Spatiotemporal characterization of G protein β and γ subunit interactors via APEX2 labeling

Jenny Lau

Degree of Master of Science

Department of Pharmacology and Therapeutics, Faculty of Medicine

McGill University

Montreal, Quebec, Canada

August 2020

A thesis submitted in partial fulfillment of the requirements of the degree of Master of Science

ACKNOWLEDGMENTS

I would first like to thank Terry Hébert for allowing me to pursue my Master degree in his lab under his amazing supervision. None of this would have been possible without his kindness, patience, and continued support which has been invaluable in challenging me and encouraging me forward, especially when things do not work out. His guidance and open-door policy are a great example of being there for his students while also giving them the independence to develop the tools for success no matter what we choose to undertake next. I am infinitely grateful to have been a part of the Hébert lab.

Thank you also to my advisor, Dr. Paul Clarke, as well as the other members of my committee, Dr. Jean-François Trempe and Dr. Jason Tanny, for their guidance and feedback, providing an outside perspective for improvements to my project. Thank you to the Department of Pharmacology, in particular Dr. Nicholas Audet, for helping me with the imaging platform.

Thank you especially to Darlaine Pétrin for making the constructs and developing the protocol for the APEX2 labeling assay. A big thank you to Andrew Bayne from the Trempe lab for running all my mass spectrometry samples and answering my constant flow of questions while optimizing the protocol and afterwards, when I received the results. Thank you also to Iulia Pirvulescu for teaching me Co-IPs and Célia Bouazza for teaching me the biotinylation assay. Thank you to all the members of the Hébert lab for being a source of moral support and often a great source of entertainment, while helping me with the many techniques used throughout my time in the lab. Thank you also to the "real adults" in the lab, Phan Trieu and Dr. Dominic Devost. A special thanks to my student, Sofia Paoli, for teaching me how to teach.

Finally, thank you to all my family and friends for their support and encouragement, without whom this endeavour would not have been possible.

TABLE OF CONTENTS

ABSTRACT

G protein-coupled receptors (GPCRs) represent a large family of membrane receptors involved in important physiological functions through regulation of myriad downstream signalling pathways. Many pathways work via heterotrimeric G proteins. In addition to the important role played by Gα subunits, recent work has described non canonical roles for Gβγ subunits in downstream signalling of GPCRs in organelles such as the nucleus, Golgi apparatus and mitochondria. Our lab has used tandem-affinity purification (TAP) to identify cytosolic and nuclear Gβγ-interacting proteins. Nonetheless, TAP and other methods to study protein interactions require them to be stable or require long incubation periods, missing transient and/or weak interactions. Furthermore, techniques that provide adequate temporal information often focus on one pathway at a time, requiring a large array of assays. The use of APEX2, a proximity-dependent labeling technique, provides both spatial and temporal information in endogenous protein expression level, allowing for more in-depth characterization of protein networks over time. This thesis describes the validation of APEX2-fused constructs, optimization of samples for LC-MS and subsequent proteins identified from our screens. Comparisons between TAP and APEX2 screens demonstrate various DNA/RNA binding proteins in HEK 293 cells, supporting the growing body of evidence for Gβγ in regulating transcription and suggests a novel role in protein synthesis. Proteins identified from different organelles also demonstrate the expanding roles of Gβγ, particularly in the nucleus. This work contributes to previously generated LC-MS data and provides a tool to study Gβγ signaling. Further experiments using fractionated samples will allow us to identify nuclear and cytoplasmic specific interactors, and stimulated conditions will enable us to observe proteome differences with receptor activation.

RÉSUMÉ

Les récepteurs couplés aux protéines G (GPCRs) représentent une grande famille de récepteurs membranaires impliqués dans des fonctions physiologiques importantes par la régulation de diverses voies de signalisation. Plusieurs de ces voies sont à travers les protéines G hétérotrimérique. En plus des rôles importants que jouent les sous-unités Gα, des études récentes décrivent des rôles pour les sous-unités Gβγ en aval de la signalisation des GPCRs au niveau du noyau, l'Appareil de Golgi et les mitochondries. Notre laboratoire a précédemment utilisé la purification par affinité en tandem (TAP) pour identifier des protéines cytosoliques et nucléaires qui interagissent avec Gβγ. Pourtant, TAP et autres méthodes pour l'étude des interactions protéiques requièrent qu'elles soient stables ou requièrent de longues incubations ce qui évitent la détection des interactions faibles et/ou transientes. De plus, les techniques qui fournissent adéquatement l'information temporelle sont souvent axées sur une voie, et donc, requièrent une multitude d'essais. L'utilisation de APEX2, une technique d'étiquetage dépendant de la proximité, fournit autant l'information temporelle que spatiale dans des concentrations endogènes de protéines, ce qui permet la caractérisation approfondie des réseaux protéiques. Cette thèse décrit la validation des plasmides contenant le gène pour APEX2, l'optimisation des échantillons pour LC-MS et l'identification des protéines provenant de nos essais. La comparaison de nos résultats versus ceux de TAP démontrent une variété de protéines qui se lient à l'ADN et l'ARN dans les cellules HEK 293, supportant les preuves pour Gβγ dans la régulation de la transcription et suggère un rôle novateur dans la synthèse des protéines. Les protéines identifiées provenant de différentes organelles démontrent aussi la variété des rôles de Gβγ, particulièrement au niveau du noyau. Ces résultats contribuent aux données de LC-MS précédent et fournissent une méthode pour l'étude de la signalisation de Gβγ. De futures expériences qui utilisent le fractionnement cellulaire permettra

l’observation des interactions spécifiques aux cytoplasme et au noyau, et des conditions stimulées permettront de comparer les différences protéomiques avec l’activation d’un récepteur.

ABBREVIATIONS

5-HT_{1F} – 5-hydroxytryptamine 1F

α_1-AR – α_1-adrenergic receptor

α_2-AR – α_2-adrenergic receptor

β_2-AR – β_2-adrenergic receptor

AC – Adenylyl cyclase

ACN – Acetonitrile

AEBP1 – Adipocyte enhancer-binding protein

AGS – Activator of G protein signaling

AHSA1 – Activator of heat shock protein ATPase homolog 1

AP-1 – Activator protein-1

APEX – Ascorbate peroxidase

AT1R – Angiotensin II type 1 receptor

ATP – Adenosine triphosphate

BSA – Bovine serum albumine

CaM – Calmodulin binding peptide

cAMP – cyclic adenosine monophosphate

CCT – T-complex protein 1

CHO – Chinese hamster ovary

CRAPome – Contaminant repository for affinity purification mass spectrometry data

CT – C-terminus

CXCR4 – CXC chemokine receptor type 4

DAG – Diacylglycerol

DMEM – Dulbecco's modified eagle medium

DTT – Dithiothreitol

EDTA – Ethylenediaminetetraacetic acid

EIF2 – Eukaryotic initiation factor 2

ER – Endoplasmic reticulum

ERK – Extracellular signal-regulated kinase

ET-1 – Endothelin 1

ETBR – Endothelin B receptor

FA – Formic acid

FBS – Fetal bovine serum

FOXO3a – Foxhead box O3a

$GABA_BR$ – GABA B receptor

GAP – GTPase activating protein

GDI – Guanine nucleotide dissociation inhibitor

GDP – Guanine nucleotide diphosphate

GEF – Guanine nucleotide exchange factor

GIRK – G protein-coupled inwardly-rectifying potassium

GO – Gene Ontology

GPCR – G protein-coupled receptor

GPR – G protein regulatory

GR – Glucocorticoid receptor

GRK – GPCR kinase

GSK3β – Glycogen synthase kinase 3β

GTP – Guanine nucleotide triphosphate

HA – Human influenza hemagglutinin

HDAC – Histone deacetylase

HEK – Human embryonic kidney

HNRNP – Heterogenous ribonuclear protein

HRP – Horseradish peroxidase

HSP90 – Heat shock protein 90

IP3 – inositol 1,4,5-triphosphate

IP3R – IP3 receptor

JNK – c-Jun N-terminal kinase

KCTD – Potassium tetramerization domain

Kir3 – Inwardly rectifying K^+ channel

LC-MS – Liquid chromatography-Mass spectrometry

LFQ – Label free quantification

M3R – Muscarinic 3 receptor

MAPK – Mitogen-activated protein kinase

MB – Mitochondrial biogenesis

MEF – Myosin enhancer factor

Mfn1 – Mitofusin 1

NDPK – Nucleoside diphosphate kinase

NES – Nuclear exclusion signal

NLS – Nuclear localization signal

NT – N-terminus

PBS – Phosphate buffered saline

PCR – polymerase chain reaction

PFDN – Prefoldin

PGC-1α – Proliferator-activated receptor-gamma coactivator-1α

PGE2 – Prostaglandin E2

PH – Pleckstrin homology

PhLP – Phosducin-like protein

PI3K – Phosphoinositide 3-kinase

PI4P – Phosphatidylinositol 4-phosphate

PIP2 – Phosphatidylinositol 4,5-bisphosphate

PIP3 – Phosphatidylinositol (3,4,5)-triphosphate

PIXα – PAK-associated guanine nucleotide exchange factor

PKD – Protein kinase D

PLC – Phospholipase C

PM – Plasma membrane

PMSF – Phenylmethlsulfonyl fluoride

PVDF – Polyvinylidene fluoride

RACK1 – Receptor activated C kinase 1

RGS – Regulator of G protein signaling

RIPA – Radioimmunoprecipitation assay

RKTG – Raf kinase trapping to the Golgi Apparatus

RNAPII – RNA polymerase II

RTK – Receptor tyrosine kinase

SAINT – Significance analysis of interactor

STAT – Signal transducer and activator of transcription

STIP 1 – Stress-induced phosphoprotein 1

TAP – Tandem affinity purification

TBS – Tris-buffered saline

TEV – Tobacco etch virus

TFA – Trifluoroacetic acid

TGN – Trans Golgi network

TIM – Triose phosphate isomerase

TMT – Tandem mass tagging

WT APEX – Wild type ascorbate peroxidase

LIST OF FIGURES

LIST OF TABLES

1 INTRODUCTION

1.1 Classical view of GPCRs and heterotrimeric G proteins

G protein-coupled receptors (GPCRs) constitute one of the largest family of proteins in the mammalian genome, with over 800 members (1, 2). Based on phylogenetic studies, this large class of receptor can be divided into five families named: Glutamate, Rhodopsin, Adhesion, Frizzled/Taste and Secretin (the so-called GRAFS classification) (3). GPCRs can be distinguished by their seven transmembrane α helices although they diverge in structure and function, allowing for transduction of extracellular signal into intracellular responses (1). These extracellular stimuli include photons, ions, peptides, and various small molecules (4). The accessible extracellular domain of these heptahelical receptors, as well as their implication in numerous physiological functions such as cell growth and differentiation, neurotransmission, metabolism, inflammatory and immune responses make them good drug targets and account for ~27% of the global therapeutic market (5, 6).

As their name implies, GPCRs couple to heterotrimeric G proteins, consisting of the α and βγ subunits which allow the cellular interpretation of external stimuli through modulation of downstream effector proteins. Under basal conditions, Gβγ associates with Gα and the receptor, and inhibits release of GDP from Gα, acting as a guanine nucleotide dissociation inhibitor (GDI) (7, 8).

The classical view of G protein regulation is through activation of the receptor, which acts as a guanine nucleotide exchange factor (GEF) to catalyze the exchange of GDP for GTP on the α subunit and dissociation of the obligate Gβγ dimer to regulate downstream signaling cascades (9). In turn, inactivation of heterotrimeric G protein activity is regulated through Gα's GTPase activity which hydrolyzes GTP to GDP and results in the reassociation of α, β and γ subunits (10).

Although there are 16 different genes encoding Gα isoforms, they can be divided into 4 distinct families: $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$ and $G\alpha_{12/13}$ (11). Coupling of the Gα subunit to GPCRs and its effector proteins is subtype specific and includes adenylyl cyclase for cAMP formation, phospholipase C for calcium release and ion channels which amplify the external signal through downstream second messengers. Various Gα subtypes are capable of coupling to the same GPCR as evidenced in Martin et al., 2018 (12), where it was found that the α_1-adrenergic receptor (α_1-AR), previously thought to couple only to Gq, is also able to couple to Gs for cAMP formation.

Similar to Gα, distinct Gβ and Gγ subtypes have also been identified and are able to form a variety of combinations regulating numerous downstream signaling cascades. There are 5 Gβ subtypes, with $G\beta_{1\text{-}4}$ showing 79 to 90% sequence similarity and $G\beta_5$ showing only ~50% similarity towards the other Gβ subunits (13). Even though Gβγ are thought to be obligate dimers, differences in $G\beta_5$ structure allows for its function without a partner Gγ, and instead dimerize with a regulator of G protein signaling (RGS) whose role is elaborated later (14). On the other hand, the 12 different Gγ subtypes show more diversity, with 26 to 76% sequence similarity between them. The available isoforms of each subunit of the heterotrimeric G protein therefore lead to a variety of combinations, yet not all combinations are possible due to limitations in tissue specific expression patterns as well as selectivity in the binding of different subunits of the heterotrimeric G protein complex (16, 17). Coupling of the heterotrimeric G protein subunits to the receptor are also subtype dependent. This was demonstrated with the endothelin B receptor (ETBR), which was coupled to $G\alpha_i$ and $G\alpha_q$ in the presence of $G\beta_1\gamma_2$ whereas only the latter Gα was able to couple to ETBR with $G\beta_5\gamma_2$ expression. (18)

1.2 GPCR-independent G protein regulation

Adding to the complexity of regulation for each component of the GPCR-G protein-effector signaling cascade is their regulation, independently of each other (19). One example of G protein activation independent o f GPCRs are activators of G protein signaling (AGS) proteins which are divided into three distinct groups defined by their mechanism in activating G proteins. Group I functions similar to GPCRs by their GEF activity, increasing exchange of GTP on Gα (20). Groups II and III influence G protein activation by binding directly to either Gα-GDP through the G protein regulatory (GPR) motif and Gβγ, respectively (21, 22). This interaction allows stabilization of the Gα-GDP complex and frees Gβγ to activate downstream signaling pathways (23). Another example is the nucleoside diphosphate kinase (NDPK) which catalyzes the transfer of a phosphate group from a triphosphate nucleotide to a diphosphate nucleotide, often at the expense of ATP pools (24). In this way, membrane bound NDPK can phosphorylate Gα-GDP and activate G proteins independently of GPCRs. Specifically, NDPK isoform B (NDPK B) interacts directly with Gβγ, bringing it into proximity with GDP bound Gα and allows for targeted phosphorylation to Gα-GTP. The NDPK B/Gβγ complex is able to increase basal cAMP synthesis in H10 cells and contributes to cardiomyocyte contractility (25, 26, 27). Furthermore, knockdown of NDPK B in mice showed reduced expression of $G\beta_1\gamma_2$, $G\alpha_i$ and $G\alpha_s$, suggesting a role for NDPK in the proper function of G proteins *in vivo* (28).

Likewise, inhibition of G protein activity can be regulated independently of GPCR deactivation. Regulators of G protein signaling (RGS) proteins can bind directly to Gα subunits and hydrolyze GTP to GDP through their GTPase activity, allowing for rapid inactivation 10-100 times faster than the intrinsic Gα GTPase activity alone (29, 30, 31). Distinct families of RGS proteins also function as more than just GTPase activating proteins (GAPs). They have been shown

to be recruited to GPCRs, act as effector proteins for downstream signaling pathways and mutations have been linked to various cancers and other diseases (32, 33, 34)

1.3 Gβγ post-translational modifications and cellular localization

Many proteins go through co- and post-translational modifications and heterotrimeric G proteins are no exception. Gα subunits undergo N-myristoylation and/or palmitoylation while Gγ subunits undergo prenylation, covalent attachment of either a farnesyl or geranylgeranyl isoprenoid lipid (35), and carboxyl methylation. Both types of Gγ post-translational modifications are on the C-terminal cysteine residue from the Cys-aa-X (CaaX) motif which is the signal that is recognized, where aa is an aliphatic amino acid and X is any amino acid (36). Although there are 2 post-translational modifications mentioned, prenylation involves a three-step process of proteolysis of the three terminal amino acids which occurs after prenylation, prior to carboxyl methylation (37). The Gβ subunit does not undergo any known post-translational modifications and instead stays tightly bound to the Gγ subunit. As demonstrated in COS-M6 simian kidney and Neuro2A cells, prenylation was not required for Gβ and Gγ subunit interaction (38). Prenylation has been most studied for the Ras and Rho family of small GTPases who undergo both palmitoylation and prenylation and involve recognition of the CaaX motif as well (37). A combination of both post-translational modifications is necessary for localization to the plasma membrane (39). Indeed, proper targeting of the heterotrimeric G protein also requires a combination of post-translational modifications through cooperative association of palmitoylated Gα and prenylated Gβγ (40, 41, 42). Furthermore, palmitoylation of $G\alpha_{s/q}$ requires binding to Gβγ and association of the heterotrimer occurs at the Golgi apparatus which allows for translocation to the plasma membrane (43, 44). Carboxyl methylation of Gγ seems to be equally important from

studies demonstrating methylation of $G\gamma_1$ facilitating membrane association and $G\alpha/\beta\gamma$ interaction (45), reinforcing cooperation between post-translationally modified G protein subunits for membrane targeting. In this way, defects in prenylation can lead to improper localization of $G\beta\gamma$. It was demonstrated in HCT 116 cells that a mutant $G\gamma_2$ with a defective prenylation site was preferentially located in the nucleus and absent at the plasma membrane (46). To this same end, treatment with a HMG-CoA reductase inhibitor, which blocks prenylation, shifted localization of a fused $G\beta_2\gamma_2$ protein from the plasma membrane to the nucleus (46).

It was previously thought that only $G\gamma_1$ is farnesylated whereas all other $G\gamma$ subtypes are geranylgeranylated (47) however, further studies report $G\gamma_{1,9,11}$ are farnesylated while the other $G\gamma$ isoforms are geranylgernanylated (48). It is not clear whether the differences in prenyl moieties confer differences in translocation kinetics. Using Chinese hamster ovary (CHO) cells overexpressing the M2 muscarinic receptor, Saini et al., 2007 (49) demonstrated rapid translocation by all three farnesylated $G\gamma$ subtypes as well as $G\gamma_{13}$, slower translocation by $G\gamma_{5,10}$ and no translocation by the remaining $G\gamma$ subunits. Nevertheless, later studies show that all $G\gamma$ isoforms are able to translocate downstream of C-X-C chemokine receptor type 4 (CXCR4) and α_2-adrenergic receptor (α_2-AR) activation, albeit at varying times ranging from 10s to a few minutes (50). The three farnesylated $G\gamma$ still showed the most rapid translocation kinetics whereas $G\gamma_{13}$, which was considered to translocate rapidly, showed slower rates. These stark differences could be due to receptor specificity, although translocation kinetics with both CXCR4 and α_2-AR showed similar trends for the different $G\gamma$ subtypes. Another study demonstrates however that carboxyl methylation may be required for stable membrane association of farnesylated $G\gamma_1$ but not geranylgeranylated $G\gamma_2$ (44). Translocation kinetics also seem to impact effector regulation. Rapidly translocating $G\gamma_9$ showed reduced levels of PIP2 hydrolysis and Kir3 channel activation

compared to $G\gamma_3$, who showed the slowest translocation rates of all Gγ isoforms and is essentially considered non-translocating (50, 51).

These post-translational modifications on Gγ are not only important for membrane-protein interactions but also for protein-protein interactions to regulate downstream effectors. Many studies have shown that prenylation of the Gγ subunit is necessary for interaction with PLCβ2, a common downstream effector, and this regulation does not need carboxyl methylation, although stimulation was lower without it (52, 53, 54).

1.4 Signaling of Gβγ near the plasma membrane

1.4.1 Regulation of Kir3 channels

Previously, the function of Gβγ was thought to simply regulate coupling of Gα to the receptor, acting as a GDI and regulating Gα activity. However, upon activation of the heterotrimeric G protein, free Gβγ can activate shared Gα effector proteins and various ion channels at the plasma membrane. The first Gβγ effector discovered was a muscarinic-gated potassium channel in the heart, later found to be members of the Kir3 (or GIRK) family of inwardly rectifying potassium channels (55, 56). Since then, many studies have supported these findings and have shown direct interaction between Gβγ and the ion channels at the N- and C-terminal ends (57). These direct interactions were further validated using co-immunoprecipitation assays (58). It was also found that overexpression of Gβγ in *Xenopus* oocytes leads to an increase in current whereas sequestration of Gβγ through varying methods led to an inhibition in ligand induced Kir3 channel activation (Reviewed in 15, 59, 60). Additional studies have also demonstrated the importance of co-expression of the Gγ subunit with Gβ which stimulated or inhibited Kir3 channel currents depending on the presence or absence, respectively, of Gγ (61). Kir3 channel currents

seem to be specific to certain Gβ (62), where each residue of a specific region on $G\beta_1$ was mutated for its $G\beta_5$ counterpart and resulted in significant reductions in channel activity.

1.4.2 Regulation of voltage-gated Ca^{2+} channels

These ion channels allow passage of Ca^{2+} across the plasma membrane upon depolarization. Gβγ is found to interact with various classes of these calcium channels, however interaction of Gβγ with Cav2 is the best understood. Voltage-dependent Ca^{2+} channels are oligomers composed of various subunits: α_1 which is the pore forming subunit (Cav2 belonging to group 2 of the pore-forming unit), Cavβ which is the cytoplasmic subunit, as well as Cavγ and Cavα_2δ. Upon membrane depolarization and rapid opening of Ca^{2+} channels, binding of Gβγ allows slowing of activation kinetics of the channels. However, depolarization pulses can impede on the inhibitory effects of Gβγ and restore rapid opening of the channels (63). Even though each Cav2 channel only contains one binding site for Gβγ, there are various binding sites, and this depends on the Gβγ dimer subtype, the state of the channel and the protein environment (64). Similar to findings in Kir3 channels, differences in Gβγ combinations produce varying degrees of inhibition and this inhibition requires association of the Gγ subunit. Expression of either Gβ or Gγ alone did not produce inhibition of channel current (65), although it was demonstrated that all 5 Gβ isoforms were able to inhibit the voltage-dependent Ca^{2+} channels depending on the Gγ subtype it was paired with (66). Efficacy of Gβγ inhibition also depends on the associated Cavβ isoform that make up the ion channel (67), as it contains a Gβγ binding consensus sequence (QXXER) found on other Gβγ effectors such as type 2 adenylyl cyclase (15).

1.4.3 Regulation of adenylyl cyclase

Adenylyl cyclase (AC) is the enzyme that converts ATP into cyclic AMP (cAMP). AC is a well-established canonical effector for Gα subunits; activation of $G\alpha_s$ activates AC for formation of cAMP whereas activation of $G\alpha_i$ inhibits AC activity. However, the various AC isoforms are differentially regulated by more than just the Gα subtypes, including G protein heterotrimers, Gβγ dimers, Ca^{2+}-calmodulin and phosphorylation (68, 69). There are nine identified AC isoforms which are either inhibited or stimulated by G proteins, the majority of them being regulated by both Gα and Gβγ, as shown in **Table 1.1** (adapted from 70).

Table 1.1 – *Adenylyl cyclase isoforms and their respective regulation by G proteins*

	AC Isoform	G Protein	
		Stimulatory	Inhibitory
Group I	AC1	$G\alpha_s$	$G\alpha_{i/o/z}$; Gβγ
	AC3	$G\alpha_s$	Gβγ
	AC8	$G\alpha_s$	Gβγ
Group II	AC2	$G\alpha_s$; Gβγ	
	AC4	$G\alpha_s$; Gβγ	
	AC7	$G\alpha_s$; Gβγ	
Group III	AC5	$G\alpha_s$; Gβγ	$G\alpha_{i/z}$
	AC6	$G\alpha_s$; Gβγ	$G\alpha_{i/z}$
Group IV	AC9	$G\alpha_s$	

For some isoforms, Gα but not Gβγ alone can regulate AC activity, yet the presence of Gβγ enhances activation of the enzyme by $G\alpha_s$ in a synergistic way (71). Regulation of AC2 has also been shown to be Gβγ subtype dependent. Studies comparing efficacy of $G\beta_1\gamma_2$ versus $G\beta_5\gamma_2$ showed that $G\beta_5$ subtype is less effective in stimulating AC2 with an EC50 of 25 nM versus 700

nM respectively (9). The adenylyl cyclase interaction sites for binding of Gβγ are not well understood, however the general structure for AC consists of 2 transmembrane domains, M1 and M2, as well as 2 cytoplasmic regions (C1 and C2) which form the catalytic domain (72). Interaction of C1 and C2 allow binding of ATP, therefore the binding interface of the catalytic domains is often where regulators bind to modulate its activity. Additionally, it has been recently appreciated that the N-terminus (NT) of AC5/6 is required for interaction and regulation by Gβγ (73). The NT of AC5 was also shown to be a binding site for the inactive form of the heterotrimeric $G\alpha_s$-Gβγ protein (69).

1.4.4 Regulation of PLC

Phospholipase C (PLC) is another well studied and common effector of activated G proteins. Upon PLC activation, it hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol (DAG) which stimulates Protein kinase C (PKC) and inositol 1,4,5-triphosphate (IP3) which translocates to the endoplasmic reticulum (ER) for binding to the IP3R and release of intracellular stores of Ca^{2+}. There are a total of 13 identified PLC isoforms classified into 6 families: PLC β, PLC γ, PLC δ, PLC ε, PLC ζ and PLC η, that all have a conserved core containing an N-terminal PH domain, EF hands, a catalytic triose phosphate isomerase (TIM) barrel containing X and Y boxes, and a C-terminal C2 domain, with additional domains that vary among the isoform (74). PLC β family is a well-known effector downstream of $G\alpha_q$ and was also found to be activated by $G\alpha_i$ coupled Gβγ. There are 4 PLC β isozymes that are differentially activated by Gβγ with more prominent effects observed with PLC β2/3, less stimulation of PLC β1 by Gβγ and PLC β4 having very minimal effects (74, 75, 76). There is also a suppra-additive activation on PLC β3 when stimulated by both $G\alpha_q$ and Gβγ together (77). In addition, PLC ε and PLC η were

found to be activated by Gβγ. In COS-7 cells, PLC ε seemed to be activated to equal levels by $G\beta_{1,2,4}$ when paired with $G\gamma_{1,2,3,14}$, less active with $G\beta_3$ and inactive with $G\beta_5$ (78). Later, observations by Zhou et al., 2005 (79) showed stimulation of PLC η2 by $G\beta_1\gamma_2$. The other 3 PLC families have not been shown to be regulated by Gβγ.

1.4.5 Regulation of PI3K

Phosphoinositide 3-kinase (PI3K) isoforms are involved in many important aspects of cell and tissue biology as well as various diseases such as cancer and diabetes. There are three PI3K classes each with distinct roles, through phosphorylation of phosphoinositides, and posses a signature structure composed of a C2 domain, a helical domain, and a catalytic kinase domain (80). Only class I PI3K subtypes will be discussed here since other classes are not relevant to Gβγ regulated signaling. PI3K class I enzymes are heterodimers made up of a catalytic (p110α, β, γ, δ) and regulatory subunit. The p110 α, β, δ subtypes all bind to the p85 regulatory subunit whereas p110 γ does not have a clear p85 binding domain, and binds instead to p101 or p87, and are therefore separated into class IA and class IB respectively (81). Class I PI3Ks are highly regulated by cell surface receptors such as receptor tyrosine kinases (RTKs) and GPCRs, most often linked to phosphorylation of PIP2 to PIP3 for downstream activation of effectors such as protein kinase B. There are currently 2 isoforms of class I PI3K demonstrated to interact with Gβγ: PI3Kβ from class IA and PI3Kγ from class IB. Gβγ interaction with PI3Kγ is much better characterised although there is no consensus on specific binding sites for Gβγ, where interaction sites on p110γ were found at both N- and C-termini (82). The need for a regulatory subunit is also debated since Gβγ was first discovered to activate PI3Kγ without binding to p85 regulatory subunit (83), although a later study demonstrated that binding to p101 increased enzyme activity by 100-fold

(84). Further studies showed that p101 requirement for Gβγ interaction depends on localization. Recruitment of Gβγ from the cytosol to the membrane requires the PI3Kγ dimer whereas membrane-bound p110γ activity was regulated by Gβγ without the need for p101 (85). Similar to PI3Kγ, PI3Kβ activity is found to be stimulated in the presence and absence of the regulatory p85 subunit (86). Gβγ was also found to bind directly to p110β and mutations in the binding site blocked Gβγ-dependent activation of PI3Kβ (87). As with many Gβγ effectors, PI3K activity is dependent on the Gβ subtype. In both PI3Kβ and PI3Kγ, $G\beta_1\gamma_2$ but not $G\beta_5\gamma_2$ stimulated the kinase activity of the enzyme (88).

1.4.6 Regulation of MAPK pathway

Mitogen-activated protein kinases (MAPK) are activated by a three-step sequential phosphorylation and protein kinase cascade to regulate cellular processes through transcription. There are currently four mammalian MAPK cascades identified and named after their primary downstream kinase components: extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK), p38 and ERK5 (reviewed in 89). Gβγ activation of MAPK was first noted for the ERK signaling cascade in COS-7 cells through a Ras-dependent mechanism and is specific to $G\alpha_i$-coupled free Gβγ (90, 91, 92). Further studies showed that regulation of JNK MAPK pathways are also activated by Gβγ through a Ras-dependent pathway whereas activation of p38 MAPK by M2 muscarinic and β-adrenergic receptors are mediated by Gβγ, with no indication of Ras dependency (93, 94).

1.4.7 Gβγ and GRKs

Specific GPCR kinases (GRKs) (reviewed in 95) were first discovered to phosphorylate activated GPCRs: GRK1 for rhodopsin receptor and GRK2 for β2-AR, leading to rapid desensitization of the receptor through uncoupling from G proteins and subsequent internalization. We now know there are 7 distinct GRKs that are grouped into 3 families: visual GRK 1/7 containing a prenylated C-tail; GRK2/3 containing a PH domain; GRK4/5/6 containing a palmitoylated C-tail. The PH domain allows GRK2/3 to bind Gβγ and only this family will be further discussed. Since GRK2/3 do not contain post-translationally modified C-tails which enable their recruitment to the PM, they instead bind free Gβγ upon receptor activation for membrane targeting. Other Gβγ downstream 2nd messengers are also able to bind PH domains such as phosphoinositide lipids like PIP2. Gβγ and PIP2 can therefore both bind GRK2, enhancing, synergistically, receptor phosphorylation and membrane recruitment of the kinase (96). There also seems to be isoform specificity between GRK and Gβγ interaction. Daaka et al., 1997 (97) demonstrated an interaction between GRK2 and $G\beta_{1,2}$ but not $G\beta_3$ while GRK3 is able to bind all three Gβ isoforms.

