

**The *IRF8* and *IRF1* Genes as Important Regulators of Immune
Response to Mycobacteria and Bone Metabolism**

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ABSTRACT

This thesis examines the role of the transcription factor, Interferon regulatory factor 8 (IRF8), in susceptibility to mycobacteria and development of mononuclear phagocytes in humans. We identified mutation *IRF8*^{K108E} in an infant who was hospitalized following routine BCG vaccination at birth due to disseminated mycobacterial infection and the inability to thrive. Analysis of PBMCs surprisingly revealed that this infant lacked all circulating monocytes and DCs. We also identified mutation *IRF8*^{T80A} in two unrelated patients suffering from MSMD who showed a selective depletion of CD11c⁺ / CD1c⁺ DCs. Functional testing revealed that both mutations significantly affect transcriptional function and DNA binding of the IRF8 proteins. RNA-sequencing on PBMCs of patient K108E compared to healthy controls revealed downregulation of genes involved in immune response and upregulation of genes involved in cell cycle and division. This analysis shows that specific cell gene signatures associated with antigen presentation were severely depleted while signatures associated with neutrophils and naïve T cells were abnormally enriched. Furthermore, we observed that genes downregulated in patient K108E are significantly associated with direct IRF8 binding sites. Further biochemical characterization revealed that the K108E mutation causes defective transcriptional regulation and nuclear translocation. The E108 mutant protein is significantly less stable and rapidly degraded via the proteasome compared to wild type K108. This is concomitant with increased ubiquitination and SUMOylation of E108 compared to K108. We identified a novel role for Irf1, the major binding partner of Irf8, in regulation of bone metabolism. We demonstrated that Irf1 negatively regulates resorption activity of osteoclasts as well as mineralization by osteoblasts while promoting proliferation of osteoblast precursors. We observed that the overall effect of *Irf1* deficiency in mice is increased stiffness as well as increased force and work to failure of femurs due to the increased mineral deposition by osteoblasts, indicating a dominant role for Irf1 in osteoblasts. In summary, the studies in this thesis identify two forms of human *IRF8* deficiencies affecting the development of monocytes and DCs and uncover a novel MSMD-causing gene. Further work based on this thesis will help to better understand the complex genetic basis of susceptibility infection with intracellular pathogens in general and particularly, mycobacteria.

RÉSUMÉ

Cette thèse étudie le rôle du facteur de transcription, Interferon regulatory 8 (IRF8) dans la susceptibilité aux infections mycobactériennes et dans le développement des phagocytes mononucléaires chez les humains. Nous avons identifié la mutation *IRF8*^{K108E} chez une fillette âgée de 10 mois qui a été hospitalisée suite à une vaccination de routine de BCG à la naissance qui a causé une infection mycobactérienne disséminée mettant gravement sa vie en danger. L'analyse de ses cellules périphériques mononucléaires sanguines (PBMCs) a montré un déficit total de monocytes et cellules dendritiques (DCs). Nous avons aussi identifié la mutation *IRF8*^{T80A} chez deux patients non-liés souffrant de la susceptibilité mendélienne aux maladies mycobactériennes (MSMD). Ces deux patients manquaient d'un sous-ensemble spécifique de cellules dendritiques, CD11c⁺ / CD1c⁺. Une évaluation fonctionnelle a montré que ces deux mutations affectent significativement la fonction transcriptionnelle et la liaison à l'ADN d'IRF8. L'analyse du séquençage de l'ARN des PBMCs de la patiente K108E en comparaison à des contrôles sains a révélé une baisse de l'expression de gènes qui contrôlent la réponse immunitaire et une augmentation d'expression de gènes qui contrôlent le cycle et la division cellulaire. Les signatures génétiques spécifiques aux cellules qui sont associées à la présentation d'antigènes sont sévèrement réduites, tandis que les signatures associées aux neutrophiles et les cellules T naïves sont anormalement enrichies. De plus, on a observé que les gènes ayant expression réduite chez la patiente K108E sont significativement associés aux sites de liaisons directes d'IRF8. Des caractérisations biochimiques additionnelles ont montré que la mutation K108E cause une translocation nucléaire et une activité transcriptionnelle défectueuses. La protéine mutante E108 est beaucoup moins stable et est dégradée plus rapidement par le protéasome en comparaison à la protéine sauvage K108. Ceci est concomitant avec une augmentation de l'ubiquitination et de la SUMOylation de la protéine mutante. Nous avons également identifié un rôle nouveau d'Irf1, un partenaire de liaison majeur d'Irf8, dans la régulation du métabolisme osseux chez les souris. Nous avons démontré qu'Irf1 régule négativement la résorption osseuse par les ostéoclastes et la minéralisation osseuse par les ostéoblastes et en même temps, favorise la prolifération des cellules précurseurs des ostéoblastes. Nous avons observé que l'effet global dû à l'absence d'Irf1 chez les souris est une augmentation de rigidité de leurs fémurs à cause de l'augmentation de dépôt de minéraux par les ostéoblastes. Ceci indique que le rôle d'Irf1 chez les ostéoblastes est plus dominant que son rôle chez les

ostéoclastes. En résumé, les études de cette thèse ont identifié deux catégories de déficiences humaines d'*IRF8* qui affectent le développement des monocytes et cellules dendritiques et ont aussi permis de découvrir un nouveau gène qui cause le MSMD. Des études futures basées sur cette thèse aideront à mieux comprendre la base génétique complexe de la susceptibilité aux infections par des mycobactéries et plus généralement par des pathogènes intracellulaires.

PREFACE

The work described in chapters 1, 2, 3 and 4 of this thesis is published as follows:

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*Equal contribution.

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Chapter 4: Salem S., Gao C., Ailian L., et al. A novel role for Interferon regulatory factor 1 (IRF1) in regulation of bone metabolism. *J Cell Mol Med.* 2014. 18(8):1588-98

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CONTRIBUTION OF AUTHORS

Chapter 2: The studies in chapter 2 were performed in collaboration with the following: Sophie Hambleton, Venetia Bigley, Muzlifah Haniffa and Matthew Collin performed cytokine production and FACS related studies on Subject 1. Jacinta Bustamante, Stephanie Boisson-Dupuis and Frederic Geissmann performed cytokine production and FACS related studies on Subjects 2 and 3. Karina Butler was the physician treating Subject 1. David Burk and Albert Berghuis performed the molecular modeling. All other authors contributed clinical samples to the collection of MSMD patients. I sequenced the IRF8 gene in Subject 1, her family members, the 450 MSMD patients and the family members of Subject 2 and 3 and finally a panel of 1200 CEPH control DNA samples. I performed all the biochemical assays including transactivation experiments, chromatin immunoprecipitations (ChIPs), co-transfections and western blot experiments. I also helped in preparing and writing the manuscript with Sophie Hambleton and Jean-Laurent Casanova who wrote and edited the final manuscript.

Chapter 3: The studies in chapter 3 were performed in collaboration with colleagues and collaborators as follows: David Langlais performed all RNA sequencing related experiments and performed bioinformatics analyses. Francois Lefebvre and Guillaume Bourque performed bioinformatic analysis of RNA sequencing datasets. Venetia Bigley and Muzlifah Haniffa obtained and handled initial clinical patient material. Karina Butler and Timothy Ronan Leahy conducted initial immunophenotyping of the patient material. David Burk and Albert Berghuis performed molecular modeling. I performed all biochemical assays including transactivation, nuclear localization, cellular fractionation, stability, ubiquitination and SUMOylation assays. I also prepared and wrote the first draft of the manuscript. Sophie Hambleton performed immunophenotyping of patient material and edited the manuscript. Jean-Laurent Casanova edited the manuscript.

Chapter 4: The studies in chapter 4 were performed with the help of our collaborators as follows: Chan Gao performed the micro CT analysis of the femurs. Ailian Li and Huifen Wang sectioned the bones for histological analysis and performed osteoblast proliferation and mineral deposition assays. Loan Nguyen-Yamamoto provided the necessary technical help and reagents. I analyzed the micro CT, histomorphometric data and histological sections and performed protein expression experiments. I also performed the osteoclast differentiation assay, the bone resorption pit formation assay, real time PCR experiments. Finally, I prepared and wrote the first draft of the manuscript. David Goltzman provided suggestions to the manuscript and technical help. Janet E. Henderson provided technical help and edited the manuscript.

My thesis supervisor, Philippe Gros, provided guidance and strategies for experimental design throughout all the above mentioned studies and edited all final manuscripts.

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OBJECTIVES AND RATIONALE

Individuals suffering from Mendelian susceptibility to mycobacterial disease (MSMD) are extremely susceptible and develop severe clinical symptoms following exposure to weakly virulent mycobacteria such as non-tuberculous environmental mycobacteria (NTM) and the BCG vaccine^{1,2}. MSMD patients were found to carry inborn mutations in gene products of the IL-12/IFN- γ circuit essential for host response to mycobacteria. Unfortunately half of MSMD patients do not have a known genetic etiology and still suffer from disseminated mycobacterial disease³. In our laboratory, we have identified and used BXH-2 mice that carry a partial loss of function mutation in the *Irf8* gene (*Irf8*^{R294C}) as a model to study susceptibility to mycobacteria. Studies on BXH-2 mice have demonstrated that *Irf8* is essential for development of dendritic cells, type I cytokine production and overall immune response to intracellular pathogens including mycobacteria in mice^{4,5}. This indicated that *IRF8* could be an ideal candidate gene to investigate in MSMD patients.

The main objective of the study in chapter 2 was to investigate the role of human *IRF8* in susceptibility to mycobacteria. We identified an infant girl who was hospitalized following routine vaccination at birth with BCG. We sequenced this patient and identified a homozygous mutation in the *IRF8* gene (*IRF8*^{K108E}). We also sequenced the *IRF8* gene in a group of 450 MSMD patients and identified a heterozygous mutation in two unrelated MSMD patients (*IRF8*^{T80A}). These two mutations were found to be the cause for the extreme mycobacterial susceptibility seen in these patients and the defects in mononuclear phagocyte development.

The objectives of the study in chapter 3 were to further characterize the effect of the K108E mutation on both biological and biochemical functions of IRF8. We performed RNA-sequencing to investigate differential gene expression due to *IRF8* deficiency of our infant patient compared to healthy controls. In addition, we compared the RNA-sequencing gene list with another gene list generated by ChIP-sequencing on IFN- γ activated myeloid cells to identify direct IRF8 target genes that are depleted in our patient. We then tested the effect of the K108E mutation on transactivation, nuclear translocation, protein stability and post-translational modifications of IRF8 protein.

Recently, a role for Irf8 in regulation of bone metabolism in mice has been identified⁶. Irf8 was found to maintain bone integrity and prevent bone breakdown by suppressing differentiation of bone resorbing osteoclasts⁶. Irf1 is a major binding partner of Irf8 and together they function in similar pathways to activate expression of genes important for cell development and immune response. The main objective in chapter 4 was to investigate a possible novel role for Irf1 in bone metabolism. We analyzed long bones of *Irf1*^{-/-} mice by radiology to study bone morphology and aimed to uncover the effect of *Irf1* deficiency on differentiation and activity of bone marrow derived osteoclasts as well as the effect on proliferation and mineral deposition of osteoblasts. This study demonstrated that Irf1 plays important regulatory roles in both bone cell lineages.

Chapter 1:

Introduction and Literature Review

Infectious diseases as a constant world health problem

In the 1970s, with the advances in vaccines and antibiotics and elimination of smallpox, scientists were optimistic that infectious diseases would no longer be a global health problem⁷. Nevertheless, infectious diseases are currently the third leading cause of death in the United States and the leading global cause accounting for 17 million deaths annually^{8,9}. Developing countries are particularly heavily burdened by HIV/AIDS, malaria, diarrheal diseases and tuberculosis⁸. Widespread resistance to antibiotics, emergence of new virulent strains and increased worldwide travel are just a few reasons for the increased severity of the problem^{10,11}. In order to develop novel therapeutic strategies and vaccines to combat infectious diseases, host defense mechanisms including critical genetic components that regulate immune response need to be better studied. The following paragraphs will discuss the pathogen that causes one of the most prevalent infectious diseases, tuberculosis (TB).

1. Mycobacterial infections

Mycobacterium species are facultative intracellular bacteria that share a characteristic thick, hydrophobic cell wall rich in mycolic acids¹². The majority of mycobacterial species are found in the environment, in contaminated soil and water and are not transmitted from person-to-person. These species are termed non-tuberculous mycobacteria (NTM) and include *Mycobacterium intracellulare* and *Mycobacterium avium*¹³⁻¹⁵. All humans are exposed to environmental mycobacteria and approximately 85% of children worldwide are vaccinated with live BCG, an attenuated strain of *Mycobacterium bovis*, as an attempt to control TB³. NTM and BCG are normally non-pathogenic but can cause severe human disease in immunocompromized hosts such as patients with primary immunodeficiencies (PIDs)¹⁶⁻¹⁸. Finally, other mycobacterial species are highly pathogenic and cause serious diseases in humans such as leprosy caused by infection with *Mycobacterium leprae* and tuberculosis caused by virulent *Mycobacterium tuberculosis* (MTB)¹⁹.

1.1 Tuberculosis (TB)

Three people in the world die of TB every minute²⁰. TB remains the largest cause of morbidity and mortality in the world from a single infectious agent²¹. Approximately one third of the world population has been infected with TB in addition to causing 8.6 million new cases and 1.3 million deaths per year worldwide²². The TB problem is worsened by the emergence of multidrug-resistant TB (MDR-TB) and by the interaction of MTB with HIV increasing the death toll as well as the risk of reactivating latent disease by 20 fold^{23,24}. Although TB can be present worldwide, the most endemic regions are south east Asia and sub-Saharan Africa (**Figure 1**) where numbers can reach up to 700 cases per 100, 000 individuals and the highest mortality is in the African region due to the synergy with HIV^{22,25}.

TB is primarily a pulmonary disease that can spread to other organs in the body. Transmission is via inhalation of aerosol droplets from lungs of infected individuals with active disease²⁵. A key to the success of MTB as a pathogen is its ability to survive and replicate in the host macrophages for long periods of time leading to different clinical outcomes²³. Primary disseminated TB is seen in the majority of pediatric cases where clinical symptoms develop within the first 2 years following infection. The secondary manifestation, observed in adults, is chronic and slowly progressive TB where clinical symptoms develop more than 2 years after infection. An effective host immune response prevents development of active disease resulting in latent and asymptomatic infection in approximately 90% of the cases. Reactivation of TB can be caused by several factors including immunosuppression, alcoholism and nutritional deficiency²⁶.

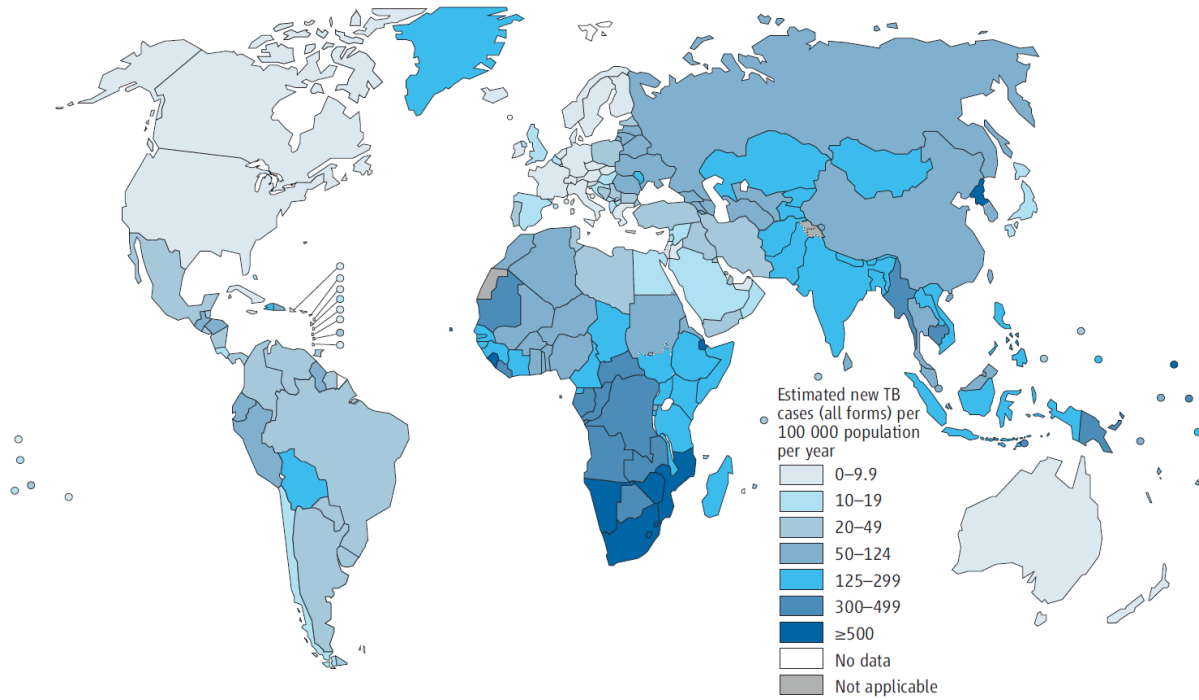


Figure 1. Estimated global TB incidence²².

The number of TB cases relative to the population in 2012. The lowest rates are found mainly in Canada and the United States of America while highest rates are found in Africa.

1.2 Host immune response to mycobacterial infection

The immune response to MTB is complex and requires coordinated interaction between cells of the innate (mononuclear phagocytes) and adaptive (T, B lymphocytes) immune system.

MTB enters the host through the mucosal surfaces via the lung and is phagocytosed by resident macrophages and dendritic cells (DCs) (**Figure 2**) resulting in cell activation and maturation which is characterized by increased expression of major histocompatibility complex (MHC) class II and co-stimulatory molecules including CD40, CD80 and CD86²⁷. Activated macrophages and DCs will migrate to the peripheral lymph nodes and secrete type 1 cytokines IL-12, IL-18 and IL-1 to prime lymphocytes and induce differentiation of T-helper 1 (Th1) cells²⁸. CD4⁺ Th1 and CD8⁺ cytotoxic T cells will recognize mycobacterial antigens presented by DCs in association with class I and class II MHC molecules, migrate to the site of infection and secrete interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α) to activate macrophage anti-microbial functions and initiate formation of protective granulomas^{27,28}. Activated macrophages produce bacteriostatic and bactericidal molecules such as reactive oxygen radicals (ROS), nitric oxide (NO) and iron chelators essential for pathogen control. Furthermore, these activated cells also show increased maturation of the MTB phagosomes including increased acidification and delivery of lysosomal enzymes. Finally T cells also produce perforin and granulysin that additionally contribute to anti-mycobacterial activity^{23,27,29}.

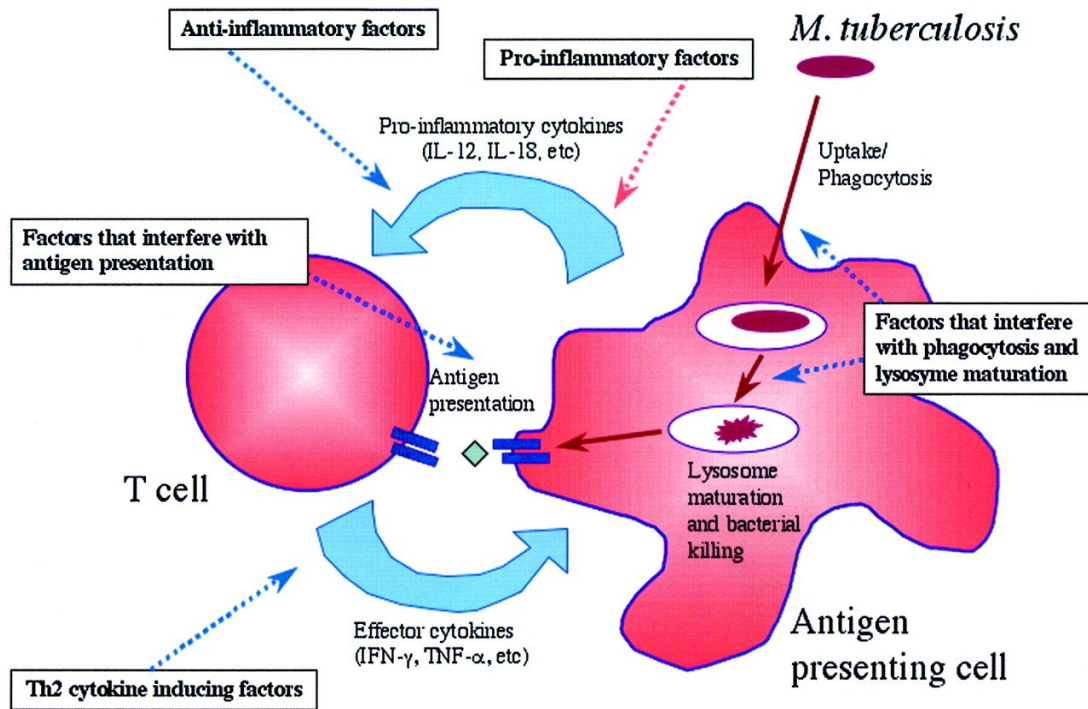


Figure 2. Pathogenesis of MTB infection³⁰.

Immune response to MTB requires interaction between infected phagocytes and antigen-specific T cells. Critical cytokines produced are IL-12, IL-18 by phagocytes and IFN- γ and TNF- α by CD4⁺ Th1 cells.

This immune response is robust and efficient as only 10% of individuals exposed to MTB develop active disease. Approximately half of these susceptible individuals will develop active TB within a year. The majority (90%) of individuals are considered naturally resistant and able control the infection to a level that does not cause disease. This is said to be latent TB which is capable of reactivation and causing outbreaks at a later time²⁷. Most susceptible humans with active TB do develop MTB-specific Th1 immunity in the form of delayed-type hypersensitivity (DTH). The expression of DTH in these patients is confirmed by the formation of protective granulomas composed primarily of macrophages at the site of lung infection. Th1 immunity is therefore critical for protecting resistant humans but also for allowing immunocompetent susceptible humans with active disease to restrict the growth of MTB and its pathological damage²⁷.

1.3 Formation of granulomas

The persistent nature of mycobacterial infections requiring constant activation of the host immune response, results in the development of protective granulomas³¹. Granulomas are structures consisting of central infected macrophages with peripheral recruited lymphocytes and a fibrous capsule allowing for both the containment of the infection but also the survival of MTB and protection from IFN- γ -producing T cells. Both cytokines, TNF- α and IFN- γ , are essential for the organized development of granulomas due to their leukocyte recruitment, chemokine production and anti-microbial functions³². Another cytokine, IL-17 has also been shown to be important for early granuloma formation by recruiting neutrophils which produce chemokines such as CXCL9 that are necessary for proper granuloma formation³¹. The granuloma structure is maintained by the constant activation of the immune system due to constant presence of antigens and immunostimulatory lipids occurring during DTH. This therefore allows the MTB to survive within the granuloma but remain in a dormant and asymptomatic state³². During active TB, the center of the granuloma undergoes necrosis and the high lipid content of the macrophages results in formation of caseum which contains highly contagious bacilli. As the lung architecture deteriorates and becomes more necrotic, MTB can disseminate and replicate in other organs³³.

The success of TB disease despite a robust immune response is in part due to the evolution of MTB to evade host macrophage defenses. Briefly, MTB has been shown to interfere with phagosomal maturation and acidification, inhibit antigen presentation by DCs and inhibiting apoptosis of macrophages while promoting sustained membrane damage and necrosis³⁴⁻³⁶. Some pathogenic mycobacteria can also escape the phagosomes and replicate in the cytosol of macrophages³⁷.

The host innate and adaptive immune defenses that MTB must defeat to persist for years in infected cells are still poorly understood and can manifest themselves as genetic determinants of innate resistance or susceptibility to infections in mouse models and in human populations^{33,38,39}.

2. Genetic determinants of response to mycobacterial infections

Association studies with candidate genes and genome wide linkage analyses have shown that the various aspects of human immune responses to mycobacterial infections are genetically complex and further modulated by different microbial pathogenicity determinants^{33,39-42}.

2.1 Genetic susceptibility to virulent MTB in humans

Since the nineteenth century, it has been believed that genetic factors play important roles in susceptibility to MTB infection. Epidemiological studies showing sex and racial differences in susceptibility, geographical distribution and familial segregation of disease as well as population studies in endemic areas have all shown evidence for a large genetic component in susceptibility to TB^{43,44}. Moreover, twin studies showed higher concordance rates of TB in monozygotic than in dizygotic twin pairs, further supporting a genetic hypothesis for TB susceptibility⁴⁵.

Studies in populations that are highly exposed to TB have attempted to identify host genetic factors that determine susceptibility to MTB infection in humans with the majority of the analyses being association studies to investigate the role of specific candidate genes. A number of these genes were investigated since their murine orthologs were established to play important roles in immune response to mycobacterial infections in mice.

The first example of such is *NRAMP1* (*SLC11A1*), the human ortholog of murine *Nramp1* which plays a key role in murine response to mycobacteria^{46,47}. Polymorphisms at or near the *NRAMP1* gene were identified and show strong association with TB disease in endemic areas of Gambia and South-East Asia as well as in linkage studies of a large aboriginal population in northern Canada⁴⁸⁻⁵⁰. *NRAMP1* alleles were also shown to represent a major risk factor for pediatric TB in a cohort of 200 ethnically diverse families from the Houston area⁵¹. Interestingly, a study has shown that monocytes derived from patients carrying variant *NRAMP1* alleles that were previously associated with TB susceptibility, show defective NRAMP1 protein function and reduced maturation of *Salmonella*-infected vacuoles⁵². These studies clearly demonstrate the essential role of Nramp1 in mycobacterial defenses in both mice and humans. Nramp1 has been shown to act as a metal ion pump at the phagosomal membrane where its activity reduces the availability of essential nutrients, thereby restricting replication of intracellular pathogens such as mycobacteria^{53,54}. Association studies with other candidate genes have identified a long list of polymorphic variants that behave as risk factors for susceptibility to TB in humans. This list includes the *SP110* gene, the human ortholog of murine *Ipr1* in which alterations causes susceptibility to pulmonary TB in mice, and the human *IRGM1* (human ortholog of murine *Lrg-47*) gene coding for an interferon-inducible, phagosomal membrane-associated GTPase. *Lrg-47* deficient mice are susceptible to TB⁵⁵⁻⁵⁹. Other TB susceptibility associated polymorphisms identified include the HLA class II, *DR2* and *DQB1*, Vitamin D receptor, mannose-binding protein, *CD20* (DC-SIGN), *CD209*, the chemokine *MCP-1*, *TLR1*, the lysosomal cysteine proteinase cathepsin Z, the promoter of the melanocortin receptor (*MC3R*) and finally the ATP receptor *P2X7*⁶⁰⁻⁶⁸. Furthermore polymorphic variants in prostaglandin-associated genes including leukotriene A4 hydrolase and 5-lipoxygenase have also been associated with

susceptibility to TB^{69,70}. Finally, a functional polymorphism mapping to the NFκB site in the *IFNG* gene has been validated in several populations as causing susceptibility to TB^{71,72}.

Genetic linkage analyses by whole genome scan in affected siblings from Gambia and from South Africa identified suggestive linkages on chromosomes 15q and Xq⁷³. Finally, another genome-wide linkage study in a Moroccan population identified a major locus that is inherited in a dominant manner on chromosome 8q12-q13 which predisposes to adult pulmonary TB⁷⁴.

2.2 Genetic susceptibility to weakly virulent mycobacteria – MSMD

In the early twentieth century, in order to fight against TB, Albert Calmette and Camille Guérin developed an attenuated and avirulent strain of a bovine tuberculous bacillus, termed Bacille Calmette-Guérin (BCG), through serial passages of virulent *Mycobacterium bovis*³². This BCG vaccine is effective in preventing childhood TB but has variable and lower efficacy in adults⁷⁵. Almost every human has encountered NTM during their lifetime and worldwide vaccination policies of newborns have ensured exposure to BCG, both of which normally do not result in manifestation of disease. Severe and disseminated mycobacterial disease due to NTM and BCG is mostly identified in immunocompromized patients such as those suffering from HIV/AIDS, patients with inherited disorders resulting in PIDs such as severe combined immunodeficiency (SCID), chronic granulomatous disease (CGD) and a small group of patients suffering from Mendelian Susceptibility to Mycobacterial (MSMD)^{17,76-78}. MSMD (MIM 209950) is a rare, congenital syndrome characterized by susceptibility to infection with weakly virulent mycobacteria (BCG, NTM) in otherwise healthy individuals^{3,77}. Affected individuals are also extremely susceptible to the more virulent MTB and salmonellosis (typhoidal or non-typhoidal) is seen in nearly half of MSMD patients^{26,79}. To date approximately 500 patients were identified worldwide and large scale candidate gene approaches have uncovered ten genetic disorders contributing to the vast clinical heterogeneity of this disease^{2,18,76}. Over the past 15 years, Casanova and colleagues have identified mutations (**Figure 3**) in genes coding for IL-12p40 (*IL12B*), its receptor IL-12Rβ1 (*IL12RB1*), both receptors for receptor IFN-γ; IFN-γR1 and IFN-γR2 (*IFNGR1, IFNGR2*), the IFN-γR signaling partner STAT1 (*STAT1*), the NFκB

regulator I κ ky (*NEMO*) and recently, a type I IFN induced ubiquitin-like protein ISG-15 (*ISG15*) (reviewed in^{2,18,33,76}). Immunodeficiency associated with these mutations is inherited as either autosomal recessive (*IL12B*, *IL12RB1*, *IFNGR1*, *IFNGR2*, *ISG15*), autosomal dominant (*IFNGR1*, *STAT1*) or X-linked recessive (*NEMO*). All mutated gene products play critical roles in the IL-12/IFN- γ signaling circuit (**Figure 3**) that is essential for macrophage function and activation as well as T cell proliferation and differentiation into CD4⁺ Th1 cells. This IL-12/IFN- γ signaling circuit has been well established to be essential for host defense against mycobacterial infections and other intracellular pathogens in both mice and humans^{18,27,28}. More recently, mutations in the gene encoding the gp91^{phox} subunit of NADPH oxidase (*CYBB*) that selectively impairs enzyme activity and macrophages' respiratory burst were identified in MSMD patients further highlighting the importance of this pathway⁸⁰. Unfortunately, in nearly half of MSMD patients, the underlying genetic defect is still unknown.

Mice as models for host response to mycobacteria

Over the years, mice have proven to be very effective in studying various aspects of host response to mycobacteria such as pathogenesis, innate and adaptive immune responses and predisposing genetic effects where important molecules were identified by studying knock-out mice (**Table I**) (summarized in⁸²). Mice infected with MTB develop fatal pulmonary disease that resembles the pathology seen in humans and require the same immune responses including protective roles of CD4⁺ T cells, IFN- γ , TNF- α and IL-12²⁷.

2.3 Genetic susceptibility to virulent MTB in mice

Two main genetic approaches are often used in order to identify genes underlying host response to mycobacterial disease. The reverse genetics approach studies the role of individual genes by infecting mice bearing a loss of function mutation at a particular locus, whereas forward genetics studies the differential phenotypic susceptibility of different inbred mouse strains following infection, and without prior knowledge of the gene involved^{33,38}.

Aerosol infection of mice with small doses (10^2 CFU) of MTB results in a physiologic form of TB that is similar to the pulmonary disease observed in human TB²⁷. Susceptibility to MTB (H37Rv) of inbred mouse strains is measured by the extent of pulmonary replication and survival following infection. A survey was conducted that classified the CBA, DBA/2, C3H and 129/Sv strains as susceptible (S) and the C57BL/10, C57BL/6 and BALB/c strains as more resistant (R) following infection via intravenous and aerosol routes⁸³. In a number of F1 hybrids tested, it was demonstrated that resistance was inherited in a dominant fashion. Susceptible mouse strains cannot control bacterial replication in the lungs and have inflammatory reactions causing pulmonary consolidation, reducing the lung surface area available for oxygen exchange which ultimately results in death. These mice also do not develop organized and lymphocyte rich granulomas but rather contain foamy macrophages filled with mycobacteria and neutrophils. Resistant mice limit bacterial growth and form lymphocyte aggregates to develop protective granulomas that prevent massive tissue injury^{83,84}.

In order to uncover the genes responsible for the differences in host response of two susceptible mouse strains DBA/2J (S) and C57BL/6J (R), whole genome scanning in informative F2 mice (DBA/2J X C57BL/6J) was carried out following intravenous infection with MTB H37Rv (10^5 CFU) and survival was monitored. This study identified two significant linkages on distal chromosome 1 termed *Trl-1* (LOD 4.8), proximal chromosome 7 termed *Trl-3* (LOD 4.7) and proximal chromosome 3 *Trl-2* (LOD 3.9). Homozygosity for B6 alleles at each locus was associated with increased resistance to infection⁸⁵. An additional genome scan was performed in F2 mice (DBA/2J X C57BL/6J) following aerosol exposure to MTB H37Rv (10^2 CFU) and lung CFUs were counted at day 90 to determine susceptibility. A strong linkage on central chromosome 19 termed, *Trl-4* (LOD 5.6), was identified with B6 alleles being associated with increased resistance and inherited in a co-dominant manner. A strong additive effect was detected between *Trl-3* and *Trl-4* (LOD 10.4) where mice homozygous for B6 alleles at both *Trl-3* and *Trl-4* were completely resistant to MTB infection⁸⁶. Furthermore, *Trl-3* was shown to modulate the ability of activated macrophages to restrict mycobacterial replication rather than affecting the initiation of Th1 response⁸⁷. Another genome-wide scan using a backcross between highly susceptible I/St mice and resistant A/Sn mice [(A/Sn X I/St) X I/St] using loss in body weight following MTB infection (5×10^5 , intravenous) identified in females significant linkages on proximal chromosome 9 (LOD 6.7) and distal chromosome 3 (LOD 3.9)⁸⁸. Additional studies in an (A/Sn X I/St) F2 cross confirmed linkages on distal chromosome 3 termed *Tbs1* (LOD 1.8) and chromosome 9 termed *Tbs2* (LOD 2.6 in males, LOD 1.9 in females)⁸⁹. A previous study showed that a new coding mutation in the *Tnfa* leader sequence carried by the susceptible I/St mice, that causes increased secretion of soluble Tnfa, may be the underlying mechanism of the suggestive linkage identified on chromosome 17⁹⁰. The high susceptibility to pulmonary TB in C3HeB/FeJ mice was demonstrated to be controlled by a complex locus on chromosome 1, termed super-susceptibility TB (*sst1*), caused by the rearrangement in the Intracellular pathogen resistance 1 (*Ipr1*) gene which codes for Ifi75 (Interferon-induced protein 75) that regulates macrophage apoptotic response following phagocytosis of MTB^{55,91,92}. In addition, the *sst-1* locus was shown to control macrophage resistance to another intracellular pathogen, *Listeria monocytogenes*⁵⁵. To date, a large number of genetically mutated or knocked-out mouse strains have been tested and classified as resistant or susceptible following infection with MTB (**Table I**) (reviewed in⁹³).

Table I. Responses of mouse mutants to MTB
(Adapted from Salem S and Gros P⁹³)

Mouse Strain	Mycobacterial Strain	Infection (dose)	Phenotype	Cytokine production following MTB infection
B2m Beta2-microglobulin	Erdman Strain	Intravenous (10^6)	-Rapid Death Uncontrolled MTB growth	Normal production
Card-9 Caspase Recruitment Domain containing protein 9	H37Rv	Aerosol (450)	-Rapid death Uncontrolled MTB growth	Lower production of TNF α , IL-6, CCL5, IL-12
Card15/Tlr2 Caspase Recruitment Domain 15 / Toll-like Receptor 2	H37Rv	Aerosol (100)	-Normal MTB load	Normal production
Ccr2 Chemokine (C-C motif) Receptor 2	H37Rv	Intravenous ($3.3-8.0 \times 10^5$)	-Rapid Death High MTB load	Lower early IFN- γ production
Cd4 Cluster of Differentiation 4	H37Rv	Aerosol (100)	-Altered granuloma Defect in late response to MTB	Normal IFN- γ production
CD8α Cluster of Differentiation 8 alpha	Erdman Strain	Intravenous (10^5 , 10^6)	-Decreased survival Uncontrolled MTB growth	N/A
Cd36 Cluster of Differentiation 36	H37Rv	MOI (10:1)	-Reduced MTB load	Normal production
CIita Class II, major histocompatibility complex Transactivator	Erdman Strain	Aerosol (100)	-Decreased survival High MTB load	Lower IFN- γ production
Cr3 Complement Receptor 3	Erdman Strain	Intravenous (2×10^5)	-Normal survival	Normal IL-12, IFN- γ production
Clec7a C-type lectin domain family 7 member A	H37Rv	Aerosol (100)	-Reduced MTB load Normal survival	Normal production

Cxcr3 CXC Chemokine Receptor 3	H37Rv	Aerosol (10-100)	-Normal survival -Normal MTB load	Normal production
Dap10 DNAX Activation Protein 10	H37Rv	Aerosol (100)	-Normal survival -Normal MTB load	Normal production
Fcy Fragment Crystallizable gamma Chain			-Decreased survival -High MTB load	Higher IL-10 production
FcyRiib Fragment Crystallizable gamma Receptor II B	Erdman Strain	Aerosol (150)	-Reduced MTB load	Higher IFN- γ , IL-12p40 production
Go Granzyme b	Erdman Strain	Aerosol (100)	-Normal MTB growth	Normal IFN- γ production
Gbp1 Guanylate binding protein 1	M.bovis BCG	Intravenous (10^5)	-Normal granuloma formation	Normal IFN- γ production, NO release
Ighm Ig mu Chain C region	H37Rv	Aerosol (100)	-Normal MTB load	Normal IFN- γ production
Irga6 Immunity Related GTPase A6	H37Rv	Aerosol (100)	-Normal MTB load	N/A

Irgd Immunity Related GTPase D	Erdman Strain	Aerosol (5×10^2)	-Normal MTB growth	
		Intravenous (10^5)	-Normal MTB load	N/A
Irgm1 Immunity Related GTPase M1	Erdman Strain	Aerosol (5×10^2)	-Decreased survival Uncontrolled MTB growth	Normal IFN- γ , TNF α , NOS2 production
		Intravenous (10^5)		
Irgm3 Immunity Related GTPase M3	Erdman Strain	Aerosol (5×10^2)		
		Intravenous (10^5)	-Normal MTB load	N/A
	Erdman Strain	Intravenous (10^5)	-Decreased survival -Uncontrolled MTB growth -Decreased survival	Higher production of IFN- γ , TNF α No production of NO
iNos Inducible Nitric Oxide Synthase	Erdman Strain	Aerosol (50)	-Uncontrolled MTB growth -Decreased survival	Lower RNI production
			-Uncontrolled MTB growth	
Itgal Integrin Alpha L	Erdman Strain	Aerosol (7.2×10^1)	-Altered granuloma	Lower IFN- γ production
Itgb2 Integrin Beta-2	Erdman Strain	Aerosol (2.43×10^2)	-Decreased survival	N/A
			-Decreased survival	
	Erdman Strain	Intravenous (10^6)	-Uncontrolled MTB growth	No production of ROI
Ifnγ Interferon- gamma	Erdman Strain	Aerosol (5×10^1)	-Uncontrolled MTB growth	N/A
IfnγR Interferon- gamma Receptor	M.bovis BCG	Intravenous (10^7)	-Decreased survival -Altered granuloma	Lower TNF α production Normal IFN- γ , TNF α production Lower NOS2, IFN α production Higher IL-6, IL-18 production
Irf1 Interferon Regulatory Factor 1	Kurono Strain	Aerosol (10^6)	-Rapid death -High MTB load	

Irf8 Interferon Regulatory Factor 8 [R294C]		Intravenous (10 ⁴)	-Rapid death -Uncontrolled MTB growth	
	H37Rv	Aerosol (100)	-Uncontrolled MTB growth	No production of IL-12p40, IFN- γ
Il1α/β Interleukin 1 alpha/beta	H37Rv	Aerosol (10 ⁶)	-Normal survival -Normal MTB Load -Larger granulomas	Normal production
Il1β Interleukin -1 beta	H37Rv	Aerosol (100)	-Decreased survival -High MTB load	Higher TNF α , IFN- γ , IL-1 α production Lower IL-12p40 production
Il1R Interleukin 1 Receptor	H37Rv	Intranasal (2x10 ²)	-Uncontrolled MTB growth -Altered granuloma	Lower IL-12p40 production
Il4 Interleukin 4	H37Rv	Aerosol (100)	-Normal defense against MTB -Decreased survival	N/A Lower IFN- γ , IL-12 production
Il6 Interleukin 6	H37Rv	Intravenous (10 ⁶)	-Uncontrolled MTB growth	Higher IL-4, TNF α production
Il10 Interleukin 10	H37Rv	Aerosol (100)	-Normal defense against MTB	N/A
Il12a Interleukin 12 subunit a (Il- 12p35)	Erdman Strain	Aerosol (50)	-Uncontrolled MTB growth	Normal production of IL- 23p19
Il12b Interleukin 12 subunit b (Il- 12p40)	Erdman Strain	Intravenous (10 ⁵)	-Decreased survival -Uncontrolled MTB growth -High MTB load	Lower IFN- γ , TNF α production
Il17a Interleukin 17 A	H37Rv	Intratracheal (10 ³)	-Altered granuloma -Normal survival	N/A
	Kurono Strain	Aerosol (10 ⁵ ,10 ⁶)	-Larger granulomas -Decreased survival	Lower IFN- γ production
Il18 Interleukin 18	H37Rv	Aerosol (100)	-High MTB load	Lower IFN- γ production
Il18R Interleukin 18 Receptor	H37Rv	Intranasal (2x10 ²)	-Normal defense against MTB	Normal production

Il23-p19 Interleukin 23 subunit p19	H37Rv	Aerosol (100)	-Normal MTB load	Normal IFN- γ , NOS2 production Lower IL-17 production
Il27R Interleukin 27 Receptor	H37Rv	Aerosol (100)	-Reduced MTB load in lung	Higher TNF α , IL-12p40, IFN- γ production
Icam1 Intracellular Adhesion Molecule 1	Erdman Strain	Aerosol (100)	-Decreased survival -Defective granuloma	N/A
Icam1 Intracellular Adhesion Molecule 1 [del Exon 5]	Erdman Strain	Aerosol (100)	-Normal survival -Normal MTB load	Normal production of IFN- γ , IL-12p40
Mcp1 Monocyte Chemoattractant Protein 1	H37Rv	Intravenous (10^5)	-Normal MTB load	N/A
Mrp1 Multidrug Resistance Protein 1	H37Rv	Intranasal (10^5)	-Defective early granuloma formation -Normal survival at late stage	Lower early IFN- γ production Higher IFN- γ , TNF α , IL-12 production Slightly decreased production of NOS2, IL-18
MyD88 Myeloid Differentiation primary response 88	Kurono Strain	Aerosol (10^6)	-Normal defense against MTB -Decreased survival	Lower IL-12p40, IFN- γ , TNF α , NOS2 production
	H37Rv	Intravenous (20-50)	-High MTB load	
Nalp3 NACHT, LRR and PYD containing Protein 3	H37Rv	Aerosol (10^2 , 10^3)	-Normal MTB load in lungs -Normal control of MTB growth	Normal production of IL- 12p40, IL-1 α
Nramp1 Natural Resistance- Associated Macrophage Protein 1	H37Rv	Intravenous (10^4)	-Normal survival	Normal production
	M.bovis BCG	Aerosol (2×10^2)	-Normal survival	Normal production
		Intravenous (2×10^4)	-Uncontrolled BCG growth	N/A

Nfkb1p50 Nuclear Factor kB 1	Kurono Strain	Aerosol (10 ⁶)	-Decreased survival -High MTB load	Lower IFN- γ , TNF α , IL-12, NOS2 production Higher IL-6 production
Nod2/Card15 Nucleotide-binding Oligomerization Domain containing 2 / Caspase Recruitment Domain 15	H37Rv	Aerosol (35-1.5x10 ³)	-Normal MTB load in lungs	Normal production
	H37Rv	Aerosol (100)	-Normal MTB load in lungs -Defective adaptive immunity	Lower IFN- γ , TNF α , NO production Normal IL-12p40, IL-10 production
Po Perforin	Erdman Strain	Aerosol (100)	-Normal MTB growth	Normal IFN- γ production
p47phox Phagocyte Oxidase p47 subunit	Erdman Strain	Aerosol (100)	-Normal survival -High MTB load in early response	Increased IFN- γ response
Pd1 Programmed Death 1	H37Rv	Aerosol (50-320)	-Rapid death -High MTB load in lungs	Higher TNF α , IL-6, IL-17, IFN- γ , IL-12 production
Pges Prostaglandin E Synthase	H37Rv	Aerosol (100)	-High MTB load in lungs	N/A
Stat1 Signal Transducer and Activator of Transcription 1	Kurono Strain	Aerosol (10 ⁶)	-Decreased survival -High MTB load	Lower IL-12p40, IFN- γ , TNF α , IL-18, NOS2 production
SpA Surfactant Associated Protein A	Erdman Strain	Aerosol (100) Aerosol (10 ³)	-Normal survival -Normal MTB load in lungs -High MTB load in lungs	Normal IFN- γ production
SpD Surfactant Associated Protein D	Erdman Strain	Aerosol (100) Aerosol (10 ³)	-Normal survival -Normal MTB load in lungs -High MTB load in lungs	Normal IFN- γ production
TcRa/β T cell Receptor alpha/beta	H37Rv	Aerosol (100)	-Decreased survival -Uncontrolled MTB growth	Lower IFN- γ , NOS2 production

TcRβ T cell Receptor beta	H37Rv	Intravenous (1x10 ⁵ , 5x10 ⁶)	-Decreased survival -Uncontrolled MTB growth	Lower IFN-γ production
TcRγ/δ T cell Receptor gamma/delta	H37Rv	Aerosol (100)	-Normal MTB growth	Normal production Lower NOS2, TNFα, TGFβ, IL-1β, IL-2 production Higher IL-4, IL-6 production
Tlr2 Toll-Like Receptor 2	Kurono Strain	Aerosol (10 ⁶)	-Normal defense against MTB	Higher IL-4, IL-6 production
Tlr2/9 Toll-like Receptor 2/9	H37Rv	Aerosol (50-100)	-Decreased survival -High MTB load in lungs	Lower IFN-γ, IL-13 production
Tlr2/4/9 Toll-like Receptor 2/4/9	H37Rv	Aerosol (100)	-Control of MTB growth	Normal TNFα, IL- 12/23p40, IFN-γ, NOS2 production
Tlr4 Toll-Like Receptor 4 [P712H]	H37Rv	Intranasal (10 ⁵)	-Decreased survival -High MTB load	Higher IFN-γ production Lower IL-4 production Lower NOS2, TNFα, TGFβ, IL-1β, IL-2 production Higher IL-4, IL-6 production
Tlr6 Toll-Like Receptor 6	Kurono Strain	Aerosol (10 ⁶)	-Normal defense against MTB	Higher IL-4, IL-6 production
Tlr9 Toll-like Receptor 9	H37Rv	Aerosol (50-100) Aerosol (5x10 ²)	-Normal survival -Normal MTB load in lungs -Decreased survival -High MTB load in lungs	Lower IFN-γ, IL-12 production
Tap1 Transporter Associated With Antigen Processing 1	Erdman Strain	Intravenous (10 ⁶)	-Decreased survival -Uncontrolled MTB growth	N/A
Tnfa Tumor Necrosis Factor	H37Rv	Aerosol (100)	-Decreased survival -High MTB load -Altered granuloma	Normal RNI production Normal IFN-γ production
TnfaR1 Tumor Necrosis Factor Receptor 1	M.bovis BCG	Intravenous (10 ⁷)	-Decreased Survival -High BCG load -Altered granuloma	N/A

Tnf α R2

Tumor Necrosis
Factor Receptor

2

-Normal defense N/A

-Enhanced
survival

-Reduction in
MTB load

-Decreased
inflammation

Higher IL-12p40, IFN- γ
production

Enhanced NOS2
production

5-Lo

5-lipoxygenase H37Rv

Intranasal (50-300)

2.4 Genetic susceptibility to weakly virulent mycobacteria in mice

The less virulent strain, *Mycobacterium bovis* Bacille Calmette-Guérin (BCG), is also used as a model organism to study host response to virulent MTB. Genetic studies in inbred mice are conducted using infection with low dose BCG ($1-5 \times 10^4$ CFU) via the intravenous route where bacillary replication occurs in the spleen and liver during the first three weeks followed by a curative phase concomitant with a T-cell mediated response, granuloma formation and finally elimination of the bacterial load⁹⁴.

Over thirty years ago, several laboratories observed interstrain differences in inbred mice for susceptibility to infection with *Salmonella typhimurium* (*S.typhimurium*), *Leishmania donovani* (*L.donovani*) and *Mycobacterium bovis* BCG (*M.bovis*). Segregation analyses suggested that the differences in susceptibility were controlled by a single gene named *Bcg/Ity/Lsh* and all three loci were mapped to chromosome 1⁹⁵⁻⁹⁷. In vitro experiments showed that the *Bcg/Ity/Lsh* locus controls intracellular microbial replication in macrophages^{98,99}. A positional cloning approach identified the gene responsible to be *Nramp1* (Natural resistance-associated macrophage protein 1: also termed *Slc11a1*) which, as previously mentioned, encodes for a membrane phosphoglycoprotein expressed in lysosomes of macrophages and gelatinous granules of neutrophils that are recruited to the membrane of microbe-infected phagosomes^{46,47,100}. *Nramp1* functions as a pH-dependent efflux pump for Fe^{+2} and Mn^{+2} ions at the phagosomal membrane to inhibit intraphagosomal bacterial replication resulting in increased acidification and enhanced recruitment of lysosomal enzymes to *Nramp1*-positive infected phagosomes^{101,102}. Studies in inbred mouse strains demonstrated that susceptibility (*Bcg^S*) is caused by a single Gly169Asp (G169D) mutation in the predicted transmembrane 4 (TM4) region that impairs protein folding, targeting and maturation resulting in a non-functional *Nramp1* protein in macrophages of these strains^{103,104}. The function of *Nramp1* was validated by a knock-out mouse strain on the 129Sv background, demonstrating that this otherwise resistant strain becomes susceptible to *S.typhimurium*, *L.donovani* and *M.bovis* infections due to deletions of *Nramp1*¹⁰⁵. Furthermore, transfer of the *Nramp1*^{G169} allele on to the C57BL/6J (*Nramp1*^{D169}) background restores resistance to infection¹⁰⁶.

Additional genetic studies using either *M.bovis* or *S.typhimurium* infection models identified the presence of genetic effects that can further modulate Nrampl-mediated resistance. For example, the wild-derived strain *Mus spretus* (SPRET/EiJ) shows an intermediate level of susceptibility to *M.bovis* BCG despite carrying the resistant *Nrampl*^{G169} allele. Whole genome scanning in a [(C57BL/6J X SPRET/EiJ) X C57BL/6J] cross and a linkage mapping approach revealed a strong interaction between Nrampl and the MHC on chromosome 17¹⁰⁷. Another example is the wild-derived strain *Mus musculus molossimus* (MOLF/Ei) that is extremely susceptible to *S.typhimurium* infection despite presence of resistant *Nrampl*^{G169} alleles. Using (C57BL/6J X MOLF/Ei) F2 mice, two loci on chromosomes 1 (*Ity3*) and chromosome 11 (*Ity2*) were found to control *Salmonella* infection in an *Nrampl*-independent manner¹⁰⁸. Loss of function mutations at the erythrocyte pyruvate kinase (*pklr*) also causes susceptibility to *Salmonella* infection despite presence of resistance *Nrampl*^{G169} allele¹⁰⁹. Finally, the same phenomenon is observed in the BXH-2 mouse strain (discussed in detail below) which is extremely susceptible to mycobacterial infections due to a partial loss of function mutation in a transcription factor, Irf8 (Interferon regulatory factor 8), that positively regulates transcription of the *Nrampl* gene^{5,87}.

Altogether, these studies demonstrate a strong evidence for complex genetic components in human and mouse susceptibility to TB. Further work in our laboratory has focused on identifying additional and novel genes responsible for human immunity against mycobacteria in particular and TB in general.

