK9_CANLF	PLCB3	139 ...		24	11	4	14	9			
288			Receptor protein-tyrosine kinase OS=Canis lupus familiaris OX=9615 GN=ERBB2 PE=3 SV=1	F1PIQ9_CANLF	ERBB2	138 ...	★	3	1	2	1	2	1	6	0
289			Afadin, adherens junction formation factor OS=Canis lupus familiaris OX=9615 GN=AFDN PE=4 SV=1	F1PSU6_CANLF	AFDN	203 ...		21	7	10	2	12	1		
290			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	F1PB89_CANLF		138 ...	★	20	15	3	8	12	4	3	5
291			40S ribosomal protein S8 OS=Canis lupus familiaris OX=9615 GN=RPS8 PE=3 SV=1	E2RF80_CANLF	RPS8	24 k...		10	6	9	8	7	7	3	16
292			Glutamyl-prolyl-tRNA synthetase OS=Canis lupus familiaris OX=9615 GN=EPRS PE=3 SV=1	E2QUR2_CANLF	EPRS	170 ...	★	6	3	2	2	14	2	10	14
293			Platelet-activating factor acetylhydrolase OS=Canis lupus familiaris OX=9615 GN=PLA2G7 PE=4 SV=1	F1P9E5_CANLF	PLA2G7	50 k...		23	8	5	1	16	5	2	5
294			IST1, ESCRT-III associated factor OS=Canis lupus familiaris OX=9615 GN=IST1 PE=4 SV=1	E2RPE3_CANLF	IST1	40 k...		18	12	7	18	9	1	1	1
295			Peroxisedoxin 6 OS=Canis lupus familiaris OX=9615 GN=PRDX6 PE=4 SV=1	F1PC59_CANLF	PRDX6	25 k...		11	4	2	13	9	6	7	6
296			40S ribosomal protein S3a OS=Canis lupus familiaris OX=9615 GN=RPS3A PE=3 SV=1	F2Z4Q5_CANLF	RPS3A	30 k...	★	10	5	4	11	12	5	5	12
297			Gelsolin OS=Canis lupus familiaris OX=9615 GN=GSN PE=4 SV=1	F6Y3P9_CANLF	GSN	85 k...		6	6	6	8	6	1	10	11
298			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=PAICS PE=3 SV=2	F1PPT7_CANLF	PAICS	50 k...		14	3	2	15	16	5	9	1
299			Latent transforming growth factor beta binding protein 1 OS=Canis lupus familiaris OX=9615 GN=LTBP1 PE=4 SV=1	F1PLA1_CANLF	LTBP1	170 ...		1	1	2	1	5	2	18	26
300			Adaptor related protein complex 2 alpha 2 subunit OS=Canis lupus familiaris OX=9615 GN=AP2A2 PE=4 SV=1	F1PD77_CANLF	AP2A2	95 k...	★	26	3	1	10	14	0	0	1
301			Discs large MAGUK scaffold protein 1 OS=Canis lupus familiaris OX=9615 GN=DLG1 PE=4 SV=1	E2RD72_CANLF	DLG1	109 ...		20	8	5	6	18	2		
302			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC484960 PE=4 SV=2	F1P707_CANLF	LOC484960	187 ...		0	1	0	0	2	2	6	7
303			F11 receptor OS=Canis lupus familiaris OX=9615 GN=F11R PE=4 SV=2	F1PP71_CANLF	F11R	33 k...		15	7	6	20	15	1	2	4
304			Glucose-6-phosphate isomerase OS=Canis lupus familiaris OX=9615 GN=GPI PE=3 SV=1	E2R2C3_CANLF	GPI	63 k...		18	4	1	11	10	0	3	2
305			Caveolin-1 OS=Canis lupus familiaris OX=9615 GN=CAV1 PE=1 SV=1	CAV1_CANLF	CAV1	21 k...		18	4	10	13	9	2	2	
306			40S ribosomal protein S18 OS=Canis lupus familiaris OX=9615 GN=RPS18 PE=1 SV=3	RS18_CANLF	RPS18	18 k...		5	7	4	13	11	4	13	20
307			Tubulin beta chain OS=Canis lupus familiaris OX=9615 GN=TUBB4B PE=3 SV=1	L7N0I7_CANLF	TUBB4B	50 k...	★	95	36	19	76	91	37	111	120
308			Nucleoside diphosphate kinase A OS=Canis lupus familiaris OX=9615 GN=NME1 PE=2 SV=1	NDKA_CANLF	NME1	17 k...	★	13	9	2	19	8	1		2
309			Annexin OS=Canis lupus familiaris OX=9615 GN=ANXA4 PE=3 SV=2	F1PXG4_CANLF	ANXA4	36 k...		17	7	11	12	9		2	0
310			RAB5B, member RAS oncogene family OS=Canis lupus familiaris OX=9615 GN=RAB5B PE=4 SV=1	E2QW69_CANLF	RAB5B	24 k...	★	17	4	8	15	12	3	5	4
311			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=RPL7 PE=4 SV=2	F1Q0Z2_CANLF	RPL7	29 k...	★	15	4	7	6	8	3	5	3
312			Capping actin protein of muscle Z-line beta subunit OS=Canis lupus familiaris OX=9615 GN=CAPZB PE=4 SV=1	F1PQ53_CANLF	CAPZB	41 k...		13	5	3	10	12	2	4	6
313			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=RPL5 PE=3 SV=2	F1P7B0_CANLF	RPL5	34 k...		9	5	0	2	7	2	2	30
314			Tetraspanin OS=Canis lupus familiaris OX=9615 GN=TSPAN6 PE=3 SV=1	F1PMP0_CANLF	TSPAN6	31 k...		17	2	9	10	19	3	5	
315			Septin 9 OS=Canis lupus familiaris OX=9615 GN=SEPT9 PE=3 SV=2	F1PB08_CANLF	SEPT9	64 k...		9	4	1	15	8	1	5	6
316			Argininosuccinate synthase 1 OS=Canis lupus familiaris OX=9615 GN=ASS1 PE=3 SV=1	E2QU08_CANLF	ASS1	47 k...			0	0	0	9	6	9	12
317			Serine/threonine-protein phosphatase OS=Canis lupus familiaris OX=9615 GN=PPP2CB PE=4 SV=1	F6X958_CANLF	PPP2CB	36 k...		9	3	4	6	17	5	10	9
318			Glycyl-tRNA synthetase OS=Canis lupus familiaris OX=9615 GN=GARS PE=4 SV=2	F1Q332_CANLF	GARS	89 k...		8	2	2	9	14	4	8	4
319			Aminopeptidase OS=Canis lupus familiaris OX=9615 GN=NPEPPS PE=3 SV=2	F1P7M0_CANLF	NPEPPS	97 k...		7	2		7	10	6	13	10
320			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=MUC1 PE=4 SV=2	F1Q3G6_CANLF	MUC1	56 k...		16	2	3	15	15	1	2	
321			Copine 3 OS=Canis lupus familiaris OX=9615 GN=CPNE3 PE=4 SV=1	E2QZQ6_CANLF	CPNE3	60 k...	★	18	8	11	11	7	1		2
322			Ubiquitin specific peptidase 42 OS=Canis lupus familiaris OX=9615 GN=USP42 PE=3 SV=2	E2RHU7_CANLF	USP42	147 ...			1	0		1	3	0	0
323			Cathepsin D OS=Canis lupus familiaris OX=9615 GN=CTSD PE=2 SV=1	CATD_CANLF	CTSD	44 k...		6	3	8	6	15	6	3	3
324			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=2	F1PD65_CANLF		53 k...	★	10	1	1	12	8	2	5	8
325			Myosin IE OS=Canis lupus familiaris OX=9615 GN=MYO1E PE=3 SV=2												

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
			Probability Legend:												
			over 95%												
			80% to 94%												
			50% to 79%												
			20% to 49%												
			0% to 19%												
426			Myosin VI OS=Canis lupus familiaris OX=9615 GN=MYO6 PE=3 SV=2	E2QUR8_CANLF	MYO6	145 ...		10	2	1	10	7		2	5
427			Stress induced phosphoprotein 1 OS=Canis lupus familiaris OX=9615 GN=STIP1 PE=4 SV=...	F1P7V6_CANLF	STIP1	63 k...		8	1	1	5	7		0	1
428			Protein tyrosine phosphatase, receptor type F OS=Canis lupus familiaris OX=9615 GN=PT...	E2RE81_CANLF	PTPRF	212 ...	★	13	4	3	2	6		1	6
429			ARP2 actin related protein 2 homolog OS=Canis lupus familiaris OX=9615 GN=ACTR2 PE...	E2QXY7_CANLF	ACTR2	45 k...		3	1	1	5	6	2	9	11
430			Fermitin family member 2 OS=Canis lupus familiaris OX=9615 GN=FERMT2 PE=4 SV=2	F1PF84_CANLF	FERMT2	72 k...		9	3	2	2	12	1	1	5
431			Claudin-3 OS=Canis lupus familiaris OX=9615 GN=CLDN3 PE=2 SV=1	CLD3_CANLF	CLDN3	23 k...	★	8	2	5	7	18	5	9	11
432			Copine 8 OS=Canis lupus familiaris OX=9615 GN=CPNE8 PE=4 SV=2	E2RNP3_CANLF	CPNE8	64 k...	★	12	2	6	7	7		1	1
433			Glypican 1 OS=Canis lupus familiaris OX=9615 GN=GPC1 PE=3 SV=1	F1PPA1_CANLF	GPC1	56 k...		13	2	4	7	6	1		
434			Inner centromere protein OS=Canis lupus familiaris OX=9615 GN=INCENP PE=4 SV=1	F6XU88_CANLF	INCENP	104 ...		2	3	3	4	5	4	7	7
435			60S ribosomal protein L15 OS=Canis lupus familiaris OX=9615 GN=RPL15 PE=1 SV=1	RL15_CANLF	RPL15	24 k...		6	3	2	4	4	1	5	3
436			Proteasome 26S subunit, ATPase 2 OS=Canis lupus familiaris OX=9615 GN=PSMC2 PE=3 ...	F1PPH7_CANLF	PSMC2	49 k...		7	2	1	1	5	1	9	3
437			Programmed cell death 6 OS=Canis lupus familiaris OX=9615 GN=PDCD6 PE=4 SV=2	F1Q1H3_CANLF	PDCD6	22 k...		13	5	7	11	7		1	2
438			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC100855730 PE=4 SV...	F6UL10_CANLF	LOC100855730	39 k...	★	6		1	8	3	0	6	4
439			Desmoglein 2 OS=Canis lupus familiaris OX=9615 GN=DSG2 PE=4 SV=1	F1PGX1_CANLF	DSG2	123 ...		14	3	5	4	6			
440			Ephrin B1 OS=Canis lupus familiaris OX=9615 GN=EFNB1 PE=3 SV=1	E2QS85_CANLF	EFNB1	35 k...		13	2	5	4	12	1	2	1
441			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC100855913 PE=4 SV...	J9NJK7_CANLF	LOC100855913	35 k...		10	3	10	10	8	2	0	5
442			Proteasome 26S subunit, non-ATPase 11 OS=Canis lupus familiaris OX=9615 GN=PSMD11 ...	E2RJ26_CANLF	PSMD11	47 k...		8	3	2	0	5	1	14	2
443			Eukaryotic translation initiation factor 5A OS=Canis lupus familiaris OX=9615 GN=EIF5A ...	F1Q264_CANLF	EIF5A	17 k...		12	6	4	8	8	2		1
444			Proliferating cell nuclear antigen OS=Canis lupus familiaris OX=9615 GN=PCNA PE=3 SV=1	E2R0D6_CANLF	PCNA	29 k...		6	1		7	7	1	4	10
445			Cationic amino acid transporter 1 OS=Canis lupus familiaris OX=9615 GN=SLC7A1 PE=2 ...	E2QU34_CANLF	SLC7A1	68 k...		14	4	4	7	7	1	1	
446			Syndecan 4 OS=Canis lupus familiaris OX=9615 GN=SDC4 PE=4 SV=1	J9NZ79_CANLF	SDC4	22 k...		18	8	5	3	12	0	3	
447			Core histone macro-H2A OS=Canis lupus familiaris OX=9615 GN=H2AFY PE=4 SV=1	E2RCZ7_CANLF	H2AFY	40 k...		19	3	4	11	8		1	0
448			N-Myc downstream regulated gene 1 OS=Canis lupus familiaris OX=9615 GN=NDRG1 PE...	E2RMB9_CANLF	NDRG1	42 k...		15	5	4	7	10			3
449			Transient receptor potential cation channel subfamily V member 4 OS=Canis lupus familia...	F6PLL5_CANLF	GLTP	103 ...		9	3	7	8	10			1
450			Galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase OS=Canis lupus famil...	F1Q4I9_CANLF	B3GAT3	37 k...		1		1		10	6	10	14
451			Hepatocyte growth factor-regulated tyrosine kinase substrate OS=Canis lupus familiaris ...	E2QWE3_CANLF	HGS	86 k...		2	2	5	20	1			3
452			Rac GTPase activating protein 1 OS=Canis lupus familiaris OX=9615 GN=RACGAP1 PE=4 SV...	E2R3Q1_CANLF	RACGAP1	72 k...		5	4	5	10	7	0		
453			Plexin A2 OS=Canis lupus familiaris OX=9615 GN=PLXNA2 PE=4 SV=2	F1PHZ1_CANLF	PLXNA2	221 ...	★	20	3	2	7	9		0	
454			Tetraspanin OS=Canis lupus familiaris OX=9615 GN=TSPAN9 PE=3 SV=2	E2R806_CANLF	TSPAN9	30 k...		15	4	4	6	15	2	2	1
455			DnaJ heat shock protein family (Hsp40) member C5 OS=Canis lupus familiaris OX=9615 ...	E2RJ89_CANLF	DNAJC5	22 k...		9	4	3	5	10	3	1	0
456			S-adenosylmethionine synthase OS=Canis lupus familiaris OX=9615 GN=MAT2A PE=3 SV...	E2R431_CANLF	MAT2A	44 k...	★	4		2	4	3	3	7	13
457			Serpin family D member 1 OS=Canis lupus familiaris OX=9615 GN=SERPIND1 PE=3 SV=1	E2RLN1_CANLF	SERPIND1	57 k...		0	0	0	0	0	0	5	8
458			CUB domain containing protein 1 OS=Canis lupus familiaris OX=9615 GN=CDCP1 PE=4 SV...	F1PV63_CANLF	CDCP1	92 k...		11	4	3	5	7	1		
459			Lipase G, endothelial type OS=Canis lupus familiaris OX=9615 GN=LIPG PE=3 SV=2	E2R774_CANLF	LIPG	57 k...		1	0			10	6	8	13
460			Betaine--homocysteine S-methyltransferase OS=Canis lupus familiaris OX=9615 GN=BH...	E2RSW1_CANLF	BHMT	45 k...	★					1	1	18	28
461			40S ribosomal protein S11 OS=Canis lupus familiaris OX=9615 GN=RPS11 PE=3 SV=1	F2Z4N5_CANLF	RPS11	18 k...		2	2	2	5	7	2	1	13
462			Adenine phosphoribosyltransferase OS=Canis lupus familiaris OX=9615 GN=APRT PE=3 SV...	E2QYQ0_CANLF	APRT	20 k...		3	2		5	11	2	4	12
463			Proteasome endopeptidase complex OS=Canis lupus familiaris OX=9615 GN=PSMA7 PE=...	F1PUB5_CANLF	PSMA7	25 k...	★	11	3	3	7	8	1		2
464			Peptidyl-prolyl cis-trans isomerase OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9NV93_CANLF		17 k...	★	21	17	14	19	22	15		10
465			Keratin 5 OS=Canis lupus familiaris OX=9615 GN=KRT5 PE=3 SV=2	E2R8Z5_CANLF	KRT5	63 k...	★	17	5	9	5	6	3		
466			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC100855540 PE=3 SV...	J9JHF7_CANLF (...	LOC100855540	15 k...		1	2	2	2	3	2	3	4
467			Actin-related protein 2/3 complex subunit OS=Canis lupus familiaris OX=9615 GN=ARPC...	E2RMT4_CANLF	ARPC18	41 k...	★	4	2	1	8	4	1	1	9
468			Regucalcin OS=Canis lupus familiaris OX=9615 GN=RGN PE=4 SV=1	E2R2G3_CANLF	RGN	33 k...		1	3	2		7	6	16	8
469			Guanine nucleotide-binding protein subunit alpha-11 (Fragment) OS=Canis lupus familia...	GNA11_CANLF	GNA11	23 k...	★	15	4	4	7	6		1	1
470			Eukaryotic translation initiation factor 3 subunit A OS=Canis lupus familiaris OX=9615 GN...	E2RSR5_CANLF	EIF3A	163 ...		7	5	0	4	13	5	0	3
471			Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2 OS=Canis lup...	A0A1W2NCW3_...	RPN2	71 k...		14	8	15	4	4	1		
472			Annexin OS=Canis lupus familiaris OX=9615 GN=ANXA7 PE=3 SV=2	E2QXD1_CANLF	ANXA7	53 k...		15	2	6	9	12			
473			Beta-2-glycoprotein 1 OS=Canis lupus familiaris OX=9615 GN=APOH PE=2 SV=1	APOH_CANLF	APOH	38 k...		5	5	2	4	5	5	2	9
474			DEAD-box helicase 3, X-linked OS=Canis lupus familiaris OX=9615 GN=DDX3X PE=3 SV...	E2RRQ7_CANLF	DDX3X	78 k...		10	3	4	5	5	1	6	4
475			Ribosomal protein S5 OS=Canis lupus familiaris OX=9615 GN=RP55 PE=3 SV=1	E2RIQ8_CANLF	RP55	23 k...		4	2	1	3	8	3	2	11
476			Threonyl-tRNA synthetase OS=Canis lupus familiaris OX=9615 GN=TARS PE=3 SV=1	E2RHW7_CANLF	TARS	84 k...		4	2		4	9	4	4	9
477			Surfactant protein D OS=Canis lupus familiaris OX=9615 GN=SFTPD PE=4 SV=1	E2QWRO_CANLF	SFTPD	38 k...		3	2		20	9		2	9
478			Protein tweety homolog OS=Canis lupus familiaris OX=9615 GN=TTYH3 PE=3 SV=1	J9P3X8_CANLF	TTYH3	57 k...		11	4	2	5	11			2
479			Plakophilin 2 OS=Canis lupus familiaris OX=9615 GN=PKP2 PE=4 SV=1	E2RHY0_CANLF	PKP2	93 k...		11	2	8	7	10		1	
480			Protein S100-A4 OS=Canis lupus familiaris OX=9615 GN=S100A4 PE=3 SV=1	S10A4_CANLF	S100A4	12 k...		7	4	2	12	6	1	2	3
481			Prostaglandin reductase 1 OS=Canis lupus familiaris OX=9615 GN=PTGR1 PE=4 SV=2	E2R002_CANLF	PTGR1	36 k...		8	3	1	4	10	4	1	2
482			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	F1PRF6_CANLF		772 ...		0	1	0	0	1	1	0	
483			Calcium binding protein 39 OS=Canis lupus familiaris OX=9615 GN=CAB39 PE=4 SV=1	E2R8W7_CANLF	CAB39	40 k...		9	4	5	4	7	1		
484			Claudin OS=Canis lupus familiaris OX=9615 GN=CLDN7 PE=3 SV=2	E2R5S3_CANLF	CLDN7	22 k...		5		2	2	17	1	4	
485			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9PAJ6_CANLF		16 k...		4	6	10		3	6	2	
486			Transmembrane protein 43 OS=Canis lupus familiaris OX=9615 GN=TMEM43 PE=4 SV=1	F1PPC9_CANLF	TMEM43	45 k...		12	3	12	5	4		0	
487			Testis expressed 15, meiosis and synapsis associated OS=Canis lupus familiaris OX=961...	F1PRB7_CANLF	TEX15	363 ...		1	8	2		0	3	5	
488			DNA topoisomerase I OS=Canis lupus familiaris OX=9615 GN=TOP1 PE=4 SV=2	F1PUE0_CANLF	TOP1	91 k...		13	2		7	5			1
489			Talin 2 OS=Canis lupus familiaris OX=9615 GN=TLN2 PE=4 SV=2	E2RSC5_CANLF	TLN2	272 ...	★	22	2	7	3	9		8	
490			Proprotein convertase subtilisin/kexin type 5 OS=Canis lupus familiaris OX=9615 GN=PC...	J9PB88_CANLF	PCSK5	199 ...						5	5	3	17
491			Inter-alpha-trypsin inhibitor heavy chain family member 4 OS=Canis lupus familiaris OX...	H9GWY1_CANLF	ITIHA	119 ...		1	6	6	3	4	5	3	7
492			60S ribosomal protein L3 OS=Canis lupus familiaris OX=9615 GN=RPL3 PE=3 SV=1	F1PLN2_CANLF ...	RPL3	46 k...		13	0	3	2	3		1	1
493			Staphylococcal nuclease and tudor domain containing 1 OS=Canis lupus familiaris OX=96...	E2RH91_CANLF	SND1	102 ...		2		1	3	3	3	6	11
494			Carnosine dipeptidase 2 OS=Canis lupus familiaris OX=9615 GN=CNDP2 PE=4 SV=2	E2R134_CANLF	CNDP2	53 k...		2			1	6	1	9	12
495			Histone H2A OS=Canis lupus familiaris OX=9615 GN=H2AFX PE=3 SV=1	E2RNB0_CANLF	H2AFX	15 k...	★	30	11	10	12	12			11
496			Nucleolin OS=Canis lupus familiaris OX=9615 GN=NCL PE=4 SV=2	F1Q0B0_CANLF	NCL	77 k...		15	4	0	10	5	3	1	
497			Dynamin 1 like OS=Canis lupus familiaris OX												

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
								Probability Legend:							
								over 95%							
								80% to 94%							
								50% to 79%							
								20% to 49%							
								0% to 19%							
511			Leukotriene A(4) hydrolase OS=Canis lupus familiaris OX=9615 GN=LTA4H PE=3 SV=1	F6Y290_CANLF	LTA4H	79 k...		9	1		3	9	3		5
512			EGF containing fibulin like extracellular matrix protein 1 OS=Canis lupus familiaris OX=9615 GN=LOC611500 PE=3 SV=2	E2R612_CANLF	EFEMP1	55 k...			1		1	4	6	6	24
513			Deoxyribonuclease OS=Canis lupus familiaris OX=9615 GN=DNASE1L1 PE=3 SV=2	F1PWT3_CANLF	DNASE1L1	33 k...		17	4	3	5	8	0		
514			Spondin 1 OS=Canis lupus familiaris OX=9615 GN=SPON1 PE=4 SV=1	F6XC28_CANLF	SPON1	91 k...		4	7		17	5	0		2
515			Collagen type XII alpha 1 chain OS=Canis lupus familiaris OX=9615 GN=COL12A1 PE=4 SV=1	J9P6G0_CANLF	COL12A1	333 k...		0	22		0	0	1	0	1
516			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=RPS25 PE=4 SV=2	E2RS49_CANLF	RPS25	14 k...		5	9	5	8	7	1	1	4
517			Phospholipid scramblase OS=Canis lupus familiaris OX=9615 GN=LOC611500 PE=3 SV=2	F1PIU9_CANLF	LOC611500	38 k...		8	3	3	5	6	2	0	1
518			Calpain 2 OS=Canis lupus familiaris OX=9615 GN=CAPN2 PE=3 SV=2	F1P975_CANLF	CAPN2	76 k...		12	1	0	6	3	1	3	1
519			Trifunctional purine biosynthetic protein adenosine-3 OS=Canis lupus familiaris OX=9615 GN=LOC100856570 PE=3 SV=1	F1PTG8_CANLF	LOC100856570	112 k...		5	1		9	6	1	0	5
520			Echinoderm microtubule associated protein like 2 OS=Canis lupus familiaris OX=9615 GN=LOC611500 PE=3 SV=2	E2RII4_CANLF	EMIL2	89 k...		0		1	3	4	5	8	14
521			Phosphoinositide phospholipase C OS=Canis lupus familiaris OX=9615 GN=PLCD1 PE=4 SV=1	F1P729_CANLF	PLCD1	97 k...		14	2	1	5	8		0	
522			Tropomyosin 4 OS=Canis lupus familiaris OX=9615 GN=TPM4 PE=3 SV=1	F6X7L0_CANLF	TPM4	33 k...	★	8	5	2	9	5		0	
523			Farnesyl diphosphate synthase OS=Canis lupus familiaris OX=9615 GN=FDPS PE=3 SV=2	F1P926_CANLF	FDPS	48 k...		9	1	1	6	3	0	4	1
524			Inter-alpha-trypsin inhibitor heavy chain 3 OS=Canis lupus familiaris OX=9615 GN=ITIH3 PE=3 SV=1	H9GWG4_CANLF	ITIH3	100 k...		3	7	9		3	2	2	5
525			Tight junction protein ZO-2 OS=Canis lupus familiaris OX=9615 GN=TJP2 PE=1 SV=1	ZO2_CANLF	TJP2	132 k...	★	7	1	2	1	11	1	1	
526			Carboxypeptidase B2 OS=Canis lupus familiaris OX=9615 GN=CPB2 PE=4 SV=1	E2RN74_CANLF	CPB2	49 k...		1	2	2		6	4	3	7
527			Ubiquitin specific peptidase 14 OS=Canis lupus familiaris OX=9615 GN=USP14 PE=3 SV=2	E2RPS0_CANLF	USP14	61 k...		3	1		1	5	0	10	5
528			Tight junction protein ZO-1 OS=Canis lupus familiaris OX=9615 GN=TJP1 PE=1 SV=1	ZO1_CANLF	TJP1	198 k...		7	3	2	0	16	1		
529			Tubulin alpha chain OS=Canis lupus familiaris OX=9615 GN=TUBA4A PE=3 SV=1	E2R8C3_CANLF	TUBA4A	50 k...	★	50	30	34	56	76	47	58	72
530			Importin 7 OS=Canis lupus familiaris OX=9615 GN=IPO7 PE=4 SV=1	F6X4J2_CANLF	IPO7	120 k...		8	2	1	5	9	2	1	5
531			EP58 like 2 OS=Canis lupus familiaris OX=9615 GN=EP58L2 PE=4 SV=2	E2RIE6_CANLF	EP58L2	63 k...		10	2	2	6	4	1	0	
532			Anion exchange protein OS=Canis lupus familiaris OX=9615 GN=SLC4A7 PE=3 SV=2	F1PTY0_CANLF	SLC4A7	138 k...		11	5	11	8	5	1	0	
533			PPFIA binding protein 1 OS=Canis lupus familiaris OX=9615 GN=PPFIBP1 PE=4 SV=1	E2QXJ7_CANLF	PPFIBP1	114 k...		9	2	0	6	10	0	0	1
534			Mitogen-activated protein kinase kinase 1 OS=Canis lupus familiaris OX=9615 GN=MAP2K1 PE=3 SV=1	J9P5W6_CANLF	MAP2K1	43 k...		0	0	0	0	2	1	10	3
535			Folate receptor beta OS=Canis lupus familiaris OX=9615 GN=FOLR2 PE=4 SV=1	E2QXF0_CANLF	FOLR2	30 k...		15	1	1	2	15	0		
536			3-hydroxy-3-methylglutaryl coenzyme A synthase OS=Canis lupus familiaris OX=9615 GN=LOC611500 PE=3 SV=2	E2QX73_CANLF	HMGCS1	57 k...		5	5	1	2	4	2	7	9
537			Poly(rC) binding protein 1 OS=Canis lupus familiaris OX=9615 GN=PCBP1 PE=4 SV=1	J9P7Y9_CANLF	PCBP1	37 k...	★	8	2	3	8	3	3	4	2
538			Afamin OS=Canis lupus familiaris OX=9615 GN=AFM PE=4 SV=2	E2R4E7_CANLF	AFM	70 k...		1	9	4		3	4	3	4
539			Proteasome 26S subunit, ATPase 5 OS=Canis lupus familiaris OX=9615 GN=PSMC5 PE=4 SV=1	E2RM61_CANLF	PSMC5	28 k...		2		1	2	6	4	8	5
540			Proteasome subunit alpha type OS=Canis lupus familiaris OX=9615 GN=PSMA6 PE=3 SV=1	E2RMN2_CANLF	PSMA6	27 k...		12	4	3	5	8	1		1
541			Proteasome 26S subunit, non-ATPase 3 OS=Canis lupus familiaris OX=9615 GN=PSMD3 PE=3 SV=1	E2QUY7_CANLF	PSMD3	61 k...		9	1		5	6	1	7	8
542			Leucine aminopeptidase 3 OS=Canis lupus familiaris OX=9615 GN=LAP3 PE=3 SV=2	E2RLY5_CANLF	LAP3	56 k...		4	1		5	2	1	14	8
543			Aldehyde dehydrogenase 9 family member A1 OS=Canis lupus familiaris OX=9615 GN=ALDH9A1 PE=3 SV=1	F1PAB7_CANLF	ALDH9A1	54 k...		2		0	1	2	3	9	14
544			Exportin 1 OS=Canis lupus familiaris OX=9615 GN=XPO1 PE=4 SV=2	E2R9K4_CANLF	XPO1	123 k...		6			1	3	2	6	6
545			ATP binding cassette subfamily E member 1 OS=Canis lupus familiaris OX=9615 GN=ABCE1 PE=3 SV=1	F6X725_CANLF	ABCE1	67 k...		2		3		7	1	2	14
546			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC477558 PE=4 SV=1	J9NUJ7_CANLF	LOC477558	28 k...		3			5	8	0	2	9
547			Alpha-centractin OS=Canis lupus familiaris OX=9615 GN=ACTR1A PE=2 SV=1	ACTZ_CANLF	ACTR1A	43 k...	★	4	1	0	1	4	1	4	11
548			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=EIF253 PE=4 SV=1	E2RMK6_CANLF	EIF253	51 k...		4	0		3	5	2	5	7
549			ATPase H+ transporting accessory protein 2 OS=Canis lupus familiaris OX=9615 GN=ATP6A2 PE=3 SV=1	E2RJH2_CANLF	ATP6A2	39 k...		3	3	6	8	3		0	4
550			Biglycan OS=Canis lupus familiaris OX=9615 GN=BGN PE=3 SV=1	G1K2D8_CANLF...	BGN	42 k...		2	1		1	7	2	6	17
551			Dynamin 2 OS=Canis lupus familiaris OX=9615 GN=DNM2 PE=3 SV=2	E2RNC3_CANLF	DNM2	98 k...		2	0		1	4	3	4	6
552			Mitogen-activated protein kinase OS=Canis lupus familiaris OX=9615 GN=MAPK1 PE=3 SV=1	E2R2N2_CANLF	MAPK1	41 k...	★	2	0	0	2	8	3	9	6
553			Protein kinase C and casein kinase substrate in neurons 3 OS=Canis lupus familiaris OX=9615 GN=PKCIP3 PE=3 SV=1	F1Q436_CANLF	PACSN3	49 k...	★	8	5	2	9	7	0		2
554			Signal transducing adaptor molecule OS=Canis lupus familiaris OX=9615 GN=STAM PE=4 SV=1	E2RNS0_CANLF	STAM	59 k...	★	8	3		9	2	1		4
555			Interleukin-13 receptor subunit alpha-2 OS=Canis lupus familiaris OX=9615 GN=IL13RA2 PE=3 SV=1	IL13R2_CANLF	IL13RA2	45 k...		7	2	4	9	3	2	1	3
556			Eukaryotic translation initiation factor 3 subunit L OS=Canis lupus familiaris OX=9615 GN=EIF3L PE=3 SV=1	F1PHR6_CANLF	EIF3L	67 k...		4	1		1	3	4	3	12
557			Ubiquitinyl hydrolase 1 OS=Canis lupus familiaris OX=9615 GN=USP5 PE=3 SV=2	F1P9P5_CANLF	USP5	96 k...	★	9	1		2	4	1	6	3
558			Ras-related protein Rab-8A OS=Canis lupus familiaris OX=9615 GN=RAB8A PE=1 SV=1	RAB8A_CANLF	RAB8A	24 k...	★	10	6	9	18	10		1	8
559			Desmoglein-3 OS=Canis lupus familiaris OX=9615 GN=DSG3 PE=2 SV=1	DSG3_CANLF	DSG3	108 k...		9	4	4	7	3	2	0	
560			Galactokinase OS=Canis lupus familiaris OX=9615 GN=GALK1 PE=2 SV=1	GALK1_CANLF	GALK1	42 k...			1	2	0	5	6	8	7
561			Septin 6 OS=Canis lupus familiaris OX=9615 GN=SEPT6 PE=3 SV=2	F1PM93_CANLF	SEPT6	50 k...	★	9	3	1	12	4		6	8
562			Desmoplakin OS=Canis lupus familiaris OX=9615 GN=DSP PE=4 SV=2	E2RNG4_CANLF	DSP	332 k...		7	0	4	1	4	1	1	
563			Proteasome subunit alpha type OS=Canis lupus familiaris OX=9615 GN=PSMA6 PE=3 SV=1	E2R1C3_CANLF		28 k...		16	4	2	2	11	1		
564			RNA binding motif protein 6 OS=Canis lupus familiaris OX=9615 GN=RBM6 PE=4 SV=1	E2R8Z4_CANLF	RBM6	128 k...		0	2	2		1	4	1	
565			Coagulation factor IX OS=Canis lupus familiaris OX=9615 GN=F9 PE=1 SV=1	FA9_CANLF (+1)	F9	51 k...		1	0		0	12	6	6	8
566			Tyrosine-protein kinase receptor OS=Canis lupus familiaris OX=9615 GN=IGF1R PE=3 SV=1	F1PXU6_CANLF	IGF1R	155 k...		8	1		2	7	1		
567			Armado repeat containing 2 OS=Canis lupus familiaris OX=9615 GN=ARMC2 PE=4 SV=2	F1PD78_CANLF	ARMC2	96 k...		5	0	0		12	0		
568			Vasorin OS=Canis lupus familiaris OX=9615 GN=VASN PE=4 SV=1	E2RRN8_CANLF	VASN	71 k...		6	1	2	0	6	4	6	7
569			Arginyl-tRNA synthetase OS=Canis lupus familiaris OX=9615 GN=RARS PE=3 SV=1	E2QRR5_CANLF	RARS	76 k...		5	3		4	8	2	1	9
570			Hepsin OS=Canis lupus familiaris OX=9615 GN=HPN PE=3 SV=2	E2RKP5_CANLF	HPN	45 k...		10	3	2	2	8	1	1	
571			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=TNS3 PE=4 SV=2	F1PQ78_CANLF	TNS3	153 k...	★	12	4	3	3	6	1	1	
572			Chromosome segregation 1 like OS=Canis lupus familiaris OX=9615 GN=CSE1L PE=4 SV=1	F1Q1V0_CANLF	CSE1L	111 k...		3	1		0	4	4	2	6
573			Nidogen 1 OS=Canis lupus familiaris OX=9615 GN=NID1 PE=4 SV=2	F1PKX3_CANLF	NID1	136 k...		1	4	3	21	1		1	3
574			Pre-mRNA processing factor 19 OS=Canis lupus familiaris OX=9615 GN=PRPF19 PE=4 SV=1	E2R8L3_CANLF	PRPF19	56 k...		0	0	0	1	6	0	3	8
575			Voltage dependent anion channel 1 OS=Canis lupus familiaris OX=9615 GN=VDAC1 PE=4 SV=1	F1PC72_CANLF	VDAC1	32 k...		8	5	10	4	1			
576			Claudin OS=Canis lupus familiaris OX=9615 GN=CLDN6 PE=3 SV=1	L7N016_CANLF	CLDN6	23 k...	★	8	3	5	6	16	7	7	9
577			Actin-related protein 2/3 complex subunit 4 OS=Canis lupus familiaris OX=9615 GN=ARPC4 PE=3 SV=1	E2QWU0_CANLF	ARPC4	20 k...		7	2	1	7	3	2	2	6
578			60S ribosomal protein L12 OS=Canis lupus familiaris OX=9615 GN=RPL12 PE=1 SV=1	RL12_CANLF	RPL12	18 k...		4	1	1	4	3	3	5	13
579			Ankyrin 3 OS=Canis lupus familiaris OX=9615 GN=ANK3 PE=4 SV=2	F1PJ98_CANLF	ANK3	480 k...		7	1	2	1	1		1	1
580			Reticulon OS=Canis lupus familiaris OX=9615 GN=RTN4 PE=4 SV=1	J9POR7_CANLF	RTN4	131 k...		4	0	6	2	5	0	2	0
581															