Additionally, GRK interaction with Gβγ plays a role in desensitization of Kir3 channels independently of their kinase activity. As mentioned previously, binding of Gβγ to Kir3 channels allows for activation of the channels. However, binding of GRKs to Gβγ sequesters Gβγ away from the channels, leading to rapid Kir3 channel inactivation, observed downstream of adenosine type 1 receptor but not muscarinic acetylcholine type 4 receptor (98).

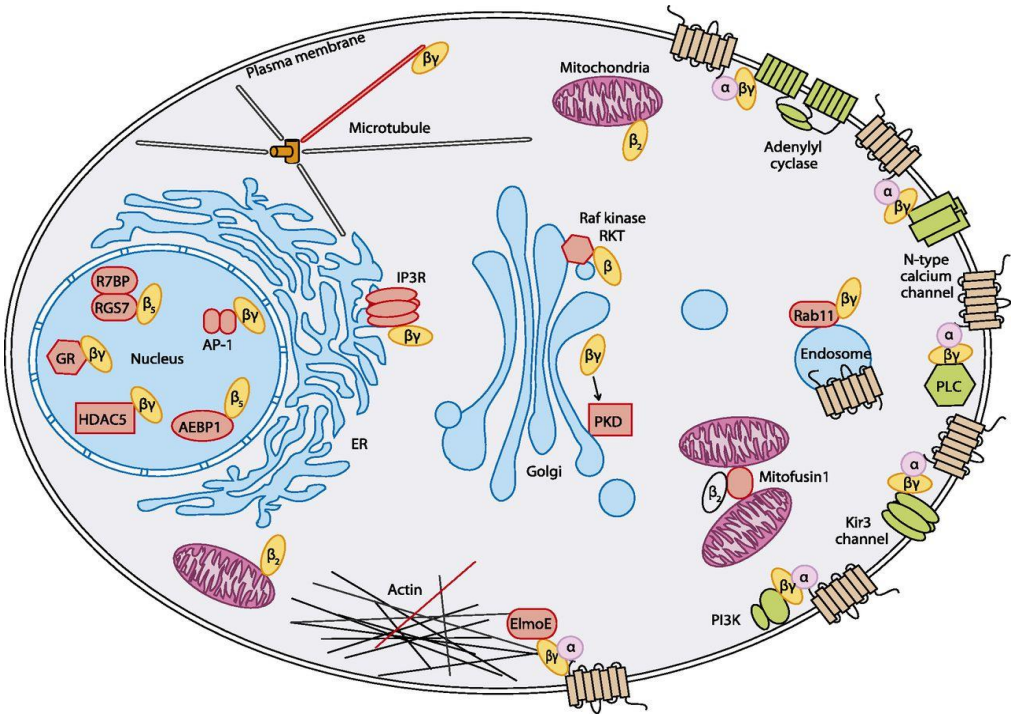


Figure 1.1 – *Gβγ signaling at the membrane and other cellular compartments*

Although no doubt incomplete, this figure provides a good overview of the myriad Gβγ effector proteins at the plasma membrane, endomembranes and various other cellular compartments. Canonical Gβγ effectors are represented in green while non canonical effectors are represented in pink. Figure taken from (14)

1.5 Gβγ signaling in other cellular compartments

1.5.1 Gβγ in the nucleus

Not only is Gβγ able to traffic from the Golgi apparatus to the plasma membrane, Gβγ is also able to translocate to various other cellular compartments upon membrane receptor activation. These compartments include the nucleus, mitochondria, Golgi and ER, where Gβγ interacts with a wide array of proteins to achieve a myriad of regulatory activities.

In the nucleus, Gβγ has been shown to directly interact with various transcription factors and coregulators to regulate gene expression. An early report of this was from a yeast two-hybrid screen which identified direct interaction between adipocyte enhancer-binding protein (AEBP1) and $G\gamma_5$ which attenuated AEBP1 transcriptional repression activity (99), although no specific Gβ was reported. Later, $G\beta_5$ was found in the nucleus and this localization was dependent on RGS proteins, specifically RGS7 (100). A mutant $G\beta_5$ preferentially dimerized to Gγ did not show localization in nuclear fractions of HEK 293 cells (101). Further screens showed the formation of a $G\beta_1\gamma_2$-histone deacetylase (HDAC5) complex, with inhibition of this interaction leading to inhibition of myosin enhancer factor (MEF) 2C activity (102). Additionally, Gβγ is able to interact with Fos proteins which dimerize with Jun proteins to form activator protein-1 (AP-1) to inhibit transcriptional activity through the recruitment of HDACs (103)

Gβγ is also able to translocate to the nucleus with glucocorticoid receptor (GR) to suppress its transcriptional activity with the migration direction dependent on receptor stimulation. Stimulation with somatostatin or dexamethasone leads to comigration of Gβγ-GR complexes to the plasma membrane or nucleus respectively (46).

The family of signal transducer and activator of transcription 3(STAT3) proteins are activated through phosphorylation, regulated by many different Gβγ dimer pairs, although no direct interaction was reported (104). However, STAT5B was shown to co-immunoprecipitate with Gβγ and activated c-Src kinase, facilitating phosphorylation of STAT5B for transcriptional regulation (105).

Other studies show that $G\beta_2$ is also involved in regulation of MEF2 transcriptional activity which was dependent on activation of the angiotensin II type 1 receptor (AT1R). Nuclear $G\beta_2$ levels increased with specific stimulation of AT1R. A $G\beta_2$-binding sequence was found on

transcription factors, which account for binding to approximately 2% of genes and demonstrate a wide-range of effects for $G\beta_2$ (106). ChIP-on-chip experiments found that $G\beta_1$ is able to localize to promoters of over 700 genes (107), and found to regulate a variety of genes, with knockdown of $G\beta_1$ followed by AngII stimulation showing trends for increase in expression of 37 genes (108). Further studies examined Gβγ interaction with a protein complex ubiquitously involved in transcription, specifically RNA polymerase II (RNAPII). The authors demonstrated that Gβγ interacts directly with Rpb1, the largest subunit of RNAPII, and the interaction increased with Ang II stimulation (108).

1.5.2 Gβγ in the Golgi Apparatus

Gβγ plays an important role in the organization of the Golgi apparatus. Early studies demonstrated complete fragmentation of Golgi membranes with treatment of purified Gβγ (109), and this fragmentation was regulated through protein kinase D (PKD) which directly interacts with Gβγ to regulate Golgi membranes and protein secretion (110). Fragmentation was shown to be receptor-induced and dependent on Gβγ translocation, demonstrated by inhibition of fragmentation with a non-translocating mutant Gγ (111).The vesiculation of the Trans Golgi network (TGN) was later demonstrated to involve DAG and PKCη. PKD is first recruited to the TGN by binding to DAG before activation by specific Gβγ dimers via PKCη to generate transport carriers from the TGN (112). One protein that sequesters Gβγ to the Golgi is Raf kinase trapping to the Golgi apparatus (RKTG). RKTG was found to bind directly to Gβγ, tethering it to the Golgi apparatus and impeding Gβγ effector binding, decreasing recruitment of effectors from the cytosol to the plasma membrane, leading to attenuated effects of Gβγ (113).

Gβγ at the Golgi plays many other important roles and understanding these pathways can lead to disease prevention. Endothelin-1 (ET-1) stimulated cardiomyocyte hypertrophy depends

on Golgi phosphatidylinositol 4-phosphate (PI4P) hydrolysis by PLCε at the perinuclear Golgi region. Blocking Gβγ inhibited ET-1 dependent PI4P hydrolysis, demonstrating Gβγ regulation of a pathway required for hypertrophy and a potential avenue for preventing heart failure (114).

1.5.3 Gβγ in mitochondria

Mitochondria are highly dynamic organelles which maintain an equilibrium between fusion and fission, important in changing the number and size of mitochondria depending on cellular processes (115). A study demonstrated direct interaction of mitofusin 1 (mfn1) and $G\beta_2$ specifically enriched at the outer membrane playing a crucial role in mitochondrial fusion, with depletion of $G\beta_2$ resulting in fragmentation, which was rescued by exogenous $G\beta_2$ (116). Further reports show heterotrimeric $G\alpha_q/\beta\gamma$ proteins were found at the outer mitochondrial membrane while dissociated Gα was found at the inner membrane, altering regulation of mfn1 and dynamin-related GTPases (Drp1) to achieve fusion/fission equilibrium (117).

Additional evidence demonstrates Gβγ effects at the mitochondria also include induction of mitochondrial biogenesis (MB). Agonist activation of the $G_{i/o}$-coupled 5-hydroxytryptamine 1F receptor ($5\text{-}HT_{1F}$) induces Gβγ-dependent activation of Akt signaling. This leads to dual effects for activation of MB and suppression of inhibitory pathways through regulation of transcription factors peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) and foxhead box O3a (FOXO3a) respectively (118).

Interestingly, a proteomic approach to identify mitochondrial proteins in rat cerebral microvessels revealed 122 proteins in Gβγ signaling being differentially regulated between male and female rats, with the former expressing higher mitochondrial-destructive proteins and the latter expressing more proteins involved in energy production (119).

1.5.4 Gβγ and the cytoskeleton

Chemotaxis involves polarization of cells to determine the direction of cell migration dependent on chemoattractant gradient and can be divided into directionality and motility. Regulation is through binding of chemoattractants to specific G_i coupled GPCRs and activating Gβγ to interact with effectors. However, there is also evidence demonstrating free Gβγ released through binding to 12155, a small molecule, being able to drive chemotaxis independently of receptor activation (120). Polarization of the cell allows for chemotaxis to occur and this is driven by F actin polymerization at the leading edge in accordance with the chemoattractant gradient (reviewed in 121). This begins with a polarized distribution of PIP3, generated by Gβγ activation of PI3Kγ which phosphorylates PIP2 and regulates orientation of cell polarity by localizing active Cdc42 and in turn, F actin. Activation of Cdc42 is through PAK-associated guanine nucleotide exchange factor (PIXα), constitutively associated with PAK1. This dimer is recruited to the membrane and in proximity to Cdc42 by direct interaction of PAK1 with Gβγ. Activated Cdc42 in turn activates its effectors such as PAK1. In this way, PAK1 functions as a scaffold protein for Cdc42 activation while also being a downstream effector (122). Although PI3Kγ and PIXα are not required for F actin formation, F actin formation in PI3Kγ or PIXα-null cells was not in accordance to the chemoattractant gradient, showing their importance in directionality of F actin polymerization (121).

PIXα's GEF activity is also able to activate Rac, involved in both directionality and motility, with higher active Rac concentrations near the front of cells (123). Alternatively, Rac can be activated by the specific GEF P-Rex1. For this latter activation to occur, P-Rex1 is recruited synergistically by Gβγ and PI3K from the cytosol to the PM where its basal activity is higher. P-

Rex1 recruitment also allows for further activation by PIP3 and Gβγ (124). However, chemotaxis regulation differs between chemokine receptors and does not necessarily involve Gβγ. A role for Gβγ signaling was observed in CXCL11-induced but not CCL3-induced cell migration (125).

Negative regulation of chemotaxis can be achieved through sequestration of Gβγ by direct interaction with the ubiquitin-like protein PLIC-1, although this effect was specific to G_i signaling (126). Competitive binding of Gβγ to receptor activated C kinase 1 (RACK1) is also involved in inhibition of chemotaxis through inhibited stimulation of PI3Kγ by Gβγ (127).

In contrast to cell migration is regulation of cell adhesion which can be distinguished by cell-cell adhesions and cell-matrix adhesions. Rap1a is important in the cell for regulation of integrin activation through conformation changes of the extracellular regions for cell-matrix adhesion and has been shown to be activated downstream of GPCRs. Further evidence shows activated Rap1a bound to its effector Radil recruits Gβγ for formation of a protein complex able to promote cell-matrix adhesion by inside-out signaling for integrin activation (128).

Although assays were done in zebrafish, Gβγ was shown to be involved in regulation of cell-cell adhesion during gastrulation movements. Activation of prostaglandin E receptors by prostaglandin E2 (PGE2) leads to stabilization of the transcriptional repressor Snai1a, limiting E-cadherin transcription and protein synthesis for cell-cell adhesion. Pathway regulation was through PGE2 potentiation of Gβγ interaction with glycogen synthase kinase 3β (GSK3β), which inhibits phosphorylation of Snai1a for proteasomal degradation (129).

G proteins have been shown to mediate effects through tubulin and microtubule regulation however, the different G protein subunits have opposing effects (reviewed in 130). Gα stimulates tubulin's GTPase activity which destabilizes the microtubule while Gβγ promotes microtubule

stability and interacts directly with tubulin in the cytosol. This dynamic regulation enables modulation of cell growth and differentiation through alteration of cytoskeletal elements.

1.6 Thesis objectives and rationale

Gβγ effects are modulated through interactions with numerous downstream effectors throughout the various cellular compartments. Studying these effectors poses a challenge since there are a myriad of proteins with differences in interaction strength and/or stability. High throughput methods such as affinity purification have been used to study the Gβ and Gγ interactome, however they can only identify strong and stable interactions. Other methods to study Gβγ and its effectors often focus on one pathway at a time, requiring a large array of assays. Previous work from our lab has used tandem affinity purification (TAP) and Flag-tagging/immunoprecipitation to study the interactome of specific $G\beta_x\gamma_y$ combinations under basal and stimulated conditions which identified various nuclear proteins. Further studies looking into Gβ involvement in transcription also led to the identification of RNA polymerase II as an interactor, identified in TAP screens, and validated by co-immunoprecipitation.

Therefore, the objective of this project is to characterize weak or transient Gβγ interactors using a proximity-dependent labeling technique coupled with mass spectrometry. We hypothesize that Gβγ signaling will be distinct in different cellular organelles. This project contributes to our long-term goals of better understanding Gβγ binding partners in various cellular compartments, especially in the nucleus. To achieve this, either APEX2 tagged Gβ1 or Gγ5 constructs were used in HEK 293F cells to label nearby proteins with biotin. Additional constructs with nuclear exclusion and localization signals were tagged with APEX2 to provide a spatial reference and account for potential non-specific bystander proteins. Three independent experiments were done, however only the final two were using the NES and NLS-tagged constructs. The first experiment used only a Flag-APEX2 construct but further validation using immunofluorescence showed

improper biotinylation all throughout the cell, instead of the cytosol only, as shown in expression immunofluorescence assays.

The first aim is to validate proper function and expression of the tagged constructs using microscopy and western blot assays since APEX2 is a large protein relative to the different G protein subunits. We needed confirmation of proper function of both the G protein subunits and the APEX2 enzyme. APEX2 is an ascorbate peroxidase which, with the addition of hydrogen peroxide, is able to catalyze biotin-phenol into biotin phenoxy radicals. These radicals bind covalently to nearby proteins on tyrosine residues within a 20 nm radius. Biotin-tagged proteins can then be purified for protein identification using mass spectrometry.

The second aim is to use the mass spectrometry data to identify Gβγ binding partners previously identified from tandem-affinity purification and the literature to assess the spatiotemporal information provided by APEX2 proximity-dependent labeling. Further experiments aim to compare compartment specific interactions through cellular fractionation as well as differences in the interactome under basal and stimulated conditions.

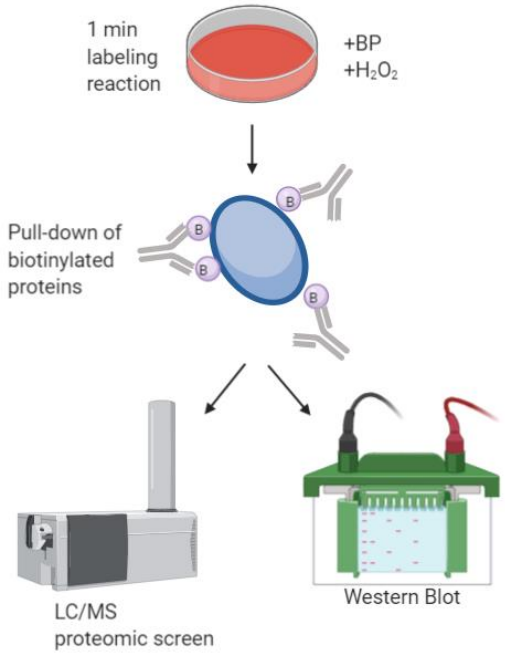


Figure 1.2 – *Overview of proximity-dependent labeling technique*

Cells expressing APEX2 constructs are incubated with biotin-phenol (BP) and hydrogen peroxide for a 1-minute reaction to label nearby proteins with biotin. Biotinylated proteins are purified using streptavidin beads for downstream analysis using LC-MS or western blot.

2 MATERIALS AND METHODS

2.1 Reagents

High glucose Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), trypsin (0.25% EDTA), penicillin/streptomycin and sodium dodecyl sulfate (SDS) were purchased from Wisent (St-Bruno, QC). Anti-Flag M2 antibody, anti-rabbit conjugated horseradish peroxidase (HRP), anti-mouse conjugated with HRP, Flag-M2 magnetic beads, 3X Flag peptide, 2-β-mercaptoethanol, potassium chloride, potassium phosphate, sodium phosphate, dimethyl sulfoxide (DMSO), tergitol/Igepal CA-630 (70% NP-40), sodium deoxycholate, Tween-20, bisbenzimide H 33342 trihydrochloride (Hoechst), protease inhibitor cocktail, paraformaldehyde (PFA), hydrogen peroxide (H_2O_2), sodium ascorbate, iodoacetamide, (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), phenylmethylsulfonyl fluoride (PMSF), urea, polyornithine, agar, paraformaldehyde and hydrogen peroxide were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Bovine serum albumin (BSA), Tris (hydroxymethyl) aminomethane (Tris), glycine, ethylene diamine tetraacetic acid (EDTA), ethylene glycol bis (2-aminoethyl) tetra-acetic acid (EGTA), sodium azide (Na_2N_3), ampicillin, G418 sulfate and skim milk powder were purchased from BioShop Canada (Burlington, ON). Lipofectamine 2000, Alexa Fluor 647 anti-mouse, streptavidin Alexa Fluor 488, anti-β-tubulin and anti-Streptavidin conjugated with HRP were purchased from Invitrogen (Carlsbad, CA, USA). Biorad protein assay dye reagent (Bradford), Acrylamide 40%, polyvinylidene difluoride (PVDF) membrane and Sypro Ruby Stain were purchased from BioRad (Hercules, CA, USA). Sodium Chloride (NaCl), ammonium bicarbonate and methanol were purchased from Fisher Scientific (Hampton, NH, USA). BLUelf prestained protein ladder was purchased from FroggaBio (North York, ON). Amersham ECL Select Western Blotting Detection Reagent and Streptavidin Sepharose High performance beads were purchased

from GE Healthcare (Chicago, IL, USA). Biotinyl tyramide (Biotin-phenol) was purchased from Toronto Research Company (TRC) (Toronto, ON). NheI and BamHI restriction enzymes and CutSmart were purchased from New England BioLabs (NEB) (Ipswich, MA, USA). The Qiagen Gel Extraction kit and Qiagen Miniprep kit were purchased from Qiagen (Hilden, Germany). T4 DNA ligase enzyme and T4 DNA ligase buffer were purchased from Promega (Madison, WI, USA). NeutrAvidin tetramethylrhodamine conjugate (TritC) and Phusion High-Fidelity Taq DNA polymerase enzyme were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Yeast extract was purchased from EMD Chemicals Inc (Darmstadt, Germany). RedSafe nucleic acid staining solution was purchased from Intron Biotechnology (South Korea). Anti-$G\alpha_{q/11}$ was purchased from Santa Cruz (Dallas, TX, USA).

2.2 Cell culture

HEK 293F cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% (v/v) Fetal Bovine Serum (FBS) and 1% (v/v) penicillin/streptomycin and incubated at 37°C with 5% CO_2.

2.2.1 Transient transfection

HEK 293F cells were plated the previous day to allow for cells to attach and reach 40% confluency. Plasmid DNA was transfected into cells using a 1 μg:2 μL DNA to Lipofectamine 2000 ratio following manufacturer's protocol. Dilutions were done in pure DMEM, without supplementation. Transfection media was removed 5 hours later and replaced with 5% FBS and 1% antibiotic supplemented DMEM. Cells were incubated for 48-72 hours to reach 90% confluency.

2.2.2 Stable cell line generation

Cells were transfected as mentioned above but with a few changes. 48 hours after transfection, neomycin (G418) is added to cells at a concentration of 1 mg/mL to select for cells that have expressed the plasmid. Cells are grown for 2 weeks, with continuous addition of G418. Stable cells are maintained with 500 μg/mL of G418.

2.2.3 Monoclonal cell line generation

Cells stably expressing the plasmid of interest are diluted and plated in a 96-well plate to achieve 1 cell per well in 100 μL of supplemented DMEM. Cells are grown until a colony has formed and can be passed into a 6-well plate and further scaled up for experiments. Continuous addition of G418 is added to maintain stable monoclonal cells.

2.3 Cloning

Nuclear exclusion signal (NES) and nuclear localization signal (NLS) were incorporated into pcDNA3.1(+) vector expressing Flag-APEX2 using PCR. Reactions were set up in a total of 50 μL containing 20 ng of DNA template, 1x high fidelity buffer, 0.5 μL of Phusion, 200 μM dNTPs and 1μM primer pair (**Table 2.1**). PCR was initiated with denaturation at 98°C for 3 minutes followed by 35 cycles of amplification with each cycle consisting of denaturation at 98°C for 10 seconds, annealing at 68°C for 20 seconds and elongation at 72°C for 20 seconds. Final elongation was done at 72°C for 5 minutes. PCR products were run on 1% agarose gel in 1x TAE (40 mM Tris; 20 mM acetic acid; 1 mM EDTA) at 120V for 35 minutes. Bands of interest were cut from gel and DNA was extracted using QIAGEN gel extraction kit. PCR product and DNA template were digested separately for 2 hours at 37°C in a total volume of 30 μL containing 1 μL each of NheI and BamHI restriction enzyme, 10% Cutsmart buffer and either 5 μg of DNA template or 15

μL of PCR product. Digested products were run on 1% agarose gel and bands were extracted from gel as previously mentioned. Digested PCR product and DNA template were ligated in a total volume of 10 μL containing 3 μL insert, 0.5 μL vector, 1 μL ligase buffer and 0.5 μL ligase for 3 hours at room temperature and then overnight at 4°C. Ligated vectors were transformed into competent DH5α *E.coli* cells and incubated overnight at 37°C on ampicillin agar plates. Colonies were selected and grown individually at 37°C for 5 hours with shaking in 4 mL of TB enriched with ampicillin. Crude mini-preps were done for each colony and then run on 1% agarose gel to confirm insertion. QIAGEN Miniprep Kit was used for positive clones and was sent for sequencing to further validate insertion.

Table 2.1 – *Primer pairs used for cloning*

Flag-APEX2-NES	Forward	caagctggctagcgccaccatggat
	Reverse	tagtggatccttagtccagggtcaggcgctccaggggaggcagctgcagggcatcagcaaaccca
Flag-APEX2-NLS	Forward	caagctggctagcgccaccatggat
	Reverse	tagtggatccttagtctgcgtcctctacctttcgtttctttttggctgcagggcatcagcaaac

2.4 Western blotting

Previously prepared protein sample were loaded with a molecular weight ladder onto a polyacrylamide gel in running buffer. Gel was run for one and a half hour at 120V before transfer on PVDF membrane which was activated for 30-45 seconds in methanol prior to transfer and left in transfer buffer to not dry out. Transfer was done at 100V for 1 hour in transfer buffer. Membranes were blocked for non-specific proteins in blocking buffer (5% milk in TBS-T) for 1 hour at room temperature (RT). After blocking, membranes were incubated at 4°C overnight on a rotator in blocking buffer containing primary antibody (anti-Flag M2 1:10,000; anti-$G\alpha_{q/11}$ 1:2000; anti-β-tubulin 1:3000). The next day, membranes were washed in 1x TBS-T three times for 10

minutes each before incubation at room temperature for 1 hour in blocking buffer and secondary antibody (anti-mouse 1:20,000; anti-rabbit 1:10,000). They were then washed in 1x TBS-T three times for 10 minutes each wash before imaging. Membranes that are being blotted against Streptavidin-HRP were blocked in 3% BSA in TBS-T at 4°C overnight on a rotator. The next day, membranes were incubated at RT for 1 hour in anti-streptavidin-HRP 1:20,000 in 3% BSA in TBS-T. They were washed 4 times in 1x TBS-T for 10 minutes each wash before imaging.

2.5 APEX2 labeling

Transfected or stable cells are grown until 90% confluent. Supplemented DMEM is removed and replaced with pure DMEM containing 500 μM biotin. Cells are treated for 30 to 60 minutes before removing from incubator. Then, 1 mM of hydrogen peroxide is added to cells for exactly 60 seconds and cells are placed on ice. Cells are washed three times with ice cold quenching buffer (5 mM Trolox; 10 mM Sodium Ascorbate; 10 mM Na_2N_3 in 1X PBS) before continuing with cell lysis with 1x RIPA (1% NP-40; 50 mM Tris-HCl pH 7.4; 150 mM NaCl; 1 mM EDTA; 1 mM EGTA; 0.1% SDS; 0.5% sodium deoxycholate; 1x protease inhibitor cocktail) buffer and Bradford quantification.

2.6 Immunoprecipitation Assays

2.6.1 Flag co-immunoprecipitation

Previously transfected or stable HEK 293F cells are grown on 10 cm dishes until 90% confluent and washed twice with cold 1x PBS (137 mM NaCl; 2.7 mM KCl; 10 mM Na_2HPO_4; 1.8 mM KH_2PO_4). Cells are lysed with 1x RIPA and scraped off. Cell lysates are then frozen, thawed on ice and lysed using the Misonix Sonicator 3000 at 4°C with 2x10 second bursts, inverting tubes twice between each burst. Insoluble fraction is pelleted by centrifugation at 13,200 rpm for 15

minutes at 4°C, supernatant is collected and then quantified by Bradford assay. Total lysate samples are prepared for western blotting with 40 µg of lysate and 1x Laemmli buffer, denatured for 15 minutes at 65°C. For immunoprecipitation, 500 µg of lysate is incubated overnight on a rotator at 4°C with pre-washed Flag M2 magnetic beads. The following day, supernatant is carefully removed, and magnetic beads are washed three times with 1x RIPA buffer. Flag-tagged proteins are eluted from beads using Flag peptide diluted in 1x TBS and incubated for 3 hours at 4°C on a rotator. Supernatant is collected and prepared for western blotting with addition of 1x Laemmli buffer and denaturation as mentioned above.

2.6.2 Streptavidin immunoprecipitation

After APEX2 labeling and Bradford quantification, cell lysate is incubated for at least 1 hour with pre-washed streptavidin agarose beads at 4°C on a rotator. The beads are washed with 1x RIPA buffer twice, followed by one wash each of 1 M KCl, 0.1 M $NaCO_3$, 2 M urea and two washes of 1x RIPA buffer. Between each wash, beads are pelleted at 2000 rpm for 2 minutes at 4°C to remove supernatant. For western blotting, 70 µL of 4x Laemmli buffer is added to beads and denatured at 95°C for 5 minutes. Samples for mass spectrometry are further washed twice with 1x PBS before continuing with mass spectrometry sample preparation.

2.7 Immunofluorescence

In triplicates, 12 000 cells per well were plated in polyornithine-coated 96-well optical-bottom plates for imaging with the microscope. Cells were grown for 24 hours before continuing with labeling assay following the APEX2 labeling protocol above. After washing with cold quenching buffer, cells were fixed with 2% PFA for 15 minutes at RT. PFA was aspirated and cold methanol was added to permeabilize cells for 10 minutes at 4°C. Cells were washed twice with 1X PBS and

blocked for 1 hour at RT in 3% BSA. Cells were then incubated with anti-Flag M2 (1:1000) overnight at 4°C. The next day, cells were washed with 1X PBS and incubated in secondary antibody using both Alexa Fluor 647 anti-mouse (1:1000) and either streptavidin Alexa Fluor 488 (1:1000) or NeutrAvidin TritC (1:200) for 1 hour at RT, protected from light, followed by 10 minutes of incubation with Hoechst (1: 10,000) nuclear stain at RT, protected from light. Cells were washed twice and stored in 1X PBS. The Operetta High Content Imaging system was used for imaging cells using the 20WD objective (Perkin Elmer).

2.8 Sypro ruby stain

Streptavidin immunoprecipitated samples were loaded onto a polyacrylamide gel in running buffer similar to western blot. The polyacrylamide gel was incubated in sypro ruby stain overnight at room temperature shaking. The following day, the gel was washed twice in 50 mL of 10% methanol, 7% acetic acid for 30 minutes at room temperature on a shaker before imaging using the GelDoc (BioRad).

2.9 Mass spectrometry sample preparation

After streptavidin immunoprecipitation, 10 mM DTT in 20 mM ammonium bicarbonate is added to washed beads for complete immersion in an Eppendorf tube and incubated for 30 minutes at 60°C. Samples are cooled before adding an equal volume of 15 mM iodoacetamide in 20 mM ammonium bicarbonate and incubated in the dark for 1 hour at room temperature. Then, DTT is spiked-in for a final concentration of 15mM to quench iodoacetamide and incubated for 10 minutes. Trypsin in 10% acetonitrile (ACN) is added at a concentration of 100 ng/1 mg of protein for complete immersion of beads and incubated overnight at 37°C. The following day, trypsin digestion is stopped by acidification with addition of trifluoroacetic acid (TFA) to a final

concentration of 1%. Supernatant is transferred to a new Eppendorf tube and beads are resuspended in 60% ACN-0.1% formic acid (FA) and incubate at room temperature for 5 minutes. Beads are spun down and supernatant is pooled with the previous one. This is repeated once more using water. Dry pooled supernatant using a SpeedVac vacuum and then resuspend sample in 0.5% FA-5% ACN. Centrifuge sample at 13,000 rpm for 10 minutes at RT. Collect supernatant and sonicate in water bath sonicator for 5 minutes. Continue with clean up using the Pierce C18 Spin Columns from Thermo Scientific (Rockford, IL, USA) following manufacture's protocol. Final elution was done using 50% ACN-0.1% FA.

2.10 Processing of mass spectrometry data

Eluted peptide samples were given to Andrew Bayne from Dr. Jean-François Trempe's laboratory and analyzed by LC-MS using electrospray ionization quadruple time-of-flight (ESI-QTOF) by Bruker (Billerica, MA, USA). The resulting spectras were searched against a FASTA file containing the human NCBI sequences through the MaxQuant computational proteomics platform (Max Planck Institute of Biochemistry) to identify peptides and proteins. Proteins were quantified using label-free quantification to obtain LFQ intensities through MaxQuant.

Endogenously biotinylated proteins, keratin proteins and any proteins found in the untransfected sample of each run were removed before continuing analysis. Additionally, proteins with a peptide count below 2 were removed. Only proteins identified in at least 2 out of 3 experiments were analyzed and discussed. Proteins were deemed high confidence interactors when LFQ intensity in bait sample was $\geq$ 2-fold change of LFQ intensity from control samples and deemed low confidence interactors when fold change < 2 compared to control samples from the same run. One experiment had Flag-APEX2 as a control while the other 2 runs used Flag-APEX2-NES and Flag-

APEX2-NLS as control. Panther online software was used to determine molecular function and protein class (131, 132). GOnet was used to connect proteins to various molecular functions (133). BioVenn online software was used to generate venn diagrams comparing identified proteins between different methods (155) and Pymol was used to generate a model for APEX fused Gβ1 (160).