3. IRF proteins and immune response to mycobacteria

3.1 The BXH-2 mouse strain

While investigating genetic modifiers of *Nramp1*-mediated resistance to intracellular infection, Turcotte, et al identified a particular mouse strain, BXH-2, that shows high susceptibility to *M.bovis* BCG despite carrying the *Nramp1*^{G169} resistance allele⁴. BXH-2 is a recombinant inbred strain derived from BCG-resistant C3H/HeJ (*Nramp1*^{G169}) and BCG-susceptible C57BL/6J (*Nramp1*^{D169}) parental strains. An F1 cross (C57BL/6J X BXH-2) produces mice that are uniformly resistant to *M.bovis* BCG (*Nramp1*^{D169/G169}) further demonstrating that the susceptibility in these mice is caused by another genetic factor that overrides resistance by the functional *Nramp1*^{G169} allele. BXH-2 mice are also known to develop a chronic myelogenous leukemia like (CML-like) syndrome which is fatal by one year of age and created by a two-step mutagenesis process. The first is an inherited predisposing mutation that causes myeloproliferative disorder characterized by a large infiltration of GR1⁺/Mac1⁺ granulocyte precursors into the bone marrow, lymph nodes and spleen causing splenomegaly¹¹⁰. This event was previously shown in our laboratory to be due to a locus on chromosome 8 termed *MyIs* that is inherited in a recessive manner in BXH-2 mice. The second is due to insertional mutagenesis of a replication-competent B-ecotropic retrovirus that is produced by recombination of two replication-incompetent integrated viruses (*Emv1*, *Emv2*)¹¹⁰. The recombinant virus is expressed and infects hematopoietic cells causing deregulated expression of proto-oncogenes and tumor suppressor genes thereby inducing tumor formation (CML) and resulting in death of these mice before one year of age^{110,111}. The production and replication of the B-ecotropic retrovirus in BXH-2 suggests a defect in anti-viral defenses in these mice.

Previous genetic studies performed in our laboratory using informative F2 mice from several crosses (BXH-2 X BALB/cJ), (BXH-2 X A/J) and (BXH-2 X C57BL/6J) mapped the gene responsible for the observed splenomegaly in BXH-2 mice to chromosome 8 (*MyIs*)¹¹⁰. Subsequent positional cloning experiments pinpointed the responsible gene to be *Irf8* (Interferon regulatory factor 8) and demonstrated that BXH-2 mice carry a partial loss of function mutation in the *Irf8* protein (R294C) which maps to the C-terminal interferon (IFN) association domain (IAD) that mediates protein interactions⁴.

BXH-2 mice show impaired control of BCG replication in the liver and spleen at early and late stages following infection with *M.bovis* BCG (2×10^4 , intravenous) due to the inability of these mice to form proper granulomas⁵. BXH-2 mice are also hyper-susceptible to infection with virulent MTB H37Rv and develop rapidly fatal pulmonary TB characterized by uncontrolled MTB replication, impaired granuloma formation followed by dissemination from the lung to the liver and spleen, extensive tissue necrosis and damage and rapid death compared to B6 and C3H parental controls (20 days versus 160 days for C3H and over 250 days for B6)¹¹². Furthermore, additional studies have demonstrated that BXH-2 mice are also susceptible to other intracellular pathogens including *S.thypimurium*, *Legionella pneumophila* (comparable to levels seen in *Nramp1* and *Tlr4* deficient mice) and to the malaria parasite *Plasmodium chabaudi* AS due to their inability to mount long-term protective immunity thereby suffering from recurring high blood parasitemia^{5,113}.

As previously discussed, immunity to mycobacterial infections requires normal functioning of innate phagocytic cells including DCs that will recognize the pathogen and initiate adaptive immune responses³¹. As opposed to their parental strains, BXH-2 mice show a severe depletion of CD8 α^+ DCs but normal numbers of other DC subsets like plasmacytoid DCs (pDCs)¹¹⁴. It has been well established that CD8 α^+ DCs are the main producers of Il-12 and are essential for promoting differentiation of CD4 $^+$ T helper cells thereby inducing a Th1-mediated adaptive immune response that is critical for immunity against mycobacteria^{31,115,116}. As a result, BXH-2 mice show reduced numbers and impaired priming of IFN- γ -producing CD4 $^+$ and CD8 $^+$ T cells and no detectable levels of IFN- γ and Il-12 in the serum^{18,112}.

In the next sections, I will describe the Interferon regulatory factor (IRF) family of proteins and their roles in hematopoietic cell development, cytokine production and immune response focusing on Irf8 and its heterodimerization partner Irf1.

3.2 The IRF family of proteins

Interferons (IFNs) form a group of secreted cytokines that mediate several anti-viral and anti-bacterial effects. Type I IFNs (IFN- α , IFN- β) are induced directly following viral infection by a variety of immune cells whereas type II IFNs (IFN- γ) are secreted by activated T lymphocytes and natural killer (NK) cells¹¹⁷. The IRF family of transcription factors plays important roles in early host response to pathogens as well as hematopoietic cell differentiation and function. So far, nine mammalian members (Irf1-9) have been identified that share a highly conserved N-terminal DNA-binding domain (DBD) that forms a helix turn helix motif consisting of five tryptophan repeats, three of which make direct contact with specific DNA base pairs in promoters of their target genes¹¹⁸. The consensus binding sequences are either the Interferon stimulated response element (ISRE; GAAAnnGAAA) if Irf8 heterodimerizes with another IRF family member (Irf1, Irf4) or ETS interferon composite element (EICE; GGAAnnGAAA) if Irf8 heterodimerizes with an ETS protein (Pu.1)^{119,120}.

3.2.1 Interferon regulatory factor 8 (Irf8)

Irf8 (formerly known as Interferon consensus sequence binding protein, Icsbp) was first identified as a transcription factor that binds to the ISRE motif in the promoter of the MHC class I gene (H-2L^D)¹²¹. Expression of Irf8 is mainly restricted to hematopoietic cells including monocytes, macrophages, DCs, B and T cells and is induced by IFN- γ and to a lesser extent by lipopolysaccharide (LPS) when it synergizes with IFN- γ in these cells¹²²⁻¹²⁵. Irf8 alone has a weak DNA binding affinity which is significantly increased following association with other transcription factors (Irf1, Irf4, Pu.1) via its IFN association domain (IAD)¹²⁶.

Irf8 plays critical roles in several aspects of myeloid cells and is an essential link between innate and adaptive immunity. Irf8 is required for intrinsic macrophage antimicrobial defenses following response to IFN- γ /PAMPs by activating a vast array of genes essential for elimination of intracellular pathogens such as *Nramp1*, *iNos*, *gp91^{phox}*, *p67^{phox}* as well as key surface receptors including *Fcgr1*, *Tlr4* and *Tlr9*¹²⁷⁻¹³¹. Irf8 is also required for initiation of adaptive

immunity by activating expression of both subunits of Il-12 (Il-12p40 and Il-12p35) and Il-18 which are the inducers of CD4⁺ T helper cell differentiation for mounting a Th1 mediated immune response¹³²⁻¹³⁴. Irf8 therefore plays a central role in the IL-12/IFN- γ circuit by creating a positive feedback loop, allowing for sustained IFN- γ during infection with intracellular pathogens such as mycobacteria¹³⁵.

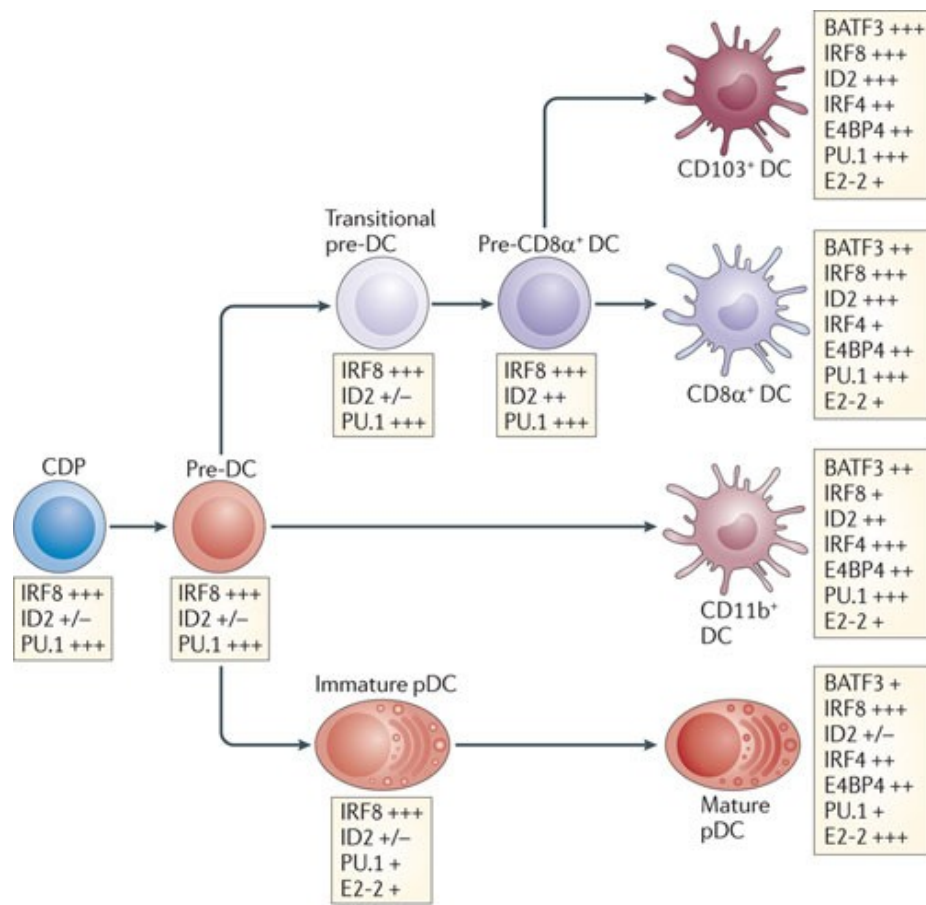
During hematopoiesis, Irf8 promotes differentiation of myeloid progenitors towards the mononuclear phagocyte lineage (monocytes, macrophage, DC) while inhibiting pathways for differentiation of granulocytes¹³⁶. Irf8 is expressed in myeloid precursors where it activates transcription of pro-apoptotic genes (*Cdkn2b*, *Nfl*, *Bax*) and inhibits anti-apoptotic genes (*Bcl2*, *Bcl-Xl*)¹³⁷⁻¹³⁹. Furthermore, Irf8 is expressed and plays an important role in the development of specific dendritic cell subsets (**Figure 4**). Irf8 is highly expressed and promotes differentiation of lymphoid-like CD11c⁺/CD8 α ⁺ conventional DCs (cDCs) which reside mainly in T cell areas of the spleen and produce high levels of Il-12 as well as CD11c⁺ / B220⁺ DCs (pDCs) which produce high levels of type I IFNs^{140,141}. The differentiation of both CD8 α ⁺ DCs and pDCs is promoted by Flt3L expression which in turn is dependent on Irf8¹³⁰. In addition, Irf8 is also required for differentiation of mouse epidermal Langerhans cells (LCs) and dermal DCs¹⁴².

Recently, Irf8 was shown to negatively regulate transcription of Ror γ t (RAR-related orphan receptor gamma t) resulting in inhibition of Th17 cell differentiation and associated immune response¹⁴³. Following heterodimerization with Pu.1, Irf8 activates transcription of the MHC II transactivator (Ciita) thereby increasing expression of MHC class II genes and enhancing antigen presentation by DCs¹⁴⁴. Irf8 is also important for B cell commitment and differentiation and has been demonstrated to restrict the size of marginal zone (MZ) and follicular (FO) B cells^{145,146}. A new role for Irf8 was shown in bone metabolism in preventing pathological bone destruction through direct transcriptional inhibition of Nfatc1 (Nuclear factor of activated T cells 1), an important inducer of osteoclastogenesis⁶. Recent studies have demonstrated that Irf8 is required for progression of neuroinflammatory disease by studying its role in experimental autoimmune encephalomyelitis (EAE), a mouse model for multiple sclerosis (MS). Mice deficient for *Irf8* (*Irf8*^{-/-}) are resistant to EAE since Irf8 expression in antigen presenting cells (APCs) leads to maintenance of Th1 cells by stimulating Il-12 which exacerbates neuroinflammation and disease progression¹⁴⁷. In addition, Berghout et al showed that BXH-2

mice are completely resistant to cerebral malaria (ECM) following infection with *Plasmodium berghei* ANKA infection. This is due to the downregulation of Irf8-regulated genes whose expression is associated with acute neuroinflammation, including *Il12b* (Il-12p40) and other myeloid-specific genes¹⁴⁸.

Irf8^{-/-} mice have been extensively studied and show similar and sometimes more severe phenotypes than BXH-2 (*Irf8*^{R294C}) mice. *Irf8*^{-/-} mice are extremely susceptible to a vast number of pathogens including *M.bovis* BCG, *M.tuberculosis*, *S.thypimurium*, *Leishmania major*, *L.monocytogenes*, *Yersinia enterocolitica*, *Toxoplasma gondii* and *Brucella abortus*^{113,135,149-153}. This high susceptibility is due to weakened anti-microbial activities of their macrophages and defective Th1-mediated immune response caused by the lack of Il-12 and IFN- γ production. These mice also develop a chronic myelogenous-like (CML-like) syndrome characterized by extensive neutrophil infiltration¹⁵⁴. In addition, *Irf8*^{-/-} mice show complete absence of both CD8 α ⁺ DCs and pDCs which further contributes to the defective Il-12 and IFN- α/β and IFN- γ production¹⁵⁵.

It has therefore clearly been demonstrated and established that in mice, Irf8 is absolutely essential for DC development and host response to intracellular pathogens such as mycobacteria.



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Figure 4. Transcription factors regulating differentiation of dendritic cell subsets¹⁵⁶

Conventional DCs (cDCs) and plasmacytoid DCs (pDCs) arise from the same common DC progenitor (CDP) and further differentiation is determined by expression of specific transcription factors. *Irf8* is shown to be required at all stages of DC formation and further differentiation of all DC subsets.

3.2.2 Interferon regulatory factor 1 (Irf1)

Irf1 was first identified as a transcriptional activator of the *Ifn-β* gene through binding to the positive regulatory domain 1 (PRD1) located in its promoter¹⁵⁷. Unlike Irf8, Irf1 is ubiquitously expressed at low levels in almost all cell types and is strongly up-regulated by several stimuli including viral infection, LPS, IFN-α/β, IFN-γ as well as IL-12^{158,159}. Irf1 is a key heterodimerization partner of Irf8 and together they bind their target consensus sequences (ISRE) through their highly conserved DBDs¹⁶⁰. Irf1 and Irf8 synergistically activate genes encoding proteins that function in macrophage activation (iNos, gp91^{phox}, p67^{phox}, caspase-1, Cox2), serve as early inflammatory cytokines (IL-12p40, IL-12p35, IL-18, Rantes, Tnfα) and genes affecting myeloid and lymphoid cell development¹⁶¹⁻¹⁶³. Therefore, along with Irf8, Irf1 also plays an important role in the IL-12/IFN-γ circuit essential for host response to intracellular pathogens.

Although Irf1 is expressed by all cell types, it plays critical roles in the development of hematopoietic cells including DCs, T cells and NK cells. Irf1 expression in myeloid precursors in the bone marrow promotes full differentiation of immature granulocytes in response to G-CSF¹⁶⁴. Furthermore, Irf1 drives differentiation of the CD8α⁺ subset of DCs, sharing this role with its binding partner, Irf8¹⁶⁵. Irf1 is essential for the development and function of NK cells which are critical for innate and adaptive immunity and are potent producers of IFN-γ^{166,167}. It has been demonstrated that the development of CD8⁺ cytotoxic T cells but not CD4⁺ T helper cells is dependent on Irf1¹⁶⁸.

In addition to playing vital roles in immune response, Irf1 is also involved in cell cycle regulation, apoptosis and autoimmunity. Overexpression of Irf1 in a variety of unrelated cell types from different mammalian species consistently lead to growth inhibition¹⁶⁹. Irf1 promotes cell cycle arrest induced by DNA damage in mouse fibroblasts as well as mitogen activated mature T lymphocytes¹⁷⁰. Finally, a study has shown that the incidence and severity of type II collagen-induced arthritis (CIA) and synovial inflammation were significantly reduced in mice deficient for *Irf1* and another study demonstrated decreased incidence of experimental allergic encephalomyelitis (EAE)^{171,172}. These studies suggest that Irf1 plays key roles in promoting inflammation and autoimmunity.

The above-mentioned functions of *Irf1* have all been identified and validated in mice deficient for *Irf1*. Similar to *Irf8*^{-/-} mice, *Irf1*^{-/-} mice are highly susceptible to intracellular pathogens including *M.tuberculosis*, *Leishmania major* and *Listeria monocytogenes*^{167,173,174}. This extreme susceptibility is due to the inability of these mice to mount a Th1-mediated immune response caused by impaired production of IL-12 by macrophages and barely detectable secretion of IFN- γ by lymphocytes¹⁶⁷. Studies *in vivo* have shown that *Irf1*^{-/-} mice have increased numbers of immature granulocyte precursors in response to both G-CSF and M-CSF¹⁷⁵. In addition, these mice have an altered and skewed DC profile characterized by reduced numbers of CD8 α ⁺ DCs and increased pDCs¹⁶⁵. *Irf1*^{-/-} mice lack NK cells and carry lineage-specific defects in thymocyte development characterized by failure in development of CD8⁺ T cells^{167,176}. Finally mouse embryonic fibroblasts (MEFs) from *Irf1*^{-/-} mice are unable to undergo DNA damage-induced cell cycle arrest similarly to MEFs lacking the tumor suppressor p53¹⁷⁷.

3.3 Genome wide association studies (GWAS) for *IRF8* and *IRF1*

Recently, several genome wide association studies have suggested roles for both *IRF1* and *IRF8* in several human inflammatory diseases further demonstrating the importance of these two proteins as inducers of inflammation. Association studies in a UK dataset identified five genes including *IRF8* causing susceptibility to systemic lupus erythematosus (SLE) with high significance. All five genes play important roles in interferon pathways which are required in the pathogenesis of SLE¹⁷⁸. Association in *IRF8* were also found for both ulcerative colitis (UC) and Crohn's disease (CD), two forms of inflammatory bowel disease (IBD) caused by defective immune responses in the gut^{179,180}. A susceptibility locus on *IRF8* was found to be associated with multiple sclerosis (MS) which is caused by initiation of inflammatory reactions against self antigens in the central nervous system (CNS)^{181,182}. One study demonstrated that the *IRF8* susceptibility allele was found to be associated with higher expression of *IRF8* mRNA and downstream IFN- β -inducible targets in MS patients¹⁸¹. Studies in the Japanese population identified a locus in *IRF8* to be associated with rheumatoid arthritis (RA), a complex autoimmune disease characterized by chronic inflammation and destruction of synovial joints¹⁸³. Furthermore, additional meta-analyses have identified *IRF1* loci to be strongly linked to IBD and CD^{184,185}. Finally a large GWAS was performed and found *IRF1* to be significantly associated to C-reactive protein (CRP), a general marker of systemic inflammation associated with cardiovascular disease¹⁸⁶.

4. Immunology and bone metabolism

Both type I and type II IFN systems have been shown to play important roles in the regulation of the skeletal system¹⁸⁷. IFN-inducible genes are therefore important factors that will regulate bone formation and destruction by acting as either inhibitors or activators of differentiation and function of bone cells. Several studies have identified these IFN-inducible genes to include *Stat1* and some members of the IRF family of transcription factors such as *Irf8*^{6,188}.

4.1 The skeleton and bone remodeling

The skeleton serves a variety of important functions including providing structural support, protecting vital internal organs, maintaining mineral homeostasis, serving as a reservoir for growth factors and cytokines and finally providing an environment for hematopoiesis in the bone marrow¹⁸⁹. For the purpose of this review, I will focus on long bones which include femurs and tibiae that are composed of a hollow shaft termed diaphysis, a cone shaped metaphyses below the growth plate and rounded epiphyses above the growth plates. The diaphysis is composed of mainly cortical bone which is dense, solid and surrounds the marrow whereas the metaphyses are composed of trabecular bone which forms a honeycomb-like meshwork in the bone marrow compartment. Cortical bone therefore plays mainly a structural role and storage of minerals (calcium) whereas trabecular bone is more suitable for metabolic activity and site for hematopoiesis¹⁹⁰.

Bone is not a metabolically inert tissue but rather undergoes constant remodeling throughout life which involves removal of old bone by bone resorbing osteoclasts and formation of new bone by osteoblasts. Bone remodeling is necessary for adapting bone strength to normal growth, changes in mechanical load, repairing mechanical damage and maintenance of blood calcium levels¹⁹⁰. This process is tightly coupled and requires precise and balanced interaction between osteoclasts and osteoblasts. The net balance in the remodeling process can be disrupted by factors that interfere in osteoblast function and osteoclast activity, or both¹⁹¹.

4.2 Bone forming Osteoblasts (OBs)

Osteoblasts develop from mesenchymal stem cells in the bone marrow that can also give rise to adipose cells. Temporal sequence of gene expression in progenitor cells has defined three stages in osteoblast development including proliferation, differentiation and mineralization¹⁹². Active proliferation of pre-osteoblasts and is characterized by high expression of cell cycle (histone H4, *cdc2*, *cdk2*) and cell growth (*c-myc*, *c-fos*) genes as well as significant expression of *Irf1*¹⁹³. During this early stage, Tgf- β (Transforming growth factor beta) is expressed at high levels and functions to recruit and stimulate osteoprogenitor cells to proliferate in order to provide a large pool of early osteoblasts. At later stages in osteoblast development, Tgf- β will act to inhibit both differentiation and mineralization¹⁹⁴. During the differentiation phase, proliferation genes are downregulated while expression of alkaline phosphatase (ALP), a byproduct of osteoblast activity and marker for active bone formation, increases more than 10-fold¹⁹². Differentiating osteoblasts express high levels of Runx2 (Runt-related transcription factor 2) in order to become mature osteoblasts. The absolute requirement for Runx2 in osteoblast differentiation was demonstrated in *Runx2*^{-/-} mice that had complete absence of osteoblasts and no bone formation¹⁹⁵. During the differentiation phase, the extracellular matrix undergoes modifications in its composition to render it competent for mineralization. The onset of the mineralization stage is reflected by increased expression osteopontin (*Opn*) which forms the organic component of bone and osteocalcin (*Ocn*) which is important for bone mineralization and calcium homeostasis¹⁹². Runx2 has been shown to physically interact with the *Ocn* promoter to induce its expression while Tgf- β inhibits the promoter during post-proliferative phases^{196,197}. At this stage, the osteoblast becomes fully encased in the bone matrix and is referred to as an osteocyte. The exact function of osteocytes is unclear but it is suggested that these cells respond to bone tissue strain and modulate bone remodeling by recruiting osteoclasts to sites where remodeling is needed¹⁹⁸.

4.3 Bone resorbing Osteoclasts (OCs)

Osteoclasts are derived from hematopoietic stem cells that can also give rise to monocytes and macrophages¹⁹¹. Osteoclasts are giant multinucleated cells that line the mineralized bone surface where they secrete lysosomal enzymes such as tartrate-resistant acid phosphatase (TRAP) and cathepsin K via their ruffled borders allowing for bone resorption¹⁹⁸. The two critical molecules for osteoclast differentiation from the bone marrow are M-CSF (macrophage colony stimulating factor) which is required for proliferation of osteoclast progenitor cells (OPCs) as well as Rankl (Receptor activator of nuclear factor kappa-B ligand) which is the major osteoclastogenesis factor. Rankl, through induction of another essential gene *Nfatc1* (Nuclear factor of activated T cells 1), will promote differentiation of OPCs to mature bone resorbing osteoclasts¹⁹¹. Osteoblasts (OBs) and activated T cells are the major producers of Rankl that will promote osteoclastogenesis which can be further enhanced in the presence of pro-inflammatory cytokines such as *Tnf* and *Il-6*¹⁹¹. In fact, *Tnf α* is a key inflammatory cytokine for the pathogenesis of autoimmune and inflammatory disease such as rheumatoid arthritis (RA) and drives pathological osteoclastogenesis and bone resorption during chronic inflammation¹⁹⁹. On the other hand, other pro-inflammatory cytokines induced by IFN- γ including *Il-12* and *Il-18* act to decrease osteoclastogenesis by inhibiting Rankl thereby contributing to maintain bone homeostasis^{200,201}. Several studies have therefore demonstrated that IFN- γ is critical for suppression of Rankl-induced osteoclastogenesis²⁰². In addition TLRs which are key sensors of microbial infection and inducers of innate immunity and inflammation have also been shown to suppress osteoclastogenesis and drive myeloid precursors to differentiate into the macrophage lineage necessary for host defense²⁰³.

4.4 Role for Irf8 in regulation of bone metabolism

Recently, a new role for Irf8 was observed in regulating bone metabolism by acting as a suppressor of osteoclastogenesis⁶. As previously discussed, Irf8 expression is induced via IFN- γ signaling as well as following co-stimulation with IFN- γ and TLRs that are known to suppress Rankl-induced osteoclastogenesis^{124,202,204}. This role was indentified through studies in *Irf8*^{-/-} mice that show severe osteoporosis caused by increased formation of osteoclasts and enhanced bone resorption in the presence of Rankl as well as pathological bone destruction in an inflammatory setting. Irf8 was shown to physically interact and inhibit the promoter of *Nfatc1*, the master transcription factor of osteoclastogenesis. This inhibition prevents Nfatc1 from binding its target genes necessary for the development of osteoclasts thereby inhibiting osteoclastogenesis and bone resorption. In addition, it was observed that Rankl downregulates levels of Irf8 protein in order to induce osteoclastogenesis (illustrated in²⁰⁵) Since Irf8 expression is restricted to hematopoietic cells (macrophages, DCs, B, T cells) that give rise to osteoclasts, the osteoporosis observed in *Irf8*^{-/-} mice was strictly due to *Irf8* deficiency in osteoclasts and not due a role in osteoblasts. Indeed, Irf8 expression is not detected in osteoblasts and differentiation and matrix calcification of osteoblasts from *Irf8*^{-/-} mice are not affected⁶.

Preface to Chapter 2

In mice, *Irf8* is essential for the development of DCs from common DC progenitors (CDP) and for immunity against intracellular pathogens such as mycobacteria. Karine Turcotte and colleagues identified an inbred mouse strain, BXH-2, that carry a partial loss of function mutation in *Irf8* (R294C) resulting in extreme susceptibility to weakly virulent (BCG) and virulent (MTB) mycobacteria, granulocytic hyperplasia and defective development of CD11c⁺ / CD8α⁺ DCs^{5,112}.

In this chapter, we aimed to study the role of IRF8 in human susceptibility to mycobacteria. We investigated the genetic basis of mycobacterial disease in an infant who was hospitalized with severe disease shortly after routine vaccination with BCG and two unrelated MSMD patients who developed milder but lifetime disease following vaccination.

We identified two novel *IRF8* mutations and characterize two forms of human *IRF8* deficiency disease.

Chapter 2

IRF8 mutations and human dendritic cell immunodeficiency

2.1 ABSTRACT

The genetic analysis of human primary immunodeficiencies has defined the contribution of specific cell populations and molecular pathways in host defence against infections. Disseminated infection caused by BCG vaccines is an early manifestation of primary immunodeficiencies such as severe combined immunodeficiency. In many patients, the etiology of disseminated BCG disease is unexplained. We investigated an infant presenting with features of severe immunodeficiency, including early-onset disseminated BCG disease, requiring hematopoietic stem cell transplantation. We also studied two otherwise healthy adults with a history of disseminated but curable BCG disease in childhood. In these patients, we characterized the monocyte and dendritic cells compartments and sequenced candidate genes, mutation of which could plausibly confer susceptibility to BCG disease. We detected two distinct disease-causing mutations affecting the transcriptional regulator IRF8. Both K108E and T80A mutations impair IRF8 transcriptional activity by disrupting IRF8 interaction with DNA. Mutation K108E caused an autosomal recessive severe immunodeficiency with a complete lack of circulating monocytes and dendritic cells. Mutation T80A was associated with an autosomal dominant milder immunodeficiency and a selective depletion of CD11c⁺ / CD1c⁺ circulating dendritic cells. These findings define a new class of human primary immunodeficiency, affecting the differentiation of mononuclear phagocytes. They also demonstrate that human IRF8 is critically required for the development of monocytes and dendritic cells and for anti-mycobacterial immunity.

2.2 INTRODUCTION

The discovery of human primary immunodeficiencies (PIDs) affecting the development of granulocytes, B cells, and T cells, has been instrumental in defining the contribution of these cell types to protective immunity^{206,207}. Monocytes, macrophages, and dendritic cells, which are all mononuclear phagocytes, have essential functions in both innate and acquired immunity. They initially recognize and engulf invading microbes, produce pro-inflammatory cytokines (such as IL-12), and process antigens for presentation to naïve T lymphocytes, which consequently secrete various lymphokines (such as IFN- γ)^{208,209}. Upon activation by cytokines secreted by T lymphocytes, mononuclear phagocytes destroy ingested micro-organisms. There are no known genetic causes of PID affecting the development of the mononuclear phagocyte.

Bacille Calmette-Guérin (BCG) vaccines and environmental mycobacteria are efficiently destroyed by T-cell activation of macrophages³⁴. However, disseminated mycobacterial disease following BCG vaccination is a sign of immunodeficiency¹. It occurs in children with severe combined immunodeficiency (absence of T lymphocytes) or with chronic granulomatous disease. While such children are vulnerable to multiple infections, patients with Mendelian Susceptibility to Mycobacterial Disease (MSMD) display a narrow vulnerability to poorly virulent mycobacteria, including BCG³. Some MSMD patients harbor genetic defects in the IL-12/IFN- γ circuit, with mutations in genes encoding IL-12p40 (encoded by *IL12B*), and its receptor (*IL12RB1*), both subunits of the receptor for IFN- γ and its signaling partner STAT1, the NF- κ B regulator IKBKG (NEMO), and the effector gp91^{phox} (*CYBB*)³.

We hypothesized that certain patients may be vulnerable to BCG because of mutations impairing the development of mononuclear phagocytes. Among the transcription factors essential to the development of mononuclear phagocytes in mice, the interferon regulatory factor 8 (IRF8) stands out as a strong candidate¹⁶⁰. It is expressed at very high levels in mononuclear phagocytes²¹⁰, and regulates granulocyte/macrophage differentiation^{136,211} and DC development^{114,141,155,212}. Acting in heterodimeric complexes with other transcription factors, IRF8 also controls the transcriptional response of mature myeloid cells to interferons (IFNs) and Toll-like receptor (TLR) agonists, a response in which IRF8 binds and trans-activates the promoters of *IL12B* and *NOS2* (*NOS2* encodes inducible nitric oxide synthase; iNOS)¹⁶⁰. Mice

bearing the R294C hypomorphic mutation (a mutation that partially compromises protein function) in *Irf8* display a specific DC phenotype with loss of CD8 α^+ lymphoid DCs and CD103 $^+$ tissue myeloid DCs, while *Irf8* knockout mice (which are devoid of Irf8) additionally lack plasmacytoid DCs (pDCs)^{114,141,212}. Mice with mutant *Irf8* are susceptible to infection with intra-macrophagic pathogens, and are hyper-susceptible to *M. bovis* BCG and *M. tuberculosis* infections^{5,112}.

2.3 MATERIAL AND METHODS

Study Subjects

A 10-week old infant presenting with disseminated BCG infection (post-vaccination), oral candidiasis, cachexia and suspected signs of immunodeficiency was admitted to hospital and was investigated (Case Description in Supplementary Information). She underwent multiple rounds of aggressive antibiotic treatments which were only partially effective in improving her health. She was successfully treated by transplantation with cord-blood stem cells. She was the second-born child to healthy unrelated Irish parents (kindred A); the elder sibling was well, and all had received BCG vaccination.

Two additional individuals diagnosed with MSMD were also enrolled in the study. Affected individual 1 (kindred B) was born in 1970 to non-consanguineous parents of Italian descent living in Brazil. He was vaccinated with BCG at birth and developed recurrent episodes of lymphadenopathies at age 15 months, 20 years and 30 years; on two such episodes, lymph node biopsies revealed presence of acid-fast bacilli and all such episodes were successfully treated with anti-mycobacterial drug regimens. His mother, father and sister were vaccinated with BCG and did not present with clinical infectious disease suggesting immunodeficiency.

Affected individual 2 (kindred C) was born in 1996 to a non consanguineous family of Italian descent living in Chile, and received BCG vaccination at birth. At one year of age, she developed lymphadenopathy with chronic granulomatous tuberculoid lesions. At two years of age, she presented with multiple lymphadenopathies and fever, requiring hospital admission. Histological analysis of lymph-node biopsy and bacterial culture identified pyrazinamide resistant *M. bovis*, and the child was successfully treated with adjusted antibiotic treatment administered for 12 months; no subsequent clinical episodes were reported. We obtained written informed consent from participants or their parents, and Institutional Review Boards approved the studies.

Genetic and Transcriptional Analyses

We sequenced the exons and selected non-coding sequences of *IRF8* from participants' genomic DNA following standard PCR amplification with sequence-specific primers (Table S3). We assayed the transcriptional activity of mutant and non-mutant *IRF8* through activation of the *IL12B* and *NOS2* promoters using reporter constructs, transiently transfected into RAW 264.7 macrophages, as previously described⁵.

Detection and Functional Characterization of IRF8 variants.

All exons and intron/exon junctions of *IRF8* were amplified by PCR (primer pairs in Table S3) from genomic DNA, followed by automated DNA sequencing. The sequence variants were confirmed by re-amplification followed by cloning and sequencing PCR products. The detected K108E variant was unique and was absent from a group of 454 individuals with mycobacterial disease that include a broad ancestry including Europe (n = 316), South America (n = 23), and the Middle East (n = 115), and in which the gene was completely sequenced (all exons and exon/intron junctions). The detected T80A variant arose twice independently as a de novo mutation in a Brazilian and Chilean pedigree. T80A was absent from a group of 1064 control DNAs (CEPH Diversity Panel)²¹³. This panel of DNAs contains controls for all ancestral ethnicity worldwide including Sub-Saharan Africa (n = 127), North Africa (n = 30), Middle East (n = 148), Asia (n = 451), Oceania (n = 39), Europe (n = 161), America (n = 108). The European samples (n = 161) include French (n = 29), Basque (n = 24), Sardinia (n = 28), Bergamo (n = 14), Tuscan (n = 8), Orcadian (n = 16), Adygei (n = 17), and Russians (n = 25). The South-American samples (n = 108) include Mexico Pima (n = 25) and Maya (n = 25), Cloumbia (n = 13), Brazil (n = 43) (41). Transcriptional activity of *IRF8* variants (re-inserted into a mouse cDNA clone) was studied in transiently transfected RAW 264.7 macrophages as described⁵, and using mutagenic primers (5')CTATAGGGAGACCCAAGCTTCCACCA(3') and (5')GATGCATGCTCGAGTTATAGACTTGC(3') for T80A and (5')TCTGAGCCATATGAAGTTTACCGA(3') and (5')TCGGTAACTTCATATGGCTC(3') for K108E. The construct for the R294C variant has been described (35). The iNOS promoter was PCR amplified using primers (5')GCATCATGCTCGAGGCTGAAATCCATAAGCTGT

(3') and (5')CCATGAGTAGATCTAGTTGACTAGGCTACTCCG(3'). Cells were transfected in the presence of Lipofectamine PLUS, with 400ng of different DNA mixtures, and 100 ng of Renilla luciferase reporter (internal control), and assayed for luciferase activity 24 hours later. Groups were compared using Student's t test, as implemented in the SAS Software version 9.1 (SAS institute, Cary, NC, USA). By this method, the differences in trans-activation activity of normal IRF8 vs. T80A, and vs. K108E were statistically different ($p < 0.0001$), and this for both the NOS2 and IL12B promoters. Likewise, the addition of mutant T80A ($p < 0.0001$) but not R294C or K108E ($p = 0.40$) caused a dominant negative effect of the transcriptional activity of the normal IRF8 protein.

Immunoblotting and Chromatin Immunoprecipitation (ChIP)

Total cell extracts from transfected RAW cells or from EBV transformed cell lines were prepared and analyzed by immunoblotting as described⁴, and using either a mouse monoclonal anti-HA (Covance Research Products) or an anti-IRF8 antibody (IRF-8 C-19; Santa Cruz Biotechnologies) with HRP-conjugated goat anti-mouse secondary antibody (Jackson ImmunoResearch Laboratories). Immune complexes were visualized using chemiluminescence. For ChIP assays, RAW cells were transfected with separate plasmids containing either the IL-12p40 promoter, IRF1 or WT or mutant IRF8. Cells were fixed with formaldehyde, and cross-linked chromatin was prepared as described²¹⁴. Precipitation of IRF8-containing chromatin complexes was carried out using a goat anti-mouse IRF8 C-19 antiserum, or control non-specific rabbit IgG (Santa Cruz Biotechnologies). DNA released from the IRF8 immunoprecipitate was used as template for real-time PCR quantification of bound IL-12p40 template with primers (5')TTCCTCTTAACCTGGGATTTCG(3') and (5')TTGCCTCCCTGAGTGCATCT(3').

Molecular Modeling Studies

A homology model of the DNA-binding domain of IRF8 was constructed using MODELLER (version9v7)²¹⁵ and coordinates of the DNA-bound IRF2 crystal structure (PDB code 2IRF)²¹⁶. The K108E and T80A models was generated in MODELLER, and subjected to energy minimization using the GROMACS molecular dynamics package and the ffAMBER force field²¹⁷.

Tissue specimens and Histology

Skin epidermal sheets and dermal cell suspensions were prepared by protease digestion, as previously described²¹⁸. Immunohistochemistry was performed on formalin-fixed paraffin-embedded tissue sections using heat-mediated antigen retrieval. Immunofluorescence and confocal microscopy was performed on acetone fixed epidermal sheets as described²¹⁸. The following antibodies (clone, supplier) were used: CD163, (10D6, Novocastra); Langerin, (12D6, Novocastra); CD68, (PGM1, Dako); CD1a, (MTB1, Ventana); anti-CD1a FITC (NA1/34; Dako).

Flow Cytometry

Purified blood mononuclear cells (PBMCs) were analyzed by flow cytometry, as described²¹⁹, and using the following antibodies (all from BD Biosciences except when indicated): HLA-DR FITC, Biotin (TU36), PERCPCy5.5 (L243); CD14 Qdot®605, APC-Alexa750 (TüK4), Pacific Blue, PE (M5E2); CD16 PE-Cy7(3G8, B73.1), PE (3G8); CD56 PE (MY31), FITC (NCAM16.2); NKp46 PE (BAB281); CD15 PE (VIMC6), CD123 APC (AC145, Miltenyi-Biotec), PE (9F5); CD11c APC (S-HCL-3), V450 (B-ly6), BDCA-2 (AC144, Miltenyi-Biotec FITC), CD1c FITC, APC (BDCA-1, AD5-8E7, Miltenyi-Biotec); CD141 FITC (BDCA-3, AD5-14H12, Miltenyi, Biotec), APC (AC145, Miltenyi-Biotec), PE (1A4); CD3 FITC, PERCPCy5.5 (SK7); CD19 FITC (4G7), PE (HIB19, e-Bioscience), PECy7 (SJ25C1); CD20 FITC (2H7), CD45 APCCy7 (2D1), CD2 RPA-2.10 (PE); CD34 PE (8G12). In certain experiments, dermal DCs were studied following staining with CD45 APCCy7 (2D1), HLA-DR PerCPCy5.5 (L243), CD1a APC (HI 149), CD14 PECy7 (M5E2), and Langerin (CD207) PE (DCGM4, Beckman Coulter). Monocytes were defined as DR⁺ CD19⁻, CD2⁻, CD56⁻, NKp46⁻, CD15⁻ cells expressing either CD14 and/or CD16. Plasmacytoid DCs were defined as DR⁺ CD14⁻, CD16⁻, CD123⁺⁺ and BDCA2⁺⁺ cells. The two subsets of Myeloid DCs were defined as DR⁺ CD11c⁺, CD19⁻, CD14⁻, CD16⁻ cells expressing either CD1c⁺ or CD141⁺.

Cytokine Assays

All cytokine assays were performed according to standard procedures. Blood was diluted 1:5 with RPMI medium and stimulated in the indicated combinations with IL-12 (20 ng/ml, R&D Systems, Abingdon, UK), IL-18 (25 ng/ml, R&D Systems), phytohaemagglutinin (PHA) (10 µg/ml, Sigma), IFN γ (5-10x10³ U/ml; Boehringer, Ingelheim, Germany), LPS (1 µg/ml, List Biochemicals, Epsom, UK) or infected with BCG. Cytokine production was measured by standard ELISA or multiplexed particle-based flow cytometry. Flt3L was assayed using a commercial reagent (Quantikine®, R & D Systems). Cytokine production by PBMCs, 4 days following BCG infection (20:1), or stimulation by PHA or PPD (5µg/ml) was also measured. Monocyte-derived dendritic cells were obtained from PBMCs, followed by incubation with CD40 ligand (ENZO Life Sciences), and determination of IL-12p40, and IL-6 production. In certain experiments, CD1c⁺ DCs, PBMC mock-depleted of CD1c⁺ DCs, and PBMC depleted of CD1c⁺ DCs were obtained by fluorescence-activated cell sorting. PBMC depleted of CD1c⁺ were obtained with an exclusion gate on CD1c⁺ CD19⁻ cells, and purified CD1c⁺ were obtained by cell sorting gated on CD1c⁺ CD19⁻ cells after depletion of CD3⁺ T cells (CD3-microbeads, Miltenyi Biotec). Cells were incubated with the TLR7/8 agonist R848 (InvivoGen, SanDiego, CA, USA, 2 µg/ml, 48 h at 37°C), and IL-12p70 in supernatants was determined by ELISA. Differences in cytokine production were compared using Student's t test, as described for trans-activation studies with the NOS2 and IL-12p40 promoters.

T lymphocyte function

In some experiments, PBMCs were incubated with PHA and pulsed with IL-2 (40 U/ml); at day 7, they were incubated with IL-12p70 (1 to 100ng/ml) and IFN γ production was measured 4 days later. Proliferative PBMC responses to indicated stimuli (PHA, 5µg/ml; IL-2 500 U/ml; concanavalin A, 5µg/ml; anti-CD3 (OKT3) 0.1µg/ml; PMA 50 ng/ml; ionomycin 0.1µg/ml) were measured by standard (³H)-thymidine incorporation. CD4⁺ T cells were also isolated from PBMC using microbeads (Miltenyi), and stimulated with PMA (10 ng/ml) and ionomycin (1µg/ml) or by exposure to CD3⁺CD28⁺ microbeads. Proliferation was assessed as above, while cytokine production was measured in supernatant using a commercial cytometric assay (CBA Human Th1/Th2/Th17 kit, BD Biosciences, UK).

CASE DESCRIPTION

Autosomal Recessive IRF8 Deficiency (K108E individual)

A female infant presented to her local children's hospital at 10 weeks of age. She was extremely unwell with failure to thrive (weight falling to 0.4th percentile), marked hepatosplenomegaly, pleural effusions, ascites, oral candidiasis, a discharging BCG scar and associated suppurative axillary lymphadenitis. She was the second-born child to healthy unrelated Irish parents; the elder sibling was well. Intracranial calcification had been noted on ultrasonographic and magnetic resonance imaging both in utero and postnatally, but the child was well at birth & no evidence of congenital infection or neurological abnormality was found. Placental histology showed normal cord vasculature, and mild focal lymphocytic and histiocytic villitis only. The infant received neonatal BCG, which is universally administered in the Republic of Ireland, and had appeared healthy until the second month of life.

Blood work-up (**Table S1**) revealed monocytopenia, anaemia & thrombocytopenia, a strikingly left-shifted neutrophil leucocytosis, lymphocytosis, raised inflammatory markers and hypoalbuminaemia. Excision biopsy of the enlarged axillary lymph node showed acid-fast bacilli on Ziehl-Nielsen stain (**Fig. S3**) and BCG was subsequently cultured. Florid myeloproliferation was also noted as described in the main text. The infant required intensive care and nutritional support and gradually improved with the institution of anti-mycobacterial as well as broad-spectrum antibacterial therapy. The working diagnosis was disseminated BCG infection with presumed underlying primary immunodeficiency. No tuberculin skin test (TST) was carried out because a microbiological diagnosis had rapidly been reached and because TST is generally nonreactive in sick infants with mycobacterial disease. Investigations for MSMD (**Fig. 1**) showed a globally abnormal profile with complete failure of IL-12 production and poor IFN γ response. She displayed an abnormal neutrophil oxidative burst with two populations of positive cells (data not shown), yet clearly did not have CGD. CD62 ligand shedding by neutrophils in response to various stimuli was also demonstrable (data not shown). Several bone marrow examinations revealed granulocytic hyperplasia (**Fig. S3A**) without evidence of myelodysplasia or malignancy, and cytogenetics were repeatedly normal. The first marrow trephine also showed some poorly formed granulomas.

Table S2 shows clinical laboratory values obtained at 6 months of age when the infant remained with moderate hepatosplenomegaly, raised inflammatory markers, & ongoing massive neutrophilia - yet she was growing and making developmental progress. Shortly after this the infant became suddenly unwell again with respiratory failure, fever, further increase in hepatosplenomegaly and worsening of cytopenias. A viral trigger for this deterioration was suspected but careful investigation revealed only Rhinovirus in respiratory secretions. In view of its life-threatening nature and a clinical picture that overlapped hemophagocytic lymphohistiocytosis (HLH), a decision was made to treat according to HLH-2004, with corticosteroids, etoposide and cyclosporine. Bone marrow examination at this time was negative for haemophagocytosis. Institution of this therapy was followed by improvement in the infant's clinical status, accompanied by amelioration of her neutrophilia and lymphocytosis. Meanwhile investigations were continued as described, resulting in the diagnosis of IRF8 deficiency.

The infant was transferred to a UK centre for children with primary immunodeficiency where she underwent a matched unrelated donor cord blood hematopoietic stem cell transplant at the age of 9 months. The decision to transplant was taken in light of the severity of her clinical course, the risk of myeloid malignancy and the expectation of cure, based on the tissue distribution of IRF8 expression and successful amelioration of murine IRF8 deficiency by bone marrow transplantation. She received modified conditioning for transplantation using treosulfan and fludarabine. Prophylaxis against graft-versus-host-disease (GVHD) was provided in the form of pre-transplant serotherapy (alemtuzumab) and post-transplant cyclosporine and mycophenolate mofetil. The infant engrafted uneventfully and no major problems were encountered in the peri-transplant period (in particular, no significant GVHD). She is currently 12 months post-transplant and thriving on minimal medication with moderate developmental delay. Analysis of peripheral blood shows full donor reconstitution including monocytes, DC and T lymphocytes (data not shown).

Sequencing of the IRF1, IFNRG1 and IFRG2 genes failed to detect the presence of mutation in this individual.

Autosomal Dominant IRF8 Deficiency (T80A individuals)

Affected individual 1 (P1, kindred B) was born in 1970 to non consanguineous parents of Italian descent living in Brazil (**Fig. 1B**). He was vaccinated with *Mycobacterium bovis*-BCG at birth. At 15 months, he developed disseminated lymphadenopathies, some of which were in the axillary region, suggesting mycobacterial disease and requiring hospitalization. He was treated with rifampin, isoniazid and pyrazinamide for six months, with a favorable outcome. At the age of 20 years, he was admitted to hospital again, for abdominal lymphadenopathies. No acid-fast bacilli were detected, and cultures for bacteria, mycobacteria and fungi were negative. He received empiric antituberculous medication (rifampin, isoniazid and pyrazinamide) for six months. P1 recovered after antibiotic treatment. At the age of 30 years, he again developed multiple peripheral and abdominal lymphadenopathies. He was prescribed rifampin, isoniazid and pyrazinamide for six months, with a partial clinical response. An axillary lymph node was excised and histologic examination showed a tuberculoid granuloma, with visible acid-fast bacilli and *Mycobacterium bovis* BCG-positive culture. The treatment was replaced by ciprofloxacin, clarithromycin and ethambutol, leading to an improvement in the clinical status of the individual. Treatment was maintained for 36 months, with a partial response. An abdominal lymph node was excised and histology showed a tuberculoid granuloma, with visible acid-fast rods. Negative culture results were obtained for mycobacteria. A tuberculin skin test (TST) with purified protein derivative was negative. He received a treatment regimen for multiresistant mycobacteria (amikacin, rifabutin, clofazimine, ofloxacin, cycloserine, ethambutol) for 30 months. The response was good, with shrinking of the abdominal mass. He had no other unusual infections. He is now 39 years old and is well, without treatment. His mother, father and sister were vaccinated with BCG and presented no clinical infectious disease or immunodeficiency.

Affected individual 2 (P2, kindred C) was born in 1996, to a non consanguineous family of Italian descent living in Chile (**Fig. 1B**). She was vaccinated with *Mycobacterium bovis*-BCG at birth. One year later, she developed a left axillary lymphadenopathy. She received no antibiotic treatment and the surgical excision of regional lymph nodes was required. Biopsy showed chronic granulomatous tuberculoid lymphadenitis. No cultures were carried out and she was considered to have BCG adenitis. At the age of two years, P2 was admitted to the hospital

with fever and multiple lymphadenopathies (axillary and cervical, 3-5 cm). A biological inflammatory syndrome was noted, with a normal chest X ray. Ultrasound scans revealed the presence of several lymph nodes (retroperitoneal and celiac hilum), tuberculin tests were negative and the VCA Epstein Barr IgM test was positive. Her fever dissipated during the study period, with a normalization of inflammatory marker levels. Three months later, P2 was admitted to hospital with a second episode of fever (14 days) and an enlargement of multiple peripheral lymphadenopathies (axillary, cervical and inguinal). A biological inflammatory reaction was observed, with a normal result in the tetrazolium blue test, a normal immunoglobulin count, negative results for HIV testing, a CD4 count of 1165 (47%), and a CD8 count of 445 (18%). Surgical excision of a right axillary lymph node was performed. Histologic analysis showed a well developed granuloma and the Ziehl-Neelsen-positive lesions on the lymph node biopsy were multibacillary. Isoniazid, rifampin and pyrazinamide were administered as the initial treatment. Mycobacterium bovis BCG resistant to pyrazinamide was isolated from the lymph tissue. Pyrazinamide treatment was stopped. Antibiotics were administered for 12 months and a favorable clinical progression was observed. P2 has had no other unusual infections. She has three sisters, all of whom were vaccinated with BCG with no adverse effect. P2 is now 14 years old and is well with no treatment.

Sequencing of the IFNRG1, IFRG2, IRF1 and STAT1 genes failed to detect the presence of mutation in these two affected individuals.

2.4 RESULTS

Autosomal Recessive IRF8 Deficiency

We evaluated a 10-week-old infant presenting with disseminated BCG disease (post-vaccination), cachexia and oral candidiasis (see **Case Description**). The blood count revealed a strikingly abnormal myeloid compartment with absence of monocytes and basophils and a very high neutrophil count (**Fig. 1; Table S1**). Examination of peripheral blood using flow cytometry confirmed severe depletion of the CD3/19/56⁻ (lin⁻) HLA-DR⁺ cellular compartment, with total absence of both CD14⁺ and CD16⁺ monocytes (**Fig. 1A-C**). Furthermore, we could not detect DCs in the blood, whether CD11c⁺ myeloid DCs (CD1c⁺ or CD141⁺) or CD123⁺ plasmacytoid DCs (**Fig. 1A-D**). The only lin⁻DR⁺ cells proved to be circulating CD34⁺ progenitor cells, which were elevated (**Fig. S1A**) and correlated with elevated serum levels of Fms-like tyrosine kinase-3 ligand (Flt3L) (**Fig. S1B**). In contrast, B and NK cells were present in normal numbers (**Table S2 and Fig. S2**), thus excluding the recently described syndrome of autosomal dominant and sporadic monocytopenia^{220,221}.

Through assays using whole blood, we observed that IL-12 production in response to BCG, phytohaemagglutinin (PHA) and lipopolysaccharide was completely absent and IFN- γ production was poor, with similarly poor production of TNF- α , IL-10 and IL-6 (**Fig. 1F-H**). Preincubation of the infant's cells with IL-12 could partially restore IFN- γ production in response to the same stimuli (**Fig. 1G**). Biopsies of the infant's bone marrow and axillary lymph node (**Fig. S3**) showed striking myeloid hyperplasia, and the presence of acid-fast bacilli within granulomata in a lymph node. Despite the subject's profound peripheral monocytopenia, the diseased node was positive for histiocytic markers CD68 and CD163 and bone trabeculae showed evidence of normal osteoclast activity (**Fig. S4**). In dermal tissues, the density of CD1a⁺ and CD14⁺ DCs was remarkably low (**Fig. 1C-D**). In contrast, we observed epidermal Langerhans cells (LCs) to be at normal density (**Fig. 1E**), and detected the occasional Langerin⁺/CD1a⁺ DC in lymph node (**Fig. S4**). We conclude that the subject had a profound deficit of tissue DCs, blood DCs and monocytes, a variable deficit of tissue macrophages, and normal levels of LCs.