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
								Probability Legend:							
								over 95%							
								80% to 94%							
								50% to 79%							
								20% to 49%							
								0% to 19%							
596			Family with sequence similarity 49 member B OS=Canis lupus familiaris OX=9615 GN=FA...	E2RCY1_CANLF	FAM49B	37 k...		10	3	3	7	6	0		
597			Aminopeptidase OS=Canis lupus familiaris OX=9615 GN=ENPEP PE=3 SV=1	F6XRM5_CANLF	ENPEP	109 ...		6	2	1	5	4			
598			N(alpha)-acetyltransferase 15, NatA auxiliary subunit OS=Canis lupus familiaris OX=9615...	F1PFN4_CANLF	NAA15	99 k...			0			1	0	4	4
599			Adaptor related protein complex 2 mu 1 subunit OS=Canis lupus familiaris OX=9615 GN=...	F1PQ79_CANLF	AP2M1	50 k...		8	0	0	1	3			
600			Transcription elongation factor spt6 OS=Canis lupus familiaris OX=9615 GN=SUPT6H PE=...	E2R4A3_CANLF	SUPT6H	199 ...		1	2	1	3	2	2	7	6
601			Galactose-3-O-sulfotransferase 2 OS=Canis lupus familiaris OX=9615 GN=GAL3ST2 PE=...	F1P199_CANLF	GAL3ST2	46 k...		1	1	1	1	10	5	2	8
602			Serine incorporator 5 OS=Canis lupus familiaris OX=9615 GN=SERINC5 PE=4 SV=1	F6Y470_CANLF	SERINC5	51 k...		6	0	5	7	6	1	3	3
603			Calmodulin 1 OS=Canis lupus familiaris OX=9615 GN=CALM1 PE=4 SV=1	E2REK6_CANLF	CALM1	17 k...		7	4	1	7	6	2	1	
604			Feline leukemia virus subgroup C cellular receptor 1 OS=Canis lupus familiaris OX=9615 ...	E2RSI2_CANLF	FLVCR1	60 k...		9	5	4	5	5	0		1
605			Proteasome 26S subunit, non-ATPase 13 OS=Canis lupus familiaris OX=9615 GN=PSMD1...	E2RSN5_CANLF	PSMD13	43 k...		4	0	0	0	2	1	10	2
606			Serine hydroxymethyltransferase OS=Canis lupus familiaris OX=9615 GN=SHMT1 PE=3 S...	E2RIV3_CANLF	SHMT1	53 k...		2			1	5	2	2	13
607			Parvin alpha OS=Canis lupus familiaris OX=9615 GN=PARVA PE=4 SV=1	J9NV14_CANLF	PARVA	43 k...		10	3	2	5	6		2	
608			Proteasome subunit beta type OS=Canis lupus familiaris OX=9615 GN=PSMB5 PE=3 SV=1	E2R3R2_CANLF	PSMB5	29 k...		13	2	2	3	9	1		
609			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=1	E2RNQ7_CANLF		21 k...	★	22	8	8	10	16	8	3	10
610			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=RPL14 PE=4 SV=2	F1PF85_CANLF	RPL14	23 k...		8	2	2	2	5	2	6	3
611			Serpin family F member 2 OS=Canis lupus familiaris OX=9615 GN=SERPINF2 PE=3 SV=1	F6Y470_CANLF	SERPINF2	55 k...		3	3	3	1	5	3	4	8
612			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC610164 PE=3 SV=1	J9P7P2_CANLF	LOC610164	31 k...	★	4	2		8	3	3	2	9
613			Malate dehydrogenase OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9NY79_CANLF		35 k...		5	3	9	2	1	3	3	
614			NRAS proto-oncogene, GTPase OS=Canis lupus familiaris OX=9615 GN=NRAS PE=4 SV=2	F1PHV7_CANLF	NRAS	21 k...	★	8	4	2	9	4		1	1
615			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9P3R3_CANLF		43 k...	★	32	10	15	43	22	13	16	16
616			Tubulin beta chain OS=Canis lupus familiaris OX=9615 GN=TUBB6 PE=3 SV=1	E2QYC2_CANLF	TUBB6	50 k...	★	31	18	6	32	42		49	56
617			Solute carrier family 7 member 5 OS=Canis lupus familiaris OX=9615 GN=SLC7A5 PE=4 S...	F1PH98_CANLF	SLC7A5	56 k...	★	5	3	3	4	2		0	2
618			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	H9GWE2_CANLF		34 k...		4	4		8	4	0	7	4
619			Ras homolog family member G OS=Canis lupus familiaris OX=9615 GN=RHOGE PE=4 SV=2	E2R020_CANLF	RHOG	28 k...	★	12	3	5	6	7		5	1
620			Alanyl-tRNA synthetase OS=Canis lupus familiaris OX=9615 GN=AARS PE=3 SV=2	F1P7C2_CANLF	AARS	107 ...		3	1		1	7	1	5	0
621			Asparaginyl-tRNA synthetase OS=Canis lupus familiaris OX=9615 GN=NARS PE=4 SV=1	E2QV97_CANLF	NARS	64 k...		4			2	4	2	6	7
622			Collagen alpha-1(I) chain OS=Canis lupus familiaris OX=9615 GN=COL1A1 PE=1 SV=1	CO1A1_CANLF (...COL1A1		139 ...		3	6	3	0	6			2
623			Carboxypeptidase N subunit 1 OS=Canis lupus familiaris OX=9615 GN=CPN1 PE=4 SV=1	E2RNG3_CANLF	CPN1	52 k...		0		1	1	6	2	14	10
624			Proteasome subunit alpha type OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9P9G4_CANLF		29 k...	★	10	1		2	9	0	0	1
625			Amyloid beta precursor like protein 2 OS=Canis lupus familiaris OX=9615 GN=APLP2 PE=...	E2RPM5_CANLF	APLP2	87 k...		4	7	4		3	1		
626			Mitogen-activated protein kinase kinase kinase 4 OS=Canis lupus familiaris OX=9...	F1P5C1_CANLF	MAP4K4	152 ...	★	10	2	5	1	2			
627			Protein disulfide-isomerase A4 OS=Canis lupus familiaris OX=9615 GN=PDIA4 PE=3 SV=2	E2R7L1_CANLF	PDIA4	72 k...		8	2	10		2	1		
628			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=FARS8 PE=4 SV=1	F6XQ20_CANLF	FARS8	66 k...		1			0	2	0	5	13
629			Fibroblast growth factor binding protein 1 OS=Canis lupus familiaris OX=9615 GN=FGFBP...	E2RLM9_CANLF	FGFBP1	29 k...		15	2			7	2		
630			Heterogeneous nuclear ribonucleoprotein K OS=Canis lupus familiaris OX=9615 GN=HNR...	E2RA50_CANLF	HNRNPK	48 k...	★	1	2	2	5	6	2	4	5
631			Carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase OS=...	E2RAV2_CANLF	CAD	244 ...	★	4	2	1	1	4	1	2	7
632			Actin-related protein 2/3 complex subunit 3 OS=Canis lupus familiaris OX=9615 PE=3 SV...	J9P309_CANLF		21 k...		5	1	0	2	3	1	2	3
633			Mannose-6-phosphate receptor, cation dependent OS=Canis lupus familiaris OX=9615 G...	E2R4C1_CANLF	M6PR	43 k...		7	4	5	4	5	2	3	
634			40S ribosomal protein S7 OS=Canis lupus familiaris OX=9615 GN=RPS7 PE=3 SV=2	E2R8A8_CANLF	RPS7	21 k...		4	5	1	2	2	2		1
635			Ras-related protein Rab-2A OS=Canis lupus familiaris OX=9615 GN=RAB2A PE=4 SV=2	G1K288_CANLF...	RAB2A	26 k...		8		3	7	3	3	1	5
636			Polypeptide N-acetylgalactosaminyltransferase OS=Canis lupus familiaris OX=9615 GN=...	F1P835_CANLF	GALNT2	61 k...	★	5	1	5	5	4		1	1
637			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=PLOD1 PE=4 SV=2	F1P9Z8_CANLF	PLOD1	84 k...	★		5		3	4	2	2	15
638			Prohibitin 2 OS=Canis lupus familiaris OX=9615 GN=PHB2 PE=4 SV=2	E2RGN6_CANLF	PHB2	33 k...		5	5	16	1	1		0	
639			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=CSNK2A1 PE=3 SV=1	E2RHZ1_CANLF	CSNK2A1	45 k...		11	2	2	3	2	1		
640			Acetyl-CoA carboxylase alpha OS=Canis lupus familiaris OX=9615 GN=ACACA PE=4 SV=2	E2RL01_CANLF	ACACA	265 ...		5	0	0		6	1	1	4
641			LLGL1, scribble cell polarity complex component OS=Canis lupus familiaris OX=9615 GN=...	E2RPS3_CANLF	LLGL1	125 ...		5	3	1	4	4		0	
642			Disco interacting protein 2 homolog B OS=Canis lupus familiaris OX=9615 GN=DIP2B PE...	F6X907_CANLF	DIP2B	172 ...		8	1	7	3	4			
643			Stress-70 protein, mitochondrial OS=Canis lupus familiaris OX=9615 GN=HSPA9 PE=3 SV...	E2RAU5_CANLF	HSPA9	74 k...	★	8	0	12	0		0	0	
644			60S ribosomal protein L18a OS=Canis lupus familiaris OX=9615 GN=RPL18A PE=3 SV=1	E2RL34_CANLF ...	RPL18A	21 k...		5	1	3	2	3	0	1	2
645			Kinesin-like protein OS=Canis lupus familiaris OX=9615 GN=KIF5B PE=3 SV=1	E2QTN0_CANLF	KIF5B	110 ...	★	9	2	2	4	4	2	1	
646			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC100687259 PE=3 SV...	J9P1N0_CANLF	LOC100687259	45 k...		5	2		2	3	2	9	1
647			Glutathione S-transferase omega 1 OS=Canis lupus familiaris OX=9615 GN=GSTO1 PE=4 ...	F1PUM3_CANLF	GSTO1	27 k...		7	2		8	3	1	3	2
648			Golgin A7 OS=Canis lupus familiaris OX=9615 GN=GOLGA7 PE=4 SV=1	E2R2A1_CANLF	GOLGA7	16 k...		11	1	5	4	5	2		0
649			Lactoylglutathione lyase OS=Canis lupus familiaris OX=9615 GN=GLO1 PE=3 SV=1	J9NRV6_CANLF	GLO1	21 k...		2			1	3	2	3	3
650			Ribosomal protein OS=Canis lupus familiaris OX=9615 GN=RPL10A PE=3 SV=2	E2RGV0_CANLF	RPL10A	25 k...		2		0	2	4	1	10	8
651			EPH receptor B4 OS=Canis lupus familiaris OX=9615 GN=EPHB4 PE=3 SV=2	F1PRQ8_CANLF	EPHB4	108 ...	★	7	2	2		4	0	1	
652			Syntaxin binding protein 3 OS=Canis lupus familiaris OX=9615 GN=STXB3 PE=3 SV=1	E2QUA6_CANLF	STXB3	68 k...		9	0	0	2	5			
653			Nucleoside diphosphate kinase B OS=Canis lupus familiaris OX=9615 GN=NME2 PE=2 SV...	NDKB_CANLF	NME2	17 k...	★	10	3		11	2	0		
654			Amino acid transporter OS=Canis lupus familiaris OX=9615 GN=SLC1A1 PE=3 SV=1	F1PUW7_CANLF	SLC1A1	57 k...		18	2	1	0	8			
655			Olfactomedin like 3 OS=Canis lupus familiaris OX=9615 GN=OLFM3 PE=4 SV=2	F1PQV4_CANLF	OLFM3	46 k...						1	4	8	10
656			Hypoxanthine phosphoribosyltransferase OS=Canis lupus familiaris OX=9615 GN=HPRT1 ...	F1PLF4_CANLF (...HPRT1		25 k...		7	5	1	5	6	4	2	2
657			60S ribosomal protein L13a OS=Canis lupus familiaris OX=9615 GN=RPL13A PE=1 SV=2	RL13A_CANLF	RPL13A	23 k...		4	2	1	4	2	2	3	4
658			Tissue specific transplantation antigen P35B OS=Canis lupus familiaris OX=9615 GN=TST...	E2RM30_CANLF	TSTA3	36 k...		6	2		4	2	1	1	7
659			Coagulation factor XIII A chain OS=Canis lupus familiaris OX=9615 GN=F13A1 PE=4 SV=2	F1PKX3_CANLF	F13A1	83 k...		1		1	7	2	1	4	5
660			Claudin domain containing 1 OS=Canis lupus familiaris OX=9615 GN=CLDND1 PE=4 SV=2	E2RRA0_CANLF	CLDND1	37 k...		10	4	3	4	4	2		
661			Charged multivesicular body protein 2A OS=Canis lupus familiaris OX=9615 GN=CHMP2A...	E2RIU2_CANLF	CHMP2A	25 k...		5	3	2	6	1		1	
662			Cytoplasmic FMR1-interacting protein OS=Canis lupus familiaris OX=9615 GN=CYFIP2 PE...	F1PMM7_CANLF	CYFIP2	152 ...	★	31	8	11	12	22	2		
663			Ornithine aminotransferase OS=Canis lupus familiaris OX=9615 GN=OAT PE=3 SV=1	F1Q2A2_CANLF	OAT	48 k...		3	3	11	0	1		4	0
664			Aspartyl aminopeptidase OS=Canis lupus familiaris OX=9615 GN=DNPEP PE=3 SV=2	F1PVI2_CANLF	DNPEP	52 k...		9	1	0	1	6			1
665			Dolichyl--diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit OS=Can...	F1PVU1_CANLF ...	DDOST	51 k...		10	3	6	3	4			
666			ST3 beta-galactoside alpha-2,3-sialyltransferase 4 OS=Canis lupus familiaris OX=9615 G...	E2RD90_CANLF	ST3GAL4	38 k...			1			7	4	6	9
667			Microtubule-actin crosslinking factor 1 OS=Canis lupus familiaris OX=9615 GN=MACF1 P...	J9PAW7_CANLF	MACF1	843 ...	★	5	0	0	0				

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
								Probability Legend:							
								over 95%							
								80% to 94%							
								50% to 79%							
								20% to 49%							
								0% to 19%							
681			Histone H3 OS=Canis lupus familiaris OX=9615 GN=LOC483172 PE=3 SV=1	J9P7X1_CANLF	LOC483172	15 k...	★	38	3		8	10			
682			Histone H2B OS=Canis lupus familiaris OX=9615 GN=LOC483170 PE=3 SV=1	J9NZE4_CANLF (...LOC483170		15 k...	★	77	15	17	37	45	3	12	20
683			Phospholipid transfer protein OS=Canis lupus familiaris OX=9615 GN=PLTP PE=4 SV=2	F1P8V0_CANLF	PLTP	57 k...		1		2	1	3	3	9	9
684			Platelet-activating factor acetylhydrolase IB subunit alpha OS=Canis lupus familiaris OX=...	E2QY31_CANLF	PAFAH1B1	47 k...		2		1	2	4	1	3	3
685			Toll like receptor 8 OS=Canis lupus familiaris OX=9615 GN=TLR8 PE=3 SV=2	F1PEB2_CANLF	TLR8	119 ...		2	2	3	6	2	2	3	
686			Proteasome subunit alpha type OS=Canis lupus familiaris OX=9615 GN=PSMA3 PE=3 SV=1	E2RKR4_CANLF	PSMA3	28 k...		6	2	1	1	5	1	2	
687			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=AKR1B1 PE=4 SV=2	E2RAU6_CANLF	AKR1B1	36 k...		2		1	1	1	3	4	9
688			Proteasome subunit beta type OS=Canis lupus familiaris OX=9615 GN=PSMB3 PE=3 SV=1	E2QX17_CANLF	PSMB3	23 k...		7	3	2	4	4	0	0	
689			Peptidyl-prolyl cis-trans isomerase OS=Canis lupus familiaris OX=9615 GN=PPIB PE=3 S...	J9NVL9_CANLF	PPIB	26 k...		5	3	4	2	1		2	
690			SH3 domain containing GRB2 like 1, endophilin A2 OS=Canis lupus familiaris OX=9615 G...	E2R273_CANLF	SH3GL1	44 k...		7	1	2	5	4		1	
691			Abl interactor 1 OS=Canis lupus familiaris OX=9615 GN=ABI1 PE=4 SV=2	E2RM13_CANLF	ABI1	55 k...		6	3	3	5	4		1	
692			Serine incorporator 1 OS=Canis lupus familiaris OX=9615 GN=SERINC1 PE=4 SV=1	F1PL20_CANLF	SERINC1	51 k...		5	5	2	8	4			1
693			Glutathione S-transferase OS=Canis lupus familiaris OX=9615 GN=GSTM3 PE=3 SV=1	E2R5B9_CANLF	GSTM3	27 k...	★		1		1	8	9	3	2
694			Phosphoinositide phospholipase C OS=Canis lupus familiaris OX=9615 GN=PLCL2 PE=4 ...	F1PL99_CANLF	PLCL2	114 ...		0	0	2		1		2	2
695			Non-specific serine/threonine protein kinase OS=Canis lupus familiaris OX=9615 GN=M...	F1PVU3_CANLF	MARK2	86 k...	★	8	1	2	2	7			
696			Integrin subunit alpha 7 OS=Canis lupus familiaris OX=9615 GN=ITGA7 PE=3 SV=2	F1PIU6_CANLF	ITGA7	130 ...		14	2	1	1	6			
697			Polypyrimidine tract binding protein 1 OS=Canis lupus familiaris OX=9615 GN=PTBP1 PE...	F1PCJ7_CANLF	PTBP1	60 k...		8	0		6	5		0	
698			Fetuin B OS=Canis lupus familiaris OX=9615 GN=FETUB PE=4 SV=1	E2R9B6_CANLF	FETUB	42 k...		3	5	3	2	2	6	3	3
699			26S proteasome non-ATPase regulatory subunit 1 OS=Canis lupus familiaris OX=9615 G...	F1PX57_CANLF	PSMD1	106 ...		4	1	1	2	4	1	4	1
700			Ferritin heavy chain OS=Canis lupus familiaris OX=9615 GN=FTH1 PE=2 SV=3	FRIH_CANLF	FTH1	21 k...		3	1	0	0	4	0	2	2
701			Low density lipoprotein receptor OS=Canis lupus familiaris OX=9615 GN=LDLR PE=4 SV=1	J9NXB0_CANLF	LDLR	95 k...		9	4	2	3	4	2	1	
702			Synaptotagmin binding cytoplasmic RNA interacting protein OS=Canis lupus familiaris OX...	F6XIK8_CANLF	SYNCRIP	63 k...	★	7	4	1	4	4	1		3
703			RB binding protein 7, chromatin remodeling factor OS=Canis lupus familiaris OX=9615 G...	E2RM67_CANLF	RBBP7	52 k...	★	5	2		0	4	2	8	4
704			Spectrin repeat containing nuclear envelope protein 2 OS=Canis lupus familiaris OX=9615...	F1Q3V9_CANLF	SYNE2	801 ...		0	0	0		1	0	2	0
705			Cofilin 2 OS=Canis lupus familiaris OX=9615 GN=CFL2 PE=3 SV=1	J9P2G7_CANLF	CFL2	19 k...	★	20	15		17	9	5	1	
706			WAS protein family member 2 OS=Canis lupus familiaris OX=9615 GN=WASF2 PE=4 SV=1	E2RR96_CANLF	WASF2	54 k...		6	4	2	4	5		0	
707			Calcium dependent secretion activator OS=Canis lupus familiaris OX=9615 GN=CADPS PE...	E2RLM0_CANLF	CADPS	145 ...		0	1	0		0	0	0	
708			Leucyl-tRNA synthetase OS=Canis lupus familiaris OX=9615 GN=LARS PE=3 SV=2	F1PZP6_CANLF	LARS	134 ...		3	0			2	1	3	8
709			Voltage dependent anion channel 2 OS=Canis lupus familiaris OX=9615 GN=VDAC2 PE=4...	E2R948_CANLF	VDAC2	32 k...		4	4	7	3	1			
710			ELKS/RAB6-interacting/CAST family member 1 OS=Canis lupus familiaris OX=9615 GN=E...	F6XUT8_CANLF	ERC1	128 ...		8	2	2	1	4			
711			Tropomyosin 3 OS=Canis lupus familiaris OX=9615 GN=TPM3 PE=3 SV=2	E2RBW4_CANLF	TPM3	33 k...	★	10	5	4	15	7			
712			Prolyl 4-hydroxylase subunit alpha 1 OS=Canis lupus familiaris OX=9615 GN=P4HA1 PE=...	E2RLA2_CANLF	P4HA1	61 k...		1		2		1		10	9
713			LDL receptor related protein 1 OS=Canis lupus familiaris OX=9615 GN=LRP1 PE=4 SV=1	J9P315_CANLF	LRP1	505 ...		1	0	8				4	4
714			Ras suppressor protein 1 OS=Canis lupus familiaris OX=9615 GN=RSU1 PE=4 SV=1	E2R9R1_CANLF	RSU1	32 k...		4	2	0	1	5			0
715			Nuclear receptor corepressor 1 OS=Canis lupus familiaris OX=9615 GN=NCOR1 PE=4 SV=2	E2RKW5_CANLF	NCOR1	270 ...		0			1	0	0	0	0
716			Protein prenyltransferase alpha subunit repeat containing 1 OS=Canis lupus familiaris OX...	J9P4Q0_CANLF	PTAR1	49 k...		0	1			5	7		
717			Dynein light chain OS=Canis lupus familiaris OX=9615 GN=DYNLL1 PE=3 SV=1	E2RK00_CANLF	DYNLL1	10 k...		2	1	1	5	3	1	1	2
718			Thioredoxin OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9NWJ5_CANLF		11 k...		4	3	2	5	5	2	1	
719			Tubulin alpha chain OS=Canis lupus familiaris OX=9615 GN=TUBA3C PE=3 SV=1	J9NXT1_CANLF	TUBA3C	50 k...	★	38		21	52	51	41	53	61
720			Tyrosine-protein kinase OS=Canis lupus familiaris OX=9615 GN=LYN PE=3 SV=2	E2RBB8_CANLF	LYN	59 k...	★	11	6	4	8	10		2	
721			Cell cycle control protein OS=Canis lupus familiaris OX=9615 GN=TMEM30A PE=3 SV=2	F1PKF1_CANLF	TMEM30A	51 k...	★	8	1	2	2	4			
722			Trinucleotide repeat containing 6B OS=Canis lupus familiaris OX=9615 GN=TNRC6B PE=4...	J9JHC8_CANLF	TNRC6B	238 ...		3			2	3		5	4
723			Ras-related protein Rab-21 OS=Canis lupus familiaris OX=9615 GN=RAB21 PE=4 SV=2	F1PGI1_CANLF (...RAB21		26 k...		10	2	1	7	3			
724			Growth factor receptor bound protein 2 OS=Canis lupus familiaris OX=9615 GN=GRB2 PE...	J9P9Z6_CANLF	GRB2	25 k...		6	1	1	1	6			
725			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=ASL PE=3 SV=2	F1PPR8_CANLF	ASL	53 k...						5	4	7	6
726			Eukaryotic translation elongation factor 1 delta OS=Canis lupus familiaris OX=9615 GN=E...	E2R9U8_CANLF	EEF1D	70 k...		3	1	1	8	3	1	1	5
727			Transportin 1 OS=Canis lupus familiaris OX=9615 GN=TNPO1 PE=4 SV=2	F1PZL7_CANLF	TNPO1	97 k...		6	1	1	1	5		3	1
728			RAP2B, member of RAS oncogene family OS=Canis lupus familiaris OX=9615 GN=RAP2B P...	J9NZ45_CANLF	RAP2B	17 k...		7	3	3	3	5	1		1
729			Eukaryotic translation initiation factor 3 subunit F OS=Canis lupus familiaris OX=9615 G...	F1PU02_CANLF	EIF3F	35 k...		6	2		1	6	4	3	2
730			Proteasome 26S subunit, ATPase 4 OS=Canis lupus familiaris OX=9615 GN=PSMC4 PE=3 S...	E2RH48_CANLF	PSMC4	47 k...		5	1	0	0	3	1	4	1
731			Kirre like nephrin family adhesion molecule 1 OS=Canis lupus familiaris OX=9615 GN=KL=...	F1Q051_CANLF	KIRREL1	83 k...		9	4	3	1	5	2		
732			DEXH-box helicase 9 OS=Canis lupus familiaris OX=9615 GN=DHX9 PE=4 SV=1	E2RNT3_CANLF	DHX9	141 ...		1			1	4	2	1	11
733			Coronin OS=Canis lupus familiaris OX=9615 GN=CORO1B PE=3 SV=2	F1Q473_CANLF	CORO1B	54 k...		3	1	0	8	3		0	3
734			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=CD55 PE=4 SV=1	J9NTG7_CANLF	CD55	60 k...		6	1	0	1	2		0	0
735			Coatomer subunit beta OS=Canis lupus familiaris OX=9615 GN=COPB1 PE=4 SV=2	E2R9A2_CANLF	COPB1	107 ...		5			2	2	0	2	5
736			Casein kinase 1 alpha 1 OS=Canis lupus familiaris OX=9615 GN=CSNK1A1 PE=3 SV=2	F1PUU0_CANLF	CSNK1A1	42 k...		4	1	4	2	2			
737			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=SIRPA PE=4 SV=2	F1PK00_CANLF	SIRPA	55 k...		9	2	5	2	5			
738			Tankyrase 1 binding protein 1 OS=Canis lupus familiaris OX=9615 GN=TNKS18P1 PE=4 S...	E2R6L1_CANLF	TNKS18P1	181 ...		4	2	1		0	0	0	0
739			Coiled-coil domain containing 80 OS=Canis lupus familiaris OX=9615 GN=CCDC80 PE=4...	E2RGR7_CANLF	CCDC80	92 k...		9	9		1	3	1		
740			Fibrous sheath interacting protein 2 OS=Canis lupus familiaris OX=9615 GN=FSIP2 PE=4 ...	J9NXK6_CANLF	FSIP2	179 ...		0	0	1		0	0	1	
741			Cadherin 2 OS=Canis lupus familiaris OX=9615 GN=CDH2 PE=4 SV=2	F1PEC4_CANLF	CDH2	100 ...		10	6	1		4		0	
742			Valyl-tRNA synthetase OS=Canis lupus familiaris OX=9615 GN=VARS PE=3 SV=2	E2RTJ7_CANLF	VARS	140 ...		1			0	3	2		15
743			Galectin OS=Canis lupus familiaris OX=9615 GN=LGALS1 PE=4 SV=1	J9P3N4_CANLF	LGALS1	19 k...						2	3	8	3
744			Alpha-1,4 glucan phosphorylase OS=Canis lupus familiaris OX=9615 GN=PYGB PE=3 SV=2	F1PSM2_CANLF	PYGB	120 ...	★					8	6	12	17
745			Cytoskeleton associated protein 4 OS=Canis lupus familiaris OX=9615 GN=CKAP4 PE=4 SV=...	F1PEI2_CANLF	CKAP4	65 k...		8		9					
746			40S ribosomal protein S6 OS=Canis lupus familiaris OX=9615 GN=RP56 PE=3 SV=1	E2RP05_CANLF ... RP56		29 k...		3	2	1	5	4	0	1	5
747			Collagen type VI alpha 1 chain OS=Canis lupus familiaris OX=9615 GN=COL6A1 PE=4 SV...	F1PFM6_CANLF	COL6A1	109 ...		1	2	2		3	4	2	4
748			Tubulin beta chain OS=Canis lupus familiaris OX=9615 GN=TUBB4A PE=3 SV=2	E2RFV2_CANLF	TUBB4A	50 k...		76	29		63	83	31	86	101
749			Aldo-keto reductase family 1 member A1 OS=Canis lupus familiaris OX=9615 GN=AKR1...	F1PK43_CANLF	AKR1A1	37 k...	★	2	3		2	7	1	6	3
750			ADP ribosylation factor 4 OS=Canis lupus familiaris OX=9615 GN=ARF4 PE=3 SV=1	J9P5N6_CANLF	ARF4	21 k...	★	22	6	7	15	11	10	3	7
751			Heterogeneous nuclear ribonucleoprotein D OS=Canis lupus familiaris OX=9615 GN=HNR...	E2RIX2_CANLF	HNRNPD	38 k...		3	1		7	3	1	2	3
752			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=FAM234A PE=4 SV=2	F1PCK9_CANLF	FAM234A	60 k...		7	1	3	4	4		0	1
753			Tyrosine-protein phosphatase non-receptor type OS=Canis lupus familiaris OX=9615												

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
			Probability Legend:												
			over 95%												
			80% to 94%												
			50% to 79%												
			20% to 49%												
			0% to 19%												
766			Myosin heavy chain 14 OS=Canis lupus familiaris OX=9615 GN=MYH14 PE=3 SV=2	F1PYS6_CANLF	MYH14	232 ...	★	12	4	2	12	7	1	5	6
767			Cystathionine gamma-lyase OS=Canis lupus familiaris OX=9615 GN=CTH PE=3 SV=1	E2RDY8_CANLF	CTH	44 k...		5	2		6	4	2	1	1
768			Serpin family A member 7 OS=Canis lupus familiaris OX=9615 GN=SERPINA7 PE=3 SV=2	F1PB85_CANLF	SERPINA7	47 k...		1	5	2		3	3	0	1
769			Lysine--tRNA ligase OS=Canis lupus familiaris OX=9615 GN=KARS PE=3 SV=1	E2RSP4_CANLF	KARS	72 k...		1	1	1	1	1		6	2
770			Importin subunit alpha OS=Canis lupus familiaris OX=9615 GN=KPNA4 PE=3 SV=2	E2RPB3_CANLF	KPNA4	58 k...	★	6	3	1	1	7	3	1	
771			Tryptophanyl-tRNA synthetase OS=Canis lupus familiaris OX=9615 GN=WARS PE=3 SV=1	E2RGG9_CANLF	WARS	54 k...		1		1	2	1	1	4	5
772			Calpain small subunit 1 OS=Canis lupus familiaris OX=9615 GN=CAPNS1 PE=4 SV=1	J9NT23_CANLF	CAPNS1	28 k...		6	2		2	4		1	3
773			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=PRPS1 PE=3 SV=1	E2RBG1_CANLF	PRPS1	35 k...	★	1			2	3	2	5	2
774			Phospholipid scramblase OS=Canis lupus familiaris OX=9615 GN=PLSCR3 PE=3 SV=2	F1PZK6_CANLF	PLSCR3	31 k...		6	2	1	4	3	1		
775			RAB35, member RAS oncogene family OS=Canis lupus familiaris OX=9615 GN=RAB35 PE=3 SV=1	E2RLP0_CANLF	RAB35	23 k...	★	8	3	3	9	5		1	
776			Serine incorporator 3 OS=Canis lupus familiaris OX=9615 GN=SERINC3 PE=4 SV=1	E2RL38_CANLF	SERINC3	52 k...		9	1	4	1	6		0	
777			Transmembrane 9 superfamily member OS=Canis lupus familiaris OX=9615 GN=TM9SF2 ...	E2RSZ5_CANLF	TM9SF2	76 k...		4	1	3	4	0			
778			X-prolyl aminopeptidase 1 OS=Canis lupus familiaris OX=9615 GN=XPNPEP1 PE=3 SV=2	E2RO97_CANLF	XPNPEP1	75 k...		1				2	0	1	7
779			Peroxiredoxin 2 OS=Canis lupus familiaris OX=9615 GN=PRDX2 PE=4 SV=2	F1PCG4_CANLF	PRDX2	22 k...	★	11	3	1	11	6			3
780			Ectonucleoside triphosphate diphosphohydrolase 3 OS=Canis lupus familiaris OX=9615 GN=ENTPD3	E2RE57_CANLF	ENTPD3	59 k...		6		1	1	3			
781			STE20 like kinase OS=Canis lupus familiaris OX=9615 GN=SLK PE=4 SV=1	E2RFZ4_CANLF	SLK	144 ...	★	7	1	0	0	2	0	0	
782			S100 calcium binding protein A14 OS=Canis lupus familiaris OX=9615 GN=S100A14 PE=...	E2RP88_CANLF	S100A14	11 k...		4	4	2	2	6	1	1	1
783			Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B OS=Canis lupus fa...	E2RJ06_CANLF	PPP2R2A	53 k...	★	3	1	1	1	5	3	1	2
784			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=PCBP2 PE=4 SV=2	E2RCF9_CANLF	PCBP2	35 k...	★	6	1	2	5	4	4	6	5
785			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=1	H9GW49_CANLF		71 k...	★	180		114	171	148	45	96	125
786			Heterogeneous nuclear ribonucleoprotein H1 OS=Canis lupus familiaris OX=9615 GN=HN...	E2R8D6_CANLF	HNRNPH1	48 k...	★	5	1	1	2	3		3	1
787			Protein S100 OS=Canis lupus familiaris OX=9615 GN=S100A16 PE=3 SV=1	E2RP76_CANLF	S100A16	12 k...		8	3	1	2	5			1
788			Actin-related protein 2/3 complex subunit OS=Canis lupus familiaris OX=9615 GN=ARPC...	F1PT97_CANLF	ARPC1A	45 k...	★			1	5	3	0	4	2
789			Isoelectyl-tRNA synthetase OS=Canis lupus familiaris OX=9615 GN=IARS PE=3 SV=2	F1PLJ9_CANLF	IARS	144 ...		1	0			2	1	2	4
790			Sorcin OS=Canis lupus familiaris OX=9615 GN=SRI PE=4 SV=1	J9P758_CANLF	SRI	35 k...		6	3	0	5	5			
791			Eukaryotic translation initiation factor 4A3 OS=Canis lupus familiaris OX=9615 GN=EIF4A...	E2RDZ4_CANLF	EIF4A3	47 k...	★	3				12	6	9	14
792			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC100855485 PE=4 SV...	J9P333_CANLF	LOC100855485	20 k...		3	0	1	2	2	0		
793			Scribbled planar cell polarity protein OS=Canis lupus familiaris OX=9615 GN=SCRIB PE=4 ...	F1PKY2_CANLF	SCRIB	173 ...	★	6	1	2		7			
794			Multivesicular body subunit 12B OS=Canis lupus familiaris OX=9615 GN=MVB12B PE=4 S...	F1PM32_CANLF	MVB12B	34 k...		10		1		9			1
795			Metalloendopeptidase OS=Canis lupus familiaris OX=9615 GN=BMP1 PE=4 SV=2	E2RJJ6_CANLF	BMP1	112 ...						10	3		4
796			MOB kinase activator 1B OS=Canis lupus familiaris OX=9615 GN=MOB1B PE=4 SV=2	F1Q0B2_CANLF	MOB1B	25 k...		6	2	1	4	3	1	1	1
797			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=RPL9 PE=4 SV=1	E2R149_CANLF	RPL9	22 k...		2	2		2	5	2	1	5
798			60S ribosomal protein L27 OS=Canis lupus familiaris OX=9615 GN=RPL27 PE=3 SV=1	F2Z4N3_CANLF ...	RPL27	16 k...		3		1	2	3	1	2	5
799			Valyl-tRNA synthetase 2, mitochondrial OS=Canis lupus familiaris OX=9615 GN=VAR52 P...	F6USV0_CANLF	VAR52	120 ...		1	2	2	0	1	2	2	0
800			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=RPL30 PE=4 SV=1	J9NU88_CANLF	RPL30	12 k...		2	1			6	1	2	8
801			Lysosomal associated membrane protein 2 OS=Canis lupus familiaris OX=9615 GN=LAMP...	E2RNJ3_CANLF	LAMP2	45 k...		7	1	5	3	3		1	
802			40S ribosomal protein S17 OS=Canis lupus familiaris OX=9615 GN=RPS17 PE=2 SV=2	RS17_CANLF	RPS17	16 k...		5	3			7	1	2	1
803			RAS related 2 OS=Canis lupus familiaris OX=9615 GN=RRAS2 PE=4 SV=2	F1P9Z4_CANLF	RRAS2	21 k...	★	10	1	2	3	6			1
804			Pyrophosphatase (inorganic) 1 OS=Canis lupus familiaris OX=9615 GN=PPA1 PE=4 SV=2	F1PIJ6_CANLF	PPA1	32 k...		3	0		3	4	1	4	1
805			Cadherin 6 OS=Canis lupus familiaris OX=9615 GN=CDH6 PE=4 SV=2	E2R0Z0_CANLF	CDH6	89 k...		5	3	1		3		1	1
806			Neuroplastin OS=Canis lupus familiaris OX=9615 GN=NPTN PE=4 SV=2	F1PDQ0_CANLF	NPTN	41 k...		5	0	2	2	4	1		0
807			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=PA2G4 PE=4 SV=1	E2QW49_CANLF	PA2G4	44 k...		1		0		4	1	6	7
808			Chromosome 2 open reading frame 72 OS=Canis lupus familiaris OX=9615 GN=C25H2orf...	F1PM72_CANLF	C25H2orf72	29 k...		8	3	2	2	4		0	
809			Proteasome subunit beta type OS=Canis lupus familiaris OX=9615 GN=PSMB2 PE=3 SV=1	E2QW34_CANLF	PSMB2	23 k...		5	2	0	1	4	1		
810			Palmitoyltransferase OS=Canis lupus familiaris OX=9615 GN=ZDHHC20 PE=3 SV=2	E2RKX3_CANLF	ZDHHC20	49 k...		10	2	2	4	1			
811			Triokinase and FMN cyclase OS=Canis lupus familiaris OX=9615 GN=TKFC PE=4 SV=2	F1Q0K5_CANLF	TKFC	59 k...		1				4	2	6	5
812			Non-specific serine/threonine protein kinase OS=Canis lupus familiaris OX=9615 GN=PA...	E2RE16_CANLF	PAK4	64 k...		5	1	0	2	2			
813			Proteasome endopeptidase complex OS=Canis lupus familiaris OX=9615 GN=PSMA5 PE=...	E2R4H4_CANLF	PSMA5	20 k...		8	1			6	0	1	
814			Phosphatidylinositol-4-phosphate 5-kinase type 1 gamma OS=Canis lupus familiaris OX...	F1PZY5_CANLF	PIPSK1C	76 k...	★	0	1	2		1	0	0	
815			Protocadherin 1 OS=Canis lupus familiaris OX=9615 GN=PCDH1 PE=4 SV=2	E2QX93_CANLF	PCDH1	133 ...		4	1	3		3			
816			Multivesicular body subunit 12A OS=Canis lupus familiaris OX=9615 GN=MVB12A PE=4 S...	E2RJN6_CANLF	MVB12A	29 k...		10		2		6			1
817			Microtubule associated protein RP/EB family member 2 OS=Canis lupus familiaris OX=96...	E2RFG1_CANLF	MAPRE2	42 k...						2		9	4
818			40S ribosomal protein S26 OS=Canis lupus familiaris OX=9615 GN=RPS26 PE=3 SV=1	J9P4R8_CANLF	RPS26	14 k...		5	2	1	2	4	1	1	1
819			Cysteine rich transmembrane module containing 1 OS=Canis lupus familiaris OX=9615 G...	E2R299_CANLF	CYSTM1	11 k...		4	1	3	4	2	1	1	3
820			Sodium/potassium-transporting ATPase subunit alpha OS=Canis lupus familiaris OX=96...	F1PJF0_CANLF	ATP1A4	114 ...	★	52	26	25	37	38	3	5	11
821			Claudin-2 OS=Canis lupus familiaris OX=9615 GN=CLDN2 PE=2 SV=1	CLD2_CANLF	CLDN2	25 k...		3	2	0	3	6	2	1	3
822			Ubiquitin conjugating enzyme E2 D2 OS=Canis lupus familiaris OX=9615 GN=UBE2D2 PE...	J9NS22_CANLF	UBE2D2	17 k...		2	0	1	3	4	1	1	
823			Myelin protein zero like 1 OS=Canis lupus familiaris OX=9615 GN=MPZL1 PE=4 SV=1	J9NS27_CANLF	MPZL1	36 k...		5	0	3	3	6	2	1	
824			ATP-dependent 6-phosphofructokinase OS=Canis lupus familiaris OX=9615 GN=PFKP PE...	F1PKS3_CANLF	PFKP	85 k...	★	4			2	6	1	3	3
825			Transmembrane protein 59 OS=Canis lupus familiaris OX=9615 GN=TMEM59 PE=4 SV=1	E2R386_CANLF	TMEM59	37 k...		7	2	1	2	5			1
826			Thyroid hormone receptor interactor 10 OS=Canis lupus familiaris OX=9615 GN=TRIP10 P...	F1PIY9_CANLF	TRIP10	68 k...		6	2	1	2	4		0	
827			Twinfilin actin binding protein 1 OS=Canis lupus familiaris OX=9615 GN=TWIF1 PE=4 SV=2	F1PFK0_CANLF	TWIF1	41 k...		5	4	2	4	3			0
828			Ubiquitin protein ligase E3 component n-recogin 4 OS=Canis lupus familiaris OX=9615 ...	E2RF02_CANLF	UBR4	573 ...		2	0	1		3	0	1	
829			40S ribosomal protein S15a OS=Canis lupus familiaris OX=9615 GN=RPS15A PE=4 SV=2	E2RPP2_CANLF	RPS15A	27 k...	★	4	0		1	6		2	4
830			Exostosin glycosyltransferase 2 OS=Canis lupus familiaris OX=9615 GN=EXT2 PE=4 SV=2	E2RKC6_CANLF	EXT2	84 k...				1		1	1	3	10
831			K-Cl cotransporter OS=Canis lupus familiaris OX=9615 GN=SLC12A4 PE=2 SV=1	Q5KU49_CANLF	SLC12A4	121 ...	★	1	1	0	0	0	0		
832			Calcium-transporting ATPase OS=Canis lupus familiaris OX=9615 GN=ATP2B1 PE=3 SV=2	E2R7S1_CANLF	ATP2B1	135 ...	★	9		5	3	7	1	0	
833			Casein kinase 1 gamma 1 OS=Canis lupus familiaris OX=9615 GN=CSNK1G1 PE=3 SV=2	E2QU92_CANLF	CSNK1G1	53 k...	★	3		2	4	3			1
834			Hemicentin 1 OS=Canis lupus familiaris OX=9615 GN=HMCN1 PE=4 SV=2	F1PXF9_CANLF	HMCN1	613 ...		0	0	0	0	1	0	0	0
835			Sulfurtransferase OS=Canis lupus familiaris OX=9615 GN=MPST PE=4 SV=1	E2RJF7_CANLF	MPST	33 k...				0		5	4	6	4
836			Importin subunit alpha OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9P8A6_CANLF		58 k...		4	1	0	2	2			
837			Charged multivesicular body protein 6 OS=Canis lupus familiaris OX=9615 GN=CHMP6 PE...	F1PRE3_CANLF	CHMP6	21 k...									