3 RESULTS

The following chapter describes validation, optimization, and results from APEX2 proximity dependent labeling technique to identify Gβγ interactors. All lab work was done by me except for the experimental design of APEX2-fused constructs and biotin labeling assay. Additionally, all mass spectrometry runs were done by Andrew Bayne from Jean-François Trempe's lab.

3.1 Flag- and APEX2-tagged proteins are expressed and properly localized

Plasmid constructs containing the APEX2 enzyme with Gβ1 and Gγ5 tagged with Flag were previously made by Darlaine Pétrin. An extra plasmid was also made with Flag tag and APEX2 enzyme alone which would be used as a cytoplasmic spatial reference. The next step was validation of proper expression and function in HEK 293F cells. To validate for expression of the plasmids, western blots were done using an antibody against the Flag tag (**Figure 3.1**). However, western blots only show presence or absence of our proteins of interest without regards to proper localization. Therefore, we proceeded to using immunofluorescence which showed protein expression in the cytoplasm and nucleus, co-stained with Hoechst nuclear stain to detect individual cell expression (**Figure 3.2**). Stable cells expressing the protein of interest were used and showed varying levels of expression for different biological replicates so monoclonal stable cell lines were generated to account for this discrepancy (data not shown).

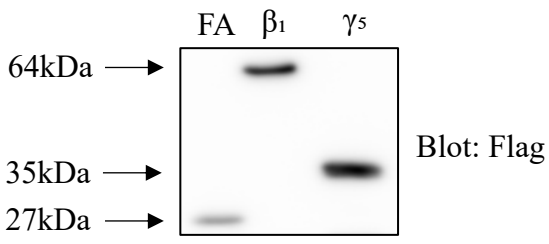


Figure 3.1 – *Validation of expression of APEX2-fused constructs*

Flag blot shows expression of Flag and APEX2-tagged constructs expressed in HEK 293F cells. This image is representative of 3 independent experiments.

FA: Flag-APEX2; β1: Flag-APEX2-Gβ1; γ5: Flag-APEX2-Gγ5

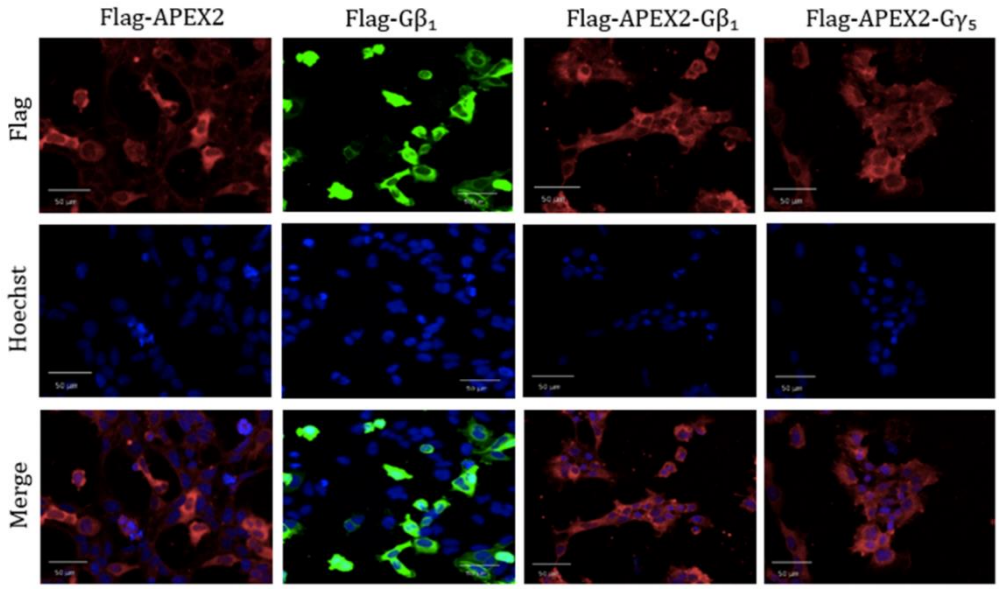


Figure 3.2 – *Validation of localization of APEX2-fused constructs*

Immunofluorescence images show localization of APEX2-fused Gβ1 and Gγ5 is similar to WT Flag-tagged Gβ1 (images by Iulia Pirvulescu). Flag-APEX2 is found exclusively in the cytosol and is used as a cytosolic spatial reference to identify bystander proteins. Scale bars represent 50 μm. These images are representative of 4 independent experiments.

3.2 Tagged proteins are functional

3.2.1 G proteins are able to interact with a known interactor

Since the APEX2 enzyme is relatively large (27 kDa) compared to Gγ5 (7 kDa) and the larger Gβ1 (37 kDa) subunits, we wanted to verify that APEX2 does not hinder the interaction of the G protein subunits with its effectors. Since inactivated G proteins are found in their

heterotrimeric form, $G\alpha_q$ was transfected into HEK 293F cells stably expressing the protein of interest and co-immunoprecipitated using magnetic Flag beads against the Flag-tagged proteins. As expected, pull-down of Gβ1 and Gγ5 but not Flag-tagged APEX2 alone co-immunoprecipitated $G\alpha_q$ (**Figure 3.3**).

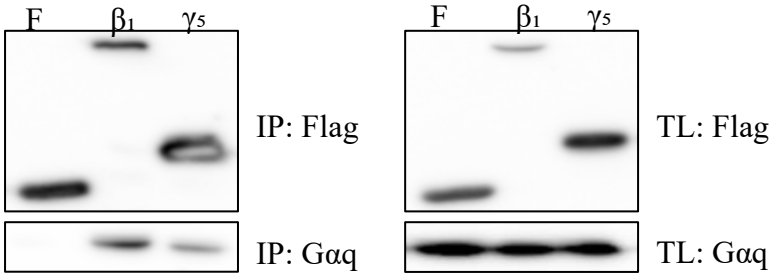


Figure 3.3 – *APEX2 tagged Gβ1 and Gγ5 subunits interact with $G\alpha_q$*

Cell lysates are immunoprecipitated with magnetic Flag beads which co-immunoprecipitated $G\alpha_q$ demonstrating interaction between G protein subunits and proper function of constructs. This image is representative of 3 independent experiments.

3.2.2 APEX2 enzyme from tagged constructs is functional

Next, we validated the function of the APEX2 enzyme, demonstrated through detection of streaks of biotinylated proteins using anti-streptavidin antibody from western blot assays (**Figure 3.4**). APEX2 is an ascorbate peroxidase that produces, with the addition of hydrogen peroxide, short lived biotin-phenoxy radicals which bind covalently to tyrosine residues of proteins within a 20nm radius (134). Due to the nature of this assay, any proteins that come within the labeling radius of the bait protein (APEX2-tagged protein) will be biotinylated, even if it does not directly interact with it. For this reason, the spatial references, used as negative controls, play an important role since proteins are dynamic and may be biotin-labeled while translocating inside the cell. Using

spatial references allows us to determine proteins that were simply in the vicinity versus proteins that are enriched in pulldowns because of proximity to the bait proteins.

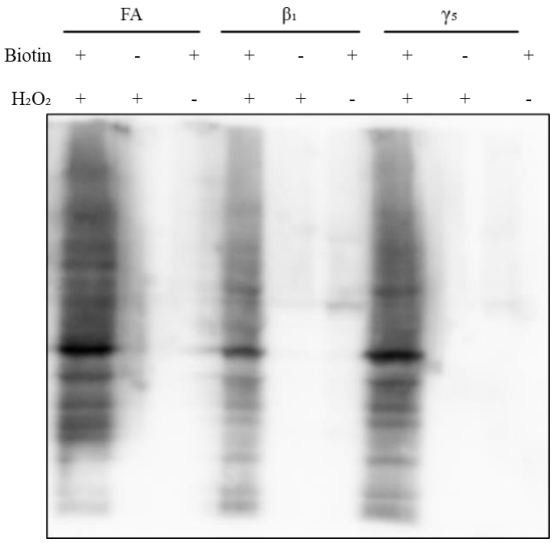


Figure 3.4 – *Proteins are biotinylated with the addition of both biotin-phenol and hydrogen peroxide*

The streptavidin blot shows streaks of biotinylated proteins only when both biotin-phenol and hydrogen peroxide are added to HEK 293F cells expressing APEX2 constructs. This image is representative of 3 independent experiments.

Biotinylation assays are also done prior to immunofluorescence to observe proper biotinylation in the cytoplasm and the nucleus. Microscopy images showed that the cytosolic spatial reference (Flag-APEX2), although expressed solely in the cytoplasm, was biotinylating mostly nuclear proteins (**Figure 3.5**).

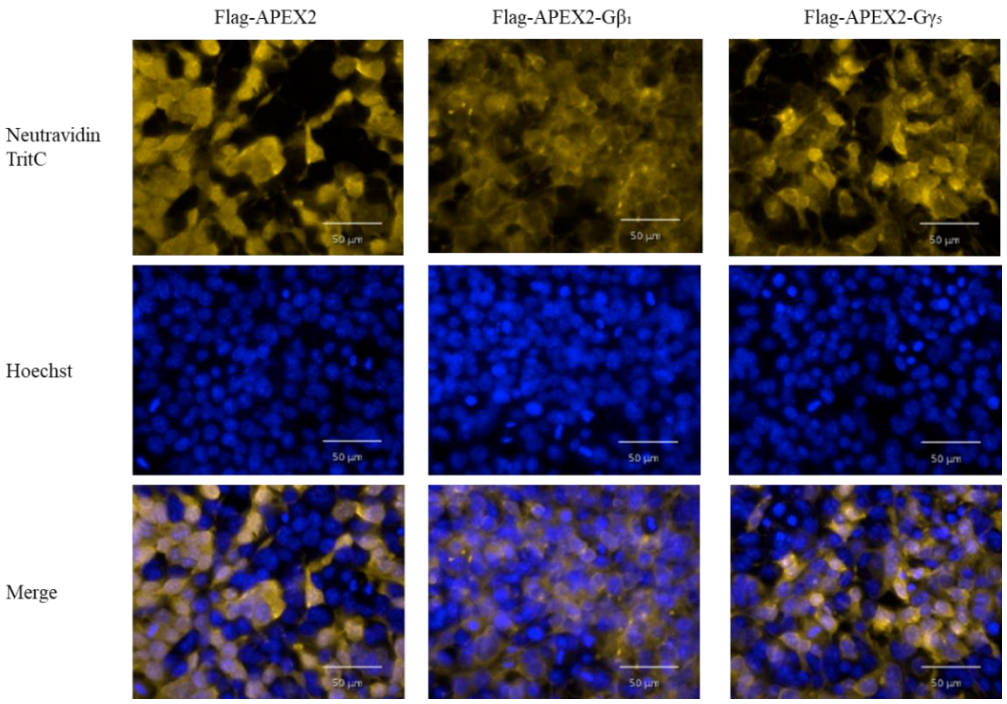


Figure 3.5 – *Validation of biotinylation patterns*

Immunofluorescence images show biotinylation by APEX2-fused Gβ and Gγ constructs is everywhere in the cells due to their localization. However, Flag-APEX2 is biotinylating most prominently in the nucleus but should be biotinylating in the cytoplasm for use as a spatial reference. Scale bars represent 50 µm. This image is representative of 5 independent experiments.

For this reason, PCR was used to add a nuclear localization signal (NLS) and a nuclear exclusion signal (NES) to the C-terminus of the Flag-APEX2 construct. The two new plasmids were validated as previously mentioned and showed proper biotinylation in the cytoplasm and nucleus for the NES and NLS constructs respectively (**Figure 3.6**).

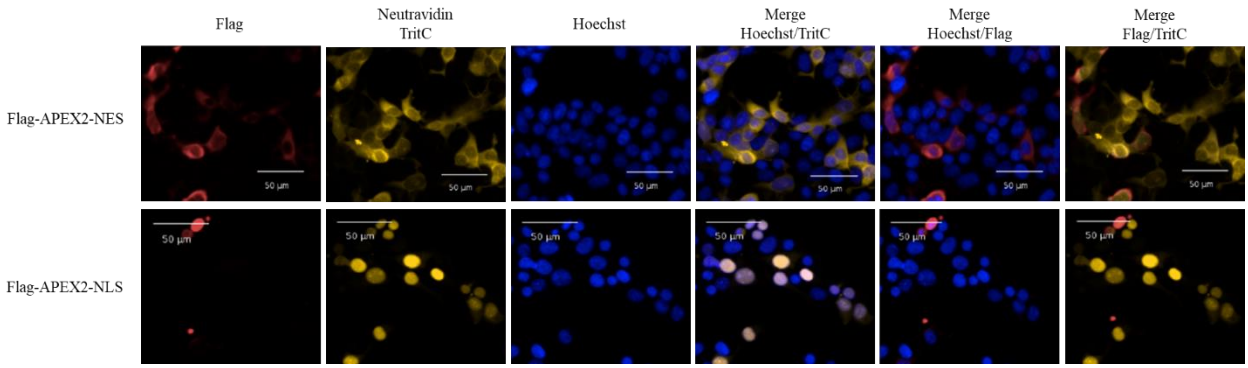


Figure 3.6 – *Immunofluorescence of APEX2 plasmids with NES and NLS tag*

Images show proper localization and biotinylation patterns of Flag-APEX2 plasmids when either NES or NLS is added to the C-terminus. With addition of a nuclear exclusion signal, localization and biotinylation is in the cytoplasm while a nuclear localization signal results in localization and biotinylation in the nucleus. Scale bars represent 50 µm. These images are representative of 3 independent experiments.

Red: Flag; Yellow: Neutravidin-TritC (biotin); Blue: Hoechst

3.3 Biotinylated proteins are purified using streptavidin beads

After APEX2 labeling assays, biotinylated proteins need to be purified and this is done using streptavidin conjugated agarose beads. Sypro ruby staining was first used to determine the optimal amount of beads to immunoprecipitate biotinylated proteins without non-specific binding (**Figure 3.7**). Sypro ruby stain is used since it is much more sensitive, with a lower detection limit of 0.25 to 1 ng (135). This allows observation of differences in immunoprecipitated proteins using streptavidin beads either from non-specific binding or biotinylated proteins.

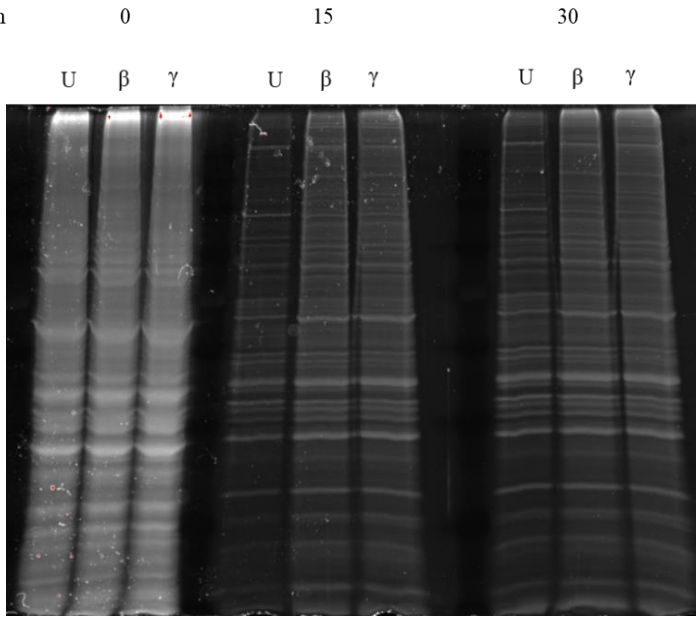


Figure 3.7 – *Sypro ruby stain of immunoprecipitated proteins from different concentrations of streptavidin agarose beads*

500 μg of biotinylated protein samples are immunoprecipitated with 0 to 30 μL of streptavidin agarose beads. 15 μL of beads per 500 μg of cell lysate is used for further experiments since there was less non-specific protein binding to the beads in untransfected HEK 293F cells compared to 30 μL of beads per 500 μg of cell lysate. This image is representative of 2 independent experiments.

U: Untransfected cells; β: Flag-APEX2-Gβ1; γ: Flag-APEX2-Gγ5

Next, immunoprecipitated proteins are visualized using western blotting (**Figure 3.8**). It is much less sensitive than sypro ruby stain so eluted proteins that can be visualized by western blot should be sufficient for mass spectrometry.

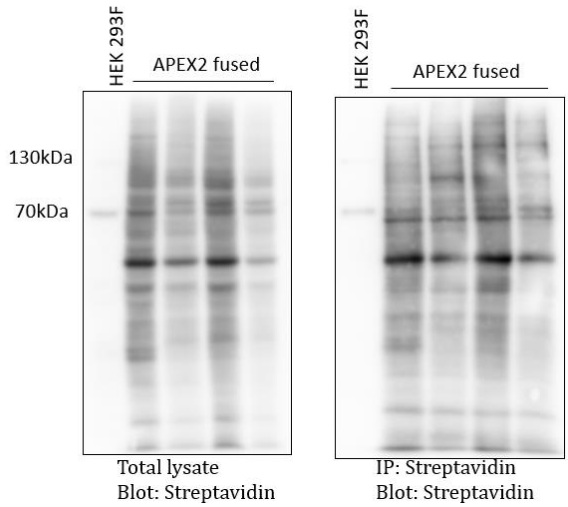


Figure 3.8 – *Biotinylated proteins are purified using streptavidin agarose beads*
Streaks of biotinylated proteins are observed with cells transfected with APEX2-tagged constructs and can be purified using streptavidin beads. Faint bands are seen at 70 kDa and 130 kDa in untransfected HEK 293F samples because cells contain endogenously biotinylated proteins, which are also detected with anti-streptavidin antibody. This image is representative of 3 independent experiments.

3.4 Gβγ interactors identified from proteomic screens

As mentioned previously, spatial references are especially important to determine if biotinylated proteins are more likely to be background proteins or potential interactors. Proteins that are more enriched to the bait should be more biotinylated compared to proteins simply in the vicinity of APEX2, for which spatial references can give a baseline quantification for these bystander proteins. For this reason, we used quantitative mass spectrometry to determine peptide intensities which allowed for protein quantification. Proteins specifically enriched to the bait protein will be more biotinylated and more abundant which translates to higher intensities in mass spectrometry results. Proteins can be quantified either through label-free methods or stable isotope-

based/isobaric labeling. Although isobaric labeling with tandem mass tagging (TMT) has been shown to have higher precision, detecting changes that reached statistical significance more often, both methods achieved comparably accurate quantification estimates (136). Protein quantities are measured by label-free quantification (LFQ) intensities which are normalized protein intensities and are better for comparison between samples of the same run (137).

However, cells also contain abundant proteins that may have high LFQ intensities in both control samples as well as samples containing the bait protein. The identified proteins may or may not be true interactors, as with all identified proteins, since APEX2 is a proximity-dependent labeling technique, so potential interactors would need to be validated using other methods to confirm interactions. Indeed, most of the analysis from the mass spectrometry results are from proteins identified with an LFQ intensity ≥2 fold change compared to control samples, herein deemed high confidence interactors, but includes results where LFQ intensity of proteins is <2 fold change compared to control samples as well, deemed low confidence interactors. Other criteria include proteins identified in at least two out of three experiments where the peptide count was higher than 1, as outlined in the methods. Additionally, 2 out of 3 screens uses the NES and NLS tagged constructs while the first run uses the Flag-APEX2 construct as control. Fold change is calculated from the control of each respective run and therefore, remain adequate comparisons between sample runs.

Using gene ontology annotations to group proteins by molecular function and protein class allow us to better interpret the dynamic picture from APEX2 labeling assays since direct interaction between bait and prey are not necessary for identification. In this way, proteins that are known interactors as well as co-factors of these interactors may also be identified in mass spectrometry screens. A full list of identified proteins can be found in the **Appendix**.

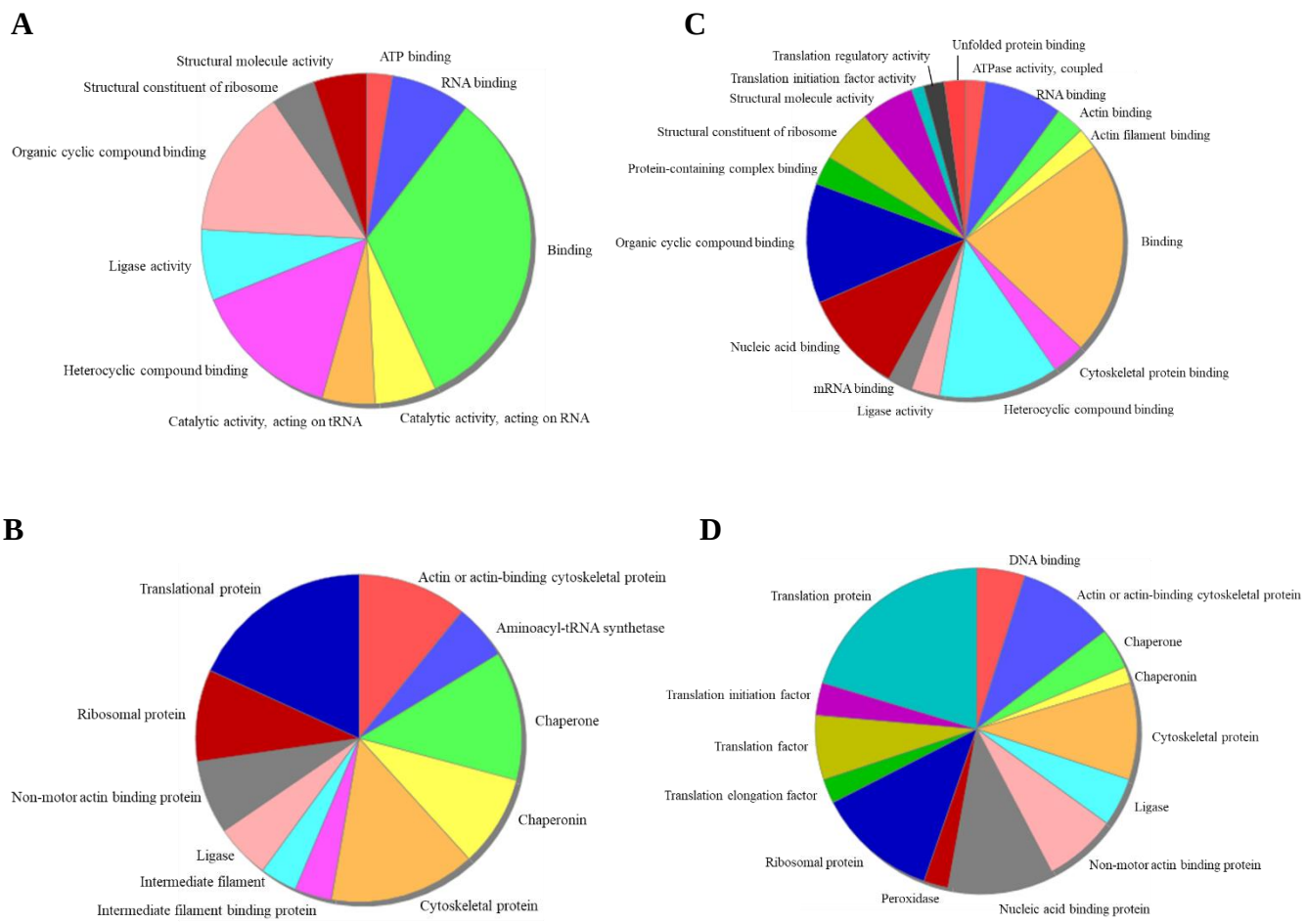


Figure 3.9 – *Identified proteins organized by molecular function and protein class*

Biotinylated proteins identified from mass spectrometry with APEX2-tagged Gβ1 and Gγ5 and organized by molecular function and protein class using the Panther online software. **A)** and **B)** Molecular function and protein class, respectively, of high confidence interactors. **C)** and **D)** Molecular function and protein class, respectively, of low confidence interactors.

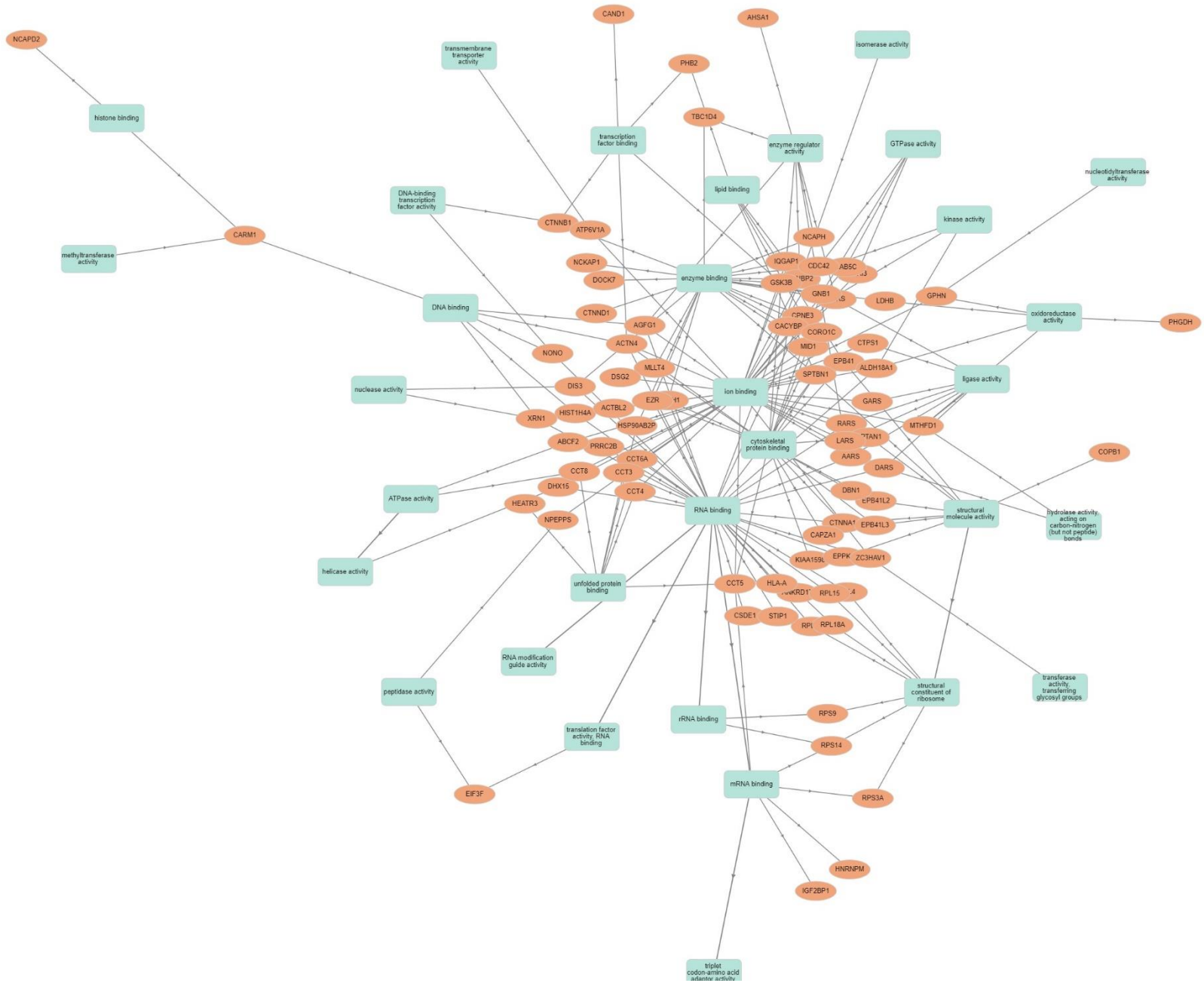

NCAPD2
histone binding
CARM1
methyltransferase activity
CAND1
AHSA1
transmembrane transporter activity
isomerase activity
PHB2
TBC1D4
transcription factor binding
enzyme regulator activity
GTPase activity
nucleotidyltransferase activity
lipid binding
DNA-binding transcription factor activity
kinase activity
CTNNB1
ATP6V1A
NCAPH
NCKAP1
IQGAP1
CDC42
DOCK7
enzyme binding
GSK3B
GNB1
LDHB
GPHN
DNA binding
CTNND1
AGFG1
CACYBP
CORO1C
oxidoreductase activity
PHGDH
ACTN4
MID1
CTPS1
NONO
MLLT4
EPB41
ALDH18A1
DSG2
SPTBN1
ligase activity
nuclease activity
DIS3
ion binding
GARS
ACTBL2
HIST1H4A
XRN1
HSP90AB2P
RARS
MTHFD1
ABCF2
PRRC2B
cytoskeletal protein binding
LARS
COPB1
CCT6A
AARS
CCT8
CCT3
DARS
ATPase activity
DHX15
CCT4
DBN1
HEATR3
RNA binding
structural molecule activity
hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds
NPEPPS
CAPZA1
helicase activity
KIAA159L
unfolded protein binding
CCT5
HLA-A
RPL15
CSDE1
STIP1
RPL18A
RNA modification guide activity
transferase activity, transferring glycosyl groups
peptidase activity
structural constituent of ribosome
rRNA binding
RPS9
translation factor activity, RNA binding
RPS14
mRNA binding
EIF3F
RPS3A
HNRNPM
IGF2BP1
triplet codon-amino acid adaptor activity

Figure 3.10 – *Interconnectivity of identified proteins organized by molecular function*

Molecular function annotation analysis of proteins identified from APEX2-tagged Gβ1 and Gγ5 using GOnet online software (133), connecting proteins to various molecular functions with the Compound Spring Embedder layout.

These GO term annotation analyses provide an overall view of common protein functions in a large set of data and easier organization for downstream analyses. **Figure 3.9** was generated using the GO slim setting in the Panther online software which groups proteins into broader functions while capturing as many annotations as possible. This resulted in a large group of proteins falling into the binding category. There were ~25% more low confidence interactors compared to high confidence interactors, demonstrated in **Figure 3.9C** which contains a larger diversity for binding subgroups than **Figure 3.9A** and leading to a smaller proportion of proteins simply being defined as binding. **Figure 3.10** allows for a more detailed analysis of specific molecular functions from the list of high confidence interactors, especially those falling into the binding category, including unfolded protein binding, cytoskeletal protein binding, ion binding, enzyme binding, DNA binding, transcription factor binding and lipid binding. This visualization also maintains the hierarchical structure which denotes each GO molecular function in relation to the parental molecular function, that can also include more than one. In **Figure 3.9A**, there is a set of identified proteins implicated in RNA binding and we observe this parental molecular function being defined in **Figure 3.10** to involve various nodes such as rRNA binding, translation factor activity (RNA binding), RNA modification guide activity and mRNA binding, further specified as triplet codon-amino acid adaptor activity. From these diagrams, we can easily determine molecular functions which have the most identified proteins and can further elaborate on specific proteins.