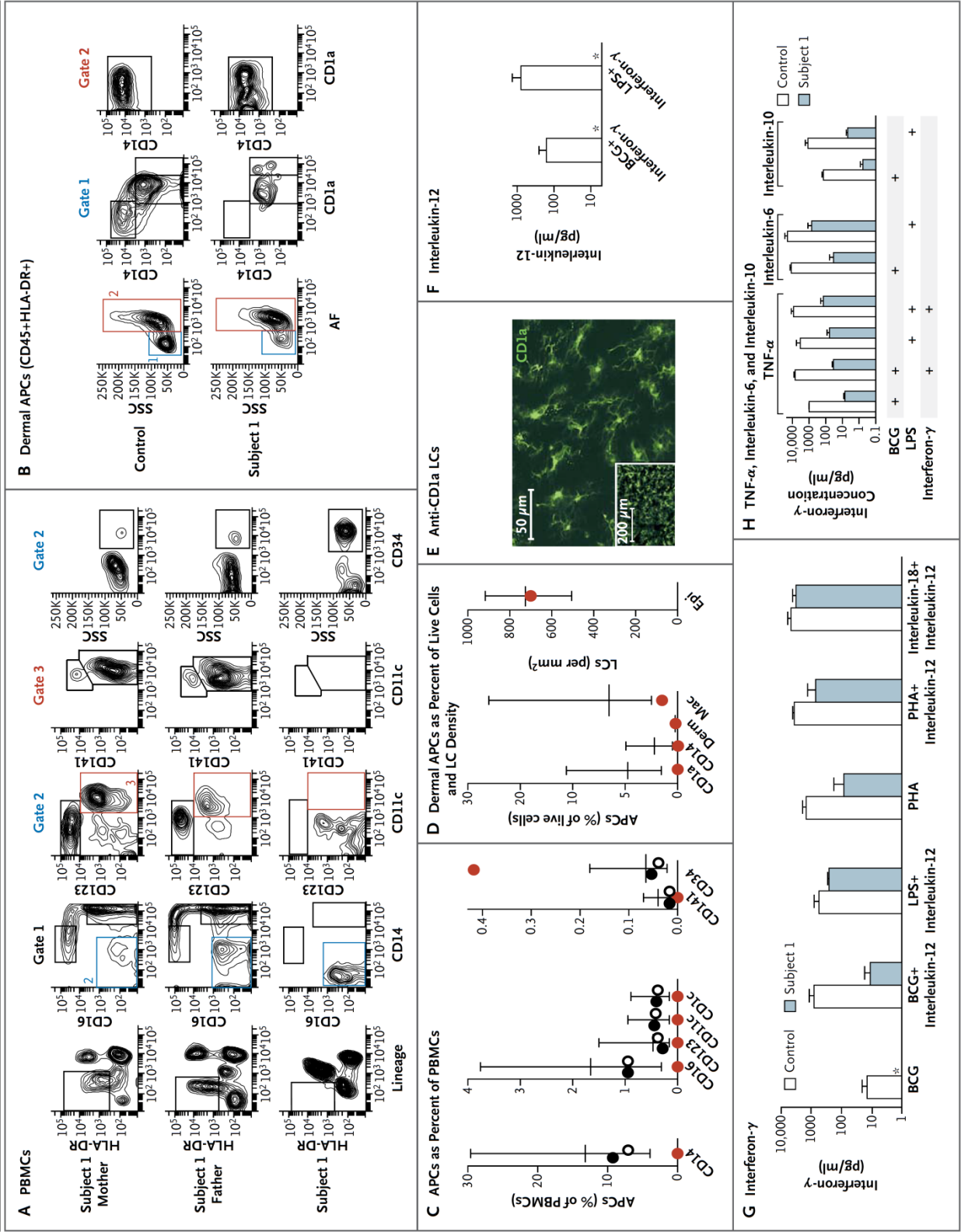


Figure 1. Severe depletion of the antigen presenting cell (APC) compartment in autosomal recessive IRF8 deficiency. (A) Flow cytometry of peripheral blood of affected individual P1 and parents. (B) Flow cytometry of CD45+HLA-DR+ mononuclear cells from collagenase digested dermis of P1 and control. (C) Quantification of peripheral blood APC subsets as % of mononuclear cells in P1 (red circle), mother (filled circle) and father (open circle) compared with normal adult range (n=28). (D) Quantification of dermal APC subsets as % of live cells, compared with normal adult range (n=22) and Langerhans cells per mm² compared with normal range (n=12). (E) Affected P1 Langerhans Cells (LC) in an epidermal sheet revealed by anti-CD1a immunofluorescence. (F-H) Whole blood cytokine responses measured *in vitro*. (F) IL-12 production in response to BCG or LPS plus exogenous IFN- γ . (G) IFN- γ production following indicated stimuli. (H) Production of TNF α , IL-6 and IL-10 in response to indicated combinations of BCG, LPS and exogenous IFN- γ . Control, empty bars; affected individual, filled bars; *, undetectable.

This combination of immunodeficiency^{136,211}, DC deficiency^{114,141,155,222} and myeloproliferation¹⁵⁴ is strikingly similar to the phenotype of mice harboring loss-of-function mutations in *Irfs*. Upon sequencing *IRF8* in the subject, we observed a homozygous missense variant predicted to cause a lysine to glutamic acid substitution at position 108 (K108E) (**Fig. 2A**). Both parents were heterozygous for K108E and an unaffected sibling lacked the variant allele (**Fig. 2C**). We sequenced *IRF8* in 454 unrelated persons with clinical susceptibility to mycobacterial infection and did not detect the variant or any other homozygous variants.

Amino-acid position 108 is within the DNA-binding domain of IRF8 (**Fig. 2A**); the lysine residue is invariant in IRF8 orthologs (**Fig. 2B**) and is highly conserved among human IRF family members (data not shown). We expressed the mutant IRF8 in cultured mouse macrophages. Immunoblotting studies showed similar expression levels of normal (K108) and mutant (K108E) variants, and a comparatively slower electrophoretic mobility of the mutant variant (**Fig. 3E**), suggesting that the mutation affects overall protein structure/folding. We tested the ability of normal and mutant isoforms to activate transcription of IRF8 targets — the promoters of *IL12B* (**Fig. 3A**) and *NOS2* (**Fig. 3B**) — in mouse macrophages. Although the combination of normal IRF8 and its co-activator IRF1 induced a dose-dependent stimulation of *IL12B* and *NOS2* promoters, the K108E variant was almost inactive (**Fig. 3A- B**), suggesting that K108E abrogates the IRF1-dependent transcriptional activity of IRF8. Moreover, the mutant IRF8 variant bound the *IL12B* promoter far more weakly than did the normal variant (**Fig. 3D**).

Molecular modeling using the structure of DNA-bound IRF2²¹⁶ places K108 in a short β strand that runs parallel to the major DNA-binding α helix, suggesting that it makes a critical hydrogen bond with the DNA sugar backbone (3.3Å) that facilitates IRF8 docking onto DNA (**Fig. 2E subpanel a**). Replacement of a positively-charged amino acid (lysine) by one that is negatively charged (glutamic acid) at position 108 would likely prevent the formation of a hydrogen bond (**Fig. 2E subpanel b**), and cause local repulsion of the supporting IRF8 β strand away from DNA, allowing a water molecule to fill the space. This model predicts loss of DNA binding and thus loss of transactivation.

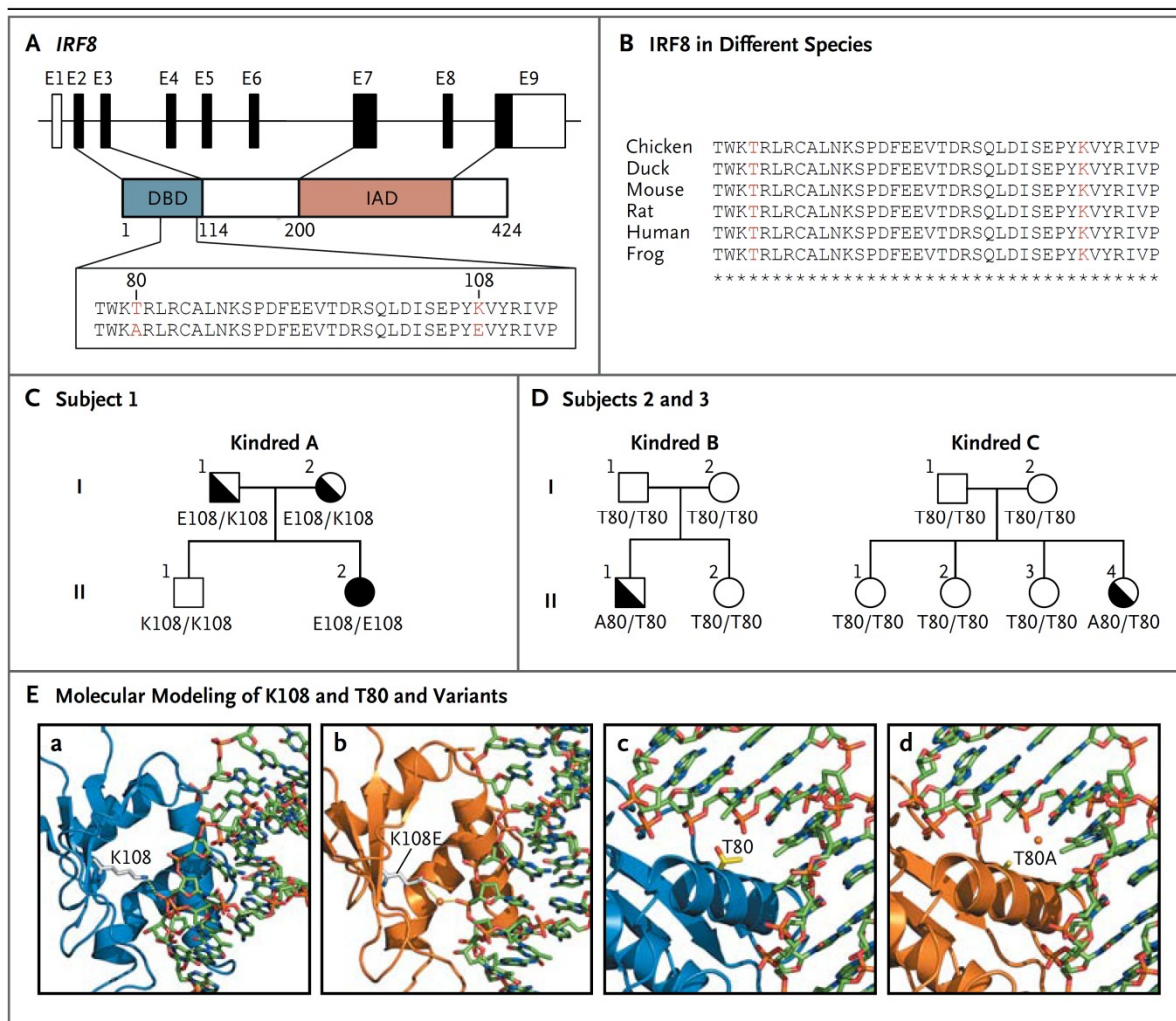


Figure 2. Genetic analysis of autosomal recessive (AR) and autosomal dominant (AD) IRF8 deficiency. (A) Schematic representation of the *IRF8* gene, including the 9 non-coding (empty) and coding (filled) exons, with the major structural features of the protein shown (DBD, DNA Binding Domain; IAD, IRF Association Domain). The position of the T80A and K108E mutations (bold) in the DBD is shown. (B) Multiple sequence alignment of IRF8 from different species (T80 and K108 are shown in red; stars identify invariant residues). (C) Segregation of K108E in the proband family. (D) T80A is a *de novo* mutation that has arisen independently in two unrelated MSMD families from Chile and Brazil. (E) Molecular modeling of wild type K108 (panel 1), and T80 (panel 3) and mutant variants E108 (panel 2) and A80 (panel 4) on the three-dimensional structure of DNA-bound IRF8. The hydrogen bond formed between the side chain amino group of K108 and the sugar backbone of DNA is interrupted by E108. The replacement of Thr80 with an Ala residue in the key DNA-binding helix (inserted into the major groove) alters the hydrophobic interface between the protein and the DNA.

These findings show that the subject carries a loss-of-function mutation in IRF8. The heterozygous parents were healthy despite having received BCG as neonates, and had normal numbers of peripheral blood monocytes and DCs (**Fig. 1**), consistent with the autosomal recessive (AR) inheritance of the IRF8^{K108E} allele.

Autosomal Dominant IRF8 Deficiency

In parallel with our analysis of the infant with recessive disease, we sequenced IRF8 in 454 persons with MSMD of unknown genetic etiology (we ruled out the possibility that these persons carried mutations in several of the genes in which alterations are known to cause MSMD; see **Case Description**). Two unrelated persons from Chile and Brazil and suffering from recurrent episodes of disseminated BCG disease, were each found to carry the same de novo heterozygous mutation, predicted to effect a threonine-to-alanine substitution at position 80 (T80A) of IRF8 (**Fig. 2A, D**). We genetically confirmed reported biological paternity. That each mutation was de novo suggests that the same T80A allele arose independently on two occasions. We did not detect the T80A variant in 1064 healthy controls²¹³ of diverse relevant ancestry. Amino-acid position 80 of IRF8 is within the DNA-binding domain (**Fig. 2A**); the threonine residue is strictly conserved across IRF8 orthologs (**Fig. 2B**) and human paralogous genes (data not shown).

The T80A mutation had no effect on level and stability of IRF8 in immortalized B cell lines derived from the subjects (**Fig. 3E**), or following expression in transfected macrophages (data not shown). When tested for its ability to transactivate the promoters of *IL12B* (**Fig. 3A**) and *NOS2* (**Fig. 3B**) in mouse macrophages, the T80A mutant showed relatively low activity (**Fig. 3A-B**), similar to that of the hypomorphic R294C Irf8 variant of Irf8-deficient BXH2 mutant mice (**Fig. 3A-B**). The T80A mutant showed weak recruitment to the *IL12B* promoter (20% of the amount of the non-mutant IRF8 protein), suggesting that the mutation interferes with DNA binding (**Fig. 3D**).

Homology modeling shows that T80 maps to the DNA-binding α helix of IRF8 that fits into the major groove, with its side-chain directed towards the DNA bases (**Fig. 2E subpanel c**). Molecular dynamics simulations indicate that T80A alters the hydrophobic interface between the protein and DNA, and possibly modulates DNA-binding specificity of IRF8 (**Fig. 2E subpanel d**). Alanine has a smaller side chain than does threonine, and so may allow entry of a water molecule at the DNA: IRF8 interface. Although the functional data suggest that T80A is severely hypomorphic, we investigated whether the T80A variant is negatively dominant (that is, it interferes with the function of the non-mutant IRF8 protein). We co-expressed the mutant and non-mutant alleles in macrophages (**Fig. 3C**) and observed decreasing activation of the *IL12B* (not shown) and *NOS2* (**Fig. 3C**) promoters with increasing levels of mutant (T80A) IRF8 against fixed levels of non-variant IRF8. The effect was T80A-specific; we did not observe it to be associated with K108E (data not shown) or R294C mutant proteins. We conclude that the T80A mutation has a dual effect on IRF8 function and causes autosomal-dominant MSMD.

Figure 3. Functional characterization of the mutant IRF8 variants. (A, B) RAW macrophages were transiently transfected with various amounts (indicated) of the IRF8 variants, with and without IRF1, and IRF8-dependent transactivation potential was monitored with IL-12p40 (A) or iNOS (B) promoter constructs linked to a luciferase reporter gene. (C) The ability of T80A and R294C to suppress transactivation of the iNOS promoter by WT IRF8 was evaluated by co-transfecting RAW macrophages with various amounts of the T80A and R294C variants and fixed amounts of WT IRF8. (D) The relative binding affinity of IRF8 variants for the IL-12p40 promoter was estimated by chromatin immunoprecipitation in transfected RAW macrophages. (E) Western blot analysis of cell extracts from RAW macrophages expressing wild-type, or the T80A or the K108E mutant IRF8 variants, with detection based on an HA epitope tag inserted in-frame in all constructs.

We observed subtle deficits in the peripheral blood mononuclear cells of the two T80A affected adults. There was no deficiency of circulating lymphocytes and granulocytes (data not shown), monocyte subsets, or BDCA2⁺ CD123⁺ plasmacytoid DC (**Fig. 4D**). However, within the CD11c⁺ myeloid DC subset, which is normally divided into minor CD141⁺ and major CD1c⁺ subsets, there was marked loss of CD1c⁺ DCs (**Fig. 4A**), while the CD141⁺ subset and total number of CD11c⁺ DCs remained intact (**Fig. 4B**). CD1c⁺ DCs from unaffected persons can produce large amounts of IL-12 when stimulated with the TLR7/8 ligand R848 (compared to PBMC; student t test, $p < 0.02$) whereas their depletion from PMBCs was associated with comparatively low levels of IL-12 production by the remaining PMBCs ($p < 0.02$) (**Fig. 4E**). PMBCs harboring IRF8^{T80A} produced one-third the amount of IL-12 produced by control cells in response to R848 ($p < 0.004$) (**Fig. 4E**). We suggest that depletion of IL-12-producing CD1c⁺ DCs contributes to susceptibility to mycobacterial disease in these individuals. In vitro assays using whole blood from the affected persons showed no detectable impact on production of IFN- γ (**Fig. S6**), or in the subjects' PBMCs in response to stimulation by PPD (purified protein derivative), BCG or PHA (**Fig. S7**), or by PHA-driven T-cell blasts stimulated with IL-12 (data not shown). Production of IL-12 in a whole blood assay in response to BCG (**Fig. S6**), by monocyte-derived DCs stimulated with CD40L (**Fig. S8A**), and by EBV-B cells stimulated with phorbol dibutyrate (**Fig. S8B**) was also normal, indicating that T80A does not cause a generalized defect in IL-12 production.

Because the two healthy heterozygous parents of the infant with autosomal-recessive IRF8 deficiency had normal levels of functional DCs, the marked deficit of CD1c⁺ CD11c⁺ DCs was probably caused by the dominant-negative effect of the T80A mutant protein.

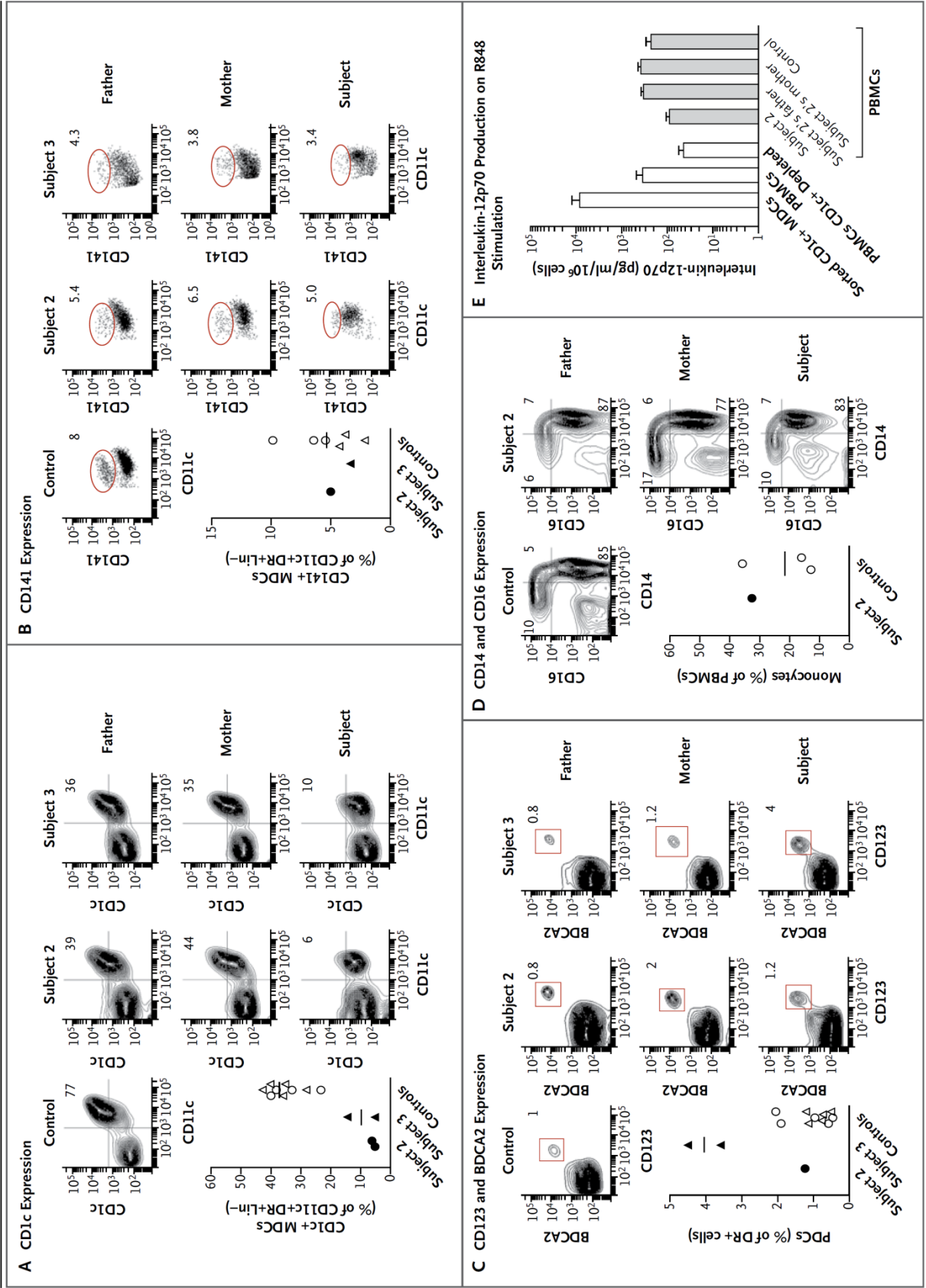


Figure 4. Selective depletion of dendritic cells in autosomal dominant IRF8 deficiency. (A) Blood CD1c⁺ DCs are gated on HLA-DR⁺ Lin⁻ (CD14⁻ CD16⁻ CD19⁻) cells, and expression of CD11c⁺ and CD1c⁺ is shown. Percentage of DR⁺ CD11c⁺ Lin⁻ cells that are positive for CD1c⁺ is shown for the T80A individuals (P1 ●, P2 ▲), their relatives and travel controls (○, △) (mean —). Numbers on contour plots represent the mean of two independent samples. (B) Blood CD141⁺ DCs are gated on HLA-DR⁺ Lin⁻ (CD14⁻ CD16⁻ CD19⁻) cells, and expression of CD11c and CD141 is shown. Percentage of DR⁺ CD11c⁺ Lin⁻ cells that are CD141^{bright} is shown. (C) PDCs are gated on HLA-DR⁺ Lin⁻ (CD14⁻ CD16⁻) cells, and expression of CD123 and BDCA2 is shown. Percentage of DR⁺ cells that are positive for CD123 and BDCA2 is shown. (D) Monocytes are gated on HLA-DR⁺ Lin⁻ (CD2⁻ CD15⁻ CD19⁻ Nkp46⁻) cells, and expression of CD14 and CD16 is shown. Numbers on contour plots represent the percentage of each subset (CD14⁺, CD14^{dim} and CD14⁺CD16⁺) among total monocytes. Percentage of PBMCs that are positive for CD14 or CD16 is shown for P1, his relatives and travel control. (E) Production of IL-12p70 (mean ± SD) in response to R848 by FACS-sorted blood CD1c⁺ (sorted CD1c⁺ MDC, n=4), PBMCs depleted of CD1c⁺ DCs (PBMC CD1c⁺depleted, n=3) or mock-depleted (PBMC, n=3) from healthy controls, and PBMCs from P1, his relatives and a control collected at the same time (grey bars).

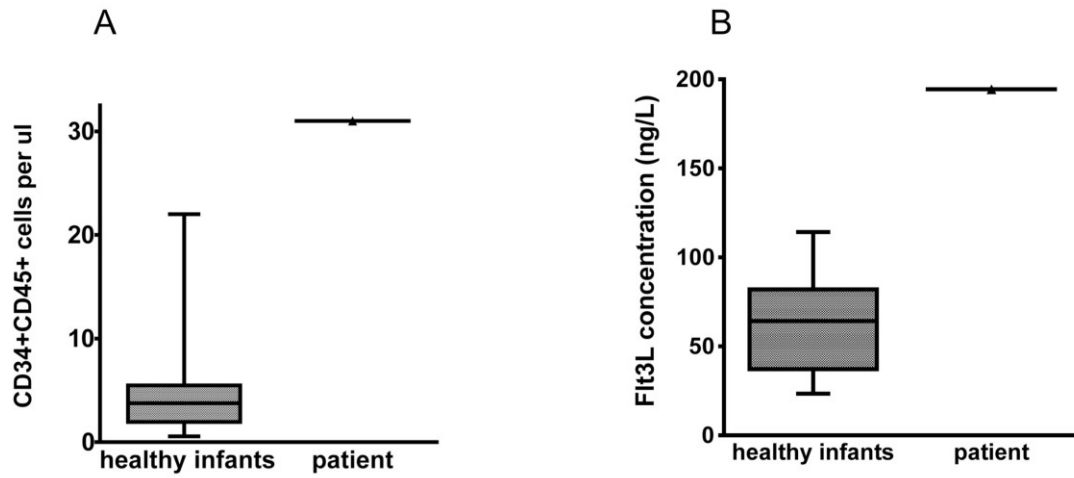


Figure S1. Myeloproliferative phenotype in the K108E individual. (A) Frequency of CD34⁺CD45⁺ cells in patient peripheral blood, compared to healthy controls. (B) Serum levels of Flt3L in the patient, compared to healthy controls.

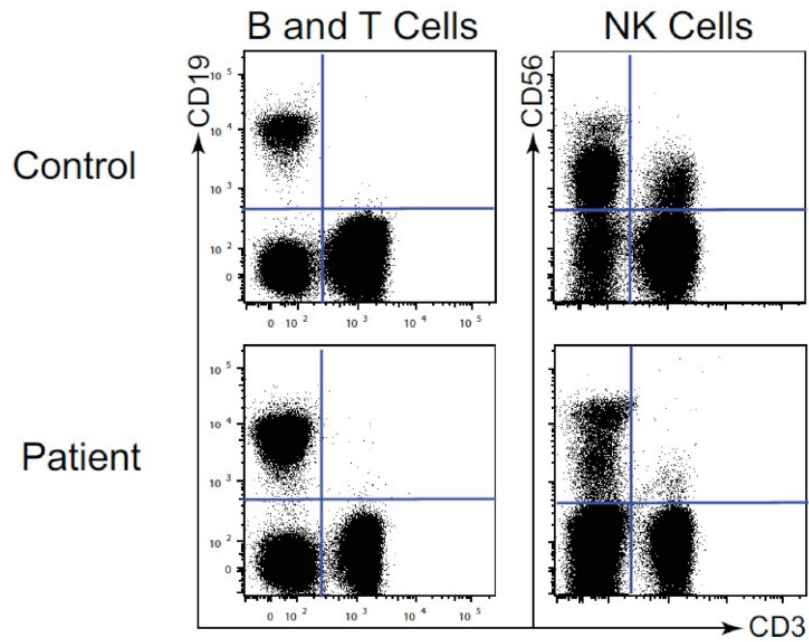


Figure S2. Flow cytometric analysis of peripheral blood lymphocytes from the K108E individual. Flow cytometry shows staining for B cells (CD19⁺), T cells (CD3⁺) and NK cells (CD56⁺) of K108E compared to control.

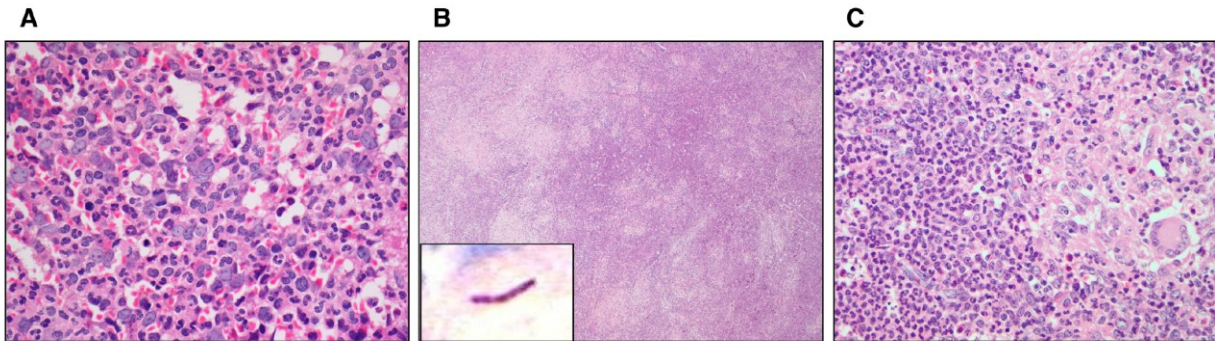


Figure S3. Histological features of bone marrow and lymph node at presentation in the K108E individual. (A) Bone marrow trephine, hematoxylin and eosin stain (H&E, x600), showing granulocytic hyperplasia. (B) Lymph node biopsy, (H&E, x40), showing effacement of normal architecture and granuloma formation; inset zoom Ziehl-Nielsen stain shows acid-fast bacillus. (C) x400 field within lymph node as in (B), showing marked extramedullary myelopoeisis, and, right, a granuloma. Lymphoid follicles were absent.

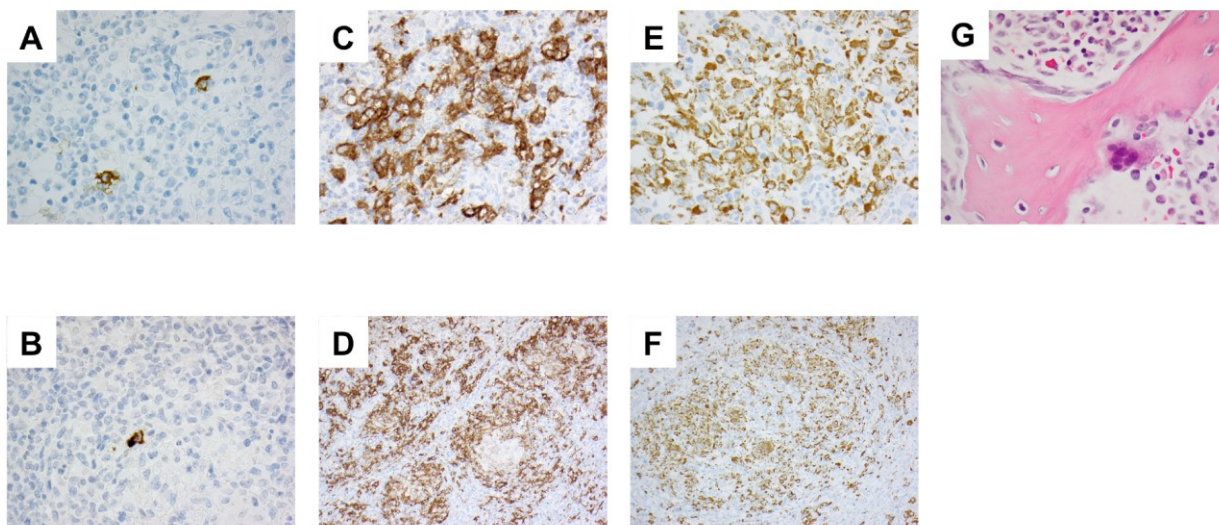


Figure S4. Immunohistochemical analysis of lymph node and bone marrow from the K108E individual. A-F, Lymph node immunohistochemistry; original magnification x600 except **D, F** (x200). (**A**) CD1a. (**B**) langerin. (**C, D**) CD163. (**E, F**) CD68. (**G**), bone marrow trephine showing osteoclast activity (x400), H&E stain.

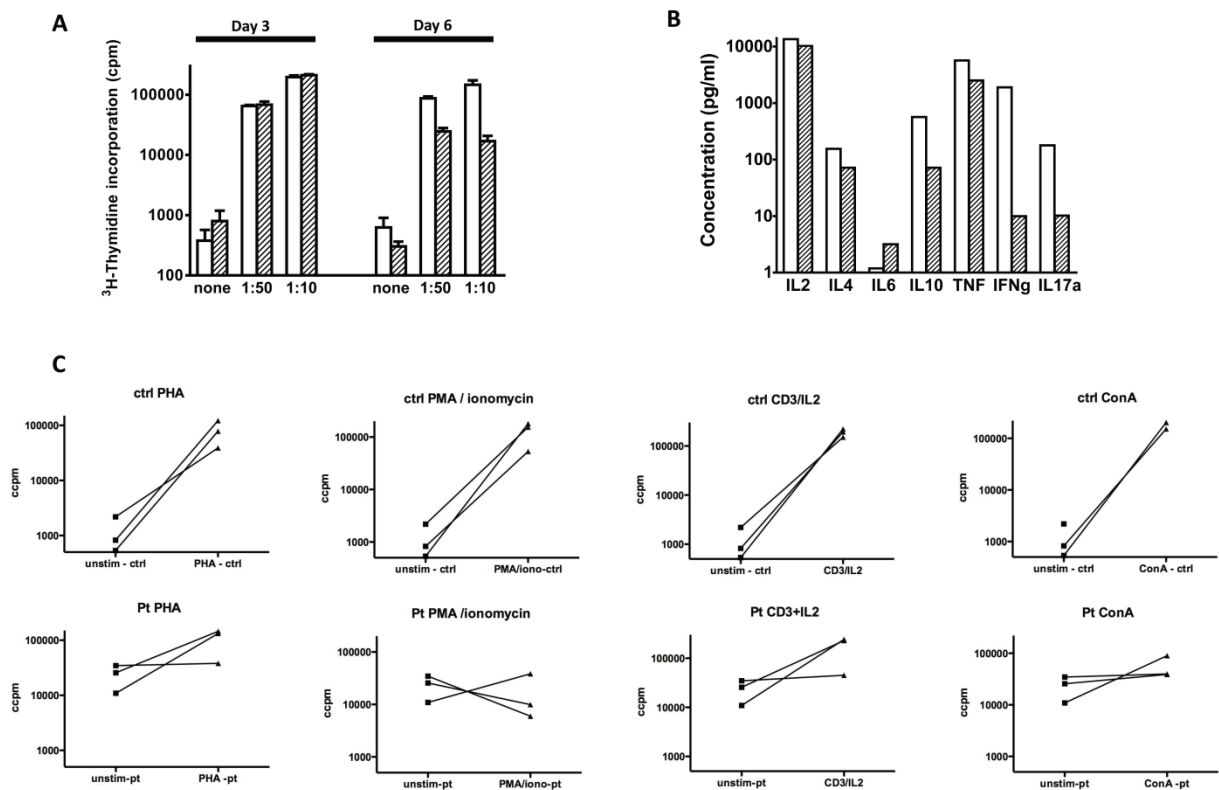


Figure S5. T cell proliferative and cytokine responses of the K108E individual and control. (A-B) Responsiveness of isolated CD4⁺ T cells to CD3⁺CD28⁺ beads. Empty bars, control; filled bars, patient. (A) CD3⁺ CD28⁺ bead-stimulated proliferation measured on day 3 and day 6 by tritiated thymidine incorporation. Bars show the mean and standard deviation of triplicate wells. (B) Cytokine concentrations in supernatant harvested after 48 hours of stimulation by CD3⁺CD28⁺ beads at a 1:10 bead:cell ratio. Similar results were obtained at a 1:50 bead:cell ratio and at 5 days (data not shown). (C) Proliferative responses of PBMC measured on day 3 by titrated thymidine incorporation. This analysis was performed on three separate occasions. Graphs show three sets of paired data (unstimulated and stimulated wells) for either control (upper plots) or patient (lower plots) for the indicated stimulus.

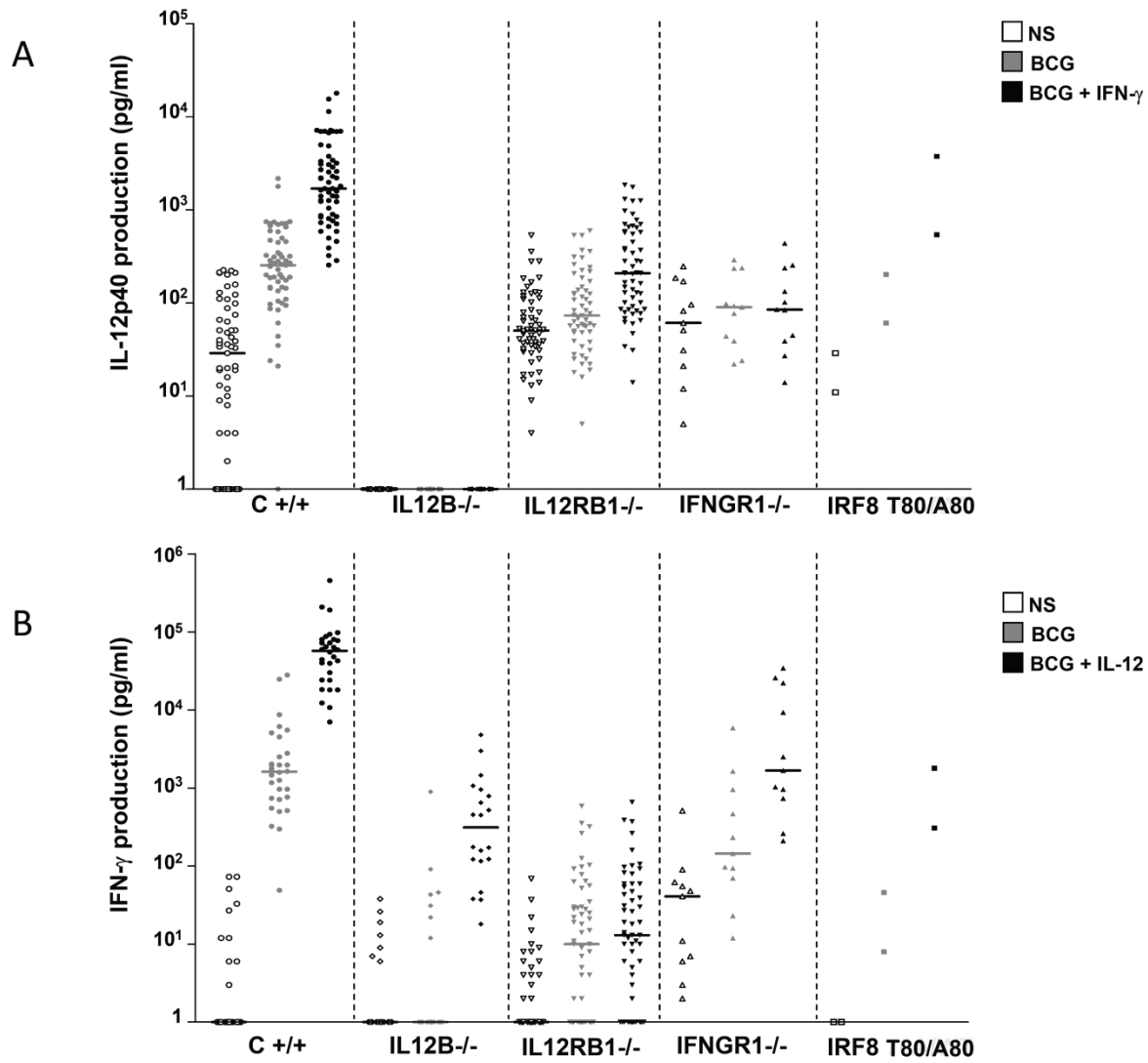


Figure S6. Cytokine production by cells from the T80A individuals (whole blood assay). Measurement, by ELISA, of **(A)** IL-12p40 production after activation with BCG or BCG plus IFN-γ, and IFN-γ secretion after stimulation with BCG or BCG plus IL-12, based on an analysis of whole-blood supernatants from healthy controls (C +/+), patients with complete IL-12 deficiency (*IL12B*^{-/-}), patients with complete IL-12Rβ1 deficiency (*IL12RB1*^{-/-}), patients with complete IFN-γR1 deficiency (*IFNGR1*^{-/-}), and individuals heterozygous for the *IRF8* T80A allele (IRF8 T80/A80). **(B)** IFN-γ production, assessed by classical ELISA, after 4 days of stimulation with PPD, BCG or PHA, in PBMCs from healthy controls (C +/+, n = 6) and IRF8 affected (n = 2).

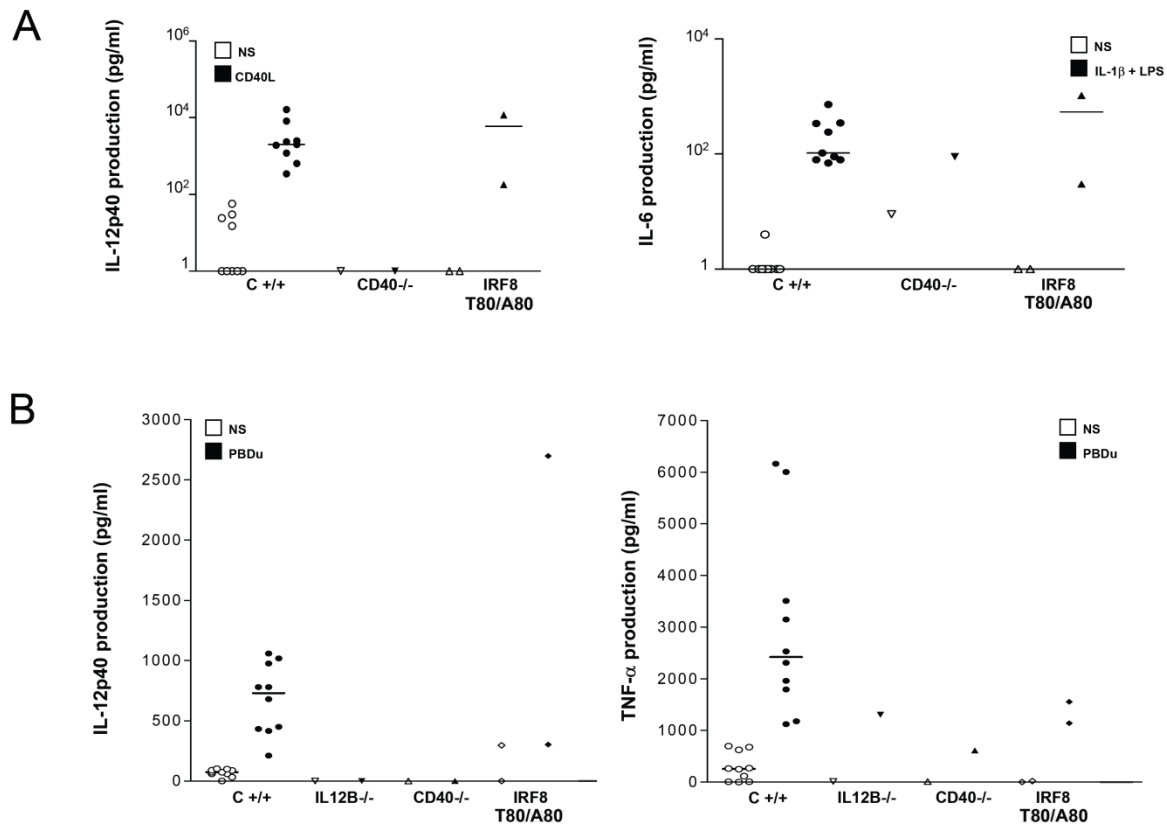


Figure S8. Production of IL-12 by monocyte-derived dendritic cells (MDDCs) and by EBV-transformed cells from the T80A individuals. (A) CD40L response in MDDCs. MDDCs were obtained from healthy controls (C +/+, n = 9), IRF8 affected (n = 2) and a CD40-deficient individual (CD40^{-/-}, n = 1). IL-12p40 and IL-6 were determined by classical ELISA. **(B)** The production of IL-12p40 and TNF- α by EBV-B cells was also measured by ELISA, after 24 hours of activation with PBDu (10^{-7} M), in cells from healthy controls (C +/+, n = 9), a patient with complete IL-12 deficiency (IL12B^{-/-}, n = 1), and the IRF8 affected (IRF8 T80A/WT, n = 2). These results represent two independent experiments.

Table S1. Complete blood count at presentation of the K108E individual

Parameter (cells x 10⁹/L)	Value	Normal
Hemoglobin (g/dL)	4.6	10.5 - 13.5
Platelets	66	150 - 450
White blood cells	125.5	6.0 - 17.5
Total neutrophils	108	1.0 - 8.5
Lymphocytes	13.8	4 - 13.5
Monocytes	0.0	0.7 - 1.5
Eosinophils	3.8	0.3 - 0.8
Basophils	0.0	0.0 - 0.1

Table S2. Clinical laboratory values of K108E individual (6 months of age)

Parameter (units)	Value at 6 months	Normal range (infant)
Hemoglobin (g/dL)	7.1	10.5 – 13.5
Platelets (x 10 ⁹ /L)	68	150-450
White blood cells (x 10 ⁹ /L)	98.2	6.0-17.5
Neutrophils (x 10 ⁹ /L)	77.2	1.0 – 8.5
Lymphocytes (x 10 ⁹ /L)	17.0	4.0 – 13.5
Monocytes	**	0.7 - 1.5
Eosinophils (x 10 ⁹ /L)	2.02	0.3 – 0.8
Basophils (x 10 ⁹ /L)	**	0.0 – 0.1
T lymphocytes (/μl)	9185	2300 – 6900
CD8+ T lymphocytes (/μl)	1729	400 – 2200
CD3+CD4-CD27+CD45RA+(/μl)	1837	
CD4+ T lymphocytes (/μl)	7402	1400 – 5300
CD3+CD4-CD27+CD45RA+(/μl)	5878	
TCR αβ+ T cells	99%	
TCR γδ+ T cells	1%	
NK lymphocytes (/μl)	2521	100 – 1400
B lymphocytes (/μl)	11598	600 – 3000
CD19+CD27+IgM-IgD-	<15%	
IgG (g/L)	3.3	3.0 – 9.0
IgM (g/L)	1.19	0.4 – 1.6
sIgA (g/L)	<0.1	0.1 – 0.5
Beta-2-microglobulin	3.0	0.7 – 1.8
C-reactive protein (mg/L)	33	0 - 5
Erythrocyte sedimentation rate (mm/hr)	63	0 - 15
Renal function	normal	
Liver function	normal	
Lactate dehydrogenase (U/L)	619	0 – 450
Ferritin (μg/L)*	2542	60 - 300
Triglycerides (mmol/L)	2.2	(non-fasting)

Table S3. PCR primer pairs

Primers for sequencing the human IRF-8 gene				
Exon	Forward Primer	T _m (°C)	Reverse Primer	T _m (°C)
2	TTG CTG CAA ACC TCT GAG TT	54.9	TGT CAC TCA ACA TCT CCA CT	53.3
3	GAT GTG TGG GGT CCT GGT CA	59.3	CAA GTT CTG TGG TGA AGG CT	55.2
4	AGA GTA GAT TAT GGC TTC AG	48.6	CAG ATC TGT ACA GGT GGA GA	53.1
5	GTT TTA CTT ACA CAT GAA CTG T	48.8	GTG GAA TGC CGT AAA CCC TT	55.6
6	AAT GGC TCT CTG CTC TGG TG	57.2	CTA GAC TTC CAC ACG AGG TC	54.3
7	TCA AAG TGG TTC AAG ACA CA	51.9	CTG CAA ATG GCA CAC AGA TA	53.3
8	TGC TGC GGG AGT TGG AGG	60.5	AAA AAG CTG CCC ATA CTT AC	50.7
9	CAC TGG GGT CAT CAG AGG AC	57.2	GGA GAT GGA TGC AGG ACG CA	59.8

2.5 DISCUSSION

We have described three subjects, one with autosomal recessive (AR), and two with autosomal dominant (AD) primary IRF8 deficiency, characterized by a complete or selective loss of mononuclear phagocyte subsets, respectively. The *IRF8* mutation underlying the AR disorder (K108E) effects an impairment of DNA binding and trans-activation potential of IRF8. This defect produced a life-threatening pediatric syndrome, characterized by absence of blood monocytes and DCs, myeloproliferation of granulocyte precursors, and severe opportunistic infections, together requiring stem cell transplantation in infancy and suggesting that that DC/monocyte deficiency of any cause will produce susceptibility to various infectious pathogens. On the other hand, AD IRF8 deficiency is associated, in the two persons we investigated, with a heterozygous mutation resulting in a dominant-negative *IRF8* allele (T80A) that suppresses the trans-activation potential of non-mutant IRF8 *in vitro*. The associated syndrome was less severe than that of the child with AR disease, and was characterized by an abnormal peripheral blood myeloid DC phenotype with a marked loss of CD11c⁺CD1c⁺ DCs. AD IRF8 deficiency causes selective susceptibility to mycobacterial infections, and represents a novel, albeit rare, etiology of MSMD. Together, these results establish a critical role for IRF8 in the ontogeny of the human mononuclear phagocyte lineage and of circulating monocytes and DCs in particular.

Cultivation of circulating stem cells from the child with autosomal-recessive IRF8 deficiency with growth factors that support granulocyte and monocyte/macrophage colony formation showed that myeloid colonies formed were almost exclusively granulocytic (>98%), thus establishing that IRF8 is critical in the differentiation of myeloid progenitors into monocytes. *Irf8*-deficiency in mice is also associated with myeloproliferation of granulocyte precursors^{136,155,211} and very low levels of circulating monocytes (F. Ginhoux and M. Merad, personal communication). That tissue macrophages and Langerhans cells are well-represented in autosomal-recessive IRF8 deficiency suggests heterogeneity within the mononuclear phagocyte compartment with respect to IRF8-independence and/or potential for local self-renewal^{209,223}. Further work will be required to establish the relative capacity of IRF8-deficient human CD34⁺ progenitors to produce fully functional macrophages and DCs *in vitro*²²⁴. Studies in IRF8-deficient mice also show normal numbers of F4/80⁺ tissue macrophages (P. Gros, unpublished), although these cells are abnormally susceptible to infection with intracellular pathogens *in*

vitro^{113,149}. Finally, we observed that although CD4⁺ T cells from the child with autosomal-recessive IRF8 deficiency proliferated normally in response to TCR stimulation with CD3 and CD28 (**Fig. S5A, C**), they poorly secreted effector cytokines IFN- γ and IL-17, and to a lesser extent IL-10 (**Fig. S5B**), with a similar pattern detected upon *ex vivo* stimulation with PMA/ionomycin (data not shown). These results strongly suggest a defect in Th function that may be caused by abnormal T cell differentiation in an environment deficient in antigen-presenting cells.

That autosomal-dominant IRF8 deficiency is associated with a loss of IL-12 producing CD1c⁺ CD11c⁺ myeloid DCs suggests that these cells are essential for protective immunity to mycobacteria in humans. The marked reduction in CD1c⁺ CD11c⁺ myeloid DCs may be caused by altered ontogeny and maturation of this subset of CD11c⁺ cells, linked to target-specific or global transcriptional effects of the IRF8^{T80A} variant²²⁵. Despite the fact that mutant whole-blood cells produced normal amounts of IL-12 upon BCG stimulation *in vitro*, specific impairment of IL-12 secretion by CD1c⁺ DCs could contribute to mycobacterial susceptibility. Alternatively, the lack of CD1c⁺ CD11c⁺ DCs may also contribute to MSMD by other mechanisms, such as impairment of cellular trafficking between draining lymph nodes and infected tissues, or of priming of CD1c-restricted T cells for subsequent activation of infected macrophages. Indeed, CD1c⁺ - restricted T cells specific for a mycobacterial phospholipid antigen have been reported^{226,227}. We observed normal levels of CD141⁺ (BDCA3⁺) DCs subset recently proposed to be the functional equivalent of mouse CD8 α ⁺ DCs (that are absent in *Irf8*-deficient mice) in the persons with autosomal-dominant disease²²⁸⁻²³¹. Cells other than CD1c⁺ DCs may be additionally involved in the pathogenesis of MSMD, and it is possible that IRF8 deficiency impairs effector function of tissue macrophages.

These findings additionally suggest that mutations in hetero-dimerization partners of IRF8 (for example, IRF1 and PU.1) or specific transcriptional targets of such complexes²²⁵ may also impair anti-mycobacterial immunity. Finally, our study illustrates the value of genetic studies in mouse models of infection to identify candidate genes for human primary immunodeficiencies³³.