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
								Probability Legend:							
								over 95%							
								80% to 94%							
								50% to 79%							
								20% to 49%							
								0% to 19%							
851			Adipocyte plasma membrane associated protein OS=Canis lupus familiaris OX=9615 GN=...	E2RPE9_CANLF	APMAP	46 k...		1	0	3		1	1	0	1
852			Collagen type IV alpha 2 chain OS=Canis lupus familiaris OX=9615 GN=COL4A2 PE=4 SV=...	F1Q129_CANLF	COL4A2	165 ...		6	2	1		1	1	0	
853			F-actin-capping protein subunit alpha-2 OS=Canis lupus familiaris OX=9615 GN=CAPZA...	CAZA2_CANLF	CAPZA2	33 k...	★	7	1	2	10	6		3	1
854			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=CFB PE=3 SV=2	E2RS80_CANLF	CFB	141 ...			1	6		1	1	1	3
855			Amyloid-beta A4 protein OS=Canis lupus familiaris OX=9615 GN=APP PE=4 SV=2	F1P603_CANLF	APP	87 k...		2	2	1	1	4	0		
856			Vang-like protein OS=Canis lupus familiaris OX=9615 GN=VANGL1 PE=3 SV=2	F1Q1G9_CANLF	VANGL1	60 k...		4	3	2	3	2			
857			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=2	F1PYX5_CANLF		17 k...		4	1	1	6	3			
858			Dystonin OS=Canis lupus familiaris OX=9615 GN=DST PE=4 SV=1	J9P0W8_CANLF	DST	883 ...	★	1	1	0	2			0	
859			Apolipoprotein D OS=Canis lupus familiaris OX=9615 GN=APOD PE=3 SV=1	E2RNM1_CANLF	APOD	24 k...		1	0	0	0	0	0	0	0
860			DEAH-box helicase 15 OS=Canis lupus familiaris OX=9615 GN=DHX15 PE=4 SV=1	E2R2U3_CANLF	DHX15	91 k...		1	0			3	0	4	5
861			Epithelial membrane protein 1 OS=Canis lupus familiaris OX=9615 GN=EMP1 PE=3 SV=1	J9JHV7_CANLF	EMP1	18 k...		1	0	1	0	0	0	0	0
862			Prohibitin OS=Canis lupus familiaris OX=9615 GN=PHB PE=4 SV=1	F1PAR8_CANLF	PHB	30 k...		3	5	7	3	0			
863			Peroxisiredoxin 4 OS=Canis lupus familiaris OX=9615 GN=PRDX4 PE=4 SV=1	E2RNL3_CANLF	PRDX4	30 k...	★	9	7	6			3		
864			Phenylalanyl-tRNA synthetase alpha subunit OS=Canis lupus familiaris OX=9615 GN=FA...	E2QU31_CANLF	FARSA	57 k...						0	0	2	10
865			Dynein axonemal heavy chain 8 OS=Canis lupus familiaris OX=9615 GN=DNAH8 PE=4 SV=...	E2REK9_CANLF	DNAH8	505 ...		0	0		0		1	0	1
866			Matrix metallopeptidase 2 OS=Canis lupus familiaris OX=9615 GN=MMP2 PE=3 SV=2	F1PMK7_CANLF	MMP2	74 k...		4	4	1	3	3	1		1
867			Interleukin enhancer binding factor 2 OS=Canis lupus familiaris OX=9615 GN=ILF2 PE=4 ...	E2R9T6_CANLF	ILF2	43 k...		2	1		1	4	1	3	3
868			Non-specific serine/threonine protein kinase OS=Canis lupus familiaris OX=9615 GN=PA...	E2RJA0_CANLF	PAK2	58 k...		3		1	1	3	2	3	0
869			Cyclin dependent kinase 1 OS=Canis lupus familiaris OX=9615 GN=CDK1 PE=3 SV=1	E2RGJ9_CANLF	CDK1	34 k...	★	7	4	2		5	3	2	
870			Guanine nucleotide-binding protein G(q) subunit alpha OS=Canis lupus familiaris OX=96...	GNAQ_CANLF	GNAQ	42 k...	★	3	1	2	4	2		1	
871			CD47 molecule OS=Canis lupus familiaris OX=9615 GN=CD47 PE=4 SV=2	F1P6D7_CANLF	CD47	35 k...		7	3	2	5	2	1		
872			Annexin OS=Canis lupus familiaris OX=9615 GN=ANXA6 PE=3 SV=2	E2RCI8_CANLF	ANXA6	76 k...		3	2	4	4	1		1	
873			Eukaryotic translation initiation factor 3 subunit E OS=Canis lupus familiaris OX=9615 G...	E2RIJ6_CANLF	EIF3E	52 k...		1	1		0	5		1	1
874			DEAD-box helicase 17 OS=Canis lupus familiaris OX=9615 GN=DDX17 PE=3 SV=2	F1PID8_CANLF	DDX17	81 k...	★	3			2	2		3	6
875			Phosphate transporter OS=Canis lupus familiaris OX=9615 GN=SLC20A1 PE=3 SV=2	E2QUQ6_CANLF	SLC20A1	75 k...		4	2	3	5	3			
876			Aldo-keto reductase family 1 member C21 OS=Canis lupus familiaris OX=9615 GN=AKR1...	E2QXQ4_CANLF	AKR1C3	37 k...			1			3	4	4	6
877			Ribosomal protein L10 like OS=Canis lupus familiaris OX=9615 GN=RPL10L PE=4 SV=1	J9PBD3_CANLF	RPL10L	25 k...		3	0		1	5			1
878			Superoxide dismutase [Cu-Zn] OS=Canis lupus familiaris OX=9615 GN=SOD1 PE=3 SV=2	F1Q462_CANLF	SOD1	16 k...		3	1		5	6			2
879			Cytochrome b reductase 1 OS=Canis lupus familiaris OX=9615 GN=CYBRD1 PE=4 SV=1	J9P922_CANLF	CYBRD1	31 k...		7	2	0	4	2			0
880			Early endosome antigen 1 OS=Canis lupus familiaris OX=9615 GN=EEA1 PE=4 SV=1	F1PZD3_CANLF	EEA1	162 ...		0	0	0	0	0		1	
881			Flotillin 1 OS=Canis lupus familiaris OX=9615 GN=FLOT1 PE=4 SV=1	E2QSE3_CANLF	FLOT1	47 k...		4	2	4	5		0		
882			Proteasome 26S subunit, non-ATPase 7 OS=Canis lupus familiaris OX=9615 GN=PSMD7 P...	J9P6R1_CANLF	PSMD7	37 k...		4				2		10	1
883			Y-box binding protein 1 OS=Canis lupus familiaris OX=9615 GN=YBX1 PE=4 SV=2	E2RIA0_CANLF	YBX1	40 k...		8	4		4	1			
884			Hephaestin OS=Canis lupus familiaris OX=9615 GN=HEPH PE=3 SV=2	F1P7M1_CANLF	HEPH	132 ...		5		0	0	4		0	5
885			Rho-associated protein kinase OS=Canis lupus familiaris OX=9615 GN=ROCK1 PE=1 SV=2	F1PU60_CANLF	ROCK1	160 ...	★	6	1			3		1	
886			Glutaminyl-tRNA synthetase OS=Canis lupus familiaris OX=9615 GN=QARS PE=3 SV=1	E2QRQ8_CANLF	QARS	88 k...	★					6	0	4	8
887			Coronin OS=Canis lupus familiaris OX=9615 GN=CORO1A PE=3 SV=1	E2QSZ5_CANLF	CORO1A	51 k...								4	14
888			L-lactate dehydrogenase OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9JH20_CANLF		36 k...	★	17	7	7	17	39	20	34	27
889			C-type lectin domain family 3 member B OS=Canis lupus familiaris OX=9615 GN=CLEC3...	E2RPM8_CANLF	CLEC3B	19 k...		3	2	2	1	3	2	1	2
890			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC100856137 PE=3 SV=...	F1PTQ6_CANLF	LOC100856137	31 k...		5	1	2	3	4	1	1	
891			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=RPL27A PE=3 SV=2	F1Q0S5_CANLF	RPL27A	18 k...		3	2	1	2	0		3	7
892			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9PT27_CANLF		51 k...	★	3		1	3	2		2	0
893			Parkinsonism associated deglycase OS=Canis lupus familiaris OX=9615 GN=PARK7 PE=4 ...	E2QS13_CANLF	PARK7	20 k...		5		1	3	1	0	0	1
894			Thioredoxin related transmembrane protein 1 OS=Canis lupus familiaris OX=9615 GN=TM...	E2RK67_CANLF	TMX1	32 k...		3	2	3	3	2		0	
895			Guanine nucleotide-binding protein subunit gamma OS=Canis lupus familiaris OX=9615 ...	E2RG15_CANLF	GNG5	7 kDa		2	1	0	3	3	1		
896			Vesicle trafficking 1 OS=Canis lupus familiaris OX=9615 GN=VTA1 PE=4 SV=2	F1PBM2_CANLF	VTA1	34 k...		5	2	1	3	3	0		
897			Syntaxin 4 OS=Canis lupus familiaris OX=9615 GN=STX4 PE=3 SV=1	F6V9G5_CANLF	STX4	34 k...		4	3	2	0	2			
898			Receptor-type tyrosine-protein phosphatase OS=Canis lupus familiaris OX=9615 GN=PT...	E2RSJ7_CANLF	PTPRA	90 k...		6	1	1	2	2			
899			Fructose-bisphosphatase 1 OS=Canis lupus familiaris OX=9615 GN=FBP1 PE=3 SV=1	E2RAN6_CANLF	FBP1	37 k...	★		1	0		4	1	4	6
900			Transmembrane p24 trafficking protein 10 OS=Canis lupus familiaris OX=9615 GN=TME...	E2QYL0_CANLF	TMED10	25 k...		6	2	2		1	0	0	
901			Alpha-1-microglobulin/bikunin precursor OS=Canis lupus familiaris OX=9615 GN=AMBP...	E2R796_CANLF	AMBP	39 k...		0	0	3	0	0		3	2
902			Damage specific DNA binding protein 1 OS=Canis lupus familiaris OX=9615 GN=DDB1 PE...	E2R9E3_CANLF	DDB1	127 ...		2				1	0	3	1
903			Phosphatidylinositol binding clathrin assembly protein OS=Canis lupus familiaris OX=96...	E2RCL7_CANLF	PICALM	77 k...		6	0	1	3	5			
904			Anillin actin binding protein OS=Canis lupus familiaris OX=9615 GN=ANLN PE=4 SV=2	F1PZ90_CANLF	ANLN	124 ...		3	6	1	6	0			
905			S-adenosylmethionine synthase OS=Canis lupus familiaris OX=9615 GN=MAT1A PE=3 SV=...	E2RS57_CANLF	MAT1A	44 k...	★					4	3	8	14
906			TNF alpha induced protein 2 OS=Canis lupus familiaris OX=9615 GN=TNFAIP2 PE=4 SV=2	E2RPL0_CANLF	TNFAIP2	77 k...		8	1	1		4			
907			Proteasome subunit beta OS=Canis lupus familiaris OX=9615 GN=PSMB4 PE=3 SV=1	E2RBR6_CANLF	PSMB4	29 k...		7		1	2	4			
908			2'-5'-oligoadenylate synthetase 2 OS=Canis lupus familiaris OX=9615 GN=OAS2 PE=4 S...	F1PTR7_CANLF	OAS2	81 k...		9	0	0	0	5		0	
909			TRAF2 and NCK interacting kinase OS=Canis lupus familiaris OX=9615 GN=TNIK PE=4 SV=...	F1PPZ5_CANLF	TNIK	148 ...	★			4		1		0	
910			Pre-mRNA processing factor 8 OS=Canis lupus familiaris OX=9615 GN=PRPF8 PE=4 SV=2	E2R4X9_CANLF	PRPF8	274 ...		5				3			1
911			Ligand of numb-protein X 2 OS=Canis lupus familiaris OX=9615 GN=LNKX2 PE=4 SV=1	E2REB2_CANLF	LNKX2	76 k...						12		0	1
912			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9P897_CANLF		15 k...		3	1	2	1	3	2	2	2
913			Proteolipid protein 2 OS=Canis lupus familiaris OX=9615 GN=PLP2 PE=4 SV=2	F1Q413_CANLF	PLP2	16 k...		2	2	2	3	3	2	1	2
914			Ribosomal protein L35a OS=Canis lupus familiaris OX=9615 GN=RPL35A PE=3 SV=1	E2QYD8_CANLF	RPL35A	13 k...		1	0		0	1	1	1	1
915			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=NAP1L1 PE=3 SV=2	F1PRE1_CANLF	NAP1L1	45 k...	★	4	5		5	2	0	3	1
916			Solute carrier family 39 member 14 OS=Canis lupus familiaris OX=9615 GN=SLC39A14 PE...	F1PMK4_CANLF	SLC39A14	54 k...		5	5	2	2	3		1	
917			Proteasome 26S subunit, non-ATPase 8 OS=Canis lupus familiaris OX=9615 GN=PSMD8 P...	E2QY43_CANLF	PSMD8	40 k...		3	0		1	1		1	2
918			Protein kinase cAMP-dependent type II regulatory subunit alpha OS=Canis lupus familiari...	E2RRD1_CANLF	PRKAR2A	45 k...	★	1			1	1	1	4	7
919			Eukaryotic translation initiation factor 2 subunit alpha OS=Canis lupus familiaris OX=961...	E2RL17_CANLF	EIF2S1	36 k...		1			2	2	1	3	6
920			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	F1P7J0_CANLF		71 k...	★	1	0		3	3	0	1	3
921			Thimet oligopeptidase 1 OS=Canis lupus familiaris OX=9615 GN=THOP1 PE=3 SV=1	E2R2V4_CANLF	THOP1	78 k...		2			0	4	1	1	1
922			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	F1PZQ3_CANLF		40 k...	★	14	6	3	4	11	2		
923			Solute carrier family 44 member 4 OS=Canis lupus familiaris OX=9615 GN=SLC44A4 PE=...	F1P9U5_CANLF	SLC44A4	79 k...		5	1		3	5	1	1	
924			Basic leucine zipper and W2 domains 2 OS=Canis lupus familiaris OX=9615 GN=BZW2 PE...	F1											

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
			Probability Legend:												
			over 95%												
			80% to 94%												
			50% to 79%												
			20% to 49%												
			0% to 19%												
936			Chloride intracellular channel protein OS=Canis lupus familiaris OX=9615 PE=3 SV=1	F1PTB9_CANLF		28 k...	★	19	9	8	17	22	6	12	10
937			Beta-1,4-galactosyltransferase 1 OS=Canis lupus familiaris OX=9615 GN=B4GALT1 PE=4...	F1PGZ1_CANLF	B4GALT1	30 k...		1	1	1		2	3	2	4
938			3'(2'), 5'-bisphosphate nucleotidase 1 OS=Canis lupus familiaris OX=9615 GN=BPNT1 PE...	J9P1R5_CANLF	BPNT1	40 k...		3	1		3	2		1	2
939			AP complex subunit beta OS=Canis lupus familiaris OX=9615 GN=AP1B1 PE=3 SV=2	E2RRJ6_CANLF	AP1B1	104 ...	★	14	7	4	15	9	1		
940			Non-specific serine/threonine protein kinase OS=Canis lupus familiaris OX=9615 GN=ST...	E2R001_CANLF	STK38L	54 k...		1	3	1		3	0	1	
941			Gamma-glutamyl hydrolase OS=Canis lupus familiaris OX=9615 GN=GGH PE=4 SV=2	E2QUT9_CANLF	GGH	36 k...			1	0		4	2	7	3
942			BCL2 associated athanogene 5 OS=Canis lupus familiaris OX=9615 GN=BAG5 PE=4 SV=2	E2RAG6_CANLF	BAG5	52 k...		5	1	1	2	3		0	
943			Eva-1 homolog A, regulator of programmed cell death OS=Canis lupus familiaris OX=961...	J9P003_CANLF	EVA1A	16 k...		7	1		1	3	2		1
944			RAB31, member RAS oncogene family OS=Canis lupus familiaris OX=9615 GN=RAB31 PE...	E2R5A5_CANLF	RAB31	22 k...		7	1	1	2	1			
945			Rho guanine nucleotide exchange factor 7 OS=Canis lupus familiaris OX=9615 GN=ARHG...	E2R427_CANLF	ARHGEF7	82 k...		6	1	2	2	2			
946			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=VPS4A PE=3 SV=2	E2RA71_CANLF	VPS4A	49 k...	★	6		2	7	5		2	
947			Proteasome subunit beta type OS=Canis lupus familiaris OX=9615 GN=PSMB1 PE=3 SV=2	F1PF02_CANLF	PSMB1	20 k...		3	1		4	7	1		
948			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=GMPS PE=4 SV=2	F1P9U2_CANLF	GMPS	78 k...		1				7	1	2	1
949			Exportin 7 OS=Canis lupus familiaris OX=9615 GN=XPO7 PE=4 SV=1	J9P5X6_CANLF	XPO7	125 ...		2			1	4	3		1
950			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=PRG4 PE=4 SV=2	F1PXD6_CANLF	PRG4	94 k...			0			2	2	3	2
951			Transmembrane 9 superfamily member OS=Canis lupus familiaris OX=9615 PE=3 SV=2	F1PAK4_CANLF		93 k...		4	3	0	2	3			
952			Peptidoglycan recognition protein 2 OS=Canis lupus familiaris OX=9615 GN=PGLYRP2 PE...	F6XZU1_CANLF	PGLYRP2	61 k...		0				3	1	5	7
953			Bromodomain PHD finger transcription factor OS=Canis lupus familiaris OX=9615 GN=BP...	J9JHE8_CANLF	BPTF	316 ...		2	0	0	3		1	0	
954			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=UBE2L3 PE=3 SV=1	E2RK79_CANLF	UBE2L3	18 k...		5	2	0	3	2			
955			Thrombospondin 4 OS=Canis lupus familiaris OX=9615 GN=THBS4 PE=4 SV=2	F1P5S2_CANLF	THBS4	96 k...	★		0	4	1			3	5
956			Syndecan OS=Canis lupus familiaris OX=9615 GN=SDC1 PE=3 SV=1	E2RT70_CANLF	SDC1	33 k...		6	1	2		3			
957			DNA helicase OS=Canis lupus familiaris OX=9615 GN=MCM2 PE=3 SV=2	F1PR47_CANLF	MCM2	107 ...						6	3	3	5
958			Proprotein convertase subtilisin/kexin type 6 OS=Canis lupus familiaris OX=9615 GN=PC...	F1PUS1_CANLF	PCSK6	102 ...						5	1	2	6
959			UDP-N-acetylglucosamine pyrophosphorylase 1 OS=Canis lupus familiaris OX=9615 GN=...	F1P7W7_CANLF	UAP1	59 k...	★					4	2	2	5
960			Tetraspanin OS=Canis lupus familiaris OX=9615 GN=TSPAN15 PE=3 SV=1	E2RS68_CANLF	TSPAN15	33 k...		7		1	1	5			
961			Coiled-coil domain containing 191 OS=Canis lupus familiaris OX=9615 GN=CCDC191 PE...	E2R8T9_CANLF	CCDC191	108 ...		0	0	1		0	0	0	0
962			Calpain 1 OS=Canis lupus familiaris OX=9615 GN=CAPN1 PE=3 SV=1	F6UZV8_CANLF	CAPN1	82 k...		3		0	3	0	0		
963			TBC1 domain family member 32 OS=Canis lupus familiaris OX=9615 GN=TBC1D32 PE=4 ...	F1P518_CANLF	TBC1D32	152 ...						0	2	1	3
964			AP-2 complex subunit alpha OS=Canis lupus familiaris OX=9615 GN=AP2A1 PE=3 SV=2	F1PE67_CANLF	AP2A1	108 ...	★	16		1	6	8			
965			Protein transport protein Sec61 subunit alpha isoform 1 OS=Canis lupus familiaris OX=96...	F1PNM2_CANLF	SEC61A1	53 k...		0	0	4				0	
966			Solute carrier family 44 member 2 OS=Canis lupus familiaris OX=9615 GN=SLC44A2 PE=...	F1PG85_CANLF	SLC44A2	80 k...		5			2	4			
967			Creatine kinase M-type OS=Canis lupus familiaris OX=9615 GN=CKM PE=1 SV=3	KCRM_CANLF	CKM	43 k...						1	1	0	
968			60S ribosomal protein L11 isoform 2 OS=Canis lupus familiaris OX=9615 GN=RPL11 PE=...	E2R9G7_CANLF	RPL11	20 k...		2	1	1	3	2		1	5
969			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	E2QUJ4_CANLF		26 k...		1	2		1	6	2	1	2
970			Serine/threonine-protein phosphatase PP1-beta catalytic subunit OS=Canis lupus familia...	PP1B_CANLF	PPP1CB	37 k...	★	10	8		9	12	9	7	14
971			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=NUCB1 PE=4 SV=1	E2RSV9_CANLF	NUCB1	53 k...		3	2	2	2	1		1	
972			Septin 11 OS=Canis lupus familiaris OX=9615 GN=SEPT11 PE=3 SV=1	E2QTP2_CANLF	SEPT11	43 k...	★	8	5	2	9			6	10
973			Dipeptidyl peptidase 3 OS=Canis lupus familiaris OX=9615 GN=DPP3 PE=3 SV=1	J9P4M5_CANLF	DPP3	79 k...		2			3	4	1	5	1
974			Fructose-bisphosphate aldolase OS=Canis lupus familiaris OX=9615 GN=ALDOC PE=3 SV...	F1P582_CANLF	ALDOC	48 k...	★	4		1	5		2	7	5
975			Vesicular integral-membrane protein VIP36 OS=Canis lupus familiaris OX=9615 GN=LMA...	LMAN2_CANLF	LMAN2	40 k...		7	1	1	1	0		1	1
976			Phospholipase A2 group III OS=Canis lupus familiaris OX=9615 GN=PLA2G3 PE=3 SV=2	F1Q3B4_CANLF	PLA2G3	57 k...		1	1			7	4	2	2
977			LIM and senescent cell antigen-like-containing domain protein OS=Canis lupus familia...	E2RKQ1_CANLF	LIMS1	46 k...		4		1	1	3		3	3
978			Polyadenylate-binding protein OS=Canis lupus familiaris OX=9615 GN=PABPC1 PE=3 SV...	J9P326_CANLF	PABPC1	71 k...	★	4	2		1	1		1	3
979			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=RPL24 PE=4 SV=2	F1PGD7_CANLF	RPL24	18 k...		1	3		2	3	2	2	
980			Vesicle associated membrane protein 8 OS=Canis lupus familiaris OX=9615 GN=VAMP8 P...	E2QU12_CANLF	VAMP8	12 k...		5		1	4	2		0	1
981			MARCKS like 1 OS=Canis lupus familiaris OX=9615 GN=MARCKSL1 PE=4 SV=1	F6Y4A3_CANLF	MARCKSL1	20 k...		6	2	1	2	3	0		
982			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9NRY1_CANLF		16 k...		2	1	0	0	1		1	3
983			HEAT repeat containing 5B OS=Canis lupus familiaris OX=9615 GN=HEATR5B PE=4 SV=2	E2RIA5_CANLF	HEATR5B	225 ...		0	0	0	0	1	1	2	3
984			Calsynenin 1 OS=Canis lupus familiaris OX=9615 GN=CLSTN1 PE=4 SV=1	J9JHQ1_CANLF	CLSTN1	108 ...		2	5	0	2	1			1
985			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9P7C2_CANLF		15 k...	★	2	0		0	2		2	6
986			Eukaryotic peptide chain release factor subunit 1 OS=Canis lupus familiaris OX=9615 GN...	E2RBM3_CANLF	ETF1	49 k...		4			2	3	1	0	4
987			Eukaryotic translation initiation factor 3 subunit I OS=Canis lupus familiaris OX=9615 GN...	F1PPR0_CANLF	EIF3I	36 k...		1	0			3	1	2	6
988			Eukaryotic translation initiation factor 3 subunit C OS=Canis lupus familiaris OX=9615 G...	E2RQP6_CANLF	EIF3C	106 ...		3	0			2	1	2	3
989			DEAD-box helicase 1 OS=Canis lupus familiaris OX=9615 GN=DDX1 PE=4 SV=1	F6V659_CANLF	DDX1	82 k...		1	0			3	2	1	4
990			Transmembrane 9 superfamily member OS=Canis lupus familiaris OX=9615 GN=TM9SF4 ...	F1PWE3_CANLF	TM9SF4	74 k...		1	0	3	2	1			
991			Anionic trypsin OS=Canis lupus familiaris OX=9615 PE=2 SV=1	TRY2_CANLF		26 k...	★	0	1	2		1	1	0	
992			BUB3, mitotic checkpoint protein OS=Canis lupus familiaris OX=9615 GN=BUB3 PE=4 SV=2	F1PNG1_CANLF	BUB3	28 k...			0	1		6	0	1	4
993			Protein kinase C and casein kinase substrate in neurons 2 OS=Canis lupus familiaris OX=...	F1PUE2_CANLF	PACSIN2	56 k...	★	5	3	2	4	4			
994			RAP1A, member of RAS oncogene family OS=Canis lupus familiaris OX=9615 GN=RAP1A ...	F6XEB5_CANLF	RAP1A	22 k...	★	21	11		17	10			6
995			Ras-related protein Rab-5A OS=Canis lupus familiaris OX=9615 GN=RAB5A PE=1 SV=1	RAB5A_CANLF	RAB5A	24 k...	★	10	3	2	6	6	2		
996			Protein kinase cAMP-dependent type I regulatory subunit alpha OS=Canis lupus familia...	E2QZV5_CANLF	PRKAR1A	57 k...		1			1	1		5	6
997			Caveolae associated protein 1 OS=Canis lupus familiaris OX=9615 GN=CAVIN1 PE=4 SV=1	J9P923_CANLF	CAVIN1	43 k...		4	3	1	5	1			
998			Heme binding protein 1 OS=Canis lupus familiaris OX=9615 GN=HEBP1 PE=4 SV=1	E2RNV4_CANLF	HEBP1	21 k...		6	0	2	2	2			1
999			Proteasome subunit beta type OS=Canis lupus familiaris OX=9615 GN=PSMB6 PE=3 SV=1	E2R0B6_CANLF	PSMB6	25 k...		5	2	1	4	3			
1000			Uridine monophosphate synthetase OS=Canis lupus familiaris OX=9615 GN=UMPS PE=3 ...	E2RRY7_CANLF	UMPS	52 k...		2			2	1		0	2
1001			Hyaluronan binding protein 2 OS=Canis lupus familiaris OX=9615 GN=HABP2 PE=3 SV=2	F1Q1K9_CANLF	HABP2	63 k...		1	0	0		0		0	5
1002			Sterol carrier protein 2 OS=Canis lupus familiaris OX=9615 GN=SCP2 PE=3 SV=1	J9P746_CANLF	SCP2	58 k...		2	0	5		3		1	
1003			RAB23, member RAS oncogene family OS=Canis lupus familiaris OX=9615 GN=RAB23 PE...	E2QWS2_CANLF	RAB23	27 k...		5	1	1	0	3			
1004			FAM20C, golgi associated secretory pathway kinase OS=Canis lupus familiaris OX=9615 ...	F6XLD3_CANLF	FAM20C	57 k...						1	1	6	4
1005			Elongation factor Tu GTP binding domain containing 2 OS=Canis lupus familiaris OX=961...	F1PUB9_CANLF	EFTUD2	109 ...	★	5	2					1	4
1006			Tsukushi, small leucine rich proteoglycan OS=Canis lupus familiaris OX=9615 GN=TSKU ...	F1PB05_CANLF	TSKU	39 k...						3	1	3	8
1007			Leucine rich repeat containing 8 VRAC subunit A OS=Canis lupus familiaris OX=9615 GN=...	E2RQD5_CANLF	LRRC8A	94 k...		3	0	4	3				
1008			N-ethylmaleimide sensitive factor, vesicle fusing ATPase OS=Canis lupus familiaris OX=9...	E2RFV4_CANLF	NSF	89 k...						2	1	4	3
1009			Plakophilin 4 OS=Canis lupus familiaris OX=9615 GN=PKP4 PE=4 SV=2	F1PQ49_CANLF	PKP4	132 ...	★	6		2					

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
								Probability Legend:							
								over 95%							
								80% to 94%							
								50% to 79%							
								20% to 49%							
								0% to 19%							
1021			VPS37C, ESCRT-I subunit OS=Canis lupus familiaris OX=9615 GN=VPS37C PE=4 SV=2	F1PIT2_CANLF	VPS37C	39 k...		2	1	1	0	6		1	1
1022			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	F6XPR6_CANLF		46 k...		1	1	1	1	2	1	2	1
1023			Lysosomal associated membrane protein 1 OS=Canis lupus familiaris OX=9615 GN=LAMP...F1Q260_CANLF	LAMP1		45 k...		3	3	3	3	2			
1024			60S ribosomal protein L13 OS=Canis lupus familiaris OX=9615 GN=RPL13 PE=3 SV=1	E2R4F5_CANLF	RPL13	24 k...		5	1	1	2			5	2
1025			Retinol-binding protein OS=Canis lupus familiaris OX=9615 GN=RBP4 PE=3 SV=1	F1Q4D9_CANLF	RBP4	24 k...		1	2	2	0	3	0		3
1026			Cdc42 GTPase-activating protein OS=Canis lupus familiaris OX=9615 GN=ARHGAP1 PE=2...	A4GT58_CANLF	ARHGAP1	51 k...		4			1	3	1	1	3
1027			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=RPL26 PE=3 SV=2	E2QUE7_CANLF	RPL26	17 k...		2	0	2	0	2		0	1
1028			Histone H2A OS=Canis lupus familiaris OX=9615 PE=3 SV=1	F6X834_CANLF		14 k...	★	18	5	4	12	4			6
1029			Protein tyrosine kinase 2 (Fragment) OS=Canis lupus familiaris OX=9615 GN=PTK2 PE=4 ...	E2RGP8_CANLF	PTK2	121 ...		4	3	2	2	3		0	
1030			Abhydrolase domain containing 148 OS=Canis lupus familiaris OX=9615 GN=ABHD148 P...	E2RLZ9_CANLF	ABHD148	22 k...		1	2		0	1	1	1	
1031			Histone H2A OS=Canis lupus familiaris OX=9615 GN=HIST2H2AC PE=3 SV=1	J9POL5_CANLF	HIST2H2AC	14 k...	★	52	18	15	26	22			15
1032			Dicarbonyl and L-xylulose reductase OS=Canis lupus familiaris OX=9615 GN=DCXR PE=3...	J9P4U9_CANLF	DCXR	27 k...		2			1	3	4	0	2
1033			Reticulon OS=Canis lupus familiaris OX=9615 GN=RTN3 PE=4 SV=2	F1PVP5_CANLF	RTN3	113 ...		3	0	3	1	1	0	1	
1034			Ubiquitin like modifier activating enzyme 6 OS=Canis lupus familiaris OX=9615 GN=UBA...	E2R529_CANLF	UBA6	118 ...		1				3	1	0	3
1035			Protein phosphatase 2 scaffold subunit Abeta OS=Canis lupus familiaris OX=9615 GN=PP...	J9P7X5_CANLF	PPP2R1B	77 k...	★	15		3	8	10		1	4
1036			Cystathionine beta-synthase OS=Canis lupus familiaris OX=9615 PE=3 SV=2	E2QXN9_CANLF		60 k...		2	0		3			2	2
1037			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	F1P631_CANLF		25 k...		3			2	6	0		1
1038			Proteasome 26S subunit, non-ATPase 4 OS=Canis lupus familiaris OX=9615 GN=PSMD4 P...	F1P1RW0_CANLF	PSMD4	41 k...		1	1	1	0	0			1
1039			Histone-lysine N-methyltransferase OS=Canis lupus familiaris OX=9615 GN=KMT2B PE=...	F1PTZ8_CANLF	KMT2B	296 ...			1			0	1	0	0
1040			Phosphoglycerate mutase OS=Canis lupus familiaris OX=9615 GN=BPGM PE=3 SV=1	E2QYX1_CANLF	BPGM	30 k...					3	2	4	4	
1041			Non-POU domain containing octamer binding OS=Canis lupus familiaris OX=9615 GN=N...	F1PTP3_CANLF (...NONO		54 k...	★	1			8	1	1		
1042			Lipase H OS=Canis lupus familiaris OX=9615 GN=LIPH PE=3 SV=2	F1PG57_CANLF	LIPH	51 k...		0			2	1			4
1043			Myelin protein zero like 2 OS=Canis lupus familiaris OX=9615 GN=MPZL2 PE=4 SV=1	E2RGD9_CANLF	MPZL2	25 k...		2	0	2	1	2			
1044			Tubulin folding cofactor D OS=Canis lupus familiaris OX=9615 GN=TBGD PE=4 SV=1	H9GWC8_CANLF	TBGD	127 ...		2		0	4			2	
1045			V-type proton ATPase subunit OS=Canis lupus familiaris OX=9615 GN=ATP6VOD1 PE=3 ...	E2RJQ8_CANLF	ATP6VOD1	40 k...		7		2	2	1			
1046			Centromere protein E OS=Canis lupus familiaris OX=9615 GN=CENPE PE=3 SV=2	E2R200_CANLF	CENPE	315 ...		0	0		0	1	1	0	1
1047			Carboxypeptidase B OS=Canis lupus familiaris OX=9615 GN=CPB1 PE=4 SV=1	F1PIR8_CANLF	CPB1	48 k...					4	2	3	6	
1048			Carbohydrate sulfotransferase OS=Canis lupus familiaris OX=9615 GN=CHST11 PE=3 SV...	J9P4N9_CANLF	CHST11	41 k...	★	1			3			2	2
1049			Myosin heavy chain 10 OS=Canis lupus familiaris OX=9615 GN=MYH10 PE=3 SV=2	F1PVV7_CANLF	MYH10	237 ...	★	16		2	19	16			
1050			Lamin B1 OS=Canis lupus familiaris OX=9615 GN=LMNB1 PE=3 SV=1	J9P3G1_CANLF	LMNB1	54 k...	★	8			3	5	0		
1051			Transmembrane emp24 domain-containing protein 7 precursor OS=Canis lupus familiaris...	E2R3T9_CANLF	TMED7	47 k...		5	0	5		1			
1052			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=HNRNPA1 PE=4 SV=1	F1Q3G7_CANLF	HNRNPA1	40 k...	★	3			4	1			
1053			Enolase 2 OS=Canis lupus familiaris OX=9615 GN=ENO2 PE=3 SV=2	E2RAS8_CANLF	ENO2	51 k...	★	20			18				13
1054			Dedicator of cytokinesis 9 OS=Canis lupus familiaris OX=9615 GN=DOCK9 PE=3 SV=2	F1PNV3_CANLF	DOCK9	236 ...		7				1		0	
1055			Spectrin repeat containing nuclear envelope family member 3 OS=Canis lupus familiaris O...	E2RIL1_CANLF	SYNE3	105 ...			0			0		1	1
1056			Lipase C, hepatic type OS=Canis lupus familiaris OX=9615 GN=LIPC PE=3 SV=2	F1PCC4_CANLF	LIPC	57 k...					4				10
1057			Transmembrane BAX inhibitor motif containing 1 OS=Canis lupus familiaris OX=9615 GN...	F1P9Q3_CANLF	TMBIM1	30 k...		2	1	2	4	3	1	1	1
1058			Phytanoyl-CoA 2-hydroxylase interacting protein like OS=Canis lupus familiaris OX=961...	E2RS47_CANLF	PHYH1PL	42 k...		2	1	1	1	3	1	1	
1059			Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2 OS=Canis lupus familiaris OX=9615 ...	F1PZL5_CANLF	PLOD2	88 k...		2	1	1		1		1	3
1060			Glutathione S-transferase OS=Canis lupus familiaris OX=9615 GN=LOC474938 PE=3 SV=1	J9NXX4_CANLF	LOC474938	26 k...		1	1			1	1	2	2
1061			Caveolin-2 OS=Canis lupus familiaris OX=9615 GN=CAV2 PE=1 SV=2	CAV2_CANLF	CAV2	18 k...		2	1	2	2	2	0		
1062			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=ERP44 PE=4 SV=2	F1PQG3_CANLF	ERP44	46 k...		3	1	3		0		1	1
1063			Sjogren syndrome antigen B OS=Canis lupus familiaris OX=9615 GN=SSB PE=4 SV=2	E2RH09_CANLF	SSB	47 k...		5	1		3	0	1	2	
1064			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC475497 PE=3 SV=2	H9GWC7_CANLF	LOC475497	22 k...		2	1	1	4	1	0		
1065			Trophoblast glycoprotein OS=Canis lupus familiaris OX=9615 GN=TPBG PE=4 SV=1	E2QX16_CANLF	TPBG	46 k...		3	2		2	1	1	0	
1066			Thrombospondin 3 OS=Canis lupus familiaris OX=9615 GN=THBS3 PE=4 SV=1	E2QYM7_CANLF	THBS3	104 ...	★	0	1		2		2	1	2
1067			LHFPL tetraspan subfamily member 2 OS=Canis lupus familiaris OX=9615 GN=LHFPL2 PE...	J9P9A6_CANLF	LHFPL2	20 k...		2	1	1	3	3			
1068			Aldehyde dehydrogenase 7 family member A1 OS=Canis lupus familiaris OX=9615 GN=A...	E2RQ99_CANLF	ALDH7A1	59 k...					0	0	1		5
1069			Guanine nucleotide-binding protein subunit gamma OS=Canis lupus familiaris OX=9615 ...	J9P702_CANLF	GNG12	8 kDa		4	1	1	3	2			
1070			Claudin OS=Canis lupus familiaris OX=9615 GN=CLDN1 PE=3 SV=1	J9P0K9_CANLF	CLDN1	23 k...		2	1		1	6	1		
1071			Ceruloplasmin OS=Canis lupus familiaris OX=9615 GN=CP PE=3 SV=2	F1PAX2_CANLF	CP	123 ...			1	0		1	1	2	4
1072			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	F1PPA0_CANLF		54 k...		1	2		2	5	3		
1073			Protein S100 OS=Canis lupus familiaris OX=9615 GN=S100A6 PE=3 SV=1	E2R5P5_CANLF	S100A6	10 k...		2	1	2	3	2			
1074			Synaptophysin like 1 OS=Canis lupus familiaris OX=9615 GN=SYPL1 PE=4 SV=2	E2RC10_CANLF	SYPL1	25 k...		4	2	3	4	1			
1075			Complement C5 OS=Canis lupus familiaris OX=9615 GN=C5 PE=4 SV=2	F1P7J4_CANLF	C5	189 ...			0	0	0	0	0	1	6
1076			ENAH, actin regulator OS=Canis lupus familiaris OX=9615 GN=ENAH PE=4 SV=1	J9JHC4_CANLF	ENAH	96 k...		5	1	1	0	4	0		
1077			Tetraspanin OS=Canis lupus familiaris OX=9615 GN=TSPAN3 PE=3 SV=1	F1PNT6_CANLF	TSPAN3	28 k...		3	2	0	0	1			
1078			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=2	E2RDX0_CANLF		32 k...		5	2	0	0	2			
1079			Proteasome endopeptidase complex OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9P915_CANLF		29 k...		8		1	2	7		0	
1080			Transmembrane p24 trafficking protein 9 OS=Canis lupus familiaris OX=9615 GN=TMED...	F6XD36_CANLF	TMED9	28 k...	★	4		1	1	4			
1081			ADAM metalloproteinase with thrombospondin type 1 motif 13 OS=Canis lupus familiaris ...	E2QUY9_CANLF	ADAMTS13	163 ...						2	2	3	4
1082			BRCA2, DNA repair associated OS=Canis lupus familiaris OX=9615 GN=BRCA2 PE=4 SV=1	F1Q0X3_CANLF	BRCA2	387 ...			1	0	0	0	0	0	0
1083			TSP0 associated protein 1 OS=Canis lupus familiaris OX=9615 GN=TSP0AP1 PE=4 SV=2	F1PQ44_CANLF	TSP0AP1	199 ...				0	2		0	0	2
1084			Sulfotransferase OS=Canis lupus familiaris OX=9615 GN=LOC482182 PE=3 SV=2	F1Q1N2_CANLF	LOC482182	35 k...		0				0	1	3	5
1085			Diaphanous related formin 1 OS=Canis lupus familiaris OX=9615 GN=DIAPH1 PE=4 SV=2	F1PZES_CANLF	DIAPH1	142 ...		2	0			0	0	2	1
1086			Cytochrome P450 family 51 subfamily A member 1 OS=Canis lupus familiaris OX=9615 G...	E2R898_CANLF	CYP51A1	57 k...		2	0	8		1			
1087			Exostosin like glycosyltransferase 2 OS=Canis lupus familiaris OX=9615 GN=EXTL2 PE=4...	J9P983_CANLF	EXTL2	38 k...						2	1	1	
1088			Atlastin GTPase 3 OS=Canis lupus familiaris OX=9615 GN=ATL3 PE=4 SV=2	F1PU09_CANLF	ATL3	59 k...		1		5		1			
1089			Eukaryotic translation initiation factor 3 subunit B OS=Canis lupus familiaris OX=9615 G...	F1P969_CANLF	EIF3B	92 k...						4		1	5
1090			Membrane bound transcription factor peptidase, site 1 OS=Canis lupus familiaris OX=961...	F1PER6_CANLF	MBTPS1	117 ...						1	1		6
1091			Proteasome subunit beta type OS=Canis lupus familiaris OX=9615 GN=PSMB7 PE=3 SV=2	E2RPC6_CANLF	PSMB7	31 k...		5			1	5			
1092			Catalase OS=Canis lupus familiaris OX=9615 GN=CAT PE=1 SV=3	CATA_CANLF	CAT	60 k...					4	0		1	
1093			Phosphoinositide kinase, FYVE-type zinc finger containing OS=Canis lupus familiaris OX=...	F1PXC7_CANLF	PIKFYVE	237 ...		0		0		1		0	
1094			Leucine rich repeat containing 7 OS=Canis lupus familiaris OX=9615 GN=LRRC7 PE=4 SV...	J9NSR9_CANLF	LRRC7	174 ...									