3.4.1 Interactors involved in protein folding

We can observe a proportion of the high confidence interactors involved in unfolded protein binding, which we can link to protein classes chaperone and chaperonin in **Figure 3.9B**, which play a role assisting protein folding. Reported chaperones that are Gβγ interactors include T-complex protein 1 (CCT) as well as heat shock protein 90 (HSP90) (138, 139).

The 8 subunits of CCT proteins form a ring-like complex, and with phosducin-like protein (PhLP), work together in Gβγ assembly, where a nascent Gβ forms a complex with CCT and PhLP, without Gγ (140). From our proteomic screens, we have identified seven of the eight CCT subunits as high and low confidence interactors, with Gβ1 as the bait protein for all except one, compared to Gγ5 which only pulled down two of the seven identified CCT proteins, in-line with the current knowledge of CCT-PhLP- Gβ complex which excludes Gγ. The remaining CCT subunit, CCT7, was identified only in one experiment.

Proteins associated with HSP90 function identified from the screen include activator of heat shock protein ATPase homolog 1 (AHSA1), putative heat shock protein HSP90 beta-1 (HSP90AB2P), heat shock protein HSP90-alpha (HSP90AA1), and the HSP90 co-chaperone, stress-induced phosphoprotein 1 (STIP1).

Table 3.1 – *Identified proteins involved in protein folding*

High (black) and low (blue) confidence interactors identified in at least two out of three experiments with a role in protein folding.

Bait	Protein name	Gene ID
Gγ5	Activator of 90 kDa heat shock protein ATPase homolog 1	AHSA1
Gβ1	T-complex protein 1 subunit alpha	TCP1
$G\beta_{1,}\ G\gamma_{5}$	T-complex protein 1 subunit beta	CCT2

Gβ1	T-complex protein 1 subunit gamma	CCT3
Gβ1	T-complex protein 1 subunit delta	CCT4
Gγ5	T-complex protein 1 subunit epsilon	CCT5
Gβ1	T-complex protein 1 subunit zeta	CCT6A
$G\beta_1$, $G\gamma_5$	T-complex protein 1 subunit theta	CCT8
$G\beta_1$, $G\gamma_5$	Heat shock protein HSP 90-alpha	HSP90AA1
Gβ1	Putative heat shock protein HSP 90-beta 2	HSP90AB2P
Gγ5	Stress-induced-phosphoprotein 1	STIP1

Previous work in the laboratory, done by Rhiannon Campden used tandem affinity purification (TAP) and Flag immunoprecipitation to study the Gβγ proteome. TAP works by having 2 different tags separated by a tobacco etch virus (TEV) cleavage site, and linked to the bait protein. In this way, there is a 2-step purification, with cleavage of one tag possible before the second purification step. Purified proteins can then be used for mass spectrometry experiments to identify the protein complexes formed between the bait and its preys. The TAP screens were able to identify a plethora of proteins involved in protein folding, including the various CCT subunits but only 1 protein related to HSP90 were identified. Instead, prefoldins (PFDN), chaperone proteins which bind CCT proteins to transfer target proteins to them (141), were identified in the TAP screen. Using APEX2, PFDN6 was found, but only in 1 experiment. However, the LFQ intensity had a fold change ≥ 2 compared to control, a positive result that it is not a bystander protein. There are no reported interactions between Gβγ and PFDN, yet PFDNs bind CCTs which could explain the potential biotinylation of PFDN due to proximity.

3.4.2 Cytoskeletal protein interactors

Proteins associated with the cytoskeleton are also a dominant protein class among high and low confidence interactors, including proteins related to the 3 cytoskeletal elements: thin (actin) filaments, intermediate filaments, and microtubules. Gβγ interacts and activates various proteins involved in the many functions of the cytoskeleton such as chemotaxis and cell adhesion, which contributes to the many cytoskeleton-related proteins identified. The role of Gβγ in chemotaxis is well known and include activation of various proteins which were identified. An early event in chemotaxis is Gβγ activation of PI3K. KIAA1598 was identified in our screens which plays a role in chemotaxis by regulating localization of PI3K. PI3K can then phosphorylate PIP2 which localizes active Cdc42 and in turn, F actin polymerization for chemotaxis. Although Cdc42 is not a direct interactor, we identified it in our screens as a high confidence interactor probably due to its proximity with PAK1, which can interact directly with Gβγ and act as a scaffold protein for Cdc42 (120). We also identified IQGAP1, a protein involved in cadherin cell-cell adhesion and functions as an effector for Cdc42 and Rac1, regulating polarization and migration (142). PIXα's GEF activity allows for Cdc42 activation but it can also activate Rac1, an important player in directionality. Our screens identified CORO1C and DOCK7, 2 proteins involved in Rac1 activation. Interestingly, an assay conducted in zebrafish demonstrated an interaction between Gβγ and GSK3β playing a role in cell-cell adhesion (127), and this protein was found in our screens as a high confidence interactor, suggesting this interaction may be relevant in mammalian cell types as well. Other cytoskeletal-related proteins identified from our screens are listed in **Table 3.2** below.

Table 3.2 – *High confidence protein interactors related to the cytoskeleton*

Identified proteins which are deemed high confidence interactors related to the cytoskeleton and the roles it plays in chemotaxis and cell adhesion.

Bait	Protein name	Gene ID
Gβ1	Beta-actin-like protein 2	ACTBL2
$G\beta_{1,}$ $G\gamma_5$	Alpha-actinin-4	ACTN4
Gγ5	F-actin-capping protein subunit alpha-1	CAPZA1
Gγ5	Cell division control protein 42 homolog	CDC42
Gγ5	Coronin-1C;Coronin	CORO1C
Gγ5	Copine-3	CPNE3
Gγ5	Catenin alpha-1	CTNNA1
Gγ5	Catenin beta-1	CTNNB1
$G\beta_{1,}$ $G\gamma_5$	Catenin delta-1	CTNND1
Gγ5	Drebrin	DBN1
Gβ1	Protein diaphanous homolog 1	DIAPH1
Gγ5	Dedicator of cytokinesis protein 7	DOCK7
$G\beta_{1,}$ $G\gamma_5$	Desmoglein-2	DSG2
Gγ5	Protein 4.1	EPB41
Gγ5	Band 4.1-like protein 2	EPB41L2
Gγ5	Band 4.1-like protein 3;Band 4.1-like protein 3, N-terminally processed	EPB41L3
Gβ1	Epiplakin	EPPK1
Gγ5	Ezrin	EZR
Gγ5	Gephyrin;Molybdopterin adenylyltransferase;Molybdopterin molybdenumtransferase	GPHN
Gγ5	Glycogen synthase kinase-3 beta	GSK3B
$G\beta_{1,}$ $G\gamma_5$	Ras GTPase-activating-like protein IQGAP1	IQGAP1
Gγ5	Shootin-1	KIAA1598
Gγ5	Afadin	MLLT4
Gγ5	Myosin light polypeptide 6;Myosin light chain 6B	MYL6;MYL6B
Gγ5	Nck-associated protein 1	NCKAP1
Gγ5	Spectrin alpha chain, non-erythrocytic 1	SPTAN1

Although the previously done TAP screens identified cytoskeletal proteins, they were tubulins (TUB), which were only identified in 1 screen using APEX2, and did not include any of these cytoskeletal proteins. This may be explained since the proteins identified here are related to known Gβγ interactors, indicating they may come into proximity with Gβγ while not directly interacting with our bait proteins.

3.4.3 Identified nuclear proteins

The majority of identified nuclear proteins are low confidence interactors, with only ~1/3 of identified proteins being high confidence interactors (shown in **Table 4**). This may be due to G protein trafficking to the nucleus being minimal under basal conditions which could be increased upon agonist stimulation, explaining the small number of high confidence interactors. Furthermore, there is evidence of nuclear GPCRs activating a pool of nuclear G proteins (153). This nuclear pool of G proteins could be contributing to the identification of low confidence interactors, where the interactome of G protein fused APEX2 under untreated conditions are similar to our control NLS-fused APEX2 construct. In our untreated samples, we were still able to detect previously reported nuclear proteins, however they only showed up in one experiment. These include the various subunits of RNAPII, STAT3, a c-Jun interacting protein and a member of the SWI/SNF family, SMARCA5 (**Appendix**). Additionally, many importin and exportin proteins were also identified in one experiment (listed in the **Appendix**).

Table 3.3 – *Nuclear proteins identified in at least 2 out of 3 experiments*

High (black) and low (blue) confidence nuclear interactors identified from our proteomic screens.

Bait	Protein name	Gene ID
Gβ1	Protein argonaute	AGO

Gβ1	Ankyrin repeat domain-containing protein 17	ANKRD17
$G\beta_1$, $G\gamma_5$	Annexin A11	ANXA11
Gγ5	Putative annexin A2-like protein;Annexin A2;Annexin	ANXA2P2;ANXA2
$G\beta_1$, $G\gamma_5$	Annexin A7	ANXA7
Gβ1	CAD protein;Glutamine-dependent carbamoyl-phosphate synthase;Aspartate carbamoyltransferase	CAD
$G\beta_1$, $G\gamma_5$	Histone-arginine methyltransferase CARM1	CARM1
Gγ5	Cyclin-dependent kinase 1	CDC2;CDK1
Gβ1	Exportin-2	CSE1L
Gβ1	CTP synthase 1;CTP synthase	CTPS1
Gβ1	Probable ATP-dependent RNA helicase DDX17	DDX17
Gβ1	Pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	DHX15
Gγ5	ATP-dependent RNA helicase A	DHX9
Gβ1	Exosome complex exonuclease RRP44	DIS3
Gγ5	Alpha-enolase	ENO1
Gγ5	Histone H1.2;Histone H1.4;Histone H1.3;Histone H1t;Histone H1.1	HIST1H1C;HIST1H1E; HIST1H1D;HIST1H1T;HIST1H1A
Gβ1	Histone H4	HIST1H4A
Gγ5	Histone H4	HIST1H4A
Gβ1	Heterogeneous nuclear ribonucleoprotein H	HNRNPH1
$G\beta_1$, $G\gamma_5$	Heterogeneous nuclear ribonucleoprotein K	HNRNPK
Gγ5	Heterogeneous nuclear ribonucleoprotein M	HNRNPM
Gγ5	Importin-7	IPO7
$G\beta_1$, $G\gamma_5$	Far upstream element-binding protein 2	KHSRP
$G\beta_1$, $G\gamma_5$	Importin subunit alpha-1	KPNA2
Gβ1	Importin subunit alpha-7	KPNA6
Gγ5	Importin subunit beta-1	KPNB1
Gβ1	DNA replication licensing factor MCM3	MCM3
Gβ1	DNA helicase;DNA replication licensing factor MCM5	MCM5
Gγ5	Mini-chromosome maintenance complex-binding protein	MCMBP
$G\beta_1$, $G\gamma_5$	Condensin complex subunit 1	NCAPD2

$G\beta_{1,}\ G\gamma_5$	Condensin complex subunit 2	NCAPH
Gβ1	Non-POU domain-containing octamer-binding protein	NONO
$G\beta_{1,}\ G\gamma_5$	Nucleophosmin	NPM1
Gγ5	Poly [ADP-ribose] polymerase 1	PARP1
$G\beta_{1,}\ G\gamma_5$	Poly(rC)-binding protein 1	PCBP1
$G\beta_{1,}\ G\gamma_5$	Prohibitin-2	PHB2
$G\beta_{1,}\ G\gamma_5$	DNA-dependent protein kinase catalytic subunit	PRKDC
Gβ1	RNA-binding protein 14	RBM14
Gγ5	U5 small nuclear ribonucleoprotein 200 kDa helicase	SNRNP200
Gβ1	TAR DNA-binding protein 43	TARDBP;TDP43
Gβ1	5-3 exoribonuclease 1	XRN1

3.4.4 RNA binding proteins

A portion of high and low confidence interactors can be attributed to translational proteins as show in **Figure 3.9B** and **D**, including components of the 60S and 40S ribosomal proteins, aminoacyl-tRNA ligases and various translation initiation factors (shown in **Table 5**). These interactions may be occurring during translation of Gβ1 and Gγ5 and consequently being labeled by APEX2. However, previous work in our lab using TAP coupled to LC-MS also identified proteins involved in RNA stability and processing, particularly, members of the heterogenous ribonuclear proteins (HNRNPs). These proteins showed an increase in identification when cells were treated with carbachol stimulation, supporting evidence of a role for Gβγ in transcriptional regulation. Additional evidence from proteomic screens done in yeast demonstrate an interaction between eukaryotic initiation factor 2 (EIF2) and GBB, the G protein β subunit found in *Saccharomyces cerevisiae*, further supporting a possible role for Gβγ in protein synthesis (143).

Table 3.4 – *Identified proteins related to RNA binding*

High (black) and low (blue) confidence interactors identified in at least 2 out of 3 LC/MS experiments that play a role in RNA processing.

Bait	Protein name	Gene ID
Gγ5	Alanine--tRNA ligase, cytoplasmic	AARS
Gγ5	Aspartate--tRNA ligase, cytoplasmic	DARS
$G\beta_1$, $G\gamma_5$	Bifunctional glutamate/proline--tRNA ligase;Glutamate--tRNA ligase;Proline--tRNA ligase	EPRS
Gβ1	Glycine--tRNA ligase	GARS
$G\beta_1$, $G\gamma_5$	Isoleucine--tRNA ligase, cytoplasmic	IARS
Gβ1	Leucine--tRNA ligase, cytoplasmic	LARS
Gγ5	Glutamine--tRNA ligase	QARS
Gβ1	Arginine--tRNA ligase, cytoplasmic	RARS
Gγ5	Threonine--tRNA ligase, cytoplasmic	TARS
$G\beta_1$, $G\gamma_5$	Cytoplasmic FMR1-interacting protein 1;Cytoplasmic FMR1-interacting protein 2	CYFIP1;CYFIP2
Gβ1	Elongation factor 1-delta	EEF1D
$G\beta_1$, $G\gamma_5$	Elongation factor 1-gamma	EEF1G
$G\beta_1$, $G\gamma_5$	Elongation factor 2	EEF2
Gγ5	Eukaryotic translation initiation factor 3 subunit D	EIF3D
Gγ5	Eukaryotic translation initiation factor 3 subunit L	EIF3L
$G\beta_1$, $G\gamma_5$	Eukaryotic initiation factor 4A-I	EIF4A1
Gβ1	Eukaryotic translation initiation factor 4 gamma 1	EIF4G1
$G\beta_1$, $G\gamma_5$	Eukaryotic translation initiation factor 5A;Eukaryotic translation initiation factor 5A-2;Eukaryotic translation initiation factor 5A-1-like	EIF5A;EIF5A2;EIF5AL1
Gγ5	Eukaryotic peptide chain release factor GTP-binding subunit ERF3A	GSPT1;GSPT2
Gβ1	Heterogeneous nuclear ribonucleoprotein H	HNRNPH1
$G\beta_1$, $G\gamma_5$	Heterogeneous nuclear ribonucleoprotein K	HNRNPK
Gγ5	Heterogeneous nuclear ribonucleoprotein M	HNRNPM
$G\beta_1$, $G\gamma_5$	60S ribosomal protein L11	RPL11

Gγ5	60S ribosomal protein L12	RPL12
Gβ1	60S ribosomal protein L13	RPL13
Gβ1	60S ribosomal protein L13a;Putative 60S ribosomal protein L13a protein RPL13AP3	RPL13a;RPL13A;RPL13AP3
Gγ5	Ribosomal protein L15;60S ribosomal protein L15	RPL15
Gβ1	60S ribosomal protein L18	RPL18
Gβ1	60S ribosomal protein L18a	RPL18A
Gβ1	60S ribosomal protein L22	RPL22
Gγ5	60S ribosomal protein L24	RPL24
Gβ1	60S ribosomal protein L27a	RPL27A
Gβ1	60S ribosomal protein L28	RPL28
Gβ1	60S ribosomal protein L4	RPL4
Gβ1	60S ribosomal protein L7	RPL7
Gβ1	60S ribosomal protein L9	RPL9
$G\beta_1, G\gamma_5$	40S ribosomal protein S11	RPS11
Gβ1	40S ribosomal protein S12	RPS12
Gγ5	40S ribosomal protein S14	RPS14
$G\beta_1, G\gamma_5$	40S ribosomal protein S3	RPS3
Gβ1	40S ribosomal protein S3a	RPS3A
$G\beta_1, G\gamma_5$	40S ribosomal protein S4, X isoform	RPS4X
$G\beta_1, G\gamma_5$	40S ribosomal protein S7	RPS7
$G\beta_1, G\gamma_5$	40S ribosomal protein S9	RPS9
Gβ1	40S ribosomal protein SA	RPSA
Gγ5	U5 small nuclear ribonucleoprotein 200 kDa helicase	SNRNP200

3.4.5 Other identified proteins

Additional proteins identified in our APEX2 runs and found in the TAP proteomic screens include ATPases in the cytoplasm and mitochondria as well as players in degradation pathways including BTB/POZ domain-containing protein, KCTD12, various E3 ligases and components of the 26S proteasome (list in **Appendix**). However, these proteins represent only a small proportion of high and low confidence interactors, and are mostly found in only 1 out of 3 experiments. Yet,

they were also identified in previous TAP screens and further explored in Jennifer Sung, MSc thesis, where 4 members of the KCTD protein family (KCTD2, 5, 12, 17) were identified. Specifically, Gβγ interaction with KCTD5 was further validated and a knockout stable cell line was generated using the Clustered Regularly Interspaced Short Palindromic Repeats (Crispr) and CRISPR-associated 9 (Cas9) gene editing technique to understand its effects on Gβγ signaling. Additionally, recent evidence has demonstrated direct interaction of KCTD12 and Gβγ downstream of the $GABA_B$ receptor ($GABA_BR$). The authors show the dual interaction of KCTD12 with $GABA_BR$ and Gβγ, sequestering Gβγ from Kir3 channels and inducing rapid desensitization (144).

Mitochondrial proteins were also identified, although they only account for a small portion of high and low confidence interactors or were found in 1 out of 3 experiments, and included translational proteins and a mitochondrial RNA polymerase. Previous TAP screens have identified various mitochondrial ATPases, translation proteins and chaperones under basal and stimulated conditions. However, many studies describe the presence of Gβγ solely at the outer mitochondrial membrane (117), including direct interaction of $Gβ_2$ and mfn1 in regulating mitochondrial fusion (116). Further validation of Gβγ interaction with mitochondrial transcription and translation proteins would need to be done to confirm a possible novel role for Gβγ inside the mitochondria.

4 DISCUSSION

4.1 General discussion

While a great deal of research has been focused on G protein signaling mediated via Gα subunits, there has also been continued interest in the obligate Gβγ dimer. Our understanding has evolved from its simple role as a GDI for inhibition of Gα signaling (7, 8), to an independent protein with its own effectors all throughout the cell. These roles include various effectors found at the plasma membrane, including common Gα effectors. The first Gβγ effector discovered was the Kir3 family of inwardly rectifying potassium channels (55), and this regulation was dependent on the presence of Gγ subunits as well as the Gβ subtype (61, 62). A second type of ion channel that is regulated by Gβγ are voltage-gated Ca^{2+} channels, which allows for the slowing of activation kinetics (63). Although all Gβ subtypes are able to inhibit Ca^{2+} channels, efficacy can vary depending on the Gγ subtype (66). Well established common effectors with Gα include adenylyl cyclase which converts ATP into cAMP, with the majority of the isoforms being regulated by both Gα and Gβγ (70). Phospholipase C, which hydrolyzes PIP2 into DAG and IP3, is also a common effector which leads to activation of PKC and release of intracellular Ca^{2+} stores, respectively. The various PLC isoforms can be classified into 6 distinct families but only 3 of these families have been shown to be regulated by Gβγ (74, 79). Interestingly, suppra-additive activation has been observed with PLC β3 when stimulated by both $G\alpha_q$ and Gβγ (77). PI3Ks phosphorylate various phosphoinositides and specifically class I PI3Ks are regulated by Gβγ. Similar to many Gβγ effectors, PI3K regulation is dependent on Gβ subtype, with $G\beta_1$ but not $G\beta_5$ stimulating kinase activity (88).

Not only are the effects of Gβγ mediated through effectors found at the plasma membrane, Gβγ can also translocate to different cellular compartments upon membrane receptor activation.

In the nucleus, Gβγ binds HDACs, GR and various transcription factors as well as RNAP II, a protein complex ubiquitously involved in transcription (46, 99, 102, 103, 105, 106, 108). A study also demonstrated the ability for $G\beta_1$ to localize to promoters of over 700 genes (107). At the Golgi Apparatus, Gβγ plays an important role in fragmentation, with treatment of purified Gβγ leading to complete fragmentation of the Golgi (109). These effects were mediated through PKD and involved upstream 2nd messengers such as DAG and PKC (112). Better understanding of Gβγ at the Golgi could also be a potential avenue for heart failure prevention through blocking of ET-1 dependent PI4P hydrolysis and subsequent inhibition of a hypertrophic pathway (114). At the mitochondria, Gβγ mediated effects involve regulation of fusion and fission equilibrium through binding to mfn1 (116) as well as induction of mitochondrial biogenesis (118). Further studies show various roles for Gβγ at the cytoskeleton involving regulation of chemotaxis through activation of PI3Kγ (reviewed in 121), cell-matrix adhesion (128), and dynamic regulation of tubulin and microtubules through opposing effects with Gα (reviewed in 130).

Using affinity purification coupled to LC-MS, our lab has previously identified a wide array of Gβγ interactors in the cytoplasm and the nucleus under basal and stimulated conditions (154). Tandem affinity purification (TAP) relies on strong interaction between the bait and prey proteins, identifying stable interaction partners with Gβγ. The current work provides an approach using the APEX2 proximity-dependent biotinylation assay coupled to LC-MS to study more transient or weak interactions.

APEX2-fused Gβ1 and Gγ5 constructs with a Flag tag were first validated for proper function, both in terms of the G protein and the APEX2 enzyme. Using Flag beads, immunoprecipitation of both constructs were capable of co-immunoprecipitation with $G\alpha_q$, demonstrating that the APEX2 enzyme adduct did not hinder interaction with a known interactor.

Next, biotinylation was validated using the labeling assay to ensure that the APEX2 moiety was functional. NLS and NES were added to Flag-APEX2 plasmids and used as controls for detection of bystander proteins, proteins that may be in the vicinity of APEX2 and therefore tagged with biotin without being true interactors. A protocol was then developed to prepare samples for LC-MS, including pull-down of biotinylated proteins using streptavidin agarose beads and tryptic digestion into peptides. Identified proteins were categorized into high or low confidence interactors, proteins with peptide count ≥2, appearing in at least 2 out of 3 experiments and having a fold change LFQ intensity ≥ or < 2 compared to control samples, respectively. Another aspect to note is the differences in controls, with one of the experiments having Flag-APEX2 as a control and the remaining 2 experiments using the modified NES and NLS constructs. Although a large overlap between prey proteins identified in Gβ1 and Gγ5 screens would indicate our assay is intrinsically functional, HEK 293F cells express the majority of Gβ and Gγ subtypes (151). Therefore, we only saw minimal overlap of identified proteins since we cannot know which dimers are being formed and the differences in signaling dynamics from each pair.

The results presented here using the above criteria are in concordance with previously presented data from affinity purification methods coupled to LC-MS from our lab as well as confirmed signaling pathways involving Gβγ. It encompasses chaperones for protein folding like CCTs and HSP90, cytoskeletal proteins for chemotaxis and cell adhesion, nuclear proteins for gene transcription and various ribosomal proteins and elongation factors, suggesting a possible role in translation as well. However, the filtering criteria excluded less abundant proteins that are potential and validated interactors including RNAPII, transcription factors, exportin and importin, direct interactors in cell polarization and mitochondrial proteins. Additionally, comparison of LFQ intensities from bait and control samples may not always show large differences for highly

abundant proteins such as tubulin, a validated Gβγ interactor, where LFQ intensities for bait and control samples did not have a fold change ≥ 2.

A comparison between 3 different LC-MS screens done in our lab, Flag-tagged Gβ1 and TAP-tagged Gβ1, and the current results from APEX2-fused Gβ1, demonstrate variabilities in identified proteins, shown in **Figure 4.1**.

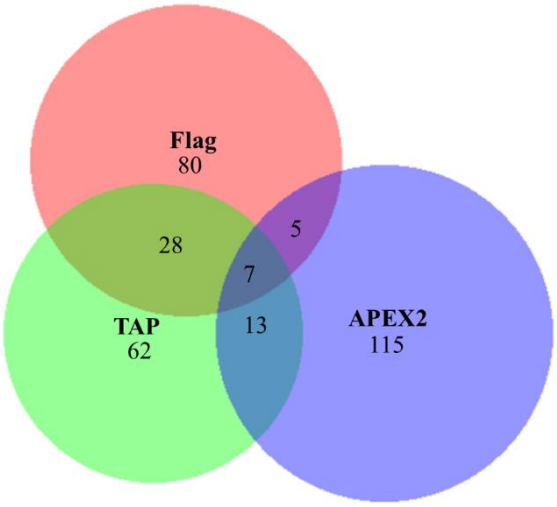


Figure 4.1 – *Number of proteins identified from 3 different methods for interactor identification*

Three different methods are used to identify Gβ1 interactors. Affinity purification from Flag tag alone identified a total of 120 proteins, TAP-tagged Gβ1 screens resulted in 110 identified proteins, and APEX2-fused Gβ1 identified a total of 140 proteins (high and low confidence interactors only). Numbers under each technique represent the number of unique proteins identified. Diagram was made using BioVenn (155).

The Venn diagram above shows that even between affinity purification techniques (Flag and TAP pulldowns), there are distinct proteins identified from each method. The lower number of total proteins identified from TAP screens may be due to the 2-step purification involved, which leads

to harsher wash conditions and elution of weaker interactions, compared to single tag purification using Flag tag alone. Another reason for the variability in interactors could be the tag size, shown in **Table 4.1**, that may cause steric and/or physical constraints between Gβ1 and its interactors. This is especially noted in results presented earlier, **Figure 3.3**, which show co-immunoprecipitation of overexpressed $G\alpha_q$ with pull-down of APEX2-fused Gβ1 but no identification of $G\alpha_q$ from our LC-MS screens.

Table 4.1 – *Tag sizes from the 3 different methods used to identify Gβ1 interactors*

APEX2 is the largest protein with a molecular weight of 27 kDa (134) compared to the total TAP tag which is only 12 kDa (156). The Flag tag is a mere 1 kDa (156), making it the smallest and least likely to interfere with the binding surface of $G\beta_1$.

Method	Tag	Size	Total tag size
Affinity purification	Flag	1kDa	1kDa
TAP	Streptavidin	1kDa	12kDa
	TEV	5kDa	
	HA	1kDa	
	CaM	5kDa	
APEX2	Flag	1kDa	28kDa
	APEX2	27kDa	

Using Pymol to model APEX2-fused Gβ1 bound to heterotrimeric $G\alpha_q$, we can observe possible physical constraints that may explain the lack of G proteins identified in our APEX2 screens, which were identified in previous TAP and Flag experiments. The structural model fuses the C-terminus (CT) of wild-type ascorbate peroxidase (WT APEX) (157) to the N-terminus of $G\beta_1$ (isolated from 158). We can then align a heterotrimeric $G\alpha_q$ protein (158) to the fused model (**Figure 4.2**). We observe that WT APEX does not block the interaction surface between $G\alpha_q$ and $G\beta_1$. However,

due to the size of both $G\alpha_q$ and WT APEX, they are superimposed in the model which could potentially block proper binding of $G\alpha_q$, and other Gα isoforms, to $G\beta_1$. Yet, APEX2 is a proximity dependent labeling assay which does not require direct interaction for detection of proteins which leads us to believe there may not only be physical constraints but also steric constraints which explain many well studied Gβγ interactors not being identified in our screen.

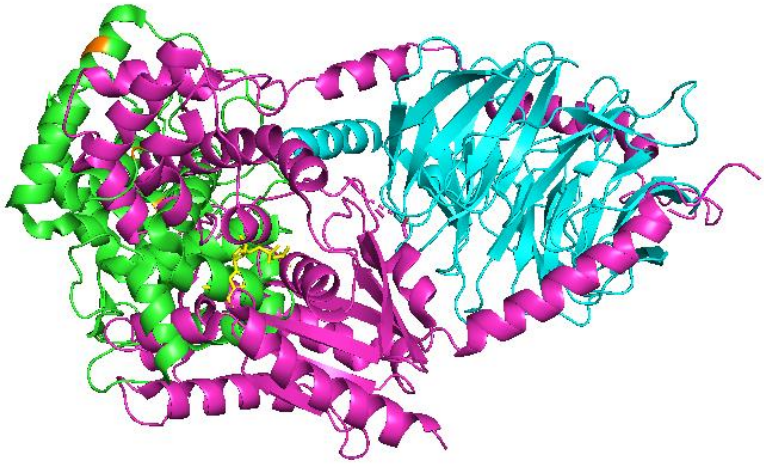


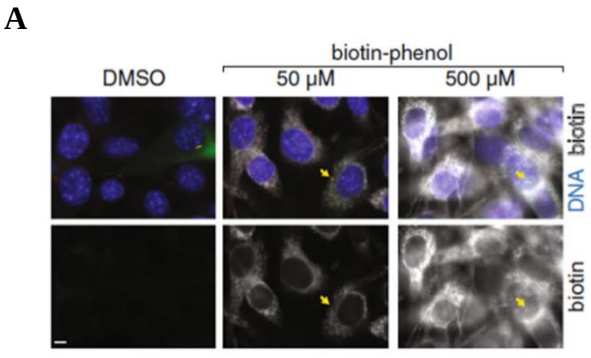
Figure 4.2 – *Structural modeling of fused WT APEX and Gβ1 aligned with heterotrimeric* $G\alpha_q\beta_1\gamma_2$

WT APEX (green) fused to Gβ1 (blue) is aligned with a crystal structure of heterotrimeric $G\alpha_q\beta_1\gamma_2$ (purple; β_1 hidden; GDP in yellow). APEX2 contains 4 mutations at K14D, W41F, E112K and A134P on WT APEX (shown in orange) (159). In the model, WT APEX is directly fused to Gβ1 whereas our constructs contain a short linker of 8 amino acids, not shown here. Structures are obtained from Protein Data Bank and the model was made using Pymol (160).

The Contaminant Repository for Affinity Purification Mass Spectrometry Data (CRAPome) would be a great tool to use for data analysis, allowing the comparison of quantified proteins against data from thousands of studies to better characterize background proteins (145). Due to the

instrument available to us providing quantification in intensities, we were not able to use CRAPome, which requires quantification in spectral counts. Another consideration is using analysis tools specific to proximity-dependent labeling such as Significance Analysis of Interactome (SAINT) or the more user-friendly SAINTexpress, which have increasingly become more common for data analysis since it uses user provided control and can include CRAPome data to assign a probability score for true interaction between the bait and prey protein (146, 147). The analysis in this thesis was not done using SAINT because the control for one of the experiments was different from the other two experiments and did not allow for an adequate comparative analysis.