ACKNOWLEDGEMENTS

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Preface to Chapter 3

In chapter 2, we identified a homozygous mutation in *IRF8*^{K108E} in an infant with a severe primary immunodeficiency characterized by a lack of monocytes and DCs as well as extreme susceptibility to mycobacteria following routine BCG vaccination. In the following chapter, we continue to functionally characterize this K108E mutation. We investigate the effect of *IRF8* deficiency on immune cell development, activation and function as well as transcriptional regulation of direct downstream IRF8 target genes. Furthermore, we test the effect of the K108E mutation on biochemical functions of IRF8 such as nuclear targeting, stability and post-translational modifications.

Chapter 3

Functional characterization of the human dendritic cell immunodeficiency associated with the IRF8^{K108E} mutation

3.1 ABSTRACT

We have previously reported on a unique patient in whom homozygosity for a mutation at *IRF8* ($\text{IRF8}^{\text{K108E}}$) causes a severe immunodeficiency. Laboratory evaluation revealed a highly unusual myeloid compartment, remarkable for the complete absence of CD14^+ and CD16^+ monocytes, absence of CD11c^+ conventional dendritic cells (DCs) and of $\text{CD11c}^+/\text{CD123}^+$ plasmacytoid DCs (pDCs), and striking granulocytic hyperplasia. The patient initially presented with severe disseminated mycobacterial and mucocutaneous fungal infections and was ultimately cured by cord blood transplant. Sequencing RNA from the $\text{IRF8}^{\text{K108E}}$ patient's primary blood cells prior to transplant shows not only depletion of IRF8-bound and -regulated transcriptional targets, in keeping with the distorted composition of the myeloid compartment, but also a paucity of transcripts associated with activated CD4^+ and CD8^+ T lymphocytes. This suggests that T cells reared in the absence of a functional antigen-presenting compartment in $\text{IRF8}^{\text{K108E}}$ are anergic. Biochemical characterization of the $\text{IRF8}^{\text{K108E}}$ mutant *in vitro* shows that loss of the positively charged side chain at K108 causes loss of nuclear localization and loss of transcriptional activity, which is concomitant with decreased protein stability, increased ubiquitination, increased SUMOylation, and enhanced proteasomal degradation. These findings provide functional insight into the molecular basis of immunodeficiency associated with loss of IRF8.

3.2 INTRODUCTION

Primary immunodeficiencies sometimes present with disseminated mycobacterial infection following neonatal vaccination with live *Bacillus Calmette-Guérin* (BCG)¹. In many cases, patients suffer from Mendelian Susceptibility to Mycobacterial Disease (MSMD), a syndrome caused by infection with weakly virulent mycobacteria such as BCG, with environmental mycobacteria, and/or recurrent infections with virulent mycobacteria (*M. tuberculosis*) over the life course³. Approximately half of MSMD patients have been shown to carry mutations in a small subset of genes involved in IFN- γ dependent early immune responses^{3,232}. Other patients develop disseminated BCG disease as part of a broader pattern of susceptibility to infection, for example severe combined immunodeficiency.

We have previously reported an infant presenting with severe disseminated BCG infection, oral candidiasis and severe respiratory viral infection²³³. Evaluation of patient's blood cellular profile revealed an aberrant myeloid compartment; notably a complete absence of CD14⁺ or CD16⁺ monocytes, of CD11c⁺ conventional dendritic cells (DCs) and of CD11c⁺/CD123⁺ plasmacytoid DCs (pDCs), and prominent granulocytic hyperplasia²³³. The peripheral blood B lymphocyte count was also increased in this patient. Production of IL-12 and IFN- γ by blood cells in response to bacterial products or non-specific stimuli was impaired²³³. The patient was cured by cord blood transplant. The phenotype and immunological features of the patient resembled that of mice bearing partial or complete loss of function mutations in *Irf8*^{130,149,212}, including susceptibility to infection with intracellular pathogens such as *Mycobacteria*^{132,149,150}, partial (BXH-2; *Irf8*^{R294C}) or complete (*Irf8*^{-/-}) absence of CD11c⁺ CD8 α ⁺ DCs and pDCs^{114,130,212}, and lack of IL-12p40 and IFN- γ production following infection^{4,132}. Sequencing of *IRF8* in this patient identified homozygosity for a unique variant at IRF8 (*IRF8*^{K108E}). The K108E variant was present in both healthy parents in a heterozygous state and maps to the DNA binding domain of IRF8. This unique patient defines a novel syndrome designated recessive IRF8 dendritic cell immunodeficiency.

IRF8 is a member of the interferon regulatory factor (IRF) family, and plays essential roles in host defense, hematopoietic differentiation and immune response, including transcriptional activation in response to interferons¹¹⁸. IRF proteins share a highly conserved amino terminal DNA binding domain of the helix-turn-helix type (aa1-115) consisting of five

tryptophan (W) residues that contact DNA at GAAA and AANNNGAAA consensus sequence motifs termed interferon stimulated response element (ISRE), found near interferon-regulated genes²³⁴. IRF8 also has an IRF association domain (IAD), serving as a recruitment module for other transcription factors, including members of the IRF (e.g. IRF1), or ETS (e.g. PU.1) families, to activate or repress gene expression^{126,132} including genes encoding proteins involved in macrophage anti-microbial functions^{127,129,160,235}, early Th1 polarization of the immune response¹³³⁻¹³⁵, antigen presentation¹⁴⁴, promoting differentiation of myeloid progenitors towards mononuclear lineages, and inducing apoptosis of the granulocytic lineage¹³⁶. Finally, IRF8 is a key regulator of pathological inflammation: inactivation of *Irf8* protects against neuroinflammation in *Plasmodium berghei* infected mice²³⁶, while association studies have identified *IRF8*-associated polymorphisms as a key genetic risk for low monocyte counts²³⁷, rheumatoid arthritis²³⁸, systemic lupus erythematosus¹⁷⁸, ulcerative colitis¹⁸⁰, Crohn's disease^{179,239} and multiple sclerosis¹⁸¹ in humans. Hence, characterizing the genes that are bound and regulated by IRF8 in human cells may shed important light on proteins and pathways that underlie pathological inflammation in humans.

The K108E patient is the only known example of recessive *IRF8* deficiency in humans. Comparative studies in primary cells from this individual offer a unique window of opportunity to identify and characterize the transcriptional targets of IRF8 *in vivo* and in the context of its normal physiological function in human cells. These studies may identify proteins and pathways that are required for ontogeny and maturation of myelomonocytic cells including effective anti-microbial defenses, and pathways activated during pathological inflammation. Finally, biochemical characterization of the mechanistic basis of loss of function in K108E may provide valuable information of pathogenesis of DC deficiency in this patient.

3.3 MATERIALS AND METHODS

Plasmid construction

A 500bp portion of the *Isg15* gene promoter region containing an ISRE (interferon sensitive response element) was PCR amplified from genomic DNA using oligonucleotide primers 5'-ACTGACTCGAGTGC TGGGATCAAAGGTGTGC- 3' and 5'-GTCAGAAGCTTGAGTCTGCTTCTGGCTGCTT-3', and cloned into the luciferase reporter vector pGL3 (Promega, Madison, WI) using Xho I and Hind III restriction sites. The IL-12p40-pGL3, IRF8-HA-pcDNA3 and IRF1-pcDNA3 vectors were generated as previously described⁵. The V5-SUMO3 and HA-SENPI plasmids were made as previously described²⁴⁰, and kindly provided by Keiko Ozato (National Institutes of Health, USA).

Cell culture, transfections, luciferase assays and Western blots

RAW 264.7 and HEK 293T cells were maintained in DMEM (Wisent) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Wisent). For luciferase assays, RAW cells (1×10^5 cells/well) were transfected using Lipofectamine PLUS (Invitrogen Life Technologies) with 400ng luciferase pGL3 reporter, 100ng *Renilla* internal control and variable amounts of pcDNA3 expression vectors. Luciferase activity was assayed 24h following transfection using a dual luciferase assay system (Promega). Whole cell extracts were analyzed by SDS-PAGE and incubated with mouse anti-HA monoclonal antibody (1:100; Covance Research Products, Princeton, NJ) followed by washing and incubation with an anti-mouse secondary antibody conjugated to HRP (1:20 000, GE Healthcare).

Immunoprecipitation, Ubiquitination and SUMOylation assays

HEK 293T were transfected and washed with cold phosphate-buffered saline (PBS) and lysed for 30min on ice in lysis buffer (50mM Tris-HCl pH 7.5, 150mM NaCl, 1% Triton X-100, 0.1% SDS) supplemented with protease inhibitors. Lysates (500µg) were incubated with 2µg anti-IRF8 antibody C-19 (Santa Cruz) overnight at 4°C. Supernatants were collected and incubated with 50µl of recombinant Protein G Agarose beads at 4°C for 4h. Immune complexes were resuspended in 50µl Laemmli sample buffer (60mM Tris-HCl pH 6.8, 2% SDS, 10% Glycerol, 5% β-Mercaptoethanol, 0.02% Bromophenol blue) and heated at 95°C for 5min. Samples were subjected to SDS-PAGE and immunoblotted either with anti-Ub P4D1 (1:250;

Santa Cruz Biotechnology Inc.), anti-IRF8 C-19 (1:250; Santa Cruz Biotechnology Inc., Santa Cruz, CA) or anti-V5 (1:5000; Invitrogen Life Technologies).

Immunofluorescence

Stably transfected RAW macrophage clones were seeded onto glass coverslips (1×10^5 cells/well). Cells were washed with PBS 24h later and fixed in 4% Paraformaldehyde (PFA) in PBS for 15min, and then permeabilized with 0.1% Triton X-100 for 15min. Coverslips were blocked with 0.1% bovine serum albumin (BSA) in PBS for 1h, then incubated with mouse anti-HA mAb (1:200) for 1h, and washed with 0.1% BSA in PBS. The coverslips were then incubated for 1h with goat anti-mouse Cy3-conjugated secondary antibody (1:100) and washed with 0.1% BSA in PBS and then with DAPI in PBS. Coverslips were mounted on microscope slides using Permafluor Aqueous Mounting Medium (Thermo Scientific). Confocal microscopy was conducted with a Zeiss LSM5Pascal laser scanning confocal microscope. All image analyses were performed using LSM5 Image.

Cellular fractionation

Stably transfected RAW macrophage clones were treated or not with IFN- γ (400U/ml; 3 hrs, 37°C). Cells were washed with PBS, resuspended in buffer A (10mM HEPES pH 7.9, 10mM KCl, 0.1mM EDTA, 0.1mM EGTA) and incubated on ice for 15 minutes. NP-40 (Sigma) was added at a final concentration of 0.6%, vortexed for 10 seconds and centrifuged for 1 minute ($13,000 \times g$, 4°C). The supernatant containing the cytoplasmic fraction was collected. The pellet was then washed twice with PBS and resuspended in buffer B (20mM HEPES pH 7.9, 25% glycerol, 400mM NaCl, 1mM EDTA, 1mM EGTA) and incubated at 4°C on a rotator for 40 minutes. Samples were centrifuged for 5 minutes ($13,000 \times g$, 4°C) and the supernatant containing the nuclear fraction was collected. Both buffers were supplemented with 2mM DTT and protease inhibitors. Fractions were separated by SDS-PAGE, immunoblotted with anti-IRF8 antibody C-19 (Santa Cruz), anti-RNA Pol II sc-899 (Santa Cruz) and anti-GAPDH 6C5 (Santa Cruz).

RNA sequencing (RNA-seq)

Total peripheral blood mononuclear cells (PBMC) RNA was extracted using RNeasy columns (Qiagen), and integrity was assessed on a Bioanalyzer RNA pico chip. The RNA-seq libraries and sequenced on a HiSeq 2000 sequencer at the McGill and Genome Quebec Innovation Center. Using the Trimmomatic software²⁴¹, raw read sequences were trimmed for minimum quality at the 3' end (phred score of at least 30), cleaned of adapter traces and filtered for a final minimum length of 32bp. Alignment to the UCSC hg19 reference genome (Illumina iGenomes) sequence was performed using TopHat2²⁴², while gene expression was quantified by counting the number of uniquely mapped reads with HTSeq (<http://www-huber.embl.de/users/anders/HTSeq/>). Normalization and differential expression analysis between patient and controls was conducted using the edgeR Bioconductor package²⁴³. We retained genes that had an expression level of at least 1 count per million reads (CPM) in at least one of the 6 samples. The K108E sample was first compared to all control samples; we also compared the Ctl1 sample to all other control samples. Differentially expressed transcripts were identified as showing a >3 fold difference between the K108E and Ctl1 samples (p value ≤ 0.05 for K108E vs all Ctls), while correcting for minor variations amongst all control samples (p value ≥ 0.05). Data are shown as heatmap (Log2 CPM) created using MeV²⁴⁴. Gene ontology enrichment analysis was performed using the DAVID website²⁴⁵. The differential gene expression profile was also subjected to gene set enrichment analysis using GSEA²⁴⁶ with MSigDB public immunologic gene signatures. Amongst the enriched gene signatures, eight representative were selected for graphical representation (GSE22886_neutrophil_vs_monocyte_up, GSE22886_neutrophil_vs_monocyte_dn, GSE22886_neutrophil_vs_DC_dn, GSE9988_LPS_vs_vehicle_treated_up, GSE22886_Ctrl_vs_LPS_24h_DC_dn, GSE22886_naive_CD4_Tcell_vs_48h_act_Th1_up, Goldrath_naive_vs_Eff_CD8_Tcell_dn, GSE11057_naive_vs_memory_CD4_Tcell_dn, GSE13411_plasma_cell_vs_memory_Bcell_up, GSE22668_Naive_Bcell_vs_BM_Plasma_cell_UP)

3.4 RESULTS

Hematological profile of the *IRF8*^{K108E} patient

A three month old infant patient homozygous for a loss of function mutation at *IRF8* (K108E) presented with symptoms of immunodeficiency which included disseminated BCG infection (following neonatal BCG vaccination), oral candidiasis and severe respiratory viral infection. Examination of blood profile and FACS assisted immunophenotyping (**Fig. 1**), showed persistent and extreme neutrophilia (note the log scale), absolute monocytopenia (CD14⁺, CD16⁺ cells), anemia and thrombocytopenia. The patient was not lymphopenic, but rather developed a marked B lymphocytosis (note log scale), and to a lesser extent NK lymphocytosis, as her clinical status evolved towards an increasingly inflammatory picture (**Fig. 1**). This B lymphocytosis was accompanied by elevated plasma immunoglobulins (**Fig. S1**) with strikingly high levels of IgM (1.2-5.6g/L). This was treated as haemophagocytic lymphohistiocytosis using a modified version of the HLH-2004 protocol (**Fig.1, blue triangle**) resulting in decline in neutrophils, B and NK cell counts, accompanied by clinical stabilization. The patient moved rapidly into conditioned hematopoietic stem cell transplant (**Fig. 1, red triangle**) at 9 months of age, following which all hematologic parameters were ultimately normalized, including appearance of circulating monocytes. Immunologic reconstitution was fully achieved including normal humoral responses to vaccines.

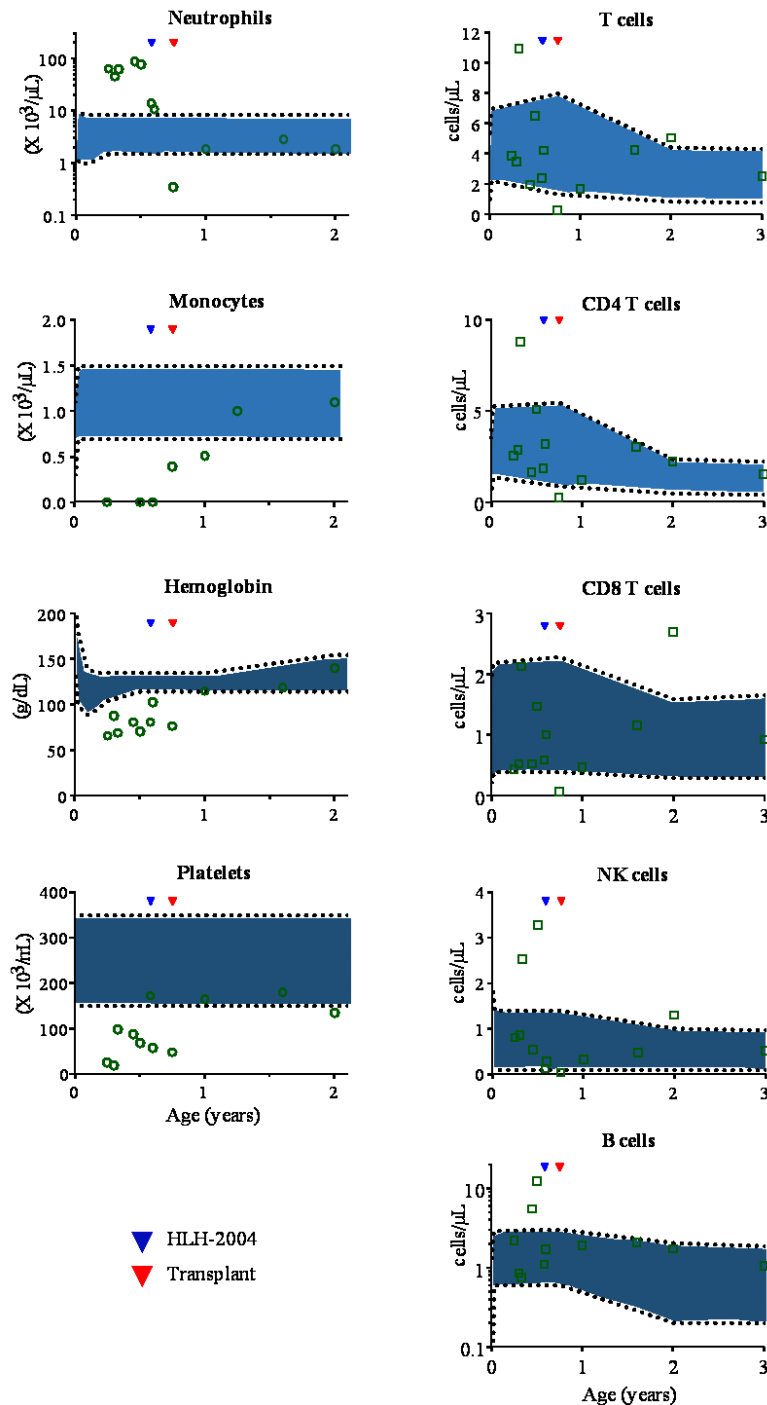


Figure 1. Blood cellular profile of patient K108E prior to and post stem cell transplant. Hematological profiles were obtained by standard automated measurements (neutrophils, platelets, hemoglobin), and immune cell populations were established by FACS using cell-specific markers. The patient was treated as haemophagocytic lymphohistiocytosis using a modified version of the HLH-2004 protocol (blue triangle) with resulting decline in neutrophil, B and NK cell counts, accompanied by clinical stabilization. The patient then moved rapidly into conditioned hematopoietic stem cell transplant (red triangle; 9 months of age).

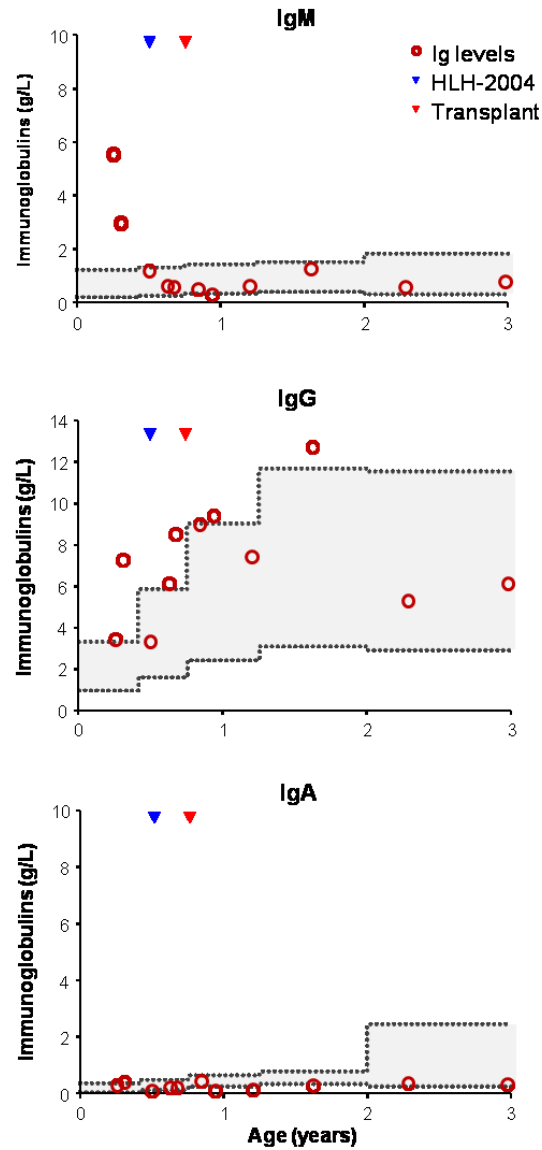
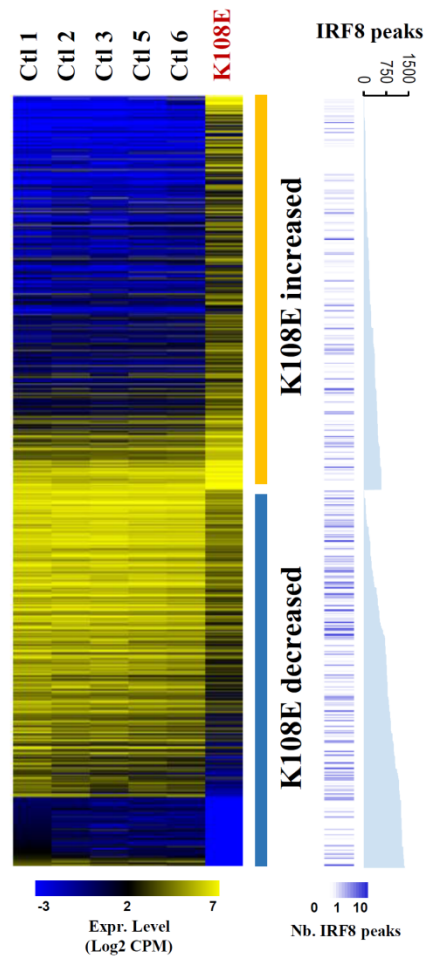


Figure S1. Serological immunoglobulin profile of patient K108E. The levels of circulating IgM, IgG, and IgA from patient K108E were measured over time prior to and following curative cord blood stem cell transplant. The shaded portion of each graph identifies normal daily values. Blue triangle corresponds to induction of haemophagocytic lymphohistiocytosis, and red triangle identifies the conditioned hematopoietic stem cell transplant.

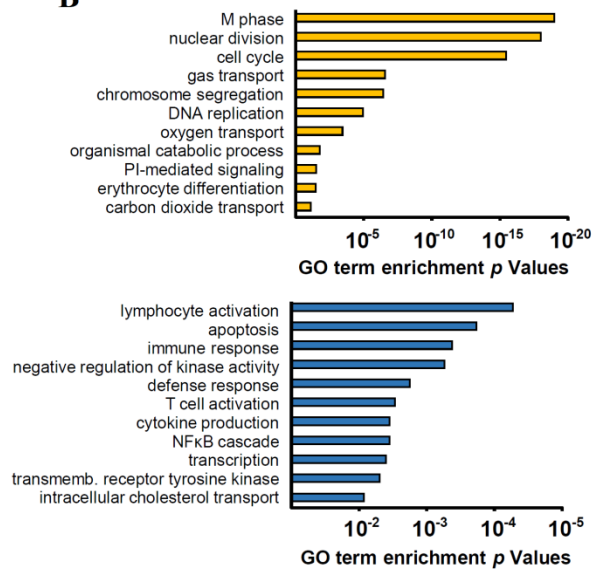
Expression Studies in Blood Cells from the IRF8^{K108E} Patient Identify Defects in Antigen Presenting Cells and in T Cell Activation

To gain insight into functional consequences of loss of IRF8 and to identify IRF8 transcriptional targets in primary human cells *in vivo*, we used RNA sequencing (RNA-seq) to compare expression profiles of peripheral blood mononuclear cells (PBMCs) from 5 healthy controls to that of the K108E patient obtained prior to curative cord blood transplant. This analysis (**Appendix 1**) detected 724 genes with increased expression, and 694 genes with decreased expression in K108E PBMCs compared to controls (**Fig. 2A**). *A priori*, these lists are expected to contain: a) genes that are directly bound and transcriptionally regulated by IRF8 (and which expression is lost in the patient), and b) genes that are differentially expressed among cell types that are more or less numerous in patient vs. controls, the ontogeny and numbers of which are regulated by IRF8. Gene ontology annotations (**Fig. 2B**) indicated that transcripts elevated in the K108E patient were associated predominantly with cell cycle and cell division, in agreement with the increased number of CD34⁺ hematopoietic progenitors, and with the myeloproliferative profile (granulocytic hyperplasia) of the patient²³³. On the other hand, transcripts linked to innate immunity were relatively depleted in the K108E patient, in agreement with the patient's immunodeficiency phenotype and lack of monocytes and dendritic cells.

A



B



C

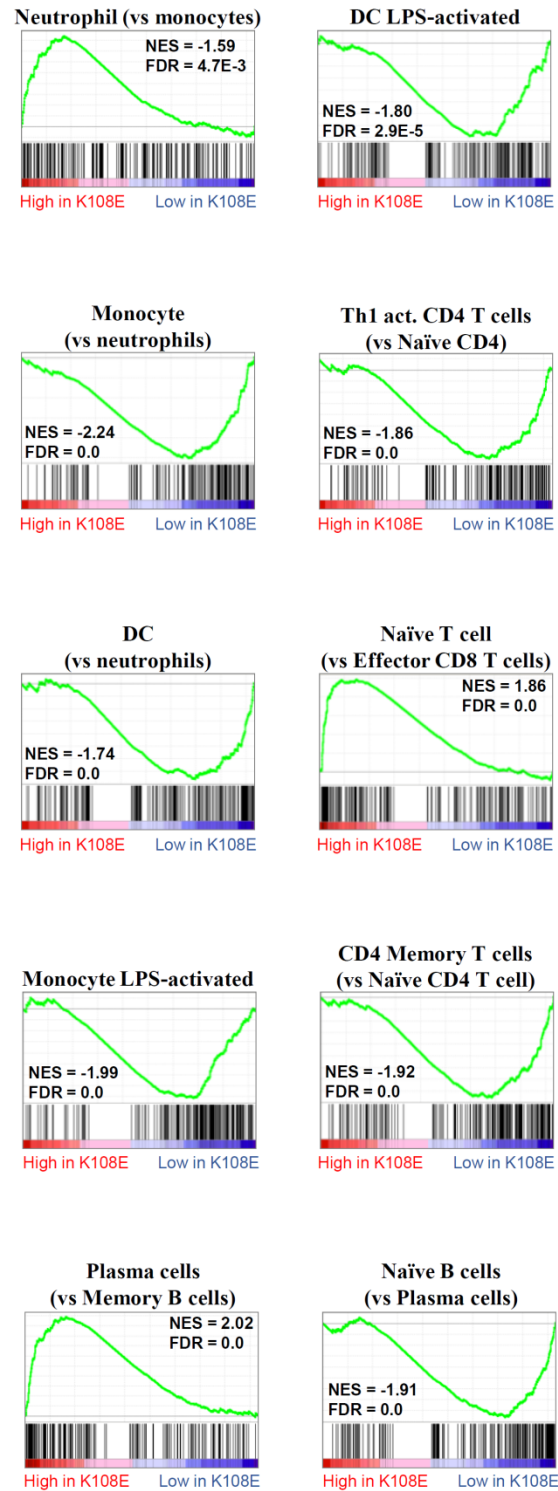


Figure 2. IRF8^{K108E} mutation modifies global gene expression profile of blood cells. (A) RNA extracted from peripheral blood mononuclear cells of 5 healthy individuals and from the IRF8^{K108E} patient were sequenced (RNA-seq). The expression level of significantly dys-regulated genes in the K108E sample, shown as Log2 of sequence read counts per millions reads (CPM), are presented in a heatmap together with the expression level of the control samples. There are 724 genes that exhibit increased and 694 genes that have decreased expression in K108E PBMCs sample. The number of IRF8 binding sites found in the vicinity of each dys-regulated gene is represented by blue bars. For the two groups of genes, a graph shows the cumulative number of IRF8 binding sites found in proximity of these genes in chromatin from activated macrophages. (B) Gene ontology (GO) enrichment analysis of genes that show either increased (yellow) or decreased (blue) expression in K108E compared to controls; the degree of statistical significance is shown. (C) The K108E expression profile was subjected to gene set enrichment analysis (GSEA)²⁴⁶ to identify specific immune cells signatures either over-represented or depleted in K108E compared to controls. Immune cell signatures were produced by pair-wise comparisons of public datasets (see Materials and Methods), and are indicated on top of each graph. GSEA graphs illustrate the cumulative enrichment score in each specific gene signature comparison; the occurrence of the cell signatures genes is identified as individual black lines over the distribution of the K108E patient gene profile. Normalized enrichment scores (NES) and false-discovery rate (FDR) are shown for each displayed analysis.

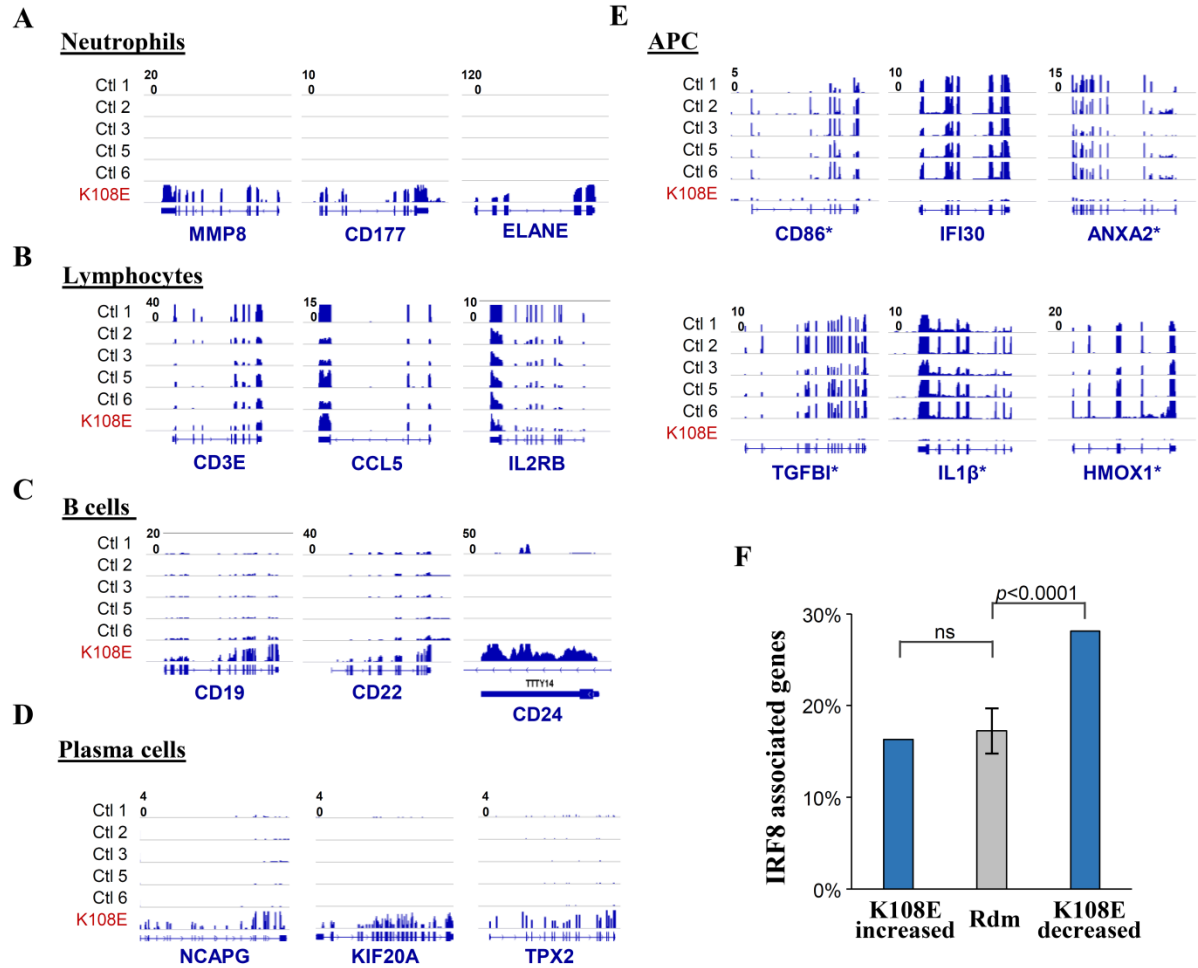


Figure 3. IRF8 directly controls gene expression in antigen-presenting cells. The RNA-seq data was queried for hematopoietic cell-specific genes, and the density of sequence reads was mapped to individual gene exons and is shown for 5 controls and for K108E. **(A)** Neutrophil genes are enriched in K108E while T lymphocytes genes **(B)** in controls and patient K108E. B-lymphocyte **(C)** and plasma cell **(D)** specific genes are enriched in patient K108E. **(E)** Transcripts expressed in antigen presenting cells (APC) are depleted in K108E in agreement with published cellular immunophenotyping data of the K108E patient²³³. The asterisk (*) identifies genes that were found to be direct IRF8 targets in ChIP-seq experiments. **(F)** The list of genes differentially expressed in K108E was compared to a list of direct IRF8 targets (containing an IRF8 binding site within 20kb from the transcription start site) and obtained by chromatin immunoprecipitation and sequencing (ChIP-Seq) that we recently published²³⁶. Genes bearing IRF8 binding sites are significantly enriched in the list of genes depleted in K108E, compared to genes of increased expression in K108E, or to a set of 10 randomly generated gene sets. The *p* value is calculated using the Fisher's Exact Test.

To study the effect of the IRF8^{K108E} variant on ontogeny and activity of individual blood cell populations, we compared the K108E-associated gene expression profile to discrete immunological cell-specific transcript signatures obtained by gene set enrichment analyses (GSEA)²⁴⁶. As expected from the extreme neutrophilia seen in the K108E patient, the GSEA analysis shows strong enrichment of neutrophil-specific signatures exemplified by neutrophil specific transcripts MMP8, CD177, and ELANE (**Fig. 2C, 3A**). Moreover, and in agreement with the B cell lymphocytosis noted in the patient, a B cell-specific signature (e.g CD19, CD22, CD24) was over-represented (**Fig. 3C**); more specifically, a plasma cell signature (e.g NCAPG, KIF20A and TPX2) was enriched compared to naïve B or memory B cells (**Fig. 3D**), perhaps in keeping with elevated plasma Ig in the patient. A close examination the immunoglobulin heavy chain locus confirms the predominance of IgM expression (**Fig. S1**). However, most striking was a decrease in monocyte and dendritic cells specific signatures, and in LPS-responsive transcripts in the patient (**Fig. 2C**), with CD86, IFI30, ANXA2, TGFBI, IL1B, and HMOX1 providing the clearest examples of this myeloid signature (**Fig. 3E**). This is in agreement with the known emptiness of the antigen presenting compartment in this patient (**Fig. 1**). Surprisingly, this analysis also clearly showed that the patient's T cells are in a more naïve state, with a striking depletion of Th1 activated CD4⁺ T cell, and of CD4⁺ and of CD8⁺ antigen-experienced memory T cell gene signatures (**Fig. 2C**). This suggests that the primary defect of antigen presenting cells in K108E is associated with a secondary defect in lymphoid cells.

We also compared the list of transcripts altered in the K108E patient (**Appendix 1**) to a list of direct transcriptional targets of IRF8 that we identified by sequencing of IRF8-specific chromatin (ChIP-seq) immunoprecipitated from a mouse macrophage cell line treated with IFN- γ ²³⁶. We noted a strong and significant depletion of transcripts from genes close to IRF8 binding sites in the K108E patient (**Fig. 2A, 3F**). Such genes represent major and direct IRF8 transcriptional targets *in vivo* in myeloid cells, expression of which is abrogated in the patient. This list defines the true IRF8 myeloid transcriptome (**Table S1**) and includes pro-inflammatory molecules (IL1 β , C1qA, C2, CCL3, CCL4) and receptors (CCR5, CCR7, CSF1R, TGFBR2), as well as cell surface proteins that define myeloid cells (CD14, CD163, CD300e). These results show that in humans, loss of IRF8 activity is associated with depletion of myelomonocytic cells, impaired T cell function, in addition to loss of IRF8-dependent transcription in these and other blood cells.

Impaired Nuclear Targeting, Decreased Stability, and Altered Post-Translational Modification of the IRF8^{K108E} Variant

IRF8 can repress *Isg15* gene expression, acting to inhibit IRF1-dependent activation of certain genes, including *Isg15*²⁴⁷. Although wild type IRF8 induces dose-dependent repression of the *Isg15* promoter activity, this activity is lost in the E108 mutant (**Fig. 4A**). IRF8 also inhibits IRF1-dependent transcriptional activation of the *Isg15* reporter, and this inhibitory effect is lost in E108 (**Fig. 4B**). Conversely, and as opposed to K108, the E108 variant fails to promote IRF1-dependent transactivation of an *IL-12p40* luciferase reporter construct (**Fig. 4C**)²³³. To gain insight into the structural requirements at K108 that are required for function, we tested other mutants at position 108. The Q108 variant, in which the positively charged side chain of lysine is eliminated (uncharged glutamine), cannot transactivate the *IL-12p40* reporter, while, a R108 variant that maintains a positively charged side chain of similar size retains transcriptional activity (**Fig. 4C**). Finally, H108 which harbors an imidazole ring and a partial positive charge shows significant transcriptional activity (**Fig. 4C**). Immunoblotting experiments show that all variants are expressed at similar levels in transfected cells. Also, the R108 mutant shows electrophoretic mobility (a function of charge and folding) similar to K108, but distinct from E108 (**Fig. 4D**). K108 maps to a short β -strand making a hydrogen bond (3.3Å) and docking IRF8 onto DNA; mutation to E108 creates repulsion, allowing for a water molecule to fill the space and to destabilize the binding³. Molecular modeling of the additional mutants reveals that H108 has a protonable side chain (pK ~7) which can act as a donor for hydrogen bonding depending on the environment (**Fig. S2**). This is consistent with the partial loss of transactivation seen in this mutant. Finally, R108 is structurally similar to K108 and is fully functional (**Fig. S2**). These results indicate that it is the loss of positive charge, rather than the gain of a negative charge, that is responsible for the loss of transcriptional function of E108.

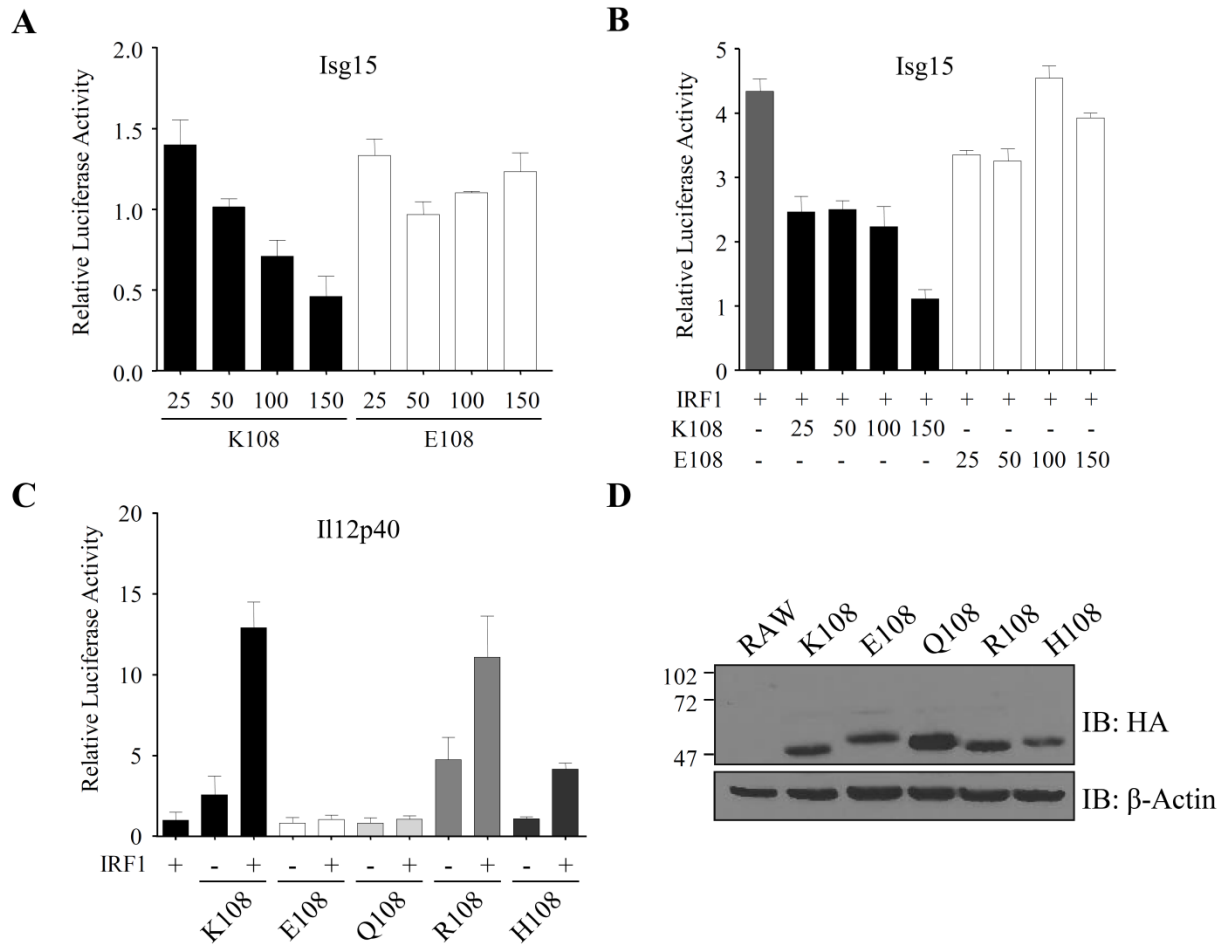


Figure 4. Loss of transcriptional function in the IRF8^{K108E} mutant. (A) RAW macrophages were transiently transfected with increasing amounts of WT (K108) or IRF8^{K108E} variant (E108) along with a firefly luciferase reporter construct containing the *Isg15* promoter and a *Renilla* luciferase internal control, and luciferase activity was assayed 24h later. (B) RAW cells were transiently transfected as in (A) with the addition of IRF1. (C) RAW cells were transiently transfected with expression vectors coding for K108 or with the E108, R108, Q108, and H108 mutants, along with or without IRF1 and an IL-12p40-pGL3 luciferase reporter construct and a *Renilla* luciferase internal control. Results are represented as means $\pm$ SD of three independent experiments. (D) Immunoblots showing similar expression level of K108, and of the E108, R108, Q108, and H108 mutants in transfected cells, and using a mouse anti-HA monoclonal antibody. Actin expression was evaluated as a loading control.

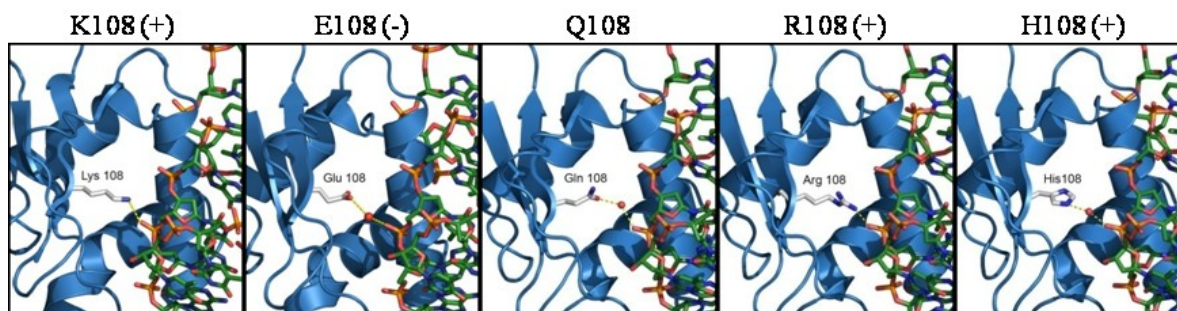


Figure S2. Molecular modeling of K108 and additional mutants. Molecular modeling of the three dimensional structure of DNA-bound Irf8 show that the hydrogen bond formed between the amino group of K108 and the DNA backbone is lost in E108 and Q108. Hydrogen bonding is fully restored in R108 and partially in H108.

At steady state, IRF8 is expressed at low levels in the cytoplasm. In response to exposure to microbial products or to IFN- γ , IRF8 expression is induced and IRF8 is recruited to the nucleus¹³³. We generated macrophages stably expressing HA-tagged K108 or variants at this position, and monitored protein expression and sub-cellular localization by immunofluorescence, and by biochemical cell fractionation. In resting macrophages, all variants are found in the cytoplasm (**Fig. 5A, S2**). Following stimulation with IFN- γ for 3h, K108 and the structurally similar R108 are both translocated to the nucleus where they co-localize with DAPI staining while H108 showed reduced nuclear translocation. This IFN- γ -induced nuclear translocation is lost in both the E108 and Q108 variants, which are mainly retained in the cytoplasm (**Fig. 5A, S3**). Cytoplasmic retention of E108 was further confirmed by cell fractionation experiments (**Fig. 5B**). In these experiments, we used an anti-IRF8 antibody that distinguishes the slower migrating transfected E108 mutant, from the faster migrating endogenous K108 protein. These experiments also allowed us to monitor a possible effect of the mutant variant on the localization of wild type protein when expressed in the same cell. Following IFN- γ stimulation, E108 is predominantly retained in the cytoplasm and barely detected in nuclear fractions, while K108 is efficiently translocated into the nucleus. Expression of the E108 mutant does not affect localization or nuclear translocation of endogenous K108 present in RAW cells (**Fig. 5B**). Loss of nuclear translocation likely contributes to the abrogated transcriptional activity of the E108 and Q108 variants (**Fig 4**) by preventing access of these proteins to DNA.

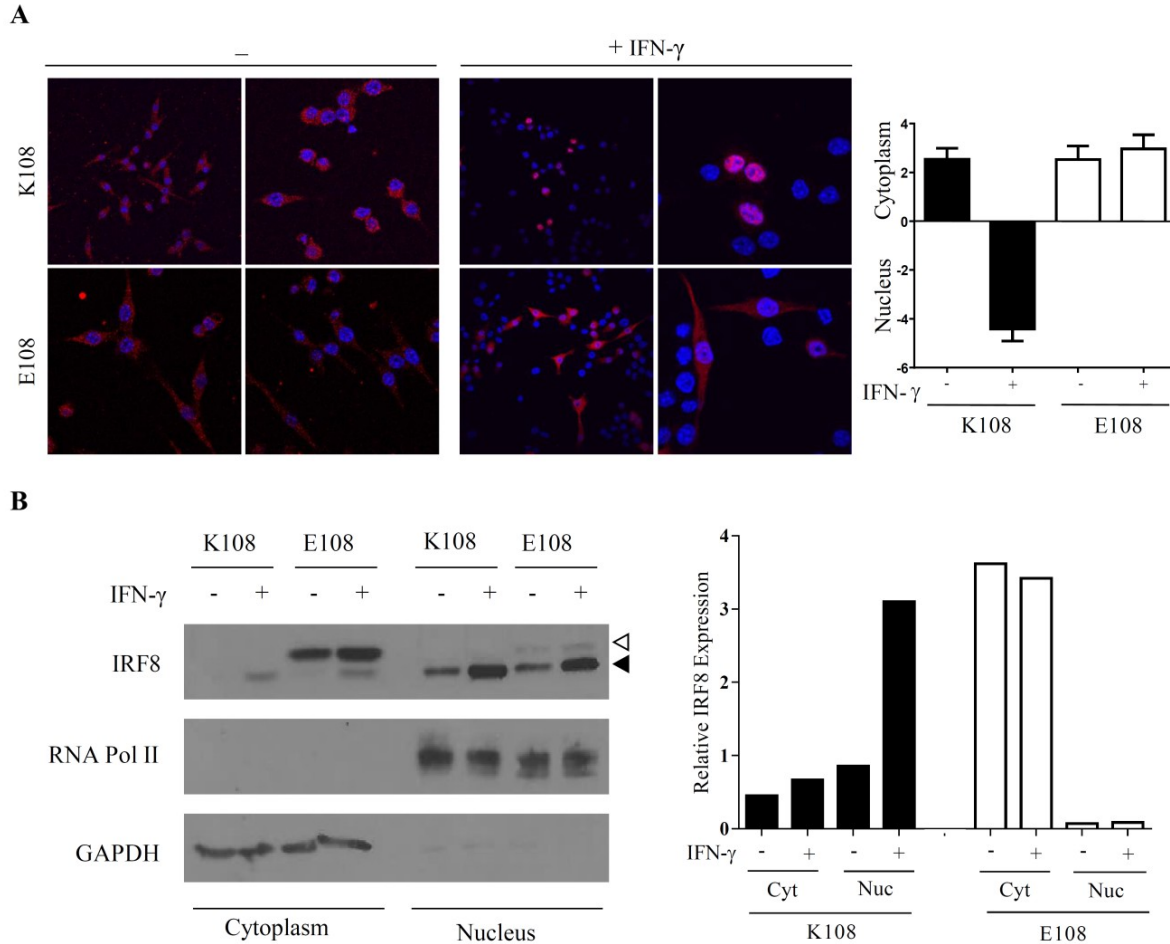


Figure 5. Defective nuclear translocation of the IRF8^{K108E} mutant. (A) RAW macrophages stably expressing HA-tagged K108 and E108 variants were stimulated or not with IFN- γ (400U/ml) for 3h. Cells were fixed, permeabilized and incubated with mouse anti-HA mAb followed by mouse Cy3-conjugated secondary antibody (red) and then with DAPI to stain nuclei (blue). Cellular localization was analyzed by confocal microscopy using a Zeiss LSM5 Pascal laser scanning confocal microscope. All image analyses were performed using LSM5 Image. Two different images of each condition are shown. Images are representative of three independent experiments. Mean fluorescence intensity of Irf8 (red) staining was measured using Image J. Ratios of cytoplasmic over nuclear fluorescence are represented as means $\pm$ SD of ten independent measurements. (B) RAW macrophages stably expressing HA-tagged K108, E108 were stimulated or not with IFN- γ (400U/ml) for 3h. Cell lysates were subjected to cellular fractionation to separate the cytoplasmic and nuclear fractions and immunoblotted with anti-IRF8, anti-RNA Pol II (nuclear control) and anti-GAPDH (cytoplasmic control) antibodies. White triangle represents transfected E108 and black triangle represents endogenous K108. The relative nuclear and cytoplasmic distribution of the total K108 and E108 proteins was evaluated and is shown.

Reduced transactivation function and defective nuclear translocation as well as altered electrophoretic mobility suggested that the K108E mutation may also affect protein folding, with pleiotropic consequences on stability, subcellular targeting, and overall function. Stability of the K108 and E108 variants was investigated in cells stably expressing each protein, and following treatment with the protein synthesis inhibitor cycloheximide (CHX). Levels of each protein were monitored over time in CHX-treated cells by immunoblotting (**Fig. 6A**). Over a 6h period, K108 protein was stable in CHX-treated cells, suggesting a half-life of > 6-8hrs; in the same conditions, there was a rapid disappearance of E108 variant, with less than 10% protein remaining at 6h (**Fig. 6A**). Enhanced degradation of E108 was proteasome-dependent and sensitive to the proteasome inhibitor MG132 (**Fig. 6B, C**). The results suggest reduced stability and increased degradation of the E108 variant.

IRF8 is targeted for proteasome degradation following ubiquitination by the E3 ubiquitin ligase Cbl²⁴⁸. In addition SUMOylation via SUMO2 and SUMO3 keeps IRF8 in a repressed functional state and can also target IRF8 for proteasomal degradation²⁴⁰. Following activation of macrophages, IRF8 is deSUMOylated by SENP1 which releases IRF8 for transcriptional activity²⁴⁰. We investigated the effect of K108E on regulatory post-translational modification of IRF8 (**Fig. 6D, E**). In transfected HEK 293T cells, we observed for both wild type and K108E mutant, a high molecular mass smear typical of ubiquitinated proteins but this smear was more intense for E108, suggesting increased ubiquitination of the mutant compared to WT (**Fig. 6D**). To test the effect of K108E on the state of IRF8 SUMOylation, HEK 293T cells were transfected with either K108 or with E108, and with V5-tagged SUMO3, with or without the deSUMOylation enzyme SENP1. SUMO3-dependent and SENP1-sensitive SUMOylation of K108 was detected in transiently transfected HEK293T cells. However, for the E108 variant, there was a strong increase in SUMO3-dependent and SENP1-sensitive conjugation compared to K108, with appearance of higher molecular weight forms unique to the E108 variant, with all forms being sensitive to SENP1 (**Fig. 6E**). Together, these results indicate increased ubiquitination and increased SUMOylation of the K108E variant, which is concomitant to decreased protein stability and increased proteasome-dependent degradation.