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
								Probability Legend:							
								over 95%							
								80% to 94%							
								50% to 79%							
								20% to 49%							
								0% to 19%							
1106			Collagen alpha-2(I) chain OS=Canis lupus familiaris OX=9615 GN=COL1A2 PE=4 SV=1	F1PHY1_CANLF	COL1A2	129 ...		1	3	1		1			1
1107			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=KRT6A PE=3 SV=1	F1PTS8_CANLF	KRT6A	61 k...	★	9	5	6	1		1		
1108			Abnormal spindle-like microcephaly-associated protein homolog OS=Canis lupus familia...	ASPM_CANLF	ASPM	408 ...		1	0	0	1	0	0	1	1
1109			Prolyl endopeptidase OS=Canis lupus familiaris OX=9615 GN=PREP PE=4 SV=2	F1PHX2_CANLF	PREP	81 k...					2	2		2	3
1110			Secreted phosphoprotein 1 OS=Canis lupus familiaris OX=9615 GN=SPPI PE=4 SV=1	E2R161_CANLF	SPPI	33 k...		3	4	2	2				
1111			Heat shock protein family A (Hsp70) member 13 OS=Canis lupus familiaris OX=9615 GN=...	J9NU25_CANLF	HSPA13	52 k...						2	1	2	1
1112			Cell adhesion molecule 1 OS=Canis lupus familiaris OX=9615 GN=CADM1 PE=4 SV=2	F1P7M7_CANLF	CADM1	50 k...		4	2	1		5			
1113			NOP56 ribonucleoprotein OS=Canis lupus familiaris OX=9615 GN=NOP56 PE=4 SV=1	E2QU53_CANLF	NOP56	66 k...		2				4		4	1
1114			Leucine rich repeat and coiled-coil centrosomal protein 1 OS=Canis lupus familiaris OX=9...	F1PGJ8_CANLF	LRRCC1	119 ...		0	0	2	0	0	0		
1115			Tumor protein, translationally-controlled 1 OS=Canis lupus familiaris OX=9615 GN=TPT1...	E2RMA1_CANLF	TPT1	20 k...		2			1	2		0	
1116			Fatty acid binding protein 3 OS=Canis lupus familiaris OX=9615 GN=FABP3 PE=3 SV=1	E2R507_CANLF	FABP3	15 k...		3	0		1	5			
1117			Transmembrane 9 superfamily member OS=Canis lupus familiaris OX=9615 GN=TM9SF3 ...	E2RSD0_CANLF	TM9SF3	68 k...		1	0	3	2				
1118			Solute carrier family 5 member 6 OS=Canis lupus familiaris OX=9615 GN=SLC5A6 PE=3 S...	E2R9T1_CANLF	SLC5A6	69 k...		3			5	3			
1119			Niemann-Pick C1-like protein 1 precursor OS=Canis lupus familiaris OX=9615 GN=NPC1...	H9GW62_CANLF	NPC1L1	57 k...		4			1	4			
1120			Protein phosphatase 1 regulatory subunit 12B OS=Canis lupus familiaris OX=9615 GN=PP...	E2RHS3_CANLF	PPP1R12B	110 ...				0		0		3	1
1121			Spectrin beta, non-erythrocytic 5 OS=Canis lupus familiaris OX=9615 GN=SPTBN5 PE=4 S...	E2RGC7_CANLF	SPTBN5	413 ...		0	1	0				0	
1122			Adhesion G protein-coupled receptor L2 OS=Canis lupus familiaris OX=9615 GN=ADGRL...	E2RKR8_CANLF	ADGRL2	159 ...		5				2			0
1123			Hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha OS=C...	F1PIPO_CANLF	HADHA	82 k...		1		8					
1124			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=1	F1PTC0_CANLF		33 k...			0		0				13
1125			Thrombospondin type 1 domain containing 4 OS=Canis lupus familiaris OX=9615 GN=TH...	J9JHI3_CANLF	THSD4	83 k...									14
1126			Mitochondrial intermediate peptidase OS=Canis lupus familiaris OX=9615 GN=MIPEP PE=...	F1PWG7_CANLF	MIPEP	71 k...			2	1	1	1	1	2	2
1127			Heat shock protein 27 kDa beta-1 OS=Canis lupus familiaris OX=9615 GN=HSPB1 PE=3 S...	F1PYE3_CANLF (...HSPB1		23 k...		1	1			2	3	2	3
1128			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=RPLP2 PE=3 SV=1	E2R9Y9_CANLF	RPLP2	12 k...		1	2			2	4	2	1
1129			Ribosomal protein L35 OS=Canis lupus familiaris OX=9615 GN=RPL35 PE=3 SV=2	E2RPE5_CANLF	RPL35	15 k...		2	2		2	1		1	2
1130			Adhesion G protein-coupled receptor E5 OS=Canis lupus familiaris OX=9615 GN=ADGRE...	Q2Q423_CANLF	ADGRE5	92 k...		2	2	1		2			1
1131			USO1 vesicle transport factor OS=Canis lupus familiaris OX=9615 GN=USO1 PE=4 SV=1	J9P5F1_CANLF	USO1	109 ...		1				1	1	2	1
1132			Inosine-5'-monophosphate dehydrogenase OS=Canis lupus familiaris OX=9615 GN=IMP...	F6UXI8_CANLF	IMPDH2	53 k...		3	1		3	3	1		
1133			Synaptosomal-associated protein OS=Canis lupus familiaris OX=9615 GN=SNAP23 PE=3 ...	E2R516_CANLF	SNAP23	23 k...		4		1	1	4		1	
1134			Ribosomal protein L19 OS=Canis lupus familiaris OX=9615 GN=RPL19 PE=3 SV=1	A0A0A0MPD2_...	RPL19	23 k...		2		1	1	1	0	0	
1135			Tetraspanin OS=Canis lupus familiaris OX=9615 GN=TSPAN33 PE=3 SV=2	F1PEJ5_CANLF	TSPAN33	32 k...		5	0		1	3	1		
1136			E1A binding protein p400 OS=Canis lupus familiaris OX=9615 GN=EP400 PE=4 SV=1	L7N089_CANLF	EP400	338 ...		2	0	1		2	0	1	
1137			Collagen type VI alpha 3 chain OS=Canis lupus familiaris OX=9615 GN=COL6A3 PE=4 SV...	F1PR31_CANLF	COL6A3	342 ...			1	3			0	3	1
1138			Delta-like protein OS=Canis lupus familiaris OX=9615 GN=JAG1 PE=4 SV=1	E2QU00_CANLF	JAG1	134 ...		4	0	1		2		1	
1139			Protein lin-7 homolog OS=Canis lupus familiaris OX=9615 GN=LIN7A PE=3 SV=2	F1PLC3_CANLF	LIN7A	23 k...		4	1	0		1	2		
1140			Small nuclear ribonucleoprotein U1 subunit 70 OS=Canis lupus familiaris OX=9615 GN=S...	F6V5G7_CANLF	SNRNP70	52 k...		3	2		0	0			1
1141			Glutamine--fructose-6-phosphate transaminase 1 OS=Canis lupus familiaris OX=9615 G...	F1PY49_CANLF	GFPT1	80 k...		2				3		3	2
1142			Band 4.1-like protein 5 OS=Canis lupus familiaris OX=9615 GN=EPB41L5 PE=4 SV=2	F1PAI1_CANLF	EPB41L5	82 k...	★	3	2	2	2				
1143			Glucosylceramidase OS=Canis lupus familiaris OX=9615 GN=GBA PE=3 SV=1	E2R018_CANLF	GBA	60 k...						5	2	1	0
1144			Eukaryotic translation initiation factor 4 gamma 1 OS=Canis lupus familiaris OX=9615 GN...	F1PNF3_CANLF	EIF4G1	157 ...		3			0	4			1
1145			Alpha-amylase OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9P3C4_CANLF (...		58 k...			4	1			1		4
1146			Phosphoglucomutase 3 (Fragment) OS=Canis lupus familiaris OX=9615 GN=PGM3 PE=4 S...	E2R529_CANLF	PGM3	63 k...		0			0	1		5	3
1147			tRNA-splicing ligase RtcB homolog OS=Canis lupus familiaris OX=9615 GN=RTCB PE=3 S...	E2RCD6_CANLF	RTCB	55 k...		0				3		2	5
1148			F-box protein 8 OS=Canis lupus familiaris OX=9615 GN=FBXO8 PE=4 SV=1	E2QX81_CANLF	FBXO8	37 k...		1	0	0			0	3	
1149			EPH receptor A1 OS=Canis lupus familiaris OX=9615 GN=EPHA1 PE=3 SV=1	E2R6A6_CANLF	EPHA1	107 ...		4		2		2			
1150			Gap junction protein OS=Canis lupus familiaris OX=9615 GN=GJB1 PE=3 SV=1	E2QYR1_CANLF	GJB1	32 k...		2		2	2				
1151			Pyruvate kinase OS=Canis lupus familiaris OX=9615 GN=PKLR PE=3 SV=1	H9KUV5_CANLF...	PKLR	63 k...	★					3	2	4	8
1152			Acetyltransferase component of pyruvate dehydrogenase complex OS=Canis lupus familia...	E2RQ59_CANLF	DLAT	69 k...		1	1	5					
1153			Sulfotransferase OS=Canis lupus familiaris OX=9615 GN=SULT1C4 PE=3 SV=2	E2RAM9_CANLF	SULT1C4	35 k...					0	1	0	5	0
1154			GCN1, eIF2 alpha kinase activator homolog OS=Canis lupus familiaris OX=9615 GN=GCN...	F1PUY0_CANLF	GCN1	293 ...		1	0			1	0		
1155			Centrosomal protein 152 OS=Canis lupus familiaris OX=9615 GN=CEP152 PE=4 SV=1	J9P002_CANLF	CEP152	203 ...		0	0			1		0	
1156			GIPC PDZ domain containing family member 1 OS=Canis lupus familiaris OX=9615 GN=GI...	E2R071_CANLF	GIPC1	36 k...		5	0	0	2				
1157			Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 OS=Canis lupus familiaris OX=961...	F1AT2A_CANLF	ATP2A2	110 ...		1		7					
1158			Phospholipid-transporting ATPase OS=Canis lupus familiaris OX=9615 GN=ATP11C PE=3...	J9P0C5_CANLF	ATP11C	129 ...		2				1			
1159			Dynein axonemal heavy chain 3 OS=Canis lupus familiaris OX=9615 GN=DNAH3 PE=4 SV...	J9NWH9_CANLF	DNAH3	468 ...				0			1	0	
1160			Pyruvate carboxylase OS=Canis lupus familiaris OX=9615 GN=PC PE=4 SV=2	F1P6G9_CANLF	PC	130 ...						1		0	
1161			Platelet activating factor acetylhydrolase 1b catalytic subunit 3 OS=Canis lupus familiaris ...	E2RLA8_CANLF	PAFAH1B3	26 k...		1	1		2	2	1	2	1
1162			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=AIMP2 PE=4 SV=1	E2QVF9_CANLF	AIMP2	35 k...		2	0	1	0	2	1	2	
1163			ATPase H+ transporting V1 subunit B2 OS=Canis lupus familiaris OX=9615 GN=ATP6V1B...	E2RAC6_CANLF	ATP6V1B2	57 k...		3			1	2	1	3	1
1164			FXD domain-containing ion transport regulator OS=Canis lupus familiaris OX=9615 GN...	J9NRW7_CANLF	FXD5	23 k...		2	2	1	2	2	1		
1165			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=RP520 PE=3 SV=1	J9P927_CANLF	RP520	13 k...		1	1		1	1	1		3
1166			Tubulin beta chain OS=Canis lupus familiaris OX=9615 GN=TUBB2A PE=3 SV=1	E2RFJ7_CANLF	TUBB2A	50 k...	★	81		19	68	74		97	104
1167			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9P7B1_CANLF		9 kDa		2	1			2	1	0	1
1168			Palmitoyltransferase ZDHHC5 OS=Canis lupus familiaris OX=9615 GN=ZDHHC5 PE=2 SV=...	1ZDHC5_CANLF	ZDHHC5	77 k...		2	2	2	2	3	0		
1169			Nedd4 family interacting protein 2 OS=Canis lupus familiaris OX=9615 GN=NDFIP2 PE=4 ...	F1P7Q9_CANLF	NDFIP2	35 k...		3	2	1	1	2			
1170			Coiled-coil domain containing 89 OS=Canis lupus familiaris OX=9615 GN=CCDC89 PE=4...	F1PU71_CANLF	CCDC89	44 k...		1	0	1		1	0	1	
1171			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=PTGES3 PE=4 SV=1	J9NT37_CANLF	PTGES3	53 k...		3	1			2	1	1	0
1172			Structural maintenance of chromosomes protein OS=Canis lupus familiaris OX=9615 GN=...	E2R774_CANLF	SMC3	142 ...		2		0		2	1	1	
1173			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=DBNL PE=4 SV=2	H9GW54_CANLF	DBNL	48 k...		5	2	1	0	1		0	
1174			NSF attachment protein gamma OS=Canis lupus familiaris OX=9615 GN=NAPG PE=4 SV=1	E2R561_CANLF	NAPG	35 k...		2	1	1	2	2			
1175			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=C1QTNF3 PE=4 SV=2	F1PPL9_CANLF	C1QTNF3	35 k...		1		3		1		1	0
1176			Adenosylhomocysteinase OS=Canis lupus familiaris OX=9615 GN=AHCYL1 PE=3 SV=1	F1P711_CANLF	AHCYL1	58 k...		2		1	6			5	5
1177			Calcylin binding protein OS=Canis lupus familiaris OX=9615 GN=CACYBP PE=4 SV=1	J9P4U8_CANLF	CACYBP	41 k...		1			1	1	0		0
1178			Non-specific serine/threonine protein kinase OS=Canis lupus familiaris OX=9615 GN=RP...	E2RP04_CANLF	RP56KA3	81 k...	★	1				2	1	0	3
1179			KN motif and ankyrin repeat domains 4 OS=Canis lupus familiaris OX=9615 GN=KANK4 P...	J9NY97_CANLF	KANK4	109 ...		3			1	1	1	0	
1180															

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
								Probability Legend: over 95% 80% to 94% 50% to 79% 20% to 49% 0% to 19%							
1195			Ninein OS=Canis lupus familiaris OX=9615 GN=NIN PE=4 SV=2	E2R9R8_CANLF	NIN	248 ...			1	2	0		1	0	
1192			HSPA (Hsp70) binding protein 1 OS=Canis lupus familiaris OX=9615 GN=HSPBP1 PE=4 SV=...	E2QZB3_CANLF	HSPBP1	39 k...		2			0	4		0	1
1193			RAN binding protein 1 OS=Canis lupus familiaris OX=9615 GN=RANBP1 PE=4 SV=1	J9P3T9_CANLF	RANBP1	27 k...		1	1		4	1			
1194			Keratin, type II cytoskeletal 2 epidermal OS=Canis lupus familiaris OX=9615 GN=KRT2 PE...	K22E_CANLF	KRT2	65 k...	★		5	13	3	3	2		
1195			Sulfotransferase OS=Canis lupus familiaris OX=9615 GN=CHST4 PE=3 SV=2	F1PIE6_CANLF	CHST4	45 k...		1				4		1	5
1196			TAO kinase 3 OS=Canis lupus familiaris OX=9615 GN=TAOK3 PE=4 SV=2	E2RM17_CANLF	TAOK3	105 ...	★	1		1		1		0	
1197			Integrin beta OS=Canis lupus familiaris OX=9615 GN=ITGB6 PE=3 SV=2	E2RBL9_CANLF	ITGB6	88 k...		4		1	0	2			
1198			UEV and lactate/malate dehydrogenase domains OS=Canis lupus familiaris OX=9615 GN=...	F1PQX2_CANLF	UEVLD	57 k...		2	3	0	4	0			
1199			Leucine rich repeat neuronal 2 OS=Canis lupus familiaris OX=9615 GN=LRRN2 PE=4 SV=1	J9PAI9_CANLF	LRRN2	79 k...		0	0			1	1	1	0
1200			Gem nuclear organelle associated protein 5 OS=Canis lupus familiaris OX=9615 GN=GEMI...	E2RIE8_CANLF	GEMIN5	168 ...				0		0	0	1	3
1201			Methyltransferase like 25 OS=Canis lupus familiaris OX=9615 GN=METTL25 PE=4 SV=1	E2QZ15_CANLF	METTL25	69 k...		0	0	1	0	0	0	1	
1202			Integrin subunit alpha 5 OS=Canis lupus familiaris OX=9615 GN=ITGA5 PE=3 SV=2	F1PY05_CANLF	ITGA5	114 ...		4	1		0	2			
1203			Homer scaffolding protein 1 OS=Canis lupus familiaris OX=9615 GN=HOMER1 PE=4 SV=1	E2RT37_CANLF	HOMER1	40 k...	★	2		1		1		0	
1204			Amidophosphoribosyltransferase OS=Canis lupus familiaris OX=9615 GN=PPAT PE=3 SV...	E2RF42_CANLF	PPAT	57 k...		0				3		1	6
1205			Ras-related protein Rab-13 OS=Canis lupus familiaris OX=9615 GN=RAB13 PE=1 SV=2	RAB13_CANLF	RAB13	23 k...		8				14	7		
1206			Collagen type IV alpha 1 chain OS=Canis lupus familiaris OX=9615 GN=COL4A1 PE=4 SV...	F1Q133_CANLF	COL4A1	156 ...				1		1			
1207			WNK lysine deficient protein kinase 4 OS=Canis lupus familiaris OX=9615 GN=WNK4 PE=...	E2RNNW6_CANLF	WNK4	174 ...		2		0		3	0	0	
1208			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC485485 PE=4 SV=1	E2REI4_CANLF	LOC485485	28 k...				0	0			1	0
1209			Kinesin family member 17 OS=Canis lupus familiaris OX=9615 GN=KIF17 PE=3 SV=2	F1PW14_CANLF	KIF17	112 ...				0	0		2	1	
1210			Signal transducer and activator of transcription OS=Canis lupus familiaris OX=9615 GN=...	E2RI87_CANLF	STAT1	136 ...								3	6
1211			Lipoprotein lipase OS=Canis lupus familiaris OX=9615 GN=LPL PE=3 SV=2	F1PA62_CANLF	LPL	53 k...								5	4
1212			Ubiquitin like modifier activating enzyme 2 OS=Canis lupus familiaris OX=9615 GN=UBA...	E2R837_CANLF	UBA2	71 k...		1				1	2	1	1
1213			Serpin family F member 1 OS=Canis lupus familiaris OX=9615 GN=SERPINF1 PE=3 SV=1	F2Z4Q7_CANLF	SERPINF1	47 k...		2	2	0	1	0		1	2
1214			Glutaredoxin 3 OS=Canis lupus familiaris OX=9615 GN=GLRX3 PE=4 SV=2	F1PFB3_CANLF	GLRX3	37 k...		3	2	1	1	1	0	1	
1215			Lysophospholipase II OS=Canis lupus familiaris OX=9615 GN=LYPLA2 PE=4 SV=1	J9PAN4_CANLF	LYPLA2	25 k...		2	1			1	2	3	1
1216			Family with sequence similarity 84 member B OS=Canis lupus familiaris OX=9615 GN=FA...	F1PW16_CANLF	FAM84B	27 k...		2	1	1	1	2			
1217			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9NYB5_CANLF		15 k...		4	2		3	2			1
1218			Dihydropyrimidinase OS=Canis lupus familiaris OX=9615 GN=DPYS PE=4 SV=2	E2RSE5_CANLF	DPYS	56 k...	★		0	1		1	2	1	2
1219			Serine incorporator 2 OS=Canis lupus familiaris OX=9615 GN=SERINC2 PE=4 SV=1	F1PZZ8_CANLF	SERINC2	51 k...		2	1	0	2	1	0		
1220			SEC13 homolog, nuclear pore and COPII coat complex component OS=Canis lupus famili...	F1PDM3_CANLF	SEC13	36 k...		2	1			1		1	1
1221			Glutamate-cysteine ligase modifier subunit OS=Canis lupus familiaris OX=9615 GN=GCL...	F1P7D3_CANLF	GCLM	26 k...		1	1			1	0	2	2
1222			NOP58 ribonucleoprotein OS=Canis lupus familiaris OX=9615 GN=NOP58 PE=4 SV=2	E2RQV7_CANLF	NOP58	61 k...		0				2	0	3	2
1223			PEAK1 related, kinase-activating pseudokinase 1 OS=Canis lupus familiaris OX=9615 GN...	F1PK38_CANLF	PRAG1	152 ...		3	0	1	1	1			
1224			NDRG family member 3 OS=Canis lupus familiaris OX=9615 GN=NDRG3 PE=4 SV=1	E2QSD6_CANLF	NDRG3	43 k...		3	1	1		2	0		
1225			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9P051_CANLF		48 k...	★			6	15	6	5	10	6
1226			Guanine nucleotide-binding protein subunit gamma OS=Canis lupus familiaris OX=9615 ...	E2R9G5_CANLF	GNG10	7 kDa		2	1	0		2	1		
1227			Quinoid dihydropteridine reductase OS=Canis lupus familiaris OX=9615 GN=QDPR PE=4 ...	E2RLU9_CANLF	QDPR	26 k...						2	2	3	1
1228			Aldehyde dehydrogenase 3 family member B1 OS=Canis lupus familiaris OX=9615 GN=A...	F1PXN6_CANLF	ALDH3B1	58 k...	★	3		1	3	2			
1229			Ras-related protein Rab-9A OS=Canis lupus familiaris OX=9615 GN=RAB9A PE=1 SV=2	RAB9A_CANLF	RAB9A	23 k...		6	1			1	1		
1230			Prenylcysteine oxidase 1 like OS=Canis lupus familiaris OX=9615 GN=PCYOX1L PE=4 SV=2	F1PU59_CANLF	PCYOX1L	46 k...						1	3	1	5
1231			GLI pathogenesis related 2 OS=Canis lupus familiaris OX=9615 GN=GLIPR2 PE=3 SV=1	J9NX46_CANLF	GLIPR2	17 k...		3	2			5	1		
1232			Protein kinase C OS=Canis lupus familiaris OX=9615 GN=PRKCI PE=3 SV=2	F1PG28_CANLF	PRKCI	79 k...		3	1	0	0	3			
1233			Eukaryotic translation initiation factor 6 OS=Canis lupus familiaris OX=9615 GN=EIF6 PE...	E2R1S5_CANLF	EIF6	26 k...		2		1		3			1
1234			Eukaryotic translation initiation factor 3 subunit M OS=Canis lupus familiaris OX=9615 G...	E2QWG8_CANLF	EIF3M	43 k...		1				7	1	1	
1235			Proteasome activator subunit 3 OS=Canis lupus familiaris OX=9615 GN=PSME3 PE=4 SV=2	E2RPP9_CANLF	PSME3	31 k...		1	0			2	0		2
1236			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=CD59 PE=4 SV=1	J9PN44_CANLF	CD59	16 k...		2		0	1	3		0	
1237			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	F1PZ36_CANLF		33 k...			0	0	0		0	1	
1238			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=SLC25A3 PE=3 SV=2	F1PXY4_CANLF	SLC25A3	40 k...		3	2	4				0	
1239			Solute carrier family 2, facilitated glucose transporter member 3 OS=Canis lupus famili...	F1PPK3_CANLF	SLC2A3	54 k...		1	1	0	3				
1240			Laminin subunit beta 2 OS=Canis lupus familiaris OX=9615 GN=LAMB2 PE=4 SV=1	E2R4J1_CANLF	LAMB2	196 ...		2	1	0	2			0	
1241			Acyl-CoA synthetase short chain family member 2 OS=Canis lupus familiaris OX=9615 G...	E2ROC6_CANLF	ACSS2	81 k...		0		0			0	2	1
1242			Aldehyde dehydrogenase 8 family member A1 OS=Canis lupus familiaris OX=9615 GN=A...	E2QZS0_CANLF	ALDH8A1	53 k...					0		1	5	2
1243			MACC1, MET transcriptional regulator OS=Canis lupus familiaris OX=9615 GN=MACC1 PE...	F6X7M2_CANLF	MACC1	96 k...		4		0	1	2			
1244			Rho associated coiled-coil containing protein kinase 2 OS=Canis lupus familiaris OX=961...	F1PE53_CANLF	ROCK2	152 ...	★	4	0	1		2			
1245			GDP-mannose pyrophosphorylase B OS=Canis lupus familiaris OX=9615 GN=GMPPB PE=...	F6X690_CANLF	GMPPB	43 k...						2	0	6	1
1246			Prolylcarboxypeptidase OS=Canis lupus familiaris OX=9615 GN=PRCP PE=4 SV=2	F1PWK3_CANLF	PRCP	56 k...						1	0	4	1
1247			CRK proto-oncogene, adaptor protein OS=Canis lupus familiaris OX=9615 GN=CRK PE=4 ...	E2QWD3_CANLF	CRK	42 k...		2	1		0	2			
1248			Target of myb1 like 1 membrane trafficking protein OS=Canis lupus familiaris OX=9615 ...	E2RAY7_CANLF	TOM1L1	53 k...		3			1	2		0	
1249			STEAP3 metalloredutase OS=Canis lupus familiaris OX=9615 GN=STEAP3 PE=4 SV=2	F1P8R0_CANLF	STEAP3	58 k...		2		3	2				
1250			PTPRF interacting protein alpha 1 OS=Canis lupus familiaris OX=9615 GN=PPFIA1 PE=4 S...	J9NTF0_CANLF	PPFIA1	142 ...		2	1	0		0			
1251			Alpha-L-fucosidase 2 OS=Canis lupus familiaris OX=9615 GN=FUCA2 PE=4 SV=1	F1PBG4_CANLF	FUCA2	47 k...						5	0	1	0
1252			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC100682773 PE=4 SV...	J9P8L6_CANLF	LOC100682773	27 k...				0		0		3	2
1253			Tubulin alpha chain OS=Canis lupus familiaris OX=9615 PE=3 SV=2	F1PLM3_CANLF		57 k...	★					18		16	
1254			Galectin OS=Canis lupus familiaris OX=9615 GN=LGALS9 PE=4 SV=2	E2QXK6_CANLF	LGALS9	40 k...		2				6			
1255			Leucine rich pentatricopeptide repeat containing OS=Canis lupus familiaris OX=9615 GN=...	F6X6K8_CANLF	LRPPRC	158 ...		1		4					
1256			Regulator of chromosome condensation 1 OS=Canis lupus familiaris OX=9615 GN=RCC1 ...	J9NTA0_CANLF	RCC1	52 k...				0		0			2
1257			Mortality factor 4 like 1 OS=Canis lupus familiaris OX=9615 GN=MORF4L1 PE=4 SV=2	E2RJF6_CANLF	MORF4L1	41 k...			0	1			0	0	
1258			Hydroxysteroid dehydrogenase like 2 OS=Canis lupus familiaris OX=9615 GN=HSDL2 PE=...	F1Q3R2_CANLF	HSDL2	46 k...				3		0		0	
1259			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC611363 PE=4 SV=1	J9NX38_CANLF	LOC611363	47 k...								1	
1260			CCR4-NOT transcription complex subunit 1 OS=Canis lupus familiaris OX=9615 GN=CNO...	E2R990_CANLF	CNOT1	267 ...						1			
1261			Vesicle associated membrane protein 3 OS=Canis lupus familiaris OX=9615 GN=VAMP3 P...	J9P1V7_CANLF	VAMP3	12 k...		2	1	1	2	1		0	
1262			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=RP510 PE=4 SV=1	E2RGQ5_CANLF...	RP510	19 k...		1	1		2	1		0	1
1263			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9NVG6_CANLF		20 k...		1	2		0	2	1		2
1264			Small nuclear ribonucleoprotein-associated protein OS=Canis lupus familiaris OX=9615 G...	E2R1C4_CANLF	SNRPB	24 k...		1	1		1	1	0		1
1265			Lysyl oxidase like 1 OS=Canis lupus familiaris OX=9615 GN=LOXL1 PE=4 SV=1	J9NVY4_CANLF	LOXL1	62 k...			1	0		1	2	2	2
1266															

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
			Probability Legend:												
			over 95%												
			80% to 94%												
			50% to 79%												
			20% to 49%												
			0% to 19%												
1276			EH domain containing 3 OS=Canis lupus familiaris OX=9615 GN=EHD3 PE=3 SV=2	F1PXX1_CANLF	EHD3	61 k...	★			15	17		5	10	14
1277			Acyl-CoA thioesterase 7 OS=Canis lupus familiaris OX=9615 GN=ACOT7 PE=4 SV=2	F1PWR0_CANLF	ACOT7	47 k...		2			2	3			2
1278			Vacuolar protein sorting-associated protein 29 OS=Canis lupus familiaris OX=9615 GN=V...	E2R8A2_CANLF	VP529	20 k...		0				2		3	3
1279			Cathepsin F OS=Canis lupus familiaris OX=9615 GN=CTSF PE=3 SV=2	E2RR02_CANLF	CTSF	51 k...					0	1	2	1	
1280			Beta-2-microglobulin OS=Canis lupus familiaris OX=9615 GN=B2M PE=1 SV=2	E2RN10_CANLF	B2M	14 k...		3	1			3	2		
1281			Insulin like growth factor binding protein acid labile subunit OS=Canis lupus familiaris O...	F1PYM4_CANLF	IGFALS	70 k...						0	1	2	3
1282			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	F1PNI3_CANLF		40 k...	★	14			15	14		12	
1283			Solute carrier family 39 member 10 OS=Canis lupus familiaris OX=9615 GN=SLC39A10 PE...	F1PA89_CANLF	SLC39A10	55 k...		5	1	1		3			
1284			SPEG complex locus OS=Canis lupus familiaris OX=9615 GN=SPEG PE=4 SV=1	F6X4X4_CANLF	SPEG	354 ...		0		0	0	0	1	0	
1285			Histidyl-tRNA synthetase OS=Canis lupus familiaris OX=9615 GN=HARS PE=3 SV=1	E2QZ69_CANLF	HARS	57 k...		2	0		1	3			
1286			Plexin domain containing 2 OS=Canis lupus familiaris OX=9615 GN=PLXDC2 PE=4 SV=2	F1PNK7_CANLF	PLXDC2	59 k...						1	1	2	2
1287			Phosphoribosyl pyrophosphate synthetase associated protein 1 OS=Canis lupus familiaris...	J9NVA0_CANLF	PRPSA1	42 k...	★	2				2		1	2
1288			Phospholipase OS=Canis lupus familiaris OX=9615 GN=PLD2 PE=3 SV=2	F1Q2H6_CANLF	PLD2	104 ...		2	2		2	1			
1289			Methylmalonic aciduria (cobalamin deficiency) cblA type OS=Canis lupus familiaris OX=96...	F1Q137_CANLF	MMAA	47 k...			1	1			1	1	
1290			CDC42 binding protein kinase beta OS=Canis lupus familiaris OX=9615 GN=CDC42BPB P...	E2RPP6_CANLF	CDC42BPB	194 ...		2				1		2	1
1291			Adaptor related protein complex 1 mu 2 subunit OS=Canis lupus familiaris OX=9615 GN=...	F1PFJ0_CANLF	AP1M2	48 k...		3			2	1	1		
1292			Small nuclear ribonucleoprotein D1 polypeptide OS=Canis lupus familiaris OX=9615 GN=...	E2RIW7_CANLF	SNRPD1	13 k...		2			0	1			1
1293			Serpin family B member 6 OS=Canis lupus familiaris OX=9615 GN=SERPINB6 PE=3 SV=2	E2RGP2_CANLF	SERPINB6	43 k...		1	0		3	2		0	
1294			Histone H2A OS=Canis lupus familiaris OX=9615 GN=LOC106558252 PE=3 SV=1	J9NRH7_CANLF ...	LOC106558252	14 k...	★	47	17		24	22			17
1295			Cordon-bleu WH2 repeat protein like 1 OS=Canis lupus familiaris OX=9615 GN=COBL1 ...	J9P829_CANLF	COBL1	126 ...		4		0	2	1	0		
1296			Obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF OS=Canis lupus familiaris ...	J9JHM0_CANLF	OBSN	862 ...		0		0	0	0		0	1
1297			Zyxin OS=Canis lupus familiaris OX=9615 GN=ZYX PE=4 SV=2	F1P7F8_CANLF	ZYX	60 k...		3		1	0	3			
1298			Secretory carrier-associated membrane protein OS=Canis lupus familiaris OX=9615 PE=3 ...	J9NT25_CANLF		38 k...		4			2	2			
1299			ATP synthase peripheral stalk-membrane subunit b OS=Canis lupus familiaris OX=9615 ...	E2QW06_CANLF	ATP5PB	29 k...		2	1	4					
1300			EFR3 homolog A OS=Canis lupus familiaris OX=9615 GN=EFR3A PE=4 SV=2	E2RCW6_CANLF	EFR3A	93 k...		4		1		1			
1301			COP9 signalosome subunit 4 OS=Canis lupus familiaris OX=9615 GN=COP54 PE=4 SV=1	E2RT55_CANLF	COP54	46 k...		1						5	3
1302			LIM domain and actin binding 1 OS=Canis lupus familiaris OX=9615 GN=LIMA1 PE=4 SV=2	E2QW54_CANLF	LIMA1	85 k...		2		1	3				
1303			Semaphorin 3E OS=Canis lupus familiaris OX=9615 GN=SEMA3E PE=3 SV=2	F1Q4C1_CANLF	SEMA3E	85 k...						0	2		4
1304			Misshapen like kinase 1 OS=Canis lupus familiaris OX=9615 GN=MINK1 PE=4 SV=2	F1PZ09_CANLF	MINK1	111 ...	★	4		3		0			
1305			Angiopoietin like 3 OS=Canis lupus familiaris OX=9615 GN=ANGPTL3 PE=4 SV=1	E2QZ98_CANLF	ANGPTL3	54 k...			0			3	1	0	
1306			Major histocompatibility complex, class II, DQ alpha precursor OS=Canis lupus familiaris ...	F1PTR6_CANLF	DLA-DQA1	48 k...	★	5		4		4			
1307			Timeless circadian regulator OS=Canis lupus familiaris OX=9615 GN=TIMELESS PE=4 SV=1	F6XA56_CANLF	TIMELESS	140 ...		0				0	1	0	
1308			Glycylpeptide N-tetradecanoyltransferase OS=Canis lupus familiaris OX=9615 GN=NMT1 ...	E2RP29_CANLF	NMT1	57 k...				0		3	0		2
1309			RAS protein activator like 2 OS=Canis lupus familiaris OX=9615 GN=RASAL2 PE=4 SV=2	F1PV47_CANLF	RASAL2	144 ...		1	0			2			0
1310			Dolichyl--diphosphooligosaccharide--protein glycosyltransferase subunit STT3A OS=Canil...	A0A1W2NC67_...	STT3A	90 k...	★	2	0	1					
1311			Replication protein A subunit OS=Canis lupus familiaris OX=9615 GN=RPA1 PE=3 SV=1	E2R436_CANLF	RPA1	68 k...		1				4			
1312			Uroporphyrinogen decarboxylase OS=Canis lupus familiaris OX=9615 GN=UROD PE=3 SV...	E2RCH3_CANLF	UROD	41 k...								4	5
1313			Solute carrier family 19 member 1 OS=Canis lupus familiaris OX=9615 GN=SLC19A1 PE=...	F1PFN8_CANLF	SLC19A1	51 k...		4				3			
1314			Transcobalamin 2 OS=Canis lupus familiaris OX=9615 GN=TCN2 PE=4 SV=2	E2RFH1_CANLF	TCN2	48 k...						6	1		
1315			Sodium/potassium-transporting ATPase subunit alpha OS=Canis lupus familiaris OX=96...	F1PL53_CANLF	ATP1A2	112 ...	★	69			62			8	
1316			Ras-related C3 botulinum toxin substrate 1 OS=Canis lupus familiaris OX=9615 GN=RAC...	RAC1_CANLF	RAC1	21 k...	★	19	7	10	16	20		10	10
1317			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9NT16_CANLF		25 k...		1	1		2	2	1	0	1
1318			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=ATP6V1E1 PE=3 SV=1	E2R1R4_CANLF	ATP6V1E1	26 k...		1			2	2	1	1	1
1319			Protein arginine N-methyltransferase 5 OS=Canis lupus familiaris OX=9615 GN=PRMT5 P...	E2R9J8_CANLF	PRMT5	73 k...		2	1		0	2	1		1
1320			Prolyl 4-hydroxylase subunit alpha 2 OS=Canis lupus familiaris OX=9615 GN=P4HA2 PE=...	F1PTP6_CANLF	P4HA2	61 k...		1		1		0	1	1	1
1321			Ferritin OS=Canis lupus familiaris OX=9615 PE=3 SV=2	E2RM23_CANLF...		20 k...	★	2	2	1		2			1
1322			Calponin OS=Canis lupus familiaris OX=9615 GN=CNN3 PE=3 SV=1	F1P9W2_CANLF	CNN3	34 k...		3	1	1	1	2			
1323			Glucosamine-phosphate N-acetyltransferase 1 OS=Canis lupus familiaris OX=9615 GN=G...	E2RJW4_CANLF	GNPNAT1	21 k...		1			1	4		1	1
1324			Sulfotransferase 1A1 OS=Canis lupus familiaris OX=9615 GN=SULT1A1 PE=1 SV=1	ST1A1_CANLF	SULT1A1	34 k...		0			0	1	1	0	2
1325			G3BP stress granule assembly factor 1 OS=Canis lupus familiaris OX=9615 GN=G3BP1 PE...	E2RN45_CANLF	G3BP1	50 k...		3	0		2	3			1
1326			6-phosphogluconolactonase OS=Canis lupus familiaris OX=9615 GN=PGLS PE=4 SV=1	E2RLQ6_CANLF	PGLS	28 k...		1			1	1	0		2
1327			Carboxymethylenebutenolidase homolog OS=Canis lupus familiaris OX=9615 GN=CMBL ...	E2RF91_CANLF	CMBL	28 k...					2	2	2	2	3
1328			Discoidin domain receptor tyrosine kinase 1 OS=Canis lupus familiaris OX=9615 GN=DD...	J9P5N9_CANLF	DDR1	104 ...		3	1	1		1			
1329			UPF1, RNA helicase and ATPase OS=Canis lupus familiaris OX=9615 GN=UPF1 PE=4 SV=2	E2RL81_CANLF	UPF1	124 ...					2	1	2	2	
1330			Oxidative stress responsive 1 OS=Canis lupus familiaris OX=9615 GN=OXSR1 PE=4 SV=2	E2RAI8_CANLF	OXSR1	58 k...		3		1	2	1			
1331			ADAM metalloproteinase domain 17 OS=Canis lupus familiaris OX=9615 GN=ADAM17 PE...	F1PFZ9_CANLF	ADAM17	93 k...		3				1		1	1
1332			Reactive intermediate imine deaminase A homolog OS=Canis lupus familiaris OX=9615 G...	E2QS94_CANLF	RIDA	14 k...		3	1		2	3			
1333			Signal recognition particle subunit SRP72 OS=Canis lupus familiaris OX=9615 GN=SRP72 ...	SRP72_CANLF	SRP72	74 k...		2				2		1	1
1334			N-acetylneuraminase synthase OS=Canis lupus familiaris OX=9615 GN=NANS PE=4 SV=1	E2QWN4_CANLF	NANS	40 k...					1	1		2	3
1335			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=SNRPD3 PE=4 SV=1	E2RQ80_CANLF ...	SNRPD3	14 k...		2	1			2	1		
1336			Golgi transport 1B OS=Canis lupus familiaris OX=9615 GN=GOLT1B PE=4 SV=1	F6V493_CANLF	GOLT1B	16 k...		1	2	5	2				
1337			DNA helicase OS=Canis lupus familiaris OX=9615 GN=MCM3 PE=3 SV=2	E2RF73_CANLF	MCM3	91 k...		1				3	1	0	2
1338			Protein tyrosine phosphatase type IVA, member 2 OS=Canis lupus familiaris OX=9615 GN...	E2R341_CANLF	PTP4A2	19 k...		3	1	1		2			
1339			RAP2A, member of RAS oncogene family OS=Canis lupus familiaris OX=9615 GN=RAP2A ...	J9P6K0_CANLF	RAP2A	21 k...	★	13	2		1	8			
1340			Fibrinogen beta chain OS=Canis lupus familiaris OX=9615 GN=FGB PE=4 SV=2	F1PW65_CANLF	FGB	56 k...				2	2			0	2
1341			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=PBDC1 PE=4 SV=2	E2RSR4_CANLF	PBDC1	25 k...		3	1		0	0	0		
1342			Charged multivesicular body protein 1A OS=Canis lupus familiaris OX=9615 GN=CHMP1A...	E2RTK6_CANLF	CHMP1A	22 k...		2	1	2	3				
1343			Metalloproteinase inhibitor 2 OS=Canis lupus familiaris OX=9615 GN=TIMP2 PE=4 SV=2	F1Q4F9_CANLF	TIMP2	38 k...		0	0		1	1		0	
1344			Phosphoribosyl pyrophosphate synthetase 2 OS=Canis lupus familiaris OX=9615 GN=PRP...	E2R0X3_CANLF	PRPS2	35 k...	★	5			2	2		3	2
1345			Axin interactor, dorsalization associated OS=Canis lupus familiaris OX=9615 GN=AIDA PE...	E2QVQ1_CANLF	AIDA	35 k...		2	1	0		1			
1346			Twinfilin actin binding protein 2 OS=Canis lupus familiaris OX=9615 GN=TWf2 PE=4 SV=2	E2RGD3_CANLF	TWf2	39 k...		3			2	3	0		
1347			Splicing factor 3b subunit 3 OS=Canis lupus familiaris OX=9615 GN=SF3B3 PE=4 SV=2	E2RR33_CANLF	SF3B3	136 ...		1				1	0		3
1348			DEAD-box helicase 6 OS=Canis lupus familiaris OX=9615 GN=DDX6 PE=3 SV=1	E2RR01_CANLF	DDX6	54 k...		0		0	4			1	1
1349			Ras homolog family member B OS=Canis lupus familiaris OX=9615 GN=RHOB PE=4 SV=1	J9NUD2_CANLF	RHOB	22 k...	★	8	8			6			
1350			Histone deacetylase OS=Canis lupus familiaris OX=9												