Furthermore, the concentration of biotin-phenol used in the labeling assay may not be well optimized. The majority of APEX2 studies use biotin-phenol at a final concentration of 500 μM, and this is the concentration we have used in all our experiments. However, one recent study on APEX2 labeling has shown that a concentration of 50 μM biotin-phenol is sufficient to label nearby proteins, and the use of 500 μM of biotin-phenol results in a large amount of background biotinylation (148), which may have been the reason for excess biotinylation with our Flag-APEX2 construct without a localization signal.



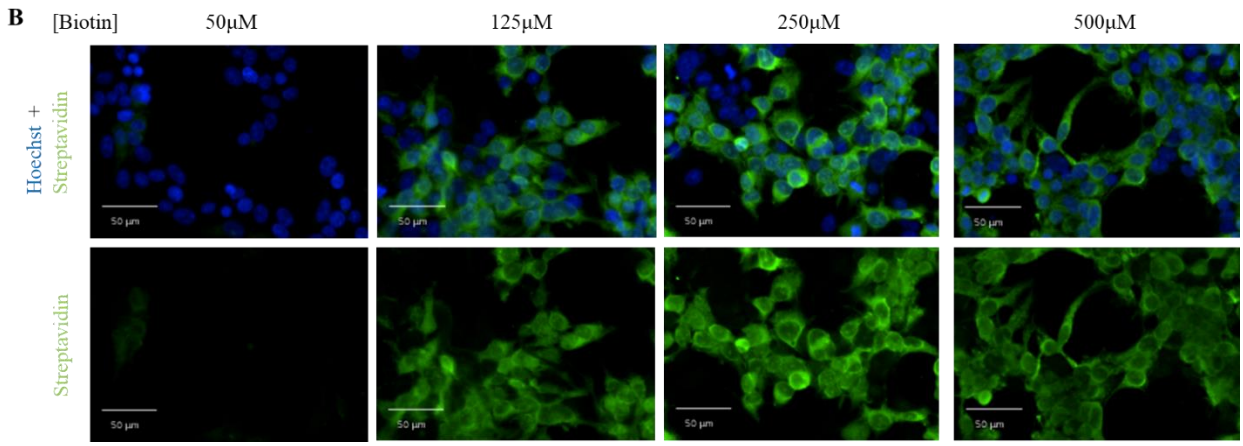


Figure 4.3 – *Labeling assay using various concentrations of biotin-phenol*

A) Figure taken from Mick DU, 2019 (148) revealing background biotinylation with 500 μM biotin-phenol **B)** Immunofluorescence images using Flag-APEX2-NES construct labeled with 50 μM to 500 μM biotin-phenol. Our results demonstrate 50 μM is not sufficient for biotinylation of proteins. However, 125 μM is sufficient for effective biotinylation, suggesting the concentration used in our assays may be causing an excess of biotinylated proteins in our proteomic screens.

4.2 Future directions

4.2.1 Subcellular fractionation of samples

One simple way to study proteins from different cellular compartments is through cellular fractionation. Also, since less proteins are present in each sample, less abundant proteins may be more easily detected through LC-MS, especially since LFQ intensities are normalized quantifications so low abundant proteins may have a calculated intensity but the corresponding LFQ intensity is 0. A protocol for this has been validated in HEK 293F cells.

For biotinylated samples that will be separated into cytosolic and nuclear fractions, quenching buffer is made using 1x TBS instead of 1x PBS. After biotinylation, cells are lysed with

hypotonic buffer #1 (20 mM Tris pH7.4; 1.5 mM $MgCl_2$; 10 mM KCl; 0.5 mM DTT; 0.1% IGEPAL CA630; 1 mM PMSF added fresh; 10 mM NaF; protease inhibitor cocktail added fresh). Cells are then scraped off and put into an Eppendorf tube. Then cell lysate is incubated at 4°C on a rotator for 15 minutes before being centrifuged at 4400 x g for 5 minutes at 4°C. Supernatant, which contains the cytosolic fraction, is transferred into a new Eppendorf. Pellet is resuspended in hypotonic buffer #2 (20 mM Tris pH7.4; 1.5 mM $MgCl_2$; 10 mM KCl; 0.1% IGEPAL CA630; 1 mM PMSF added fresh; 10 mM NaF; 1 mM Na_3VO_4 added fresh; protease inhibitor cocktail added fresh) and incubated for 5 minutes on the rotator at 4°C then centrifuged at 800 x g for 5 minutes at 4°C. Supernatant is discarded and hypotonic buffer #2 is added to resuspend the pellet before centrifugation at 800 x g for 5 minutes at 4°C. Supernatant is discarded and pellet is resuspended in modified RIPA lysis buffer to lyse pellet using the Misonix Sonicator 3000 at 4°C with 2x 5 second bursts. Prior to rotating incubation at 4°C for 1 hour 30 minutes, $MgCl_2$ is spiked in to a final concentration of 2 mM and 250 U of benzonase is added. After incubation, EDTA and sodium deoxycholate are spiked-in to a final concentration of 2 mM and 0.5% respectively, and then centrifuged at 16,100 x g for 15 minutes at 4°C. Supernatant containing nuclear proteins is transferred to a new Eppendorf tube for Bradford assay to quantify proteins. Streptavidin immunoprecipitation and mass spectrometry sample preparation are done as mentioned in the methods.

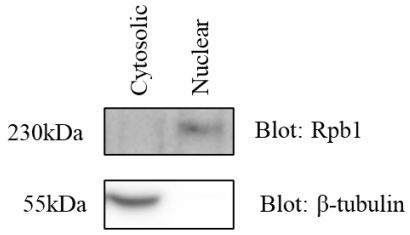


Figure 4.4 – *Separation of cytosolic and nuclear fractions*

Using the protocol described above, we can see proper separation of nuclear and cytosolic fractions. β-tubulin, a cytosolic protein, is only found in the cytosolic fraction while Rpb1, a nuclear protein, is only found in the nuclear fraction.

4.2.2 Studying changes in the proteome under agonist stimulation

All results presented in the thesis were done under basal conditions, limiting the effects that Gβγ could have under agonist-stimulated conditions with activated GPCRs. A validated pathway in HEK293F cells is stimulation of the M3R using carbachol. Our lab has previously demonstrated an increased interaction between Gβγ and RNAPII when cells were stimulated with carbachol for 45 minutes (108). These conditions may be more optimal in observing RNAPII as a high confidence interactor in our screens since it was only identified in 1 out of 3 experiments, although the LFQ intensity was much higher than that of control samples. Our lab has also shown that carbachol stimulation resulted in a net increase of Gβγ in the nuclear fraction and net decrease in the cytosolic fraction, suggesting a potential for Gβγ proteome differences and identification of candidate nuclear interactors with Gβγ.

4.2.3 APEX2-fused $G\beta_1$ mutants

There have been reports of disease causing $G\beta_1$ mutations including GNB1 encephalopathy (GNB1-E), characterized by moderate to profound developmental delay or intellectual disability

(149) and various cancers. Previous work from our lab by Iulia Pirvulescu has demonstrated gains and losses of function of different $G\beta_1$ mutants in rescuing the increase of intracellular calcium release under carbachol stimulation, and interacting with $G\alpha_{q/11}$ and RNAPII. These divergent effects in signaling pathways and interactions related to single nucleotide mutations suggests there may be wider differences. The use of APEX2 would enable us to study more widespread proteome differences instead of studying single pathways or interactions.

4.2.4 Caspex

Caspex is the fusion of a catalytically dead RNA-guided endonuclease (dCas9) and APEX2 (150). Using this tool, genome-based interactomes of loci of interests can be studied. Since previous studies in our lab has demonstrated occupancy of $G\beta_1$ at various promoters, targeting of Caspex to specific gene loci could help in determining association or not of $G\beta_1$ and other related G proteins. Single guide RNAs targeting the Akt1 promoter and GNB4 CpG island have been designed and stable cell lines have been generated for future proteomic screens.

4.2.5 Split-APEX2

HEK 293 cells express the majority of G protein isoforms: 12 of 16 Gα subtypes, all 5 Gβ subtypes and 10 of 12 Gγ subtypes (151). As previously mentioned, not all combinations of Gβγ dimers are possible, however because a large majority of the isoforms are expressed in the cells used in our experiments, we do not know specifically which Gβγ dimer pairs are being formed and the proteome differences related to varying Gβγ dimers. One way to ensure dimerization of specific Gβγ subtypes is through split-APEX2 (sAPEX2). Using this tool, we could fuse half of the APEX2

enzyme (AP) to Gβ and the 2nd half of the enzyme (EX) to Gγ. The enzyme can then be reconstituted with the interaction of Gβ and Gγ and remain functional under these conditions (152).

4.3 Conclusions

The results presented in this thesis demonstrate the use of APEX2 proximity-dependent labeling coupled to LC-MS as a means to study G protein signaling. Our lab has been particularly interested in signaling pathways involving Gβγ, specifically its role in regulating transcription in the nucleus. Previous work from our lab has demonstrated the localization of Gβγ to the promoters of over 700 genes, likely through the interaction between Gβγ and RNAPII. Over the years, studies have also demonstrated the interaction of Gβγ with a multitude of transcription factors.

To better understand the signaling dynamics of Gβγ, our lab has used TAP screens to study the Gβγ proteome and differences resulting from receptor stimulation as well as fractionated samples to identify cytoplasmic and nuclear proteins separately. Using TAP, stable interactors were identified, however there are transient interactions that are missed. For this reason, we sought to use proximity-dependent labeling, which does not depend on direct interaction between bait and prey proteins. We chose specifically APEX2 due to its high potential to capture spatiotemporal information about interacting proteins, requiring only a 1-minute labeling time.

Our APEX2 screens identified a wide array of proteins implicated in protein folding, chemotaxis and cell adhesion, transcription and translation. Although there is little evidence demonstrating Gβγ involvement in translation to date, TAP screens also identified candidate proteins for RNA binding and translation, and previous LC-MS screens done in yeast also show association of Gβ with EIF2 (143).

Future study should be focused on fractionated samples to understand specific cytoplasmic and nuclear protein interactions, studying proteome differences under stimulated conditions as well as using data analysis tools specific to proximity-dependent labeling assays such as SAINT to increase the probability of identifying true interactors. Validation of candidate DNA/RNA binding and mitochondrial interactors could provide insight into novel roles for Gβγ at diverse cellular compartments.

REFERENCES

1. Lin WZ, Xiao X, Chou KC. GPCR-GIA: a web-server for identifying G-protein coupled receptors and their families with grey incidence analysis. Protein Eng Des Sel. 2009 Nov;22(11):699-705.
2. Pierce KL, Premont RT, Lefkowitz RJ. Seven-transmembrane receptors. Nat Rev Mol Cell Biol. 2002 Sep;3(9):639-50. Review.
3. Schiöth HB, Fredriksson R. The GRAFS classification system of G-protein coupled receptors in comparative perspective. Gen Comp Endocrinol. 2005 May 15;142(1-2):94-101. Epub 2005 Feb 5. Review.
4. Stevens RC, Cherezov V, Katritch V, Abagyan R, Kuhn P, Rosen H, Wüthrich K. The GPCR Network: a large-scale collaboration to determine human GPCR structure and function. Nat Rev Drug Discov. 2013 Jan;12(1):25-34. Epub 2012 Dec 14. Review.
5. Hauser AS, Attwood MM, Rask-Andersen M, Schiöth HB, Gloriam DE. Trends in GPCR drug discovery: new agents, targets and indications. Nat Rev Drug Discov. 2017 Dec;16(12):829-842. Epub 2017 Oct 27. Review.
6. Hu GM, Mai TL, Chen CM. Visualizing the GPCR Network: Classification and Evolution. Sci Rep. 2017 Nov 14;7(1):15495.
7. Siderovski DP, Willard FS. The GAPs, GEFs, and GDIs of heterotrimeric G-protein alpha subunits. Int J Biol Sci. 2005 Apr 1;1(2):51-66.
8. Higashijima T, Ferguson KM, Sternweis PC, Smigel MD, Gilman AG. Effects of Mg2+ and the beta gamma-subunit complex on the interactions of guanine nucleotides with G proteins. J Biol Chem. 1987 Jan 15;262(2):762-6.
9. Arshavsky VY, Lamb TD, Pugh EN Jr. G proteins and phototransduction. Annu Rev Physiol. 2002;64:153-87. Review.
10. Birnbaumer L. The discovery of signal transduction by G proteins: a personal account and an overview of the initial findings and contributions that led to our present understanding. Biochim Biophys Acta. 2007 Apr;1768(4):756-71. Epub 2006 Oct 4.

11. Milligan G, Kostenis E. Heterotrimeric G-proteins: a short history. Br J Pharmacol. 2006 Jan;147 Suppl 1:S46-55. Review.

12. Martin RD, Sun Y, Bourque K, Audet N, Inoue A, Tanny JC, Hébert TE. Receptor-and cellular compartment-specific activation of the cAMP/PKA pathway by α(1)-adrenergic and ETA endothelin receptors. Cell Signal. 2018 Apr;44:43-50.

13. Watson AJ, Katz A, Simon MI. A fifth member of the mammalian G-protein beta-subunit family. Expression in brain and activation of the beta 2 isotype of phospholipase C. J Biol Chem. 1994 Sep 2;269(35):22150-6.

14. Khan SM, Sleno R, Gora S, Zylbergold P, Laverdure JP, Labbé JC, Miller GJ, Hébert TE. The expanding roles of Gβγ subunits in G protein-coupled receptor signaling and drug action. Pharmacol Rev. 2013 Feb 13;65(2):545-77.

15. Simonds WF, Zhang JH. New dimensions in G protein signalling: G beta 5 and the RGS proteins. Pharm Acta Helv. 2000 Mar;74(2-3):333-6. Review.

16. Spring DJ, Neer EJ. A 14-amino acid region of the G protein gamma subunit is sufficient to confer selectivity of gamma binding to the beta subunit. J Biol Chem. 1994 Sep 9;269(36):22882-6.

17. Pronin AN, Gautam N. Interaction between G-protein beta and gamma subunit types is selective. Proc Natl Sci USA. 1992 Jul;89:6220-6224.

18. Lindorfer MA, Myung CS, Savino Y, Yasuda H, Khazan R, Garrison JC. Differential activity of the G protein beta5 gamma2 subunit at receptors and effectors. J Biol Chem. 1998 Dec 18;273(51):34429-36.

19. Wettschureck N, Offermanns S. Mammalian G proteins and their cell type specific functions. Physiol Rev. 2005 Oct;85(4):1159-204. Review.

20. Cismowski MJ, Takesono A, Ma C, Lizano JS, Xie X, Fuernkranz H, Lanier SM, Duzic E. Genetic screens in yeast to identify mammalian nonreceptor modulators of G-protein signaling. Nat Biotechnol. 1999 Sep;17(9):878-83.

21. Cismowski MJ, Takesono A, Bernard ML, Duzic E, Lanier SM. Receptor-independent activators of heterotrimeric G-proteins. Life Sci. 2001 Apr 6;68(19-20):2301-8. Review.

22. Blumer JB, Cismowski MJ, Sato M, Lanier SM. AGS proteins: receptor-independent activators of G-protein signaling. Trends Pharmacol Sci. 2005 Sep;26(9):470-6. Review.

23. Lanier SM. AGS proteins, GPR motifs and the signals processed by heterotrimeric G proteins. Biol Cell. 2004 Jun;96(5):369-72. Review.

24. Kimura N. Role of Nucleoside Diphosphate Kinase in G-Protein Action. In: Dickey B.F., Birnbaumer L. (eds) GTPases in Biology II. Handbook of Experimental Pharmacology. 1993;vol 108 / 2.

25. Hippe HJ, Lutz S, Cuello F, Knorr K, Vogt A, Jakobs KH, Wieland T, Niroomand F. Activation of heterotrimeric G proteins by a high energy phosphate transfer via nucleoside diphosphate kinase (NDPK) B and Gbeta subunits. Specific activation of Gsalpha by an NDPK B.Gbetagamma complex in H10 cells. J Biol Chem. 2003 Feb 28;278(9):7227-33. Epub 2002 Dec 16.

26. Hippe HJ, Luedde M, Lutz S, Koehler H, Eschenhagen T, Frey N, Katus HA, Wieland T, Niroomand F. Regulation of cardiac cAMP synthesis and contractility by nucleoside diphosphate kinase B/G protein beta gamma dimer complexes. Circ Res. 2007 Apr 27;100(8):1191-9. Epub 2007 Mar 15.

27. Wieland T. Interaction of nucleoside diphosphate kinase B with heterotrimeric G protein betagamma dimers: consequences on G protein activation and stability. Naunyn Schmiedebergs Arch Pharmacol. 2007 Feb;374(5-6):373-83. Epub 2007 Jan 3. Review.

28. Hippe HJ, Wolf NM, Abu-Taha I, Mehringer R, Just S, Lutz S, Niroomand F, Postel EH, Katus HA, Rottbauer W, Wieland T. The interaction of nucleoside diphosphate kinase B with Gbetagamma dimers controls heterotrimeric G protein function. Proc Natl Acad Sci U S A. 2009 Sep 22;106(38):16269-74. Epub 2009 Sep 4.

29. Berman DM, Wilkie TM, Gilman AG. GAIP and RGS4 are GTPase-activating proteins for the Gi subfamily of G protein alpha subunits. Cell. 1996 Aug 9;86(3):445-52.

30. Watson N, Linder ME, Druey KM, Kehrl JH, Blumer KJ. RGS family members: GTPase-activating proteins for heterotrimeric G-protein alpha-subunits. Nature. 1996 Sep 12;383(6596):172-5.

31. Hepler JR, Berman DM, Gilman AG, Kozasa T. RGS4 and GAIP are GTPase-activating proteins for Gq alpha and block activation of phospholipase C beta by gamma-thio-GTP-Gq alpha. Proc Natl Acad Sci U S A. 1997 Jan 21;94(2):428-32.

32. De Vries L, Gist Farquhar M. RGS proteins: more than just GAPs for heterotrimeric G proteins. Trends Cell Biol. 1999 Apr;9(4):138-44. Review.

33. Hepler JR. RGS protein and G protein interactions: a little help from their friends. Mol Pharmacol. 2003 Sep;64(3):547-9. Review.

34. O'Brien JB, Wilkinson JC, Roman DL. Regulator of G protein signaling (RGS) proteins as drug targets: Progress and future potentials. J Biol Chem. 2019 Dec 6;294(49):18571-18585.

35. Casey PJ. Mechanisms of protein prenylation and role in G protein function. Biochem Soc Trans. 1995 Feb;23(1):161-6. Review.

36. Fukada Y. Prenylation and carboxylmethylation of G-protein gamma subunit. Methods Enzymol. 1995;250:91-105. Review.

37. Clarke S. Protein isoprenylation and methylation at carboxyl-terminal cysteine residues. Annu Rev Biochem. 1992;61:355-86. Review.

38. Muntz KH, Sternweis PC, Gilman AG, Mumby SM. Influence of gamma subunit prenylation on association of guanine nucleotide-binding regulatory proteins withbmembranes. Mol Biol Cell. 1992 Jan;3(1):49-61.

39. Hancock JF, Paterson H, Marshall CJ. A polybasic domain or palmitoylation is required in addition to the CAAX motif to localize p21ras to the plasma membrane. Cell. 1990 Oct 5;63(1):133-9.

40. Iiri T, Backlund PS Jr, Jones TL, Wedegaertner PB, Bourne HR. Reciprocal regulation of Gs alpha by palmitate and the beta gamma subunit. Proc Natl Acad Sci U S A. 1996 Dec 10;93(25):14592-7.

41. Morales J, Fishburn CS, Wilson PT, Bourne HR. Plasma membrane localization of G alpha z requires two signals. Mol Biol Cell. 1998 Jan;9(1):1-14.

42. Fishburn CS, Herzmark P, Morales J, Bourne HR. Gbetagamma and palmitate target newly synthesized Galphaz to the plasma membrane. J Biol Chem. 1999 Jun 25;274(26):18793-800.

43. Evanko DS, Thiyagarajan MM, Wedegaertner PB. Interaction with Gbetagamma is required for membrane targeting and palmitoylation of Galpha(s) and Galpha(q). J Biol Chem. 2000 Jan 14;275(2):1327-36.

44. Michaelson D, Ahearn I, Bergo M, Young S, Philips M. Membrane trafficking of heterotrimeric G proteins via the endoplasmic reticulum and Golgi. Mol Biol Cell. 2002 Sep;13(9):3294-302.

45. Fukada Y, Matsuda T, Kokame K, Takao T, Shimonishi Y, Akino T, Yoshizawa T. Effects of carboxyl methylation of photoreceptor G protein gamma-subunit in visual transduction. J Biol Chem. 1994 Feb 18;269(7):5163-70.

46. Kino T, Kozasa T, Chrousos GP. Statin-induced blockade of prenylation alters nucleocytoplasmic shuttling of GTP-binding proteins gamma2 and beta2 and enhances their suppressive effect on glucocorticoid receptor transcriptional activity. Eur J Clin Invest. 2005 Aug;35(8):508-13.

47. Wedegaertner PB, Wilson PT, Bourne HR. Lipid modifications of trimeric G proteins. J Biol Chem. 1995 Jan 13;270(2):503-6. Review.

48. Escribá PV, Wedegaertner PB, Goñi FM, Vögler O. Lipid-protein interactions in GPCR-associated signaling. Biochim Biophys Acta. 2007 Apr;1768(4):836-52.

49. Saini DK, Kalyanaraman V, Chisari M, Gautam N. A family of G protein βγ subunits translocate reversibly from the plasma membrane to endomembranes on receptor activation. J Biol Chem. 2007 Aug 17;282(33):24099-108.

50. Ajith Karunarathne WK, O'Neill PR, Martinez-Espinosa PL, Kalyanaraman V, Gautam N. All G protein βγ complexes are capable of translocation on receptor activation. Biochem Biophys Res Commun. 2012 May 11;421(3):605-11.

51. Chisari M, Saini DK, Cho JH, Kalyanaraman V, Gautam N. G protein subunit dissociation and translocation regulate cellular response to receptor stimulation. PLoS One. 2009 Nov 11;4(11):e7797.

52. Dietrich A, Meister M, Brazil D, Camps M, Gierschik P. Stimulation of phospholipase C-beta 2 by recombinant guanine-nucleotide-binding protein beta gamma dimers produced in a baculovirus/insect cell expression system. Requirement of gamma-subunit isoprenylation for stimulation of phospholipase C. Eur J Biochem. 1994 Jan 15;219(1-2):171-8.

53. Dietrich A, Brazil D, Jensen ON, Meister M, Schrader M, Moomaw JF, Mann M, Illenberger D, Gierschik P. Isoprenylation of the G protein gamma subunit is both necessary and sufficient for beta gamma dimer-mediated stimulation of phospholipase C. Biochemistry. 1996 Dec 3;35(48):15174-82

54. Fogg VC, Azpiazu I, Linder ME, Smrcka A, Scarlata S, Gautam N. Role of the gamma subunit prenyl moiety in G protein beta gamma complex interaction with phospholipase Cbeta. J Biol Chem. 2001 Nov 9;276(45):41797-802.

55. Logothetis DE, Kurachi Y, Galper J, Neer EJ, Clapham DE. The beta gamma subunits of GTP-binding proteins activate the muscarinic K+ channel in heart. Nature. 1987 Jan 22-28;325(6102):321-6.

56. Schoots O, Wilson JM, Ethier N, Bigras E, Hebert TE, Van Tol HH. Co-expression of human Kir3 subunits can yield channels with different functional properties. Cell Signal. 1999 Dec;11(12):871-83.

57. Huang CL, Slesinger PA, Casey PJ, Jan YN, Jan LY. Evidence that direct binding of G beta gamma to the GIRK1 G protein-gated inwardly rectifying K+ channel is important for channel activation. Neuron. 1995 Nov;15(5):1133-43.

58. Krapivinsky G, Krapivinsky L, Wickman K, Clapham DE. G beta gamma binds directly to the G protein-gated K+ channel, IKACh. J Biol Chem. 1995 Dec 8;270(49):29059-62.

59. Mark MD, Herlitze S. G-protein mediated gating of inward-rectifier K+ channels. Eur J Biochem. 2000 Oct;267(19):5830-6. Review.

60. Zylbergold P, Ramakrishnan N, Hebert T. The role of G proteins in assembly and function of Kir3 inwardly rectifying potassium channels. Channels (Austin). 2010 Sep-Oct;4(5):411-21.

61. Peng L, Mirshahi T, Zhang H, Hirsch JP, Logothetis DE. Critical determinants of the G protein gamma subunits in the Gbetagamma stimulation of G protein-activated inwardly rectifying potassium (GIRK) channel activity. J Biol Chem. 2003 Dec 12;278(50):50203-11.

62. Mirshahi T, Robillard L, Zhang H, Hébert TE, Logothetis DE. Gbeta residues that do not interact with Galpha underlie agonist-independent activity of K+ channels. J Biol Chem. 2002 Mar 1;277(9):7348-55.

63. Diversé-Pierluissi M, Goldsmith PK, Dunlap K. Transmitter-mediated inhibition of N-type calcium channels in sensory neurons involves multiple GTP-binding proteins and subunits. Neuron. 1995 Jan;14(1):191-200.

64. De Waard M, Hering J, Weiss N, Feltz A. How do G proteins directly control neuronal Ca2+ channel function? Trends Pharmacol Sci. 2005 Aug;26(8):427-36. Review.

65. Ikeda SR. Voltage-dependent modulation of N-type calcium channels by G-protein beta gamma subunits. Nature. 1996 Mar 21;380(6571):255-8.

66. Zhou JY, Siderovski DP, Miller RJ. Selective regulation of N-type Ca channels by different combinations of G-protein beta/gamma subunits and RGS proteins. J Neurosci. 2000 Oct 1;20(19):7143-8.

67. Feng ZP, Arnot MI, Doering CJ, Zamponi GW. Calcium channel beta subunits differentially regulate the inhibition of N-type channels by individual Gbeta isoforms. J Biol Chem. 2001 Nov 30;276(48):45051-8.

68. Tang WJ, Gilman AG. Adenylyl cyclases. Cell. 1992 Sep 18;70(6):869-72. Review.

69. Sadana R, Dascal N, Dessauer CW. N terminus of type 5 adenylyl cyclase scaffolds Gs heterotrimer. Mol Pharmacol. 2009 Dec;76(6):1256-64.

70. Sadana R, Dessauer CW. Physiological roles for G protein-regulated adenylyl cyclase isoforms: insights from knockout and overexpression studies. Neurosignals. 2009;17(1):5-22.

71. Tang WJ, Hurley JH. Catalytic mechanism and regulation of mammalian adenylyl cyclases. Mol Pharmacol. 1998 Aug;54(2):231-40. Review.

72. Hurley JH. Structure, mechanism, and regulation of mammalian adenylyl cyclase. J Biol Chem. 1999 Mar 19;274(12):7599-602. Review.

73. Gao X, Sadana R, Dessauer CW, Patel TB. Conditional stimulation of type V and VI adenylyl cyclases by G protein betagamma subunits. J Biol Chem. 2007 Jan 5;282(1):294-302.

74. Gresset A, Sondek J, Harden TK. The phospholipase C isozymes and their regulation. Subcell Biochem. 2012;58:61-94.

75. Camps M, Carozzi A, Schnabel P, Scheer A, Parker PJ, Gierschik P. Isozyme-selective stimulation of phospholipase C-beta 2 by G protein beta gamma-subunits. Nature. 1992 Dec 17;360(6405):684-6.

76. Clapham DE, Neer EJ. G protein beta gamma subunits. Annu Rev Pharmacol Toxicol. 1997;37:167-203. Review.

77. Philip F, Kadamur G, Silos RG, Woodson J, Ross EM. Synergistic activation of phospholipase C-beta3 by Galpha(q) and Gbetagamma describes a simple two-state coincidence detector. Curr Biol. 2010 Aug 10;20(15):1327-35.

78. Wing MR, Houston D, Kelley GG, Der CJ, Siderovski DP, Harden TK. Activation of phospholipase C-epsilon by heterotrimeric G protein betagamma-subunits. J Biol Chem. 2001 Dec 21;276(51):48257-61.

79. Zhou Y, Wing MR, Sondek J, Harden TK. Molecular cloning and characterization of PLC-eta2. Biochem J. 2005 Nov 1;391(Pt 3):667-76.

80. Jean S, Kiger AA. Classes of phosphoinositide 3-kinases at a glance. J Cell Sci. 2014 Mar 1;127(Pt 5):923-8.

81. Vanhaesebroeck B, Leevers SJ, Panayotou G, Waterfield MD. Phosphoinositide 3-kinases: a conserved family of signal transducers. Trends Biochem Sci. 1997 Jul;22(7):267-72. Review.

82. Leopoldt D, Hanck T, Exner T, Maier U, Wetzker R, Nürnberg B. Gbetagamma stimulates phosphoinositide 3-kinase-gamma by direct interaction with two domains of the catalytic p110 subunit. J Biol Chem. 1998 Mar 20;273(12):7024-9.

83. Stoyanov B, Volinia S, Hanck T, Rubio I, Loubtchenkov M, Malek D, Stoyanova S, Vanhaesebroeck B, Dhand R, Nürnberg B, et al. Cloning and characterization of a G protein-activated human phosphoinositide-3 kinase. Science. 1995 Aug 4;269(5224):690-3.

84. Stephens LR, Eguinoa A, Erdjument-Bromage H, Lui M, Cooke F, Coadwell J, Smrcka AS, Thelen M, Cadwallader K, Tempst P, Hawkins PT. The G beta gamma sensitivity of a PI3K is dependent upon a tightly associated adaptor, p101. Cell. 1997 Apr 4;89(1):105-14.

85. Brock C, Schaefer M, Reusch HP, Czupalla C, Michalke M, Spicher K, Schultz G, Nürnberg B. Roles of G beta gamma in membrane recruitment and activation of p110 gamma/p101 phosphoinositide 3-kinase gamma. J Cell Biol. 2003 Jan 6;160(1):89-99.

86. Maier U, Babich A, Nürnberg B. Roles of non-catalytic subunits in gbetagamma-induced activation of class I phosphoinositide 3-kinase isoforms beta and gamma. J Biol Chem. 1999 Oct 8;274(41):29311-7.

87. Dbouk HA, Vadas O, Shymanets A, Burke JE, Salamon RS, Khalil BD, Barrett MO, Waldo GL, Surve C, Hsueh C, Perisic O, Harteneck C, Shepherd PR, Harden TK, Smrcka AV, Taussig R, Bresnick AR, Nürnberg B, Williams RL, Backer JM. G protein-coupled receptor-mediated activation of p110β by Gβγ is required for cellular transformation and invasiveness. Sci Signal. 2012 Dec 4;5(253):ra89.

88. Maier U, Babich A, Macrez N, Leopoldt D, Gierschik P, Illenberger D, Nurnberg B. Gbeta 5gamma 2 is a highly selective activator of phospholipid-dependent enzymes. J Biol Chem. 2000 May 5;275(18):13746-54.