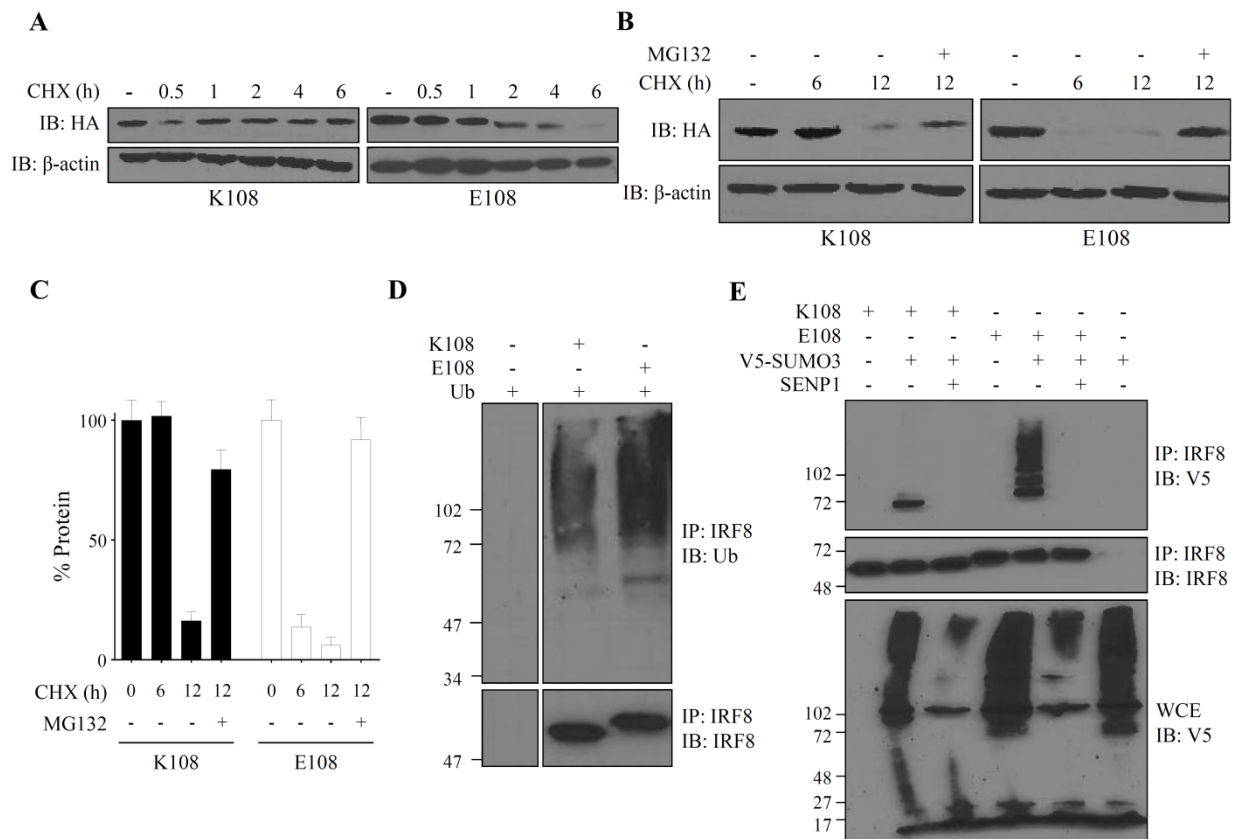


Figure 6. Decreased stability of the IRF8^{K108E} mutant. (A) RAW macrophages stably expressing HA-tagged K108 or E108 variant were treated with cycloheximide (CHX) for the indicated periods of time (h) and for up to 6h. Cell lysates were subjected to SDS-PAGE and immunoblotted with a mouse anti-HA mAb. Actin was used as an internal loading control. (B) RAW macrophages stably expressing HA-tagged K108 or E108 were treated with cycloheximide for 6h and 12h with or without the proteasome inhibitor MG132 (10 μ M) and cell lysates were analyzed by immunoblotting. (C) Relative protein expression was assessed by densitometry using ImageJ. Results are represented as means $\pm$ SD of three independent experiments. (D) HEK 293T cells were transiently transfected with K108 or E108 variant, along with Ubiquitin, and 24h later, cells were treated with MG132. IRF8 was immunoprecipitated from cell lysates followed by immunoblotting with anti-ubiquitin antibody. IRF8 was also detected by immunoblotting from total cell lysates. The left and right panels come from the same experiment, membrane and film, and thus can be used for comparison. (E) HEK 293T cells were transiently transfected with K108 or E108 variant, with or without V5-tagged SUMO3, and/or HA-tagged SENP1. Cell lysates were immunoprecipitated with anti-IRF8 antibody and immunoblotted with anti-V5 antibody. Total cell extracts were also immunoblotted with anti-V5 antibody to show SUMOylation of other proteins and SENP1 de-SUMOylating activity.

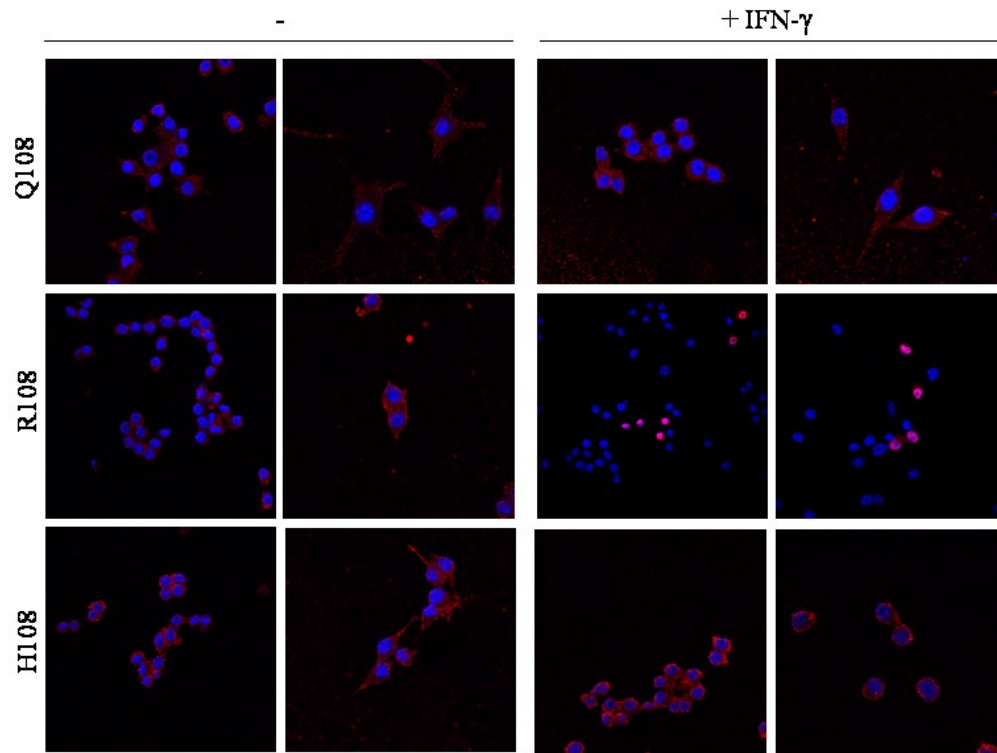


Figure S3. Nuclear translocation of Q108, R108 and H108 variants. RAW macrophages stably expressing HA-tagged Q108, R108 and H108 variants were stimulated or not with IFN- γ (400U/ml) for 3h. Cells were fixed, permeabilized and incubated with mouse anti-HA monoclonal Ab followed by mouse Cy3-conjugated secondary antibody (red) and then with DAPI to stain nuclei (blue). Cellular localization was analyzed by confocal microscopy using a Zeiss LSM5 Pascal laser scanning confocal microscope. All image analyses were performed using LSM5 Image. Two different images of each condition are shown. Images are representative of three independent experiments.

Table S1. Genes depleted in K108E with at least 1 IRF8 binding site within 20Kb of TSS

Gene symbol	Gene name	Chr	Fold change	p Value
ABCG1	ATP-binding cassette, sub-family G (WHITE), member 1	chr17	-12.0	0.0
ADAM12	ADAM metalloproteinase domain 12	chr7	-392.3	0.0
ADNP	activity-dependent neuroprotector homeobox	chr2	-3.7	0.0
ADRB2	adrenoceptor beta 2, surface	chr18	-12.5	0.0
AIM2	absent in melanoma 2	chr1	-25.5	0.0
AKT3	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma)	chr1	-5.5	0.0
ALDH9A1	aldehyde dehydrogenase 9 family, member A1	chr1	-3.3	0.0
ANG	angiogenin, ribonuclease, RNase A family, 5	chr14	-392.3	0.0
ANKRD40	ankyrin repeat domain 40	chr11	-3.3	0.0
ANKRD44	ankyrin repeat domain 44	chr1	-3.8	0.0
ANXA2	annexin A2	chr9	-12.2	0.0
APH1A	anterior pharynx defective 1 homolog A (C. elegans)	chr3	-4.3	0.0
ARID1B	AT rich interactive domain 1B (SWI1-like)	chr17	-3.5	0.0
ASGR1	asialoglycoprotein receptor 1	chr11	-9.1	0.0
ASGR2	asialoglycoprotein receptor 2	chr11	-30.2	0.0
ATAD1	ATPase family, AAA domain containing 1	chr19	-3.8	0.0
ATAD2B	ATPase family, AAA domain containing 2B	chr12	-3.5	0.0
ATF7IP	activating transcription factor 7 interacting protein	chr6	-3.6	0.0
ATM	ataxia telangiectasia mutated	chr9	-3.6	0.0
ATP11B	ATPase, class VI, type 11B	chr3	-3.4	0.0
ATP1A1	ATPase, Na ⁺ /K ⁺ transporting, alpha 1 polypeptide	chr3	-3.5	0.0
BCL9	B-cell CLL/lymphoma 9	chr3	-8.0	0.0
BCL9L	B-cell CLL/lymphoma 9-like	chr9	-4.3	0.0
BIRC6	baculoviral IAP repeat containing 6	chr17	-4.8	0.0
BTBD9	BTB (POZ) domain containing 9	chr17	-3.6	0.0
C1QA	complement component 1, q subcomponent, A chain	chr4	-1542.3	0.0
C2	complement component 2	chr17	-699.4	0.0
CADM1	cell adhesion molecule 1	chr9	-874.0	0.0
CASKIN2	CASK interacting protein 2	chr11	-284.0	0.0
CASP7	caspase 7, apoptosis-related cysteine peptidase	chr19	-3.8	0.0
CCDC109B	coiled-coil domain containing 109B	chr3	-4.2	0.0
CCDC134	coiled-coil domain containing 134	chr15	-8.5	0.0
CCDC39	coiled-coil domain containing 39	chr3	-560.9	0.0
CCDC71	coiled-coil domain containing 71	chr9	-6.0	0.0
CCL3	chemokine (C-C motif) ligand 3	chr11	-35.2	0.0
CCL4	chemokine (C-C motif) ligand 4	chr11	-24.8	0.0
CCR5	chemokine (C-C motif) receptor 5 (gene/pseudogene)	chr9	-146.8	0.0
CCR7	chemokine (C-C motif) receptor 7	chr11	-5.6	0.0
CD14	CD14 molecule	chr18	-14.3	0.0
CD163	CD163 molecule	chr6	-6045.7	0.0
CD300E	CD300e molecule	chr11	-63.5	0.0
CD4	CD4 molecule	chr6	-10.4	0.0
CD48	CD48 molecule	chr1	-5.2	0.0
CD86	CD86 molecule	chr16	-10.3	0.0
CDC14A	cell division cycle 14A	chr3	-3.4	0.0
CDC42SE2	CDC42 small effector 2	chr11	-4.8	0.0
CHN2	chimerin 2	chr6	-7.6	0.0
CLIC5	chloride intracellular channel 5	chr17	-1048.6	0.0
CLIP4	CAP-GLY domain containing linker protein family, member 4	chr17	-16.4	0.0
CMTM3	CKLF-like MARVEL transmembrane domain containing 3	chr8	-3.2	0.0
COG1	component of oligomeric golgi complex 1	chr11	-3.8	0.0
CPVL	carboxypeptidase, vitellogenic-like	chr6	-21.0	0.0
CSF1R	colony stimulating factor 1 receptor	chr18	-18.1	0.0
CSNK1E	casein kinase 1, epsilon	chr15	-4.1	0.0
CST3	cystatin C	chr2	-9.5	0.0
CTSO	cathepsin O	chr3	-6.9	0.0
CTTNBP2NL	CTTNBP2 N-terminal like	chr3	-633.2	0.0

CXCR6	chemokine (C-X-C motif) receptor 6	chr9	-16.7	0.0
DDX28	DEAD (Asp-Glu-Ala-Asp) box polypeptide 28	chr8	-4.3	0.0
DNAJC7	DnaJ (Hsp40) homolog, subfamily C, member 7	chr11	-4.8	0.0
DOCK10	dedicator of cytokinesis 10	chr1	-4.1	0.0
DOCK8	dedicator of cytokinesis 8	chr19	-4.8	0.0
DUSP11	dual specificity phosphatase 11 (RNA/RNP complex 1-interacting)	chr6	-4.2	0.0
EDEM1	ER degradation enhancer, mannosidase alpha-like 1	chr6	-3.3	0.0
EHD4	EH-domain containing 4	chr2	-6.3	0.0
ELF1	E74-like factor 1 (ets domain transcription factor)	chr14	-6.0	0.0
ELMO1	engulfment and cell motility 1	chr13	-4.2	0.0
EPSTI1	epithelial stromal interaction 1 (breast)	chr14	-5.2	0.0
ERCC5	excision repair cross-complementing rodent repair deficiency, complementation group 5	chr1	-1295.4	0.0
EVI2A	ecotropic viral integration site 2A	chr11	-6.6	0.0
FKBP5	FK506 binding protein 5	chr17	-4.4	0.0
FLNB	filamin B, beta	chr14	-3.9	0.0
FNBP1	formin binding protein 1	chr2	-4.4	0.0
FYN	FYN oncogene related to SRC, FGR, YES	chr10	-4.5	0.0
GEMIN5	gem (nuclear organelle) associated protein 5	chr11	-5.9	0.0
GIMAP6	GTPase, IMAP family member 6	chr6	-18.5	0.0
GIMAP7	GTPase, IMAP family member 7	chr6	-18.1	0.0
GLUD1	glutamate dehydrogenase 1	chr14	-3.4	0.0
GNA11	guanine nucleotide binding protein (G protein), alpha 11 (Gq class)	chr10	-11.9	0.0
GORASP2	golgi reassembly stacking protein 2, 55kDa	chr2	-3.5	0.0
GPR137B	G protein-coupled receptor 137B	chr13	-5.0	0.0
GPR19	G protein-coupled receptor 19	chr6	-290.0	0.0
GPR68	G protein-coupled receptor 68	chr12	-6.5	0.0
HEG1	HEG homolog 1 (zebrafish)	chr16	-4.6	0.0
HERPUD2	HERPUD family member 2	chr9	-3.3	0.0
HIVEP2	human immunodeficiency virus type I enhancer binding protein 2	chr10	-6.7	0.0
HMOX1	heme oxygenase (decycling) 1	chr8	-15.1	0.0
HPS6	Hermansky-Pudlak syndrome 6	chr19	-5.4	0.0
HS3ST3B1	heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1	chr11	-3589.3	0.0
IL10RA	interleukin 10 receptor, alpha	chr9	-5.0	0.0
IL1B	interleukin 1, beta	chr2	-17.0	0.0
IL7	interleukin 7	chr3	-284.0	0.0
INPP4B	inositol polyphosphate-4-phosphatase, type II, 105kDa	chr8	-11.7	0.0
ITK	IL2-inducible T-cell kinase	chr11	-4.9	0.0
ITPR1	inositol 1,4,5-trisphosphate receptor, type 1	chr6	-3.5	0.0
KCNA3	potassium voltage-gated channel, shaker-related subfamily, member 3	chr3	-9.2	0.0
KCNK3	potassium voltage-gated channel, Shaw-related subfamily, member 3	chr7	-554.9	0.0
KHDRBS1	KH domain containing, RNA binding, signal transduction associated 1	chr4	-3.1	0.0
KIF21A	kinesin family member 21A	chr15	-13.6	0.0
KLHL9	kelch-like family member 9	chr4	-5.5	0.0
KYNU	kynureninase	chr2	-18.8	0.0
LARGE	like-glycosyltransferase	chr8	-17.3	0.0
LEPROTL1	leptin receptor overlapping transcript-like 1	chr8	-5.9	0.0
LNPEP	leucyl/cystinyl aminopeptidase	chr17	-5.0	0.0
LTBR	lymphotoxin beta receptor (TNFR superfamily, member 3)	chr6	-4.7	0.0
LY6E	lymphocyte antigen 6 complex, locus E	chr15	-4.0	0.0
MALT1	mucosa associated lymphoid tissue lymphoma translocation gene 1	chr18	-3.6	0.0
MAN1A2	mannosidase, alpha, class 1A, member 2	chr3	-6.0	0.0
MAN2A1	mannosidase, alpha, class 2A, member 1	chr17	-3.3	0.0
MAN2B2	mannosidase, alpha, class 2B, member 2	chr5	-3.7	0.0
MAPK13	mitogen-activated protein kinase 13	chr17	-3.5	0.0
MARCO	macrophage receptor with collagenous structure	chr1	-34.7	0.0
MDGA1	MAM domain containing glycosylphosphatidylinositol anchor 1	chr17	-11.7	0.0
MED29	mediator complex subunit 29	chr7	-3.5	0.0
MESDC1	mesoderm development candidate 1	chr7	-5.7	0.0
MEX3C	mex-3 homolog C (C. elegans)	chr18	-4.5	0.0

MLLT4	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 4	chr17	-6.8	0.0
MRPL49	mitochondrial ribosomal protein L49	chr19	-3.3	0.0
MS4A7	membrane-spanning 4-domains, subfamily A, member 7	chr19	-6.8	0.0
MTMR11	myotubularin related protein 11	chr3	-37.3	0.0
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	chr15	-3.8	0.0
MYCL1	v-myc myelocytomatosis viral oncogene homolog 1, lung carcinoma derived (avian)	chr4	-4992.1	0.0
NAAA	N-acylethanolamine acid amidase	chr5	-6.4	0.0
NDFIP1	Nedd4 family interacting protein 1	chr18	-3.2	0.0
NEFL	neurofilament, light polypeptide	chr14	-1343.6	0.0
NEU3	sialidase 3 (membrane sialidase)	chr7	-7.6	0.0
NEURL	neuralized homolog (Drosophila)	chr19	-10.5	0.0
NFATC2	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2	chr2	-14.5	0.0
NFXL1	nuclear transcription factor, X-box binding-like 1	chr5	-4.9	0.0
NPAT	nuclear protein, ataxia-telangiectasia locus	chr9	-6.9	0.0
NPC2	Niemann-Pick disease, type C2	chr12	-3.6	0.0
NRCAM	neuronal cell adhesion molecule	chr12	-1759.0	0.0
OLFM1	olfactomedin 1	chr2	-422.4	0.0
OXR1	oxidation resistance 1	chr15	-4.3	0.0
PBRM1	polybromo 1	chr14	-3.8	0.0
PDE4A	phosphodiesterase 4A, cAMP-specific	chr9	-4.6	0.0
PGS1	phosphatidylglycerophosphate synthase 1	chr11	-3.2	0.0
PLEKHA1	pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 1	chr7	-4.5	0.0
PLXNB2	plexin B2	chr15	-4.5	0.0
PLXND1	plexin D1	chr6	-3.3	0.0
PPM1J	protein phosphatase, Mg2+/Mn2+ dependent, 1J	chr3	-12.1	0.0
PPP1R11	protein phosphatase 1, regulatory (inhibitor) subunit 11	chr17	-4.2	0.0
PRDM1	PR domain containing 1, with ZNF domain	chr10	-13.9	0.0
PRRC1	proline-rich coiled-coil 1	chr18	-3.6	0.0
PRSS23	protease, serine, 23	chr7	-13.9	0.0
PTPRC	protein tyrosine phosphatase, receptor type, C	chr1	-5.0	0.0
RAPGEF6	Rap guanine nucleotide exchange factor (GEF) 6	chr11	-7.3	0.0
REST	RE1-silencing transcription factor	chr5	-4.1	0.0
RGMB	RGM domain family, member B	chr17	-5.0	0.0
RNASE6	ribonuclease, RNase A family, k6	chr14	-10.6	0.0
S100A10	S100 calcium binding protein A10	chr3	-4.1	0.0
SACS	spastic ataxia of Charlevoix-Saguenay (sacsin)	chr14	-6.1	0.0
SAMHD1	SAM domain and HD domain 1	chr2	-9.3	0.0
SART3	squamous cell carcinoma antigen recognized by T cells 3	chr5	-3.1	0.0
SCT	secretin	chr7	-296.0	0.0
SGPL1	sphingosine-1-phosphate lyase 1	chr10	-3.9	0.0
SHD	Src homology 2 domain containing transforming protein D	chr17	-374.3	0.0
SIPA1L3	signal-induced proliferation-associated 1 like 3	chr7	-5.0	0.0
SLAMF7	SLAM family member 7	chr1	-5.3	0.0
SLC38A1	solute carrier family 38, member 1	chr15	-4.8	0.0
SLC7A7	solute carrier family 7 (amino acid transporter light chain, y+L system), member 7	chr14	-7.0	0.0
SLFN5	schlafen family member 5	chr11	-13.9	0.0
SMCR8	Smith-Magenis syndrome chromosome region, candidate 8	chr11	-5.3	0.0
SNX30	sorting nexin family member 30	chr4	-5.2	0.0
SP4	Sp4 transcription factor	chr12	-4.3	0.0
SPATA13	spermatogenesis associated 13	chr14	-3.5	0.0
SSR2	signal sequence receptor, beta (translocon-associated protein beta)	chr3	-3.8	0.0
SYS1	SYS1 Golgi-localized integral membrane protein homolog (S. cerevisiae)	chr2	-4.0	0.0
TAGAP	T-cell activation RhoGTPase activating protein	chr17	-4.6	0.0
TAP1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	chr17	-4.2	0.0
TBC1D14	TBC1 domain family, member 14	chr5	-6.7	0.0
TDP1	tyrosyl-DNA phosphodiesterase 1	chr12	-3.9	0.0
TGFB1	transforming growth factor, beta-induced, 68kDa	chr13	-178.4	0.0

TGFBFR2	transforming growth factor, beta receptor II (70/80kDa)	chr9	-7.1	0.0
TIAM1	T-cell lymphoma invasion and metastasis 1	chr16	-3.5	0.0
TICAM1	toll-like receptor adaptor molecule 1	chr17	-7.5	0.0
TLR3	toll-like receptor 3	chr8	-277.9	0.0
TMED10	transmembrane emp24-like trafficking protein 10 (yeast)	chr12	-3.5	0.0
TMEM176A	transmembrane protein 176A	chr6	-4853.6	0.0
TMEM176B	transmembrane protein 176B	chr6	-61.0	0.0
TMEM66	transmembrane protein 66	chr8	-4.9	0.0
TMEM87B	transmembrane protein 87B	chr2	-3.9	0.0
TRERF1	transcriptional regulating factor 1	chr17	-4.2	0.0
TRPS1	trichorhinophalangeal syndrome I	chr15	-5.5	0.0
TXNIP	thioredoxin interacting protein	chr3	-7.3	0.0
USP25	ubiquitin specific peptidase 25	chr16	-3.3	0.0
VEZF1	vascular endothelial zinc finger 1	chr11	-4.4	0.0
VGLL4	vestigial like 4 (Drosophila)	chr6	-4.5	0.0
VPS13C	vacuolar protein sorting 13 homolog C (S. cerevisiae)	chr9	-4.6	0.0
VPS13D	vacuolar protein sorting 13 homolog D (S. cerevisiae)	chr4	-3.8	0.0
XBP1	X-box binding protein 1	chr11	-3.9	0.0
YPEL2	yippee-like 2 (Drosophila)	chr11	-4.0	0.0
ZBTB38	zinc finger and BTB domain containing 38	chr9	-14.5	0.0
ZFP106	zinc finger protein 106 homolog (mouse)	chr2	-3.2	0.0
ZNF710	zinc finger protein 710	chr7	-3.5	0.0

3.5 DISCUSSION

Patient IRF8^{K108E} is the only reported case of recessive *IRF8*-deficiency in humans to date. This was characterized by complete absence of circulating blood dendritic cells and monocytes in an overall myeloproliferative picture, together with striking susceptibility to infection including mycobacterial disease. This unique patient offers the rare occasion to study the role of monocytes and dendritic cells *in vivo*, and to characterize possible pleiotropic effects of their absence on the activity of other cell types of the immune system. Critically, comparative transcript profiling studies in *IRF8*-deficient primary human cells from this patient offer the exceptional opportunity to identify IRF8 transcriptional targets of relevance *in vivo*.

Although the patient was cured by cord blood transplant, we were able to analyze one archival sample of peripheral blood mononuclear cells, cryo-preserved prior to transplant. RNA sequencing and gene profiling studies showed that, as expected, IRF8^{K108E} was associated with depletion of transcripts associated with mononuclear phagocytes while it was enriched for transcripts from the granulocytic lineage, in agreement with the immunological profile. A novel finding was the depletion of gene signatures associated with T lymphocyte activation in the patient, and this for both the CD4⁺ and the CD8⁺ lineages which rather displayed signatures characteristic of naïve T cells. This occurred despite normal numbers of circulating T cells in the patient, and despite intense antigenic exposure taking place during the multiple episodes of infection (bacterial, viral and fungal) in this patient. These results extend *in vitro* studies with cultured T cells from the patient showing poor responsiveness of these cells to microbial products, and to non-specific stimulation (PMA/ionomycin)²³³. The detected deficiency in CD4⁺ and CD8⁺ T cell activation in this patient suggests that T cells reared in the absence of an intact APC compartment are anergic. Such an anergic T cell phenotype was not observed in recently described patients with GATA2 haplo-insufficiency who also develop deficiency of monocytes and DCs as well as progressive loss of B and NK lymphocytes, termed DCML deficiency^{221,249}. Although symptomatic GATA2-deficient patients also show impaired production of IL-12 and IFN- γ in whole blood assays, this may well reflect the absence of monocytes and NK cells rather than any T cell autonomous defect. Furthermore, DCML patients can live well into adulthood before becoming ill²⁵⁰ whereas the K108E patient was hospitalized before the age of 3 months²³³ and thus more strongly resembled infants with SCID who lack T cell function altogether. This therefore highlights the uniqueness and severity of the congenital DC immunodeficiency

associated with mutations in *IRF8*. Interestingly, we observed that the K108E patient had increased expression of genes and markers for mature B lymphocytes, and more specifically plasma cells (**Fig. 2C, 3C-D**). This is in agreement with her hematological profile showing increase in B cells compared to the normal values (**Fig. 1**). In mice, *Irf8* deficiency (*Irf8*^{-/-}) has been shown to result in increased levels of mature marginal zone (MZ) and follicular (FO) B cells in the spleen²⁵¹. Although this remains to be formally demonstrated, it is tempting to speculate that the role of IRF8 in restricting the expansion and maturation of B cells is conserved between mice and humans.

Using comparative RNA sequencing and chromatin immunoprecipitation (ChIP-sequencing), we further characterized the genes that are bound and regulated by IRF8 in human myeloid cells *in vivo*. This list of genes is presented in **Table S1**. These results support the interpretation that IRF8 functions primarily as a transcriptional activator in myeloid cells. This list is validated by the presence of well-known myeloid-specific genes and proteins (CD14, CD163, CD300e), as well as well-known pro-inflammatory molecules (IL1 β , CCL3, CCL4, C1QA) and their receptors (CCR5, CCR7, CSF1R, TGFBR2). The list also contains a number of other genes of unknown function that constitute novel IRF8 targets in human antigen presenting cells. The study of the role of these poorly characterized genes in the ontogeny and function of myeloid cells promises to provide novel insight into the anti-microbial and immune function of these cells.

With respect to the pathological nature of the IRF8^{K108E} mutation itself, we demonstrate a clear effect on IRF8 stability, which was unexpected. The decreased stability of the protein has pleiotropic effects on IRF8 targeting to the nucleus, post-translational modification, and transactivation potential. These results strongly suggest that K108 plays an additional critical role in normal folding of the DBD of IRF8 in presence or in absence of a DNA template, and that its replacement to glutamic acid causes significant mis-folding of the protein and increased proteasomal degradation following ubiquitination and SUMOylation of the misfolded variant. It is also possible that the K108R variant has a more subtle effect on IRF8 structure that may affect access to regulatory enzymes such as Cbl/DUBs (for ubiquitination) and E2I/SEN1P1 (for SUMOylation), thereby displacing the regulatory equilibrium between active IRF8 and inactive SUMOylated/ubiquitinated protein^{240,248}. Finally, studies in cells expressing both the K108E

mutant, and the wild type protein demonstrate that K108E does not behave as a dominant negative, and does not alter targeting of the wild type protein to the nucleus. This is in agreement with the recessive mode of inheritance of the immunodeficiency and granulocytic hyperplasia phenotypes associated with the K108E mutation in this family.

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Preface to chapter 4

A previous study identified a new role for Irf8 as an inhibitor of bone breakdown by osteoclasts in mice. Irf8 was shown to negatively regulate differentiation of mature osteoclasts from precursor cells in the bone marrow⁶. Irf1 is the major binding partner of Irf8 and together they heterodimerize to transcriptionally activate critical genes functioning in similar pathways¹¹⁸. Furthermore, mice deficient for *Irf8* have almost identical phenotypes as mice deficient for *Irf1*¹¹⁸. In the following chapter, we aim to investigate a possible role for Irf1 in regulation of bone metabolism in mice. We test the effect of *Irf1* deficiency on differentiation and function of both bone-resorbing osteoclasts and bone-forming osteoblasts.

Chapter 4

A novel role for interferon regulatory factor 1 (Irf1) in regulation of bone metabolism

4.1 ABSTRACT

Increased risk of bone fractures is observed in patients with chronic inflammatory conditions such as inflammatory bowel disease and rheumatoid arthritis. Members of the Interferon Response Factor family of transcriptional regulators, IRF1 and IRF8, have been identified as genetic risk factors for several chronic inflammatory and autoimmune diseases. We have investigated a potential role for the *Irf1* gene in bone metabolism. Here we report that *Irf1*^{-/-} mutant mice show altered bone morphology in association with altered trabecular bone architecture and increased cortical thickness and cellularity. *Ex vivo* studies on cells derived from bone marrow stimulated with Rank ligand revealed an increase in size and resorptive activity of tartrate resistant acid positive cells from *Irf1*^{-/-} mice compared to wild type control mice. *Irf1* deficiency was also associated with decreased proliferation of bone marrow derived osteoblast precursors *ex vivo*, concomitant with increased mineralization activity compared to control cells. We show that *Irf1* plays a role in bone metabolism and suggest that *Irf1* regulates the maturation and activity of osteoclasts and osteoblasts. The altered bone phenotype of *Irf1*^{-/-} mutants is strikingly similar to that of *Stat1*^{-/-} mice, suggesting that the two interacting proteins play a critical enabling role in the common regulation of these two cell lineages.

4.2 INTRODUCTION

Interferon regulatory factor 1 (IRF1) is a transcriptional activator belonging to the IRF family of transcription factors. This family consists of nine members (IRF1-9) that play critical roles in immune and inflammatory responses and in host defenses against infections, as well as regulating the development and apoptosis of immune cells²⁵². IRF proteins share significant sequence similarity in the amino terminal portions (aa1-115) that contain their defining DNA-binding domain (DBD). IRF1 also has a carboxyl terminal domain that serves as a recruitment module for other transcription factors including other members of the IRF (e.g. IRF8), or ETS (e.g. PU.1) families, to activate or repress gene expression²⁵³.

IRF1 is expressed broadly and is required for the ontogeny and function of the lymphoid and myeloid compartments of the immune system, including T lymphocytes, natural killer (NK) cells and macrophages²⁵³. *Irf1*^{-/-} mice are immuno-deficient, and susceptible to infections. They carry lineage-specific defects in thymocyte development characterized by a marked reduction in CD8⁺ T cells¹⁷⁶, a decrease in NK cell numbers with associated impaired cytolytic activity¹⁶⁷ as well as reduced numbers of CD8α⁺ dendritic cells (DCs) concomitant with a skewed differentiation of CD11b⁺ cells towards plasmacytoid DCs (pDCs)¹⁶⁵. IRF1 is an important regulator of the myeloid and lymphoid-mediated inflammatory response. Human *IRF1* has been detected as a genetic risk factor for IBD¹⁸⁴ and has also been associated with the incidence and severity of synovial inflammation in type II collagen-induced arthritis¹⁷¹. IRF1 mRNA and protein expression are regulated in a cell cycle dependent fashion, and *Irf1* deficient mouse embryo fibroblasts do not undergo DNA damage-induced apoptosis²⁵⁴. IRF1 also acts as a negative growth regulator, and loss of IRF1 function has been associated with transformation in several cancers, including pre-leukemic myelo-dysplastic syndromes²⁵⁵. IRF8 is another member of the IRF family of transcription factors that is expressed exclusively in cells of the immune system including B and T lymphocytes, macrophages and DCs²⁵⁶. Like *Irf1*^{-/-} mice, *Irf8*^{-/-} mice lack both CD8⁺ DCs and pDCs, have impaired T cell function and defective IL-12-dependent Th1 polarization of the early immune response^{257,258}. In humans, loss of function mutations in *IRF8* cause a severe form of DC immunodeficiency²³³. At the molecular level, IRF8 and IRF1 synergize in the IFNγ-dependent activation of intrinsic macrophage anti-microbial defenses (iNOS, gp91^{phox}, p67^{phox}, caspase-1, Cox2), of inflammatory cytokines activating early immune response (IL-12p40, IL-18, RANTES, TNFα) and of genes affecting maturation of myeloid

(DCs) and lymphoid (NK, CD8⁺ T cells) cells^{129,133,235}. Several of these direct IRF8/IRF1 downstream target genes play critical roles in bone metabolism by promoting (Cox-2, TNF α) or inhibiting (IL-12, IL-18, RANTES) bone resorption^{200,201,259-262}.

Recent genome wide association studies have pointed to a role for both *IRF1* and *IRF8* in genetically complex and heterogeneous human inflammatory diseases including ulcerative colitis and Crohn's disease^{179,184,185,239}. Increased risk of bone fractures occurs in patients with chronic inflammatory conditions such as IBD, in which IRF1 and IRF8 have been identified as genetic risks^{263,264}. A role for *Irf8* in bone metabolism was recently demonstrated when *Irf8*^{-/-} mice displayed bone defects due to enhanced osteoclastogenesis, resulting in increased bone resorption and reduced trabecular bone density⁶. Considering the critical and complementary roles played by *Irf8* and *Irf1* in myeloid lineage cell maturation and in the etiology of chronic inflammatory diseases, we investigated a possible role of *Irf1* in bone metabolism. In this study, we show that *Irf1*^{-/-} mice have an abnormal bone phenotype due to altered development and function of both bone resorbing osteoclasts and bone forming osteoblasts, thereby uncoupling their tightly regulated interaction. Our results suggest a novel role for *Irf1* as a regulator of bone metabolism in mice.

4.3 MATERIALS AND METHODS

Western Blotting

Irf1 protein was evaluated in cultures of osteoclasts that were derived from bone marrow cells of 8 week old wild type mice as described above and in MC3T3 osteoblast-like cells. Cells were washed with cold phosphate-buffered saline (PBS) and lysed for 30min on ice in 0.5ml of lysis buffer (50mM Tris-HCl pH 7.5, 150mM NaCl, 1% Triton X-100, 0.1% SDS) supplemented with protease inhibitors. Cell lysates were clarified by centrifugation (15min, 4°C, 13, 000 rpm). Whole cell extracts (50µg) were separated on 12% SDS-polyacrylamide gel and transferred by electro-blotting onto 0.45µm Protran BA 85 membrane (GE Healthcare). Equal loading of extract and transfer of protein were verified by staining with Ponceau S red (Sigma-Aldrich). Blots were incubated with rabbit anti-IRF1 M-20 antibody (1:250; Santa Cruz) in TBST (10mM Tris-HCl pH 8, 150mMNaCl, 0.05% Tween 20) plus 5% skim milk (16h at 4°C), followed by washing and incubation with an anti-rabbit secondary antibody conjugated to HRP (1:20 000, GE Healthcare). Chemiluminescence was used for the detection of immune complexes on the immunoblot (SuperSignal West Pico, Thermo Scientific).

Analysis of bone phenotypes

Eight-week-old male *Irf1*^{-/-} mutant mice and wild type (WT) C57BL/6J controls were used for all experiments, except when otherwise indicated. *Irf1*^{-/-} mice were purchased as breeding pairs from the Jackson Laboratory (Bar Harbor, ME). The mutant stock name is B6.129S2-Irf1^{tm1Mak}/J. Male mice were used for all experiments to avoid age-related variability that is greater in females than in males for the C57BL/6J strain²⁶⁵. Femurs were dissected at necropsy and fixed in 70% ethanol; they were then subjected to radiologic imaging using a Faxitron® MX-20 instrument at a voltage of 21kV and current of 550µA. Micro-computed tomographic imaging (Micro-CT) was used to investigate trabecular bone micro-architecture and was performed using a Skyskan 1072 instrument. Bone mineral density of femurs was performed using the GE Lunar Piximus instrument. All the above imaging was performed at the Centre for Bone and Periodontal Research at McGill University. Qualitative and quantitative Micro-CT analysis of cortical and trabecular bone was performed at the Bone Engineering Labs at the Research Institute-McGill University Health Centre using a Skyscan 1172 instrument (Bruker Corp.). Analysis of cortical bone was performed on a 1.1 mm long

region of the diaphysis extending from the distal end of third femoral trochanter. Analysis of trabecular bone was performed separately on growth plate proximal and growth plate distal regions each measuring 1.65mm in length. Radiology performed on 7 mice per strain (n=7) and significance determined using a student's t-test.

Histology and Histomorphometry

Femurs from 8-week or 4-month old male mice were isolated and fixed overnight in 4% paraformaldehyde, then rinsed three times in phosphate-buffered saline (PBS). Tissues were embedded in poly-methyl methacrylate (PMMA) and 4-6µm sections were prepared. Tissue section were then stained with Hematoxylin and Eosin (H&E) for general morphology, with von Kossa/Toluidine blue to distinguish mineralized from soft tissue, for tartrate resistant acid phosphatase (TRAP) activity to identify osteoclasts, and alkaline phosphatase (ALP) activity for hypertrophic chondrocytes and osteoblasts. Images were captured using a Leica DMR microscope and histomorphometric data was obtained using Bioquant Nova Prime image analysis software and ImageJ software. Histology and Histomorphometry performed on 5 mice per strain (n=5).

Calcein double labeling

Eight week-old male mice were given an intra-peritoneal injection of 100µl/20g of calcein solution (0.25% calcein-AM and 2% NaHCO₃ dissolved in 0.15M NaCl) at day 10 and day 3 prior to euthanasia on day 0. Femurs were harvested, fixed in 70% ethanol and embedded in poly-methyl methacrylate (PMMA). Sections were cut at 10 microns and examined by fluorescence microscopy. Mineral apposition rate (MAR) and bone formation rate (BFR) were calculated using ImageJ where mineralizing surface (MS) = $dLS + sLS / 2$; dLS is the double labeling surface perimeter and sLS is the single labeling perimeter. MAR = distance between the 2 labels/time (7days) and bone formation rate (BFR) = MAR x (MS/BS); BS is the total bone surface. Calcein double labelling was performed on 5 mice per strain (n=5).

MSC proliferation and osteoblast differentiation

Whole bone marrow was harvested from 8-week-old male mice and adherent mesenchymal stromal cells (MSC) isolated as described previously²⁶⁶. The soft tissue and the knee ends of the femora and tibiae were removed under aseptic conditions before placing the bones in Eppendorf tubes and centrifuging at low speed to dislodge the marrow. For proliferation studies, cells were seeded at a density of 10,000 cells /cm², cultured in DMEM and the cells trypsinized and counted with a haemocytometer on days 3, 6 and 9. For differentiation studies the cells were seeded in individual 3cm tissue culture dishes at a density of 50,000 cells /cm². Osteoblast differentiation was initiated by cultivation in osteogenic DMEM medium (Wisent) supplemented with 10% FBS, 50mg/ml ascorbic acid and 10mM β -glycerophosphate for 10 and 15 days, changing the medium every 3 days. Cultures were terminated by fixation in 4% paraformaldehyde and rinsing with PBS. Alkaline phosphatase activity was detected by immersing the dishes in ALP reaction buffer (0.6 mg/ml Fast Red Violet LB salt; Sigma-Aldrich), 0.1 M Tris-HCl pH 8.3, 0.1 mg/ml naphthol AS-MX phosphate disodium salt and 0.4% N,N-dimethylformamide for 15 minutes at 20°C. Dishes were then stained with 1% silver nitrate solution (von Kossa stain) under ultraviolet light for 20 minutes to identify mineral deposits, washed 3 times with dH₂O and images acquired using a light microscope. MSC proliferation and differentiation experiments were performed on 3 mice per strain (n=3). Significance was determined using a student's t-test.

Reverse transcription and quantitative real-time PCR

Total RNA was isolated from cells cultured in osteogenic medium as described above using commercial Trizol reagent (Invitrogen). Aliquots of 1 μ g total RNA were used to generate cDNA using reverse transcriptase (MMLV-RT; Invitrogen) and priming with a combination of oligodT and random hexamers. For quantitative real-time PCR (RT-PCR), all reactions were carried out using PerfeCTa® SYBR® Green Supermix, ROX (Quanta BioSciences Inc.) with a melting temperature of 95°C for 15 seconds, an annealing temperature of 60°C for 15 seconds and an extension temperature of 72°C for 30 seconds (for 40–50 cycles). The following primer pairs were used for lineage-specific transcripts *Ocn*: 5'-GCCTTCATGTCCAAGCAGGA-3' and 5'-GCGCCGGAGTCTGTTCACATA-3', *Opn*: 5'-GATGATGATGACGATGGAGACC-3' and 5'-CGACTGTAGGGACGATTGGAG-3', *Mgp*:

5'-CCTGTGCTACGAATCTCACGAA-3' and 5'-TCGCAGGCCTCTCTGTTGAT-3', *Rankl*: 5'-CACAGCGCTTCTCAGGAGCT-3' and 5'-CATCCAACCATGAGCCTTCC-3'. The level of mRNA expression of each target gene was normalized using *Gapdh* as an internal house keeping control gene. RNA expression performed on 3 mice per strain (n=3).

In vitro osteoclast differentiation

Whole bone marrow harvested from the femurs of 8-week-old male *Irf1*^{-/-} mutant and WT control mice was plated in tissue culture dishes containing α -MEM medium (Wisent) supplemented with 10% heat inactivated fetal bovine serum (FBS Wisent) and 10% L-cells conditioned medium (LCCM)²⁶⁷, and cultured for 5 days with one change of medium at day 2. The immature bone marrow derived macrophages (BMDMs) obtained were then used to initiate osteoclasts cultures. Briefly, plastic-adherent BMDMs were harvested by scraping, and seeded in 6-well tissue culture dishes at a density of 2.5×10^4 cells/well, and cultured in α -MEM supplemented with 10%FBS, 5% LCCM and 50-100ng/ml RANKL (Peprotech) for 6 days, changing medium every 2 days, until multinucleated cells(>3 nuclei)appeared. TRAP staining was performed using the acid phosphatase leukocyte kit (Sigma-Aldrich) according to the manufacturer's instructions and the number of TRAP-positive cells was recorded. Osteoclast differentiation was performed using 3 mice per strain (n=3). Significance was determined using a student's t-test.

Resorption pit assay

BMDMs were produced as described above, and seeded in substrate-coated six-well tissue culture plates (Osteo Assay surface plates; Corning) at a density of 2×10^4 cells/well and incubated for 6 days in α -MEM medium containing 10%FBS, 5% LCCM and 50-100ng/ml RANKL to induce osteoclast differentiation, with medium changes every 2 days. After 6 days, 10% bleach was added to the OsteoAssay surface plates for 5 minutes at 20°C to terminate the assay, followed by extensive washing with distilled water. Resorption pits were identified and counted using a microscope and the relative resorption area calculated using ImageJ software. Resorption pit assay was performed using 3 mice per strain (n=3). Significance was determined using a student's t-test.

Three Point Bending Assay

Biomechanical testing was performed on femurs of 8 week old male *Irf1*^{-/-} mice and WT littermates at the Centre for Bone and Periodontal Research of McGill University (Montreal, Canada) using a three-point bending test on a Mach-1TM Micromechanical Systems device. Failure loads were analyzed using the Mach-1TM Motion and Analysis software (version 3.0.2, Bio SyntechCanada). A load-displacement curve was generated using this software to measure biomechanical parameters including stiffness (N/mm), ultimate force (N), ultimate displacement (μm), and work to failure (N*mm). Biomechanical testing was performed on 5 mice per strain (n=5). Significance was determined using a student's t-test.

4.4 RESULTS

To investigate a possible role of *Irf1* in bone metabolism, we first investigated *Irf1* protein expression in bone cells. Immunoblotting studies using the MC3T3 osteoblast-like cell line and primary osteoclasts derived from bone marrow *ex vivo* identified *Irf1* protein expression in both osteoblast and osteoclast cell lineages (**Fig. S1**). A possible role of *Irf1* in bone metabolism in general, and in bone cell function in particular, was then investigated by comparative analyses of tissues and cells from normal mice and from mice carrying a null mutation at *Irf1* (*Irf1*^{-/-}).

***Irf1* deficiency results in altered bone morphology and mineral content**

Radiologic imaging (**Fig. 1**) was first used to screen for differences in the appendicular skeletons of WT and *Irf1*^{-/-} mutant mice. High resolution x-rays (**Fig. 1A**) revealed shorter femurs with increased distal radiolucency in the *Irf1*^{-/-} mice. Bone mineral density (**Fig. 1B**) was anomalously increased in the mutant compared with WT mice. Micro CT analyses (**Fig. 1C, D**) confirmed a reduction in trabecular bone in the proximal (removed from the growth plate), but not distal (adjacent to the growth plate) metaphysis (M) of *Irf1*^{-/-} mice and increased cortical bone width in the diaphysis (D). Quantitative micro CT analyses (**Table 1**) of bone structure and composition was performed separately on trabecular bone adjacent to, and removed from, the growth plate and on cortical bone. There were no significant differences in quantitative data in trabecular bone adjacent to the growth plate except for an increase in the number of closed pores, which represent spaces occupied by marrow, in *Irf1*^{-/-} compared to WT mice. In contrast, the total amount of bone (BV/TV) and the number of trabeculae (Tb. N) were reduced while trabecular thickness (Tb. Th) was increased, and the spaces between trabeculae increased (Tb. Sp) in the mutant compared with WT mice. The total volume (TV) of cortical bone was increased but the surface (BS/TV) decreased in the mutant compared with wild type mice, confirming the apparent increase in cortical width seen in the 2D micro CT sections (**Fig. 1**). The number and volume of closed pores, which represent osteocytes or vascular channels in cortical bone, were also significantly increased in the mutant mice. The altered structure model index (SMI) values in mutant mice (**Table 1**) are indicative of osteopenia in trabecular bone and of increased connectivity in cortical bone.

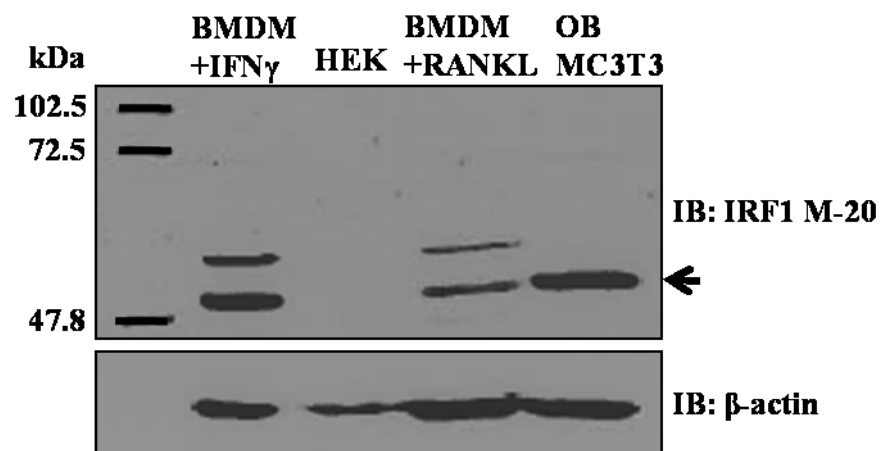


Figure S1. Detection of Irf1 expression in bone cells. Total cell lysates of osteoclasts derived from bone marrow (BM) cells of B6 mice and MC3T3 cells were analyzed by SDS-PAGE and immunoblotting with anti-IRF1 M-20 antibody. Irf1 expression was compared to bone marrow derived macrophages (BMDMs) stimulated with Interferon- γ (IFN- γ) which was used as a positive control and HEK 293T cells used as a negative control.

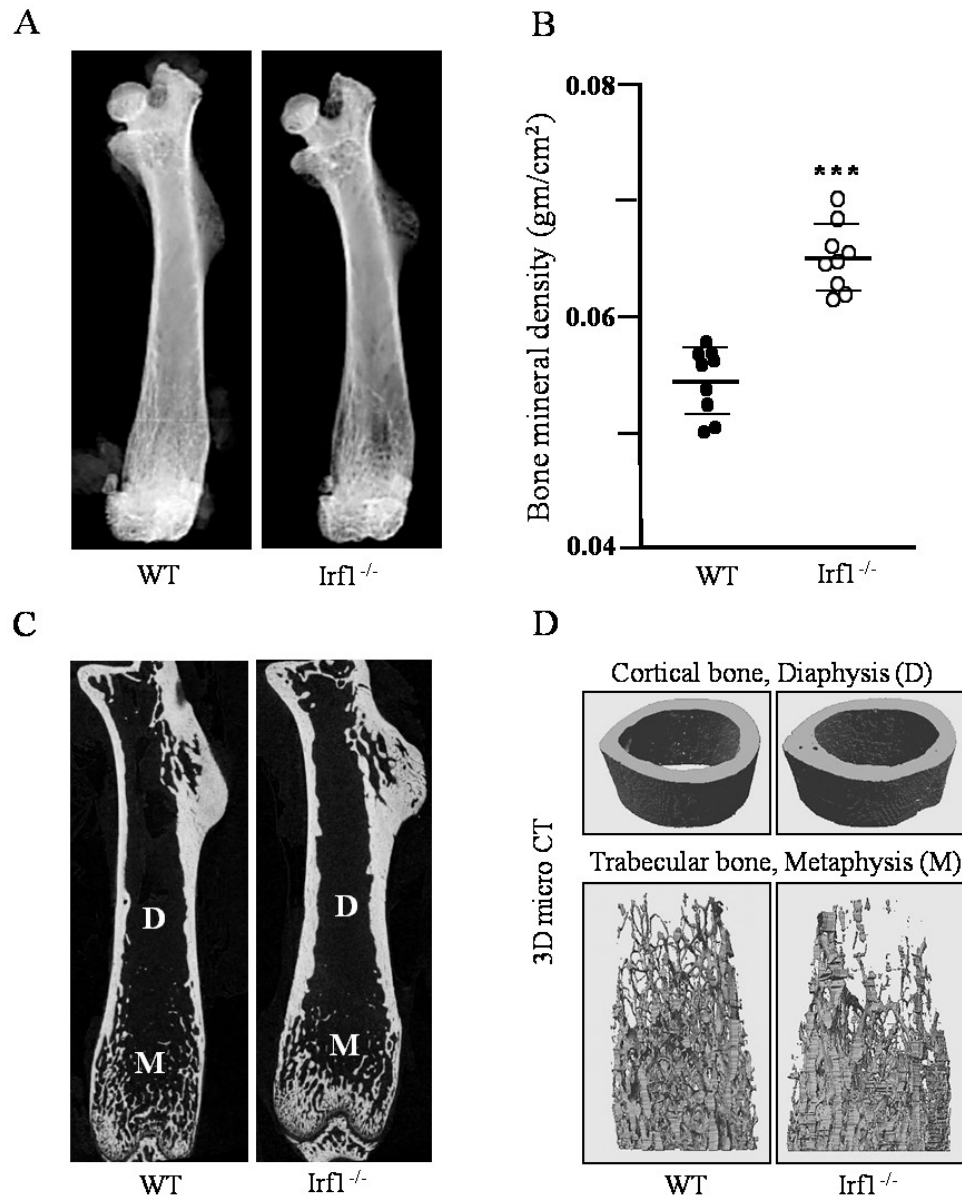


Figure 1. Radiologic analysis of femurs from control and *Irfl*^{-/-} mice. High resolution X-ray (A), bone mineral density (B) 2D (C) and 3D (D) micro CT from representative 8 week old wild type (B6) and mutant (*Irfl*^{-/-}) are shown. Panel B *** p<0.001.