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
			Probability Legend:												
			over 95%												
			80% to 94%												
			50% to 79%												
			20% to 49%												
			0% to 19%												
1365			DENN domain containing 3 OS=Canis lupus familiaris OX=9615 GN=DENND3 PE=4 SV=2	F1PQX0_CANLF	DENND3	139 ...			1	1	0		1		
1365			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	E2R0C4_CANLF		8 kDa		0		1		0	1		
1365			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	F1PRS3_CANLF		213 ...		2	0	0			0		
1365			Signal recognition particle subunit SRP68 OS=Canis lupus familiaris OX=9615 GN=SRP68 ...	F1P142_CANLF	SRP68	70 k...		1						0	2
1365			Hexokinase 2 OS=Canis lupus familiaris OX=9615 GN=HK2 PE=3 SV=2	F1PAZ2_CANLF	HK2	99 k...						0		5	2
1365			Eva-1 homolog B OS=Canis lupus familiaris OX=9615 GN=EVA1B PE=4 SV=1	J9NTS3_CANLF	EVA1B	18 k...		4	1			4			
1365			Vacuolar protein sorting 13 homolog A OS=Canis lupus familiaris OX=9615 GN=VPS13A P...	J9P283_CANLF	VPS13A	365 ...			0	0			0	1	
1365			Collagen type XXIV alpha 1 chain OS=Canis lupus familiaris OX=9615 GN=COL24A1 PE=4...	E2R8U5_CANLF	COL24A1	175 ...			1			0	1		
1365			Lectin, mannose binding 1 OS=Canis lupus familiaris OX=9615 GN=LMAN1 PE=4 SV=2	E2QW60_CANLF	LMAN1	58 k...		1		2		0			
1370			3'-phosphoadenosine 5'-phosphosulfate synthase 1 OS=Canis lupus familiaris OX=9615...	E2QZ13_CANLF	PAPS51	71 k...			0					1	3
1375			Phosphodiesterase 4D interacting protein OS=Canis lupus familiaris OX=9615 GN=PDE4D...	J9NW79_CANLF	PDE4DIP	295 ...			1				0	0	0
1375			Spectrin beta chain OS=Canis lupus familiaris OX=9615 GN=SPTB PE=3 SV=2	F1Q253_CANLF	SPTB	268 ...		1		0	0			0	0
1375			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=2	E2RRD2_CANLF		23 k...	★	19				10			
1375			Aldehyde dehydrogenase 3 family member A2 OS=Canis lupus familiaris OX=9615 GN=A...	E2RPP8_CANLF	ALDH3A2	67 k...	★	3		3					
1375			EGF containing fibulin extracellular matrix protein 2 OS=Canis lupus familiaris OX=9615 ...	E2RNX7_CANLF	EFEMP2	50 k...								3	7
1376			Calpain 13 OS=Canis lupus familiaris OX=9615 GN=CAPN13 PE=3 SV=2	E2QWT7_CANLF	CAPN13	77 k...		2				3			
1377			Carbonic anhydrase 12 OS=Canis lupus familiaris OX=9615 GN=CA12 PE=4 SV=2	F1Q441_CANLF	CA12	40 k...		5				1			
1378			Glycogen debranching enzyme OS=Canis lupus familiaris OX=9615 GN=AGL PE=2 SV=1	GDE_CANLF	AGL	175 ...								1	6
1378			Methylthioribose-1-phosphate isomerase OS=Canis lupus familiaris OX=9615 GN=MRI1 ...	E2R7C2_CANLF	MRI1	39 k...						1			2
1380			Ryanodine receptor 2 OS=Canis lupus familiaris OX=9615 GN=RYR2 PE=4 SV=2	F1PEE8_CANLF	RYR2	564 ...						1	0		
1380			Sorting nexin 2 OS=Canis lupus familiaris OX=9615 GN=SNX2 PE=4 SV=2	F1PMB5_CANLF	SNX2	46 k...		1				1	1	1	2
1382			Bone morphogenetic protein 7 OS=Canis lupus familiaris OX=9615 GN=BMP7 PE=3 SV=1	J9P6P0_CANLF	BMP7	49 k...		1				3	2	1	1
1383			Protein XRP2 OS=Canis lupus familiaris OX=9615 GN=RP2 PE=3 SV=1	E2RK13_CANLF	RP2	39 k...		1	1	1	1	2			
1384			40S ribosomal protein S24 OS=Canis lupus familiaris OX=9615 GN=RPS24 PE=3 SV=2	E2QW78_CANLF...	RPS24	15 k...		3	1			1	0		2
1385			BRICK1, SCAR/WAVE actin nucleating complex subunit OS=Canis lupus familiaris OX=961...	F1PCT1_CANLF	BRK1	12 k...		2	1		2	2			
1386			Tissue alpha-L-fucosidase OS=Canis lupus familiaris OX=9615 GN=FUCA1 PE=4 SV=2	F1PAF0_CANLF ...	FUCA1	54 k...						2	1	1	1
1387			Actin like 6A OS=Canis lupus familiaris OX=9615 GN=ACTL6A PE=3 SV=1	F1Q0F8_CANLF	ACTL6A	48 k...	★					1	1	2	3
1388			DnaJ heat shock protein family (Hsp40) member C7 OS=Canis lupus familiaris OX=9615 ...	E2QWR5_CANLF	DNAJC7	56 k...		2	1	0		1		1	
1388			Chitinase, acidic OS=Canis lupus familiaris OX=9615 GN=CHIA PE=3 SV=1	E2R8B1_CANLF	CHIA	53 k...			2	2				2	2
1390			SBD5, ribosome maturation factor OS=Canis lupus familiaris OX=9615 GN=SBD5 PE=4 SV...	E2R2W6_CANLF	SBD5	29 k...		1			0	4	2	1	
1391			Lumican OS=Canis lupus familiaris OX=9615 GN=LUM PE=4 SV=1	E2R416_CANLF	LUM	38 k...			1	0		0	2	1	1
1392			Signal transducer and activator of transcription OS=Canis lupus familiaris OX=9615 GN=S...	E2R8B2_CANLF	STAT3	88 k...		1				0		2	1
1393			Eukaryotic translation initiation factor 3 subunit D OS=Canis lupus familiaris OX=9615 G...	E2REK3_CANLF	EIF3D	64 k...		1				4	0	3	
1394			Osteoclast stimulating factor 1 OS=Canis lupus familiaris OX=9615 GN=OSTF1 PE=4 SV=2	F1P8W3_CANLF	OSTF1	24 k...		2	1		0	1	0		
1395			Multiple EGF like domains 10 OS=Canis lupus familiaris OX=9615 GN=MEGF10 PE=4 SV=1	E2RQ32_CANLF	MEGF10	123 ...		3	0	0	1	1			
1396			Selenium binding protein 1 OS=Canis lupus familiaris OX=9615 GN=SELENBP1 PE=4 SV=2	F1PNH6_CANLF	SELENBP1	55 k...						1	1	0	4
1397			Phosphoribosylformylglycinamide synthase OS=Canis lupus familiaris OX=9615 GN=PF...	E2QYQ9_CANLF	PFAS	145 ...						3	0	1	3
1398			Adhesion G protein-coupled receptor G6 OS=Canis lupus familiaris OX=9615 GN=ADGRG...	F1PB00_CANLF	ADGRG6	134 ...		0	0				0	1	1
1399			Glycogenin 1 OS=Canis lupus familiaris OX=9615 GN=GYG1 PE=4 SV=2	F1PVC0_CANLF	GYG1	37 k...						3	1	0	1
1400			Thioredoxin interacting protein OS=Canis lupus familiaris OX=9615 GN=TXNIP PE=4 SV=1	E2QX20_CANLF	TXNIP	44 k...		3	0		1	3			
1401			Dynein regulatory complex subunit 7 OS=Canis lupus familiaris OX=9615 GN=DRC7 PE=...	F6V8E4_CANLF	DRC7	102 ...		0		1		0	0	1	
1402			Complement C7 OS=Canis lupus familiaris OX=9615 GN=C7 PE=4 SV=2	E2RG01_CANLF	C7	95 k...				1		0	1		3
1403			Ras homolog family member D OS=Canis lupus familiaris OX=9615 GN=RHOD PE=4 SV=1	F6Y507_CANLF	RHOD	24 k...		2	0	2		2			
1404			ADP ribosylation factor like GTPase 8B OS=Canis lupus familiaris OX=9615 GN=ARL8B PE...	E2RE13_CANLF	ARL8B	22 k...		1			2	1			
1405			Microtubule-associated protein RP/EB family member 1 OS=Canis lupus familiaris OX=96...	F6Y5Z5_CANLF	MAPRE1	33 k...		2			2	0			
1406			Solute carrier family 38 member 5 OS=Canis lupus familiaris OX=9615 GN=SLC38A5 PE=...	F1PJV8_CANLF	SLC38A5	48 k...		3		2	2				
1407			Ras homolog family member C OS=Canis lupus familiaris OX=9615 GN=RHOC PE=4 SV=2	E2RMM5_CANLF	RHOC	23 k...	★	20		10		19			
1408			Beta-hexosaminidase OS=Canis lupus familiaris OX=9615 GN=HEXA PE=3 SV=2	E2RIM8_CANLF	HEXA	61 k...						1	1	1	
1409			Apolipoprotein B mRNA editing enzyme catalytic subunit 2 OS=Canis lupus familiaris OX...	E2RD17_CANLF	APOBEC2	26 k...						1	2	0	
1410			Signal sequence receptor subunit 4 OS=Canis lupus familiaris OX=9615 GN=SSR4 PE=4 S...	E2QY54_CANLF	SSR4	19 k...		5	1	2					
1411			Mannan binding lectin serine peptidase 1 OS=Canis lupus familiaris OX=9615 GN=MASP1...	F1PQ85_CANLF	MASP1	86 k...				2	2			1	
1412			Acetyl-CoA acyltransferase 1 OS=Canis lupus familiaris OX=9615 GN=ACAA1 PE=3 SV=1	E2REH5_CANLF	ACAA1	44 k...		2		1		1			
1413			Versican OS=Canis lupus familiaris OX=9615 GN=VCAN PE=4 SV=2	F1PGL1_CANLF	VCAN	369 ...			2			1	2		
1414			TRK-fused gene OS=Canis lupus familiaris OX=9615 GN=TFG PE=4 SV=1	E2REP2_CANLF	TFG	43 k...		1	1		2				
1415			Family with sequence similarity 53 member C OS=Canis lupus familiaris OX=9615 GN=FA...	E2RGK4_CANLF	FAM53C	43 k...			2	2			2	0	
1416			Fibroblast activation protein alpha OS=Canis lupus familiaris OX=9615 GN=FAP PE=3 SV=2	E2RD94_CANLF	FAP	88 k...						1		2	4
1417			Ubiquitin conjugating enzyme E2 V1 OS=Canis lupus familiaris OX=9615 GN=UBE2V1 PE...	J9P1M6_CANLF	UBE2V1	18 k...		0	0		1	0		0	
1418			Discs large MAGUK scaffold protein 5 OS=Canis lupus familiaris OX=9615 GN=DLG5 PE=...	F6X5Z8_CANLF	DLG5	214 ...		1	0	0		0	0		
1419			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC476006 PE=4 SV=2	F1Q0I8_CANLF	LOC476006	24 k...	★	5			4	5			8
1420			10-formyltetrahydrofolate dehydrogenase OS=Canis lupus familiaris OX=9615 GN=ALDH...	E2RC62_CANLF	ALDH1L2	102 ...	★		0				0	4	4
1421			Cartilage oligomeric matrix protein OS=Canis lupus familiaris OX=9615 GN=COMP PE=4 ...	E2RJEO_CANLF	COMP	87 k...	★		1					1	5
1422			Desmocollin 3 OS=Canis lupus familiaris OX=9615 GN=DSC3 PE=4 SV=2	F1PEF0_CANLF	DSC3	98 k...	★	1		2	0			0	
1423			Fanconi anemia complementation group M OS=Canis lupus familiaris OX=9615 GN=FANCM...	J9P777_CANLF	FANCM	232 ...		0		0		0		1	
1424			Treacle ribosome biogenesis factor 1 OS=Canis lupus familiaris OX=9615 GN=TCOF1 PE=...	E2R7J9_CANLF	TCOF1	145 ...		1	1					0	
1425			Pre-mRNA processing factor 40 homolog A OS=Canis lupus familiaris OX=9615 GN=PRPF...	E2RSV6_CANLF	PRPF40A	109 ...		2	0			1			
1426			Platelet derived growth factor D OS=Canis lupus familiaris OX=9615 GN=PDGFD PE=4 SV...	J9PM86_CANLF	PDGFD	42 k...						3		0	1
1427			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=TMED2 PE=3 SV=1	E2RGZ5_CANLF	TMED2	23 k...		4		1		0			
1428			Collagen type II alpha 1 chain OS=Canis lupus familiaris OX=9615 GN=COL2A1 PE=4 SV=1	F1PS24_CANLF	COL2A1	142 ...	★		1			2			0
1429			IQ motif containing GTPase activating protein 3 OS=Canis lupus familiaris OX=9615 GN=...	F6Y6X6_CANLF	IQGAP3	166 ...		1	0	0	0		0		
1430			Four and a half LIM domains 2 OS=Canis lupus familiaris OX=9615 GN=FHL2 PE=4 SV=2	F1PU47_CANLF	FHL2	32 k...		3		0	1				
1431			Coiled-coil domain containing 177 OS=Canis lupus familiaris OX=9615 GN=CCDC177 PE...	E2QXU0_CANLF	CCDC177	79 k...		1		0	2			0	
1432			Myosin VIIA OS=Canis lupus familiaris OX=9615 GN=MYO7A PE=3 SV=2	F1PB31_CANLF	MYO7A	251 ...					0	1			0
1433			Notch 3 OS=Canis lupus familiaris OX=9615 GN=NOTCH3 PE=4 SV=2	F1Q2J4_CANLF	NOTCH3	216 ...	★	0						1	5
1434			Fibrinogen gamma chain OS=Canis lupus familiaris OX=9615 GN=FGG PE=4 SV=2	F1P8G0_CANLF	FGG	49 k...				3				1	
1435			Membrane palmitoylated protein 5 OS=Canis lupus familiaris OX=9615 GN=MPP5 PE=3 S...	E2QY99_CANLF	MPP5	77 k...		4			1				
1436			Synemin OS=Canis lupus familiaris OX=9615 GN=SYNM PE=3 SV=2	E2R151_CANLF	SYNM	169 ...									

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated				
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4	
				Probability Legend:												
				over 95%												
				80% to 94%												
				50% to 79%												
				20% to 49%												
				0% to 19%												
1446			Thiopurine S-methyltransferase OS=Canis lupus familiaris OX=9615 GN=TPMT PE=2 SV=1	TPMT_CANLF	TPMT	28 k...							1	1	1	1
1447			Transportin 3 OS=Canis lupus familiaris OX=9615 GN=TNPO3 PE=4 SV=2	E2REK5_CANLF	TNPO3	104 ...		1	1		0	1				1
1448			Presenilin OS=Canis lupus familiaris OX=9615 GN=PSEN1 PE=3 SV=2	F1PHD2_CANLF	...PSEN1	52 k...		3	1			1	1			
1449			Methionyl-tRNA synthetase OS=Canis lupus familiaris OX=9615 GN=MARS PE=3 SV=1	F6XD28_CANLF	MARS	101 ...		1				3			1	2
1450			Endonuclease domain containing 1 OS=Canis lupus familiaris OX=9615 GN=ENDOD1 PE=...	F1PQA0_CANLF	ENDOD1	37 k...		3	1	1		1				
1451			G protein pathway suppressor 1 OS=Canis lupus familiaris OX=9615 GN=GP51 PE=4 SV=2	F1P996_CANLF	GP51	49 k...		1				1			2	1
1452			Tetraspanin OS=Canis lupus familiaris OX=9615 GN=TSPAN1 PE=3 SV=2	E2RPE6_CANLF	TSPAN1	32 k...		2			1	1	2			
1453			DLA class II histocompatibility antigen, DR-1 beta chain OS=Canis lupus familiaris OX=9...	HB2D_CANLF		30 k...	★		11	10	24	25	6			
1454			Lysophospholipase I OS=Canis lupus familiaris OX=9615 GN=LYPLA1 PE=4 SV=2	F1PQ50_CANLF	LYPLA1	23 k...		1	0			2	1			1
1455			C1GALT1 specific chaperone 1 OS=Canis lupus familiaris OX=9615 GN=C1GALT1C1 PE=4...	E2RMM2_CANLF	C1GALT1C1	36 k...		1				3	1		2	
1456			Fibulin 5 OS=Canis lupus familiaris OX=9615 GN=FBLN5 PE=4 SV=2	F1PJM1_CANLF	FBLN5	51 k...					1	1	0	0	1	
1457			Lysine methyltransferase 2D OS=Canis lupus familiaris OX=9615 GN=KMT2D PE=4 SV=1	J9P0X8_CANLF	KMT2D	609 ...		2	1	1	0			1		
1458			Transforming growth factor beta receptor 3 OS=Canis lupus familiaris OX=9615 GN=TGF...	F1PIG0_CANLF	TGFBF3	94 k...						1	0	0	2	
1459			Spermine synthase OS=Canis lupus familiaris OX=9615 GN=SMS PE=4 SV=1	F1PF82_CANLF	SMS	41 k...		1	1		1	1				
1460			ER lipid raft associated 2 OS=Canis lupus familiaris OX=9615 GN=ERLIN2 PE=4 SV=1	E2QY23_CANLF	ERLIN2	38 k...		3	1	2		0				
1461			RELT like 1 OS=Canis lupus familiaris OX=9615 GN=RELL1 PE=4 SV=2	E2QUZ0_CANLF	RELL1	29 k...		3		0		3	1			
1462			Heterogeneous nuclear ribonucleoprotein R OS=Canis lupus familiaris OX=9615 GN=HNR...	E2RE36_CANLF	HNRNPR	71 k...	★	3	2			3				
1463			UDP-glucose glycoprotein glucosyltransferase 1 OS=Canis lupus familiaris OX=9615 GN=...	E2RR16_CANLF	UGGT1	177 ...			0	1		1		0		
1464			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC607207 PE=4 SV=2	E2QY85_CANLF	...LOC607207	20 k...		3				2	2			
1465			Prefoldin subunit 3 OS=Canis lupus familiaris OX=9615 GN=VBP1 PE=3 SV=1	F6XQ49_CANLF	VBP1	22 k...		1				2	2			
1466			E3 ubiquitin-protein ligase OS=Canis lupus familiaris OX=9615 GN=WWP2 PE=4 SV=2	E2RBA6_CANLF	WWP2	100 ...	★	4		2		1				
1467			Heterogeneous nuclear ribonucleoprotein A2/B1 OS=Canis lupus familiaris OX=9615 GN=...	F1Q1K6_CANLF	HNRNPA2B1	37 k...		2				3	1			
1468			FERM, ARH/RhoGEF and pleckstrin domain protein 2 OS=Canis lupus familiaris OX=9615 ...	E2R9U9_CANLF	FARP2	117 ...			1	2		2				
1469			TNF receptor superfamily member 12A OS=Canis lupus familiaris OX=9615 GN=TNFRSF1...	E2R8M4_CANLF	TNFRSF12A	13 k...		2	1			3				
1470			Phosphoserine phosphatase OS=Canis lupus familiaris OX=9615 GN=PSPH PE=4 SV=2	E2RPN5_CANLF	PSPH	26 k...						1	1	2		
1471			Cullin 1 OS=Canis lupus familiaris OX=9615 GN=CUL1 PE=3 SV=2	E2R1V2_CANLF	CUL1	90 k...		1				2				1
1472			Interleukin enhancer binding factor 3 OS=Canis lupus familiaris OX=9615 GN=ILF3 PE=4 ...	F1PG86_CANLF	ILF3	96 k...								1	1	
1473			Desmocollin 2 OS=Canis lupus familiaris OX=9615 GN=DSC2 PE=4 SV=2	F1PEF1_CANLF	DSC2	101 ...	★	2		1		2				
1474			Adhesion G protein-coupled receptor G1 OS=Canis lupus familiaris OX=9615 GN=ADGRG...	F6UZU1_CANLF	ADGRG1	79 k...		1		1	2					
1475			Translocin-associated protein subunit alpha OS=Canis lupus familiaris OX=9615 GN=SS...	A0A0A0MPC0_...	SSR1	33 k...		3		3		2				
1476			Adenylate kinase 2, mitochondrial OS=Canis lupus familiaris OX=9615 GN=AK2 PE=3 SV...	E2RE39_CANLF	AK2	27 k...		3			1	1				
1477			COP9 signalosome subunit 8 OS=Canis lupus familiaris OX=9615 GN=COPS8 PE=4 SV=1	J9NYE0_CANLF	COPS8	23 k...		1						1	3	
1478			Acyl-CoA-binding protein OS=Canis lupus familiaris OX=9615 GN=DBI PE=1 SV=2	ACBP_CANLF	DBI	10 k...		2	1			0	1			
1479			Proteasome subunit alpha type OS=Canis lupus familiaris OX=9615 GN=PSMA8 PE=3 SV=2	E2RF52_CANLF	PSMA8	28 k...	★	7				3	6			
1480			Glutathione peroxidase OS=Canis lupus familiaris OX=9615 GN=GPX3 PE=3 SV=1	J9P028_CANLF	GPX3	17 k...						2	1			2
1481			cAMP-dependent protein kinase catalytic subunit alpha OS=Canis lupus familiaris OX=96...	KAPCA_CANLF	PRKACA	41 k...						1	0	1	0	2
1482			Intercellular adhesion molecule 1 OS=Canis lupus familiaris OX=9615 GN=ICAM1 PE=4 S...	F1PB95_CANLF	ICAM1	58 k...	★	21	10			15				
1483			RAD23 homolog A, nucleotide excision repair protein OS=Canis lupus familiaris OX=9615...	F1PTL1_CANLF	RAD23A	40 k...	★	1	1			1				
1484			NADH-cytochrome b5 reductase OS=Canis lupus familiaris OX=9615 GN=CYB5R3 PE=3 S...	F1PS26_CANLF	(...CYB5R3	36 k...					4	1			1	
1485			Cysteine sulfinic acid decarboxylase OS=Canis lupus familiaris OX=9615 GN=CSAD PE=3 ...	F1PKT4_CANLF	CSAD	56 k...							1		3	1
1486			Endothelin converting enzyme 1 OS=Canis lupus familiaris OX=9615 GN=ECE1 PE=4 SV=2	F1PK66_CANLF	ECE1	85 k...		4		1			0			
1487			Flap endonuclease 1 OS=Canis lupus familiaris OX=9615 GN=FEN1 PE=3 SV=1	J9PB88_CANLF	FEN1	43 k...		0	0			1	0	0	0	0
1488			Ring finger protein 17 OS=Canis lupus familiaris OX=9615 GN=RNF17 PE=4 SV=1	J9NYU9_CANLF	RNF17	183 ...					1	2		0		
1489			Ubiquitin conjugating enzyme E2 M OS=Canis lupus familiaris OX=9615 GN=UBE2M PE=3...	E2RIU5_CANLF	UBE2M	21 k...		2				1	1			
1490			Sodium- and chloride-dependent betaine transporter OS=Canis lupus familiaris OX=9615...	56A12_CANLF	SLC6A12	69 k...	★	1		1	0					
1491			RB binding protein 4, chromatin remodeling factor OS=Canis lupus familiaris OX=9615 G...	E2QXR8_CANLF	RBBP4	48 k...	★					2	2	5	6	
1492			CD276 molecule OS=Canis lupus familiaris OX=9615 GN=CD276 PE=4 SV=2	F1PDPS_CANLF	CD276	57 k...		2				1	1			
1493			Glycosyltransferase 8 domain containing 1 OS=Canis lupus familiaris OX=9615 GN=GLT8...	F1PTP8_CANLF	GLT8D1	45 k...				0		0		2	1	
1494			Echinoderm microtubule associated protein like 4 OS=Canis lupus familiaris OX=9615 GN...	F1PY01_CANLF	EML4	109 ...					2	0	0	0	1	
1495			Regulator of chromosome condensation 2 OS=Canis lupus familiaris OX=9615 GN=RCC2 ...	E2QWR3_CANLF	RCC2	47 k...				1				0		1
1496			Chromosome 1 open reading frame 216 OS=Canis lupus familiaris OX=9615 GN=C15H1o...	E2QW37_CANLF	C15H1orf216	25 k...		1		0					0	
1497			Proteasome activator subunit 1 OS=Canis lupus familiaris OX=9615 GN=PSME1 PE=4 SV=1	F6UY11_CANLF	PSME1	27 k...		0				1	1			0
1498			Isocitrate dehydrogenase [NADP] OS=Canis lupus familiaris OX=9615 GN=IDH2 PE=3 SV=2	F1PAM3_CANLF	IDH2	47 k...	★			1			2	4		
1499			CD2 associated protein OS=Canis lupus familiaris OX=9615 GN=CD2AP PE=4 SV=2	F1PUU5_CANLF	CD2AP	73 k...		1				1	1			
1500			Glutathione peroxidase OS=Canis lupus familiaris OX=9615 GN=GPX1 PE=3 SV=1	F6XH46_CANLF	GPX1	17 k...						1	1			0
1501			Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial OS=Canis lupu...	F1Q1X9_CANLF	SDHA	79 k...					3		1	0		
1502			Triacylglycerol lipase OS=Canis lupus familiaris OX=9615 GN=PNLIPRP2 PE=3 SV=2	F1PHZ2_CANLF	PNLIPRP2	53 k...			0	1			0	1		
1503			Aspartate aminotransferase OS=Canis lupus familiaris OX=9615 GN=GOT2 PE=4 SV=1	A0A0B4J194_C...	GOT2	47 k...		3		3			0			
1504			Niemann-Pick C1-like protein 1 precursor OS=Canis lupus familiaris OX=9615 GN=NPC1...	J9PB86_CANLF	NPC1L1	37 k...		4		0		2				
1505			Coiled-coil domain containing 170 OS=Canis lupus familiaris OX=9615 GN=CCDC170 PE...	F1PGL3_CANLF	CCDC170	81 k...			0	0				0	1	
1506			Coiled-coil domain containing 88A OS=Canis lupus familiaris OX=9615 GN=CCDC88A PE...	E2R498_CANLF	CCDC88A	216 ...			1			0	0			
1507			DeXD-box helicase 21 OS=Canis lupus familiaris OX=9615 GN=DDX21 PE=4 SV=1	F6V8G4_CANLF	DDX21	88 k...				0		0			0	3
1508			V-type proton ATPase subunit A OS=Canis lupus familiaris OX=9615 GN=TCIRG1 PE=3 SV...	E2QXJ5_CANLF	TCIRG1	93 k...		1		3		0				
1509			Glyceraldehyde-3-phosphate dehydrogenase OS=Canis lupus familiaris OX=9615 GN=GA...	G3P_CANLF	GAPDH	36 k...	★	59				87	32			156
1510			Coiled-coil domain containing 88C OS=Canis lupus familiaris OX=9615 GN=CCDC88C PE...	F1PMI5_CANLF	CCDC88C	227 ...		1		1		0				
1511			Thioredoxin domain containing 5 OS=Canis lupus familiaris OX=9615 GN=TXNDC5 PE=3 ...	F1PHP1_CANLF	TXNDC5	36 k...		2		3						
1512			Transcription factor BTF3 OS=Canis lupus familiaris OX=9615 GN=BTF3 PE=3 SV=1	F1PI57_CANLF	BTF3	15 k...		4				3				
1513			Eukaryotic translation initiation factor 1A, X-linked OS=Canis lupus familiaris OX=9615 G...	J9NYQ8_CANLF	EIF1AX	16 k...		5				2				
1514			Vacuolar protein sorting 36 homolog OS=Canis lupus familiaris OX=9615 GN=VPS36 PE=...	E2QV84_CANLF	VPS36	44 k...		1				2				
1515			Ribosomal L1 domain containing 1 OS=Canis lupus familiaris OX=9615 GN=RSL1D1 PE=4...	E2R755_CANLF	RSL1D1	55 k...		3				1				
1516			DNA-directed RNA polymerase subunit OS=Canis lupus familiaris OX=9615 GN=POLR2A ...	F1PG50_CANLF	POLR2A	217 ...						2	1			
1517			Centrosomal protein 128 OS=Canis lupus familiaris OX=9615 GN=CEP128 PE=4 SV=2	E2RR71_CANLF	CEP128	136 ...						1	0	1		
1518			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=TNNI3K PE=4 SV=2	F1P6V7_CANLF	TNNI3K	93 k...				0		1	1			

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
								Probability Legend: over 95% 80% to 94% 50% to 79% 20% to 49% 0% to 19%							
1533			Small nuclear ribonucleoprotein D2 polypeptide OS=Canis lupus familiaris OX=9615 GN=...	F1PSI7_CANLF	SNRPD2	14 k...		1	2	0	1	1	1		
1533			Eukaryotic translation initiation factor 2 subunit beta OS=Canis lupus familiaris OX=9615...	F1PZ47_CANLF	EIF2S2	38 k...		1			1	2			1
1533			Oncostatin M receptor OS=Canis lupus familiaris OX=9615 GN=OSMR PE=4 SV=1	E2QWS7_CANLF	OSMR	109 ...		0		1	1	1			
1534			S-phase kinase-associated protein 1 OS=Canis lupus familiaris OX=9615 GN=SKP1 PE=3...	E2RRW0_CANLF	SKP1	19 k...		2	1		1	1			
1535			Heterogeneous nuclear ribonucleoprotein F OS=Canis lupus familiaris OX=9615 GN=HNR...	E2RB90_CANLF	HNRNPf	46 k...	★	4	1	1	2	2			
1536			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	E2R4V7_CANLF		28 k...		1	0		3			1	
1533			Secretion associated Ras related GTPase 1B OS=Canis lupus familiaris OX=9615 GN=SAR1...	E2RRQ3_CANLF	SAR1B	22 k...		1			2	1	1		0
1536			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=PIP4K2A PE=4 SV=2	F1PPJ1_CANLF	PIP4K2A	46 k...		0	1	1	1	0			
1535			Complement C9 OS=Canis lupus familiaris OX=9615 GN=C9 PE=4 SV=2	E2RFV9_CANLF	C9	62 k...			1	2	2				2
1540			Serine/threonine-protein phosphatase OS=Canis lupus familiaris OX=9615 GN=PPP1CC P...	E2QXE0_CANLF	PPP1CC	39 k...	★				8	13	9	7	16
1543			Dynein cytoplasmic 1 intermediate chain 2 OS=Canis lupus familiaris OX=9615 GN=DYNC...	E2RM92_CANLF	DYNC112	68 k...		1			0	1		2	
1543			ADP ribosylation factor like GTPase 3 OS=Canis lupus familiaris OX=9615 GN=ARL3 PE=3...	J9NTN4_CANLF	ARL3	22 k...		1	1		1	0	0		
1543			Sulfhydryl oxidase OS=Canis lupus familiaris OX=9615 GN=QSOX2 PE=4 SV=2	F1PCH0_CANLF	QSOX2	78 k...		0		1				0	2
1544			O-linked N-acetylglucosamine (GlcNAc) transferase OS=Canis lupus familiaris OX=9615 ...	E2QSQ5_CANLF	OGT	117 ...				0		2		1	1
1545			Quinone oxidoreductase OS=Canis lupus familiaris OX=9615 GN=CRYZ PE=4 SV=2	E2R3I8_CANLF	CRYZ	36 k...		0		1		1		2	
1546			Carbohydrate sulfotransferase OS=Canis lupus familiaris OX=9615 GN=CHST12 PE=3 SV...	J9NS69_CANLF	CHST12	49 k...	★	0				1	2	2	
1543			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	E2R118_CANLF		16 k...		1			1			1	0
1548			Tetraspanin OS=Canis lupus familiaris OX=9615 GN=UPK1B PE=3 SV=1	E2QUQ7_CANLF	UPK1B	30 k...		2	1		0	0			
1545			Transmembrane protein 33 OS=Canis lupus familiaris OX=9615 GN=TMEM33 PE=4 SV=1	E2RMY0_CANLF	TMEM33	28 k...		2	2	1		0			
1556			Developmentally regulated GTP binding protein 1 OS=Canis lupus familiaris OX=9615 GN...	J9P0A6_CANLF	DRG1	41 k...		1			0		1		1
1553			Sorbitol dehydrogenase OS=Canis lupus familiaris OX=9615 GN=SORD PE=3 SV=2	F1PXG0_CANLF	SORD	38 k...						0	1	1	3
1552			Enolase 3 OS=Canis lupus familiaris OX=9615 GN=ENO3 PE=3 SV=1	E2R5B2_CANLF	ENO3	47 k...	★					20	12	9	
1553			Major prion protein (Fragment) OS=Canis lupus familiaris OX=9615 GN=PRNP PE=2 SV=1	Q1W2J9_CANLF	PRNP	28 k...		2	1		0	2			
1554			Serine/threonine-protein kinase PLK OS=Canis lupus familiaris OX=9615 GN=PLK1 PE=3 ...	J9P0Q4_CANLF	PLK1	68 k...		2	1			1		0	
1555			Casein kinase II subunit beta OS=Canis lupus familiaris OX=9615 GN=CSNK2B PE=3 SV=2	E2RP71_CANLF	CSNK2B	34 k...		3			2				1
1556			Endoplasmic reticulum oxidoreductase 1 alpha OS=Canis lupus familiaris OX=9615 GN=E...	E2RWN5_CANLF	ERO1A	54 k...		2		2			1		
1553			Biliverdin reductase A OS=Canis lupus familiaris OX=9615 GN=BLVRA PE=3 SV=2	F1PCM1_CANLF	BLVRA	34 k...							1	4	2
1554			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=USP9X PE=3 SV=2	E2RBU9_CANLF	USP9X	190 ...			1			0		2	1
1555			Notchless homolog 1 OS=Canis lupus familiaris OX=9615 GN=NLE1 PE=4 SV=1	J9NRM1_CANLF	NLE1	59 k...						1		1	3
1560			Purine rich element binding protein A OS=Canis lupus familiaris OX=9615 GN=PURA PE=...	E2R2A4_CANLF	PURA	56 k...		2	0			1			
1563			Cyclin and CBS domain divalent metal cation transport mediator 4 OS=Canis lupus familia...	F1PJ05_CANLF	CNNM4	87 k...		1	1			2			
1562			COP9 signalosome subunit 3 OS=Canis lupus familiaris OX=9615 GN=COP3 PE=4 SV=1	E2RD70_CANLF	COP3	48 k...		0				1	0	1	3
1563			Malic enzyme OS=Canis lupus familiaris OX=9615 GN=ME1 PE=3 SV=1	J9NZX7_CANLF	ME1	55 k...		3			1				2
1564			Xylulokinase OS=Canis lupus familiaris OX=9615 GN=XYLB PE=4 SV=2	F1PI40_CANLF	XYLB	58 k...						1		2	2
1565			Ecm29 proteasome adaptor and scaffold OS=Canis lupus familiaris OX=9615 GN=ECPAS P...	F1PYA6_CANLF	ECPAS	203 ...		0	0	1		0		0	1
1566			Dystroglycan OS=Canis lupus familiaris OX=9615 GN=DAG1 PE=3 SV=1	DAG1_CANLF	DAG1	97 k...		0	1			1			
1563			Oncoprotein induced transcript 3 OS=Canis lupus familiaris OX=9615 GN=OIT3 PE=4 SV=2	E2RAS2_CANLF	OIT3	37 k...							0	1	3
1563			Citrate synthase OS=Canis lupus familiaris OX=9615 GN=CS PE=3 SV=2	F1PV92_CANLF	CS	52 k...		1		2			1		0
1565			Calcium and integrin binding 1 OS=Canis lupus familiaris OX=9615 GN=CIB1 PE=4 SV=1	E2RRH5_CANLF	CIB1	22 k...		3			1	1			
1570			NPC intracellular cholesterol transporter 1 OS=Canis lupus familiaris OX=9615 GN=NPC1 ...	F1PB50_CANLF	NPC1	142 ...		3		0	1				
1573			Tubulin folding cofactor E OS=Canis lupus familiaris OX=9615 GN=TBCE PE=4 SV=2	E2R359_CANLF	TBCE	61 k...				0	0	2		1	0
1573			Microtubule-associated protein OS=Canis lupus familiaris OX=9615 GN=MAP4 PE=4 SV=2	F1PCX8_CANLF	MAP4	119 ...					1		1		
1573			Aspartate beta-hydroxylase OS=Canis lupus familiaris OX=9615 GN=ASPH PE=4 SV=2	F1Q257_CANLF	ASPH	86 k...		1		1	0		0		0
1574			Aldo-keto reductase family 1 member E2 OS=Canis lupus familiaris OX=9615 GN=AKR1E...	J9NSI8_CANLF	AKR1E2	36 k...	★					1	1	5	2
1574			Four jointed box 1 OS=Canis lupus familiaris OX=9615 GN=FJX1 PE=4 SV=1	J9P056_CANLF	FJX1	42 k...						0		2	3
1576			Piccolo presynaptic cytomatrix protein OS=Canis lupus familiaris OX=9615 GN=PCLO PE=...	F1Q2N0_CANLF	PCLO	530 ...				0		1	0	0	
1573			Kallikrein B1 OS=Canis lupus familiaris OX=9615 GN=KLKB1 PE=2 SV=2	F1PNV5_CANLF	KLKB1	71 k...				1		0			3
1574			Leucine rich repeat containing 1 OS=Canis lupus familiaris OX=9615 GN=LRRC1 PE=4 SV...	E2RRN2_CANLF	LRRC1	59 k...	★	3	0			2			
1575			Kinesin associated protein 3 OS=Canis lupus familiaris OX=9615 GN=KIFAP3 PE=4 SV=2	E2RMU6_CANLF	KIFAP3	91 k...			0		1			0	
1580			Structural maintenance of chromosomes protein OS=Canis lupus familiaris OX=9615 GN=...	E2R7R8_CANLF	SMC2	135 ...		1	0			0	0	1	
1583			Mahogunin ring finger 1 OS=Canis lupus familiaris OX=9615 GN=MGRN1 PE=4 SV=1	F1Q078_CANLF	MGRN1	60 k...		3				2			
1582			Glutaminyl-peptide cyclotransferase OS=Canis lupus familiaris OX=9615 GN=QPCT PE=4 ...	F1PZF4_CANLF	QPCT	41 k...						2		3	
1583			Phenylalanine hydroxylase OS=Canis lupus familiaris OX=9615 GN=PAH PE=4 SV=1	F6XY00_CANLF	PAH	65 k...								2	1
1584			Elongation factor Tu OS=Canis lupus familiaris OX=9615 GN=TUFM PE=3 SV=1	E2RSU3_CANLF	TUFM	49 k...			2	5					
1585			Peptidylprolyl isomerase OS=Canis lupus familiaris OX=9615 GN=FKBP10 PE=4 SV=1	E2QZA8_CANLF	FKBP10	64 k...		1			3				
1586			Hyaluronidase OS=Canis lupus familiaris OX=9615 GN=HYAL2 PE=3 SV=2	E2R560_CANLF	HYAL2	54 k...		1				1			
1583			Cytoskeleton associated protein 5 OS=Canis lupus familiaris OX=9615 GN=CKAP5 PE=4 ...	E2RSW0_CANLF	CKAP5	225 ...		3						0	
1584			Ladinin 1 OS=Canis lupus familiaris OX=9615 GN=LAD1 PE=4 SV=1	F6XZH6_CANLF	LAD1	50 k...		4				1			
1585			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=HNRNPUL2 PE=4 SV=1	J9PAS2_CANLF	HNRNPUL2	86 k...		1			0				
1590			Sad1 and UNC84 domain containing 2 OS=Canis lupus familiaris OX=9615 GN=SUN2 PE=...	E2RL48_CANLF	SUN2	85 k...		1		1					
1593			LCA5, lebercilin OS=Canis lupus familiaris OX=9615 GN=LCA5 PE=4 SV=2	E2R449_CANLF	LCA5	79 k...				2		1			
1593			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=DDX52 PE=4 SV=2	E2RB54_CANLF ...	DDX52	68 k...			0	1					0
1593			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=KNG1 PE=4 SV=2	E2R886_CANLF	KNG1	47 k...				3					2
1594			Kinesin family member C2 OS=Canis lupus familiaris OX=9615 GN=KIFC2 PE=3 SV=2	E2RN60_CANLF	KIFC2	85 k...		0	0	1					
1595			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=HSPE1 PE=4 SV=1	E2R841_CANLF	HSPE1	26 k...						1		1	
1596			Mov10 RISC complex RNA helicase OS=Canis lupus familiaris OX=9615 GN=MOV10 PE=4 ...	F1P7T4_CANLF	MOV10	114 ...				1	0				0
1597			Elongin B OS=Canis lupus familiaris OX=9615 GN=ELOB PE=4 SV=1	J9P8V5_CANLF	ELOB	13 k...		1	0			0	0		
1598			GDP-mannose pyrophosphorylase A OS=Canis lupus familiaris OX=9615 GN=GMPPA PE=...	E2R1D1_CANLF	GMPPA	46 k...		0				1	0		
1598			Gap junction protein OS=Canis lupus familiaris OX=9615 GN=GJB4 PE=3 SV=1	E2R5G3_CANLF	GJB4	30 k...		5		1					
1600			DNA helicase OS=Canis lupus familiaris OX=9615 GN=MCM5 PE=3 SV=2	F1P8U8_CANLF	MCM5	74 k...						1			4
1603			Endoplasmic reticulum-golgi intermediate compartment 1 OS=Canis lupus familiaris OX=...	E2QTE3_CANLF	ERGIC1	33 k...		2				1			
1602			Solute carrier family 12 member 6 OS=Canis lupus familiaris OX=9615 GN=SLC12A6 PE=...	F1Q2J3_CANLF	SLC12A6	128 ...	★	2		0					
1603			Histocompatibility minor 13 OS=Canis lupus familiaris OX=9615 GN=HM13 PE=4 SV=2	E2RB96_CANLF	HM13	47 k...		1			1				
1604			Dynein axonemal heavy chain 1 OS=Canis lupus familiaris OX=9615 GN=DNAH1 PE=4 SV...	F1PDQ9_CANLF	DNAH1	492 ...			0		0			1	
1605			Transcription factor 25 OS=Canis lupus familiaris OX=9615 GN=TCF25 PE=4 SV=2	E2R5D7_CANLF	TCF25	77 k...				1				0	
1606			Tight junction protein ZO-3 OS=Canis lupus familiaris OX=9615 GN=TJP3 PE=3 SV=2	F1Q080_CANLF	TJP3	114 ...				0		1		0	
1607			Pre-mRNA processing factor 4 OS=Canis lupus familiaris OX=9615 GN=PRPF4 PE=4 SV=2	F1PZB0_CANLF	PRPF4	60 k...								0	4
1608			Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3B OS=Canil...	STT3B_CANLF	STT3B	94 k...	★	0		2					