89. Plotnikov A, Zehorai E, Procaccia S, Seger R. The MAPK cascades: signaling components, nuclear roles and mechanisms of nuclear translocation. Biochim Biophys Acta. 2011 Sep;1813(9):1619-33.

90. Crespo P, Xu N, Simonds WF, Gutkind JS. Ras-dependent activation of MAP kinase pathway mediated by G-protein beta gamma subunits. Nature. 1994 Jun 2;369(6479):418-20.

91. Faure M, Voyno-Yasenetskaya TA, Bourne HR. cAMP and beta gamma subunits of heterotrimeric G proteins stimulate the mitogen-activated protein kinase pathway in COS-7 cells. J Biol Chem. 1994 Mar 18;269(11):7851-4.

92. Koch WJ, Hawes BE, Allen LF, Lefkowitz RJ. Direct evidence that Gi-coupled receptor stimulation of mitogen-activated protein kinase is mediated by G beta gamma activation of p21ras. Proc Natl Acad Sci U S A. 1994 Dec 20;91(26):12706-10.

93. Coso OA, Teramoto H, Simonds WF, Gutkind JS. Signaling from G protein-coupled receptors to c-Jun kinase involves beta gamma subunits of heterotrimeric G proteins acting on a Ras and Rac1-dependent pathway. J Biol Chem. 1996 Feb 23;271(8):3963-6.

94. Yamauchi J, Nagao M, Kaziro Y, Itoh H. Activation of p38 mitogen-activated protein kinase by signaling through G protein-coupled receptors. Involvement of Gbetagamma and Galphaq/11 subunits. J Biol Chem. 1997 Oct 31;272(44):27771-7.

95. Gurevich VV, Gurevich EV. GPCR Signaling Regulation: The Role of GRKs and Arrestins. Front Pharmacol. 2019 Feb 19;10:125.

96. Pitcher JA, Touhara K, Payne ES, Lefkowitz RJ. Pleckstrin homology domain-mediated membrane association and activation of the beta-adrenergic receptor kinase requires coordinate interaction with G beta gamma subunits and lipid. J Biol Chem. 1995 May 19;270(20):11707-10.

97. Daaka Y, Pitcher JA, Richardson M, Stoffel RH, Robishaw JD, Lefkowitz RJ. Receptor and G betagamma isoform-specific interactions with G protein-coupled receptor kinases. Proc Natl Acad Sci U S A. 1997 Mar 18;94(6):2180-5.

98. Raveh A, Cooper A, Guy-David L, Reuveny E. Nonenzymatic rapid control of GIRK channel function by a G protein-coupled receptor kinase. Cell. 2010 Nov 24;143(5):750-60.

99. Park JG, Muise A, He GP, Kim SW, Ro HS. Transcriptional regulation by the gamma5 subunit of a heterotrimeric G protein during adipogenesis. EMBO J. 1999 Jul 15;18(14):4004-12.

100. Zhang JH, Barr VA, Mo Y, Rojkova AM, Liu S, Simonds WF. Nuclear localization of G protein beta 5 and regulator of G protein signaling 7 in neurons and brain. J Biol Chem. 2001 Mar 30;276(13):10284-9.

101. Rojkova AM, Woodard GE, Huang TC, Combs CA, Zhang JH, Simonds WF. Ggamma subunit-selective G protein beta 5 mutant defines regulators of G protein signaling protein binding requirement for nuclear localization. J Biol Chem. 2003 Apr 4;278(14):12507-12.

102. Spiegelberg BD, Hamm HE. G betagamma binds histone deacetylase 5 (HDAC5) and inhibits its transcriptional co-repression activity. J Biol Chem. 2005 Dec 16;280(50):41769-76.

103. Robitaille M, Gora S, Wang Y, Goupil E, Pétrin D, Del Duca D, Villeneuve LR, Allen BG, Laporte SA, Bernard DJ, Hébert TE. Gbetagamma is a negative regulator of AP-1 mediated transcription. Cell Signal. 2010 Aug;22(8):1254-66.

104. Yuen JW, Poon LS, Chan AS, Yu FW, Lo RK, Wong YH. Activation of STAT3 by specific Galpha subunits and multiple Gbetagamma dimers. Int J Biochem Cell Biol. 2010 Jun;42(6):1052-9.

105. Georganta EM, Agalou A, Georgoussi Z. Multi-component signaling complexes of the delta-opioid receptor with STAT5B and G proteins. Neuropharmacology. 2010 Sep;59(3):139-48.

106. Bhatnagar A, Unal H, Jagannathan R, Kaveti S, Duan ZH, Yong S, Vasanji A, Kinter M, Desnoyer R, Karnik SS. Interaction of G-protein βγ complex with chromatin modulates GPCR-dependent gene regulation. PLoS One. 2013;8(1):e52689.

107. Khan SM, Min A, Gora S, et al. Gβ4γ1 as a modulator of M3 muscarinic receptor signalling and novel roles of Gβ1 subunits in the modulation of cellular signalling. *Cell Signal*. 2015;27(8):1597-1608.

108. Khan SM, Martin RD, Bouazza C, Jones-Tabah J, Zhang A, MacKinnon S, Trieu P, Gora S, Clarke PBS, Tanny JC, Hébert TE. A novel interaction between Gbetagamma and RNA polymerase II regulates cardiac fibrosis. BiorXiv Preprint 2018.

109. Jamora C, Takizawa PA, Zaarour RF, Denesvre C, Faulkner DJ, Malhotra V. Regulation of Golgi structure through heterotrimeric G proteins. Cell. 1997 Nov 28;91(5):617-26.

110. Jamora C, Yamanouye N, Van Lint J, Laudenslager J, Vandenheede JR, Faulkner DJ, Malhotra V. Gbetagamma-mediated regulation of Golgi organization is through the direct activation of protein kinase D. Cell. 1999 Jul 9;98(1):59-68.

111. Saini DK, Karunarathne WK, Angaswamy N, Saini D, Cho JH, Kalyanaraman V, Gautam N. Regulation of Golgi structure and secretion by receptor-induced G protein βγ complex translocation. Proc Natl Acad Sci U S A. 2010 Jun 22;107(25):11417-22.

112. Díaz Añel AM, Malhotra V. PKCeta is required for beta1gamma2/beta3gamma2- and PKD-mediated transport to the cell surface and the organization of the Golgi apparatus. J Cell Biol. 2005 Apr 11;169(1):83-91. Erratum in: J Cell Biol. 2005 May 9;169(3):539.

113. Jiang Y, Xie X, Zhang Y, Luo X, Wang X, Fan F, Zheng D, Wang Z, Chen Y. Regulation of G-protein signaling by RKTG via sequestration of the G betagamma subunit to the Golgi apparatus. Mol Cell Biol. 2010 Jan;30(1):78-90.

114. Malik S, deRubio RG, Trembley M, Irannejad R, Wedegaertner PB, Smrcka AV. G protein βγ subunits regulate cardiomyocyte hypertrophy through a perinuclear Golgi phosphatidylinositol 4-phosphate hydrolysis pathway. Mol Biol Cell. 2015 Mar 15;26(6):1188-98.

115. Scott I, Youle RJ. Mitochondrial fission and fusion. *Essays Biochem*. 2010;47:85-98. doi:10.1042/bse0470085

116. Zhang J, Liu W, Liu J, Xiao W, Liu L, Jiang C, Sun X, Liu P, Zhu Y, Zhang C, Chen Q. G-protein β2 subunit interacts with mitofusin 1 to regulate mitochondrial fusion. Nat Commun. 2010 Oct 19;1:101.

117. Benincá C, Planagumà J, de Freitas Shuck A, Acín-Perez R, Muñoz JP, de Almeida MM, Brown JH, Murphy AN, Zorzano A, Enríquez JA, Aragay AM. A new non-canonical pathway of Gα(q) protein regulating mitochondrial dynamics and bioenergetics. Cell Signal. 2014 May;26(5):1135-46.

118. Gibbs WS, Garrett SM, Beeson CC, Schnellmann RG. Identification of dual mechanisms mediating 5-hydroxytryptamine receptor 1F-induced mitochondrial biogenesis. Am J Physiol Renal Physiol. 2018 Feb 1;314(2):F260-F268.

119. Cikic S, Chandra PK, Harman JC, Rutkai I, Katakam PVG, Guidry JJ, Gidday JM, Busija DW. Sexual differences in mitochondrial proteins in rat cerebral microvessels: a proteomic approach. BiorXiv Preprint 2019.

120. Surve CR, Lehmann D, Smrcka AV. A chemical biology approach demonstrates G protein βγ subunits are sufficient to mediate directional neutrophil chemotaxis. J Biol Chem. 2014 Jun 20;289(25):17791-801.

121. Wu D. Signaling mechanisms for regulation of chemotaxis. Cell Res. 2005 Jan;15(1):52-6. Review.

122. Li Z, Hannigan M, Mo Z, Liu B, Lu W, Wu Y, Smrcka AV, Wu G, Li L, Liu M, Huang CK, Wu D. Directional sensing requires G beta gamma-mediated PAK1 and PIX alpha-dependent activation of Cdc42. Cell. 2003 Jul 25;114(2):215-27.

123. Lyda JK, Tan ZL, Rajah A, Momi A, Mackay L, Brown CM, Khadra A. Rac activation is key to cell motility and directionality: An experimental and modelling investigation. Comput Struct Biotechnol J. 2019 Nov 7;17:1436-1452.

124. Barber MA, Donald S, Thelen S, Anderson KE, Thelen M, Welch HC. Membrane translocation of P-Rex1 is mediated by G protein betagamma subunits and phosphoinositide 3-kinase. J Biol Chem. 2007 Oct 12;282(41):29967-76.

125. Kerr JS, Jacques RO, Moyano Cardaba C, Tse T, Sexton D, Mueller A. Differential regulation of chemotaxis: role of Gβγ in chemokine receptor-induced cell migration. Cell Signal. 2013 Apr;25(4):729-35.

126. N'Diaye EN, Brown EJ. The ubiquitin-related protein PLIC-1 regulates heterotrimeric G protein function through association with Gbetagamma. J Cell Biol. 2003 Dec 8;163(5):1157-65.

127. Chen S, Lin F, Shin ME, Wang F, Shen L, Hamm HE. RACK1 regulates directional cell migration by acting on G betagamma at the interface with its effectors PLC beta and PI3K gamma. Mol Biol Cell. 2008 Sep;19(9):3909-22.

128. Ahmed SM, Daulat AM, Meunier A, Angers S. G protein betagamma subunits regulate cell adhesion through Rap1a and its effector Radil. J Biol Chem. 2010 Feb 26;285(9):6538-51.

129. Speirs CK, Jernigan KK, Kim SH, Cha YI, Lin F, Sepich DS, DuBois RN, Lee E, Solnica-Krezel L. Prostaglandin Gbetagamma signaling stimulates gastrulation movements by limiting cell adhesion through Snai1a stabilization. Development. 2010 Apr;137(8):1327-37.

130. Dave RH, Saengsawang W, Yu JZ, Donati R, Rasenick MM. Heterotrimeric G-proteins interact directly with cytoskeletal components to modify microtubule-dependent cellular processes. Neurosignals. 2009;17(1):100-8.

131. Mi H, Muruganujan A, Casagrande JT, Thomas PD. Large-scale gene function analysis with the PANTHER classification system. *Nat Protoc*. 2013;8(8):1551-1566.

132. Mi H, Muruganujan A, Huang X, et al. Protocol Update for large-scale genome and gene function analysis with the PANTHER classification system (v.14.0). *Nat Protoc*. 2019;14(3):703-721.

133. Pomaznoy M, Ha B, Peters B. GOnet: a tool for interactive Gene Ontology analysis. *BMC Bioinformatics*. 2018;19(1):470.

134. Hung V, Udeshi ND, Lam SS, et al. Spatially resolved proteomic mapping in living cells with the engineered peroxidase APEX2. *Nat Protoc*. 2016;11(3):456-475.

135. Molecular Probes. *SYPRO Ruby Protein Gel Stain*. Product information. 2007.

136. O'Connell JD, Paulo JA, O'Brien JJ, Gygi SP. Proteome-Wide Evaluation of Two Common Protein Quantification Methods. *J Proteome Res*. 2018;17(5):1934-1942.

137. Cox J, Hein MY, Luber CA, Paron I, Nagaraj N, Mann M. Accurate proteome-wide label-free quantification by delayed normalization and maximal peptide ratio extraction, termed MaxLFQ. *Mol Cell Proteomics*. 2014;13(9):2513-2526.

138. Wells CA, Dingus J, Hildebrandt JD. Role of the chaperonin CCT/TRiC complex in G protein betagamma-dimer assembly. *J Biol Chem*. 2006;281(29):20221-20232.

139. Inanobe A, Takahashi K, Katada T. Association of the beta gamma subunits of trimeric GTP-binding proteins with 90-kDa heat shock protein, hsp90. *J Biochem*. 1994;115(3):486-492.

140. Lukov GL, Baker CM, Ludtke PJ, et al. Mechanism of assembly of G protein betagamma subunits by protein kinase CK2-phosphorylated phosducin-like protein and the cytosolic chaperonin complex. *J Biol Chem*. 2006;281(31):22261-22274.

141. Sahlan M, Zako T, Yohda M. Prefoldin, a jellyfish-like molecular chaperone: functional cooperation with a group II chaperonin and beyond. *Biophys Rev*. 2018;10(2):339-345.

142. Noritake J, Watanabe T, Sato K, Wang S, Kaibuchi K. IQGAP1: a key regulator of adhesion and migration.

143. Ho Y, Gruhler A, Heilbut A, et al. Systematic identification of protein complexes in Saccharomyces cerevisiae by mass spectrometry. *Nature*. 2002;415(6868):180-183.

144. Zheng S, Abreu N, Levitz J, Kruse AC. Structural basis for KCTD-mediated rapid desensitization of $GABA_B$ signalling. *Nature*. 2019;567(7746):127-131.

145. Mellacheruvu D, Wright Z, Couzens AL, et al. The CRAPome: a contaminant repository for affinity purification-mass spectrometry data. *Nat Methods*. 2013;10(8):730-736.

146. Choi H, Larsen B, Lin ZY, et al. SAINT: probabilistic scoring of affinity purification-mass spectrometry data. *Nat Methods*. 2011;8(1):70-73.

147. Teo G, Liu G, Zhang J, Nesvizhskii AI, Gingras AC, Choi H. SAINTexpress: improvements and additional features in Significance Analysis of INTeractome software. *J Proteomics*. 2014;100:37-43.

148. Mick DU. Establishing Cell Culture-Based Experimental Setups for Proximity Labeling Using Ascorbate Peroxidase (APEX). *Methods Mol Biol*. 2019;2008:29-39.

149. Revah-Politi A, Sands TT, Colombo S, et al. GNB1 Encephalopathy. 2020 Mar 5. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]Accessed on 23 May 2020.

150. Myers SA, Wright J, Peckner R, Kalish BT, Zhang F, Carr SA. Discovery of proteins associated with a predefined genomic locus via dCas9-APEX-mediated proximity labeling. *Nat Methods*. 2018;15(6):437-439.

151. Atwood BK, Lopez J, Wager-Miller J, Mackie K, Straiker A. Expression of G protein-coupled receptors and related proteins in HEK293, AtT20, BV2, and N18 cell lines as revealed by microarray analysis. *BMC Genomics*. 2011;12:14.

152. Han Y, Branon TC, Martell JD, et al. Directed Evolution of Split APEX2 Peroxidase [published correction appears in ACS Chem Biol. 2019 Dec 20;14(12):2942]. *ACS Chem Biol*. 2019;14(4):619-635.

153. Campden R, Audet N, Hébert TE. Nuclear G protein signaling: new tricks for old dogs. *J Cardiovasc Pharmacol*. 2015;65(2):110-122.

154. Campden R, Pétrin D, Robitaille M, et al. Tandem affinity purification to identify cytosolic and nuclear gβγ-interacting proteins. *Methods Mol Biol*. 2015;1234:161-184.

155. Hulsen T, de Vlieg J, Alkema W. BioVenn - a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams. *BMC Genomics*. 2008;9:488.

156. Young CL, Britton ZT, Robinson AS. Recombinant protein expression and purification: a comprehensive review of affinity tags and microbial applications. *Biotechnol J*. 2012;7(5):620-634.

157. PDB ID: 1OAG

Sharp KH, Mewies M, Moody PC, Raven EL. Crystal structure of the ascorbate peroxidase-ascorbate complex. *Nat Struct Biol*. 2003;10(4):303-307.

158. PDB ID: 3AH8

Nishimura A, Kitano K, Takasaki J, et al. Structural basis for the specific inhibition of heterotrimeric Gq protein by a small molecule. *Proc Natl Acad Sci U S A*. 2010;107(31):13666-13671.

159. Lam SS, Martell JD, Kamer KJ, et al. Directed evolution of APEX2 for electron microscopy and proximity labeling. *Nat Methods*. 2015;12(1):51-54.

160. The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC.

APPENDIX – All identified proteins organized by number of times identified and fold change

Table S1 – *High confidence interactors identified in the proteomic screens*

Proteins identified in 2 out of 3 experiments with a fold change of LFQ intensity from bait versus control samples ≥ 2.

Bait	Protein name	Gene ID
Gβ1	Beta-actin-like protein 2	ACTBL2
Gβ1	Arf-GAP domain and FG repeat-containing protein 1	AGFG1
Gβ1	Protein argonaute	AGO
Gβ1	Delta-1-pyrroline-5-carboxylate synthase;Glutamate 5-kinase;Gamma-glutamyl phosphate reductase	ALDH18A1
Gβ1	Ankyrin repeat domain-containing protein 17	ANKRD17
Gβ1	V-type proton ATPase catalytic subunit A	ATP6V1A
Gβ1	T-complex protein 1 subunit gamma	CCT3
Gβ1	T-complex protein 1 subunit delta	CCT4
Gβ1	T-complex protein 1 subunit zeta	CCT6A
Gβ1	Cold shock domain-containing protein E1	CSDE1
Gβ1	CTP synthase 1;CTP synthase	CTPS1
Gβ1	Pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	DHX15
Gβ1	Protein diaphanous homolog 1	DIAPH1
Gβ1	Exosome complex exonuclease RRP44	DIS3
Gβ1	Eukaryotic translation initiation factor 3 subunit F	EIF3F
Gβ1	Epiplakin	EPPK1
Gβ1	Glycine--tRNA ligase	GARS
Gβ1	HEAT repeat-containing protein 3	HEATR3
Gβ1	Histone H4	HIST1H4A
Gβ1	Putative heat shock protein HSP 90-beta 2	HSP90AB2P

Gβ1	Insulin-like growth factor 2 mRNA-binding protein 1	IGF2BP1
Gβ1	Importin subunit alpha-7	KPNA6
Gβ1	Leucine--tRNA ligase, cytoplasmic	LARS
Gβ1	Leucine-rich repeat-containing protein 40	LRRC40
Gβ1	Non-POU domain-containing octamer-binding protein	NONO
Gβ1	Puromycin-sensitive aminopeptidase	NPEPPS
Gβ1	Ribose-phosphate pyrophosphokinase 2;Ribose-phosphate pyrophosphokinase 1;Ribose-phosphate pyrophosphokinase 3	PRPS2;PRPS1;PRPS1L1
Gβ1	Protein PRRC2B	PRRC2B
Gβ1	E3 SUMO-protein ligase RanBP2	RANBP2
Gβ1	Arginine--tRNA ligase, cytoplasmic	RARS
Gβ1	60S ribosomal protein L13	RPL13
Gβ1	60S ribosomal protein L18a	RPL18A
Gβ1	60S ribosomal protein L4	RPL4
Gβ1	40S ribosomal protein S3a	RPS3A
Gβ1	5-3 exoribonuclease 1	XRN1
$G\beta_{1,}$ $G\gamma_5$	Alpha-actinin-4	ACTN4
$G\beta_{1,}$ $G\gamma_5$	Sodium/potassium-transporting ATPase subunit alpha-1;Sodium/potassium-transporting ATPase subunit alpha-3	ATP1A1;ATP1A3
$G\beta_{1,}$ $G\gamma_5$	Cullin-associated NEDD8-dissociated protein 1	CAND1
$G\beta_{1,}$ $G\gamma_5$	Histone-arginine methyltransferase CARM1	CARM1
$G\beta_{1,}$ $G\gamma_5$	T-complex protein 1 subunit theta	CCT8
$G\beta_{1,}$ $G\gamma_5$	Catenin delta-1	CTNND1
$G\beta_{1,}$ $G\gamma_5$	Cytoplasmic FMR1-interacting protein 1;Cytoplasmic FMR1-interacting protein 2	CYFIP1;CYFIP2
$G\beta_{1,}$ $G\gamma_5$	Desmoglein-2	DSG2
$G\beta_{1,}$ $G\gamma_5$	Ras GTPase-activating-like protein IQGAP1	IQGAP1
$G\beta_{1,}$ $G\gamma_5$	L-lactate dehydrogenase B chain;L-lactate dehydrogenase	LDHB
$G\beta_{1,}$ $G\gamma_5$	Condensin complex subunit 1	NCAPD2
$G\beta_{1,}$ $G\gamma_5$	Condensin complex subunit 2	NCAPH
$G\beta_{1,}$ $G\gamma_5$	Prohibitin-2	PHB2

$G\beta_{1,}G\gamma_5$	D-3-phosphoglycerate dehydrogenase	PHGDH
$G\beta_{1,}G\gamma_5$	40S ribosomal protein S9	RPS9
Gγ5	Alanine--tRNA ligase, cytoplasmic	AARS
Gγ5	ATP-binding cassette sub-family F member 2	ABCF2
Gγ5	Activator of 90 kDa heat shock protein ATPase homolog 1	AHSA1
Gγ5	UPF0488 protein C8orf33	C8orf33
Gγ5	Calcyclin-binding protein	CACYBP
Gγ5	F-actin-capping protein subunit alpha-1	CAPZA1
Gγ5	T-complex protein 1 subunit epsilon	CCT5
Gγ5	Cell division control protein 42 homolog	CDC42
Gγ5	Coatomer subunit beta	COPB1
Gγ5	Coronin-1C;Coronin	CORO1C
Gγ5	Copine-3	CPNE3
Gγ5	Catenin alpha-1	CTNNA1
Gγ5	Catenin beta-1	CTNNB1
Gγ5	Aspartate--tRNA ligase, cytoplasmic	DARS
Gγ5	Drebrin	DBN1
Gγ5	Dedicator of cytokinesis protein 7	DOCK7
Gγ5	Eukaryotic translation initiation factor 2 subunit 3;Putative eukaryotic translation initiation factor 2 subunit 3-like protein	EIF2S3;EIF2S3L
Gγ5	Protein 4.1	EPB41
Gγ5	Band 4.1-like protein 2	EPB41L2
Gγ5	Band 4.1-like protein 3;Band 4.1-like protein 3, N-terminally processed	EPB41L3
Gγ5	Ezrin	EZR
Gγ5	Guanine nucleotide-binding protein G(k) subunit alpha	GNAI3
Gγ5	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	GNB1
Gγ5	Gephyrin;Molybdopterin adenylyltransferase;Molybdopterin molybdenumtransferase	GPHN
Gγ5	Glycogen synthase kinase-3 beta	GSK3B
Gγ5	Eukaryotic peptide chain release factor GTP-binding subunit ERF3A	GSPT1;GSPT2

Gγ5	Histone H1.2;Histone H1.4;Histone H1.3;Histone H1t;Histone H1.1	HIST1H1C;HIST1H1E;HIST1H1D; HIST1H1T;HIST1H1A
Gγ5	HLA class I histocompatibility antigen, A-2 alpha chain	HLA-A
Gγ5	Heterogeneous nuclear ribonucleoprotein M	HNRNPM
Gγ5	Shootin-1	KIAA1598
Gγ5	E3 ubiquitin-protein ligase Midline-1	MID1
Gγ5	Afadin	MLLT4
Gγ5	C-1-tetrahydrofolate synthase, cytoplasmic	MTHFD1
Gγ5	Myosin light polypeptide 6;Myosin light chain 6B	MYL6;MYL6B
Gγ5	Nck-associated protein 1	NCKAP1
Gγ5	Glutamine--tRNA ligase	QARS
Gγ5	Ras-related protein Rab-5C	RAB5C
Gγ5	Ribosomal protein L15;60S ribosomal protein L15	RPL15
Gγ5	40S ribosomal protein S14	RPS14
Gγ5	Protein scribble homolog	SCRIB
Gγ5	Synaptosomal-associated protein;Synaptosomal-associated protein 23	SNAP23
Gγ5	Spectrin alpha chain, non-erythrocytic 1	SPTAN1
Gγ5	Spectrin beta chain, non-erythrocytic 1	SPTBN1
Gγ5	Stress-induced-phosphoprotein 1	STIP1
Gγ5	TBC1 domain family member 4	TBC1D4
Gγ5	Ubiquitin-conjugating enzyme E2 D2;Ubiquitin-conjugating enzyme E2 D3	UBE2D2;UBE2D3
Gγ5	Zinc finger CCCH-type antiviral protein 1	ZC3HAV1

Table S2 – *Low confidence interactors identified from proteomic screens*

Proteins identified in 2 out of 3 experiments with a fold change of LFQ intensity from bait versus control samples < 2

Bait	Protein name	Gene ID
Gβ1	CAD protein;Glutamine-dependent carbamoyl-phosphate synthase;Aspartate carbamoyltransferase	CAD
Gβ1	Cofilin-1	CFL1
Gβ1	Creatine kinase B-type	CKB
Gβ1	Cellular nucleic acid-binding protein	CNBP
Gβ1	Exportin-2	CSE1L
Gβ1	Catenin beta-1	CTNNB1
Gβ1	Probable ATP-dependent RNA helicase DDX17	DDX17
Gβ1	Elongation factor 1-delta	EEF1D
Gβ1	Eukaryotic translation initiation factor 4 gamma 1	EIF4G1
Gβ1	Ezrin	EZR
Gβ1	Hematological and neurological expressed 1-like protein	HN1L
Gβ1	Heterogeneous nuclear ribonucleoprotein H	HNRNPH1
Gβ1	78 kDa glucose-regulated protein	HSPA5
Gβ1	60 kDa heat shock protein, mitochondrial	HSPD1
Gβ1	Heat shock protein 105 kDa	HSPH1
Gβ1	Junction plakoglobin	JUP
Gβ1	L-lactate dehydrogenase A chain	LDHA
Gβ1	Microtubule-associated protein 4	MAP4
Gβ1	DNA replication licensing factor MCM3	MCM3
Gβ1	DNA helicase;DNA replication licensing factor MCM5	MCM5
Gβ1	Macrophage migration inhibitory factor	MIF
Gβ1	Nucleoside diphosphate kinase A;Nucleoside diphosphate kinase B	NME1;NME2;NME1-NME2;NME2P1

Gβ1	Multifunctional protein ADE2;Phosphoribosylaminoimidazole carboxylase	PAICS
Gβ1	Protein-L-isoaspartate O-methyltransferase	PCMT1
Gβ1	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	PPP2R1A
Gβ1	Polypyrimidine tract-binding protein 1	PTBP1
Gβ1	RNA-binding protein 14	RBM14
Gβ1	60S ribosomal protein L13a;Putative 60S ribosomal protein L13a protein RPL13AP3	RPL13a;RPL13A;RPL13AP3
Gβ1	60S ribosomal protein L18	RPL18
Gβ1	60S ribosomal protein L22	RPL22
Gβ1	60S ribosomal protein L27a	RPL27A
Gβ1	60S ribosomal protein L28	RPL28
Gβ1	60S ribosomal protein L7	RPL7
Gβ1	60S ribosomal protein L9	RPL9
Gβ1	40S ribosomal protein S12	RPS12
Gβ1	40S ribosomal protein SA	RPSA
Gβ1	TAR DNA-binding protein 43	TARDBP;TDP43
Gβ1	T-complex protein 1 subunit alpha	TCP1
Gβ1	Ubiquitin-associated protein 2-like	UBAP2L
Gβ1	Transitional endoplasmic reticulum ATPase	VCP
$G\beta_{1,}G\gamma_{5}$	Annexin A11	ANXA11
$G\beta_{1,}G\gamma_{5}$	Annexin A7	ANXA7
$G\beta_{1,}G\gamma_{5}$	T-complex protein 1 subunit beta	CCT2
$G\beta_{1,}G\gamma_{5}$	Clathrin heavy chain;Clathrin heavy chain 1	CLTC
$G\beta_{1,}G\gamma_{5}$	Calponin-3	CNN3
$G\beta_{1,}G\gamma_{5}$	Src substrate cortactin	CTTN
$G\beta_{1,}G\gamma_{5}$	Elongation factor 1-gamma	EEF1G
$G\beta_{1,}G\gamma_{5}$	Elongation factor 2	EEF2
$G\beta_{1,}G\gamma_{5}$	Eukaryotic initiation factor 4A-I	EIF4A1

$G\beta_{1,}G\gamma_5$	Eukaryotic translation initiation factor 5A;Eukaryotic translation initiation factor 5A-2;Eukaryotic translation initiation factor 5A-1-like	EIF5A;EIF5A2;EIF5AL1
$G\beta_{1,}G\gamma_5$	Bifunctional glutamate/proline--tRNA ligase;Glutamate--tRNA ligase;Proline--tRNA ligase	EPRS
$G\beta_{1,}G\gamma_5$	Fatty acid synthase	FASN
$G\beta_{1,}G\gamma_5$	Filamin-A	FLNA
$G\beta_{1,}G\gamma_5$	Filamin-B	FLNB
$G\beta_{1,}G\gamma_5$	Trifunctional purine biosynthetic protein adenosine-3	GART
$G\beta_{1,}G\gamma_5$	Heterogeneous nuclear ribonucleoprotein K	HNRNPK
$G\beta_{1,}G\gamma_5$	Heat shock protein HSP 90-alpha	HSP90AA1
$G\beta_{1,}G\gamma_5$	Heat shock 70 kDa protein 4	HSPA4
$G\beta_{1,}G\gamma_5$	Heat shock cognate 71 kDa protein	HSPA8
$G\beta_{1,}G\gamma_5$	Stress-70 protein, mitochondrial	HSPA9
$G\beta_{1,}G\gamma_5$	Isoleucine--tRNA ligase, cytoplasmic	IARS
$G\beta_{1,}G\gamma_5$	Far upstream element-binding protein 2	KHSRP
$G\beta_{1,}G\gamma_5$	Importin subunit alpha-1	KPNA2
$G\beta_{1,}G\gamma_5$	Nucleophosmin	NPM1
$G\beta_{1,}G\gamma_5$	Polyadenylate-binding protein 1;Polyadenylate-binding protein	PABPC1
$G\beta_{1,}G\gamma_5$	Poly(rC)-binding protein 1	PCBP1
$G\beta_{1,}G\gamma_5$	Programmed cell death 6-interacting protein	PDCD6IP
$G\beta_{1,}G\gamma_5$	Pyruvate kinase;Pyruvate kinase PKM	PKM
$G\beta_{1,}G\gamma_5$	Plastin-3	PLS3
$G\beta_{1,}G\gamma_5$	Peroxiredoxin-1	PRDX1
$G\beta_{1,}G\gamma_5$	Peroxiredoxin-2	PRDX2
$G\beta_{1,}G\gamma_5$	Peroxiredoxin-6	PRDX6
$G\beta_{1,}G\gamma_5$	DNA-dependent protein kinase catalytic subunit	PRKDC