Table 1: Quantitative micro CT

Parameter	Units	Trabecular bone adjacent to GP			Trabecular bone away from GP			Cortical bone in diaphysis		
		WT	<i>Irf1</i> ^{-/-}	<i>p</i>	WT	<i>Irf1</i> ^{-/-}	<i>p</i>	WT	<i>Irf1</i> ^{-/-}	<i>p</i>
TV		4.31	4.43	NS	2.97	2.74	NS	1.10	1.20	0.009
BV/TV	%	16.1	16.2	NS	5.75	3.41	0.008	98.9	99.0	NS
BS/TV	mm ⁻¹	11.5	11.2	NS	4.83	2.89	0.001	13.1	11.9	0.002
Tb.Sp	mm ⁻¹	0.22	0.25	NS	0.34	0.61	0.008	-	-	-
Tb.N	mm ⁻¹	2.63	2.55	NS	0.93	0.50	0.004	-	-	-
Po.N (cl)		23.5	76.5	0.001	2.00	4.75	NS	150	429	0.002
SMI				NS	2.68	3.46	0.025	0.16	-1.52	0.002

TV total volume; BV/TV bone volume/total volume; BS/TV bone surface/total volume; Tb.Sp trabecular separation; Tb.N trabecular number; Po.N (cl) number of closed pores; SMI structure model index.

Histological evidence of reduced trabecular and increased cortical bone in *Irf1*^{-/-} mice

The long bones of immature two month old mice continue to grow in length and in width. Thick sections (10 microns) of un-decalcified femurs were examined under fluorescence microscopy, to evaluate calcein labelling and stained with von Kossa and toluidine blue to assess bone micro-structure (**Fig. 2**). In von Kossa stained sections harvested from the mid-diaphysis the distal femurs were longer and cortical bone thinner in representative WT (**Fig. 2A**) compared with *Irf1*^{-/-} mice (**Fig. 2F**). Calcein (green) was deposited at the endosteal and periosteal surfaces of cortical bone with little apparent difference between WT (**Fig. 2B1-E1**) and *Irf1*^{-/-} (**Fig. 2G1-J1**) femurs. The orderly deposition of calcein along trabeculae arising from the growth plate in WT mice (**Fig. 2D1-E1**) was reflected in the well-defined structures aligned with the longitudinal axis of the bone (**Fig. 2D, E**). In contrast, von Kossa stained sections (**Fig. 2I, J**) and calcein labelled (**Fig. 2I1-J1**) sub-chondral bone in *Irf1*^{-/-} mice was both disorganised and diminished. Adjacent thin (five microns) sections stained with H&E revealed hypercellular bone marrow in some of the mutant mice (**Fig. 3D inset**), decreased alkaline phosphatase staining in *Irf1*^{-/-} mice (**Fig. 3B, E**) and no difference between WT and *Irf1*^{-/-} mice in TRAP activity (**Fig. 3C, F arrows**) in osteoclasts. Bone histomorphometric measurements confirmed a decrease in osteoblast surface in *Irf1*^{-/-}, and no difference in osteoclast number compared to WT (**Fig. 3G, H**).

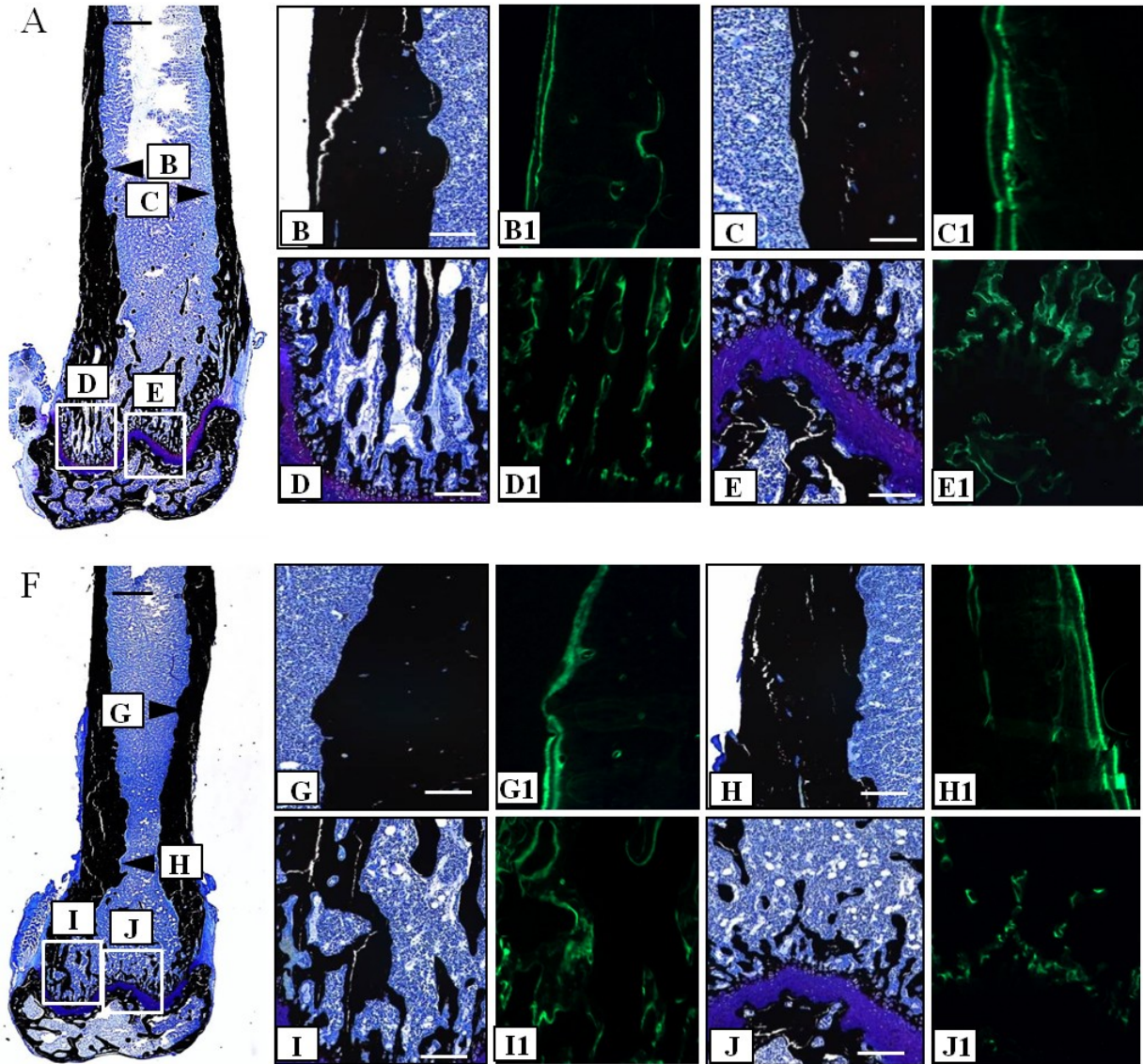


Figure 2. Histological analysis of bone apposition in control and *Irfl*^{-/-} mice. Representative B6 (A-E) and *Irfl*^{-/-} (F-J) bone was sectioned at 10 microns and imaged with a fluorescence microscope before staining with Von Kossa/Toluidine blue and re-imaging. Boxed areas (A, F) identify the regions shown at higher magnification (B, C, G, H) with the corresponding fluorescence images of calcein labelling. Arrowheads indicate the regions of cortical bone shown at higher magnification (D,E,I,J) with the corresponding fluorescence images. Scale bars represent 500 microns (A,F) and 200 microns (B-E, G-J).

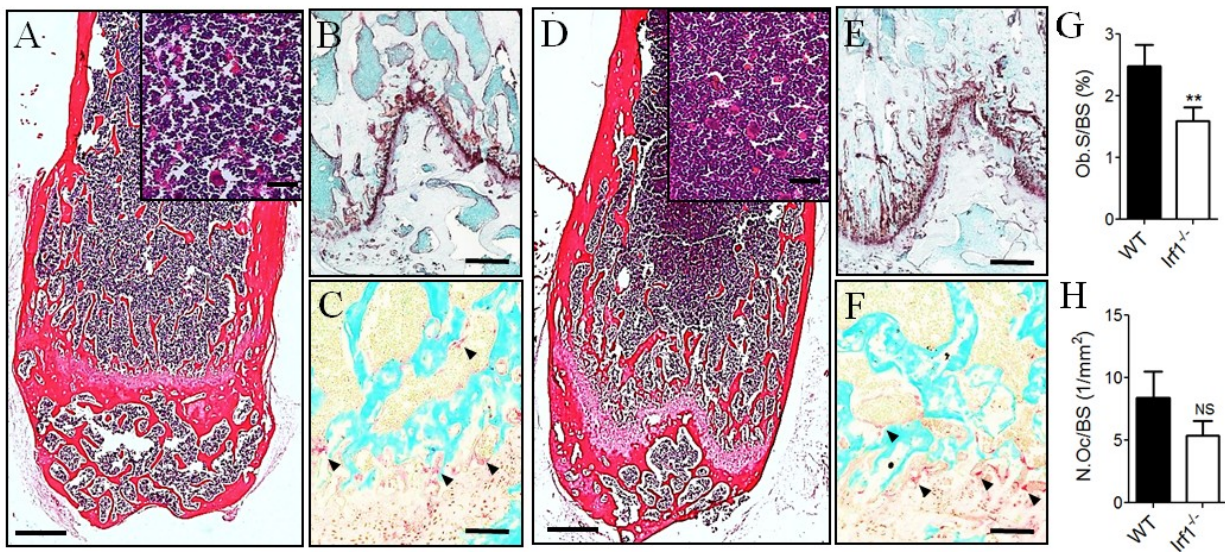


Figure 3. Histochemical staining of femurs from control and *Irfl*^{-/-} mice. Five micron sections of decalcified bone from representative B6 (A) and *Irfl*^{-/-} (D) mice were stained with hematoxylin and eosin (A, D) for bone marrow (inset). Five micron sections of un-decalcified bone were stained for alkaline phosphatase activity (brown) in hypertrophic chondrocytes and osteoblasts (B, E) or for tartrate resistant acid phosphatase activity (arrowheads) in osteoclasts (C, F). Scale bars represent 500 microns (A, D) and 50 microns (inset) or 200 microns (B, C, E, F). Bone histomorphometric measurements of osteoblast surface per bone surface (Ob. S/BS) (G) and osteoclast number per bone surface (N. Oc/BS) (H).

Irf1* deficiency results in increased MSC proliferation and differentiation *ex vivo

In order to uncover the cellular cause of the altered bone phenotype, we examined the effect of loss of function of *Irf1* on the proliferation of MSCs and their differentiation into mature osteoblasts *ex vivo*, using alkaline phosphatase activity (**Fig. 4, pink**) as an early marker and von Kossa staining as an index of mineral deposition at a later stage (**Fig. 4, black**). MSCs isolated from *Irf1*^{-/-} mutant mouse bone marrow also proliferated more slowly than those isolated from bone marrow of WT mice (**Fig. 4A**). Interestingly however, MSCs from *Irf1*^{-/-} mutant mice differentiated more rapidly and deposited significantly more mineral (black) than cells harvested from WT mice cultured under the same conditions (**Fig. 4 B, C**). Total cellular RNA isolated from the cultures on Day 6 after induction of differentiation was used to monitor expression of osteoblast lineage markers. Consistent with the more rapid differentiation and increased mineral deposition seen in the *Irf1*^{-/-} cultures, significantly higher levels of expression of mRNAs encoding Osteocalcin (*Ocn*) and Osteopontin (*Opn*), and lower levels of Matrix Gla protein (*Mgp*), were detected in *Irf1*^{-/-} compared with WT cells (**Fig. 5D**). *Irf1*^{-/-} cells also showed a 6 fold increase in expression of the mRNA for the osteoclastogenesis inducer Rank ligand (*Rankl*), when compared to WT cells, suggesting a stronger osteoblast-directed signal for bone destruction by osteoclasts in *Irf1*^{-/-} mice.

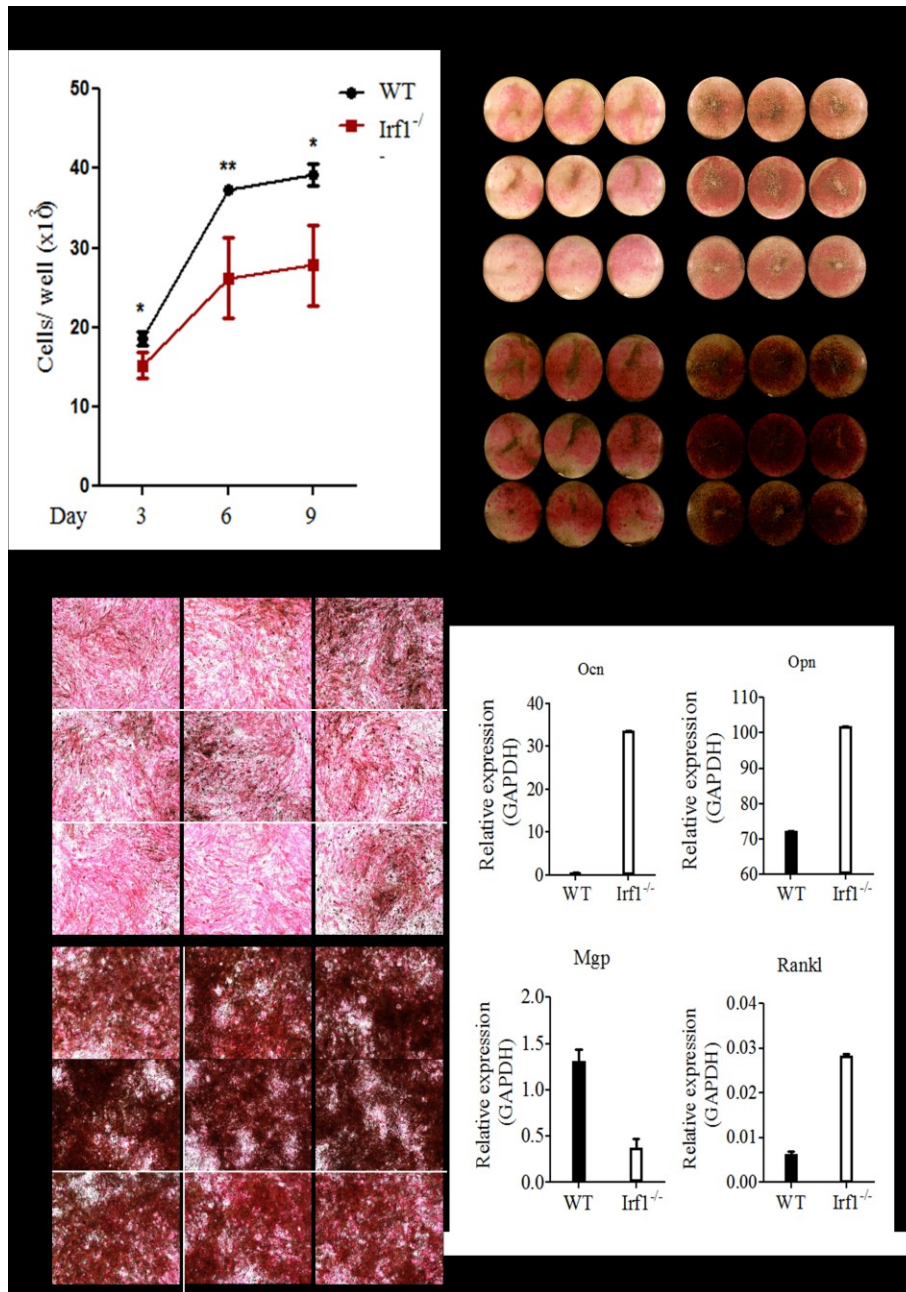


Figure 4. Altered osteoblast function in *Irfl*^{-/-} mice. (A) MSCs isolated from 8-week-old male mice were maintained in medium and cells were counted at days 3, 6 and 9. Results represented as means $\pm$ SD, n=4. ** P < 0.05. (B) Bone marrow stromal cells (MSCs) were isolated from 8-week-old male *Irfl*^{-/-} mice and WT controls and maintained in osteoblast differentiation medium consisting of ascorbic acid and β -glycerophosphate for 10 or 15 days. Cultures were fixed in 4% paraformaldehyde and stained *in situ* for ALP activity and with silver nitrate (von Kossa) to detect mineralized nodules. (C) Microscopic images of mineralized nodules (3 independent experiments; n = 3 mice/group). (D) Quantitative real-time PCR analysis of mRNA expression of Osteocalcin (*Ocn*), Osteopontin (*Opn*), Matrix gla protein (*Mgp*), Receptor activator of NF κ B ligand (*Rankl*) in cultured osteoblasts (at day 6). Expression of test genes is relative to *Gapdh* which was used as an internal control (mean of 3 experiments $\pm$ SD).

Irf1* deficiency results in enhanced osteoclast activity *ex vivo

We then examined the effect of *Irf1* loss of function on osteoclast formation and osteoclast function *ex vivo* (**Fig. 5**). Primary bone marrow derived macrophages were cultured for 7 days in the presence of RANK ligand (tested at 50 and 100ng/mL) to induce osteoclast formation *ex vivo*. These experiments did not reveal an effect of loss of *Irf1* on the total number of TRAP-positive multinucleated cells formed under these conditions, although the cells appeared larger in size than those from WT mice (**Fig. 5A, B**). The activity of the WT and *Irf1* mutant osteoclasts was assessed by their capacity to form resorption pits on a synthetic calcium phosphate bone surface. TRAP positive multi-nucleated cells derived from *Irf1*^{-/-} mouse marrow formed significantly more pits with a greater area than the equivalent cells derived from WT mouse marrow. Similar results were seen with both concentrations of RANK ligand tested (**Fig. 5 C, D**). These results suggested *Irf1* regulates osteoclast maturation and/or activity, which could impact on bone metabolism *in vivo*. In agreement with these findings, we detected higher serum levels of calcium and phosphorus in *Irf1* mutants than controls, however no differences in serum TRAP or osteocalcin (**Fig. S2 A-D**).

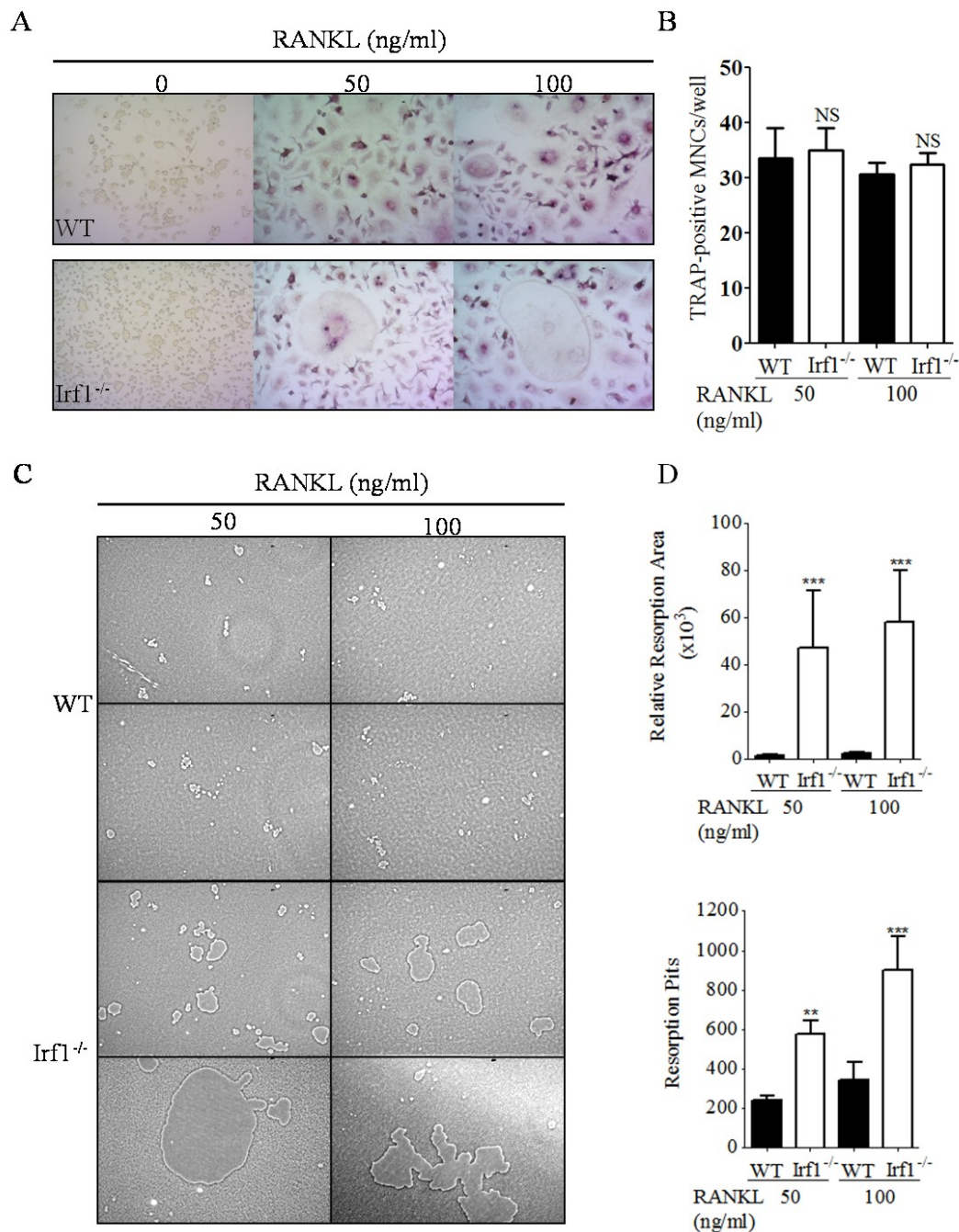


Figure 5. Enhanced osteoclast activity and bone resorption in *Irf1*^{-/-} mice. (A) Bone marrow derived macrophages (BMMs) from 8-week-old *Irf1*^{-/-} mice and WT controls were stimulated with RANKL in the presence of M-CSF to produce osteoclasts. After 7 days, TRAP staining was performed. TRAP-positive cells appear as large, multinucleated cells (MNCs). (B) The number of TRAP-positive MNCs containing at least 3 nuclei were quantitated. (C) BMMs were plated on OsteoAssay plates coated with inorganic synthetic followed by stimulation with RANKL for 7 days to induce formation of resorption pits. (D) The number of resorption pits, and the total relative resorption area and were counted using Image J software. Results represented as means from 9 independent experiments ($\pm$ SD). *** P <0.0001, ** P <0.001. NS, no statistical significance.

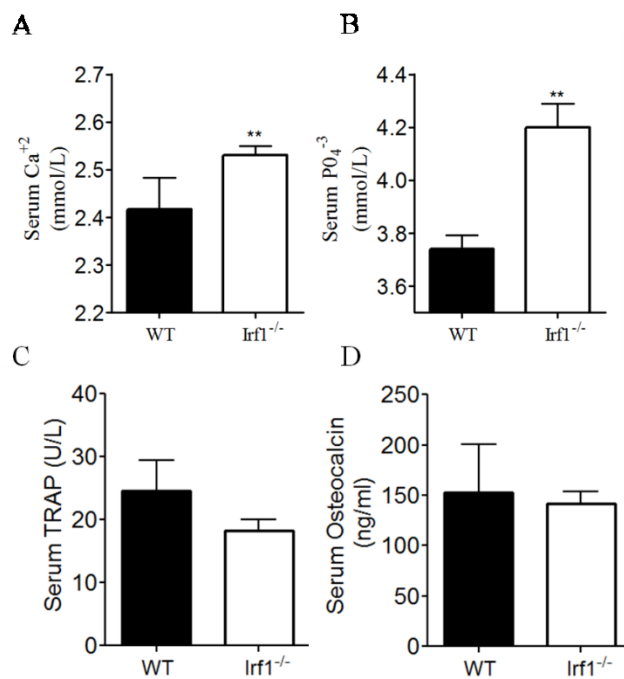


Figure S2. Serum biochemistry. Serum from *Irfl*^{-/-} mice and WT littermates was analyzed for calcium, phosphorous, TRAP and Osteocalcin levels.

***Irf1* deficiency leads to stiffer long bones**

The increase in trabecular and cortical thickness, enhanced osteoblast differentiation and higher mineralization led us to examine the strength of the long bones from age matched *Irf1*^{-/-} mice and WT littermates (**Fig. 6**). Biomechanical testing by a 3 point bending assay revealed that femurs from *Irf1*^{-/-} mice were stiffer (K) than those of WT mice, with a larger ultimate force (N) and more work to failure (U) (**Fig. 6 A-D**). These results suggest that *Irf1* deficiency leads to stronger bones, due to the enhanced osteoblast differentiation and mineral deposition.

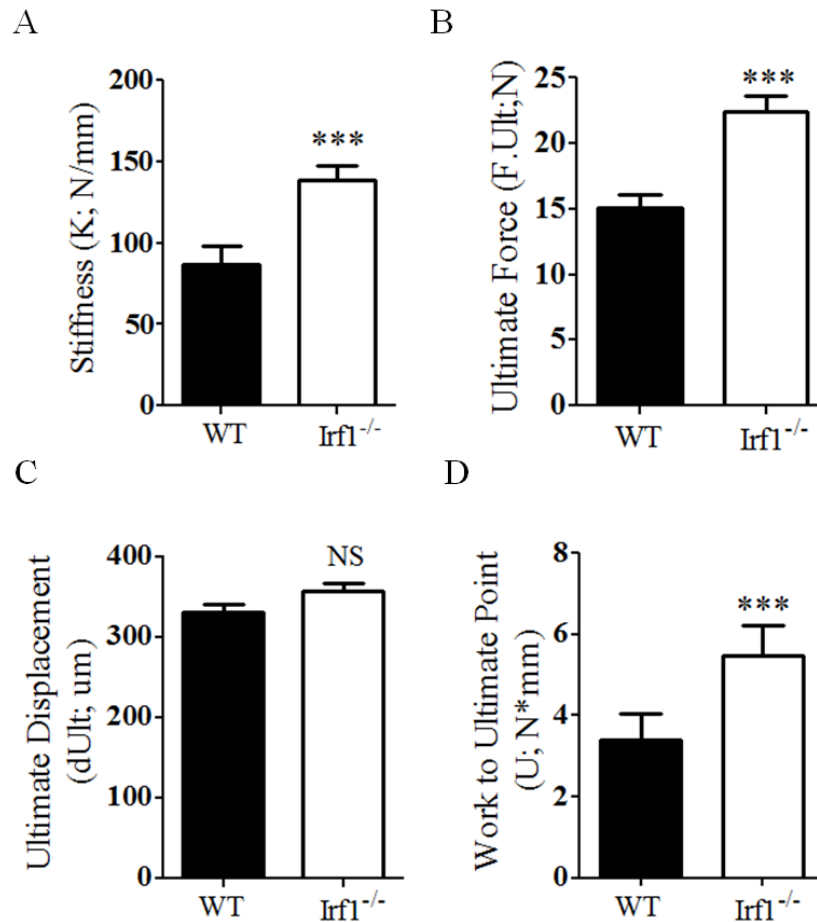


Figure 6. Biomechanical testing reveals stronger bones in *Irfl*^{-/-} mice. Biomechanical testing by a 3 point bending assay was performed and analysis demonstrated stiffness (A), force (B), ultimate displacement (d.Ult) and ultimate force to point (D). n=5. Results represented as means ($\pm$ SD). *** $P < 0.0001$. NS, no statistical significance.

4.5 DISCUSSION

This study provides evidence for a novel role for *Irf1* in regulating long bone development, which is critically dependent on the orderly and co-ordinated activity of osteoblasts and osteoclasts²⁶⁸. *Irf1*^{-/-} mice exhibit alterations in femoral morphology, micro-structure and bone cell activity. X-ray and quantitative micro CT analyses revealed shorter femurs with altered trabecular architecture resulting from fewer, wider trabeculae in the mutant compared with WT mice. The changes in bone micro-structure were confirmed by histological analyses of un-decalcified bone, which showed no apparent differences in ALP activity in osteoblasts or TRAP activity in osteoclasts lining the trabeculae. The reduction in bone length and altered trabecular bone architecture are therefore attributed to changes in the turnover of cartilage to bone in the growth plates. Cortical bone width and cellularity were also increased in the *Irf1*^{-/-} mice, which accounted for the increase in BMD and enhanced biomechanical strength of their long bones compared with those of WT control. Histological analysis confirmed the increase in cortical bone width in the mutant mice, but with no apparent change in the rate of deposition (calcein labeling) suggesting it represents an adaptive mechanism to maintain mechanical strength in the face of decreased and disorganised trabecular bone architecture²⁶⁹.

Bone remodeling is achieved through a precise balance between the activity of bone-resorbing osteoclasts and bone-forming osteoblasts. The proliferation of osteoblast progenitor cells and their differentiation into mineralizing osteoblasts is tightly regulated during bone development and in the adult skeleton undergoing remodeling¹⁹⁵. In previous *in vitro* studies *Irf1* mRNA was detected in proliferating precursor cells and in differentiated osteoblasts depositing mineral¹⁹³. It was therefore not surprising that Irf1 protein was present in cultures of MC3T3 mouse osteoblast-like cells (**Fig. S1**). We now show that cultures of mesenchymal stromal cells isolated from the bone marrow of *Irf1*^{-/-} mice proliferate more slowly and differentiate into mineralizing osteoblasts more rapidly than those isolated from WT mice. These observations suggest that Irf1 may act as a negative regulator of osteoblast function, possibly by maintaining the precursor cells in a proliferative, poorly differentiated state with no capacity for matrix mineralization. Consistent with this conjecture was the significant up-regulation of *Ocn*, and down-regulation of *Matrix gla protein* mRNA in differentiating cultures of *Irf1*^{-/-} cells. OCN and MGP are non-collagenous proteins that promote and inhibit tissue mineralization respectively. Of additional interest was the increased expression of OPN in the mutant

osteoblasts, which has an un-defined role in tissue mineralization but is believed to anchor osteoclasts to bone matrix during the re-modeling cycle. Cultures of *Irf1*^{-/-} cells also expressed 6-fold more *Rankl* mRNA than WT cells. RANKL is a cell surface protein on osteoblasts that binds to its receptor RANK on osteoclast precursors and stimulates their differentiation and activity.

Osteoclasts arise from the same myeloid progenitor cells that give rise to tissue macrophages and dendritic cells, as well as circulating monocytes²⁷⁰. These cells express *Irf1* and *Irf8*, and require both factors for their maturation and for the function of fully differentiated cells^{165,253,271} (**Fig. S1**). In agreement with the *in vivo* TRAP staining data, there appeared to be no difference in the number of TRAP-positive cells in cultures of *Irf1*^{-/-} and WT control bone marrow cells stimulated with RANKL to induce osteoclastogenesis. The TRAP-positive cells from the mutant mice were however larger and resorbed more pits of a larger size in synthetic bone *ex vivo* than the cells harvested from WT control mice. A similar correlation between the size of cultured TRAP-positive cells and their *ex vivo* resorptive capacity has been shown for cells isolated from rabbits²⁷² and from genetically modified mice with synovitis and excessive sub-chondral bone resorption²⁷³. The later situation reflects that seen in patients with rheumatoid arthritis, where large mononuclear and multinucleate TRAP positive cells in the synovium are thought to contribute to both cartilage and bone destruction²⁷⁴. The fact that serum calcium and phosphorous were elevated in the mutant compared to WT mice (**Fig. S2**), in association with a reduction in trabecular bone suggest that osteoclast activity was indeed elevated in *Irf1*^{-/-} mice. Taken together the data indicate that *Irf1* may suppress the activity of differentiated osteoclasts and/or maintain them in a less differentiated state

The increase in osteoclast activity is similar to that seen in *Irf8*^{-/-} mice, which lack the dimerization partner for *Irf1*⁶. This highlights key roles for *Irf1* and *Irf8* in development and function of the myeloid lineage in general, and of osteoclasts in particular. *Irf1*^{-/-} mutant mice show immune defects in the myeloid and lymphoid cell compartments, including impaired Th1 immune response, decreased pro-inflammatory cytokine production and susceptibility to intracellular pathogens^{129,133,235,257,258}. Pro-inflammatory cytokines have been shown to contribute to regulation of bone metabolism²⁶¹. Two important cytokines, IL-12 and IL-18 are expressed in osteoblasts and act to decrease osteoclastogenesis by inhibiting *Rankl* production

and other aspects of osteoclast formation and activity^{200,201,261}. These pro-inflammatory anti-osteoclastogenic cytokines are not produced by *Irf1*^{-/-} mice¹⁷⁴. When translated to the *in vivo* situation the significant differences in *ex vivo* growth and activity of cells of both the osteoblast and osteoclast lineages would ultimately impact on the biomechanical properties of the long bones of *Irf1*^{-/-} mice. The functional consequence of altered bone cell activity was evident in a three point bending test where the force applied to the mid-diaphysis that was required to break the bone was significantly higher in the mutant compared with animals. The data suggests that the balance between enhanced osteoblast and osteoclast function was tipped in favor of osteogenesis.

The bone phenotype of *Irf1*^{-/-} mice is strikingly similar to that of mice bearing a null allele at *Stat1* (*Stat1*^{-/-}; signal transducer and activator of transcription 1). *Stat1* is a transcriptional regulator that physically and functionally interacts with *Irf1* in the regulation of gene expression downstream interferon gamma signaling^{275,276}. Similar to *Irf1* deficiency, *Stat1*^{-/-} mice show an increase in bone mass, in cortical bone thickness and in bone mineral density, due to increased osteoblast function despite having excessive osteoclastogenesis¹⁸⁸. *Stat1* plays an important regulatory role in both osteoclast and osteoblast differentiation and function. In osteoclast precursor cells, *Stat1* is required for IFN- β dependent dampening of RANKL-induced expression of c-fos, a transcription factor essential for osteoclastogenesis²⁷⁷. *Stat1* is also required for IFN- γ -dependent inhibition of RANKL expression by activated T-cells^{278,279}. In osteoblasts, *Stat1* has been shown to inhibit the function of Runx2, a transcription factor that plays a central role in bone formation¹⁸⁸. The net increase in bone formation and resulting bone mass suggests that *in vivo*, the regulatory function of *Stat1* in osteoblasts dominates over its inhibitory function in osteoclasts. *Irf1* is a well known transcriptional activator of IFN- β genes¹¹⁸ which are critical for regulating the skeletal system by inhibiting osteoclastogenesis¹⁸⁷. Thus, we propose that absence of *Irf1* would eliminate this regulatory function and enhance osteoclastogenesis. Although the mechanism of *Irf1* negative regulation of osteoblast differentiation and function remains to be elucidated, it is tempting to speculate that it may involve the formation of *Irf1*/*Stat1* heterodimers²⁷⁵.

The current study identifies a novel role for Irf1 in bone metabolism during development. Further experiments will be required to identify the transcriptional targets of Irf1 that regulate the maturation and activity of cells of the osteoclast and osteoblast lineages. Likewise, it will be interesting to investigate a possible role of IRF1 and of its hetero-dimerization partner IRF8 in human disorders characterized by osteopenia such as osteoporosis. Although genome-wide association studies have identified positive genetic associations with markers in the proximity of the *IRF1* (5q31.1) and *IRF8* (16q24.1) genes^{280,281} a role for these genes and their targets in clinical osteoporosis needs to be formally tested.

ACKNOWLEDGEMENTS

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Chapter 5

Summary and Future Perspectives

5.1 SUMMARY

The large inter-individual variability of clinical outcomes following infection with MTB, the reported familial clustering of TB cases as well as twin studies showing higher incidence of TB in monozygotic than in dizygotic twin pairs all provide substantial evidence for the contribution of human genetics to mycobacterial disease^{282,283}. Forward genetics in inbred mouse strains have helped to identify key genes, chromosomal regions and linkages controlling susceptibility to MTB or BCG³⁸. One of the most influential findings of such studies was by Vidal and colleagues who demonstrated that interstrain differences in susceptibility to BCG was controlled by a single locus named *Bcg/Ity/Lsh* and identified the gene responsible to be *Nramp1*¹⁰⁵. In parallel, association studies demonstrated that human polymorphisms near or at *NRAMP1* were found to be associated with susceptibility to TB in various populations⁴⁸⁻⁵¹. Subsequent studies of recombinant inbred mouse strains by Turcotte and colleagues identified BXH-2 mice to be extremely susceptible to infection with BCG despite carrying the resistant *Nramp1*^{G169} allele, and also displaying a myeloproliferative disorder with infiltration of immature granulocyte precursors^{4,110}. Furthermore, Marquis and colleagues demonstrated that BXH-2 mice are hypersusceptible to virulent MTB and develop fatal pulmonary TB¹¹². This abnormal susceptibility to mycobacterial infection and defective myelopoiesis in BXH-2 mice were both found to be due to a partial loss of function recessive mutation in *Irf8* (*Irf8*^{R294C})^{4,5}. These studies demonstrated that *Irf8* is required for the development of myeloid cells and for orchestrating early innate response and long-term immune protection against intracellular pathogens such as mycobacteria. The work in this thesis was undertaken to investigate the role of IRF8 in susceptibility to mycobacterial infections in humans; a parallel line of investigation that developed during these studies, was the identification of a role for *Irf1*, a major binding partner of *Irf8*, in regulation of bone development.

In chapter 2, we studied an infant (Subject 1) who was hospitalized following routine postnatal BCG vaccination and presented with disseminated BCG lesions, lymphadenopathy, splenomegaly, and failure to thrive. At presentation, the patient had shown anemia, abnormally high numbers of neutrophils and a complete depletion of circulating monocytes. She was found to lack all subsets of DCs including CD11c⁺ / CD141⁺ myeloid and CD11c⁺ / CD123⁺ plasmacytoid DCs. This phenotype is identical to *Irf8* deficient mice (*Irf8*^{-/-})^{135,155}. Sequencing of Subject 1 and her family led to the identification of a mutation in IRF8 (K108E) mapping to the

highly conserved DBD of the protein. Subject 1 is homozygous for the mutant allele while her parents are heterozygous and her brother is wild type. All family members were BCG vaccinated with no adverse reactions indicating a recessive mode of inheritance of this IRF8 deficiency in humans. Sequencing of the 450 MSMD patients also led to the discovery of another IRF8 mutation in two unrelated patients (Subject 2, 3) from South America, identified as a *de novo* heterozygous mutation (T80A) also mapping to the DBD of the protein. These patients showed a selective depletion of CD11c⁺ / CD1c⁺ DCs but normal levels of CD11c⁺ / CD141⁺ myeloid and CD11c⁺ / CD123⁺ plasmacytoid DCs, identical to what is observed in BXH-2 (Irf8^{R294C}) mice^{5,114}. We then functionally tested these IRF8 mutants and observed that T80A causes a 75% decrease in trans-activation of both Il-12p40 and iNos promoters, similarly to the R294C mutant protein, while K108E is functionally null. Both T80A and K108E show severe defects in DNA binding to target genes. Furthermore, we observed that T80A behaves as a dominant negative, affecting function of wild type Irf8 while K108E has no such effect and behaves as a loss of function mutant. These studies identified two novel diseases affecting the development of monocytes and DCs including autosomal recessive (AR) IRF8 deficiency which we showed is a severe and life-threatening susceptibility to mycobacteria and autosomal dominant (AD) IRF8 deficiency which is a milder and treatable mycobacterial susceptibility, placing IRF8 as a new MSMD-causing gene. Subject 1 was ultimately rescued by a hematopoietic stem cell transplant.

In chapter 3, we continued to study the K108E mutation and aimed to further investigate the functional consequences of IRF8 deficiency on hematopoietic cell development and function. We first followed up with the status of Subject 1 and her physicians to observe the amelioration of her condition following the stem cell transplant. We observed that her abnormally high neutrophil count and complete lack of monocytes were corrected following the successful transplant indicating that immunologic reconstitution was fully achieved including normal humoral responses to vaccines. We were fortunate to have a vial of PBMCs from Subject 1 prior to her transplant and took advantage to perform RNA sequencing on this sample and on PBMCs from five healthy controls to look at differences in gene expression. This analysis demonstrated depletion of transcripts associated with mononuclear phagocytes and enrichment of transcripts associated with the granulocytic lineage, all in agreement with her immunological profile. We interestingly observed a depletion of gene signatures associated with T cell activation in patient K108E, for both the CD4⁺ and the CD8⁺ lineages, displaying signatures that are characteristic of

naïve T cells. Importantly, this occurred despite normal numbers of circulating T cells and despite intense antigenic exposure during the multiple episodes of infection in this patient. RNA sequencing and ChIP sequencing demonstrated that genes depleted in patient K108E are significantly associated with genes that are bound and regulated by Irf8 in IFN- γ treated myeloid cells. We finally demonstrated that the K108E mutation is detrimental to IRF8 protein in terms of causing defective transcriptional function, impaired nuclear targeting, severely decreased stability of the protein and increased proteasomal degradation, all of which contribute to the pathological nature of this mutation in Subject 1.

Previously, Irf8 was reported to inhibit bone loss by negatively regulating differentiation of osteoclasts in mice⁶. In chapter 4, we identified a novel role for Irf8's binding partner Irf1 in the regulation of bone metabolism. We observed that Irf1 is expressed in both the osteoblast and osteoclast lineages in contrast to Irf8 that is restricted to osteoclasts. We demonstrated that in the osteoclast lineage, Irf1 acts as a negative regulator of osteoclast activity while in osteoblasts it functions to promote proliferation of precursor cells but inhibit differentiation and mineralization of mature osteoblasts. The net effect of the altered bone cellular functions is increased stiffness as well as increased force and work to failure in femurs of *Irf1*^{-/-} mice. This is due to the increased mineralization by these osteoblasts indicating that the role of Irf1 in osteoblasts dominates over its functions in the osteoclast lineage.

5.2 The genetic theories of infectious diseases

Infectious diseases continue to threaten human lives worldwide and increasing evidence points to a major role of host genetics in susceptibility to infection and the different clinical outcomes. An adoptee cohort study that investigated mortality due to infectious diseases in children given up for adoption found that mortality risk increased by almost 6 fold if one of the biological parents died of an infectious disease²⁸⁴. In this case, environmental factors such as exposure to pathogen differ which strengthens the case for a major genetic role on the variable outcomes of infectious disease. Further evidence for a major host genetic role in infectious disease and TB is demonstrated by twin studies, racial variation in susceptibility to TB where individuals with African ancestry show higher risk of disease than European individuals and finally from studies of MSMD patients as previously discussed²⁸⁵⁻²⁸⁷.

There are several theories that aim to explain the variability in susceptibility of human populations to infectious agents, including initial infection, colonization, response to infection and ultimate outcomes. The microbiological theory focuses on microbial variability, with microbial pathogenicity determinants conveying diseases of different intensities and outcome. The immunological theory studies variability in somatic immunity as a cause of different “vulnerability” to infections. The genetic theory is based on heritable (germ line) genetic variability in immune functions or other physiological pathways that are engaged²⁸⁸. The genetic theory has evolved in two different directions led by population and clinical geneticists. Population geneticists generally favor models in which infectious disease susceptibility is regulated in part by multiple common variants acting alone or in combination (multiple genes, one disease). In contrast, clinical geneticists favor the proposal that rare but highly penetrant mutations underlie predisposition to multiple and recurrent infections (one gene, multiple infections), often manifesting themselves early during childhood²⁸⁸. These two theories are not mutually exclusive.

5.2.1 Populations genetics and genome-wide association

Since infectious diseases are known to represent a major human selective pressure and that numerous genes are involved in host response to infectious agents, it has been proposed that human susceptibility to infection is polygenic, meaning that susceptibility is controlled by many common genetic loci^{289,290}. Population genetics therefore implements genome-wide association studies (GWAS) to detect potential association between common (minor allele frequency between 1- 5%) as well as informative genetic variations and presence/absence of common diseases in very large cohorts²⁹¹. Associations with these common variants however, usually confer small increases in disease risk of only 1-1.5 fold. The size effect is influenced by other genetic, epigenetic or environmental determinants, including the presence of the microbe triggering the clinical infection status. Furthermore, these common variants explain only a minor fraction of heritability of infectious disease, a problem which is referred to as “missing heritability”^{292,293}. Nevertheless, these identified loci often point to critical genes encoding host proteins that interact with the pathogen as well as essential molecules involved in downstream signaling cascades following host-pathogen interaction. GWAS in infectious diseases have been reported and show strong associations with specific genes/loci that largely confirm previous

association studies with candidate genes, including *DC-SIGN*, *TLR1*, *TLR2*, *TNF*, *IL1B* and *NRAMP1* (reviewed in²⁹⁴).

Unfortunately, GWAS on pulmonary tuberculosis have had limited successes. A GWAS performed on a large sample of over 11, 000 individuals from Gambia and Ghana identified a SNP with weak effect located in a gene poor region on chromosome 18q11.2 between the genes *CTAGE1* and *RBBP8*²⁹⁵. Another GWAS identified a second locus on chromosome 11p13 in the Ghanaian population which was replicated in the Gambian populations and confirmed in a South African population but barely displayed a significant association in Indonesian and Russian patients^{296,297}. Lastly, a GWAS in Asian populations identified a TB risk locus on chromosome 20q12 but only in patients defined as young cases that had an onset of TB before the age of 45²⁹⁸. GWAS in large cohort of patients infected with mycobacteria (TB) have therefore been of limited value to identify major gene effects in susceptibility to this class of infections. This suggests that the clinical outcome of TB is variable and depends on several factors, only some of which are genetic in nature.

GWAS have reduced power to detect associations in the case of genetic heterogeneity where multiple genetic effects result in similar clinical end points and associated phenotypes²⁹⁹. Furthermore, the disease-associated variants identified by GWAS explain only a fraction of heritability of disease since each individual association is of modest magnitude. Epistatic interactions between loci that might account for some of the missing genetic component are a likely contributing factor, and have been difficult to uncover²⁹⁹. In the context of infectious diseases, GWAS studies are further limited due not only to the requirement of very large sample sizes and the appropriate matched controls to achieve significance, but also because clinical end point is complex and prone to ascertainment bias^{294,299}. The degree of pathogen diversity is also believed to affect GWAS success. This can be seen by the greater number of loci identified in GWAS of leprosy (*Mycobacterium leprae*) compared to tuberculosis due to the higher diversity of *M. tuberculosis* relative to *M. leprae*^{295,300}. Finally factors such as dose and route of infection and the possibility of co-infection with other pathogens such as for example, HIV are difficult to account for in genetic association studies^{294,301}. One way to improve GWAS success in pulmonary TB would be to first ascertain presence of MTB bacilli in infected patients and absence of such bacilli in healthy controls. This is essential since 90% of individuals infected

with MTB do not develop active disease and instead enter LTBI with no clinical symptoms. It is therefore highly possible that individuals classified as healthy controls used for these GWA studies are actually LTBI cases, ultimately significantly limiting the success of GWAS for pulmonary TB³⁰².

Finally, the fact that only a fraction of human heritability of infection has been explained by GWAS indicates that a substantial proportion of other relevant genetic factors remain to be identified³⁰³.

5.2.2 Clinical genetics and linkage studies

The study of primary immunodeficiencies (PIDs) shows that severe infections occur in individuals carrying rare mutations in genes that play critical roles in defense against pathogens. These can be viewed in essence as monogenic traits, as exemplified by individuals suffering from Mendelian Susceptibility to Mycobacterial Disease (MSMD)²⁸⁸. Studies of individual MSMD patients have also aided in the understanding of the genetics of severe tuberculosis as some MSMD patients, such as those with *IFNGR1* and *IL12B* mutations, have been shown to suffer from pulmonary infection due to MTB suggesting that the tuberculosis in these patients can be monogenic. Another example is *STAT1* mutations that cause susceptibility not only to mycobacteria but also to viral disease and chronic mucocutaneous candidiasis, further demonstrating the “one gene, multiple infections” basis of clinical genetics³⁰⁴⁻³⁰⁶.

A widely used approach to identify loci linked to infectious diseases is the study of affected sibling pairs by linkage analysis. One important limitation of this approach is the difficulty of recruiting numerous multicaser families where two siblings are affected by an infectious disease^{41,307}. This approach in the context of infectious diseases has also shown poor replication due to unknown variations in host-pathogen or gene-environment interactions. Finally the candidate gene approach often has relied on existing biological hypotheses on the role of a particular gene defined from other approaches such as animal models which can be inaccurate or not conserved in humans^{294,299}.

A major limitation of using PIDs as entry points to study the genetic architecture of infectious diseases is that the number of cases is limited. In many cases, mutations are life-

threatening and therefore individuals are unlikely to pass on the mutated allele(s). Hence, although the study of PIDs provides valuable insight into the major regulators of the immune system, they do not provide a full view of more modest genetic effects that may modulate susceptibility to infections in a more subtle way, but in a larger number of individuals.

5.2.3 A bridge of the genetic theories

The studies conducted in this thesis on human *IRF8* mutations have provided further insight into the genetic aspects of susceptibility to mycobacteria and infectious disease. We demonstrated in chapter 2 that homozygosity for the K108E mutation causes a severe PID characterized by loss of monocytes and DCs as well as susceptibility to multiple infections. This resembles studies performed on other monogenic PIDs such as individuals with mutations in *STAT1* and *IL12B* and as such, follows the clinical genetics theory (one gene, multiple diseases)
2,288

Interestingly, we have observed that heterozygosity for loss of function mutations or reduced expression of a transcription factor, like IRF8, may also be responsible for genetic effects in susceptibility to infections. Indeed, by whole exome sequencing of a Turkish family suffering from severe visceral leishmaniasis, we identified a novel and rare human heterozygous mutation in the *IRF8* gene (Ile15Met; I15M) showing familial segregation where all affected members carry the *IRF8*^{I15M/WT} mutant allele. This I15M mutation maps to the highly conserved DBD and causes a partial loss of function IRF8 protein (50% decrease in transactivation). However, unlike the T80A or K108E mutations, I15M does not cause any apparent defects in myeloid or lymphoid cell numbers and therefore shows no known cellular cause of disease (Salem S, unpublished). These results suggested that the partial loss of IRF8 function is not enough to affect myeloid cell numbers but does cause a decrease in transcriptional regulation by IRF8. Furthermore, the effect of this partial loss of IRF8 transcriptional function could possibly be amplified due to additive losses in function of downstream direct transcriptional target genes acting in the same pathways. This therefore provides evidence that heterozygous mutations that do not behave as dominant negative or gain of function are still important and phenotypically

significant. Furthermore, not only is partial loss of function of an important gene significant but it may also mimic the additive effect of multiple common variants (including those in the transcription factor targets), the concept that is proposed by the population genetics theory. In agreement with this proposal are results from Berghout and colleagues that observed a significant cerebral malaria resistance phenotype in *Irf8*^{R294C/WT} F1 heterozygous mice that is similar to *Irf8*^{R294C/R294C} homozygotes¹⁴⁸. Interestingly, these *Irf8*^{R294C/WT} heterozygous mice, similarly to the *IRF8*^{I15M/WT} patients, also do not show any defects in myeloid and lymphoid cell numbers but do indeed show altered defense responses and expression of downstream target genes. The observation of a phenotype in heterozygote mice suggests a partial loss of important downstream targets in myeloid-specific and pro-inflammatory pathways that cause pathological inflammation, perhaps amplifying the effect of partial loss of function of *Irf8*¹⁴⁸. Finally, in chapter 2 we did not observe any defect in myeloid cell numbers in the parents of patient K108E who are both heterozygous (*IRF8*^{K108E/WT}) for the mutation which is similar to the I15M family and *Irf8*^{R294C/WT} mice. We will be investigating the effect of both heterozygous mutations (K108E, I15M) on the IRF8 transcriptional profile of these individuals compared to the K108E homozygous patient.