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
			Probability Legend:												
			over 95%												
			80% to 94%												
			50% to 79%												
			20% to 49%												
			0% to 19%												
1616			Histone H3 OS=Canis lupus familiaris OX=9615 GN=LOC475916 PE=3 SV=1	E2R6K5_CANLF	LOC475916	15 k...	★	23							
1617			Lipase OS=Canis lupus familiaris OX=9615 GN=LIPA PE=3 SV=2	E2QX51_CANLF	LIPA	46 k...		1		1		1	1	1	
1618			Myotrophin OS=Canis lupus familiaris OX=9615 GN=MTPN PE=3 SV=3	MTPN_CANLF	MTPN	13 k...		1				0	1	1	1
1619			Cysteine rich protein 2 OS=Canis lupus familiaris OX=9615 GN=CRIP2 PE=4 SV=2	F1PP40_CANLF	CRIP2	23 k...		2	1	1	0	1			
1620			Spermidine synthase OS=Canis lupus familiaris OX=9615 GN=SRM PE=3 SV=1	J9P2P6_CANLF	SRM	28 k...		1			1	1		2	1
1621			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9NRK5_CANLF		30 k...		2	1		1	1	0		
1622			Claudin 12 OS=Canis lupus familiaris OX=9615 GN=CLDN12 PE=4 SV=1	E2RH20_CANLF	CLDN12	27 k...		2	1	1		1			
1623			CD74 molecule OS=Canis lupus familiaris OX=9615 GN=CD74 PE=4 SV=2	E2RF90_CANLF	CD74	34 k...		2	1		2	1			
1624			Keratin 72 OS=Canis lupus familiaris OX=9615 GN=KRT72 PE=3 SV=2	E2QUT7_CANLF	KRT72	57 k...		2	1	1		1			
1625			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=KRT76 PE=3 SV=2	F1Q3U2_CANLF	KRT76	66 k...	★	8	3	5		4			
1626			Solute carrier family 16 member 3 OS=Canis lupus familiaris OX=9615 GN=SLC16A3 PE=...	E2QZD4_CANLF	SLC16A3	50 k...		2	0	1	1	1			
1627			Cullin 3 OS=Canis lupus familiaris OX=9615 GN=CUL3 PE=3 SV=1	F1PU58_CANLF	CUL3	87 k...		1		1	0	2			
1628			Cytochrome c OS=Canis lupus familiaris OX=9615 GN=CYCS PE=1 SV=2	CYC_CANLF	CYCS	12 k...		1	1		1	1			
1629			Mannose receptor C type 2 OS=Canis lupus familiaris OX=9615 GN=MRC2 PE=4 SV=1	J9NY60_CANLF	MRC2	178 ...			1	0		0	1		1
1630			Connective tissue growth factor OS=Canis lupus familiaris OX=9615 GN=CTGF PE=4 SV=1	J9NTX8_CANLF	CTGF	38 k...		0				1	2		1
1631			High mobility group protein B1 OS=Canis lupus familiaris OX=9615 GN=HMGB1 PE=2 SV...	HMGB1_CANLF	HMGB1	25 k...		1			1	1			
1632			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9NTH4_CANLF		14 k...		2	1						1
1633			RAB34, member RAS oncogene family OS=Canis lupus familiaris OX=9615 GN=RAB34 PE...	F1PDF1_CANLF	RAB34	29 k...		2		0	1	1			
1634			Ubiquitin conjugating enzyme E2 K OS=Canis lupus familiaris OX=9615 GN=UBE2K PE=3 ...	E2R6W9_CANLF	UBE2K	22 k...		2	1		0	1			
1635			Zinc finger protein 214 OS=Canis lupus familiaris OX=9615 GN=ZNF214 PE=4 SV=2	E2RJ82_CANLF	ZNF214	77 k...				0	1			0	1
1636			RAB8B, member RAS oncogene family OS=Canis lupus familiaris OX=9615 GN=RAB8B PE...	E2RRT3_CANLF	RAB8B	24 k...	★	3		3		4			
1637			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9P8U3_CANLF		27 k...				5	4	3			
1638			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=WDR77 PE=4 SV=2	E2QW05_CANLF	WDR77	47 k...		0				1	0	0	
1639			A-kinase anchoring protein 12 OS=Canis lupus familiaris OX=9615 GN=AKAP12 PE=4 SV...	F1PGL8_CANLF	AKAP12	188 ...		1	3			1			
1640			Calponin OS=Canis lupus familiaris OX=9615 GN=CNN2 PE=3 SV=1	E2R5Q1_CANLF	CNN2	33 k...		1		1	2				
1641			S-formylglutathione hydrolase OS=Canis lupus familiaris OX=9615 GN=ESD PE=3 SV=1	E2RCQ9_CANLF	ESD	31 k...					1		0	0	1
1642			Signal peptidase complex catalytic subunit SEC11A OS=Canis lupus familiaris OX=9615 G...	SC11A_CANLF	SEC11A	21 k...		2		1		1			
1643			Dynein axonemal assembly factor 5 OS=Canis lupus familiaris OX=9615 GN=DNAAF5 PE...	E2R9K0_CANLF	DNAAF5	95 k...						2	1	1	
1644			Keratin 24 OS=Canis lupus familiaris OX=9615 GN=KRT24 PE=3 SV=2	E2R150_CANLF	KRT24	56 k...	★	1	1				1		
1645			Glia maturation factor beta OS=Canis lupus familiaris OX=9615 GN=GMFB PE=4 SV=1	J9NV86_CANLF	GMFB	17 k...		1		0		1	1		
1646			ATP binding cassette subfamily C member 2 OS=Canis lupus familiaris OX=9615 GN=AB...	F1PHV0_CANLF	ABCC2	174 ...		1				1	1		
1647			Programmed cell death 10 OS=Canis lupus familiaris OX=9615 GN=PCD10 PE=4 SV=2	E2R9R6_CANLF	PCD10	21 k...		2			2	1			
1648			Signal peptidase complex subunit 2 OS=Canis lupus familiaris OX=9615 GN=SPCS2 PE=1 ...	SPCS2_CANLF	SPCS2	25 k...		2		2		1			
1649			Heterogeneous nuclear ribonucleoprotein A/B OS=Canis lupus familiaris OX=9615 GN=H...	F1PA19_CANLF	HNRNPAB	30 k...		2	1			3	2		
1650			Zinc finger protein 239 OS=Canis lupus familiaris OX=9615 GN=ZNF239 PE=4 SV=1	J9NV92_CANLF	ZNF239	46 k...				0	2			0	1
1651			Ubiquitin specific peptidase 12 OS=Canis lupus familiaris OX=9615 GN=USP12 PE=3 SV=1	E2RG22_CANLF	USP12	43 k...		2	1	0		1			
1652			Far upstream element binding protein 1 OS=Canis lupus familiaris OX=9615 GN=FUBP1 P...	E2RJQ2_CANLF	FUBP1	70 k...		1		0		1	0	0	
1653			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	E2R259_CANLF		48 k...		1	0	0	1				
1654			Serine/threonine-protein phosphatase OS=Canis lupus familiaris OX=9615 GN=PPP6C PE...	J9P6W4_CANLF	PPP6C	35 k...				1		1			1
1655			Low-density lipoprotein receptor-related protein OS=Canis lupus familiaris OX=9615 GN...	F1PXA0_CANLF	LRP5	179 ...		1				0		2	
1656			Procollagen C-endopeptidase enhancer 2 OS=Canis lupus familiaris OX=9615 GN=PCOLC...	J9P1U0_CANLF	PCOLCE2	47 k...						1	0		2
1657			PERP, TP53 apoptosis effector OS=Canis lupus familiaris OX=9615 GN=PERP PE=4 SV=1	J9NZW5_CANLF	PERP	21 k...		2		0	0	1			
1658			Notch 2 OS=Canis lupus familiaris OX=9615 GN=NOTCH2 PE=4 SV=2	F1PS94_CANLF	NOTCH2	265 ...		1				1	0		
1659			RNA polymerase I subunit C OS=Canis lupus familiaris OX=9615 GN=POLR1C PE=4 SV=1	E2RH57_CANLF	POLR1C	39 k...		0				1			2
1660			Carboxypeptidase M OS=Canis lupus familiaris OX=9615 GN=CPM PE=4 SV=2	F1PGL5_CANLF	CPM	42 k...		3		0	1				
1661			Synaptotagmin 8 OS=Canis lupus familiaris OX=9615 GN=SYT8 PE=4 SV=2	F1PECO_CANLF	SYT8	45 k...		1			0	0			
1662			Beta-1,4-glucuronyltransferase 1 OS=Canis lupus familiaris OX=9615 GN=B4GAT1 PE=4 ...	E2RM44_CANLF	B4GAT1	47 k...						0		0	2
1663			Myosin XVIIIa OS=Canis lupus familiaris OX=9615 GN=MYO18A PE=3 SV=1	F6Y037_CANLF	MYO18A	233 ...		1		0		2		0	
1664			Solute carrier family 38 member 1 OS=Canis lupus familiaris OX=9615 GN=SLC38A1 PE=...	F6XT35_CANLF	SLC38A1	54 k...		2		0	1				
1665			Pleckstrin homology domain containing B2 OS=Canis lupus familiaris OX=9615 GN=PLEK...	E2RSD9_CANLF	PLEKH82	25 k...		2			0	1			
1666			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC479912 PE=4 SV=2	F1Q3C4_CANLF	LOC479912	26 k...	★						2	1	0
1667			Sec1 family domain containing 1 OS=Canis lupus familiaris OX=9615 GN=SCFD1 PE=3 SV...	F1PLS0_CANLF	SCFD1	72 k...						1	0	2	
1668			Cytospin-A OS=Canis lupus familiaris OX=9615 GN=SPECC1L PE=2 SV=1	CYTSA_CANLF	SPECC1L	125 ...	★	0	0			1	0		
1669			Fumarate hydratase OS=Canis lupus familiaris OX=9615 GN=FH PE=3 SV=1	E2RGR9_CANLF	FH	54 k...		0		1		2			
1670			Solute carrier family 45 member 4 OS=Canis lupus familiaris OX=9615 GN=SLC45A4 PE=...	E2RGI2_CANLF	SLC45A4	85 k...		1	0			2			
1671			CTP synthase OS=Canis lupus familiaris OX=9615 GN=CTPS1 PE=3 SV=1	E2QUQ3_CANLF	CTPS1	67 k...						1			0
1672			RAB43, member RAS oncogene family OS=Canis lupus familiaris OX=9615 GN=RAB43 PE...	E2RP90_CANLF	RAB43	23 k...	★	3		1		3			
1673			COP9 signalosome subunit 2 OS=Canis lupus familiaris OX=9615 GN=COPS2 PE=4 SV=2	E2REA8_CANLF	COPS2	52 k...						0		2	1
1674			Leucine rich repeat containing 59 OS=Canis lupus familiaris OX=9615 GN=LRRCS9 PE=4 S...	F1Q3G5_CANLF	LRRCS9	37 k...		2				1		0	
1675			Polypeptide N-acetylglactosaminyltransferase OS=Canis lupus familiaris OX=9615 GN=...	E2QY58_CANLF	GALNT3	73 k...		1		2				0	
1676			Phosphoglucomutase 2 OS=Canis lupus familiaris OX=9615 GN=PGM2 PE=4 SV=1	E2QV12_CANLF	PGM2	68 k...		0				1			2
1677			Tripeptidyl-peptidase 1 OS=Canis lupus familiaris OX=9615 GN=TPP1 PE=4 SV=1	Z4YHH2_CANLF	TPP1	62 k...								1	2
1678			ERGIC and golgi 3 OS=Canis lupus familiaris OX=9615 GN=ERGIC3 PE=4 SV=2	F1PZP8_CANLF	ERGIC3	44 k...		2		2					
1679			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=NDST2 PE=4 SV=1	E2RBI5_CANLF	NDST2	101 ...								1	3
1680			Eukaryotic translation initiation factor 2B subunit delta OS=Canis lupus familiaris OX=96...	E2R8W5_CANLF	EIF2B4	60 k...						1			2
1681			Ras-related protein Rab-27A OS=Canis lupus familiaris OX=9615 GN=RAB27A PE=2 SV=1	RB27A_CANLF	RAB27A	25 k...		0				2			
1682			CRK like proto-oncogene, adaptor protein OS=Canis lupus familiaris OX=9615 GN=CRKL ...	E2RF98_CANLF	CRKL	34 k...		4				1			
1683			Vacuolar-sorting protein SNF8 OS=Canis lupus familiaris OX=9615 GN=SNF8 PE=3 SV=1	E2RTF6_CANLF	SNF8	29 k...		1				4			
1684			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=HAVCR1 PE=4 SV=2	F1PDE5_CANLF	HAVCR1	40 k...		2				1			
1685			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	L7N074_CANLF		22 k...	★				2	2			
1686			SHC binding and spindle associated 1 OS=Canis lupus familiaris OX=9615 GN=SHCBP1 PE...	F1PFR2_CANLF	SHCBP1	75 k...		1			2				
1687			GIT ArfGAP 1 OS=Canis lupus familiaris OX=9615 GN=GIT1 PE=4 SV=1	F1PJM7_CANLF	GIT1	84 k...		1			1				
1688			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=EMC2 PE=4 SV=1	E2R0P8_CANLF	EMC2	35 k...		2		2					
1689			VAMP associated protein A OS=Canis lupus familiaris OX=9615 GN=VAPA PE=4 SV=1	J9PA22_CANLF	VAPA	32 k...		2			1				
1690			Heterogeneous nuclear ribonucleoprotein A3 OS=Canis lupus familiaris OX=9615 GN=HN...	E2RFV7_CANLF	HNRNPA3	40 k...		2				1		0	
1691			Dynactin subunit 1 OS=Canis lupus familiaris OX=9615 GN=DCTN1 PE=4 SV=1	F6UMF1_CANLF	DCTN1	142 ...		1	0					0	
1692			Carbamoyl-phosphate synthase 1 OS=Canis lupus familiaris OX=9615 GN=CP51 PE=3 SV...	F1PJF3_CANLF	CP51	165 ...	★								

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated				
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4	
				Probability Legend:												
				over 95%												
				80% to 94%												
				50% to 79%												
				20% to 49%												
				0% to 19%												
1705			ATPase family, AAA domain containing 2 OS=Canis lupus familiaris OX=9615 GN=ATAD2...	E2RRW1_CANLF	ATAD2	157 ...					1			0	0	
1702			PD55 cohesin associated factor A OS=Canis lupus familiaris OX=9615 GN=PD55A PE=4 S...	E2R7R4_CANLF	PD55A	151 ...					0				0	1
1703			Acetyl-CoA acetyltransferase 1 OS=Canis lupus familiaris OX=9615 GN=ACAT1 PE=3 SV=1	J9JHD4_CANLF	ACAT1	46 k...					4					
1704			Kinesin family member 14 OS=Canis lupus familiaris OX=9615 GN=KIF14 PE=3 SV=1	E2R054_CANLF	KIF14	186 ...					1				0	
1705			Coagulation factor VIII OS=Canis lupus familiaris OX=9615 GN=F8 PE=3 SV=2	F1PFS7_CANLF (...F8		266 ...			0							2
1706			TNF receptor associated protein 1 OS=Canis lupus familiaris OX=9615 GN=TRAP1 PE=3 S...	F1PXQ9_CANLF	TRAP1	80 k...						3				
1707			Urocanate hydratase 1 OS=Canis lupus familiaris OX=9615 GN=UROC1 PE=3 SV=1	E2RT03_CANLF	UROC1	74 k...										4
1708			Sortilin related receptor 1 OS=Canis lupus familiaris OX=9615 GN=SORL1 PE=4 SV=2	E2R5F5_CANLF	SORL1	247 ...			5							
1709			Mannosyl-oligosaccharide glucosidase OS=Canis lupus familiaris OX=9615 GN=MOGS PE...	E2R8C9_CANLF	MOGS	92 k...						4				
1710			Membrane palmitoylated protein 6 OS=Canis lupus familiaris OX=9615 GN=MPP6 PE=3 S...	E2R451_CANLF	MPP6	62 k...			3							
1711			3'-phosphoadenosine 5'-phosphosulfate synthase 2 OS=Canis lupus familiaris OX=9615...	E2R1E3_CANLF	PAPS52	70 k...										2
1712			Solute carrier organic anion transporter family member OS=Canis lupus familiaris OX=96...	F1PNF5_CANLF	SLCO4A1	76 k...			1	1	1		1			
1713			Olfactomedin like 2B OS=Canis lupus familiaris OX=9615 GN=OLFML2B PE=4 SV=2	F1PAD2_CANLF	OLFML2B	80 k...							1	1	1	1
1714			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC480785 PE=3 SV=2	E2RGJ8_CANLF	LOC480785	31 k...	★		1			2	2			3
1715			ADAM metallopeptidase domain 9 OS=Canis lupus familiaris OX=9615 GN=ADAM9 PE=4 ...	J9P001_CANLF	ADAM9	93 k...			1	1		1				
1716			Mitogen-activated protein kinase OS=Canis lupus familiaris OX=9615 GN=MAPK9 PE=4 S...	F1P630_CANLF	MAPK9	48 k...					1	1	1			1
1717			Histone acetyltransferase type B catalytic subunit OS=Canis lupus familiaris OX=9615 GN...	F1PUX9_CANLF	HAT1	49 k...			1				1	1	1	
1718			Golgi reassembly stacking protein 2 OS=Canis lupus familiaris OX=9615 GN=GORASP2 PE...	E2RHJ7_CANLF	GORASP2	48 k...			1				1	1	0	
1719			Lymphotoxin beta receptor OS=Canis lupus familiaris OX=9615 GN=LTBR PE=4 SV=1	J9P9E3_CANLF	LTBR	46 k...			1	1			1			
1720			Methionine adenosyltransferase 2 subunit beta OS=Canis lupus familiaris OX=9615 GN=...	E2RT47_CANLF	MAT2B	38 k...					0		1		2	1
1721			Vesicle associated membrane protein 7 OS=Canis lupus familiaris OX=9615 GN=VAMP7 P...	E2R555_CANLF	VAMP7	25 k...			1	0		1	1			
1722			Complement C8 beta chain OS=Canis lupus familiaris OX=9615 GN=C8B PE=4 SV=1	E2R141_CANLF	C8B	67 k...						1			1	1
1723			Importin subunit alpha OS=Canis lupus familiaris OX=9615 GN=KPNA6 PE=3 SV=1	J9NST3_CANLF	KPNA6	60 k...			2				1		1	
1724			Carboxypeptidase N subunit 2 OS=Canis lupus familiaris OX=9615 GN=CPN2 PE=4 SV=1	J9PAA4_CANLF	CPN2	59 k...							1	2		1
1725			Acidic nuclear phosphoprotein 32 family member B OS=Canis lupus familiaris OX=9615 G...	F1PUN9_CANLF	ANP32B	30 k...			1				1	1		
1726			Coatomer subunit delta OS=Canis lupus familiaris OX=9615 GN=ARCN1 PE=3 SV=2	F1PRT4_CANLF	ARCN1	57 k...							1	1		1
1727			40S ribosomal protein S12 OS=Canis lupus familiaris OX=9615 GN=RPS12 PE=3 SV=1	E2R4N7_CANLF	RPS12	15 k...			2				1	1		
1728			3-hydroxyanthranilate 3,4-dioxygenase OS=Canis lupus familiaris OX=9615 GN=HAAO P...	F1PUR7_CANLF	HAAO	32 k...								1	1	1
1729			WD repeat domain 61 OS=Canis lupus familiaris OX=9615 GN=WDR61 PE=4 SV=1	E2RBV0_CANLF	WDR61	34 k...							1	2	1	
1730			Serpin family B member 1 OS=Canis lupus familiaris OX=9615 GN=SERPINB1 PE=3 SV=1	E2RKJ6_CANLF	SERPINB1	43 k...			1	1			2	0		
1731			N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase OS=Canis lupus...	J9NVR0_CANLF	NAGPA	61 k...			1					1		1
1732			Actin-related protein 2/3 complex subunit 5 OS=Canis lupus familiaris OX=9615 GN=AR...	E2RD65_CANLF	ARPC5	12 k...							2			1
1733			E3 ubiquitin-protein ligase OS=Canis lupus familiaris OX=9615 GN=SMURF1 PE=4 SV=1	F1PQT6_CANLF	SMURF1	86 k...	★		2	1			1			
1734			40S ribosomal protein S27 OS=Canis lupus familiaris OX=9615 GN=RPS27 PE=3 SV=1	E2RLE2_CANLF (...RPS27		9 kDa							1	2		1
1735			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9P098_CANLF		11 k...			3		1			1		
1736			Superoxide dismutase [Cu-Zn] OS=Canis lupus familiaris OX=9615 GN=SOD1 PE=2 SV=1	SODC_CANLF	SOD1	16 k...	★		4				4	6		
1737			Solute carrier family 52 member 3 OS=Canis lupus familiaris OX=9615 GN=SLC52A3 PE=...	E2RM06_CANLF	SLC52A3	51 k...			3	1				1		
1738			Semaphorin 3F OS=Canis lupus familiaris OX=9615 GN=SEMA3F PE=3 SV=1	F6XP73_CANLF	SEMA3F	88 k...							1	0		2
1739			Mediator of cell motility 1 OS=Canis lupus familiaris OX=9615 GN=MEMO1 PE=3 SV=2	F1PRD0_CANLF	MEMO1	32 k...			0				0		1	2
1740			Ras-related protein Rab-12 (Fragment) OS=Canis lupus familiaris OX=9615 GN=RAB12 P...	RAB12_CANLF	RAB12	24 k...	★		2	3		1				
1741			Leucyl and cystinyl aminopeptidase OS=Canis lupus familiaris OX=9615 GN=LNPEP PE=4 ...	F1PZH2_CANLF	LNPEP	119 ...			1	1				1		
1742			Apolipoprotein E OS=Canis lupus familiaris OX=9615 GN=APOE PE=1 SV=2	APOE_CANLF	APOE	35 k...						1			0	
1743			Potassium calcium-activated channel subfamily N member 4 OS=Canis lupus familiaris O...	F1PNK5_CANLF	KCNNA4	53 k...			1	1				1		
1744			Tyrosine-protein kinase OS=Canis lupus familiaris OX=9615 GN=CSK PE=3 SV=2	E2RFF7_CANLF	CSK	51 k...							1		0	1
1745			Cullin 4A OS=Canis lupus familiaris OX=9615 GN=CUL4A PE=3 SV=2	F1Q284_CANLF	CUL4A	88 k...	★		0	0			1			1
1746			Paxillin OS=Canis lupus familiaris OX=9615 GN=PXN PE=4 SV=1	F1PUT6_CANLF	PXN	64 k...			1				0	1		
1747			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9PAK1_CANLF		56 k...	★		0			3		0		
1748			Fibulin 7 OS=Canis lupus familiaris OX=9615 GN=FBLN7 PE=4 SV=1	J9P996_CANLF	FBLN7	51 k...				0					0	1
1749			Complement factor I OS=Canis lupus familiaris OX=9615 GN=CFI PE=4 SV=2	F1PY40_CANLF	CFI	69 k...				1					1	
1750			Family with sequence similarity 129 member A OS=Canis lupus familiaris OX=9615 GN=F...	J9NZV5_CANLF	FAM129A	104 ...							0		2	2
1751			Syntaxin 2 OS=Canis lupus familiaris OX=9615 GN=STX2 PE=3 SV=2	F1PR71_CANLF	STX2	34 k...			2				0	1		
1752			FGF1 intracellular binding protein OS=Canis lupus familiaris OX=9615 GN=FIBP PE=4 SV=2	E2REC6_CANLF	FIBP	41 k...			2					1	0	
1753			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	F1PBA0_CANLF		21 k...			0			0	1	2		
1754			Sorting nexin OS=Canis lupus familiaris OX=9615 GN=SNX9 PE=3 SV=2	F1PBG7_CANLF	SNX9	66 k...			2				0	2		
1755			Htra serine peptidase 3 OS=Canis lupus familiaris OX=9615 GN=HTRA3 PE=4 SV=1	F1PCX9_CANLF	HTRA3	42 k...								1	0	2
1756			NEDD8-activating enzyme E1 regulatory subunit OS=Canis lupus familiaris OX=9615 GN...	E2RE24_CANLF	NAE1	60 k...			0							1
1757			STEAP2 metalloredutase OS=Canis lupus familiaris OX=9615 GN=STEAP2 PE=4 SV=2	E2RH63_CANLF	STEAP2	53 k...			0	0			2			
1758			Eukaryotic translation initiation factor 3 subunit K OS=Canis lupus familiaris OX=9615 G...	E2R4V6_CANLF	EIF3K	25 k...			1				0	1		
1759			Nuclear receptor binding protein 1 (Fragment) OS=Canis lupus familiaris OX=9615 GN=NR...	E2REI7_CANLF	NRBP1	60 k...						0				1
1760			Extracellular sulfatase OS=Canis lupus familiaris OX=9615 GN=SULF1 PE=3 SV=2	F1Q233_CANLF	SULF1	103 ...			1	0				1		
1761			Signal recognition particle receptor subunit alpha OS=Canis lupus familiaris OX=9615 GN...	J9P788_CANLF	SRPRA	84 k...			0	0				1		0
1762			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=PROCR PE=4 SV=1	F6XR14_CANLF	PROCR	27 k...			3				0	1		
1763			Proteasome activator subunit 2 OS=Canis lupus familiaris OX=9615 GN=PSME2 PE=4 SV=2	F1PD77_CANLF	PSME2	27 k...							1	1		
1764			Testin OS=Canis lupus familiaris OX=9615 GN=TES PE=4 SV=1	F1PFX9_CANLF (...TES		48 k...			2				1	0		
1765			Alpha-1,4 glucan phosphorylase OS=Canis lupus familiaris OX=9615 GN=PYGM PE=3 SV...	F1P832_CANLF	PYGM	97 k...	★							7		4
1766			Transmembrane p24 trafficking protein 4 OS=Canis lupus familiaris OX=9615 GN=TMED...	J9P872_CANLF	TMED4	21 k...	★		2				3			
1767			GMP reductase OS=Canis lupus familiaris OX=9615 GN=GMPPR2 PE=3 SV=1	E2RBW1_CANLF	GMPPR2	38 k...	★							2		2
1768			Transmembrane protein 165 OS=Canis lupus familiaris OX=9615 GN=TMEM165 PE=4 SV...	F1PU63_CANLF	TMEM165	35 k...			2				1			
1769			Serine palmitoyltransferase long chain base subunit 1 OS=Canis lupus familiaris OX=961...	E2RKV3_CANLF	SPTLC1	53 k...			1				2			
1770			NOP2 nucleolar protein OS=Canis lupus familiaris OX=9615 GN=NOP2 PE=3 SV=2	E2RCH6_CANLF	NOP2	91 k...										1
1771			CLPTM1, transmembrane protein OS=Canis lupus familiaris OX=9615 GN=CLPTM1 PE=4 S...	F1PJ80_CANLF	CLPTM1	78 k...			1				2			
1772			Golgi membrane protein 1 OS=Canis lupus familiaris OX=9615 GN=GOLM1 PE=4 SV=2	E2RLA5_CANLF	GOLM1	46 k...			2				1			
1773			Ring finger and SPRY domain containing 1 OS=Canis lupus familiaris OX=9615 GN=RSPRY...	E2RSA4_CANLF	RSPRY1	64 k...			1				2			
1774			Alpha-galactosidase OS=Canis lupus familiaris OX=9615 GN=GLA PE=3 SV=2	F1PGD0_CANLF	GLA	48 k...								2		1
1775			STIP1 homology and U-box containing protein 1 OS=Canis lupus familiaris OX=9615 GN...	J9NRV4_CANLF	STUB1	27 k...			3				0			
1776			Calpastatin OS=Canis lupus familiaris OX=9615 GN=CAST PE=4 SV=2	E2QXA6_CANLF	CAST	84 k...			1				1			
177																

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated				
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4	
Probability Legend:																
over 95%																
80% to 94%																
50% to 79%																
20% to 49%																
0% to 19%																
1786			Cell division cycle and apoptosis regulator 1 OS=Canis lupus familiaris OX=9615 GN=CC...	E2QS34_CANLF	CCAR1	133 ...		2							1	
1787			Heat shock protein family A (Hsp70) member 2 OS=Canis lupus familiaris OX=9615 GN=H...	E2QX84_CANLF	HSPA2	70 k...	★	47				86			40	
1788			Homogentisate 1,2-dioxygenase OS=Canis lupus familiaris OX=9615 GN=HGD PE=4 SV=2	F1Q313_CANLF	HGD	50 k...									1	3
1789			ELAV-like protein OS=Canis lupus familiaris OX=9615 GN=ELAVL3 PE=3 SV=2	F1PVV1_CANLF	ELAVL3	40 k...						1		0		2
1790			YTH domain containing 2 OS=Canis lupus familiaris OX=9615 GN=YTHDC2 PE=4 SV=2	E2QZR7_CANLF	YTHDC2	155 ...				0		1				
1791			NPL4 homolog, ubiquitin recognition factor OS=Canis lupus familiaris OX=9615 GN=NPL...	F1PNX1_CANLF	NPLOC4	68 k...				0		0			1	
1792			DNA-directed RNA polymerase subunit beta OS=Canis lupus familiaris OX=9615 GN=POL...	E2RNN5_CANLF	POLR2B	134 ...				0						2
1793			EPH receptor A4 OS=Canis lupus familiaris OX=9615 GN=EPHA4 PE=3 SV=2	F1PYW2_CANLF	EPHA4	107 ...	★	2	0	0						
1794			Vesicle associated membrane protein 5 OS=Canis lupus familiaris OX=9615 GN=VAMP5 P...	F1Q136_CANLF	VAMP5	13 k...		1		0	0					
1795			Protein transport protein sec16 OS=Canis lupus familiaris OX=9615 GN=SEC16A PE=3 SV...	E2R5K7_CANLF	SEC16A	246 ...				0					0	1
1796			Macoinlin OS=Canis lupus familiaris OX=9615 GN=MACO1 PE=2 SV=1	MACO1_CANLF	MACO1	76 k...						0	1			1
1797			Protein transport protein SEC23 OS=Canis lupus familiaris OX=9615 GN=SEC23B PE=3 SV...	E2RH29_CANLF	SEC23B	86 k...	★					2			1	
1798			Glycerol-3-phosphate dehydrogenase OS=Canis lupus familiaris OX=9615 GN=GPD2 PE=...	E2RAZ8_CANLF	GPD2	80 k...		1		1						
1799			Family with sequence similarity 45 member A OS=Canis lupus familiaris OX=9615 GN=FA...	J9P9P9_CANLF	FAM45A	44 k...						1	0	0	0	
1800			ARP1 actin related protein 1 homolog B OS=Canis lupus familiaris OX=9615 GN=ACTR1B ...	E2RHX6_CANLF	ACTR1B	42 k...	★	1			0		1			4
1801			LUC7 like OS=Canis lupus familiaris OX=9615 GN=LUC7L PE=4 SV=2	E2R057_CANLF	LUC7L	44 k...		1	0						0	
1802			V-type proton ATPase subunit a OS=Canis lupus familiaris OX=9615 GN=ATP6V0A1 PE=3...	F1PVU4_CANLF	ATP6V0A1	97 k...		0				1	0			
1803			Cell division cycle 37 OS=Canis lupus familiaris OX=9615 GN=CDC37 PE=4 SV=1	E2RCN3_CANLF	CDC37	45 k...		1				0				
1804			LIM and SH3 protein 1 OS=Canis lupus familiaris OX=9615 GN=LASP1 PE=4 SV=1	J9P3M1_CANLF	LASP1	30 k...		1			0	0				
1805			7-dehydrocholesterol reductase OS=Canis lupus familiaris OX=9615 GN=DHCR7 PE=4 SV...	E2RNO5_CANLF	DHCR7	54 k...		0		2						
1806			Protocadherin related 15 OS=Canis lupus familiaris OX=9615 GN=PCDH15 PE=4 SV=2	F1PR94_CANLF	PCDH15	205 ...				0				0	1	
1807			Nuclear distribution C, dynein complex regulator OS=Canis lupus familiaris OX=9615 GN...	F6V5M4_CANLF	NUDC	38 k...		3			0				0	
1808			Rab geranylgeranyltransferase beta subunit OS=Canis lupus familiaris OX=9615 GN=RAB...	F1P6X0_CANLF	RABGGTB	37 k...						0	0	1		0
1809			Cullin 5 OS=Canis lupus familiaris OX=9615 GN=CUL5 PE=3 SV=1	J9PAX0_CANLF	CUL5	88 k...						2			0	
1810			Protein kinase C substrate 80K-H OS=Canis lupus familiaris OX=9615 GN=PRKCSH PE=4 ...	E2RKK6_CANLF	PRKCSH	60 k...		4						0		
1811			Family with sequence similarity 50 member A OS=Canis lupus familiaris OX=9615 GN=FA...	E2R0M3_CANLF	FAM50A	40 k...				2					0	
1812			Septin 10 OS=Canis lupus familiaris OX=9615 GN=SEPT10 PE=3 SV=1	J9NUL3_CANLF	SEPT10	55 k...						1	0			0
1813			Phosphoribosyl pyrophosphate synthetase associated protein 2 OS=Canis lupus familiaris...	E2RIW6_CANLF	PRPSA2	40 k...	★						0		1	3
1814			Protein tyrosine phosphatase, non-receptor type 23 OS=Canis lupus familiaris OX=9615 ...	J9P9Q0_CANLF	PTPN23	140 ...								0		1
1815			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=CD302 PE=4 SV=2	F1PGD5_CANLF	CD302	198 ...		1					0			
1816			WD repeat domain 91 OS=Canis lupus familiaris OX=9615 GN=WDR91 PE=4 SV=2	F1Q0G5_CANLF	WDR91	77 k...		0							3	
1817			FERM domain containing 8 OS=Canis lupus familiaris OX=9615 GN=FRMD8 PE=4 SV=2	E2R8F5_CANLF	FRMD8	51 k...		1		0						
1818			Single-pass membrane protein with coiled-coil domains 1 OS=Canis lupus familiaris OX=...	J9P0H3_CANLF	SMCO1	24 k...								1		0
1819			WD repeat domain 82 OS=Canis lupus familiaris OX=9615 GN=WDR82 PE=4 SV=1	E2RGF2_CANLF	WDR82	35 k...								0		3
1820			Beta-ureidopropionase 1 OS=Canis lupus familiaris OX=9615 GN=UPB1 PE=4 SV=1	J9PR81_CANLF	UPB1	43 k...								0	2	
1821			NAD(P) dependent steroid dehydrogenase-like OS=Canis lupus familiaris OX=9615 GN=N...	F6XJP3_CANLF	NSDHL	41 k...		0		2						
1822			HRas proto-oncogene, GTPase OS=Canis lupus familiaris OX=9615 GN=HRAS PE=4 SV=1	J9P550_CANLF	HRAS	27 k...	★	5		0						
1823			Golgin A4 OS=Canis lupus familiaris OX=9615 GN=GOLGA4 PE=4 SV=2	F1PCW0_CANLF	GOLGA4	262 ...									1	
1824			Ras-related protein Rab-11A OS=Canis lupus familiaris OX=9615 GN=RAB11A PE=2 SV=3	RB11A_CANLF	RAB11A	24 k...	★						9			
1825			DNA polymerase iota OS=Canis lupus familiaris OX=9615 GN=POLI PE=4 SV=2	F1PDA5_CANLF	POLI	79 k...							1			
1826			LanC like 1 OS=Canis lupus familiaris OX=9615 GN=LANCL1 PE=4 SV=1	E2RQR4_CANLF	LANCL1	45 k...										1
1827			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9NVR8_CANLF		28 k...	★				1				1	
1828			TAO kinase 1 OS=Canis lupus familiaris OX=9615 GN=TAOK1 PE=4 SV=1	E2QRV2_CANLF	TAOK1	116 ...	★	2								
1829			Phospholipid-transporting ATPase OS=Canis lupus familiaris OX=9615 GN=ATP10D PE=...	E2RG44_CANLF	ATP10D	159 ...		2								
1830			Anoctamin OS=Canis lupus familiaris OX=9615 GN=ANO6 PE=3 SV=1	J9NZU1_CANLF	ANO6	106 ...		4								
1831			Fructose-bisphosphate aldolase OS=Canis lupus familiaris OX=9615 GN=ALDOB PE=3 SV...	F6XUY6_CANLF	ALDOB	40 k...	★								2	
1832			MICOS complex subunit MIC60 OS=Canis lupus familiaris OX=9615 GN=IMMT PE=3 SV=1	F6Y8P9_CANLF	IMMT	87 k...				3						
1833			Zinc finger and BTB domain containing 41 OS=Canis lupus familiaris OX=9615 GN=ZBTB...	E2QXV9_CANLF	ZBTB41	105 ...				2						
1834			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC489992 PE=4 SV=2	F1PB46_CANLF	LOC489992	130 ...				2						
1835			Peptidylprolyl isomerase OS=Canis lupus familiaris OX=9615 GN=FKBP9 PE=4 SV=2	F1Q1F5_CANLF	FKBP9	59 k...				1						
1836			Zinc finger protein 280D OS=Canis lupus familiaris OX=9615 GN=ZNF280D PE=4 SV=2	F1Q263_CANLF	ZNF280D	109 ...				1						
1837			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=METT126 PE=4 SV=2	E2R5L1_CANLF	METT126	21 k...		1	1			1	1			
1838			Glutathione reductase OS=Canis lupus familiaris OX=9615 GN=GSR PE=3 SV=2	F1PY21_CANLF	GSR	56 k...		1	1	1			1			
1839			THO complex 2 OS=Canis lupus familiaris OX=9615 GN=THOC2 PE=4 SV=1	J9PB31_CANLF	THOC2	183 ...		1	1				1	0		
1840			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9P5A1_CANLF		12 k...		1					1	1		0
1841			Thioredoxin like 1 OS=Canis lupus familiaris OX=9615 GN=TXNL1 PE=4 SV=2	E2R5M7_CANLF	TXNL1	32 k...		1					1			
1842			Aspartate aminotransferase OS=Canis lupus familiaris OX=9615 GN=GOT1 PE=4 SV=1	A0A0B4J195_C...	GOT1	46 k...					1	1				
1843			Ras homolog, mTORC1 binding OS=Canis lupus familiaris OX=9615 GN=RHEB PE=4 SV=1	E2RFF0_CANLF	RHEB	20 k...		1	1				1			
1844			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=KRT42 PE=3 SV=1	E2R7W6_CANLF	KRT42	50 k...		7					6			
1845			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC606772 PE=4 SV=1	J9P093_CANLF	LOC606772	14 k...		1		2		1				
1846			Doublecortin like kinase 1 OS=Canis lupus familiaris OX=9615 GN=DCLK1 PE=4 SV=2	F1PWY9_CANLF	DCLK1	82 k...		1	1			1				
1847			Farnesyltransferase, CAAX box, alpha OS=Canis lupus familiaris OX=9615 GN=FNTA PE=4...	E2RHY8_CANLF	FNTA	45 k...		1				2			1	
1848			PDZ and LIM domain 5 OS=Canis lupus familiaris OX=9615 GN=PDLIM5 PE=4 SV=1	E2RBA8_CANLF	PDLIM5	64 k...			0			1	1			
1849			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	E2QY54_CANLF ...		58 k...		2	1					1		
1850			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=1	A0A075B5H7_C...		12 k...		1					1			1
1851			tRNA methyltransferase subunit 11-2 OS=Canis lupus familiaris OX=9615 GN=TRMT112 ...	E2RGM1_CANLF	TRMT112	14 k...		1				1	1			
1852			Family with sequence similarity 216 member B OS=Canis lupus familiaris OX=9615 GN=F...	J9NUA2_CANLF	FAM216B	18 k...							1	1	1	
1853			Glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase 1 OS=Canis lupus fam...	E2RF40_CANLF	C1GALT1	42 k...		1	1							1
1854			Nedd4 family interacting protein 1 OS=Canis lupus familiaris OX=9615 GN=NDFIP1 PE=4 ...	F1Q4C9_CANLF	NDFIP1	24 k...		0	1			1	1			
1855			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=STEAP1 PE=4 SV=1	E2RH77_CANLF	STEAP1	40 k...		1	1				1			
1856			LUC7 like 3 pre-mRNA splicing factor OS=Canis lupus familiaris OX=9615 GN=LUC7L3 PE...	E2RS55_CANLF	LUC7L3	51 k...		1	1				1			
1857			Lecithin-cholesterol acyltransferase OS=Canis lupus familiaris OX=9615 GN=LCAT PE=4 ...	F1PA63_CANLF	LCAT	50 k...								0	0	1
1858			Angiopoietin like 4 OS=Canis lupus familiaris OX=9615 GN=ANGPTL4 PE=4 SV=1	F1PLK4_CANLF	ANGPTL4	45 k...		0							2	1
1859			V-type proton ATPase subunit H OS=Canis lupus familiaris OX=9615 GN=ATP6V1H PE=3 ...	E2RHZ8_CANLF	ATP6V1H	56 k...		1					0			1
1860			Aconitate hydratase, mitochondrial OS=Canis lupus familiaris OX=9615 GN=ACO2 PE=3 S...	E2RCY8_CANLF	ACO2	86 k...		0		1					1	
1861			Torsin OS=Canis lupus familiaris OX=9615 GN=TOR1B PE=3 SV=2	F1PD54_CANLF	TOR1B	38 k...										