$G\beta_{1,}G\gamma_5$	60S ribosomal protein L11	RPL11
$G\beta_{1,}G\gamma_5$	40S ribosomal protein S11	RPS11
$G\beta_{1,}G\gamma_5$	40S ribosomal protein S3	RPS3
$G\beta_{1,}G\gamma_5$	40S ribosomal protein S4, X isoform	RPS4X
$G\beta_{1,}G\gamma_5$	40S ribosomal protein S7	RPS7
$G\beta_{1,}G\gamma_5$	Transgelin-2	TAGLN2
$G\beta_{1,}G\gamma_5$	Tropomyosin alpha-3 chain	TPM3
$G\beta_{1,}G\gamma_5$	Elongation factor Tu, mitochondrial	TUFM
$G\beta_{1,}G\gamma_5$	Ubiquitin-like modifier-activating enzyme 1	UBA1
$G\beta_{1,}G\gamma_5$	Probable ubiquitin carboxyl-terminal hydrolase FAF-X	USP9X
$G\beta_{1,}G\gamma_5$	Voltage-dependent anion-selective channel protein 2	VDAC2
$G\beta_{1,}G\gamma_5$	X-ray repair cross-complementing protein 5	XRCC5
$G\beta_{1,}G\gamma_5$	X-ray repair cross-complementing protein 6	XRCC6
$G\beta_{1,}G\gamma_5$	14-3-3 protein epsilon	YWHAE
$G\beta_{1,}G\gamma_5$	ATP-citrate synthase	ACLY
$G\beta_{1,}G\gamma_5$	Fructose-bisphosphate aldolase;Fructose-bisphosphate aldolase A	ALDOA
$G\beta_{1,}G\gamma_5$	Angiomotin	AMOT
$G\gamma5$	Adenylosuccinate synthetase isozyme 2	ADSS
$G\gamma5$	Adenosylhomocysteinase	AHCY
$G\gamma5$	Putative annexin A2-like protein;Annexin A2;Annexin	ANXA2P2;ANXA2
$G\gamma5$	ATP synthase subunit alpha, mitochondrial	ATP5A1
$G\gamma5$	F-actin-capping protein subunit beta	CAPZB
$G\gamma5$	Cyclin-dependent kinase 1	CDC2;CDK1
$G\gamma5$	Drebrin-like protein	DBNL
$G\gamma5$	ATP-dependent RNA helicase A	DHX9
$G\gamma5$	Developmentally-regulated GTP-binding protein 2	DRG2
$G\gamma5$	Eukaryotic translation initiation factor 3 subunit D	EIF3D

Gγ5	Eukaryotic translation initiation factor 3 subunit L	EIF3L
Gγ5	Alpha-enolase	ENO1
Gγ5	Fatty acid desaturase 2	FADS2
Gγ5	Fragile X mental retardation syndrome-related protein 1	FXR1
Gγ5	Rab GDP dissociation inhibitor beta	GDI2
Gγ5	Histone H4	HIST1H4A
Gγ5	Importin-7	IPO7
Gγ5	BTB/POZ domain-containing protein KCTD12	KCTD12
Gγ5	Importin subunit beta-1	KPNB1
Gγ5	Mini-chromosome maintenance complex-binding protein	MCMBP
Gγ5	Poly [ADP-ribose] polymerase 1	PARP1
Gγ5	PDZ and LIM domain protein 1	PDLIM1
Gγ5	Phosphoribosylformylglycinamidine synthase	PFAS
Gγ5	Profilin-1	PFN1
Gγ5	Peptidyl-prolyl cis-trans isomerase A	PPIA
Gγ5	Protein RCC2	RCC2
Gγ5	Transforming protein RhoA;Rho-related GTP-binding protein RhoC;Rho-related GTP-binding protein RhoB	RHOA;RHOC;RHOB
Gγ5	60S ribosomal protein L12	RPL12
Gγ5	60S ribosomal protein L24	RPL24
Gγ5	U5 small nuclear ribonucleoprotein 200 kDa helicase	SNRNP200
Gγ5	Serine-threonine kinase receptor-associated protein	STRAP
Gγ5	Threonine--tRNA ligase, cytoplasmic	TARS
Gγ5	Tubulin-specific chaperone E	TBCE
Gγ5	14-3-3 protein theta	YWHAQ
Gγ5	14-3-3 protein zeta/delta	YWHAZ

Table S3 – *Proteins identified in 1 out of 3 experiments with a fold change of LFQ intensity from bait versus control samples ≥ 2*

Bait	Protein name	Gene ID
Gβ1	Alanine--tRNA ligase, cytoplasmic	AARS
Gβ1	ADP-ribosylation factor 4	ARF4
Gβ1	Sodium/potassium-transporting ATPase subunit alpha-1	ATP1A1
Gβ1	ATP synthase subunit alpha, mitochondrial	ATP5A1
Gβ1	ATP synthase subunit gamma, mitochondrial	ATP5C1
Gβ1	Autism susceptibility gene 2 protein	AUTS2
Gβ1	BMP-2-inducible protein kinase	BMP2K
Gβ1	Calcyclin-binding protein	CACYBP
Gβ1	F-actin-capping protein subunit beta	CAPZB
Gβ1	Kynurenine--oxoglutarate transaminase 3	CCBL2
Gβ1	T-complex protein 1 subunit epsilon	CCT5
Gβ1	Cyclin-dependent kinase 1	CDC2;CDK1
Gβ1	Coatomer subunit beta	COPB2
Gβ1	COP9 signalosome complex subunit 3	COPS3
Gβ1	Coronin-1C;Coronin	CORO1C
Gβ1	Copine-3	CPNE3
Gβ1	Aspartate--tRNA ligase, cytoplasmic	DARS
Gβ1	Drebrin	DBN1
Gβ1	DnaJ homolog subfamily A member 3, mitochondrial	DNAJA3
Gβ1	Band 4.1-like protein 2	EPB41L2
Gβ1	Eukaryotic peptide chain release factor subunit 1	ETF1
Gβ1	Fatty acid desaturase 2	FADS2
Gβ1	Forkhead box protein P1	FOXP1
Gβ1	Rab GDP dissociation inhibitor beta	GDI2
Gβ1	Guanine nucleotide-binding protein G(k) subunit alpha	GNAI3

Gβ1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	GNB1
Gβ1	Eukaryotic peptide chain release factor GTP-binding subunit ERF3A;Eukaryotic peptide chain release factor GTP-binding subunit ERF3B	GSPT1;GSPT2
Gβ1	Histone H3;Histone H3.2;Histone H3.3;Histone H3.1;Histone H3.1t	H3F3B;H3F3A;HIST2H3A; HIST1H3A;HIST3H3
Gβ1	Trifunctional enzyme subunit alpha, mitochondrial;Long-chain enoyl-CoA hydratase;Long chain 3-hydroxyacyl-CoA dehydrogenase	HADHA
Gβ1	Heterogeneous nuclear ribonucleoprotein M	HNRNPM
Gβ1	Heat shock protein beta-1	HSPB1
Gβ1	Plastin-2	LCP1
Gβ1	Afadin	MLLT4
Gβ1	Protein misato homolog 1	MSTO1
Gβ1	C-1-tetrahydrofolate synthase, cytoplasmic;Methylenetetrahydrofolate dehydrogenase;Methenyltetrahydrofolate cyclohydrolase;Formyltetrahydrofolate synthetase;C-1-tetrahydrofolate synthase, cytoplasmic, N-terminally processed	MTHFD1
Gβ1	Monofunctional C1-tetrahydrofolate synthase, mitochondrial	MTHFD1L
Gβ1	Myosin light polypeptide 6	MYL6
Gβ1	Nck-associated protein 1	NCKAP1
Gβ1	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 13	NDUFA13
Gβ1	Vesicle-fusing ATPase	NSF
Gβ1	Obg-like ATPase 1	OLA1
Gβ1	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial	PCK2
Gβ1	Proliferating cell nuclear antigen	PCNA
Gβ1	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	PDHB
Gβ1	Phosphoacetylglucosamine mutase	PGM3
Gβ1	Phosphatidylinositol-binding clathrin assembly protein	PICALM
Gβ1	DNA-directed RNA polymerase, mitochondrial	POLRMT
Gβ1	26S proteasome non-ATPase regulatory subunit 14	PSMD14
Gβ1	Proteasome activator complex subunit 3	PSME3
Gβ1	Ras-related protein Rab-7a	RAB7A
Gβ1	Arginine-glutamic acid dipeptide repeats protein	RERE

Gβ1	Ribosomal protein L15;60S ribosomal protein L15	RPL15
Gβ1	60S ribosomal protein L23	RPL23
Gβ1	60S ribosomal protein L26-like 1;60S ribosomal protein L26	RPL26L1;RPL26
Gβ1	60S ribosomal protein L6	RPL6
Gβ1	40S ribosomal protein S14	RPS14
Gβ1	40S ribosomal protein S2	RPS2
Gβ1	Ribosomal protein S6 kinase alpha-3	RPS6KA3
Gβ1	Protein scribble homolog	SCRIB
Gβ1	U1 small nuclear ribonucleoprotein 70 kDa	SNRNP70
Gβ1	Spectrin alpha chain, non-erythrocytic 1	SPTAN1
Gβ1	Spectrin beta chain, non-erythrocytic 1	SPTBN1
Gβ1	TATA-binding protein-associated factor 2N	TAF15
Gβ1	TBC1 domain family member 4	TBC1D4
Gβ1	Transmembrane protein 263	TMEM263
Gβ1	Titin	TTN
Gβ1	Tubulin beta-3 chain	TUBB3
Gβ1	Regulator of nonsense transcripts 1	UPF1
Gβ1	Ubiquitin carboxyl-terminal hydrolase 5	USP5
Gβ1	Vinculin	VCL
Gβ1	Serine/threonine-protein kinase WNK1	WNK1
Gβ1	YLP motif-containing protein 1	YLPM1
Gβ1	Zinc finger CCCH-type antiviral protein 1	ZC3HAV1
Gβ1	Zinc finger RNA-binding protein	ZFR
Gβ1	Zinc finger protein 503	ZNF503
$G\beta_{1,}G\gamma_{5}$	ATP-binding cassette sub-family D member 3	ABCD3
$G\beta_{1,}G\gamma_{5}$	ATP-binding cassette sub-family E member 1	ABCE1
$G\beta_{1,}G\gamma_{5}$	ATP-binding cassette sub-family F member 1	ABCF1
$G\beta_{1,}G\gamma_{5}$	Alpha-actinin-1	ACTN1
$G\beta_{1,}G\gamma_{5}$	Alpha-adducin	ADD1

$G\beta_{1,}G\gamma_5$	Aldehyde dehydrogenase family 16 member A1	ALDH16A1
$G\beta_{1,}G\gamma_5$	Annexin A6;Annexin	ANXA6
$G\beta_{1,}G\gamma_5$	AP-2 complex	AP2
$G\beta_{1,}G\gamma_5$	Asparagine synthetase [glutamine-hydrolyzing]	ASNS
$G\beta_{1,}G\gamma_5$	Adenylyl cyclase-associated protein 1;Adenylyl cyclase-associated protein	CAP1
$G\beta_{1,}G\gamma_5$	Cyclin-dependent-like kinase 5	CDK5
$G\beta_{1,}G\gamma_5$	Cip1-interacting zinc finger protein	CIZ1
$G\beta_{1,}G\gamma_5$	CTP synthase 1;CTP synthase	CTPS1
$G\beta_{1,}G\gamma_5$	Nucleolar RNA helicase 2	DDX21
$G\beta_{1,}G\gamma_5$	ATP-dependent RNA helicase DDX39A	DDX39A
$G\beta_{1,}G\gamma_5$	DnaJ homolog subfamily C member 7	DNAJC7
$G\beta_{1,}G\gamma_5$	Destrin	DSTN
$G\beta_{1,}G\gamma_5$	Elongation factor 1-alpha 1;Putative elongation factor 1-alpha-like 3;Elongation factor 1-alpha	EEF1A1;EEF1A1P5
$G\beta_{1,}G\gamma_5$	Extended synaptotagmin-1	ESYT1
$G\beta_{1,}G\gamma_5$	Phenylalanine--tRNA ligase beta subunit	FARSB
$G\beta_{1,}G\gamma_5$	RNA-binding protein FUS	FUS
$G\beta_{1,}G\gamma_5$	Translational activator GCN1	GCN1L1
$G\beta_{1,}G\gamma_5$	Guanine nucleotide-binding protein subunit beta-2-like 1;Guanine nucleotide-binding protein subunit beta-2-like 1, N-terminally processed	GNB2L1
$G\beta_{1,}G\gamma_5$	Host cell factor 1;HCF N-terminal chain 1;HCF N-terminal chain 2;HCF N-terminal chain 3;HCF N-terminal chain 4;HCF N-terminal chain 5;HCF N-terminal chain 6;HCF C-terminal chain 1;HCF C-terminal chain 2;HCF C-terminal chain 3;HCF C-terminal chain 4;HCF C-terminal chain 5;HCF C-terminal chain 6	HCFC1
$G\beta_{1,}G\gamma_5$	E3 ubiquitin-protein ligase HUWE1	HUWE1
$G\beta_{1,}G\gamma_5$	Importin-5	IPO5
$G\beta_{1,}G\gamma_5$	Importin-9	IPO9
$G\beta_{1,}G\gamma_5$	Kinesin-1 heavy chain	KIF5B

$G\beta_{1,}G\gamma_5$	La-related protein 1	LARP1
$G\beta_{1,}G\gamma_5$	Lamin-B receptor	LBR
$G\beta_{1,}G\gamma_5$	Mitogen-activated protein kinase kinase kinase 7	MAP3K7
$G\beta_{1,}G\gamma_5$	Methionine--tRNA ligase, cytoplasmic	MARS
$G\beta_{1,}G\gamma_5$	DNA replication licensing factor MCM4	MCM4
$G\beta_{1,}G\gamma_5$	DNA replication licensing factor MCM7	MCM7
$G\beta_{1,}G\gamma_5$	Serine/threonine-protein kinase Nek9	NEK9
$G\beta_{1,}G\gamma_5$	Nucleolar protein 58	NOP58
$G\beta_{1,}G\gamma_5$	Cytosolic Fe-S cluster assembly factor NUBP2	NUBP2
$G\beta_{1,}G\gamma_5$	Nuclear pore complex protein Nup153	NUP153
$G\beta_{1,}G\gamma_5$	Nuclear pore complex protein Nup88	NUP88
$G\beta_{1,}G\gamma_5$	Poly(rC)-binding protein 2	PCBP2
$G\beta_{1,}G\gamma_5$	Protein disulfide-isomerase A6	PDIA6
$G\beta_{1,}G\gamma_5$	DNA-directed RNA polymerase II subunit RPB1	POLR2A
$G\beta_{1,}G\gamma_5$	Serine/threonine-protein phosphatase;Serine/threonine-protein phosphatase PP1-gamma catalytic subunit;Serine/threonine-protein phosphatase PP1-alpha catalytic subunit;Serine/threonine-protein phosphatase PP1-beta catalytic subunit	PPP1CA;PPP1CC;PPP1CB
$G\beta_{1,}G\gamma_5$	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform	PPP2R2A
$G\beta_{1,}G\gamma_5$	26S protease regulatory subunit 6B	PSMC4
$G\beta_{1,}G\gamma_5$	26S proteasome non-ATPase regulatory subunit 3	PSMD3
$G\beta_{1,}G\gamma_5$	GTP-binding nuclear protein Ran	RAN
$G\beta_{1,}G\gamma_5$	60S ribosomal protein L10a	RPL10A
$G\beta_{1,}G\gamma_5$	60S ribosomal protein L17	RPL17;RPL17-C18orf32
$G\beta_{1,}G\gamma_5$	Ribosomal protein L19;60S ribosomal protein L19	RPL19
$G\beta_{1,}G\gamma_5$	60S ribosomal protein L21	RPL21
$G\beta_{1,}G\gamma_5$	60S ribosomal protein L5	RPL5

$G\beta_{1,}G\gamma_5$	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	RPN1
$G\beta_{1,}G\gamma_5$	40S ribosomal protein S15a	RPS15A
$G\beta_{1,}G\gamma_5$	Septin-2	SEPT2
$G\beta_{1,}G\gamma_5$	Mitochondrial dicarboxylate carrier	SLC25A10
$G\beta_{1,}G\gamma_5$	Mitochondrial 2-oxoglutarate/malate carrier protein	SLC25A11
$G\beta_{1,}G\gamma_5$	ADP/ATP translocase 2;ADP/ATP translocase 2, N-terminally processed	SLC25A5
$G\beta_{1,}G\gamma_5$	Heterogeneous nuclear ribonucleoprotein Q	SYNCRIP
$G\beta_{1,}G\gamma_5$	E3 ubiquitin-protein ligase TRIP12	TRIP12
$G\beta_{1,}G\gamma_5$	Pachytene checkpoint protein 2 homolog	TRIP13
$G\beta_{1,}G\gamma_5$	Tubulin alpha-1A chain;Tubulin alpha-3E chain	TUBA1A;TUBA3E
$G\beta_{1,}G\gamma_5$	Tubulin gamma-2 chain;Tubulin gamma-1 chain;Tubulin gamma chain	TUBG2;TUBG1
$G\beta_{1,}G\gamma_5$	Ubiquitin-like modifier-activating enzyme 6	UBA6
$G\beta_{1,}G\gamma_5$	Voltage-dependent anion-selective channel protein 3	VDAC3
$G\beta_{1,}G\gamma_5$	WD repeat-containing protein 6	WDR6
Gγ5	Arf-GAP domain and FG repeat-containing protein 1	AGFG1
Gγ5	Delta-1-pyrroline-5-carboxylate synthase;Glutamate 5-kinase;Gamma-glutamyl phosphate reductase	ALDH18A1
Gγ5	Ankyrin repeat domain-containing protein 17	ANKRD17
Gγ5	ADP-ribosylation factor 1;ADP-ribosylation factor 3;ADP-ribosylation factor 5;ADP-ribosylation factor 4	ARF1;ARF3;ARF4;ARF5
Gγ5	Isoaspartyl peptidase/L-asparaginase;Isoaspartyl peptidase/L-asparaginase alpha chain;Isoaspartyl peptidase/L-asparaginase beta chain	ASRGL1
Gγ5	ATPase family AAA domain-containing protein 3A;ATPase family AAA domain-containing protein 3B	ATAD3A;ATAD3B
Gγ5	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	ATP2A2
Gγ5	V-type proton ATPase subunit B, brain isoform	ATP6V1B2
Gγ5	Biliverdin reductase A	BLVRA
Gγ5	Mitotic checkpoint serine/threonine-protein kinase BUB1 beta	BUB1B

Gγ5	CAD protein;Glutamine-dependent carbamoyl-phosphate synthase;Aspartate carbamoyltransferase;Dihydroorotase	CAD
Gγ5	T-complex protein 1 subunit gamma	CCT3
Gγ5	T-complex protein 1 subunit delta	CCT4
Gγ5	T-complex protein 1 subunit zeta	CCT6A
Gγ5	Cat eye syndrome critical region protein 5	CECR5
Gγ5	CLIP-associating protein 2	CLASP2
Gγ5	Copine-1	CPNE1
Gγ5	CREB-regulated transcription coactivator 2	CRTC2
Gγ5	Cold shock domain-containing protein E1	CSDE1
Gγ5	Protein DEK	DEK
Gγ5	Density-regulated protein	DENR
Gγ5	Pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	DHX15
Gγ5	Putative ATP-dependent RNA helicase DHX30	DHX30
Gγ5	Protein diaphanous homolog 1	DIAPH1
Gγ5	Exosome complex exonuclease RRP44	DIS3
Gγ5	DnaJ homolog subfamily A member 1	DNAJA1
Gγ5	Eukaryotic translation initiation factor 2 subunit 3;Putative eukaryotic translation initiation factor 2 subunit 3-like protein	EIF2S3;EIF2S3L
Gγ5	Eukaryotic translation initiation factor 4E-binding protein 1	EIF4EBP1
Gγ5	Eukaryotic translation initiation factor 4 gamma 1	EIF4G1
Gγ5	Eukaryotic translation initiation factor 5	EIF5
Gγ5	Epiplakin	EPPK1
Gγ5	Fragile X mental retardation protein 1	FMR1
Gγ5	GTPase-activating protein and VPS9 domain-containing protein 1	GAPVD1
Gγ5	Gem-associated protein 5	GEMIN5
Gγ5	Glomulin	GLMN
Gγ5	Guanine nucleotide-binding protein-like 3	GNL3
Gγ5	HEAT repeat-containing protein 3	HEATR3
Gγ5	Hypoxanthine-guanine phosphoribosyltransferase	HPRT1

Gγ5	Putative heat shock protein HSP 90-beta 2	HSP90AB2P
Gγ5	Insulin-like growth factor 2 mRNA-binding protein 1	IGF2BP1
Gγ5	Inosine-5-monophosphate dehydrogenase 2	IMPDH2
Gγ5	Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 2	INPPL1
Gγ5	Kinesin-like protein KIF21A	KIF21A
Gγ5	Kinesin light chain 1	KLC1
Gγ5	Importin subunit alpha-7	KPNA6
Gγ5	GTPase KRas;GTPase KRas, N-terminally processed;GTPase NRas;GTPase HRas;GTPase HRas, N-terminally processed	KRAS;NRAS;HRAS
Gγ5	La-related protein 4	LARP4
Gγ5	Leucine--tRNA ligase, cytoplasmic	LARS
Gγ5	Ribosomal biogenesis protein LAS1L	LAS1L
Gγ5	LIM and SH3 domain protein 1	LASP1
Gγ5	Leucine-rich repeat-containing protein 40	LRRC40
Gγ5	Melanoma-associated antigen D2	MAGED2
Gγ5	Microtubule-associated protein 1B;MAP1B heavy chain;MAP1 light chain LC1	MAP1B
Gγ5	Microtubule-associated protein;Microtubule-associated protein 4	MAP4
Gγ5	Microtubule-associated protein RP/EB family member 1	MAPRE1
Gγ5	Midasin	MDN1
Gγ5	Methionine aminopeptidase 1	METAP1
Gγ5	DNA mismatch repair protein Msh6	MSH6
Gγ5	Moesin;Radixin	MSN;RDX
Gγ5	Myosin-9	MYH9
Gγ5	Unconventional myosin-Ib	MYO1B
Gγ5	Unconventional myosin-VI	MYO6
Gγ5	N-alpha-acetyltransferase 10	NAA10
Gγ5	Nicotinamide phosphoribosyltransferase	NAMPT
Gγ5	Non-POU domain-containing octamer-binding protein	NONO
Gγ5	5-nucleotidase domain-containing protein 2	NT5DC2
Gγ5	Cancer-related nucleoside-triphosphatase	NTPCR

Gγ5	Oxysterol-binding protein-related protein 11	OSBPL11
Gγ5	Serine/threonine-protein kinase PAK 4	PAK4
Gγ5	PDZ and LIM domain protein 5	PDLIM5
Gγ5	Peflin	PEF1
Gγ5	Prefoldin subunit 6	PFDN6
Gγ5	Pleckstrin homology domain-containing family A member 5	PLEKHA5
Gγ5	Mitochondrial-processing peptidase subunit beta	PMPCB
Gγ5	DNA polymerase;DNA polymerase delta catalytic subunit	POLD1
Gγ5	Liprin-alpha-1	PPFIA1
Gγ5	Protein phosphatase 1 regulatory subunit 12A	PPP1R12A
Gγ5	Serine/threonine-protein phosphatase;Serine/threonine-protein phosphatase 5	PPP5C
Gγ5	Pre-mRNA-processing factor 6	PRPF6
Gγ5	Protein PRRC2B	PRRC2B
Gγ5	Proteasome inhibitor PI31 subunit	PSMF1
Gγ5	Pumilio homolog 2	PUM2
Gγ5	Paxillin	PXN
Gγ5	Ras-related C3 botulinum toxin substrate 1;Ras-related C3 botulinum toxin substrate 3	RAC1;RAC3
Gγ5	E3 SUMO-protein ligase RanBP2	RANBP2
Gγ5	Ras-related protein Rap-1A;Ras-related protein Rap-1b;Ras-related protein Rap-1b-like protein	RAP1A;RAP1B
Gγ5	Arginine--tRNA ligase, cytoplasmic	RARS
Gγ5	60S ribosomal protein L18a	RPL18A
Gγ5	60S ribosomal protein L4	RPL4
Gγ5	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2	RPN2
Gγ5	40S ribosomal protein S3a	RPS3A
Gγ5	Ribosome-binding protein 1	RRBP1
Gγ5	Ribonucleoside-diphosphate reductase large subunit	RRM1
Gγ5	Vesicle-trafficking protein SEC22b	SEC22B
Gγ5	Protein transport protein Sec31A	SEC31A

Gγ5	Structural maintenance of chromosomes protein 1A;Structural maintenance of chromosomes protein	SMC1A
Gγ5	Structural maintenance of chromosomes flexible hinge domain-containing protein 1	SMCHD1
Gγ5	Signal recognition particle receptor subunit beta	SRPRB
Gγ5	Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial	SUCLA2
Gγ5	Surfeit locus protein 4	SURF4
Gγ5	Transforming acidic coiled-coil-containing protein 3	TACC3
Gγ5	Tubulin-specific chaperone A	TBCA
Gγ5	T-complex protein 1 subunit alpha	TCP1
Gγ5	Transferrin receptor protein 1;Transferrin receptor protein 1, serum form	TFRC
Gγ5	Talin-1	TLN1
Gγ5	Lamina-associated polypeptide 2, isoform alpha;Thymopoietin;Thymopentin;Lamina-associated polypeptide 2, isoforms beta/gamma;Thymopoietin;Thymopentin	TMPO
Gγ5	Transportin-1	TNPO1
Gγ5	Apoptosis-stimulating of p53 protein 2	TP53BP2
Gγ5	Tubulin beta-4A chain	TUBB4A
Gγ5	Gamma-taxilin;Putative gamma-taxilin 2	TXLNG;TXLNGY
Gγ5	Ubiquitin conjugation factor E4 B	UBE4B
Gγ5	Protein unc-45 homolog A	UNC45A
Gγ5	Voltage-dependent anion-selective channel protein 1	VDAC1
Gγ5	Vacuolar protein sorting-associated protein 37C	VPS37C
Gγ5	5-3 exoribonuclease 1	XRN1

Table S4 – *Proteins identified in 1 out of 3 experiments with fold change of LFQ intensity from bait versus control samples < 2*

Bait	Protein name	Gene ID
Gβ1	Actin-binding LIM protein 1	ABLIM1
Gβ1	3-ketoacyl-CoA thiolase, mitochondrial	ACAA2
Gβ1	Cytosolic acyl coenzyme A thioester hydrolase	ACOT7
Gβ1	Alpha-centractin;Beta-centractin	ACTR1A;ACTR1B
Gβ1	Activity-dependent neuroprotector homeobox protein	ADNP
Gβ1	Proteasomal ubiquitin receptor ADRM1	ADRM1
Gβ1	Annexin A2;Annexin;Putative annexin A2-like protein	ANXA2;ANXA2P2
Gβ1	AP-3 complex subunit beta-1	AP3B1
Gβ1	Rho guanine nucleotide exchange factor 2	ARHGEF2
Gβ1	AT-rich interactive domain-containing protein 1A	ARID1A
Gβ1	Atrophin-1	ATN1
Gβ1	Caprin-1	CAPRIN1
Gβ1	Hsp90 co-chaperone Cdc37	CDC37
Gβ1	Cdc42 effector protein 1	CDC42EP1
Gβ1	Coilin	COIL
Gβ1	Cytoplasmic polyadenylation element-binding protein 4;Cytoplasmic polyadenylation element-binding protein 2;Cytoplasmic polyadenylation element-binding protein 3	CPEB4;CPEB2;CPEB3
Gβ1	Cleavage and polyadenylation specificity factor subunit 6	CPSF6
Gβ1	Cleavage and polyadenylation specificity factor subunit 7	CPSF7
Gβ1	DAZ-associated protein 1	DAZAP1
Gβ1	Drebrin-like protein	DBNL
Gβ1	ATP-dependent RNA helicase DHX36	DHX36
Gβ1	ATP-dependent RNA helicase A	DHX9
Gβ1	Dihydrolipoyl dehydrogenase, mitochondrial;Dihydrolipoyl dehydrogenase	DLD
Gβ1	Elongation factor 1-beta	EEF1B2
Gβ1	116 kDa U5 small nuclear ribonucleoprotein component	EFTUD2

Gβ1	EH domain-binding protein 1	EHBP1
Gβ1	Eukaryotic translation initiation factor 3 subunit D	EIF3D
Gβ1	Eukaryotic translation initiation factor 3 subunit L	EIF3L
Gβ1	Eukaryotic translation initiation factor 4H	EIF4H
Gβ1	ELAV-like protein 1	ELAVL1
Gβ1	Alpha-enolase	ENO1
Gβ1	Enhancer of rudimentary homolog	ERH
Gβ1	Far upstream element-binding protein 1	FUBP1
Gβ1	GTPase-activating protein and VPS9 domain-containing protein 1	GAPVD1
Gβ1	GMP synthase [glutamine-hydrolyzing]	GMPS
Gβ1	Guanine nucleotide-binding protein G(s) subunit alpha isoforms XLas;Guanine nucleotide-binding protein G(s) subunit alpha isoforms short	GNAS
Gβ1	Heterogeneous nuclear ribonucleoprotein A1-like 2;Heterogeneous nuclear ribonucleoprotein A1;Heterogeneous nuclear ribonucleoprotein A1, N-terminally processed	HNRNPA1;HNRNPA1L2
Gβ1	Heterogeneous nuclear ribonucleoprotein A3	HNRNPA3
Gβ1	Heterogeneous nuclear ribonucleoprotein A/B	HNRNPAB
Gβ1	Heterogeneous nuclear ribonucleoprotein D-like	HNRNPDL
Gβ1	Heterogeneous nuclear ribonucleoprotein R	HNRNPR
Gβ1	Heterogeneous nuclear ribonucleoprotein U-like protein 1	HNRNPUL1
Gβ1	Heat shock 70 kDa protein 6;Putative heat shock 70 kDa protein 7	HSPA6;HSPA7
Gβ1	Interleukin enhancer-binding factor 3	ILF3
Gβ1	BTB/POZ domain-containing protein KCTD12	KCTD12
Gβ1	Kinesin-like protein KIF11	KIF11
Gβ1	Importin subunit beta-1	KPNB1
Gβ1	Luc7-like protein 3	LUC7L3
Gβ1	Mini-chromosome maintenance complex-binding protein	MCMBP
Gβ1	UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase 110 kDa subunit	OGT
Gβ1	Programmed cell death protein 5	PDCD5
Gβ1	PDZ and LIM domain protein 1	PDLIM1
Gβ1	Protein pelota homolog	PELO