We have also identified novel and rare heterozygous human mutations in the *IRF1* gene that are associated with susceptibility to mycobacteria by sequencing of the cohort of 450 MSMD patients (Salem S, unpublished). These two heterozygous mutations (S125L, D194N) were identified in two unrelated MSMD patients. Mutation S125L maps to the highly conserved nuclear localization sequence (NLS) while D194N maps to the heterodimerization domain (HD) of IRF1 and both cause a partial loss of function of IRF1 protein but with no apparent defects in myeloid or lymphoid cell numbers. Patient S125L suffered from two episodes of TB infection while patient D194N suffered from an *M. avium* infection (Salem S, unpublished). Once again, these phenotypes could be due to additive effects of partial loss of function of multiple IRF1 downstream targets. Further immunological and biochemical studies are required to uncover the basis of disease in these patients.

Studies, like the ones performed in this thesis, of monogenic susceptibility to a specific pathogen, leads to an idea that rare mutations of key genes and pathways in individuals may affect susceptibility to diseases in the general population²⁰⁷. Indeed, although the mutations we

identified in *IRF8* and *IRF1* are very rare, GWAS studies have demonstrated significant associations for both *IRF8* and *IRF1* in other diseases of inflammation, including SLE, MS, ulcerative colitis, IBD and Crohn's disease, as previously discussed. These identified SNPs affect the level of expression of otherwise unaltered *IRF8* or *IRF1* which is similar to what we observed in our studies of heterozygous mutations causing partial loss of function. It is therefore becoming increasingly evident that both rare and common variants in the same molecular pathway, such as for example inflammation, may underlie susceptibility to related diseases requiring a Th1 immune response such as leishmaniasis.

Collectively, the coming together of clinical and population genetics has been successful in uncovering novel genetic variants that are important in immune response to a specific pathogen but also highlighting the roles of shared signaling pathways in the pathogenesis of diverse diseases and finally continuing to identify an array of childhood deleterious mutations that confer extreme susceptibility to disease^{294,299}.

5.3 Future Perspectives

5.3.1 Future genetic approaches for studying infectious diseases

One of the future goals of the human genetics of infectious disease field is to define the relative contribution of common and rare variants to disease susceptibility at the individual and population level^{39,293,294}. In order to achieve this, next generation sequencing studies to identify rare variants will become dominant tools that will strengthen traditional genetic approaches. Next generation sequencing will help to investigate factors of disease susceptibility such as pathogen genetic variation or virulence, environmental factors as well as epistatic and epigenetic effects²⁹⁴. For the moment, whole exome sequencing in patients suffering from infectious disease appears to be a more cost effective and efficient approach instead of whole genome sequencing that can identify both rare monogenic and rare diverse variants causing susceptibility to disease^{293,299}. This proved to be successful in studies on *TLR* genes where multiple rare coding changes were identified by whole exome sequencing in patients with meningococcal disease but also in patients with TB compared to controls^{308,309}. Once whole genome sequencing becomes cost-effective, it will be essential in identifying novel and previously unidentified regulatory SNPs located in enhancers and promoters than can alter the epigenetic status of this regulatory element and/or a transcription factor binding sites³¹⁰. Furthermore, other next generation

sequencing strategies such as RNA-sequencing are important for investigating loci that mediate differential expression of genes in a cell- or tissue-specific manner but also for detecting transcription of unannotated exons^{311,312}. Indeed, loci associated with variation in gene expression in human dendritic cells following infection with MTB have been identified and can become interesting candidate loci to be tested for association with susceptibility to mycobacterial disease or TB³¹³.

In chapter 3 of this thesis, we used RNA-seq and ChIP-seq to identify cell specific genes that are lacking in patient K108E and that are direct targets of IRF8. These genes can serve as excellent candidates to uncover the underlying genetic etiology of the remaining MSMD patients, new patients or families showing abnormal susceptibility to pathogens requiring Th1-mediated immunity and also patients suffering from primary immunodeficiencies affecting the development of monocytes and dendritic cells. Indeed, we are currently using RNA-seq in the Turkish family (*IRF8*^{I15M/WT}) suffering from visceral leishmaniasis compared to healthy controls in order to investigate the contribution of a loss of one IRF8 allele on different aspects of immune response such as cell development, cell activation and cytokine production. In parallel, we will also be performing RNA-seq on the mother of patient K108E who is heterozygous for the mutation to study differential gene expression and compare this gene list to that of the Turkish family. These analyses can hopefully identify novel genes and pathways that confer susceptibility to mycobacteria.

5.3.2 Clinical applications of genetic studies on infectious diseases

Studies of host genetic factors in susceptibility to infectious diseases have many powerful clinical implications. The emergence and spread of new microbes and antibiotic resistant pathogens such as drug-resistant MTB pose new and continuous threats to humans. Genetic studies of infectious diseases allow for more accurate molecular diagnosis and genetic counseling based on the identification of deleterious genetic mutations which can then lead to more personalized treatment²⁸⁸. Indeed, studies on susceptible individuals with inborn mutations such as studies of MSMD patients lead the way to cytokine therapy to complement a specific genetic deficiency including, G-CSF and recombinant IFN- γ ³¹². High dose IFN- γ therapy has been beneficial in patients suffering from disseminated TB due to a mutation in *IL12RB1* which causes impaired IFN- γ production³¹⁴. Another consideration is that clinical trials that do not take

into account human genetic variation can possibly miss relevant effects on subgroups of individuals and perhaps lead to disappointing results²⁹⁴. This is one of the most critical issues in the field of human TB research.

Whole exome sequencing is beneficial and will continue to show important implications in several areas such as prenatal diagnostics, prognosis of preclinical individuals and especially in newborn screening procedures and treatment. The identification of genes and new biological networks will improve disease management and drug discovery, ultimately helping to improving health care³¹⁵.

Although the microbiological theory allowed for the development of powerful medicines and the immunological theory successfully uncovered adaptive immunity which led to the development of vaccines, a fact remains that some genetically deficient individuals have an inherent susceptibility that cannot be overcome by conventional vaccination and antibiotics²⁸⁸. This is particularly seen in patients carrying inborn mutations that affect the development of either mononuclear phagocytes (as seen in patient K108E) or lymphocytes resulting in a lack of innate and adaptive anti-mycobacterial immunity, as well as in patients with severe PIDs such as SCID^{16,207,250}. Vaccinating these individuals will result in disastrous and life threatening reactions due to their lack of an essential immune system. Some individuals show extreme disease susceptibility due to homozygous mutations while others show dampened immune responses due to heterozygous mutations resulting in reduced protein function, altogether adding another level of complexity for prevention and treatment options. This can be seen in the subgroup of MSMD patients as well as patient K108E who develop disseminated mycobacterial disease that is not treatable with traditional anti-mycobacterial drugs and also despite routine BCG vaccination at birth. Another subgroup of MSMD patients such as the T80A patients develop disseminated mycobacterial disease that is treatable with anti-mycobacterial therapy that is required for life. The largest and most obvious concern here is that the disseminated mycobacterial disease observed in these patients is actually caused by the BCG vaccination itself, which is supposed to prevent TB. This shows clear evidence of a need for a new vaccine to combat tuberculosis. Unfortunately, recent vaccine trials have shown disappointing results likely indicating our incomplete understanding of human genetically inherited susceptibility to mycobacteria and biological pathways and networks necessary for resistance to TB^{316,317}.

Genetic studies are therefore critical for the development of new effective TB vaccines that must take into account the genetic susceptibility of patients with disease.

5.4 Final Conclusions

Tuberculosis is still a global health problem and one of the leading causes of death due to infection with a single pathogen, despite nearly worldwide vaccination with BCG. The genetic basis for TB and mycobacterial disease is complex and our studies have identified novel human genetic mutations and primary immunodeficiencies causing extreme susceptibility to mycobacteria and paved the way for identifying future novel genetic targets and pathways critical for host immune response. Our work has also provided continued evidence for the successes of using not only mouse models to study human disease but also in highlighting the importance of human genetic factors in controlling susceptibility to mycobacteria in particular and infectious diseases in general.

ORIGINAL CONTRIBUTIONS TO KNOWLEDGE

1. Identification of a mutation in *IRF8* ($IRF8^{K108E}$) in an infant patient that causes a lack of monocyte and DC development as well as extreme susceptibility to weakly virulent mycobacteria (BCG).
2. Identification of a mutation in *IRF8* ($IRF8^{T80A}$) in two unrelated MSMD patients causing a selective depletion in $CD11c^+/CD1c^+$ DCs and high susceptibility to weakly virulent mycobacteria (BCG).
3. The $IRF8^{K108E}$ and $IRF8^{T80A}$ mutant proteins have severely defective DNA binding to target genes.
4. Establishment of *IRF8* as an MSMD-causing gene.
5. Mutation $IRF8^{K108E}$ leads to depletion of antigen presentation and DC gene signatures.
6. Mutation $IRF8^{K108E}$ causes defective $CD4^+$ T cell activation leading to anergic T cells despite normal numbers of T lymphocytes in the infant patient.
7. The $IRF8^{K108E}$ mutant protein is transcriptionally null, shows defective nuclear localization, is highly unstable and strongly targeted to proteasomal degradation following increased ubiquitination and SUMOylation.
8. Identification of a novel role for *Irf1* in regulation of bone metabolism in mice. *Irf1* deficiency causes enhanced osteoclast activity and resorption and increased mineral deposition by osteoblasts.

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Appendix 1: K108E dysregulated genes
1418 genes

Gene information				Log2 CPM						Fold change (K108E/Ctl1)	p Value (K108E vs Ctl5)	p Value (Ctl2,3,5,6)
Gene symbol	Entrez Gene ID	Chr	Gene name	Ctl1	Ctl2	Ctl3	Ctl5	Ctl6	K108E			
ABCA13	154664	7	ATP-binding cassette, sub-family A (ABC1), member 13	-0.73	-0.38	-1.85	-1.87	-0.32	4.37	34.6	1.5E-03	8.0E-01
ABCB1	5243	7	ATP-binding cassette, sub-family B (MDR/TAP), member 1	4.89	4.23	7.04	4.72	4.86	1.32	-11.9	9.1E-03	5.8E-01
ABCB10	23456	1	ATP-binding cassette, sub-family B (MDR/TAP), member 10	4.50	4.86	4.80	4.91	4.65	6.35	3.6	2.6E-02	5.6E-01
ABCB6	10058	2	ATP-binding cassette, sub-family B (MDR/TAP), member 6	2.95	1.90	2.53	1.75	2.34	6.50	11.7	1.0E-03	2.2E-01
ABCC13	150000	21	ATP-binding cassette, sub-family C (CFTR/MRP), member 13, pseudogene	-2.68	-7.11	-4.03	-4.76	-1.88	4.26	126.2	1.9E-03	3.6E-01
ABCD2	225	12	ATP-binding cassette, sub-family D (ALD), member 2	3.97	1.77	1.71	1.63	1.19	-0.98	-31.3	6.0E-03	1.3E-01
ABCG1	9619	21	ATP-binding cassette, sub-family G (WHITE), member 1	5.04	5.24	6.26	5.40	5.74	1.46	-12.0	2.3E-03	3.3E-01
ABCG2	9429	4	ATP-binding cassette, sub-family G (WHITE), member 2	-0.95	-0.54	1.93	-2.68	-2.84	3.93	29.6	3.2E-02	6.4E-01
ABTB1	80325	3	ankyrin repeat and BTB (POZ) domain containing 1	7.22	6.63	6.88	6.77	6.88	8.89	3.2	3.4E-02	3.6E-01
ACACB	32	12	acetyl-CoA carboxylase beta	4.87	4.54	4.03	4.76	4.66	2.39	-5.6	1.3E-02	4.1E-01
ACOT1	641371	14	acyl-CoA thioesterase 1	0.15	1.03	0.23	1.53	0.58	-7.11	-314.1	3.4E-02	5.3E-01
ACOT2	10965	14	acyl-CoA thioesterase 2	3.31	3.15	2.85	3.18	3.34	0.59	-6.6	3.2E-02	1.0E+00
ACOT4	122970	14	acyl-CoA thioesterase 4	1.15	0.27	0.50	0.93	-0.02	-7.11	-627.1	8.4E-03	6.3E-01
ACSL6	23305	5	acyl-CoA synthetase long-chain family member 6	4.68	2.84	2.34	3.24	2.93	6.74	4.2	4.8E-02	5.3E-02
ADAM12	8038	10	ADAM metalloproteinase domain 12	0.47	0.06	1.27	-1.58	-0.97	-7.11	-392.3	3.4E-02	8.4E-01
ADAMTS14	140766	10	ADAM metalloproteinase with thrombospondin type 1 motif, 14	-2.89	-2.02	-3.12	-2.68	-2.02	1.70	24.8	2.6E-03	5.2E-01
ADAMTS3	9508	4	ADAM metalloproteinase with thrombospondin type 1 motif, 3	-7.11	-7.11	-7.11	-7.11	-7.11	1.32	707.9	3.2E-04	5.6E-01
ADAT2	134637	6	adenosine deaminase, tRNA-specific 2	3.11	3.73	3.91	4.58	4.79	5.64	5.8	1.7E-02	2.6E-01
ADCY1	107	7	adenylate cyclase 1 (brain)	0.01	-0.46	-1.38	-0.89	-0.52	3.09	8.5	3.3E-02	2.3E-01
ADCY7	113	16	adenylate cyclase 7	8.26	7.80	8.17	8.01	7.44	6.39	-3.6	3.7E-02	6.7E-01
ADIPOR1	51094	1	adiponectin receptor 1	6.85	7.58	7.36	7.79	7.78	9.90	8.3	7.4E-04	3.2E-01
ADNP	23394	20	activity-dependent neuroprotector homeobox	6.49	6.90	7.18	6.48	6.57	4.61	-3.7	2.5E-02	5.5E-01
ADORA2A	135	22	adenosine A2a receptor	6.13	5.60	3.88	5.99	6.58	3.93	-4.6	4.5E-02	5.7E-01
ADPRH	141	3	ADP-ribosylarginine hydrolase	3.54	4.00	2.94	2.93	4.10	0.01	-11.7	6.5E-03	5.8E-01
ADRB2	154	5	adrenoceptor beta 2, surface	3.97	2.45	1.44	2.70	2.39	0.33	-12.5	2.3E-02	3.7E-01
AFAP1	60312	4	actin filament associated protein 1	4.69	2.80	4.62	3.06	2.47	1.91	-6.9	2.5E-02	1.5E-01
AGPAT2	10555	9	1-acylglycerol-3-phosphate O-acyltransferase 2	4.83	4.55	4.79	3.96	4.37	6.65	3.5	3.9E-02	7.8E-01
AGPHD1	123688	15	aminoglycoside phosphotransferase domain containing 1	0.23	-1.16	-1.48	-1.58	-0.64	-7.11	-332.1	3.0E-02	7.4E-02
AIM2	9447	1	absent in melanoma 2	2.69	1.45	1.12	0.63	0.47	-1.96	-25.5	1.3E-02	2.3E-01
AK7	122481	14	adenylate kinase 7	-5.13	-4.41	-7.11	-7.11	-4.89	1.46	110.9	5.0E-04	1.0E+00
AKAP11	11215	13	A kinase (PRKA) anchor protein 11	5.63	5.59	6.43	4.84	4.52	3.55	-4.2	4.3E-02	9.3E-01
AKAP6	9472	14	A kinase (PRKA) anchor protein 6	0.33	1.40	0.55	-0.26	0.20	-7.11	-356.2	2.6E-02	7.6E-01
AKAP8L	26993	19	A kinase (PRKA) anchor protein 8-like	6.50	6.54	7.15	6.59	6.56	8.48	3.9	1.7E-02	8.9E-01
AKR1C1	1645	10	aldo-keto reductase family 1, member C1	-2.05	-0.82	-0.87	-1.15	0.13	1.32	10.5	2.8E-02	2.9E-01
AKT3	10000	1	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, delta)	4.70	4.69	5.21	4.47	4.14	2.25	-5.5	1.3E-02	9.5E-01
ALB	213	4	albumin	-2.89	-2.27	-2.16	-1.46	-0.83	1.81	26.7	1.5E-02	5.5E-01
ALDH9A1	223	1	aldehyde dehydrogenase 9 family, member A1	5.85	5.35	5.14	5.41	5.22	4.13	-3.3	4.3E-02	6.0E-01
ALG2	85365	9	ALG2, alpha-1,3/1,6-mannosyltransferase	4.60	5.34	4.62	4.56	4.86	2.70	-3.7	4.6E-02	5.1E-01
AMT	275	3	aminomethyltransferase	3.48	4.74	4.51	4.97	5.11	5.93	5.5	2.0E-02	2.2E-01
ANG	283	14	angiogenin, ribonuclease, RNase A family, 5	0.47	-1.45	1.47	-0.60	-2.59	-7.11	-392.3	4.5E-02	9.1E-01
ANGPT2	285	8	angiopoietin 2	-1.72	-0.31	-1.19	-0.36	-0.70	2.32	16.6	1.0E-02	5.1E-01
ANKLE1	126549	19	ankyrin repeat and LEM domain containing 1	2.07	3.08	3.01	3.53	3.17	5.18	8.6	9.1E-03	3.6E-01
ANKRD1	27063	10	ankyrin repeat domain 1 (cardiac muscle)	-5.13	-1.80	-2.81	-2.99	-1.62	3.46	443.2	6.8E-06	6.6E-02
ANKRD19P	138649	9	ankyrin repeat domain 19, pseudogene	0.62	0.49	1.82	1.87	1.83	3.64	8.1	3.4E-02	5.7E-01
ANKRD22	118932	10	ankyrin repeat domain 22	-1.53	-0.93	-0.94	-1.87	-2.37	4.57	69.0	2.2E-03	2.5E-01
ANKRD30BL	554226	2	ankyrin repeat domain 30B-like	-0.12	0.36	-2.81	-1.87	-2.59	5.92	66.1	6.8E-03	2.1E-01
ANKRD30BP2	149992	21	ankyrin repeat domain 30B pseudogene 2	-2.50	-3.52	-3.12	-3.38	-2.37	0.81	10.1	3.1E-02	4.4E-01
ANKRD40	91369	17	ankyrin repeat domain 40	5.44	5.20	5.57	5.56	5.25	3.73	-3.3	4.0E-02	8.6E-01
ANKRD44	91526	2	ankyrin repeat domain 44	6.67	7.20	6.83	7.20	7.16	4.75	-3.8	1.6E-02	4.4E-01
ANLN	54443	7	anillin, actin binding protein	-0.84	0.61	-0.34	0.49	0.26	3.55	21.1	1.3E-03	3.1E-01
ANO9	338440	11	anoctamin 9	6.24	6.35	6.18	6.96	6.90	8.12	3.7	4.3E-02	8.9E-01
ANXA2	302	15	annexin A2	7.44	7.38	5.61	7.42	7.59	3.83	-12.2	1.9E-03	8.2E-01
ANXA5	308	4	annexin A5	6.97	6.73	6.11	6.64	6.78	4.93	-4.1	4.2E-02	8.4E-01
AP4B1	10717	1	adaptor-related protein complex 4, beta 1 subunit	4.37	4.86	5.18	5.27	5.43	6.29	3.8	3.0E-02	3.6E-01
APH1A	51107	1	anterior pharynx defective 1 homolog A (C. elegans)	7.46	7.27	7.44	7.14	7.36	5.36	-4.3	8.9E-03	9.2E-01
APOBEC3G	60489	22	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C	6.18	4.11	4.74	4.35	4.37	3.64	-5.8	2.7E-02	1.5E-01
APOC1	341	19	apolipoprotein C-I	-3.14	-2.58	-0.39	-2.22	-2.02	0.59	13.7	3.4E-02	3.2E-01
AQP1	358	7	aquaporin 1 (Colton blood group)	-0.63	-3.52	-1.28	-1.72	-1.13	6.68	160.0	1.1E-05	1.5E-01
AQP6	363	12	aquaporin 6, kidney specific	-0.84	-2.02	-1.59	-7.11	-4.05	2.64	11.2	4.9E-02	2.0E-01
ARAP2	116984	4	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 2	5.67	6.91	7.21	6.56	6.14	3.75	-3.8	2.2E-02	1.0E-01
ARGFXP2	503640	17	arginine-fifty homeobox pseudogene 2	-3.44	-0.63	-1.59	-3.38	-4.05	0.33	14.1	3.9E-02	3.1E-01
ARHGAP23	57636	17	Rho GTPase activating protein 23	-0.89	1.34	-0.80	1.29	1.87	3.52	21.5	5.0E-03	7.8E-02
ARHGAP31	57514	3	Rho GTPase activating protein 31	4.34	4.64	4.67	3.46	3.54	1.58	-6.8	1.6E-02	8.9E-01
ARHGAP35	2909	19	Rho GTPase activating protein 35	6.08	4.58	5.89	4.07	3.66	3.70	-5.2	4.5E-02	1.7E-01
ARHGAP5	394	14	Rho GTPase activating protein 5	4.42	6.05	6.87	5.15	5.16	2.09	-5.0	2.6E-02	5.5E-02
ARHGEF10L	55160	1	Rho guanine nucleotide exchange factor (GEF) 10-like	4.20	4.83	3.48	4.34	4.36	-7.11	-5208.8	9.2E-06	6.1E-01
ARHGEF11	9826	1	Rho guanine nucleotide exchange factor (GEF) 11	5.95	5.52	5.11	5.22	4.78	4.02	-3.8	3.5E-02	3.9E-01
ARHGEF12	23365	11	Rho guanine nucleotide exchange factor (GEF) 12	5.11	4.10	5.00	4.06	3.66	7.19	4.2	3.8E-02	2.8E-01
ARHGEF17	9828	11	Rho guanine nucleotide exchange factor (GEF) 17	-0.41	-0.05	2.96	0.71	0.60	2.90	10.0	4.7E-02	2.2E-01
ARHGEF3	50650	3	Rho guanine nucleotide exchange factor (GEF) 3	7.19	5.83	6.67	6.32	5.31	4.75	-5.4	1.6E-02	4.0E-01
ARHGEF37	389337	5	Rho guanine nucleotide exchange factor (GEF) 37	-4.33	-2.98	-0.87	-2.68	-3.14	4.04	357.8	2.9E-05	1.6E-01
ARID1A	8289	1	AT rich interactive domain 1A (SWI-like)	7.62	7.71	7.84	7.69	7.61	5.85	-3.4	2.4E-02	9.5E-01
ARID1B	57492	6	AT rich interactive domain 1B (SWI1-like)	6.63	6.99	7.02	6.79	6.55	4.82	-3.5	2.1E-02	8.3E-01
ARL4C	10123	2	ADP-ribosylation factor-like 4C	8.70	9.06	8.51	8.96	8.76	6.26	-5.4	1.2E-02	6.8E-01
ARNTL2	56938	12	aryl hydrocarbon receptor nuclear translocator-like 2	0.96	1.45	1.55	1.48	0.41	3.80	7.2	2.0E-02	7.5E-01
ARPM1	NA	NA	NA	-2.50	-1.61	-4.03	-1.87	-1.74	1.17	13.0	2.4E-02	6.6E-01
ART4	420	12	ADP-ribosyltransferase 4 (Dombrock blood group)	-7.11	-7.11	-7.11	-7.11	-7.11	2.00	1132.0	1.3E-04	1.0E+00
ASB3	51130	2	ankyrin repeat and SOCS box containing 3	-1.01	0.53	-0.15	0.58	0.16	1.91	7.6	3.9E-02	3.6E-01
ASCC2	84164	22	activating signal cointegrator 1 complex subunit 2	6.36	6.00	6.40	6.51	6.57	8.10	3.3	2.8E-02	8.5E-01
ASGR1	432	17	asialoglycoprotein receptor 1	3.51	2.64	2.94	2.24	1.85	0.33	-9.1	3.1E-02	7.0E-01
ASGR2	433	17	asialoglycoprotein receptor 2	2.94	4.55	2.92	4.19	3.90	-1.96	-30.2	2.2E-03	1.1E-01
ASH1L	55870	1	ash1 (absent, small, or homeotic)-like (Drosophila)	5.74	7.00	7.77	6.32	6.00	3.55	-4.6	3.1E-02	2.3E-01
ASPHD1	253982	16	aspartate beta-hydroxylase domain containing 1	-4.33	-2.98	-1.02	-2.22	-1.88	0.59	32.6	7.0E-03	1.3E-01
ASPM	259266	1	asp (abnormal spindle) homolog, microcephaly associated (Drosophila)	0.54	0.22	0.75	0.14	-0.14	4.46	15.2	2.2E-03	8.4E-01
ASTE1	28990	3	asteroid homolog 1 (Drosophila)	3.54	3.28	3.69	3.50	3.43	0.81	-6.7	1.6E-02	8.7E-01
ATAD1	84896	10	ATPase family, AAA domain containing 1	4.74	4.29	5.50	4.35	4.21	2.81	-3.8	4.9E-02	8.3E-01
ATAD2B	54454	2	ATPase family, AAA domain containing 2B	4.45	5.21	5.75	4.68	4.64	2.64	-3.5	4.9E-02	2.7E-01
ATAD3C	219293	1	ATPase family, AAA domain containing 3C	1.83	1.31	1.08	2.00	1.87	4.64	7.0	2.0E-02	6.0E-01
ATF7IP	55729	12	activating transcription factor 7 interacting protein	5.80	6.36	6.28	5.94	5.95	3.95	-3.6	2.0E-02	5.3E-01
ATHL1	80162	11	ATH1, acid trehalase-like 1 (yeast)	9.15	9.59	9.89	10.37	9.07	11.57	5.4	3.6E-02	6.1E-01
ATM	472	11	ataxia telangiectasia mutated	8.23	8.38	8.20	7.79	7.				

ATP1A3	478	19	ATPase, Na+/K+ transporting, alpha 3 polypeptide	3.69	2.40	2.61	2.53	2.12	0.01	-12.9	4.2E-03	1.2E-01
ATP2C2	9914	16	ATPase, Ca++ transporting, type 2C, member 2	-0.84	-3.52	-0.87	-0.81	-0.32	2.81	12.6	2.8E-02	6.5E-01
ATP9B	374868	18	ATPase, class II, type 9B	4.89	4.13	4.92	4.33	4.44	2.95	-3.8	3.5E-02	5.6E-01
ATXN2	6311	12	ataxin 2	4.45	3.46	4.58	3.52	3.44	2.09	-5.1	4.3E-02	4.3E-01
ATXN7	6314	3	ataxin 7	5.67	7.38	7.24	6.80	6.57	3.86	-3.5	3.9E-02	6.0E-02
ATXN7L3B	552889	12	ataxin 7-like 3B	7.30	7.01	6.83	6.86	6.68	5.35	-3.8	1.8E-02	6.6E-01
AURKA	6790	20	aurora kinase A	0.97	-0.54	-0.61	-1.24	-1.40	4.71	13.4	1.5E-02	3.1E-01
AURKB	9212	17	aurora kinase B	0.68	-0.24	0.95	0.29	0.41	4.39	13.1	5.4E-03	5.8E-01
AVP	551	20	arginine vasopressin	-4.33	-7.11	0.65	-2.99	-2.19	1.70	70.5	2.0E-02	1.5E-01
B3GAT1	27087	11	beta-1,3-glucuronyltransferase 1 (glucuronosyltransferase P)	2.15	2.70	0.70	2.21	0.75	-7.11	-1259.3	2.4E-03	6.4E-01
B3GNT9	84752	16	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 9	1.86	-1.30	0.32	-0.36	-1.40	-1.96	-14.4	4.3E-02	8.6E-02
BAALC	79870	8	brain and acute leukemia, cytoplasmic	0.40	-0.38	-1.85	-0.81	0.72	4.85	21.9	3.6E-03	5.6E-01
BAHD1	22893	15	bromo adjacent homology domain containing 1	6.13	5.57	5.42	5.42	5.56	4.23	-3.8	1.8E-02	3.0E-01
BAIAP2L2	80115	22	BAI1-associated protein 2-like 2	0.31	0.32	0.60	0.88	1.19	3.28	7.9	2.3E-02	8.1E-01
BAZ1A	11177	14	bromodomain adjacent to zinc finger domain, 1A	6.32	6.40	6.89	6.57	6.66	8.02	3.2	2.8E-02	5.5E-01
BBX	56987	3	bobby sox homolog (Drosophila)	5.69	6.94	7.25	6.61	6.38	3.49	-4.6	9.8E-03	1.0E-01
BCAM	4059	19	basal cell adhesion molecule (Lutheran blood group)	-5.13	-4.41	-3.50	-3.91	-2.59	5.70	2104.5	4.5E-08	3.3E-01
BCL11B	64919	14	B-cell CLL/lymphoma 11B (zinc finger protein)	7.60	6.75	8.24	7.15	6.88	4.76	-7.2	2.7E-03	7.7E-01
BCL2L1	598	20	BCL2-like 1	6.15	5.20	4.38	5.21	5.54	10.78	24.8	7.4E-06	2.5E-01
BCL2L15	440603	1	BCL2-like 15	1.65	0.65	2.07	0.93	1.43	5.15	11.3	1.0E-02	5.1E-01
BCL9	607	1	B-cell CLL/lymphoma 9	4.90	4.92	3.75	3.68	4.16	1.91	-8.0	4.5E-03	2.6E-01
BCL9L	283149	11	B-cell CLL/lymphoma 9-like	8.38	7.65	9.01	8.61	7.95	6.29	-4.3	3.0E-02	8.7E-01
BCYRN1	618	2	brain cytoplasmic RNA 1	-0.68	-0.24	-0.49	0.14	0.41	2.46	8.9	3.6E-02	7.4E-01
BHLHE41	79365	12	basic helix-loop-helix family, member e41	0.75	1.86	1.42	-0.06	0.41	-7.11	-476.6	1.6E-02	7.7E-01
BICD1	636	12	bicaudal D homolog 1 (Drosophila)	2.81	2.19	1.56	1.45	1.24	-0.40	-9.3	2.0E-02	1.6E-01
BIRC5	332	17	baculoviral IAP repeat containing 5	1.11	-0.63	-1.59	0.10	0.23	5.22	17.3	2.1E-03	1.0E-01
BIRC6	57448	2	baculoviral IAP repeat containing 6	6.89	7.70	8.21	7.15	6.96	4.63	-4.8	9.7E-03	3.5E-01
BLVRB	645	19	biliverdin reductase B (flavin reductase (NADPH))	4.88	3.63	2.44	3.72	3.88	9.11	18.7	1.3E-03	4.1E-01
BMP2K	55589	4	BMP2 inducing kinase	3.35	4.23	5.06	3.46	3.76	5.41	4.2	4.6E-02	2.8E-01
BMPRI1A	657	10	bone morphogenetic protein receptor, type IA	2.22	1.14	2.41	1.39	0.79	-1.96	-18.4	2.1E-02	6.6E-01
BPGM	669	7	2,3-bisphosphoglycerate mutase	3.70	4.71	5.01	5.04	4.72	9.96	76.6	2.5E-09	8.8E-02
BRDT	676	1	bromodomain, testis-specific	-1.44	-4.41	-2.81	-3.38	-3.14	1.58	8.2	4.9E-02	8.4E-02
BRF2	55290	8	BRF2, subunit of RNA polymerase III transcription initiation factor, B	3.87	3.87	3.17	4.07	4.12	1.70	-4.5	3.9E-02	9.9E-01
BRIP1	83990	17	BRCA1 interacting protein C-terminal helicase 1	2.23	2.51	3.06	2.10	2.16	4.84	6.1	2.6E-02	9.6E-01
BSG	682	19	basigin (Ok blood group)	7.75	6.50	6.05	6.15	6.25	10.29	5.8	1.1E-02	1.2E-01
BTBD9	114781	6	BTB (POZ) domain containing 9	4.94	5.48	5.79	5.08	4.78	3.09	-3.6	4.1E-02	5.7E-01
BTNL8	79908	5	butyrophilin-like 8	-3.82	-3.52	-4.03	0.10	-0.97	2.90	111.3	6.4E-03	2.3E-01
BUB1	699	2	BUB1 mitotic checkpoint serine/threonine kinase	1.71	0.57	0.77	0.88	0.90	4.17	5.5	3.7E-02	3.6E-01
BUB1B	701	15	BUB1 mitotic checkpoint serine/threonine kinase B	0.15	-0.63	0.70	-0.54	0.20	3.39	9.5	1.5E-02	8.7E-01
BVES	11149	6	blood vessel epicardial substance	-0.89	-2.27	-1.72	-1.87	-1.88	2.00	7.5	3.0E-02	2.1E-01
C12orf34	NA	NA	NA	0.54	0.36	1.68	1.00	0.09	-7.11	-410.4	2.9E-02	5.8E-01
C12orf35	NA	NA	NA	7.95	6.64	8.01	6.00	5.69	5.48	-5.6	3.9E-02	5.6E-01
C14orf169	79697	14	chromosome 14 open reading frame 169	4.55	3.69	3.80	4.21	3.92	0.81	-13.4	6.5E-04	4.3E-01
C14orf21	NA	NA	NA	4.09	4.77	4.67	4.22	4.02	1.46	-6.2	8.2E-03	6.1E-01
C14orf45	NA	NA	NA	1.40	0.88	0.86	0.39	0.53	4.17	6.8	2.0E-02	4.4E-01
C15orf38	348110	15	chromosome 15 open reading frame 38	0.56	2.31	-0.49	0.91	0.39	-7.11	-416.4	2.9E-02	3.6E-01
C17orf103	256302	17	chromosome 17 open reading frame 103	4.88	5.27	4.91	4.19	4.45	7.47	6.0	4.8E-03	8.7E-01
C18orf1	NA	NA	NA	5.10	4.59	5.68	4.09	4.02	2.75	-5.1	2.2E-02	8.1E-01
C19orf35	374872	19	chromosome 19 open reading frame 35	2.01	1.96	0.77	1.95	2.42	4.64	6.2	3.9E-02	9.3E-01
C19orf51	NA	NA	NA	-5.13	-2.98	-4.87	-4.76	-2.84	1.00	80.7	8.5E-03	1.6E-01
C19orf63	NA	NA	NA	6.75	6.16	5.48	6.12	6.23	4.97	-3.4	4.1E-02	4.9E-01
C19orf76	NA	NA	NA	1.41	2.07	2.20	2.89	3.26	4.57	8.9	2.1E-02	3.8E-01
C19orf77	284422	19	chromosome 19 open reading frame 77	-0.54	-1.45	-1.28	-0.21	0.79	3.80	20.4	5.2E-03	9.7E-01
C1orf105	92346	1	chromosome 1 open reading frame 105	-7.11	-4.41	-4.03	-4.76	-7.11	1.32	707.9	3.5E-04	2.6E-01
C1orf115	79762	1	chromosome 1 open reading frame 115	2.07	2.06	1.78	1.37	2.04	-7.11	-1193.1	1.4E-03	7.5E-01
C1orf135	NA	NA	NA	-0.84	1.09	-0.55	-0.31	-0.70	2.52	10.3	1.9E-02	4.8E-01
C1orf159	54991	1	chromosome 1 open reading frame 159	3.47	3.92	4.15	4.14	4.45	5.68	4.6	2.5E-02	5.4E-01
C1orf172	126695	1	chromosome 1 open reading frame 172	2.07	-0.11	1.06	0.22	-0.83	-1.96	-16.6	2.0E-02	5.7E-02
C1orf228	339541	1	chromosome 1 open reading frame 228	5.35	5.05	4.10	5.51	5.90	7.80	5.4	9.6E-03	7.8E-01
C1orf229	388759	1	chromosome 1 open reading frame 229	-0.63	-1.30	-1.28	-0.60	-0.83	3.46	17.1	3.9E-03	5.5E-01
C1orf50	79078	1	chromosome 1 open reading frame 50	3.40	3.22	2.98	3.19	3.06	1.00	-5.3	3.5E-02	7.1E-01
C1QA	712	1	complement component 1, q subcomponent, A chain	2.44	2.35	-0.15	0.39	1.79	-7.11	-1542.3	2.8E-03	7.5E-01
C2	717	6	complement component 2	1.31	2.58	2.49	2.14	2.12	-7.11	-699.4	4.9E-03	1.7E-01
C20orf194	25943	20	chromosome 20 open reading frame 194	3.04	4.84	5.43	4.23	4.02	0.59	-5.5	3.6E-02	8.9E-02
C21orf91	54149	21	chromosome 21 open reading frame 91	4.81	5.65	5.72	5.55	5.45	2.17	-6.3	4.0E-03	1.3E-01
C22orf23	84645	22	chromosome 22 open reading frame 23	2.62	2.66	2.94	2.91	3.02	-1.96	-24.4	4.0E-03	9.6E-01
C22orf26	55267	22	chromosome 22 open reading frame 26	0.66	-1.04	-0.61	-2.68	-2.02	-7.11	-446.5	2.5E-02	1.1E-01
C22orf28	51493	22	chromosome 22 open reading frame 28	5.91	5.94	5.76	5.28	5.56	4.26	-3.1	4.7E-02	8.3E-01
C3orf19	NA	NA	NA	4.44	5.46	5.10	4.87	4.68	1.91	-5.8	8.6E-03	3.2E-01
C4BPB	725	1	complement component 4 binding protein, beta	-3.14	0.01	-0.11	-2.03	-0.10	1.58	27.3	1.0E-02	1.0E-01
C5orf45	51149	5	chromosome 5 open reading frame 45	3.05	2.40	2.24	2.42	2.47	0.33	-6.6	3.4E-02	3.3E-01
C6orf132	647024	6	chromosome 6 open reading frame 132	-1.82	-0.72	-1.10	-0.97	-1.21	2.52	20.5	2.6E-03	5.5E-01
C6orf174	NA	NA	NA	0.28	1.09	1.24	0.91	0.36	-7.11	-344.2	2.5E-02	5.7E-01
C6orf48	50854	6	chromosome 6 open reading frame 48	7.58	7.02	7.30	7.82	7.88	5.74	-3.6	2.8E-02	7.9E-01
C7orf59	NA	NA	NA	6.73	5.57	5.39	5.63	6.04	8.49	3.4	2.7E-02	9.8E-02
C8orf73	NA	NA	NA	3.17	3.73	2.58	4.23	3.49	5.66	5.6	1.7E-02	6.3E-01
C9orf102	NA	NA	NA	2.43	3.58	3.52	2.91	2.58	-0.40	-7.2	3.0E-02	3.7E-01
C9orf152	401546	9	chromosome 9 open reading frame 152	-7.11	-3.52	-4.87	-7.11	-7.11	0.81	495.8	1.5E-03	3.2E-01
C9orf172	389813	9	chromosome 9 open reading frame 172	2.52	1.78	1.32	1.86	1.07	-7.11	-1620.5	5.7E-04	3.0E-01
C9orf66	157983	9	chromosome 9 open reading frame 66	0.66	1.36	0.16	0.33	1.29	3.86	9.2	1.4E-02	7.2E-01
CA1	759	8	carbonic anhydrase I	-5.13	-2.58	-7.11	-3.38	0.79	11.40	108955.0	2.1E-06	7.0E-02
CA12	771	15	carbonic anhydrase XII	-3.14	-1.30	-0.87	-1.46	-2.59	0.33	11.4	4.0E-02	2.9E-01
CA2	760	8	carbonic anhydrase II	4.98	3.90	3.26	3.42	4.00	8.19	9.2	4.5E-03	2.2E-01
CA3	761	8	carbonic anhydrase III, muscle specific	-1.28	-4.41	-4.03	-2.43	-1.62	3.09	20.8	8.9E-03	3.8E-01
CA8	767	8	carbonic anhydrase VIII	-2.19	-1.80	-2.16	-1.72	-1.62	3.21	42.7	5.9E-04	7.6E-01
CACNA2D3	55799	3	calcium channel, voltage-dependent, alpha 2/delta subunit 3	2.40	2.00	-1.38	1.42	1.47	-7.11	-1500.1	4.9E-03	7.4E-01
CADM1	23705	11	cell adhesion molecule 1	1.63	-0.82	1.57	1.56	0.36	-7.11	-874.0	5.8E-03	6.6E-01
CALHM2	51063	10	calcium homeostasis modulator 2	5.73	3.00	3.27	2.98	2.60	2.25	-11.2	3.0E-02	1.8E-01
CALML6	163688	1	calmodulin-like 6	-1.14	-2.02	-1.48	0.58	-0.18	2.32	11.1	3.3E-02	7.4E-01
CAMK2N1	55450	1	calcium/calmodulin-dependent protein kinase II inhibitor 1	1.52	0.65	1.13	1.79	1.74	-7.11	-813.8	3.9E-03	9.3E-01
CAMK4	814	5	calcium/calmodulin-dependent protein kinase IV	5.70	4.92	5.48	4.82	5.02	2.90	-7.0	2.2E-03	3.6E-01
CAMP	820	3	cathelicidin antimicrobial peptide	-1.36	-2.27	-2.56	-1.87	-0.02	9.58	1981.6	6.6E-13	7.7E-01
CAMSAP2	23271	1	calmodulin regulated spectrin-associated protein family, member 2	2.83	2.00	3.45	1.14	2.03	-0.98	-14.2	1.4E-02	7.9E-01
CAPN2	824	1	calpain 2, (mII) large subunit	7.90	8.03	6.86	7.90	7.96	6.08	-3.5	4.1E-02	9.5E-01
CASC5	57082	15	cancer susceptibility candidate 5	-0.02	2.85	1.60	0.95	1.37	3.21	9.4	2.5E-02	1.1E-01
CASD1	64921	7	CAS1 domain containing 1									

CBR3	874	21	carbonyl reductase 3	2.11	1.11	1.35	0.88	1.00	-7.11	-1223.2	1.2E-03	1.9E-01
CBX6	23466	22	chromobox homolog 6	5.49	7.24	6.18	7.08	6.71	3.09	-5.3	1.7E-02	1.3E-01
CCBE1	147372	18	collagen and calcium binding EGF domains 1	0.75	-1.04	-0.49	-0.54	-0.47	4.58	14.2	5.4E-03	5.8E-02
CCDC109B	55013	4	coiled-coil domain containing 109B	6.49	5.57	5.80	5.86	5.67	4.41	-4.2	1.4E-02	4.2E-01
CCDC122	160857	13	coiled-coil domain containing 122	0.56	-0.05	0.41	0.02	-0.22	3.32	6.8	2.7E-02	5.6E-01
CCDC134	79879	22	coiled-coil domain containing 134	2.09	3.63	2.62	2.08	2.06	-0.98	-8.5	3.9E-02	5.3E-01
CCDC149	91050	4	coiled-coil domain containing 149	1.67	3.48	1.75	3.43	2.74	-1.96	-12.5	3.5E-02	1.2E-01
CCDC154	645811	16	coiled-coil domain containing 154	2.70	2.00	2.47	1.77	2.44	5.45	6.7	1.7E-02	4.2E-01
CCDC164	92749	2	coiled-coil domain containing 164	-4.33	-7.11	-3.12	-4.76	-2.84	1.81	76.0	1.2E-03	7.6E-01
CCDC18	343099	1	coiled-coil domain containing 18	2.59	3.10	2.89	3.54	2.70	4.88	4.9	3.0E-02	5.8E-01
CCDC39	339829	3	coiled-coil domain containing 39	0.99	2.82	3.57	2.58	2.19	-7.11	-560.9	8.0E-03	5.2E-02
CCDC48	NA	NA	NA	-1.21	-3.52	-4.87	-1.46	-1.62	2.17	10.5	3.9E-02	4.6E-01
CCDC69	26112	5	coiled-coil domain containing 69	7.95	8.25	7.72	8.06	7.94	6.24	-3.3	4.3E-02	7.5E-01
CCDC71	64925	3	coiled-coil domain containing 71	4.59	2.96	3.38	2.58	2.51	2.00	-6.0	3.7E-02	2.2E-01
CCDC84	338657	11	coiled-coil domain containing 84	3.34	4.95	6.32	5.61	5.79	5.97	6.2	4.5E-02	7.8E-02
CCDC85C	317762	14	coiled-coil domain containing 85C	3.76	2.60	2.05	2.71	2.67	1.00	-6.8	2.5E-02	2.3E-01
CCDC88B	283234	11	coiled-coil domain containing 88B	8.22	7.89	7.58	8.38	8.48	10.68	5.5	1.1E-02	6.9E-01
CCL3	6348	17	chemokine (C-C motif) ligand 3	7.04	5.42	4.48	7.36	6.67	1.91	-35.2	9.4E-04	2.4E-01
CCL4	6351	17	chemokine (C-C motif) ligand 4	5.63	3.81	2.55	6.35	5.10	1.00	-24.8	1.8E-03	4.7E-01
CCNA2	890	4	cyclin A2	1.25	0.91	0.68	1.27	1.41	4.84	12.0	2.9E-03	8.1E-01
CCNB1	891	5	cyclin B1	1.69	0.32	-0.24	0.33	0.30	5.25	11.8	4.1E-03	1.2E-01
CCNB2	9133	15	cyclin B2	1.16	-0.24	-0.87	0.55	0.53	5.04	14.7	2.9E-03	1.6E-01
CCR4	1233	3	chemokine (C-C motif) receptor 4	5.10	4.09	5.51	3.38	3.12	0.81	-19.6	2.4E-03	6.7E-01
CCR5	1234	3	chemokine (C-C motif) receptor 5 (gene/pseudogene)	5.21	3.43	3.41	1.64	2.42	-1.96	-146.8	2.7E-04	2.5E-01
CCR7	1236	17	chemokine (C-C motif) receptor 7	8.81	7.75	8.82	6.44	6.72	6.33	-5.6	4.3E-02	3.6E-01
CD14	929	5	CD14 molecule	7.45	6.34	5.63	5.61	5.85	3.61	-14.3	2.0E-02	8.6E-01
CD163	9332	12	CD163 molecule	4.41	3.90	0.16	3.32	3.51	-7.11	-6045.7	1.3E-03	6.7E-01
CD177	57126	19	CD177 molecule	-7.11	-7.11	-7.11	-3.91	-2.84	6.44	24530.2	6.7E-09	1.2E-01
CD19	930	16	CD19 molecule	4.58	4.89	4.69	4.62	5.95	7.79	9.2	2.6E-03	7.3E-01
CD1D	912	1	CD1d molecule	4.89	6.11	5.47	5.80	5.52	2.81	-4.3	2.0E-02	1.2E-01
CD22	933	19	CD22 molecule	6.33	6.56	5.86	5.77	6.93	8.61	4.9	1.5E-02	7.4E-01
CD24	100133941	6	CD24 molecule	3.84	1.61	1.68	0.79	2.29	8.19	20.4	2.0E-03	8.1E-02
CD244	51744	1	CD244 molecule, natural killer cell receptor 2B4	5.64	4.37	3.78	4.16	3.79	3.04	-6.1	4.9E-02	4.1E-01
CD28	940	2	CD28 molecule	6.78	6.14	6.76	6.27	5.49	4.28	-5.7	7.4E-03	6.3E-01
CD300C	10871	17	CD300c molecule	4.28	4.06	2.08	2.47	3.88	0.33	-15.6	9.1E-03	9.4E-01
CD300E	342510	17	CD300e molecule	5.99	7.57	6.26	5.75	6.90	0.01	-63.5	1.5E-04	1.1E-01
CD4	920	12	CD4 molecule	8.86	7.51	6.57	7.16	7.03	5.48	-10.4	2.3E-03	1.1E-01
CD44	960	11	CD44 molecule (Indian blood group)	9.09	9.53	9.39	9.57	9.89	6.95	-4.4	2.7E-02	4.4E-01
CD48	962	1	CD48 molecule	9.23	8.32	7.23	8.78	8.69	6.84	-5.2	1.6E-02	3.1E-01
CD5	921	11	CD5 molecule	8.37	6.91	8.18	8.33	7.42	5.51	-7.3	4.3E-03	4.7E-01
CD72	971	9	CD72 molecule	3.91	2.59	3.61	2.73	3.50	6.24	5.0	2.1E-02	3.0E-01
CD86	942	3	CD86 molecule	4.95	6.37	6.78	5.19	5.85	1.58	-10.3	3.9E-03	5.3E-02
CD8A	925	2	CD8a molecule	7.96	5.30	6.96	6.81	6.32	4.49	-11.1	7.5E-04	8.2E-02
CDA	978	1	cytidine deaminase	3.03	4.17	2.91	4.54	3.95	6.68	12.6	4.6E-04	1.2E-01
CDC14A	8556	1	cell division cycle 14A	5.72	5.76	5.96	6.83	6.33	3.97	-3.4	4.3E-02	4.5E-01
CDC25A	993	3	cell division cycle 25A	-0.95	-0.05	-1.85	-0.06	-0.76	3.42	20.9	3.9E-03	8.2E-01
CDC25C	995	5	cell division cycle 25C	-3.14	-4.41	-4.87	-2.99	-2.37	1.70	29.6	5.6E-03	7.6E-01
CDC34	997	19	cell division cycle 34	5.21	5.02	5.56	4.80	4.71	7.75	5.8	2.5E-03	7.4E-01
CDC42EP3	10602	2	CDC42 effector protein (Rho GTPase binding) 3	6.21	7.42	5.19	7.10	7.06	3.83	-5.2	2.1E-02	2.6E-01
CDC42SE2	56990	5	CDC42 small effector 2	8.11	7.91	8.89	8.31	8.26	5.85	-4.8	2.2E-02	4.7E-01
CDC45	8318	22	cell division cycle 45	0.18	0.22	0.12	0.02	0.44	3.35	9.1	1.4E-02	9.4E-01
CDC6	990	17	cell division cycle 6	0.31	0.17	-1.38	0.69	0.86	3.67	10.3	1.5E-02	9.7E-01
CDCA2	157313	8	cell division cycle associated 2	-1.14	-0.63	-1.19	-1.46	-0.90	3.13	19.4	1.9E-03	9.6E-01
CDCA5	113130	11	cell division cycle associated 5	1.18	0.68	-0.29	0.88	1.00	4.39	9.3	1.1E-02	6.3E-01
CDCA8	55143	1	cell division cycle associated 8	1.45	1.71	0.01	1.30	0.86	4.37	7.6	2.2E-02	7.8E-01
CDH1	999	16	cadherin 1, type 1, E-cadherin (epithelial)	0.18	-0.24	1.74	-0.48	0.33	3.90	13.3	1.2E-02	5.9E-01
CDK1	983	10	cyclin-dependent kinase 1	0.38	0.36	-0.11	0.76	0.47	3.28	7.5	3.0E-02	7.5E-01
CDK5R1	8851	17	cyclin-dependent kinase 5, regulatory subunit 1 (p35)	4.81	5.33	5.99	5.90	4.64	2.70	-4.3	4.3E-02	4.0E-01
CDK5RAP3	80279	17	CDK5 regulatory subunit associated protein 3	7.36	7.44	7.75	8.03	7.98	9.28	3.8	3.3E-02	7.5E-01
CDKN1C	1028	11	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	4.94	3.44	2.58	3.44	2.24	2.09	-7.2	4.2E-02	2.5E-01
CDKN2AIP	55602	4	CDKN2A interacting protein	5.30	5.37	5.73	5.85	5.49	3.49	-3.5	3.1E-02	5.8E-01
CDKN3	1033	14	cyclin-dependent kinase inhibitor 3	-0.30	-0.38	-0.87	0.33	0.26	3.58	14.8	3.7E-03	8.4E-01
CDR2	1039	16	cerebellar degeneration-related protein 2, 62kDa	6.42	5.98	6.26	6.69	6.61	4.39	-4.1	2.2E-02	9.9E-01
CDT1	81620	16	chromatin licensing and DNA replication factor 1	1.79	0.65	-0.07	1.50	1.04	5.53	13.3	3.9E-03	2.6E-01
CEACAM1	634	19	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary g	1.27	-1.30	-1.28	-1.46	-0.52	5.58	19.9	6.3E-03	6.5E-02
CEACAM3	1084	19	carcinoembryonic antigen-related cell adhesion molecule 3	-0.05	-0.11	-3.12	0.71	0.79	5.95	64.5	4.8E-04	7.0E-01
CEACAM6	4680	19	carcinoembryonic antigen-related cell adhesion molecule 6 (non-spe	-5.13	-2.27	-4.87	-4.76	-2.84	6.88	4742.6	3.8E-10	2.7E-01
CEACAM8	1088	19	carcinoembryonic antigen-related cell adhesion molecule 8	-0.95	-0.93	-1.59	-1.46	-0.22	7.50	351.8	4.4E-09	6.8E-01
CEBPE	1053	14	CCAAT/enhancer binding protein (C/EBP), epsilon	0.97	0.36	-0.94	-0.42	-0.06	6.07	34.4	3.8E-04	5.5E-01
CECR7	100130418	22	cat eye syndrome chromosome region, candidate 7 (non-protein cod	0.07	1.42	1.80	0.39	0.23	-7.11	-296.0	4.0E-02	4.0E-01
CEL	1056	9	carboxyl ester lipase (bile salt-stimulated lipase)	-1.08	-1.16	-0.80	-0.67	-1.05	2.17	9.5	1.5E-02	9.7E-01
CELF2	10659	10	CUGBP, Elav-like family member 2	7.65	8.50	8.66	8.62	8.05	5.94	-3.3	4.5E-02	2.6E-01
CELF3	11189	1	CUGBP, Elav-like family member 3	-3.14	-2.98	-2.35	-2.22	-2.84	0.33	11.4	2.8E-02	8.1E-01
CENPA	1058	2	centromere protein A	-1.14	1.24	-0.39	0.84	0.20	2.46	12.2	2.3E-02	2.7E-01
CENPE	1062	4	centromere protein E, 312kDa	1.15	2.19	1.79	2.07	1.57	4.50	10.3	9.0E-03	2.9E-01
CENPM	79019	22	centromere protein M	1.08	-0.93	-1.38	-0.60	-0.18	3.70	6.2	4.2E-02	7.2E-02
CENPW	387103	6	centromere protein W	0.40	0.79	-1.48	0.39	0.47	3.49	8.5	2.6E-02	8.8E-01
CES1	1066	16	carboxylesterase 1	3.29	4.64	1.83	5.49	5.46	-7.11	-2764.5	2.5E-04	8.8E-02
CHI3L1	1116	1	chitinase 3-like 1 (cartilage glycoprotein-39)	-2.68	-2.58	-4.03	0.88	1.54	7.40	1107.6	6.5E-05	1.2E-01
CHIT1	1118	1	chitinase 1 (chitotriosidase)	-2.50	-1.04	-0.24	-0.81	-0.37	6.97	721.8	5.9E-07	2.3E-01
CHN1	1123	2	chimerin 1	0.01	0.01	1.98	0.26	-0.22	-7.11	-284.0	4.4E-02	4.0E-01
CHN2	1124	7	chimerin 2	3.24	3.34	2.38	2.82	2.58	0.33	-7.6	3.0E-02	9.4E-01
CHST14	113189	15	carbohydrate (N-acetyl/galactosamine 4-O) sulfotransferase 14	4.20	2.35	2.94	2.33	2.30	1.81	-5.3	4.6E-02	8.5E-02
CHST6	4166	16	carbohydrate (N-acetyl/glucosamine 6-O) sulfotransferase 6	1.53	0.94	1.44	0.76	0.77	4.67	8.8	1.3E-02	3.8E-01
CHTF18	63922	16	CTF18, chromosome transmission fidelity factor 18 homolog (S. cere	4.30	4.27	3.93	4.97	4.83	6.60	4.9	1.3E-02	9.0E-01
CHTF8	54921	16	CTF8, chromosome transmission fidelity factor 8 homolog (S. cerevi	6.76	5.16	5.36	5.10	5.20	4.94	-3.5	4.7E-02	6.6E-02
CISD2	493856	4	CDGSH iron sulfur domain 2	3.04	3.49	4.16	3.89	3.84	5.57	5.8	8.3E-03	1.7E-01
CIT	11113	12	citron (rho-interacting, serine/threonine kinase 21)	1.12	0.79	0.60	0.63	0.88	4.52	10.6	6.3E-03	5.0E-01
CKAP2L	150468	2	cytoskeleton associated protein 2-like	-1.01	-0.63	-1.28	-0.54	-0.83	3.04	16.7	2.9E-03	8.2E-01
CKS2	1164	9	CDC28 protein kinase regulatory subunit 2	2.02	2.11	1.17	1.48	2.24	4.82	7.0	1.7E-02	9.6E-01
CLC	1178	19	Charcot-Leyden crystal protein	-2.05	-1.45	-2.81	-0.97	-0.58	4.06	70.5	4.2E-04	5.8E-01
CLDN11	5010	3	claudin 11	-1.62	-1.61	-0.73	-1.46	-1.30	1.91	11.6	2.0E-02	9.7E-01
CLDN9	9080	16	claudin 9	-1.72	-2.98	-2.56	-0.31	-1.05	3.09	28.2	8.2E-03	9.9E-01
CLEC11A	6320	19	C-type lectin domain family 11, member A	2.95	3.32	-0.29	1.63	2.58	5.85	7.5	3.2E-02	5.5E-01
CLEC12B	387837	12	C-type lectin domain family 12, member B	0.86	2.16	-1.28	3.11	1.98	-7.11	-512.8	2.8E-02	4.0E-0