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
								Probability Legend: over 95% 80% to 94% 50% to 79% 20% to 49% 0% to 19%							
1875			Endoplasmic reticulum metallopeptidase 1 OS=Canis lupus familiaris OX=9615 GN=ERMP...	F1PSG1_CANLF	ERMP1	100 ...		0		1					
1875			RE1 silencing transcription factor OS=Canis lupus familiaris OX=9615 GN=REST PE=4 SV=2	F1PP47_CANLF	REST	111 ...		0	1	1					
1875			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=ID11 PE=4 SV=1	J9P2H1_CANLF	ID11	18 k...		0						1	
1875			Lysophosphatidylcholine acyltransferase 3 OS=Canis lupus familiaris OX=9615 GN=LPCA...	E2RKK9_CANLF	LPCAT3	71 k...				1					
1875			Nicotinamide phosphoribosyltransferase OS=Canis lupus familiaris OX=9615 GN=NAMPT ...	F1P6K2_CANLF	NAMPT	56 k...		2						1	
1875			Apoptosis inducing factor mitochondria associated 1 OS=Canis lupus familiaris OX=9615...	E2R541_CANLF	AIFM1	67 k...								1	
1875			Interleukin 1 receptor like 1 OS=Canis lupus familiaris OX=9615 GN=IL1RL1 PE=4 SV=1	F1PSF7_CANLF	IL1RL1	66 k...						1	1		
1875			Transmembrane channel-like protein OS=Canis lupus familiaris OX=9615 GN=TMC3 PE=...	F6UQL7_CANLF	TMC3	125 ...						1		1	
1875			Haloacid dehalogenase like hydrolase domain containing 2 OS=Canis lupus familiaris OX...	F6XJB3_CANLF	HDHD2	43 k...				1					
1885			NPC intracellular cholesterol transporter 2 OS=Canis lupus familiaris OX=9615 GN=NPC2 ...	F1PAR9_CANLF	NPC2	17 k...		0			1				
1885			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=EMG1 PE=4 SV=1	E2RKH8_CANLF	EMG1	27 k...							2		2
1885			Fibrinogen alpha chain OS=Canis lupus familiaris OX=9615 GN=FGA PE=4 SV=1	F1PBL4_CANLF	FGA	97 k...				1				1	
1885			Mut5 homolog 6 OS=Canis lupus familiaris OX=9615 GN=MSH6 PE=3 SV=1	J9P5H1_CANLF	MSH6	145 ...						1			2
1885			CTD small phosphatase 1 OS=Canis lupus familiaris OX=9615 GN=CTDSP1 PE=4 SV=2	E2RTC0_CANLF	CTDSP1	38 k...		2				1			
1885			Phosphohistidine phosphatase 1 OS=Canis lupus familiaris OX=9615 GN=PHPT1 PE=4 SV...	E2R1M6_CANLF	PHPT1	14 k...		1				2			
1885			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	E2RGNO_CANLF		26 k...		1				3			
1885			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=SNU13 PE=4 SV=2	E2RRM7_CANLF...	SNU13	14 k...		1				2			
1885			NAD(P)H-hydrate epimerase OS=Canis lupus familiaris OX=9615 GN=NAXE PE=3 SV=1	A0A0A0MPD3_...	NAXE	37 k...		1				1			
1885			Ephrin B2 OS=Canis lupus familiaris OX=9615 GN=EFNB2 PE=3 SV=2	F1Q2Q5_CANLF	EFNB2	37 k...		1				1			
1895			Dolichyl-phosphate mannosyltransferase subunit 1, catalytic OS=Canis lupus familiaris O...	J9NZX5_CANLF	DPM1	29 k...		1		1					
1895			Transmembrane serine protease 13 OS=Canis lupus familiaris OX=9615 GN=TMPRSS13 P...	J9P1Q2_CANLF	TMPRSS13	57 k...	★	1				1			
1895			Terpene cyclase/mutase family member OS=Canis lupus familiaris OX=9615 GN=LSS PE=...	F1PD82_CANLF	LSS	83 k...		2		1					
1895			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC102151683 PE=4 SV...	F1PJ50_CANLF	LOC102151683	12 k...		1				1			
1895			Coagulation factor X OS=Canis lupus familiaris OX=9615 GN=F10 PE=3 SV=2	F1Q4A3_CANLF	F10	55 k...						1		2	
1895			Tubulin gamma chain OS=Canis lupus familiaris OX=9615 GN=LOC100856176 PE=3 SV=1	E2RD85_CANLF	LOC100856176	51 k...						1		1	0
1895			DEAD-box helicase 5 OS=Canis lupus familiaris OX=9615 GN=DDX5 PE=3 SV=2	F1PEA6_CANLF	DDX5	76 k...	★					3		4	
1895			Discoidin, CUB and LCCL domain containing 2 OS=Canis lupus familiaris OX=9615 GN=D...	F1PVX2_CANLF	DCBLD2	80 k...		2				1			
1895			Syndecan binding protein 2 OS=Canis lupus familiaris OX=9615 GN=SDCBP2 PE=4 SV=2	E2RDD6_CANLF	SDCBP2	33 k...		1			2				
1895			Glucosylceramidase beta 3 (gene/pseudogene) OS=Canis lupus familiaris OX=9615 GN=G...	F6XB5Y_CANLF	GBA3	57 k...						1	1		
1905			Nicalin OS=Canis lupus familiaris OX=9615 GN=NCLN PE=3 SV=1	E2QY93_CANLF	NCLN	63 k...		1		1					
1905			Gamma-butyrobetaine hydroxylase 1 OS=Canis lupus familiaris OX=9615 GN=BBOX1 PE...	E2RKPO_CANLF	BBOX1	45 k...						0		1	1
1905			Electron transfer flavoprotein alpha subunit OS=Canis lupus familiaris OX=9615 GN=ETF...	E2RAE2_CANLF	ETFA	35 k...				1		0		0	
1905			Palmitoyltransferase OS=Canis lupus familiaris OX=9615 GN=ZDHHC3 PE=3 SV=1	E2RND4_CANLF	ZDHHC3	37 k...		2				1			
1905			Fructose-bisphosphatase 2 OS=Canis lupus familiaris OX=9615 GN=FBP2 PE=3 SV=1	E2RAN3_CANLF	FBP2	37 k...	★					1			
1905			DnaJ heat shock protein family (Hsp40) member C11 OS=Canis lupus familiaris OX=9615 ...	F1PDH1_CANLF	DNAJC11	63 k...		2		1					
1905			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=NMRAL1 PE=4 SV=2	E2R512_CANLF	NMRAL1	33 k...					1	0		1	
1905			Keratin 85 OS=Canis lupus familiaris OX=9615 GN=KRT85 PE=3 SV=2	E2R484_CANLF	KRT85	55 k...	★			1			2		
1905			Torsin 1A interacting protein 1 OS=Canis lupus familiaris OX=9615 GN=TOR1AIP1 PE=4 S...	E2RCN4_CANLF	TOR1AIP1	65 k...		2		0			0		
1905			Heparin binding growth factor OS=Canis lupus familiaris OX=9615 GN=HDGF PE=4 SV=1	J9NSE8_CANLF	HDGF	25 k...		3				1			
1915			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9P7Y8_CANLF		34 k...		1				1			
1915			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=MARVELD3 PE=4 SV=1	J9NYT4_CANLF	MARVELD3	49 k...		1				1			
1915			Signal peptide peptidase like 2B OS=Canis lupus familiaris OX=9615 GN=SPPL2B PE=4 SV...	J9PQO5_CANLF	SPPL2B	70 k...		1	0			0			
1915			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9NXO7_CANLF		35 k...						1	0	0	
1915			Protein Wnt OS=Canis lupus familiaris OX=9615 GN=WNT7A PE=3 SV=1	J9P2Q5_CANLF	WNT7A	39 k...								0	3
1915			DEAD-box helicase 47 OS=Canis lupus familiaris OX=9615 GN=DDX47 PE=3 SV=2	E2RN03_CANLF	DDX47	54 k...						1			2
1915			Chromosome 5 open reading frame 15 OS=Canis lupus familiaris OX=9615 GN=C11H5orf...	J9P0A8_CANLF	C11H5orf15	29 k...		3				0			
1915			Ryanodine receptor 1 OS=Canis lupus familiaris OX=9615 GN=RYR1 PE=4 SV=2	F1PIS0_CANLF	RYR1	567 ...							0	0	1
1915			Calcium load-activated calcium channel OS=Canis lupus familiaris OX=9615 GN=TMCO1 ...	E2RG72_CANLF	TMCO1	21 k...		0	0	2					
1915			5-oxoprolinase, ATP-hydrolysing OS=Canis lupus familiaris OX=9615 GN=OPLAH PE=4 S...	F1PGA9_CANLF	OPLAH	126 ...					0			0	1
1925			Microtubule associated scaffold protein 2 OS=Canis lupus familiaris OX=9615 GN=MTUS2...	F1PPX5_CANLF	MTUS2	150 ...							0	1	
1925			FERM, ARH/RhoGEF and pleckstrin domain protein 1 OS=Canis lupus familiaris OX=9615 ...	E2REU8_CANLF	FARP1	118 ...		1	0						
1925			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC100686524 PE=4 SV...	E2R2A8_CANLF	LOC100686524	25 k...		0				1			0
1925			Adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 OS=Ca...	E2R6J0_CANLF	APPL1	80 k...		1				0			
1925			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	E2R3X2_CANLF		47 k...		1				0			
1925			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC490986 PE=3 SV=2	E2R708_CANLF	LOC490986	51 k...	★		0					1	
1925			Septin 8 OS=Canis lupus familiaris OX=9615 GN=SEPT8 PE=3 SV=2	F1PQD4_CANLF	SEPT8	89 k...	★	4				7			2
1925			Dynactin subunit 2 OS=Canis lupus familiaris OX=9615 GN=DCTN2 PE=4 SV=1	E2QZ05_CANLF	DCTN2	44 k...		2	0				0		
1925			Signal transducer and activator of transcription OS=Canis lupus familiaris OX=9615 GN=S...	F1PAY3_CANLF	STAT6	94 k...		1		0			0		
1925			Cold shock domain containing E1 OS=Canis lupus familiaris OX=9615 GN=CSDE1 PE=4 S...	F1PG95_CANLF	CSDE1	89 k...		2						0	
1935			Complement C1q binding protein OS=Canis lupus familiaris OX=9615 GN=C1QB8 PE=4 S...	F1PR95_CANLF	C1QB8	30 k...			0	1				0	
1935			Phospholipid phosphatase 1 OS=Canis lupus familiaris OX=9615 GN=PLPP1 PE=4 SV=1	F1PVT9_CANLF	PLPP1	32 k...		3				0			
1935			Dipeptidyl peptidase 9 OS=Canis lupus familiaris OX=9615 GN=DPP9 PE=3 SV=1	E2R8Z1_CANLF	DPP9	102 ...								0	1
1935			SEC23 interacting protein OS=Canis lupus familiaris OX=9615 GN=SEC23IP PE=4 SV=2	F1PQ80_CANLF	SEC23IP	112 ...				0			1		0
1935			Arrestin beta 1 OS=Canis lupus familiaris OX=9615 GN=ARRB1 PE=4 SV=1	F1PQM2_CANLF	ARRB1	47 k...								1	0
1935			Retinol dehydrogenase 11 (all-trans/9-cis/11-cis) OS=Canis lupus familiaris OX=9615 G...	F1P957_CANLF	RDH11	35 k...		1		0					
1935			Sortilin OS=Canis lupus familiaris OX=9615 GN=SORT1 PE=4 SV=2	F1P6N4_CANLF	SORT1	97 k...		1							0
1935			ATP binding cassette subfamily A member 13 OS=Canis lupus familiaris OX=9615 GN=AB...	F1PWW4_CANLF	ABCA13	554 ...				0			1		
1935			Peroxisomal biogenesis factor 3 OS=Canis lupus familiaris OX=9615 GN=PEX3 PE=4 SV=1	E2R9C7_CANLF	PEX3	42 k...					1				0
1935			DEAD-box helicase 23 OS=Canis lupus familiaris OX=9615 GN=DDX23 PE=4 SV=1	E2RTL6_CANLF	DDX23	96 k...					1	0			
1945			Keratinocyte associated protein 3 OS=Canis lupus familiaris OX=9615 GN=KRTCAP3 PE=...	E2RKB4_CANLF	KRTCAP3	26 k...		2		0					
1945			Dihydropyrimidine dehydrogenase [NAD(P+)] OS=Canis lupus familiaris OX=9615 GN=DP...	E2RSQ9_CANLF	DPYD	112 ...									2
1945			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=2	E2R7N8_CANLF		34 k...					0			2	
1945			Protocadherin 12 OS=Canis lupus familiaris OX=9615 GN=PCDH12 PE=4 SV=2	E2QX82_CANLF	PCDH12	131 ...								1	
1945			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC100856533 PE=3 SV...	F1PIZ7_CANLF	LOC100856533	40 k...	★							1	
1945			Ubiquitinyl hydrolase 1 OS=Canis lupus familiaris OX=9615 GN=USP33 PE=3 SV=1	F1P6Y2_CANLF	USP33	107 ...					1				
1945			Ring finger protein 123 OS=Canis lupus familiaris OX=9615 GN=RNF123 PE=4 SV=2	E2R3P7_CANLF	RNF123	148 ...									3
1945			Hydroxyacyl-CoA dehydrogenase OS=Canis lupus familiaris OX=9615 GN=HADH PE=4 SV...	F6XHT8_CANLF	HADH	32 k...				2					
1945			Lipin 2 OS=Canis lupus familiaris OX=9615 GN=LPIN2 PE=4 SV=1	J9NRN1_CANLF	LPIN2	104 ...								1	
1945			Glyoxalase domain containing 4 OS=Canis lupus familiaris OX=9615 GN=GLOD4 PE=4 SV...	E2QXD6_CANLF	GLOD4	53 k...		2							
1955			Hexosyltransferase OS=Canis lupus familiaris OX=9615 GN=CHSY1 PE=3 SV=1	E2QWQ7_CANLF	CHSY1	92 k...									3
1955			DnaJ homolog subfamily C member 10 OS=Canis lupus familiaris OX=9615 GN=DNAJC10...	E2RCY4_CANLF	DNAJC10	91 k...		1							

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated				
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4	
				Probability Legend:												
				over 95%												
				80% to 94%												
				50% to 79%												
				20% to 49%												
				0% to 19%												
1956			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=PDHB PE=4 SV=1	E2R268_CANLF	PDHB	39 k...				2						
1957			SHOC2, leucine rich repeat scaffold protein OS=Canis lupus familiaris OX=9615 GN=SHOC...	E2R260_CANLF	SHOC2	65 k...							2			
1958			KIAA0319 like OS=Canis lupus familiaris OX=9615 GN=KIAA0319L PE=4 SV=1	F6XGU5_CANLF	KIAA0319L	105 ...		2								
1959			Hydroxysteroid 17-beta dehydrogenase 10 OS=Canis lupus familiaris OX=9615 GN=HSD...	E2R9F5_CANLF	HSD17B10	27 k...					3					
1960			Protein 4.1 OS=Canis lupus familiaris OX=9615 GN=EPB41 PE=2 SV=1	41_CANLF	EPB41	91 k...							1			
1961			ATP binding cassette subfamily D member 3 OS=Canis lupus familiaris OX=9615 GN=AB...	J9P7K3_CANLF	ABCD3	80 k...					1					
1962			FLII, actin remodeling protein OS=Canis lupus familiaris OX=9615 GN=FLII PE=4 SV=1	J9P2Y3_CANLF	FLII	145 ...						2				
1963			Haloacid dehalogenase like hydrolase domain containing 5 OS=Canis lupus familiaris OX...	F1Q482_CANLF	HDHD5	42 k...					1					
1964			Pyrroline-5-carboxylate reductase OS=Canis lupus familiaris OX=9615 GN=PYCR3 PE=3 S...	E2RM35_CANLF	PYCR3	30 k...									1	
1965			Strawberry notch homolog 2 OS=Canis lupus familiaris OX=9615 GN=SBN02 PE=4 SV=1	F6XG82_CANLF	SBN02	150 ...									1	
1966			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	E2R657_CANLF		32 k...		1	1			1				
1967			StAR related lipid transfer domain containing 10 OS=Canis lupus familiaris OX=9615 GN=...	F1PTT5_CANLF	STARD10	44 k...								1	1	1
1968			ADP ribosylation factor like GTPase 1 OS=Canis lupus familiaris OX=9615 GN=ARL1 PE=3...	E2RG67_CANLF	ARL1	20 k...		1				1	1			
1969			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=IFIT5 PE=4 SV=2	F1PWG0_CANLF	IFIT5	56 k...							1		1	1
1970			NDRG family member 2 OS=Canis lupus familiaris OX=9615 GN=NDRG2 PE=4 SV=2	E2RB70_CANLF	NDRG2	41 k...							1	1	1	
1971			Elastin microfibril interfacer 3 OS=Canis lupus familiaris OX=9615 GN=EMILIN3 PE=4 SV=1	E2RR98_CANLF	EMILIN3	83 k...					1				1	1
1972			RAB3D, member RAS oncogene family OS=Canis lupus familiaris OX=9615 GN=RAB3D PE...	E2RHH4_CANLF	RAB3D	25 k...	★	2			2	8				
1973			Acetylserotonin O-methyltransferase like OS=Canis lupus familiaris OX=9615 GN=ASMTL...	F1PKN7_CANLF	ASMTL	59 k...		1					1			1
1974			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9P4N2_CANLF		10 k...		1	1				1			
1975			Complement C1q C chain OS=Canis lupus familiaris OX=9615 GN=C1QC PE=4 SV=1	E2RJC2_CANLF	C1QC	26 k...							1	0	1	
1976			Unc-45 myosin chaperone A OS=Canis lupus familiaris OX=9615 GN=UNC45A PE=4 SV=2	F1PR18_CANLF	UNC45A	104 ...		1					1		1	
1977			Mannan binding lectin serine peptidase 2 OS=Canis lupus familiaris OX=9615 GN=MASP2...	F6USM4_CANLF	MASP2	75 k...				0	1					1
1978			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC482711 PE=4 SV=2	E2QVN9_CANLF	LOC482711	37 k...							1		0	1
1979			Adhesion regulating molecule 1 OS=Canis lupus familiaris OX=9615 GN=ADRM1 PE=4 SV...	E2RD84_CANLF	ADRM1	42 k...		1			0		1			
1980			Glycogen synthase kinase 3 alpha OS=Canis lupus familiaris OX=9615 GN=GSK3A PE=3 S...	F1PAE3_CANLF	GSK3A	52 k...		0					1			1
1981			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=SLC6A8 PE=4 SV=2	F1PZI9_CANLF	SLC6A8	61 k...				1	1	0				
1982			Vav guanine nucleotide exchange factor 1 OS=Canis lupus familiaris OX=9615 GN=VAV1 ...	E2RG78_CANLF	VAV1	98 k...			1					0	1	
1983			Fas cell surface death receptor OS=Canis lupus familiaris OX=9615 GN=FAS PE=4 SV=2	F1P7Q7_CANLF	FAS	36 k...		1					1			0
1984			Complement C6 OS=Canis lupus familiaris OX=9615 GN=C6 PE=4 SV=2	E2RG76_CANLF	C6	106 ...					1				1	
1985			Mannosyl (alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase OS=Canis...	E2RFM5_CANLF	MGAT1	52 k...									1	1
1986			Phosphoinositide phospholipase C OS=Canis lupus familiaris OX=9615 GN=PLCG2 PE=4 ...	E2RQM0_CANLF	PLCG2	148 ...									1	1
1987			Anthrax toxin receptor 1 OS=Canis lupus familiaris OX=9615 GN=ANTXR1 PE=4 SV=2	F1PYQ9_CANLF	ANTXR1	54 k...									1	1
1988			Sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1 OS=Canis lup...	J9P1K9_CANLF	SVEP1	397 ...									1	1
1989			SH3 domain binding protein 2 OS=Canis lupus familiaris OX=9615 GN=SH3BP2 PE=4 SV=2	E2RJ40_CANLF	SH3BP2	66 k...								0		1
1990			Isochorismatase domain containing 1 OS=Canis lupus familiaris OX=9615 GN=ISOC1 PE=...	E2R0P3_CANLF	ISOC1	32 k...			1				2			1
1991			Peptidylprolyl isomerase OS=Canis lupus familiaris OX=9615 GN=FKBP11 PE=4 SV=2	F1P9T5_CANLF	FKBP11	23 k...				1	1					
1992			Family with sequence similarity 177 member A1 OS=Canis lupus familiaris OX=9615 GN=...	E2RD87_CANLF	FAM177A1	24 k...		2					1			
1993			Prefoldin subunit 2 OS=Canis lupus familiaris OX=9615 GN=PFN2 PE=4 SV=2	F1PLU5_CANLF	PFN2	17 k...		2					1			
1994			Cerebroside sulfotransferase OS=Canis lupus familiaris OX=9615 GN=GAL3ST1 PE=2 SV=1	A1IGX9_CANLF	GAL3ST1	49 k...							1			2
1995			Torsin OS=Canis lupus familiaris OX=9615 GN=TOR2A PE=3 SV=2	F1P826_CANLF	TOR2A	36 k...							1			2
1996			Inosine triphosphate pyrophosphatase OS=Canis lupus familiaris OX=9615 GN=ITPA PE=...	J9P4A1_CANLF	ITPA	21 k...		1					2			
1997			Occludin OS=Canis lupus familiaris OX=9615 GN=OCLN PE=3 SV=1	F1PZF7_CANLF (...)	OCLN	63 k...					1	1				
1998			Glutamate-cysteine ligase catalytic subunit OS=Canis lupus familiaris OX=9615 GN=GCL...	E2RPJ4_CANLF	GCLC	73 k...		1				1				
1999			Solute carrier family 35 member B2 OS=Canis lupus familiaris OX=9615 GN=SLC35B2 PE...	J9NY30_CANLF	SLC35B2	48 k...			1				1			
2000			Chromosome 11 open reading frame 54 OS=Canis lupus familiaris OX=9615 GN=C21H11...	E2RSD8_CANLF	C21H11orf54	36 k...		1					1			
2001			Caveolae associated protein 3 OS=Canis lupus familiaris OX=9615 GN=CAVIN3 PE=4 SV=2	F1Q0L3_CANLF	CAVIN3	28 k...		1							2	
2002			Protein phosphatase 1 regulatory subunit 13 like OS=Canis lupus familiaris OX=9615 GN...	E2RDK5_CANLF	PPP1R13L	90 k...		1					1			
2003			Extended synaptotagmin 1 OS=Canis lupus familiaris OX=9615 GN=ESYT1 PE=4 SV=1	J9NRF6_CANLF	ESYT1	119 ...		1				1				
2004			Serine and arginine rich splicing factor 7 OS=Canis lupus familiaris OX=9615 GN=SRSF7 P...	J9P218_CANLF	SRSF7	27 k...		1					1			
2005			Hypoxia inducible factor 1 alpha subunit inhibitor OS=Canis lupus familiaris OX=9615 GN...	E2RDK9_CANLF	HIF1AN	40 k...		1								2
2006			N-acetylglucosamine-1-phosphate transferase gamma subunit OS=Canis lupus familiaris...	J9NW92_CANLF	GNPTG	35 k...							1			2
2007			Procollagen C-endopeptidase enhancer OS=Canis lupus familiaris OX=9615 GN=PCOLCE ...	E2RC23_CANLF	PCOLCE	49 k...			1					1		
2008			Lipase maturation factor OS=Canis lupus familiaris OX=9615 GN=LMF1 PE=3 SV=1	J9P7V8_CANLF	LMF1	63 k...		1					1			
2009			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	E2RJ54_CANLF		35 k...	★	3				3				
2010			KIAA0040 OS=Canis lupus familiaris OX=9615 GN=KIAA0040 PE=4 SV=1	J9NTG5_CANLF	KIAA0040	12 k...		2					1			
2011			Small nuclear ribonucleoprotein polypeptide A' OS=Canis lupus familiaris OX=9615 GN=S...	E2QWU2_CANLF	SNRPA1	28 k...							1			1
2012			Centromere protein Q OS=Canis lupus familiaris OX=9615 GN=CENPQ PE=4 SV=2	E2RKY1_CANLF	CENPQ	31 k...		1							1	
2013			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=C32H4orf54 PE=4 SV=1	J9P776_CANLF	C32H4orf54	190 ...		1	1	0						
2014			ATP binding cassette subfamily B member 6 OS=Canis lupus familiaris OX=9615 GN=ABC...	F1PRH1_CANLF	ABCB6	107 ...		1		1						
2015			Eukaryotic translation initiation factor 2 alpha kinase 4 OS=Canis lupus familiaris OX=96...	E2RS10_CANLF	EIF2AK4	187 ...									1	1
2016			ErbB-2 receptor tyrosine kinase 3 OS=Canis lupus familiaris OX=9615 GN=ERBB3 PE=4 S...	F1PVC5_CANLF	ERBB3	113 ...		1			1					
2017			Beta-nerve growth factor precursor OS=Canis lupus familiaris OX=9615 GN=NGF PE=3 SV...	F1PDQ3_CANLF	NGF	30 k...							1	0		1
2018			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC477556 PE=3 SV=1	E2RD21_CANLF	LOC477556	27 k...	★						1			1
2019			C1q and TNF related 4 OS=Canis lupus familiaris OX=9615 GN=C1QTNF4 PE=4 SV=1	J9P5P5_CANLF	C1QTNF4	48 k...									1	2
2020			LDL receptor related protein associated protein 1 OS=Canis lupus familiaris OX=9615 GN...	F1PAG7_CANLF	LRPAP1	42 k...			1	1						
2021			EF-hand domain family member D2 OS=Canis lupus familiaris OX=9615 GN=EFHD2 PE=4...	F1PIV8_CANLF	EFHD2	16 k...		2					1			
2022			Transmembrane protein 51 OS=Canis lupus familiaris OX=9615 GN=TMEM51 PE=4 SV=1	F6XLZ4_CANLF	TMEM51	28 k...		2					1			
2023			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=NCR3LG1 PE=4 SV=1	J9P8M5_CANLF	NCR3LG1	37 k...		1					2			
2024			Secreted phosphoprotein 2 OS=Canis lupus familiaris OX=9615 GN=SPP2 PE=4 SV=1	E2RQP5_CANLF	SPP2	23 k...									1	1
2025			Caseinolytic mitochondrial matrix peptidase chaperone subunit OS=Canis lupus familiaris...	E2QSS3_CANLF	CLPX	69 k...								1		1
2026			Sorting nexin OS=Canis lupus familiaris OX=9615 GN=SNX6 PE=3 SV=2	F1PFD0_CANLF	SNX6	47 k...							1		1	
2027			Bone morphogenetic protein 4 OS=Canis lupus familiaris OX=9615 GN=BMP4 PE=3 SV=1	F1PDL1_CANLF	BMP4	47 k...									0	2
2028			Transgelin OS=Canis lupus familiaris OX=9615 GN=TAGLN PE=3 SV=1	E2RIF3_CANLF	TAGLN	26 k...				1			1			
2029			Dual serine/threonine and tyrosine protein kinase OS=Canis lupus familiaris OX=9615 G...	F1PEI7_CANLF	DSTYK	82 k...						1			1	
2030			LMBR1 domain containing 1 OS=Canis lupus familiaris OX=9615 GN=LMBRD1 PE=4 SV=1	F6XWJ1_CANLF	LMBRD1	61 k...		1		1						
2031			ATPase H+ transporting accessory protein 1 OS=Canis lupus familiaris OX=9615 GN=ATP...	F1PWQ1_CANLF	ATP6AP1	52 k...		1					1			
2032			Phosphatidylinositol glycan anchor biosynthesis class													

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated				
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4	
								Probability Legend:								
								over 95%								
								80% to 94%								
								50% to 79%								
								20% to 49%								
								0% to 19%								
2043			Aminoacidipate-semialdehyde dehydrogenase-phosphopantetheinyl transferase OS=Canis ...	F1PHM4_CANLF	AASDHPPT	34 k...							1			0
2043			ERGIC and golgi 2 OS=Canis lupus familiaris OX=9615 GN=ERGIC2 PE=4 SV=2	E2QUT2_CANLF	ERGIC2	42 k...		0		1					0	
2043			Carboxypeptidase OS=Canis lupus familiaris OX=9615 GN=SCPEP1 PE=3 SV=2	F1PSP6_CANLF	SCPEP1	51 k...				0		1				
2044			LDL receptor related protein 10 OS=Canis lupus familiaris OX=9615 GN=LRP10 PE=4 SV=2	F1PXL5_CANLF	LRP10	79 k...		1				0				
2045			Tyrosine--tRNA ligase OS=Canis lupus familiaris OX=9615 GN=YARS PE=3 SV=2	E2RHR7_CANLF	YARS	63 k...		0								2
2046			Frizzled-6 OS=Canis lupus familiaris OX=9615 GN=FZD6 PE=3 SV=1	F1P9V1_CANLF ...	FZD6	80 k...		1	0							
2047			E3 ubiquitin-protein ligase OS=Canis lupus familiaris OX=9615 GN=WWP1 PE=4 SV=2	E2RSE2_CANLF	WWP1	105 k...	★	4							0	
2048			Eukaryotic translation initiation factor 5 OS=Canis lupus familiaris OX=9615 GN=EIF5 PE...	E2RJU3_CANLF	EIF5	49 k...		1			0					
2049			DEAH-box helicase 36 OS=Canis lupus familiaris OX=9615 GN=DHX36 PE=4 SV=1	F6V8H1_CANLF	DHX36	127 ...				0		2				
2050			STAM binding protein OS=Canis lupus familiaris OX=9615 GN=STAMBP PE=4 SV=1	J9P8N1_CANLF	STAMBP	37 k...		2		0						
2051			Solute carrier family 25 member 6 OS=Canis lupus familiaris OX=9615 GN=SLC25A6 PE=...	F1Q1J6_CANLF	SLC25A6	30 k...	★			8					0	
2052			Transmembrane protein 127 OS=Canis lupus familiaris OX=9615 GN=TMEM127 PE=4 SV...	E2RDK2_CANLF	TMEM127	27 k...		1			0					
2053			Decapping enzyme, scavenger OS=Canis lupus familiaris OX=9615 GN=DCPS PE=4 SV=2	E2RNR3_CANLF	DCPS	41 k...						0		1		
2054			Ubiquitin specific peptidase 7 OS=Canis lupus familiaris OX=9615 GN=USP7 PE=3 SV=2	F1PX83_CANLF	USP7	126 ...		0								2
2055			Anion exchange protein OS=Canis lupus familiaris OX=9615 GN=SLC4A2 PE=3 SV=2	E2RCJ6_CANLF	SLC4A2	136 ...		2							0	
2056			BRX1, biogenesis of ribosomes OS=Canis lupus familiaris OX=9615 GN=BRX1 PE=4 SV=1	E2QZ99_CANLF	BRX1	40 k...		1							0	
2057			Janus kinase and microtubule interacting protein 3 OS=Canis lupus familiaris OX=9615 G...	J9P8Z0_CANLF	JAKMIP3	96 k...					0					1
2058			RAB18, member RAS oncogene family OS=Canis lupus familiaris OX=9615 GN=RAB18 PE...	J9NRZ2_CANLF	RAB18	23 k...		1			0					
2059			Scavenger receptor cysteine rich family member with 5 domains OS=Canis lupus familiaris...	E2R0D2_CANLF	SSC5D	163 ...				1						0
2060			WASH complex subunit 5 OS=Canis lupus familiaris OX=9615 GN=WASHC5 PE=4 SV=2	F1PW21_CANLF	WASHC5	134 ...				0		2				
2061			Thymidylate synthetase OS=Canis lupus familiaris OX=9615 GN=TYMS PE=3 SV=1	F1PNL9_CANLF	TYMS	40 k...		1						0		
2062			SEL1L ERAD E3 ligase adaptor subunit OS=Canis lupus familiaris OX=9615 GN=SEL1L PE=...	F1PVX5_CANLF	SEL1L	89 k...			0	1						
2063			Myomesin 1 OS=Canis lupus familiaris OX=9615 GN=MYOM1 PE=4 SV=2	E2RNN6_CANLF	MYOM1	188 ...			0				1			
2064			Nicotinate phosphoribosyltransferase OS=Canis lupus familiaris OX=9615 GN=NAPRT PE...	J9P3W8_CANLF	NAPRT	59 k...									2	
2065			Serine/threonine-protein kinase OS=Canis lupus familiaris OX=9615 GN=PRKD2 PE=3 SV...	E2RR18_CANLF	PRKD2	97 k...									1	
2066			Zinc finger with UFM1 specific peptidase domain OS=Canis lupus familiaris OX=9615 GN...	F6UP55_CANLF	ZUP1	66 k...		0							1	
2067			Coiled-coil domain-containing protein 39 OS=Canis lupus familiaris OX=9615 GN=CCDC39 ...	E2R0D3_CANLF	CCDC39	111 ...										1
2068			Receptor protein serine/threonine kinase OS=Canis lupus familiaris OX=9615 GN=BMPR2...	F1PCC1_CANLF	BMPR2	115 ...		2								
2069			Solute carrier family 26 member 6 OS=Canis lupus familiaris OX=9615 GN=SLC26A6 PE=...	F1PIC0_CANLF	SLC26A6	83 k...		1								
2070			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=CYP4A11 PE=3 SV=2	E2RSK0_CANLF	CYP4A11	59 k...				2						
2071			Apoptosis inhibitor 5 OS=Canis lupus familiaris OX=9615 GN=API5 PE=4 SV=1	E2RHB3_CANLF	API5	59 k...						1				
2072			Glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase OS=Canis lupus...	F1PLL9_CANLF	GNE	87 k...										3
2073			Acyl-CoA dehydrogenase very long chain OS=Canis lupus familiaris OX=9615 GN=ACADVL ...	E2QXA3_CANLF	ACADVL	74 k...				2						
2074			Tubulin folding cofactor B OS=Canis lupus familiaris OX=9615 GN=TBCB PE=4 SV=2	E2QYN0_CANLF	TBCB	39 k...		3								
2075			Diaphanous related formin 2 OS=Canis lupus familiaris OX=9615 GN=DIAPH2 PE=4 SV=2	F1Q1Q1_CANLF	DIAPH2	126 ...		2								
2076			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	E2RQF8_CANLF		40 k...					2					
2077			Kynureninase OS=Canis lupus familiaris OX=9615 GN=KYNU PE=3 SV=2	E2RB73_CANLF	KYNU	53 k...										2
2078			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=SLC52A2 PE=4 SV=2	E2RP36_CANLF	SLC52A2	57 k...		3								
2079			AP complex subunit sigma OS=Canis lupus familiaris OX=9615 GN=AP1S2 PE=3 SV=2	F1PR34_CANLF	AP1S2	19 k...		2								
2080			Secretory carrier-associated membrane protein OS=Canis lupus familiaris OX=9615 GN=S...	E2RBL2_CANLF	SCAMP2	39 k...		2								
2081			Glutamine synthetase OS=Canis lupus familiaris OX=9615 GN=GLUL PE=1 SV=3	GLNA_CANLF	GLUL	42 k...		2								
2082			Acid phosphatase 2, lysosomal OS=Canis lupus familiaris OX=9615 GN=ACP2 PE=3 SV=1	E2RS03_CANLF	ACP2	48 k...		2								
2083			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=C3H3orf52 PE=4 SV=1	J9NTM4_CANLF	C3H3orf52	17 k...		2								
2084			ER membrane protein complex subunit 1 OS=Canis lupus familiaris OX=9615 GN=EMC1 P...	J9P2M7_CANLF	EMC1	113 ...				2						
2085			Eukaryotic translation initiation factor 2B subunit gamma OS=Canis lupus familiaris OX=...	E2RBJ1_CANLF	EIF2B3	50 k...							1			
2086			Clustered mitochondria protein homolog OS=Canis lupus familiaris OX=9615 GN=CLUH P...	E2R9L7_CANLF	CLUH	151 ...							1			
2087			Serine hydroxymethyltransferase OS=Canis lupus familiaris OX=9615 GN=SHMT2 PE=3 S...	E2R4L7_CANLF	SHMT2	59 k...				1						
2088			Fibromodulin OS=Canis lupus familiaris OX=9615 GN=FMOD PE=4 SV=2	F1PLN5_CANLF	FMOD	43 k...									1	
2089			HECT and RLD domain containing E3 ubiquitin protein ligase 4 OS=Canis lupus familiaris ...	E2RFV1_CANLF	HERC4	118 ...										1
2090			DNA-directed RNA polymerase subunit OS=Canis lupus familiaris OX=9615 GN=POLR1A ...	E2R0G4_CANLF	POLR1A	197 ...							1			
2091			NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial OS=Canis lupus familiari...	E2R514_CANLF	NDUFV1	51 k...				1						
2092			Tripartite motif containing 72 OS=Canis lupus familiaris OX=9615 GN=TRIM72 PE=4 SV=1	E2RTM6_CANLF	TRIM72	53 k...				1						
2093			Coagulation factor XI OS=Canis lupus familiaris OX=9615 GN=F11 PE=3 SV=2	F1PPK8_CANLF	F11	71 k...			1							
2094			Glycerol-3-phosphate dehydrogenase [NAD(+)] OS=Canis lupus familiaris OX=9615 GN=...	F1P976_CANLF	GPDI	38 k...									2	
2095			Aldehyde dehydrogenase 1 family member A3 OS=Canis lupus familiaris OX=9615 GN=A...	E2R543_CANLF	ALDH1A3	56 k...	★								5	
2096			Tudor domain containing 9 OS=Canis lupus familiaris OX=9615 GN=TDRD9 PE=4 SV=2	E2RNZ9_CANLF	TDRD9	128 ...								1		
2097			Cartilage associated protein OS=Canis lupus familiaris OX=9615 GN=CRTPA PE=4 SV=1	J9NTX5_CANLF	CRTPA	52 k...						1				
2098			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=NFS1 PE=3 SV=2	F1PAY2_CANLF	NFS1	50 k...									2	
2099			Centrobin, centriole duplication and spindle assembly protein OS=Canis lupus familiaris ...	E2RTC8_CANLF	CNTR0B	100 ...				1						
2100			FH2 domain containing 1 OS=Canis lupus familiaris OX=9615 GN=FHDC1 PE=4 SV=1	E2R006_CANLF	FHDC1	119 ...				1						
2101			Bardet-Biedl syndrome 10 OS=Canis lupus familiaris OX=9615 GN=BBS10 PE=4 SV=1	J9NVH9_CANLF	BBS10	86 k...							1			
2102			Tyrosine-protein kinase OS=Canis lupus familiaris OX=9615 GN=SYK PE=3 SV=1	F1PSC8_CANLF	SYK	72 k...									1	
2103			Protein Wnt OS=Canis lupus familiaris OX=9615 GN=WNT5B PE=3 SV=2	F1Q0S4_CANLF	WNT5B	40 k...						1				
2104			Sepiapterin reductase OS=Canis lupus familiaris OX=9615 GN=SPR PE=4 SV=2	F1PTP2_CANLF	SPR	29 k...						2				
2105			Carboxypeptidase Q OS=Canis lupus familiaris OX=9615 GN=CPQ PE=4 SV=1	E2R599_CANLF	CPQ	52 k...								1		1
2106			Dipeptidyl peptidase 7 OS=Canis lupus familiaris OX=9615 GN=DPP7 PE=4 SV=1	F6XH37_CANLF	DPP7	55 k...								1		1
2107			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9NWL3_CANLF		7 kDa								1		1
2108			Ligand of numb-protein X 1 OS=Canis lupus familiaris OX=9615 GN=LNK1 PE=4 SV=1	E2RBE8_CANLF	LNK1	81 k...							1			1
2109			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9NW96_CANLF		30 k...							1	1		
2110			Mannose-P-dolichol utilization defect 1 OS=Canis lupus familiaris OX=9615 GN=MPDU1 ...	E2R6F8_CANLF	MPDU1	27 k...		1		1						
2111			Dolichyl-phosphate mannosyltransferase subunit 3 OS=Canis lupus familiaris OX=9615 ...	E2RLU1_CANLF	DPM3	11 k...		1		1						
2112			Stearoyl-CoA desaturase OS=Canis lupus familiaris OX=9615 GN=SCD PE=3 SV=2	F1PEJ0_CANLF	SCD	42 k...		1		1						
2113			Protein RER1 OS=Canis lupus familiaris OX=9615 GN=RER1 PE=3 SV=1	F6XF58_CANLF	RER1	23 k...		1		1						
2114			Tax1 binding protein 3 OS=Canis lupus familiaris OX=9615 GN=TAX1BP3 PE=4 SV=2	E2R7R0_CANLF	TAX1BP3	14 k...		1		1						
2115			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9P2X4_CANLF		15 k...		1		1						
2116			Methylsterol monooxygenase 1 OS=Canis lupus familiaris OX=9615 GN=MSMO1 PE=3 SV...	E2R6R3_CANLF	MSMO1	35 k...		1	1							
2117			Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit DAD1 OS=Cani...	E2R4X3_CANLF	DAD1	13 k...		1					1			
2118			NSFL1 cofactor OS=Canis lupus familiaris OX=9615 GN=NSFL1C PE=4 SV=2	F1PWN6_CANLF	NSFL1C	41 k...		1					1			
2119			V-type proton ATPase subunit G OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9NTK7_CANLF ...		11 k...		1					1			
2120			Eukaryotic translation initiation factor 3 subunit H OS=Canis lupus familiaris OX=9615 G...	J9NTR8_CANLF	EIF3H	43 k...										