Gβ1	ATP-dependent 6-phosphofructokinase, muscle type	PFKM
Gβ1	Profilin-1	PFN1
Gβ1	Peptidyl-prolyl cis-trans isomerase A;Peptidyl-prolyl cis-trans isomerase A, N-terminally processed;Peptidyl-prolyl cis-trans isomerase	PPIA
Gβ1	Pumilio homolog 1	PUM1
Gβ1	RNA-binding motif protein, X chromosome;RNA-binding motif protein, X chromosome, N-terminally processed;RNA binding motif protein, X-linked-like-1	RBMX;RBMXL1
Gβ1	Rho-associated protein kinase 1	ROCK1
Gβ1	60S ribosomal protein L24	RPL24
Gβ1	Secretory carrier-associated membrane protein 3	SCAMP3
Gβ1	Protein transport protein Sec24A	SEC24A
Gβ1	Plasminogen activator inhibitor 1 RNA-binding protein	SERBP1
Gβ1	U5 small nuclear ribonucleoprotein 200 kDa helicase	SNRNP200
Gβ1	Threonine--tRNA ligase, cytoplasmic	TARS
Gβ1	14-3-3 protein zeta/delta	YWHAZ
$G\beta_{1,}G\gamma_5$	A-kinase anchor protein 12	AKAP12
$G\beta_{1,}G\gamma_5$	Anaphase-promoting complex subunit 7	ANAPC7
$G\beta_{1,}G\gamma_5$	Ankyrin repeat and KH domain-containing protein 1	ANKHD1
$G\beta_{1,}G\gamma_5$	Ataxin-10	ATXN10
$G\beta_{1,}G\gamma_5$	Chromobox protein homolog 3	CBX3
$G\beta_{1,}G\gamma_5$	T-complex protein 1 subunit eta	CCT7
$G\beta_{1,}G\gamma_5$	Cysteine and histidine-rich domain-containing protein 1	CHORDC1
$G\beta_{1,}G\gamma_5$	CCR4-NOT transcription complex subunit 2	CNOT2
$G\beta_{1,}G\gamma_5$	Coatomer subunit gamma-1	COPG1
$G\beta_{1,}G\gamma_5$	Probable ATP-dependent RNA helicase DDX17	DDX17
$G\beta_{1,}G\gamma_5$	Probable ATP-dependent RNA helicase DDX5	DDX5
$G\beta_{1,}G\gamma_5$	Eukaryotic translation initiation factor 3 subunit C;Eukaryotic translation initiation factor 3 subunit C-like protein	EIF3C;EIF3CL
$G\beta_{1,}G\gamma_5$	Eukaryotic translation initiation factor 3 subunit E	EIF3E

$G\beta_{1,}G\gamma_5$	Phenylalanine--tRNA ligase alpha subunit	FARSA
$G\beta_{1,}G\gamma_5$	Neutral alpha-glucosidase AB	GANAB
$G\beta_{1,}G\gamma_5$	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH
$G\beta_{1,}G\gamma_5$	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] 1	GFPT1
$G\beta_{1,}G\gamma_5$	E3 ubiquitin-protein ligase HECTD1	HECTD1
$G\beta_{1,}G\gamma_5$	Probable helicase with zinc finger domain	HELZ
$G\beta_{1,}G\gamma_5$	Heterogeneous nuclear ribonucleoprotein D0	HNRNPD
$G\beta_{1,}G\gamma_5$	Heterogeneous nuclear ribonucleoprotein U	HNRNPU
$G\beta_{1,}G\gamma_5$	Very-long-chain 3-oxoacyl-CoA reductase	HSD17B12
$G\beta_{1,}G\gamma_5$	Heat shock protein HSP 90-beta	HSP90AB1
$G\beta_{1,}G\gamma_5$	Heat shock 70 kDa protein 4L	HSPA4L
$G\beta_{1,}G\gamma_5$	Insulin receptor substrate 4	IRS4
$G\beta_{1,}G\gamma_5$	Kinesin-like protein;Kinesin-like protein KIF1A	KIF1A
$G\beta_{1,}G\gamma_5$	Matrin-3	MATR3
$G\beta_{1,}G\gamma_5$	DNA replication licensing factor MCM2	MCM2
$G\beta_{1,}G\gamma_5$	DNA replication licensing factor MCM7	MCM7
$G\beta_{1,}G\gamma_5$	Nuclear pore complex protein Nup155	NUP155
$G\beta_{1,}G\gamma_5$	Nuclear pore complex protein Nup214	NUP214
$G\beta_{1,}G\gamma_5$	Nuclear pore complex protein Nup93	NUP93
$G\beta_{1,}G\gamma_5$	Nuclear pore complex protein Nup98-Nup96;Nuclear pore complex protein Nup98;Nuclear pore complex protein Nup96	NUP98
$G\beta_{1,}G\gamma_5$	Ubiquitin thioesterase OTUB1	OTUB1
$G\beta_{1,}G\gamma_5$	Polyadenylate-binding protein;Polyadenylate-binding protein 4	PABPC4
$G\beta_{1,}G\gamma_5$	Prohibitin	PHB
$G\beta_{1,}G\gamma_5$	Inorganic pyrophosphatase	PPA1
$G\beta_{1,}G\gamma_5$	26S protease regulatory subunit 7	PSMC2

$G\beta_{1,}G\gamma_{5}$	60S ribosomal protein L3	RPL3
$G\beta_{1,}G\gamma_{5}$	60S ribosomal protein L8	RPL8
$G\beta_{1,}G\gamma_{5}$	40S ribosomal protein S16	RPS16
$G\beta_{1,}G\gamma_{5}$	40S ribosomal protein S27;40S ribosomal protein S27-like	RPS27;RPS27L
$G\beta_{1,}G\gamma_{5}$	Ubiquitin-40S ribosomal protein S27a;Ubiquitin;40S ribosomal protein S27a;Ubiquitin-60S ribosomal protein L40;Ubiquitin;60S ribosomal protein L40;Polyubiquitin-B;Ubiquitin;Polyubiquitin-C;Ubiquitin	RPS27A;UBB;UBC;UBA52
$G\beta_{1,}G\gamma_{5}$	40S ribosomal protein S6	RPS6
$G\beta_{1,}G\gamma_{5}$	40S ribosomal protein S8	RPS8
$G\beta_{1,}G\gamma_{5}$	RuvB-like 1	RUVBL1
$G\beta_{1,}G\gamma_{5}$	Protein transport protein Sec16A	SEC16A
$G\beta_{1,}G\gamma_{5}$	Protein transport protein Sec24C	SEC24C
$G\beta_{1,}G\gamma_{5}$	Serpin H1	SERPINH1
$G\beta_{1,}G\gamma_{5}$	Splicing factor 3B subunit 1	SF3B1
$G\beta_{1,}G\gamma_{5}$	Splicing factor, proline- and glutamine-rich	SFPQ
$G\beta_{1,}G\gamma_{5}$	ADP/ATP translocase 3;ADP/ATP translocase 3, N-terminally processed	SLC25A6
$G\beta_{1,}G\gamma_{5}$	C-Jun-amino-terminal kinase-interacting protein 4	SPAG9
$G\beta_{1,}G\gamma_{5}$	Sorcin	SRI
$G\beta_{1,}G\gamma_{5}$	Serrate RNA effector molecule homolog	SRRT
$G\beta_{1,}G\gamma_{5}$	Sjoegren syndrome/scleroderma autoantigen 1	SSSCA1
$G\beta_{1,}G\gamma_{5}$	Trinucleotide repeat-containing gene 6A protein	TNRC6A
$G\beta_{1,}G\gamma_{5}$	Trinucleotide repeat-containing gene 6B protein	TNRC6B
$G\beta_{1,}G\gamma_{5}$	DNA topoisomerase 2-alpha	TOP2A
$G\beta_{1,}G\gamma_{5}$	Transcription intermediary factor 1-beta	TRIM28
$G\beta_{1,}G\gamma_{5}$	Tubulin beta-2B chain;Tubulin beta-2A chain	TUBB2B;TUBB2A
$G\beta_{1,}G\gamma_{5}$	Tubulin beta-8 chain	TUBB8

$G\beta_{1,}G\gamma_5$	E3 ubiquitin-protein ligase UBR4	UBR4
$G\beta_{1,}G\gamma_5$	Cytochrome b-c1 complex subunit 2, mitochondrial	UQCRC2
$G\beta_{1,}G\gamma_5$	Vimentin	VIM
$G\beta_{1,}G\gamma_5$	Exportin-1	XPO1
$G\beta_{1,}G\gamma_5$	Exportin-5	XPO5
$G\beta_{1,}G\gamma_5$	5-3 exoribonuclease 2	XRN2
Gγ5	Aladin	AAAS
Gγ5	Abl interactor 1	ABI1
Gγ5	Abl interactor 2	ABI2
Gγ5	Actin-related protein 3	ACTR3
Gγ5	Double-stranded RNA-specific adenosine deaminase	ADAR
Gγ5	1-acyl-sn-glycerol-3-phosphate acyltransferase alpha	AGPAT1
Gγ5	Aminoacyl tRNA synthase complex-interacting multifunctional protein 1;Endothelial monocyte-activating polypeptide 2	AIMP1
Gγ5	A-kinase anchor protein 13	AKAP13
Gγ5	Ankyrin-3	ANK3
Gγ5	Serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit C	ANKRD52
Gγ5	Ankyrin repeat and SAM domain-containing protein 1A	ANKS1A
Gγ5	Actin-binding protein anillin	ANLN
Gγ5	AP-3 complex subunit mu-1	AP3M1
Gγ5	Adenomatous polyposis coli protein	APC
Gγ5	Adenine phosphoribosyltransferase	APRT
Gγ5	Serine/threonine-protein kinase A-Raf	ARAF
Gγ5	Brefeldin A-inhibited guanine nucleotide-exchange protein 3	ARFGEF3
Gγ5	Rho guanine nucleotide exchange factor 5	ARHGEF5
Gγ5	Activating signal cointegrator 1 complex subunit 2	ASCC2
Gγ5	Activating signal cointegrator 1 complex subunit 3	ASCC3
Gγ5	Cysteine protease ATG4B	ATG4B
Gγ5	Copper transport protein ATOX1	ATOX1

Gγ5	Ataxin-2-like protein	ATXN2L
Gγ5	BAG family molecular chaperone regulator 2	BAG2
Gγ5	Brain acid soluble protein 1	BASP1
Gγ5	Large proline-rich protein BAG6	BAT3;BAG6
Gγ5	Bromodomain adjacent to zinc finger domain protein 1A	BAZ1A
Gγ5	Breakpoint cluster region protein	BCR
Gγ5	Baculoviral IAP repeat-containing protein 6	BIRC6
Gγ5	BolA-like protein 2	BOLA2;BOLA2B
Gγ5	Mitotic checkpoint protein BUB3	BUB3
Gγ5	Calcium/calmodulin-dependent protein kinase type II subunit delta;Calcium/calmodulin-dependent protein kinase type II subunit beta;Calcium/calmodulin-dependent protein kinase type II subunit alpha;Calcium/calmodulin-dependent protein kinase type II subunit gamma	CAMK2D;CAMK2B; CAMK2A;CAMK2G
Gγ5	Calmodulin-regulated spectrin-associated protein 3	CAMSAP3
Gγ5	Calpain-1 catalytic subunit	CAPN1
Gγ5	Calpain-2 catalytic subunit	CAPN2
Gγ5	Peripheral plasma membrane protein CASK	CASK
Gγ5	Cystathionine beta-synthase	CBS
Gγ5	Cell cycle and apoptosis regulator protein 2	CCAR2
Gγ5	Non-specific serine/threonine protein kinase;Serine/threonine-protein kinase MRCK alpha	CDC42BPA
Gγ5	CDKN2AIP N-terminal-like protein	CDKN2AIPNL
Gγ5	Protein CDV3 homolog	CDV3
Gγ5	Centrosomal protein of 170 kDa;Cep170-like protein	CEP170;CEP170P1
Gγ5	Cofilin-1	CFL1
Gγ5	Chromodomain-helicase-DNA-binding protein 4	CHD4
Gγ5	Calcium homeostasis endoplasmic reticulum protein	CHERP
Gγ5	Cytoskeleton-associated protein 4	CKAP4
Gγ5	Cytoskeleton-associated protein 5	CKAP5
Gγ5	Creatine kinase B-type	CKB
Gγ5	CLIP-associating protein 1	CLASP1
Gγ5	Cellular nucleic acid-binding protein	CNBP

Gγ5	CCR4-NOT transcription complex subunit 1	CNOT1
Gγ5	Coatomer subunit gamma-2	COPG2
Gγ5	COP9 signalosome complex subunit 2	COPS2
Gγ5	Rootletin	CROCC
Gγ5	Exportin-2	CSE1L
Gγ5	Cysteine and glycine-rich protein 2	CSRP2
Gγ5	Dynactin subunit 1	DCTN1;DKFZp686E0752
Gγ5	Dynactin subunit 2	DCTN2
Gγ5	ATP-dependent RNA helicase DDX1	DDX1
Gγ5	Probable ATP-dependent RNA helicase DDX20	DDX20
Gγ5	Disks large homolog 1	DLG1
Gγ5	Dynamin-1-like protein	DNM1L
Gγ5	Dynamin-2	DNM2
Gγ5	Dihydropyrimidinase-related protein 2	DPYSL2
Gγ5	Desmoplakin	DSP
Gγ5	EF-hand domain-containing protein D2	EFHD2
Gγ5	Eukaryotic translation initiation factor 3 subunit A	EIF3A
Gγ5	Eukaryotic translation initiation factor 3 subunit B	EIF3B
Gγ5	Eukaryotic initiation factor 4A-III;Eukaryotic initiation factor 4A-III, N-terminally processed	EIF4A3
Gγ5	Emerin	EMD
Gγ5	Protein LAP2	ERBB2IP
Gγ5	Constitutive coactivator of PPAR-gamma-like protein 1	FAM120A
Gγ5	Protein Niban	FAM129A
Gγ5	Niban-like protein 1	FAM129B
Gγ5	rRNA 2-O-methyltransferase fibrillarin	FBL
Gγ5	Probable fibrosin-1	FBRS
Gγ5	Fermitin family homolog 2	FERMT2
Gγ5	Peptidyl-prolyl cis-trans isomerase;Peptidyl-prolyl cis-trans isomerase FKBP4;Peptidyl-prolyl cis-trans isomerase FKBP4, N-terminally processed	FKBP4
Gγ5	Filamin-C	FLNC

Gγ5	Far upstream element-binding protein 3	FUBP3
Gγ5	Ras GTPase-activating protein-binding protein 1	G3BP1
Gγ5	Ras GTPase-activating protein-binding protein 2	G3BP2
Gγ5	Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1	GBF1
Gγ5	Gem-associated protein 4	GEMIN4
Gγ5	PERQ amino acid-rich with GYF domain-containing protein 2	GIGYF2
Gγ5	ARF GTPase-activating protein GIT1	GIT1
Gγ5	Golgin subfamily A member 2	GOLGA2
Gγ5	Golgi-associated PDZ and coiled-coil motif-containing protein	GOPC
Gγ5	Genetic suppressor element 1	GSE1
Gγ5	Glutathione reductase, mitochondrial	GSR
Gγ5	General transcription factor 3C polypeptide 1	GTF3C1
Gγ5	Vigilin	HDLBP
Gγ5	Hexokinase;Hexokinase-2	HK2
Gγ5	Hydroxymethylglutaryl-CoA synthase, cytoplasmic	HMGCS1
Gγ5	Hematological and neurological expressed 1-like protein	HN1L
Gγ5	Heterogeneous nuclear ribonucleoprotein F;Heterogeneous nuclear ribonucleoprotein F, N-terminally processed	HNRNPF
Gγ5	Heterogeneous nuclear ribonucleoprotein H;Heterogeneous nuclear ribonucleoprotein H, N-terminally processed	HNRNPH1
Gγ5	Endoplasmin	HSP90B1
Gγ5	78 kDa glucose-regulated protein	HSPA5
Gγ5	60 kDa heat shock protein, mitochondrial	HSPD1
Gγ5	Insulin-like growth factor 2 mRNA-binding protein 3	IGF2BP3
Gγ5	Importin-4	IPO4
Gγ5	Ras GTPase-activating-like protein IQGAP2	IQGAP2
Gγ5	E3 ubiquitin-protein ligase Itchy homolog	ITCH
Gγ5	Junction plakoglobin	JUP
Gγ5	Lysine-specific demethylase 3B	KDM3B
Gγ5	KIF1-binding protein	KIAA1279

Gγ5	Protein CIP2A	KIAA1524
Gγ5	Kinesin-like protein KIF15;Kinesin-like protein	KIF15
Gγ5	Kinesin-like protein;Kinesin-like protein KIF23	KIF23
Gγ5	Kinesin-like protein KIF2C	KIF2C
Gγ5	Chromosome-associated kinesin KIF4A;Chromosome-associated kinesin KIF4B	KIF4A;KIF4B
Gγ5	DNA ligase 3	LIG3
Gγ5	LIM domain and actin-binding protein 1	LIMA1
Gγ5	Lethal(2) giant larvae protein homolog 1	LLGL1
Gγ5	LIM domain only protein 7	LMO7
Gγ5	Lipoma-preferred partner	LPP
Gγ5	Lipopolysaccharide-responsive and beige-like anchor protein;Neurobeachin	LRBA;NBEA
Gγ5	Leucine-rich PPR motif-containing protein, mitochondrial	LRPPRC
Gγ5	Leucine-rich repeat-containing protein 16A	LRRC16A
Gγ5	Leucine-rich repeat-containing protein 47	LRRC47
Gγ5	Putative RNA-binding protein Luc7-like 2	LUC7L2
Gγ5	Leucine zipper protein 1	LUZP1
Gγ5	Microtubule-actin cross-linking factor 1, isoforms 1/2/3/5	MACF1
Gγ5	Melanoma-associated antigen D1	MAGED1
Gγ5	Dual specificity mitogen-activated protein kinase kinase 1;Dual specificity mitogen-activated protein kinase kinase 2	MAP2K2;MAP2K1
Gγ5	S-adenosylmethionine synthase isoform type-2	MAT2A
Gγ5	DNA replication licensing factor MCM3	MCM3
Gγ5	DNA helicase;DNA replication licensing factor MCM5	MCM5
Gγ5	Mediator of DNA damage checkpoint protein 1	MDC1
Gγ5	Protein O-GlcNAcase	MGEA5
Gγ5	Macrophage migration inhibitory factor	MIF
Gγ5	Antigen KI-67	MKI67
Gγ5	MMS19 nucleotide excision repair protein homolog	MMS19
Gγ5	MAGUK p55 subfamily member 6	MPP6
Gγ5	Myosin phosphatase Rho-interacting protein	MPRIP

Gγ5	DNA mismatch repair protein Msh2	MSH2
Gγ5	Myotubularin-related protein 3;Myotubularin-related protein 4	MTMR3;MTMR4
Gγ5	Unconventional myosin-Ic	MYO1C
Gγ5	N-alpha-acetyltransferase 15, NatA auxiliary subunit	NAA15
Gγ5	NEDD8-activating enzyme E1 regulatory subunit	NAE1
Gγ5	Nucleosome assembly protein 1-like 1	NAP1L1
Gγ5	Nuclear autoantigenic sperm protein	NASP
Gγ5	Neuroblastoma-amplified sequence	NBAS
Gγ5	Condensin-2 complex subunit D3	NCAPD3
Gγ5	Condensin complex subunit 3	NCAPG
Gγ5	Nuclear receptor coactivator 2	NCOA2
Gγ5	Nuclear receptor corepressor 1	NCOR1
Gγ5	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial	NDUFA9
Gγ5	Neurofibromin;Neurofibromin truncated	NF1
Gγ5	NHL repeat-containing protein 2	NHLRC2
Gγ5	NHS-like protein 1	NHSL1
Gγ5	NF-kappa-B-repressing factor	NKRF
Gγ5	Nucleoside diphosphate kinase;Nucleoside diphosphate kinase B;Nucleoside diphosphate kinase A	NME1-NME2;NME1;NME2
Gγ5	Nucleolar protein 56	NOP56
Gγ5	Nardilysin	NRD1
Gγ5	tRNA (cytosine(34)-C(5))-methyltransferase	NSUN2
Gγ5	Cytosolic Fe-S cluster assembly factor NUBP1	NUBP1
Gγ5	Nuclear mitotic apparatus protein 1	NUMA1
Gγ5	Nucleoporin NUP188 homolog	NUP188
Gγ5	Nuclear pore complex protein Nup205	NUP205
Gγ5	Multifunctional protein ADE2;Phosphoribosylaminoimidazole-succinocarboxamide synthase;Phosphoribosylaminoimidazole carboxylase	PAICS
Gγ5	Poly(ADP-ribose) glycohydrolase	PARG
Gγ5	Protein-L-isoaspartate O-methyltransferase;Protein-L-isoaspartate(D-aspartate) O-methyltransferase	PCMT1

Gγ5	Sister chromatid cohesion protein PDS5 homolog A	PDS5A
Gγ5	ATP-dependent 6-phosphofructokinase, platelet type	PFKP
Gγ5	PHD finger protein 3	PHF3
Gγ5	Phosphatidylinositol 4-kinase alpha	PI4KA
Gγ5	Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit alpha	PIK3C2A
Gγ5	Phosphatidylinositol 3-kinase regulatory subunit beta	PIK3R2
Gγ5	Serine/threonine-protein kinase N2	PKN2
Gγ5	Plakophilin-2	PKP2
Gγ5	Plectin	PLEC
Gγ5	Plastin-1	PLS1
Gγ5	DNA-directed RNA polymerase;DNA-directed RNA polymerase II subunit RPB2	POLR2B
Gγ5	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	PPP2R1A
Gγ5	Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit delta isoform	PPP2R5D
Gγ5	Thioredoxin-dependent peroxide reductase, mitochondrial	PRDX3
Gγ5	Protein arginine N-methyltransferase 1	PRMT1
Gγ5	Pre-mRNA-processing-splicing factor 8	PRPF8
Gγ5	Protein PRRC2A	PRRC2A
Gγ5	Protein PRRC2C	PRRC2C
Gγ5	26S protease regulatory subunit 8	PSMC5
Gγ5	Polypyrimidine tract-binding protein 1	PTBP1
Gγ5	Tyrosine-protein phosphatase non-receptor type 11	PTPN11
Gγ5	Tyrosine-protein phosphatase non-receptor type 13	PTPN13
Gγ5	Tyrosine-protein phosphatase non-receptor type 23	PTPN23
Gγ5	Receptor-type tyrosine-protein phosphatase F	PTPRF
Gγ5	Ras-related protein Rab-7a	RAB7A
Gγ5	Ankycorbin	RAI14
Gγ5	Ran-specific GTPase-activating protein	RANBP1
Gγ5	Ran GTPase-activating protein 1	RANGAP1
Gγ5	Histone-binding protein RBBP4	RBBP4

Gγ5	Histone-binding protein RBBP7	RBBP7
Gγ5	RNA-binding protein 14	RBM14
Gγ5	RNA-binding protein 25	RBM25
Gγ5	RNA-binding protein 39	RBM39
Gγ5	Transcription factor p65	RELA
Gγ5	Rapamycin-insensitive companion of mTOR	RICTOR
Gγ5	Telomere-associated protein RIF1	RIF1
Gγ5	RING finger protein 214	RNF214
Gγ5	Ribonuclease inhibitor	RNH1
Gγ5	Aminopeptidase B	RNPEP
Gγ5	RNA polymerase II-associated protein 1	RPAP1
Gγ5	60S ribosomal protein L10;60S ribosomal protein L10-like	RPL10;RPL10L
Gγ5	60S ribosomal protein L13a	RPL13A
Gγ5	60S ribosomal protein L18	RPL18
Gγ5	60S ribosomal protein L22	RPL22
Gγ5	60S ribosomal protein L27	RPL27
Gγ5	60S ribosomal protein L27a	RPL27A
Gγ5	60S ribosomal protein L28	RPL28
Gγ5	60S ribosomal protein L7	RPL7
Gγ5	60S ribosomal protein L7a	RPL7A
Gγ5	60S ribosomal protein L9	RPL9
Gγ5	60S acidic ribosomal protein P2	RPLP2
Gγ5	40S ribosomal protein S12	RPS12
Gγ5	40S ribosomal protein SA	RPSA
Gγ5	RRP12-like protein	RRP12
Gγ5	tRNA-splicing ligase RtcB homolog	RTCB
Gγ5	Rhotekin	RTKN
Gγ5	Reticulon-3	RTN3
Gγ5	RUN and FYVE domain-containing protein 1	RUFY1

Gγ5	Protein SAAL1	SAAL1
Gγ5	Myotubularin-related protein 5	SBF1
Gγ5	Protein transport protein Sec23B	SEC23B
Gγ5	Sentrin-specific protease 3	SENP3
Gγ5	Septin-7	SEPT7
Gγ5	Splicing factor 3B subunit 2	SF3B2
Gγ5	Splicing factor 3B subunit 3	SF3B3
Gγ5	Sideroflexin-1	SFXN1
Gγ5	Tyrosine-protein kinase SgK223	SGK223
Gγ5	Serine hydroxymethyltransferase;Serine hydroxymethyltransferase, mitochondrial;Serine hydroxymethyltransferase, cytosolic	SHMT2;SHMT1
Gγ5	Protein Shroom2	SHROOM2
Gγ5	Serine/threonine-protein kinase SIK3	SIK3
Gγ5	SLAIN motif-containing protein 1	SLAIN1
Gγ5	Neutral amino acid transporter B(0);Amino acid transporter	SLC1A5
Gγ5	Calcium-binding mitochondrial carrier protein Aralar2;Calcium-binding mitochondrial carrier protein Aralar1	SLC25A13;SLC25A12
Gγ5	Zinc transporter ZIP14	SLC39A14
Gγ5	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5	SMARCA5
Gγ5	Structural maintenance of chromosomes protein 2	SMC2
Gγ5	Structural maintenance of chromosomes protein;Structural maintenance of chromosomes protein 4	SMC4
Gγ5	Sorting nexin-2	SNX2
Gγ5	Sorting nexin-9	SNX9
Gγ5	Spermatogenesis-associated protein 5	SPATA5
Gγ5	Cytospin-A	SPECC1L-ADORA2A;SPECC1L
Gγ5	Spartin	SPG20
Gγ5	Spectrin beta chain, non-erythrocytic 2	SPTBN2
Gγ5	Sequestosome-1	SQSTM1
Gγ5	SRSF protein kinase 1	SRPK1

Gγ5	Signal recognition particle receptor subunit alpha	SRPR
Gγ5	Putative protein FAM10A4;Hsc70-interacting protein;Putative protein FAM10A5	ST13;ST13P4;ST13P5
Gγ5	Signal transducer and activator of transcription;Signal transducer and activator of transcription 1-alpha/beta	STAT1
Gγ5	Signal transducer and activator of transcription;Signal transducer and activator of transcription 3	STAT3
Gγ5	Supervillin	SVIL
Gγ5	TGF-beta-activated kinase 1 and MAP3K7-binding protein 2	TAB2
Gγ5	Tubulin-specific chaperone D	TBCD
Gγ5	Transducin beta-like protein 3	TBL3
Gγ5	Transcription elongation factor B polypeptide 1	TCEB1
Gγ5	Protein TFG	TFG
Gγ5	Thyroid adenoma-associated protein	THADA
Gγ5	Mitochondrial import inner membrane translocase subunit TIM44	TIMM44
Gγ5	Tight junction protein ZO-1	TJP1
Gγ5	Thymidine kinase, cytosolic;Thymidine kinase	TK1
Gγ5	182 kDa tankyrase-1-binding protein	TNKS1BP1
Gγ5	Transportin-3	TNPO3
Gγ5	Mitochondrial import receptor subunit TOM40 homolog	TOMM40
Gγ5	Tumor suppressor p53-binding protein 1	TP53BP1
Gγ5	Heat shock protein 75 kDa, mitochondrial	TRAP1
Gγ5	E3 ubiquitin/ISG15 ligase TRIM25	TRIM25
Gγ5	Tripartite motif-containing protein 65	TRIM65
Gγ5	Tetratricopeptide repeat protein 37	TTC37
Gγ5	Dual specificity protein kinase TTK	TTK
Gγ5	Tubulin alpha-1C chain	TUBA1C
Gγ5	Tubulin beta-6 chain	TUBB6
Gγ5	Gamma-tubulin complex component 2	TUBGCP2
Gγ5	Alpha-taxilin	TXLNA
Gγ5	U2 snRNP-associated SURP motif-containing protein	U2SURP

Gγ5	Ubiquitin-associated protein 2	UBAP2
Gγ5	Ubiquitin-associated protein 2-like	UBAP2L
Gγ5	E2/E3 hybrid ubiquitin-protein ligase UBE2O	UBE2O
Gγ5	Ubiquitin-protein ligase E3C	UBE3C
Gγ5	Regulator of nonsense transcripts 1	UPF1
Gγ5	Cytochrome b-c1 complex subunit 1, mitochondrial	UQCRC1
Gγ5	Ubiquitin carboxyl-terminal hydrolase 10	USP10
Gγ5	Ubiquitin carboxyl-terminal hydrolase 15;Ubiquitin carboxyl-terminal hydrolase;Ubiquitin carboxyl-terminal hydrolase 4;Ubiquitin carboxyl-terminal hydrolase 11	USP15;USP11;USP4
Gγ5	Ubiquitin carboxyl-terminal hydrolase 24	USP24
Gγ5	Ubiquitin carboxyl-terminal hydrolase 5	USP5
Gγ5	Utrophin	UTRN
Gγ5	Valine--tRNA ligase	VARS
Gγ5	Transitional endoplasmic reticulum ATPase	VCP
Gγ5	Vacuolar protein sorting-associated protein 4B;Vacuolar protein sorting-associated protein 4A;Fidgetin-like protein 1	VPS4B;VPS4A;FIGNL1
Gγ5	Methylosome protein 50	WDR77
Gγ5	Exportin-7	XPO7
Gγ5	Exportin-T	XPOT
Gγ5	14-3-3 protein beta/alpha;14-3-3 protein beta/alpha, N-terminally processed;14-3-3 protein gamma;14-3-3 protein gamma, N-terminally processed	YWHAB;YWHAG
Gγ5	14-3-3 protein eta	YWHAH
Gγ5	Terminal uridylyltransferase 4	ZCCHC11
Gγ5	Palmitoyltransferase ZDHHC5;Palmitoyltransferase	ZDHHC5
Gγ5	Zinc finger FYVE domain-containing protein 16	ZFYVE16
Gγ5	Zinc finger MYM-type protein 4	ZMYM4
Gγ5	Zinc finger protein 428	ZNF428
Gγ5	Zyxin	ZYX
Gγ5	Methionine synthase	
Gγ5	Myb-binding protein 1A	
Gγ5	E3 ubiquitin-protein ligase MYCBP2	

Gγ5	Actin, alpha skeletal muscle	ACTA1