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated				
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4	
			Probability Legend:													
			over 95%													
			80% to 94%													
			50% to 79%													
			20% to 49%													
			0% to 19%													
2126			Fumarylacetoacetate hydrolase OS=Canis lupus familiaris OX=9615 GN=FAH PE=4 SV=2	E2RS63_CANLF	FAH	46 k...							1		1	
2127			TGF-beta receptor type-2 OS=Canis lupus familiaris OX=9615 GN=TGFBR2 PE=3 SV=2	F1PNA9_CANLF	TGFBR2	64 k...		1		1						
2128			Proteasome 26S subunit, non-ATPase 10 OS=Canis lupus familiaris OX=9615 GN=PSMD1...	E2RA22_CANLF	PSMD10	25 k...		1							1	
2129			Proteasome assembly chaperone 1 OS=Canis lupus familiaris OX=9615 GN=PSMG1 PE=3 ...	F1PF61_CANLF	PSMG1	33 k...		1					1			
2130			GrpE protein homolog OS=Canis lupus familiaris OX=9615 GN=GRPEL1 PE=3 SV=2	E2RA26_CANLF	GRPEL1	26 k...			1	1						
2131			Plastin 1 OS=Canis lupus familiaris OX=9615 GN=PLS1 PE=4 SV=2	E2QWB2_CANLF	PLS1	70 k...	*								3	4
2132			Fibronectin leucine rich transmembrane protein 3 OS=Canis lupus familiaris OX=9615 GN=...	E2RKY7_CANLF	FLRT3	73 k...		1					1			
2133			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=PRDX3 PE=4 SV=1	E2RRD4_CANLF	PRDX3	28 k...		1		1						
2134			Eukaryotic translation initiation factor 4 gamma 2 OS=Canis lupus familiaris OX=9615 GN=...	F1PAH7_CANLF	EIF4G2	105 ...			1				1			
2135			RAB3 GTPase activating protein catalytic subunit 1 OS=Canis lupus familiaris OX=9615 G...	E2RK37_CANLF	RAB3GAP1	110 ...			1	1						
2136			MANSC domain containing 1 OS=Canis lupus familiaris OX=9615 GN=MANSC1 PE=4 SV=1	J9NY08_CANLF	MANSC1	49 k...			1	1						
2137			Proline rich coiled-coil 1 OS=Canis lupus familiaris OX=9615 GN=PRRC1 PE=4 SV=2	E2RQ04_CANLF	PRRC1	44 k...		1							0	
2138			Protein disulfide isomerase family A member 5 OS=Canis lupus familiaris OX=9615 GN=P...	F1PAN3_CANLF	PDIA5	60 k...				1						0
2139			Transmembrane protein 109 OS=Canis lupus familiaris OX=9615 GN=TMEM109 PE=4 SV=...	J9P616_CANLF	TMEM109	26 k...				1			0			
2140			NADH dehydrogenase [ubiquinone] flavoprotein 2 OS=Canis lupus familiaris OX=9615 GN=...	E2QVP4_CANLF	NDUFV2	27 k...		0	1							
2141			Dipeptidyl peptidase 4 OS=Canis lupus familiaris OX=9615 GN=DPP4 PE=3 SV=2	F1PP08_CANLF	DPP4	89 k...		0				1				
2142			Small nuclear ribonucleoprotein U5 subunit 40 OS=Canis lupus familiaris OX=9615 GN=S...	E2R016_CANLF	SNRNP40	39 k...							1			0
2143			PDZ and LIM domain 1 OS=Canis lupus familiaris OX=9615 GN=PDLIM1 PE=4 SV=1	J9NXY7_CANLF	PDLIM1	30 k...		1					0			
2144			V-type proton ATPase subunit C OS=Canis lupus familiaris OX=9615 GN=ATP6V1C1 PE=...	E2RSK6_CANLF	ATP6V1C1	46 k...							1		0	
2145			Aldose 1-epimerase OS=Canis lupus familiaris OX=9615 GN=GALM PE=3 SV=1	J9P687_CANLF	GALM	38 k...								1	0	
2146			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=2	F1PSF9_CANLF		57 k...		1		0						
2147			Polyadenylate-binding protein OS=Canis lupus familiaris OX=9615 GN=PABPC4 PE=3 SV=...	E2R9I1_CANLF	PABPC4	77 k...	*							0	2	
2148			Translocase of outer mitochondrial membrane 70 OS=Canis lupus familiaris OX=9615 GN=...	E2RKG3_CANLF	TOMM70	69 k...				0				1		
2149			BCL2 associated athanogene 2 OS=Canis lupus familiaris OX=9615 GN=BAG2 PE=4 SV=1	J9P4C5_CANLF	BAG2	24 k...		1					0			
2150			60S ribosome subunit biogenesis protein NIP7 homolog OS=Canis lupus familiaris OX=9...	J9NWQ0_CANLF	NIP7	21 k...							0			1
2151			Annexin OS=Canis lupus familiaris OX=9615 GN=ANXA3 PE=3 SV=1	E2R0N3_CANLF	ANXA3	36 k...	*	0				4				
2152			RBR-type E3 ubiquitin transferase OS=Canis lupus familiaris OX=9615 GN=ARIH2 PE=3 S...	E2RSY7_CANLF	ARIH2	57 k...									0	1
2153			BCAR1, Cas family scaffolding protein OS=Canis lupus familiaris OX=9615 GN=BCAR1 PE...	F1P9X8_CANLF	BCAR1	95 k...		0		1						
2154			ATP-dependent RNA helicase DHX29 OS=Canis lupus familiaris OX=9615 GN=DHX29 PE=...	J9P6V4_CANLF	DHX29	155 ...					0				1	
2155			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC484145 PE=4 SV=1	J9P7C7_CANLF	LOC484145	69 k...								0		1
2156			Gamma-glutamyltransferase 6 OS=Canis lupus familiaris OX=9615 GN=GGT6 PE=4 SV=2	E2RKU6_CANLF	GGT6	51 k...		1					0			
2157			Peptidase M20 domain containing 1 OS=Canis lupus familiaris OX=9615 GN=PM20D1 PE=...	E2RKU5_CANLF	PM20D1	55 k...							0		1	
2158			FAM20B, glycosaminoglycan xylosylkinase OS=Canis lupus familiaris OX=9615 GN=FAM2...	E2RSG3_CANLF	FAM20B	46 k...		1		0						
2159			Gem nuclear organelle associated protein 4 OS=Canis lupus familiaris OX=9615 GN=GEMI...	F1PFG3_CANLF	GEMIN4	135 ...							1		0	
2160			1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase OS=Canis lupus familiaris OX=...	H9GW79_CANLF	ADI1	17 k...		1					0			
2161			Inosine-5'-monophosphate dehydrogenase OS=Canis lupus familiaris OX=9615 GN=IMP...	E2RID1_CANLF	IMPDH1	57 k...		1				0				
2162			PDZ domain containing 11 OS=Canis lupus familiaris OX=9615 GN=PDZD11 PE=4 SV=1	J9NRG7_CANLF	PDZD11	16 k...		1	0							
2163			Nuclear RNA export factor 1 OS=Canis lupus familiaris OX=9615 GN=NXF1 PE=4 SV=1	F6XRT0_CANLF	NXF1	71 k...		0								1
2164			Sigma non-opioid intracellular receptor 1 OS=Canis lupus familiaris OX=9615 GN=SIGMA...	J9P813_CANLF	SIGMAR1	25 k...		1		0						
2165			Protocadherin gamma subfamily B, 1 OS=Canis lupus familiaris OX=9615 GN=PCDHGB1 P...	E2R8V4_CANLF	PCDHGB1	88 k...							0			1
2166			Thioredoxin related transmembrane protein 2 OS=Canis lupus familiaris OX=9615 GN=T...	E2R415_CANLF	TMX2	34 k...				1						
2167			Ferritin OS=Canis lupus familiaris OX=9615 PE=3 SV=1	E2RRH1_CANLF		20 k...	*	1	1							
2168			Anterior pharynx defective 1 homolog A OS=Canis lupus familiaris OX=9615 GN=APH1A ...	E2R5H5_CANLF	APH1A	29 k...		2								
2169			Latent transforming growth factor beta binding protein 4 OS=Canis lupus familiaris OX=9...	F1PDK1_CANLF	LTBP4	175 ...										2
2170			Cyclin Y OS=Canis lupus familiaris OX=9615 GN=CCNY PE=3 SV=2	F1PHF7_CANLF	CCNY	35 k...		2								
2171			Prosaposin OS=Canis lupus familiaris OX=9615 GN=PSAP PE=4 SV=1	E2RLF1_CANLF	PSAP	58 k...		2								
2172			Noggin OS=Canis lupus familiaris OX=9615 GN=NOG PE=3 SV=1	J9NYY7_CANLF	NOG	25 k...		2								
2173			Splicing factor 3b subunit 6 OS=Canis lupus familiaris OX=9615 GN=SF3B6 PE=4 SV=1	E2RRA3_CANLF	SF3B6	15 k...							2			
2174			Complement C8 alpha chain OS=Canis lupus familiaris OX=9615 GN=C8A PE=4 SV=1	E2R109_CANLF	C8A	66 k...					2					
2175			Protein phosphatase methyltransferase 1 OS=Canis lupus familiaris OX=9615 GN=PPME1 PE...	E2RKQ0_CANLF	PPME1	42 k...							2			
2176			Dihydrolipoamide S-succinyltransferase OS=Canis lupus familiaris OX=9615 GN=DLST PE=...	E2R0H0_CANLF	DLST	49 k...					2					
2177			Hikeshi, heat shock protein nuclear import factor OS=Canis lupus familiaris OX=9615 GN=...	J9P373_CANLF	HIKESHI	22 k...		2								
2178			Trans-2,3-enoyl-CoA reductase OS=Canis lupus familiaris OX=9615 GN=TECR PE=4 SV=2	F1PZL2_CANLF	TECR	40 k...										
2179			COP9 signalosome subunit 7A OS=Canis lupus familiaris OX=9615 GN=COP57A PE=4 SV=...	E2RED3_CANLF	COP57A	30 k...									2	
2180			Prothymosin alpha OS=Canis lupus familiaris OX=9615 GN=PTMA PE=4 SV=1	J9NZAO_CANLF	PTMA	12 k...		2								
2181			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	E2RNY7_CANLF		19 k...					2					
2182			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=C7H1orf116 PE=4 SV=2	E2R2G0_CANLF	C7H1orf116	50 k...		1								
2183			Serum paraoxonase/arylesterase 2 OS=Canis lupus familiaris OX=9615 GN=PON2 PE=2 S...	PON2_CANLF	PON2	40 k...					1					
2184			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9NZZ6_CANLF		30 k...					2					
2185			Serine/threonine-protein phosphatase 2A activator OS=Canis lupus familiaris OX=9615 G...	F1PD37_CANLF	PTPA	37 k...		1								
2186			Solute carrier family 31 member 1 OS=Canis lupus familiaris OX=9615 GN=SLC31A1 PE=...	F1Q0F2_CANLF	SLC31A1	21 k...							1			
2187			Eukaryotic translation initiation factor 18 OS=Canis lupus familiaris OX=9615 GN=EIF18 ...	J9P1M0_CANLF	EIF18	8 kDa		1								
2188			Phosducin like 3 OS=Canis lupus familiaris OX=9615 GN=PDCL3 PE=4 SV=1	E2RF39_CANLF	PDCL3	28 k...		1								
2189			EPH receptor B1 OS=Canis lupus familiaris OX=9615 GN=EPHB1 PE=3 SV=2	F1PNA5_CANLF	EPHB1	110 ...	*				1					
2190			Endoplasmic reticulum protein 29 OS=Canis lupus familiaris OX=9615 GN=ERP29 PE=4 S...	J9P4L2_CANLF	ERP29	42 k...		1								
2191			COP9 signalosome subunit 5 OS=Canis lupus familiaris OX=9615 GN=COP55 PE=4 SV=1	E2QWEO_CANLF	COP55	38 k...										1
2192			Gap junction protein OS=Canis lupus familiaris OX=9615 GN=GJB3 PE=3 SV=1	E2R5G5_CANLF	GJB3	31 k...		1								
2193			Prostaglandin E2 receptor EP2 subtype OS=Canis lupus familiaris OX=9615 GN=PTGER2 P...	F1P776_CANLF	PTGER2	40 k...		1								
2194			Paralemmin 3 OS=Canis lupus familiaris OX=9615 GN=PALM3 PE=4 SV=2	F1PAS6_CANLF	PALM3	66 k...		1								
2195			Scellin OS=Canis lupus familiaris OX=9615 GN=SCEL PE=4 SV=2	E2RNM8_CANLF	SCEL	79 k...		1								
2196			Dehydrogenase/reductase 7B OS=Canis lupus familiaris OX=9615 GN=DHRS7B PE=3 SV=2	F1PLI4_CANLF	DHRS7B	32 k...					1					
2197			Golgi phosphoprotein 3 OS=Canis lupus familiaris OX=9615 GN=GOLPH3 PE=4 SV=2	F1PKG4_CANLF	GOLPH3	26 k...									1	
2198			Zinc finger C4H2-type containing OS=Canis lupus familiaris OX=9615 GN=ZC4H2 PE=4 S...	E2QXX5_CANLF	ZC4H2	29 k...									1	
2199			Eukaryotic translation initiation factor 4E family member 18 OS=Canis lupus familiaris OX...	F1P7M3_CANLF	EIF4E18	27 k...										1
2200			Pescadillo homolog OS=Canis lupus familiaris OX=9615 GN=PES1 PE=3 SV=1	J9P0G2_CANLF	PES1	71 k...		1								
2201			Pleckstrin homology and RUN domain containing M2 OS=Canis lupus familiaris OX=9615 ...	F1Q0U6_CANLF	PLEKHM2	113 ...									1	
2202			Superoxide dismutase [Cu-Zn] OS=Canis lupus familiaris OX=9615 GN=SOD3 PE=3 SV=2	F1PAS8_CANLF	SOD3	33 k...							1			
2203			Integral membrane protein 2C OS=Canis lupus familiaris OX=9615 GN=ITM2C PE=4 SV=1	J9NX68_CANLF	ITM2C	30 k...		1								
2204			Erythrocyte membrane protein band 4.1 like 2 OS=Canis lupus familiaris OX=9615 GN=EP...	F1PTI7_CANLF	EPB41L2	102 ...					1					

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
			Probability Legend:												
			over 95%												
			80% to 94%												
			50% to 79%												
			20% to 49%												
			0% to 19%												
2211			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9P7Q9_CANLF		63 k...		1							
2212			MORC family CW-type zinc finger 1 OS=Canis lupus familiaris OX=9615 GN=MORC1 PE=...	E2RJX9_CANLF	MORC1	110 ...							1		
2213			Cytochrome b5 OS=Canis lupus familiaris OX=9615 GN=CYB5A PE=3 SV=1	E2R119_CANLF	... CYB5A	18 k...		1							
2214			Dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex OS=C...	E2RQG4_CANLF	DBT	53 k...			1						
2215			Carbohydrate sulfotransferase 15 OS=Canis lupus familiaris OX=9615 GN=CHST15 PE=4 ...	E2RN25_CANLF	CHST15	65 k...							1		
2216			Poly [ADP-ribose] polymerase OS=Canis lupus familiaris OX=9615 GN=MPHOSPH8 PE=4 S...	E2QWJ0_CANLF	MPHOSPH8	190 ...		1							
2217			cGMP-specific 3',5'-cyclic phosphodiesterase OS=Canis lupus familiaris OX=9615 GN=PD...	PDE5A_CANLF	PDE5A	98 k...									1
2218			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=DNAJC28 PE=4 SV=1	J9NZC7_CANLF	DNAJC28	46 k...								1	
2219			Huntingtin interacting protein 1 related OS=Canis lupus familiaris OX=9615 GN=HIP1R PE...	F1PZG3_CANLF	HIP1R	119 ...								1	
2220			Mitogen-activated protein kinase kinase kinase 20 OS=Canis lupus familiaris OX=9615 G...	E2REE9_CANLF	MAP3K20	92 k...								1	
2221			Metallo-beta-lactamase domain containing 2 OS=Canis lupus familiaris OX=9615 GN=M...	J9NVD1_CANLF	MBLAC2	31 k...		1							
2222			Beta-1,4-galactosyltransferase 3 OS=Canis lupus familiaris OX=9615 GN=B4GALT3 PE=4...	J9NUD4_CANLF	B4GALT3	44 k...							1		
2223			F-box protein 6 OS=Canis lupus familiaris OX=9615 GN=FBXO6 PE=4 SV=1	J9NXM2_CANLF	FBXO6	36 k...							1		
2224			WD repeat domain 92 OS=Canis lupus familiaris OX=9615 GN=WDR92 PE=4 SV=2	F1PZ86_CANLF	WDR92	75 k...								2	
2225			Integrin subunit alpha 2b OS=Canis lupus familiaris OX=9615 GN=ITGA2B PE=3 SV=2	F1PRU9_CANLF	ITGA2B	119 ...			1						
2226			Notch 1 OS=Canis lupus familiaris OX=9615 GN=NOTCH1 PE=4 SV=2	F1PDC7_CANLF	NOTCH1	272 ...	★					1			
2227			Complement C1r OS=Canis lupus familiaris OX=9615 GN=C1R PE=3 SV=2	F1PNG7_CANLF	C1R	82 k...									1
2228			Bromodomain containing 4 OS=Canis lupus familiaris OX=9615 GN=BRD4 PE=4 SV=2	E2QW92_CANLF	BRD4	151 ...							1		
2229			RAS p21 protein activator 1 OS=Canis lupus familiaris OX=9615 GN=RASA1 PE=4 SV=2	F1PGT1_CANLF	RASA1	114 ...									1
2230			Protein tyrosine phosphatase, receptor type 5 OS=Canis lupus familiaris OX=9615 GN=PT...	E2R6Z2_CANLF	PTPRS	211 ...	★								1
2231			Glutathione S-transferase C-terminal domain containing OS=Canis lupus familiaris OX=9...	E2R8Z9_CANLF	GSTCD	71 k...			1						
2232			ST3 beta-galactoside alpha-2,3-sialyltransferase 1 OS=Canis lupus familiaris OX=9615 G...	E2RLK1_CANLF	ST3GAL1	39 k...									1
2233			Ring finger protein 25 OS=Canis lupus familiaris OX=9615 GN=RN25 PE=4 SV=1	E2RE32_CANLF	RNF25	51 k...								1	
2234			Transformer 2 beta homolog OS=Canis lupus familiaris OX=9615 GN=TRA2B PE=4 SV=2	E2RD37_CANLF	TRA2B	34 k...		1							
2235			Amidohydrolase domain containing 1 OS=Canis lupus familiaris OX=9615 GN=AMDHD1 ...	F1Q0L2_CANLF	AMDHD1	36 k...						1			
2236			Ras and Rab interactor 1 OS=Canis lupus familiaris OX=9615 GN=RIN1 PE=4 SV=2	E2RGE1_CANLF	RIN1	85 k...						1			
2237			Cytokine receptor like factor 3 OS=Canis lupus familiaris OX=9615 GN=CRLF3 PE=4 SV=2	F1PLL6_CANLF	CRLF3	49 k...									1
2238			VAMP associated protein B and C OS=Canis lupus familiaris OX=9615 GN=VAPB PE=4 SV=1	E2RRB7_CANLF	VAPB	27 k...		1							
2239			Non-specific serine/threonine protein kinase OS=Canis lupus familiaris OX=9615 GN=M...	F1P9C6_CANLF	MARK3	84 k...	★					2			
2240			Yip1 interacting factor homolog A, membrane trafficking protein OS=Canis lupus familiari...	J9P3D1_CANLF	YIF1A	32 k...				1					
2241			TBC1 domain family member 20 OS=Canis lupus familiaris OX=9615 GN=TBC1D20 PE=4 ...	J9P5A7_CANLF	TBC1D20	46 k...				1					
2242			Sorting nexin OS=Canis lupus familiaris OX=9615 GN=SNX18 PE=3 SV=1	E2RJ12_CANLF	SNX18	68 k...		1							
2243			Cleavage and polyadenylation specific factor 1 OS=Canis lupus familiaris OX=9615 GN=C...	F1PC28_CANLF	CPSF1	156 ...									1
2244			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=NECTIN1 PE=4 SV=1	F1PCB6_CANLF	NECTIN1	57 k...		1							
2245			Carbonic anhydrase 3 OS=Canis lupus familiaris OX=9615 GN=CA3 PE=4 SV=2	F1PD27_CANLF	CA3	29 k...									1
2246			Fibulin 2 OS=Canis lupus familiaris OX=9615 GN=FBLN2 PE=4 SV=1	J9P2K0_CANLF	FBLN2	131 ...									1
2247			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=1	J9P5N8_CANLF		36 k...	★								35
2248			S-methyl-5'-thioadenosine phosphorylase OS=Canis lupus familiaris OX=9615 GN=MTA...	J9P511_CANLF	MTAP	32 k...						1			
2249			Thrombomodulin OS=Canis lupus familiaris OX=9615 GN=THBD PE=4 SV=1	F1PB37_CANLF	... THBD	61 k...			1						
2250			Surfeit 4 OS=Canis lupus familiaris OX=9615 GN=SURF4 PE=4 SV=2	F1PB56_CANLF	SURF4	29 k...		1							
2251			Histone cluster 1 H1 family member A OS=Canis lupus familiaris OX=9615 GN=HIST1H1A ...	E2RR73_CANLF	HIST1H1A	22 k...	★	14							
2252			Tetraspanin OS=Canis lupus familiaris OX=9615 GN=TSPAN17 PE=3 SV=2	F1P7M4_CANLF	TSPAN17	32 k...		2							
2253			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=1	J9NRN4_CANLF		49 k...	★						9		
2254			Dispatched RND transporter family member 2 OS=Canis lupus familiaris OX=9615 GN=DI...	F1PRY8_CANLF	DISP2	152 ...						1			
2255			Dimethylarginine dimethylaminohydrolase 2 OS=Canis lupus familiaris OX=9615 GN=DD...	F1PB12_CANLF	DDAH2	30 k...								1	
2256			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	H9GWB5_CANLF		17 k...								1	
2257			Out at first homolog OS=Canis lupus familiaris OX=9615 GN=OAF PE=4 SV=1	J9NRG9_CANLF	OAF	28 k...								1	
2258			Tyrosine kinase with immunoglobulin like and EGF like domains 1 OS=Canis lupus famili...	E2QXM1_CANLF	TIE1	125 ...								1	
2259			Coatomer subunit epsilon OS=Canis lupus familiaris OX=9615 GN=COPE PE=3 SV=1	E2RAT6_CANLF	COPE	35 k...								1	
2260			Cytochrome c oxidase subunit 4I1 OS=Canis lupus familiaris OX=9615 GN=COX4I1 PE=4 ...	E2QUA8_CANLF	COX4I1	20 k...				1					
2261			Tensin 4 OS=Canis lupus familiaris OX=9615 GN=TNS4 PE=4 SV=2	E2QX58_CANLF	TNS4	76 k...				1					
2262			Solute carrier family 39 member 9 OS=Canis lupus familiaris OX=9615 GN=SLC39A9 PE=...	E2QXU2_CANLF	SLC39A9	33 k...				1					
2263			Tectonin beta-propeller repeat containing 1 OS=Canis lupus familiaris OX=9615 GN=TEC...	E2R967_CANLF	TECPR1	131 ...				1					
2264			Amine oxidase OS=Canis lupus familiaris OX=9615 GN=AOC3 PE=3 SV=2	E2RFC0_CANLF	AOC3	85 k...				1					
2265			Peptidyl-tRNA hydrolase 2 OS=Canis lupus familiaris OX=9615 GN=PTRH2 PE=4 SV=1	E2RND8_CANLF	PTRH2	19 k...				1					
2266			ATP synthase F1 subunit delta OS=Canis lupus familiaris OX=9615 GN=ATP5F1D PE=3 SV...	F1PY24_CANLF	ATP5F1D	18 k...				1					
2267			IlvB acetolactate synthase like OS=Canis lupus familiaris OX=9615 GN=ILVBL PE=3 SV=2	F1Q255_CANLF	ILVBL	68 k...				1					
2268			Cysteine and glycine rich protein 1 OS=Canis lupus familiaris OX=9615 GN=CSR1 PE=4 ...	J9NYN2_CANLF	CSR1	21 k...				1					
2269			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=HADHB PE=3 SV=1	J9NZC6_CANLF	HADHB	52 k...				1					
2270			Phosphatidylinositol glycan anchor biosynthesis class K OS=Canis lupus familiaris OX=9...	J9P6N1_CANLF	PIGK	46 k...				1					
2271			Alpha-galactosidase OS=Canis lupus familiaris OX=9615 GN=NAGA PE=3 SV=2	E2RRW9_CANLF	NAGA	46 k...								1	
2272			Arylsulfatase G OS=Canis lupus familiaris OX=9615 GN=ARSG PE=2 SV=1	ARSG_CANLF	ARSG	58 k...									1
2273			EPM2A interacting protein 1 OS=Canis lupus familiaris OX=9615 GN=EPM2AI1 PE=4 SV=2	E2QUW7_CANLF	EPM2AI1	70 k...									1
2274			Ubiquitin like modifier activating enzyme 7 OS=Canis lupus familiaris OX=9615 GN=UBA...	E2QYA0_CANLF	UBA7	111 ...									1
2275			Hexokinase 3 OS=Canis lupus familiaris OX=9615 GN=HK3 PE=3 SV=2	E2R8C2_CANLF	HK3	104 ...									1
2276			Ribosomal oxygenase 2 OS=Canis lupus familiaris OX=9615 GN=RIOX2 PE=4 SV=2	E2RD59_CANLF	RIOX2	64 k...									1
2277			Exosome component 4 OS=Canis lupus familiaris OX=9615 GN=EXOSC4 PE=4 SV=1	E2RIM3_CANLF	EXOSC4	26 k...									1
2278			SMU1, DNA replication regulator and spliceosomal factor OS=Canis lupus familiaris OX=9...	E2RLX2_CANLF	SMU1	58 k...									1
2279			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=UTP4 PE=4 SV=2	E2RPQ7_CANLF	UTP4	57 k...									1
2280			Betaine--homocysteine S-methyltransferase 2 OS=Canis lupus familiaris OX=9615 GN=B...	E2RSV3_CANLF	BHMT2	40 k...	★								5
2281			Autophagy-related protein 3 OS=Canis lupus familiaris OX=9615 GN=ATG3 PE=3 SV=2	F1PJ26_CANLF	ATG3	35 k...									1
2282			Glyoxylate and hydroxypyruvate reductase OS=Canis lupus familiaris OX=9615 GN=GRHP...	F1PJ50_CANLF	GRHPR	36 k...									1
2283			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=LOC609513 PE=4 SV=2	F1PZ03_CANLF	LOC609513	31 k...									1
2284			Follistatin like 3 OS=Canis lupus familiaris OX=9615 GN=FSTL3 PE=4 SV=2	F1Q1E3_CANLF	FSTL3	28 k...									1
2285			Autophagy protein 5 OS=Canis lupus familiaris OX=9615 GN=ATG5 PE=3 SV=1	F6V678_CANLF	ATG5	32 k...									1
2286			Pyridoxal phosphatase OS=Canis lupus familiaris OX=9615 GN=PDXP PE=3 SV=1	J9NUR4_CANLF	PDXP	32 k...									1
2287			RNA binding protein fox-1 homolog OS=Canis lupus familiaris OX=9615 GN=RBFOX3 PE...	J9PAH3_CANLF	RBFOX3	34 k...									1
2288			Tenascin XB OS=Canis lupus familiaris OX=9615 GN=TNXB PE=4 SV=1	J9PBD8_CANLF	TNXB	445 ...									1
2289			Pleckstrin OS=Canis lupus familiaris OX=9615 GN=PLEK PE=2 SV=1	PLEK_CANLF	PLEK	40 k...									1
2290			Aquaporin-1 OS=Canis lupus familiaris OX=9615 GN=AQP1 PE=2 SV=3	AQP1_CANLF	AQP1	29 k...				1					
2291			DEP domain containing 1B OS=Canis lupus familiaris OX=9615 GN=DEPDC1B PE=4 SV=2	F1PWG6_CANLF	DEPDC1B	59 k...				1					
2292			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=4 SV=2	F1PZ69_CANLF		34 k...				1					
2293			Uncharacterized protein OS=Canis lupus familiaris OX=9615 PE=3 SV=2	F1Q0L5_CANLF		12 k...				1					
2294			F2R like trypsin receptor 1 OS=Canis lupus familiaris OX=9615 GN=F2RL1 PE=2 SV=1=												

#	Visible?	Starred?	Bio View: Identified Proteins (2335/2336)	Accession Number	Alternate ID	Molecular Weight	Protein Grouping Ambiguity	control				treated			
								Control1	Control2	Control3	Control4	Treated1	Treated2	Treated3	Treated4
								Probability Legend: over 95% 80% to 94% 50% to 79% 20% to 49% 0% to 19%							
2296			Endothelial differentiation related factor 1 OS=Canis lupus familiaris OX=9615 GN=EDF1 ...	E2R2F2_CANLF	EDF1	16 k...		1							
2297			Cytochrome P450 family 1 subfamily B member 1 OS=Canis lupus familiaris OX=9615 GN...	E2R329_CANLF	CYP1B1	60 k...		1							
2298			NOC2 like nucleolar associated transcriptional repressor OS=Canis lupus familiaris OX=9...	E2R3P0_CANLF	NOC2L	87 k...		1							
2299			RNA binding motif protein 39 OS=Canis lupus familiaris OX=9615 GN=RBM39 PE=4 SV=1	E2R4L0_CANLF	RBM39	59 k...		1							
2300			ER lumen protein-retaining receptor OS=Canis lupus familiaris OX=9615 GN=KDEL3 PE...	E2RA31_CANLF	KDEL3	25 k...		1							
2301			Secretory carrier-associated membrane protein OS=Canis lupus familiaris OX=9615 GN=S...	E2RBG0_CANLF	SCAMP5	26 k...		1							
2302			WW domain binding protein 2 OS=Canis lupus familiaris OX=9615 GN=WBP2 PE=4 SV=2	E2RFA4_CANLF	WBP2	29 k...		1							
2303			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=RFFL PE=4 SV=2	E2RNX6_CANLF	RFFL	43 k...		1							
2304			Transmembrane p24 trafficking protein 5 OS=Canis lupus familiaris OX=9615 GN=TMED...	E2RR38_CANLF	TMED5	26 k...		1							
2305			AP complex subunit sigma OS=Canis lupus familiaris OX=9615 GN=AP1S1 PE=3 SV=2	E2RTH1_CANLF	AP1S1	21 k...		1							
2306			Transmembrane protein 30B OS=Canis lupus familiaris OX=9615 GN=TMEM30B PE=4 SV...	F1P8N9_CANLF	TMEM30B	24 k...	★	1							
2307			Ring finger protein 130 OS=Canis lupus familiaris OX=9615 GN=RNF130 PE=4 SV=2	F1PQP8_CANLF	RNF130	38 k...		1							
2308			Receptor protein serine/threonine kinase OS=Canis lupus familiaris OX=9615 GN=TGFBR...	F1P563_CANLF	TGFBR1	56 k...		1							
2309			Anterior gradient 2, protein disulphide isomerase family member OS=Canis lupus familiar...	F1PUR5_CANLF	AGR2	20 k...		1							
2310			Potassium two pore domain channel subfamily K member 1 OS=Canis lupus familiaris OX...	F1PWQ7_CANLF	KCNK1	29 k...		1							
2311			Leucine rich repeat containing 47 OS=Canis lupus familiaris OX=9615 GN=LRRC47 PE=4 S...	F1PYM5_CANLF	LRRC47	64 k...		1							
2312			Delta-aminolevulinic acid dehydratase OS=Canis lupus familiaris OX=9615 GN=ALAD PE...	F1PZA0_CANLF	ALAD	37 k...		1							
2313			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=IGBP1 PE=4 SV=1	F6UMY5_CANLF	IGBP1	45 k...		1							
2314			Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit OS=Canis lupus fam...	F6V7D9_CANLF	PPP2R5E	55 k...		1							
2315			Prostate transmembrane protein, androgen induced 1 OS=Canis lupus familiaris OX=9615...	J9P9R3_CANLF	PMEPA1	26 k...		1							
2316			Oligosaccharyltransferase complex subunit OSTC OS=Canis lupus familiaris OX=9615 GN...	OSTC_CANLF	OSTC	17 k...		1							
2317			SH3 domain and tetratricopeptide repeats 1 OS=Canis lupus familiaris OX=9615 GN=SH3...	E2RAQ0_CANLF	SH3TC1	146 ...							1		
2318			ST3 beta-galactoside alpha-2,3-sialyltransferase 6 OS=Canis lupus familiaris OX=9615 G...	E2RCV7_CANLF	ST3GAL6	38 k...							1		
2319			Protein kinase AMP-activated non-catalytic subunit gamma 2 OS=Canis lupus familiaris ...	E2RFC5_CANLF	PRKAG2	63 k...							1		
2320			Claudin OS=Canis lupus familiaris OX=9615 GN=CLDN16 PE=3 SV=1	E2RQW9_CANLF	CLDN16	34 k...							1		
2321			Activator of HSP90 ATPase activity 1 OS=Canis lupus familiaris OX=9615 GN=AHSA1 PE=...	E2RSU7_CANLF	AHSA1	38 k...					1				
2322			Uncharacterized protein OS=Canis lupus familiaris OX=9615 GN=PSMD5 PE=4 SV=1	F1P7L2_CANLF	PSMD5	56 k...	★						3		
2323			Beta-site APP-cleaving enzyme 2 OS=Canis lupus familiaris OX=9615 GN=BACE2 PE=3 S...	F1PA64_CANLF	BACE2	46 k...							1		
2324			Syntrophin beta 2 OS=Canis lupus familiaris OX=9615 GN=SNB2 PE=4 SV=2	F1PBV5_CANLF	SNB2	61 k...							1		
2325			Bridging integrator 1 OS=Canis lupus familiaris OX=9615 GN=BIN1 PE=4 SV=2	F1PJ63_CANLF	BIN1	71 k...							1		
2326			Nuclear cap binding protein subunit 1 OS=Canis lupus familiaris OX=9615 GN=NCBP1 PE...	F1PUP7_CANLF	NCBP1	92 k...							1		
2327			UDP-glucose ceramide glucosyltransferase OS=Canis lupus familiaris OX=9615 GN=UGC...	F1PY65_CANLF	UGCG	45 k...					1				
2328			Tyrosine-protein phosphatase OS=Canis lupus familiaris OX=9615 GN=PTPN12 PE=4 SV=2	F1PZ23_CANLF	PTPN12	84 k...							1		
2329			Small glutamine rich tetratricopeptide repeat containing alpha OS=Canis lupus familiaris ...	F6XMP7_CANLF	SGTA	34 k...					1				
2330			Probable tRNA N6-adenosine threonylcarbamoyltransferase OS=Canis lupus familiaris OX...	J9P2K2_CANLF	OSGEP	36 k...							1		
2331			Pachytene checkpoint protein 2 homolog OS=Canis lupus familiaris OX=9615 GN=TRIP13 ...	PCH2_CANLF	TRIP13	48 k...					1				
2332			Zinc finger CCHC-type containing 9 OS=Canis lupus familiaris OX=9615 GN=ZCCHC9 PE...	F6V033_CANLF	ZCCHC9	30 k...								1	
2333			Fatty acid binding protein 1 OS=Canis lupus familiaris OX=9615 GN=FABP1 PE=4 SV=1	F1P9C9_CANLF	FABP1	14 k...							1		
2334			Transmembrane protein 248 OS=Canis lupus familiaris OX=9615 GN=TMEM248 PE=4 SV...	E2R6Y5_CANLF	TMEM248	35 k...					1				
2335			Plasmalipin OS=Canis lupus familiaris OX=9615 GN=PLLP PE=4 SV=2	F1P9U7_CANLF	PLLP	20 k...					1				