CLTCL1	8218	22	clathrin, heavy chain-like 1	1.29	1.31	1.27	0.66	0.88	4.72	10.8	2.1E-02	9.4E-01
CMTM2	146225	16	CKLF-like MARVEL transmembrane domain containing 2	-0.46	-0.63	-1.19	0.58	2.15	4.66	34.7	2.1E-03	2.9E-01
CMTM3	123920	16	CKLF-like MARVEL transmembrane domain containing 3	6.61	6.78	6.04	6.73	6.55	4.93	-3.2	3.6E-02	9.6E-01
CMTM8	152189	3	CKLF-like MARVEL transmembrane domain containing 8	2.09	-0.93	0.92	0.91	0.30	-1.96	-16.8	2.6E-02	2.9E-01
CN5H6.4	150384	22	uncharacterized LOC150384	0.07	-0.17	-0.87	0.42	0.06	-7.11	-296.0	4.1E-02	6.5E-01
CNGB1	1258	16	cyclic nucleotide gated channel beta 1	-1.93	-2.98	-4.03	-3.91	-2.59	2.25	18.4	5.4E-03	1.4E-01
CNTNAP1	8506	17	contactin associated protein 1	3.65	3.19	3.21	3.36	3.18	0.59	-8.4	1.9E-02	4.4E-01
COG1	9382	17	component of oligomeric golgi complex 1	4.96	5.65	6.03	5.58	5.35	3.04	-3.8	3.4E-02	4.4E-01
COL23A1	91522	5	collagen, type XXIII, alpha 1	-2.68	-1.61	2.01	-1.24	0.47	2.25	31.2	1.4E-02	7.7E-02
COL24A1	255631	1	collagen, type XXIV, alpha 1	-1.08	-0.17	0.09	0.14	0.33	1.70	6.9	3.5E-02	3.0E-01
COL6A4P2	646300	3	collagen, type VI, alpha 4 pseudogene 2	-1.53	-1.61	-2.00	-0.02	1.13	3.00	23.3	8.0E-03	3.2E-01
COL8A2	1296	1	collagen, type VIII, alpha 2	1.03	1.31	0.86	1.30	1.11	-7.11	-579.0	1.2E-02	8.3E-01
COX16	51241	14	COX16 cytochrome c oxidase assembly homolog (S. cerevisiae)	0.68	0.06	0.47	0.49	0.75	-7.11	-452.5	1.5E-02	9.4E-01
COX4I2	84701	20	cytochrome c oxidase subunit IV isoform 2 (lung)	-7.11	-7.11	-7.11	-7.11	-4.89	0.33	354.4	1.7E-03	5.5E-01
CPLX2	10814	5	complexin 2	-4.33	-3.52	-7.11	-3.91	-7.11	0.01	21.8	2.0E-02	9.5E-01
CPLX3	594855	15	complexin 3	-2.19	-0.93	-0.73	-0.11	-0.22	0.81	8.1	4.9E-02	2.3E-01
CPM	1368	12	carboxypeptidase M	2.48	3.06	2.93	2.95	2.94	4.82	5.0	3.1E-02	2.8E-01
CPNE9	151835	3	copine family member IX	-2.68	0.12	0.19	0.10	0.65	1.00	13.1	3.6E-02	8.4E-02
CPVL	54504	7	carboxypeptidase, vitellogenic-like	6.39	8.34	3.40	7.76	7.98	2.00	-21.0	3.6E-03	1.9E-01
CPXM1	56265	20	carboxypeptidase X (M14 family), member 1	0.75	0.68	0.26	-0.16	1.18	5.00	19.0	1.7E-03	6.4E-01
CR1L	1379	1	complement component (3b/4b) receptor 1-like	-2.50	-4.41	-7.11	-7.11	-2.59	4.24	109.3	9.2E-04	2.4E-01
CRB1	23418	1	crumbs homolog 1 (Drosophila)	-4.33	-3.52	-2.35	-2.43	-2.02	1.00	43.4	1.3E-03	1.8E-01
CRB3	92359	19	crumbs homolog 3 (Drosophila)	0.94	0.82	0.23	0.55	0.33	-7.11	-542.9	1.1E-02	6.7E-01
CREB3L1	90993	11	cAMP responsive element binding protein 3-like 1	-7.11	-7.11	-7.11	-4.76	-4.05	0.33	354.4	2.5E-03	1.3E-01
CRISP2	7180	6	cysteine-rich secretory protein 2	-5.13	-3.52	-7.11	-4.76	-4.89	0.33	50.5	1.0E-02	9.3E-01
CRISP3	10321	6	cysteine-rich secretory protein 3	-7.11	-3.52	-4.87	-3.91	-3.14	6.87	33083.6	1.8E-11	8.2E-02
CRTAP	10491	3	cartilage associated protein	6.66	7.56	6.06	7.17	7.07	4.84	-3.5	4.0E-02	4.0E-01
CRY1	1407	12	cryptochrome 1 (photolyase-like)	4.93	5.52	5.93	6.24	6.22	2.46	-5.5	1.6E-02	1.4E-01
CRYGS	1427	3	crystallin, gamma S	1.13	1.36	2.48	1.63	1.74	4.00	7.3	4.4E-02	7.6E-01
CRYM	1428	16	crystallin, mu	-1.21	-0.72	-0.07	-1.05	-0.42	1.91	8.7	3.4E-02	4.3E-01
CSDA	8531	12	cold shock domain protein A	5.38	6.96	5.47	6.73	7.24	9.31	15.3	5.7E-05	6.8E-02
CSF1R	1436	5	colony stimulating factor 1 receptor	7.60	5.11	5.19	3.43	3.81	3.42	-18.1	2.3E-02	1.8E-01
CSNK1A1L	122011	13	casein kinase 1, alpha 1-like	-7.11	-7.11	-7.11	-3.91	-3.14	0.01	283.8	2.2E-03	1.4E-01
CSNK1E	1454	22	casein kinase 1, epsilon	6.17	6.66	6.45	7.09	7.11	4.15	-4.1	2.8E-02	4.9E-01
CST3	1471	20	cystatin C	8.83	8.47	6.58	8.12	8.65	5.59	-9.5	3.7E-03	6.4E-01
CSTF2T	23283	10	cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kDa, tau variant	5.67	5.75	5.25	4.72	4.95	3.52	-4.4	2.7E-02	8.9E-01
CTRC	11330	1	chymotrypsin C (caldecrin)	-1.72	0.82	1.42	1.61	2.45	1.81	11.6	4.8E-02	5.7E-02
CTSE	1510	1	cathepsin E	-7.11	-4.41	-4.87	-7.11	-4.05	3.97	4454.4	2.4E-06	2.1E-01
CTSO	1519	4	cathepsin O	4.70	5.05	4.96	4.49	4.27	1.91	-6.9	8.1E-03	5.1E-01
CTTNBP2NL	55917	1	CTTNBP2 N-terminal like	1.16	1.17	2.13	0.58	0.09	-7.11	-633.2	1.3E-02	4.8E-01
CXADR	1525	21	coxsackie virus and adenovirus receptor	0.28	0.41	-0.07	0.52	0.47	2.95	6.4	4.5E-02	8.0E-01
CXCL2	2920	4	chemokine (C-X-C motif) ligand 2	3.24	5.20	6.00	5.79	7.26	-7.11	-2668.1	1.4E-03	5.5E-02
CXCR6	10863	3	chemokine (C-X-C motif) receptor 6	4.07	1.45	2.30	-0.42	0.13	0.01	-16.7	2.9E-02	1.5E-01
CXorf69	NA	NA	NA	-2.68	-3.52	-3.50	-2.03	-2.19	0.81	11.5	3.7E-02	5.4E-01
CYP2F1	1572	19	cytochrome P450, family 2, subfamily F, polypeptide 1	-2.68	-1.16	-1.59	-1.58	-1.30	0.59	9.9	3.6E-02	4.0E-01
CYP2S1	29785	19	cytochrome P450, family 2, subfamily S, polypeptide 1	2.67	4.40	2.77	3.41	3.81	-7.11	-1801.2	2.2E-04	2.3E-01
CYP2U1	113612	4	cytochrome P450, family 2, subfamily U, polypeptide 1	3.55	3.46	3.79	3.33	3.54	0.81	-6.7	1.4E-02	9.6E-01
CYSTM1	84418	5	cysteine-rich transmembrane module containing 1	2.22	2.53	2.51	2.34	3.05	5.85	12.4	7.3E-04	5.5E-01
CYTIP	9595	2	cytohesin 1 interacting protein	7.73	8.25	8.00	7.17	7.29	5.31	-5.3	1.2E-02	7.9E-01
DAAM2	23500	6	dishevelled associated activator of morphogenesis 2	-3.82	-3.52	-3.12	-0.81	-1.30	0.33	18.6	4.5E-02	3.1E-01
DAGLA	747	11	diacylglycerol lipase, alpha	2.32	2.78	3.54	2.54	3.23	-0.98	-9.9	1.7E-02	3.4E-01
DARC	2532	1	Duffy blood group, chemokine receptor	-7.11	-7.11	-7.11	-7.11	-4.89	4.35	5797.5	1.7E-06	5.5E-01
DCAF12	25853	9	DDb1 and CUL4 associated factor 12	5.24	2.26	4.30	2.13	2.47	9.63	20.9	5.6E-03	3.1E-01
DDIT4L	115265	4	DNA-damage-inducible transcript 4-like	-2.89	-2.58	-3.50	-3.91	-1.74	0.81	13.4	2.2E-02	1.0E+00
DDX28	55794	16	DEAD (Asp-Glu-Ala-Asp) box polypeptide 28	4.92	5.14	4.35	4.94	4.93	2.81	-4.3	1.8E-02	1.0E+00
DEC2R2	26063	16	2,4-dienoyl CoA reductase 2, peroxisomal	2.12	2.75	2.82	3.08	3.43	4.41	4.9	4.0E-02	3.7E-01
DEFA4	1669	8	defensin, alpha 4, corticostatin	-5.13	-2.98	-3.50	-4.76	-1.74	7.59	7793.4	5.5E-09	2.4E-01
DENND4C	55667	9	DENN/MADD domain containing 4C	5.75	5.78	6.82	5.74	5.63	3.39	-5.1	8.8E-03	6.2E-01
DEPDC1	55635	1	DEP domain containing 1	-1.93	-2.27	-2.81	-2.68	-2.84	3.70	50.2	2.2E-04	4.8E-01
DEPDC1B	55789	5	DEP domain containing 1B	-0.63	-1.45	-2.56	-2.43	-3.14	3.13	13.6	1.7E-02	3.6E-01
DGCR11	25786	22	DiGeorge syndrome critical region gene 11 (non-protein coding)	0.96	0.94	0.44	-0.06	0.84	4.85	14.9	1.9E-03	7.0E-01
DIAPH3	81624	13	diaphanous homolog 3 (Drosophila)	-1.72	-4.41	-3.50	-3.91	-3.53	2.39	17.4	9.5E-03	2.0E-01
DISC2	27184	1	disrupted in schizophrenia 2 (non-protein coding)	-1.36	-4.41	-2.56	-2.68	-3.14	2.17	11.6	4.2E-02	5.0E-02
DKK3	27122	11	dickkopf 3 homolog (Xenopus laevis)	1.03	0.79	3.79	0.95	1.19	-7.11	-579.0	1.8E-02	3.4E-01
DMC1	11144	22	DMC1 dosage suppressor of mck1 homolog, meiosis-specific homolog	-0.68	-1.45	-2.16	-1.05	-1.13	2.46	8.9	3.0E-02	2.9E-01
DNAJC6	9829	1	DnaJ (Hsp40) homolog, subfamily C, member 6	0.33	-1.04	1.35	-0.60	-1.13	5.00	25.4	2.9E-03	8.0E-01
DNAJC7	7266	17	DnaJ (Hsp40) homolog, subfamily C, member 7	5.72	5.05	5.18	5.05	5.05	3.46	-4.8	1.6E-02	7.5E-01
DOCK10	55619	2	dedicator of cytokinesis 10	7.34	7.69	7.37	6.87	6.62	5.30	-4.1	1.4E-02	7.9E-01
DOCK8	81704	9	dedicator of cytokinesis 8	7.86	8.01	7.72	7.59	7.24	5.59	-4.8	9.8E-03	8.8E-01
DOCK9	23348	13	dedicator of cytokinesis 9	6.43	6.00	6.29	5.88	5.08	3.55	-7.4	1.4E-03	3.0E-01
DPCD	25911	10	deleted in primary ciliary dyskinesia homolog (mouse)	1.22	0.17	0.44	-0.54	0.20	3.83	6.1	3.7E-02	2.7E-01
DPM2	8818	9	dolichyl-phosphate mannosyltransferase polypeptide 2, regulatory subunit	4.66	3.34	5.56	4.14	3.86	7.11	5.5	2.1E-02	6.8E-01
DPY19L2	283417	12	dpy-19-like 2 (C. elegans)	-1.36	-0.63	-2.00	-0.16	0.86	2.52	14.9	1.7E-02	4.5E-01
DQX1	165545	2	DEAQ box RNA-dependent ATPase 1	-5.13	-2.27	-0.15	-3.38	-3.53	0.01	40.4	1.5E-02	5.8E-02
DSG3	1825	18	desmocollin 3	0.18	-0.72	-1.38	-1.58	-0.90	3.25	8.4	2.2E-02	8.5E-02
DSG3	1830	18	desmoglein 3	0.26	0.82	0.86	0.81	1.14	3.04	6.9	3.5E-02	7.0E-01
DTD1	92675	20	D-tyrosyl-tRNA deacylase 1	4.48	4.10	3.62	4.29	4.15	2.52	-3.9	4.2E-02	6.1E-01
DTL	51514	1	denticless E3 ubiquitin protein ligase homolog (Drosophila)	0.38	1.96	-1.02	0.86	1.00	3.64	9.6	3.4E-02	4.9E-01
DTNBP1	84062	6	dystrobrevin binding protein 1	4.80	3.75	3.98	3.75	4.12	2.90	-3.7	4.6E-02	2.5E-01
DTX1	1840	12	deltex homolog 1 (Drosophila)	4.12	2.28	2.89	3.26	4.26	7.00	7.3	9.3E-03	2.0E-01
DUSP11	8446	2	dual specificity phosphatase 11 (RNA/RNP complex 1-interacting)	4.53	5.12	4.84	5.13	5.01	2.46	-4.2	3.3E-02	3.0E-01
DUSP13	51207	10	dual specificity phosphatase 13	-5.13	-7.11	-4.03	-4.76	-7.11	1.91	151.2	1.3E-03	7.5E-01
DUSP16	80824	12	dual specificity phosphatase 16	6.52	4.54	6.92	4.72	4.15	3.49	-8.2	1.7E-02	4.2E-01
DUSP7	1849	3	dual specificity phosphatase 7	6.23	4.48	4.03	4.40	4.12	3.42	-7.0	1.2E-02	5.2E-02
DYRK2	8445	12	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	7.04	7.05	7.35	7.22	7.16	4.83	-4.6	5.0E-03	8.1E-01
DYRK3	8444	1	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3	-2.50	-1.61	-0.80	-1.87	-2.37	2.86	41.7	6.0E-04	3.5E-01
DYSF	8291	2	dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive)	3.25	5.19	2.62	5.23	4.61	6.32	8.4	1.5E-02	8.2E-02
DZIP1L	199221	3	DAZ interacting protein 1-like	-1.14	0.27	1.32	-0.97	-0.58	2.46	12.2	2.0E-02	3.3E-01
E2F2	1870	1	E2F transcription factor 2	3.45	2.54	0.29	1.96	2.11	7.38	15.2	1.1E-03	1.1E-01
E2F8	79733	11	E2F transcription factor 8	-1.28	-2.58	-2.56	-2.22	-2.84	2.00	9.8	3.2E-02	4.5E-01
EBF4	57593	20	early B-cell factor 4	1.95	1.47	1.24	2.58	2.34	-1.96	-15.2	1.8E-02	9.6E-01
EDARADD	128178	1	EDAR-associated death domain	1.92	-0.17	1.71	0.97	0.72	-7.11	-1072.7	2.9E-03	4.8E-01
EDEM1	9695	3	ER degradation enhancer, mannosidase alpha-like 1	7.15	8.08	8.04	7.96	8.56	5.41	-3.3	4.4E-02	2.1E-01
EFCAB4A	283229	11	EF-hand calcium binding domain 4A	0.97	1.83	0.90	2.31	3.06	4.46	1		

EIF4B	1975	12	eukaryotic translation initiation factor 4B	9.99	9.63	9.04	9.76	9.69	7.99	-4.0	4.0E-02	5.9E-01
ELANE	1991	19	elastase, neutrophil expressed	-1.93	-2.27	-2.56	-0.97	-0.27	8.56	1459.2	5.8E-12	6.0E-01
ELF1	1997	13	E74-like factor 1 (ets domain transcription factor)	7.46	9.04	8.25	8.82	8.42	4.87	-6.0	9.2E-03	1.1E-01
ELMO1	9844	7	engulfment and cell motility 1	6.25	5.91	5.79	5.52	5.49	4.19	-4.2	1.2E-02	4.2E-01
ELMO3	79767	16	engulfment and cell motility 3	2.91	1.85	3.21	2.58	2.44	5.38	5.5	2.5E-02	7.5E-01
ELOVL6	79071	4	ELOVL fatty acid elongase 6	1.59	1.06	0.05	0.10	-0.27	4.54	7.7	4.6E-02	4.9E-01
EMCN	51705	4	endomucin	-2.50	-2.58	-0.80	-1.87	-1.74	1.17	13.0	2.9E-02	6.5E-01
EME2	197342	16	essential meiotic endonuclease 1 homolog 2 (S. pombe)	2.03	3.65	3.80	3.41	3.22	-0.98	-8.1	3.1E-02	1.6E-01
EMID1	129080	22	EMI domain containing 1	-0.22	-0.82	-1.38	-1.58	0.53	3.93	17.8	6.5E-03	5.7E-01
EML5	161436	14	echinoderm microtubule associated protein like 5	2.66	3.02	3.28	2.46	2.26	-0.98	-12.6	1.2E-02	9.7E-01
EMR2	30817	19	egf-like module containing, mucin-like, hormone receptor-like 2	4.95	6.31	5.26	5.50	5.83	3.13	-3.5	5.0E-02	1.1E-01
EMX2	2018	10	empty spiracles homeobox 2	-5.13	-4.41	-4.87	-3.38	-3.14	0.59	60.6	7.7E-04	3.3E-01
ENPP5	59084	6	ectonucleotide pyrophosphatase/phosphodiesterase 5 (putative)	1.53	-1.61	-1.02	-1.87	-4.89	-7.11	-819.8	4.0E-02	5.4E-01
EPB41L3	23136	18	erythrocyte membrane protein band 4.1-like 3	3.96	5.74	7.49	4.22	3.96	-7.11	-4402.1	1.0E-04	6.1E-02
EPB42	2038	15	erythrocyte membrane protein band 4.2	-3.82	-2.02	-4.87	-1.46	0.60	8.73	6311.8	4.8E-05	1.9E-01
EPHA2	1969	1	EPH receptor A2	1.18	1.49	0.09	1.21	1.09	-7.11	-639.2	8.8E-03	9.0E-01
EPHA3	2042	3	EPH receptor A3	-3.44	-0.93	-4.03	-1.58	-2.19	0.81	19.8	2.1E-02	3.6E-01
EPHB1	2047	3	EPH receptor B1	0.89	-0.38	-2.81	-0.97	-0.10	-7.11	-524.8	2.2E-02	2.6E-01
EPHB2	2048	1	EPH receptor B2	0.31	1.11	0.82	0.49	0.06	-7.11	-350.2	3.3E-02	4.7E-01
EPOR	2057	19	erythropoietin receptor	3.49	2.61	3.19	2.93	2.68	5.70	4.6	2.4E-02	3.8E-01
EPST11	94240	13	epithelial stromal interaction 1 (breast)	3.82	3.61	3.60	3.83	3.14	1.46	-5.2	3.9E-02	7.9E-01
EPX	8288	17	eosinophil peroxidase	-0.95	-0.38	0.44	-1.87	-1.88	3.55	22.8	5.8E-03	8.8E-01
ERCC5	2073	13	excision repair cross-complementing rodent repair deficiency, complementation group 5	2.19	2.25	2.70	1.14	1.21	-7.11	-1295.4	1.5E-03	8.1E-01
ERG	2078	21	v-ets erythroblastosis virus E26 oncogene homolog (avian)	-1.93	-2.98	-2.16	-2.99	-0.32	3.75	52.1	2.2E-03	8.0E-01
ERLEC1	27248	2	endoplasmic reticulum lectin 1	5.16	5.50	5.41	5.22	5.32	3.28	-3.7	4.1E-02	4.5E-01
ERMAP	114625	1	erythroblast membrane-associated protein (Scianna blood group)	3.44	4.04	3.76	3.63	3.59	6.35	7.5	1.8E-03	5.6E-01
ERMP1	79956	9	endoplasmic reticulum metalloproteinase 1	5.20	3.44	3.55	2.70	2.78	2.64	-5.9	4.2E-02	7.7E-02
ERN1	2081	17	endoplasmic reticulum to nucleus signaling 1	5.47	5.18	5.36	5.06	4.73	2.00	-11.1	2.7E-04	7.4E-01
ESCO2	157570	8	establishment of cohesion 1 homolog 2 (S. cerevisiae)	-1.53	-2.27	-4.03	-3.38	-2.84	2.17	13.1	2.8E-02	5.0E-01
ESPL1	9700	12	extra spindle pole bodies homolog 1 (S. cerevisiae)	0.49	2.32	3.68	2.03	2.76	4.21	13.1	1.9E-02	1.3E-01
ESPN	83715	1	espin	-0.33	-1.30	-0.15	-0.48	-0.42	2.70	8.2	2.0E-02	9.0E-01
EV12A	2123	17	ecotropic viral integration site 2A	6.45	6.28	5.97	5.57	5.44	3.73	-6.6	1.8E-02	7.7E-01
FADD	8772	11	Fas (TNFRSF6)-associated via death domain	4.64	3.50	4.11	4.07	3.94	2.25	-5.3	2.3E-02	6.6E-01
FAM115C	285966	7	family with sequence similarity 115, member C	5.54	5.75	7.13	6.07	5.88	2.09	-5.5	3.4E-02	6.0E-02
FAM117B	150864	2	family with sequence similarity 117, member B	5.55	6.00	5.35	5.42	5.43	3.55	-4.0	3.5E-02	6.5E-01
FAM123C	NA	NA	NA	-1.08	-0.93	-1.72	-0.67	-0.47	2.58	12.7	1.2E-02	9.7E-01
FAM13A	10144	4	family with sequence similarity 13, member A	2.97	3.08	4.09	2.73	2.78	6.46	11.2	9.8E-04	7.2E-01
FAM168B	130074	2	family with sequence similarity 168, member B	6.98	8.15	7.49	8.38	8.30	5.06	-3.8	4.1E-02	1.5E-01
FAM174B	400451	15	family with sequence similarity 174, member B	1.31	-1.61	-1.59	-0.36	0.44	-7.11	-699.4	1.1E-02	1.6E-01
FAM198B	51313	4	family with sequence similarity 198, member B	3.12	3.74	2.85	2.65	2.10	-7.11	-2457.4	4.9E-03	2.5E-01
FAM210B	116151	20	family with sequence similarity 210, member B	5.25	4.12	2.46	4.42	4.09	8.75	11.3	2.1E-03	2.8E-01
FAM214B	80256	9	family with sequence similarity 214, member B	5.17	5.50	5.97	5.76	5.92	6.95	3.5	3.2E-02	2.3E-01
FAM40B	NA	NA	NA	0.77	1.47	2.11	1.45	1.44	4.24	11.1	5.4E-03	4.6E-01
FAM53B	9679	10	family with sequence similarity 53, member B	7.53	7.37	7.85	7.33	7.18	5.67	-3.6	2.7E-02	9.5E-01
FAM60A	58516	12	family with sequence similarity 60, member A	5.65	6.44	6.34	6.31	6.29	4.04	-3.0	4.3E-02	2.4E-01
FAM65C	140876	20	family with sequence similarity 65, member C	0.31	2.35	0.55	1.70	1.97	7.21	119.7	1.6E-06	2.4E-01
FAM69B	138311	9	family with sequence similarity 69, member B	2.76	1.94	3.36	2.44	4.10	5.32	5.9	4.1E-02	8.5E-01
FAM72B	653820	1	family with sequence similarity 72, member B	-2.68	0.41	-1.59	-0.36	0.16	1.58	19.7	7.5E-03	7.4E-02
FAM86EP	348926	4	family with sequence similarity 86, member A pseudogene	1.15	-0.82	0.01	-0.31	-1.13	-7.11	-627.1	9.6E-03	1.2E-01
FAM86HP	729375	3	family with sequence similarity 86, member A pseudogene	-0.12	-0.31	1.06	0.69	-0.06	2.52	6.3	4.8E-02	7.0E-01
FAM92A3	NA	NA	NA	-2.19	-0.46	-0.44	-0.81	-0.52	1.58	13.9	1.0E-02	2.3E-01
FASLG	356	1	Fas ligand (TNF superfamily, member 6)	3.25	0.97	0.68	1.04	0.33	-1.96	-37.6	8.9E-03	2.6E-01
FAT4	79633	4	FAT tumor suppressor homolog 4 (Drosophila)	0.23	1.57	0.58	-0.97	-1.30	-7.11	-332.1	4.5E-02	7.6E-01
FBLIM1	54751	1	filamin binding LIM protein 1	1.05	1.14	0.41	0.58	0.55	3.64	6.0	4.1E-02	4.9E-01
FBN1	2200	15	fibrillin 1	-0.59	-0.46	-2.35	-0.81	-0.64	3.13	13.2	8.3E-03	7.7E-01
FBP1	2203	9	fructose-1,6-bisphosphatase 1	4.46	5.03	6.26	4.68	4.70	2.00	-5.5	1.6E-02	2.7E-01
FBXL14	144699	12	F-box and leucine-rich repeat protein 14	5.41	3.66	4.15	4.11	3.84	2.00	-10.6	9.9E-04	7.2E-02
FBXO21	23014	12	F-box protein 21	5.18	6.15	6.05	6.16	6.20	3.28	-10.7	3.3E-02	1.8E-01
FBXO32	114907	8	F-box protein 32	5.14	6.03	6.17	6.31	5.17	2.64	-5.6	1.2E-02	3.6E-01
FBXO42	54455	1	F-box protein 42	4.96	4.44	5.15	4.21	4.04	3.04	-3.8	4.9E-02	8.1E-01
FBXO7	25793	22	F-box protein 7	6.75	7.69	7.51	8.04	8.17	9.72	7.9	2.0E-03	1.8E-01
FBXO9	26268	6	F-box protein 9	5.91	5.28	6.44	5.63	5.38	8.10	4.5	1.2E-02	9.2E-01
FCRL2	79368	1	Fc receptor-like 2	3.34	4.31	1.78	3.58	4.90	6.40	8.4	1.5E-02	5.8E-01
FCRL5	83416	1	Fc receptor-like 5	3.48	3.89	2.72	4.36	5.95	6.78	9.9	2.4E-02	4.5E-01
FDX1	2230	11	ferredoxin 1	4.15	5.17	4.39	5.19	5.60	1.70	-5.5	1.4E-02	1.4E-01
FECH	2235	18	ferrochelatase	4.47	4.12	4.09	4.32	4.15	9.30	28.5	1.2E-07	8.6E-01
FFAR2	2867	19	free fatty acid receptor 2	1.67	3.85	-0.29	4.64	5.58	5.09	10.7	3.9E-02	7.1E-02
FGF23	8074	12	fibroblast growth factor 23	-4.33	-2.27	-2.16	-3.38	-2.84	0.81	38.0	2.4E-03	2.8E-01
FGF5	2250	4	fibroblast growth factor 5	0.56	2.33	2.49	2.39	3.03	3.17	6.1	4.8E-02	1.2E-01
FGFBP2	83888	4	fibroblast growth factor binding protein 2	6.33	5.14	3.32	5.72	4.66	2.86	-11.1	7.8E-03	3.2E-01
FHDC1	85462	4	FH2 domain containing 1	-0.12	-2.02	-2.56	-1.72	-2.02	5.86	63.4	2.0E-04	2.3E-01
FIGNL2	401720	12	fidgetin-like 2	0.07	0.75	-1.10	-0.60	-0.47	-7.11	-296.0	3.9E-02	8.9E-01
FKBP5	2289	6	FK506 binding protein 5	7.23	6.29	7.61	6.77	5.05	5.11	-4.4	3.7E-02	6.0E-01
FKBP8	23770	19	FK506 binding protein 8, 38kDa	8.43	7.25	7.36	7.63	8.11	10.97	5.8	6.5E-03	1.9E-01
FLJ30403	729975	19	uncharacterized LOC729975	0.40	0.75	1.10	1.82	1.70	3.13	6.6	4.4E-02	5.1E-01
FLJ37505	400087	12	uncharacterized LOC400087	-4.33	-4.41	-4.87	-7.11	-7.11	0.81	38.0	4.0E-03	6.4E-01
FLJ38109	386627	5	uncharacterized LOC386627	-0.26	0.88	0.60	-0.26	-1.40	2.58	7.2	4.4E-02	7.9E-01
FLJ41649	401260	6	uncharacterized LOC401260	-3.44	-1.80	-4.87	-2.03	-1.30	1.32	28.2	2.8E-02	5.8E-01
FLJ45513	729220	17	uncharacterized LOC729220	0.15	0.61	0.50	1.10	0.92	3.32	9.0	1.7E-02	7.1E-01
FLNB	2317	3	filamin B, beta	6.82	6.40	7.51	6.04	5.73	4.87	-3.9	4.2E-02	5.8E-01
FLT4	2324	5	fms-related tyrosine kinase 4	3.56	2.80	3.57	3.23	2.85	0.33	-9.4	1.3E-02	5.6E-01
FLVCR1	28982	1	feline leukemia virus subgroup C cellular receptor 1	2.76	4.20	5.10	4.05	4.20	5.57	7.0	7.7E-03	9.2E-02
FLVCR2	55640	14	feline leukemia virus subgroup C cellular receptor family, member 2	2.76	3.29	3.01	3.65	3.81	-7.11	-1915.6	1.9E-04	1.9E-01
FN3K	64122	17	fructosamine 3 kinase	1.54	-0.05	0.70	-0.06	0.47	4.11	5.9	3.8E-02	1.0E-01
FNBP1	23048	9	formin binding protein 1	8.52	7.09	8.43	7.09	6.92	6.40	-4.4	2.9E-02	1.7E-01
FNDc8	54752	17	fibronectin type III domain containing 8	1.28	0.72	0.60	-0.26	0.16	-7.11	-687.3	6.4E-03	4.0E-01
FOXD2	2306	1	forkhead box D2	0.84	0.06	-0.87	-0.21	0.02	-7.11	-506.7	1.4E-02	4.5E-01
FOXM1	2305	12	forkhead box M1	1.33	1.09	1.38	0.91	0.96	4.96	12.4	2.6E-03	6.6E-01
FREM3	166752	4	FRAS1 related extracellular matrix 3	-5.13	-3.52	-4.03	-2.43	-3.14	0.33	50.5	3.8E-03	1.7E-01
FRMD4A	55691	10	FERM domain containing 4A	1.83	2.19	3.35	2.38	2.09	5.22	10.5	7.1E-03	6.0E-01
FRMD6	122786	14	FERM domain containing 6	0.40	0.88	-0.07	0.91	0.79	-7.11	-374.3	2.7E-02	5.7E-01
FRMD6-AS1	145438	14	FRMD6 antisense RNA 1	-1.72	-0.93	-1.72	-1.35	-1.13	2.25	15.8	1.1E-02	9.4E-01
FSTL3	10272	19	folliculin-like 3 (secreted glycoprotein)	2.80	2.73	0.90	2.56	2.62	5.60	7.0	1.8E-02	6.1E-01
FTCD	10841	21	formiminotransferase cyclodeaminase	-2.68	-4.41	-3.50	-1.58	-0.97	1.58	19.7	1.4E-02	4.8E-01
FTSJD1	55783	16	FtsJ methyltransferase domain containing 1	4.89	4.13	4.64	3.70	3.41	2.39	-5.		

FZD9	8326	7	frizzled family receptor 9	-5.13	-1.80	-3.12	-3.91	-4.89	1.32	100.8	1.6E-03	1.9E-01
GAB1	2549	4	GRB2-associated binding protein 1	1.47	3.04	1.99	2.43	2.75	3.95	5.6	3.4E-02	1.1E-01
GABARAPL2	11345	16	GABA(A) receptor-associated protein-like 2	5.96	4.95	4.86	4.83	5.23	7.76	3.5	4.4E-02	3.3E-01
GAD1	2571	2	glutamate decarboxylase 1 (brain, 67kDa)	-5.13	-2.27	-1.02	-4.76	-4.05	2.46	221.7	2.9E-03	1.9E-01
GALNT14	79623	2	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactose	-2.68	-1.16	0.38	-1.46	-0.76	1.46	18.0	1.2E-02	1.6E-01
GATA3	2625	10	GATA binding protein 3	6.19	5.66	5.79	6.45	6.10	4.24	-3.8	2.0E-02	6.5E-01
GCC1	79571	7	GRIP and coiled-coil domain containing 1	5.12	5.82	5.37	5.56	5.46	3.13	-4.0	2.7E-02	4.4E-01
GCC2	9648	2	GRIP and coiled-coil domain containing 2	6.17	6.43	7.82	7.12	6.65	4.00	-4.5	2.2E-02	2.9E-01
GCNT4	51301	5	glucosaminyl (N-acetyl) transferase 4, core 2	4.19	2.78	3.99	2.65	2.17	0.01	-18.3	3.6E-03	4.6E-01
GEMIN5	25929	5	gem (nuclear organelle) associated protein 5	4.64	2.81	4.21	2.88	2.44	2.09	-5.9	3.5E-02	2.3E-01
GEMIN8P4	492303	1	gem (nuclear organelle) associated protein 8 pseudogene 4	-3.14	-0.17	-0.20	-1.46	0.26	0.81	15.9	1.9E-02	5.6E-02
GFRA2	2675	8	GDNF family receptor alpha 2	1.79	0.75	-1.48	0.60	0.81	-7.11	-976.3	6.0E-03	2.4E-01
GGT5	2687	22	gamma-glutamyltransferase 5	-3.44	-1.04	-2.16	-2.03	-1.62	3.95	174.8	2.5E-05	2.3E-01
GH1	2688	17	growth hormone 1	-4.33	-2.02	-0.94	-1.46	0.02	0.59	32.6	2.0E-02	8.7E-02
GIMAP2	26157	7	GTPase, IMAP family member 2	7.08	4.13	3.25	4.66	3.98	3.80	-9.7	3.0E-02	5.0E-02
GIMAP6	474344	7	GTPase, IMAP family member 6	8.14	3.52	4.57	2.98	2.48	3.93	-18.5	3.9E-02	5.0E-02
GIMAP7	168537	7	GTPase, IMAP family member 7	8.38	4.79	6.14	3.66	3.54	4.21	-18.1	1.7E-02	5.7E-02
GINS2	51659	16	GINS complex subunit 2 (Psf2 homolog)	1.05	0.12	0.01	-0.42	0.81	3.58	5.8	4.3E-02	3.7E-01
GKN2	200504	2	gastrokine 2	-7.11	-7.11	-7.11	-7.11	-7.11	0.01	283.8	1.6E-03	1.0E+00
GLRX5	51218	14	glutaredoxin 5	4.56	3.88	4.09	4.05	4.61	7.96	10.6	1.8E-04	8.4E-01
GLS	2744	2	glutaminase	6.55	7.20	6.70	6.61	6.75	4.93	-3.1	3.9E-02	5.2E-01
GLTPD2	388323	17	glycolipid transfer protein domain containing 2	0.45	2.00	1.32	1.29	2.01	-7.11	-386.3	3.2E-02	1.5E-01
GLUD1	2746	10	glutamate dehydrogenase 1	6.73	6.61	6.72	6.65	6.83	4.95	-3.4	3.4E-02	6.5E-01
GMFR2	51292	14	guanosine monophosphate reductase 2	5.92	5.30	5.02	4.87	4.89	4.06	-3.6	4.1E-02	3.4E-01
GNA11	2767	19	guanine nucleotide binding protein (G protein), alpha 11 (Gq class)	1.60	1.27	0.90	-0.06	-0.37	-1.96	-11.9	4.7E-02	4.8E-01
GORASP2	26003	2	golgi reassembly stacking protein 2, 55kDa	6.28	6.55	6.27	6.83	6.73	4.47	-3.5	2.8E-02	5.4E-01
GPATCH8	23131	17	G patch domain containing 8	6.54	7.22	7.59	7.40	7.03	4.57	-3.9	1.8E-02	2.5E-01
GPBAR1	151306	2	G protein-coupled bile acid receptor 1	4.58	2.82	2.33	2.82	3.23	0.33	-19.1	6.3E-03	2.6E-01
GPER	2852	7	G protein-coupled estrogen receptor 1	0.64	0.94	-1.48	0.06	0.77	-7.11	-440.5	2.2E-02	6.2E-01
GPR111	222611	6	G protein-coupled receptor 111	-3.14	0.27	-4.03	-0.97	-0.14	2.86	65.9	1.4E-02	2.5E-01
GPR137B	7107	1	G protein-coupled receptor 137B	3.31	4.20	4.08	4.05	4.46	1.00	-5.0	3.4E-02	1.6E-01
GPR15	2838	3	G protein-coupled receptor 15	3.17	2.81	2.50	1.47	2.29	-7.11	-2553.7	1.2E-04	3.9E-01
GPR153	387509	1	G protein-coupled receptor 153	2.51	1.34	2.32	1.59	0.26	-0.40	-7.5	4.6E-02	3.1E-01
GPR157	80045	1	G protein-coupled receptor 157	0.89	-0.54	-0.29	-0.89	-1.88	-7.11	-524.8	1.8E-02	3.2E-01
GPR183	1880	13	G protein-coupled receptor 183	6.59	8.79	7.51	7.31	8.12	4.46	-4.4	4.7E-02	9.4E-02
GPR19	2842	12	G protein-coupled receptor 19	0.04	-1.45	0.05	-0.54	-0.90	-7.11	-290.0	4.2E-02	4.3E-01
GPR3	2827	1	G protein-coupled receptor 3	-1.36	1.82	1.39	2.40	2.52	2.32	12.9	3.8E-02	5.1E-02
GPR56	9289	16	G protein-coupled receptor 56	6.84	5.03	3.59	6.08	4.85	4.00	-7.2	3.1E-02	1.6E-01
GPR68	8111	14	G protein-coupled receptor 68	4.60	3.96	2.55	5.02	4.28	1.91	-6.5	2.1E-02	5.1E-01
GPSM2	29899	1	G-protein signaling modulator 2	0.68	-0.24	-0.15	0.29	0.02	3.75	8.4	2.0E-02	7.2E-01
GSDMB	55876	17	gasdermin B	3.77	3.49	3.15	4.50	4.59	6.42	6.3	1.9E-02	9.9E-01
GSPT1	2935	16	G1 to S phase transition 1	6.76	6.74	7.93	7.96	7.38	9.31	5.9	8.2E-03	2.6E-01
GTPBP8	29083	3	GTP-binding protein 8 (putative)	2.97	3.63	3.23	3.09	3.29	0.59	-5.2	4.3E-02	4.9E-01
GTSE1	51512	22	G-2 and S-phase expressed 1	0.21	0.36	-0.49	0.81	0.41	4.13	15.2	6.5E-03	8.5E-01
GUCA1B	2979	6	guanylate cyclase activator 1B (retina)	1.16	2.68	4.00	3.24	3.63	4.06	7.5	4.6E-02	1.2E-01
GUK1	2987	1	guanylate kinase 1	7.12	7.30	7.01	7.74	7.97	9.06	3.9	2.8E-02	6.2E-01
GUSBP11	91316	22	glucuronidase, beta pseudogene 11	4.63	5.91	5.76	6.26	6.37	6.65	4.1	3.3E-02	1.3E-01
GUSBP2	387036	6	glucuronidase, beta pseudogene 2	0.94	0.68	0.16	0.46	0.26	-7.11	-542.9	1.0E-02	6.9E-01
GUSBP3	653188	5	glucuronidase, beta pseudogene 3	-0.63	-0.38	-0.49	-1.05	-0.47	3.17	14.0	5.3E-03	8.7E-01
GUSBP4	375513	6	glucuronidase, beta pseudogene 4	0.62	0.36	0.88	0.14	-0.18	-7.11	-434.5	1.8E-02	9.7E-01
GYPA	2993	4	glycophorin A (MNS blood group)	-7.11	-7.11	-7.11	-7.11	-4.05	7.21	41990.4	1.7E-12	4.0E-01
GYPB	2994	4	glycophorin B (MNS blood group)	-7.11	-3.52	-7.11	-7.11	-7.11	6.15	20076.8	4.7E-09	4.0E-01
GYPC	2995	2	glycophorin C (Gerbich blood group)	7.07	5.72	6.51	6.52	6.46	9.18	4.3	1.3E-02	1.6E-01
GZMB	3002	14	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine	6.10	5.47	4.01	5.69	4.88	3.90	-4.6	3.6E-02	3.6E-01
GZMH	2999	14	granzyme H (cathepsin G-like 2, protein h-CCPX)	5.29	4.87	3.11	5.74	4.38	2.52	-6.8	2.1E-02	7.0E-01
H1FO	3005	22	H1 histone family, member 0	4.70	6.79	4.59	7.23	6.07	7.63	7.6	9.2E-03	9.4E-02
HAGH	3029	16	hydroxacylglutathione hydrolase	5.27	3.98	4.94	2.83	3.53	7.52	4.8	3.8E-02	9.5E-02
HARBI1	283254	11	harbinger transposase derived 1	1.64	3.59	1.60	2.63	3.01	-1.96	-12.3	3.5E-02	3.3E-01
HAS2	3037	8	hyaluronan synthase 2	-4.33	-1.80	-2.56	-2.43	-2.37	1.17	48.9	1.0E-03	1.8E-01
HBB	3043	11	hemoglobin, beta	2.64	6.04	3.44	9.99	12.96	17.51	30052.0	5.6E-03	5.2E-02
HBBP1	3044	11	hemoglobin, beta pseudogene 1	-4.33	-7.11	-7.11	-7.11	-4.89	3.80	303.6	9.3E-05	1.0E-01
HBD	3045	11	hemoglobin, delta	-2.19	-4.41	-7.11	-3.91	2.15	10.98	9380.8	1.6E-03	4.1E-01
HBE1	3046	11	hemoglobin, epsilon 1	-5.13	-7.11	-4.87	-4.76	-4.05	0.01	40.4	7.6E-03	9.8E-01
HBG1	3047	11	hemoglobin, gamma A	-5.13	-2.98	-7.11	-3.38	0.58	10.16	46306.7	8.3E-06	9.0E-02
HBG2	3048	11	hemoglobin, gamma G	-1.62	-1.80	-2.56	0.06	4.49	12.79	21916.2	7.2E-05	7.0E-02
HBQ1	3049	16	hemoglobin, theta 1	0.18	-2.58	-3.12	-2.43	0.30	5.72	46.8	1.7E-03	1.0E-01
HCN1	348980	5	hyperpolarization activated cyclic nucleotide-gated potassium chann	-2.68	-3.52	-4.03	-3.38	-4.05	1.17	14.8	3.5E-02	1.0E+00
HCP5	10866	6	HLA complex P5 (non-protein coding)	7.43	5.34	5.55	3.57	3.55	3.78	-12.6	1.1E-02	6.2E-02
HCRT	3060	17	hypocretin (orexin) neuropeptide precursor	-5.13	-4.41	-3.12	-2.99	-2.02	0.33	50.5	4.1E-03	1.9E-01
HEATR8	NA	NA	NA	-5.13	-7.11	-7.11	-3.91	-4.05	1.00	80.7	6.5E-03	9.1E-01
HEG1	57493	3	HEG homolog 1 (zebrafish)	5.01	4.79	5.49	4.53	3.95	2.81	-4.6	2.4E-02	9.7E-01
HEMGN	55363	9	hemogen	2.80	-0.24	-0.07	0.63	0.50	8.73	61.0	3.1E-04	9.9E-02
HEPACAM	220296	11	hepatic and glial cell adhesion molecule	-4.33	-3.52	-3.12	-2.43	-3.14	0.33	27.2	1.2E-02	5.1E-01
HEPACAM2	253012	7	HEPACAM family member 2	-5.13	-4.41	-7.11	-7.11	-7.11	3.28	392.8	7.9E-05	5.5E-01
HERC2P3	283755	15	hect domain and RLD 2 pseudogene 3	3.23	2.86	2.18	2.11	0.53	-0.40	-12.5	1.5E-02	1.5E-01
HERC3	8916	4	HECT and RLD domain containing E3 ubiquitin protein ligase 3	5.43	6.28	5.89	5.67	5.20	3.13	-4.9	8.8E-03	5.3E-01
HERPUD2	64224	7	HERPUD family member 2	6.37	6.39	6.18	6.52	6.21	4.63	-3.3	2.7E-02	9.5E-01
HES6	55502	2	hairy and enhancer of split 6 (Drosophila)	1.48	2.39	1.87	2.34	2.79	4.57	8.5	1.6E-02	3.2E-01
HIVEP2	3097	6	human immunodeficiency virus type I enhancer binding protein 2	7.05	5.81	7.49	5.49	5.63	4.30	-6.7	1.0E-02	3.4E-01
HJURP	55355	2	Holliday junction recognition protein	1.06	-0.31	-0.87	0.10	-0.22	4.90	14.4	3.8E-03	6.9E-02
HLA-DPA1	3113	6	major histocompatibility complex, class II, DP alpha 1	9.08	9.17	7.00	8.40	9.20	6.69	-5.2	3.2E-02	7.3E-01
HLA-DPB1	3115	6	major histocompatibility complex, class II, DP beta 1	8.58	8.87	7.86	7.97	9.00	6.46	-4.3	2.6E-02	8.1E-01
HMBS	3145	11	hydroxymethylbilane synthase	3.63	2.51	1.95	2.27	2.51	8.72	34.0	9.3E-07	1.0E-01
HMG2A	8091	12	high mobility group AT-hook 2	-3.82	-2.98	-3.50	-2.68	-3.53	0.59	22.3	4.6E-03	6.2E-01
HMGB2	3148	4	high mobility group box 2	6.48	7.86	7.45	7.48	7.69	8.57	4.3	2.2E-02	8.4E-02
HMMR	3161	5	hyaluronan-mediated motility receptor (RHAMM)	-0.46	1.59	0.23	1.48	0.39	4.17	24.7	1.9E-03	2.8E-01
HMOX1	3162	22	heme oxygenase (decycling) 1	5.91	7.54	6.08	7.22	10.60	2.00	-15.1	2.7E-02	6.7E-02
HOMER1	9456	5	homer homolog 1 (Drosophila)	0.52	-1.80	0.23	-0.54	-0.52	-7.11	-404.4	2.6E-02	4.0E-01
HPICAL4	51440	1	hippocalcin like 4	3.72	1.45	2.86	1.88	2.49	0.81	-7.5	3.8E-02	4.1E-01
HPR	3250	16	haptoglobin-related protein	-7.11	-7.11	-7.11	-4.76	-7.11	0.33	354.4	1.7E-03	5.5E-01
HPS6	79803	10	Hermansky-Pudlak syndrome 6	5.14	3.71	3.89	3.87	3.51	2.70	-5.4	2.5E-02	2.7E-01
HS3ST3B1	9953	17	heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1	3.66	2.42	2.15	1.37	2.32	-7.11	-3589.3	3.7E-04	5.3E-01
HSD3BP4	128102	1	hydroxy-delta-5-steroid dehydrogenase, 3 beta, pseudogene 4	-1.82	-2.58	-4.87	-7.11	-1.30	3.80	50.0	9.2E-03	6.6E-01
HTRA3	94031	4	HtrA serine peptidase 3	-3.44	-3.52	-3.50	-3.91	-1.74	4.70	293.1	1.3E-05	7.4E-01
HYAL3	8372	3	hyaluronoglucosaminidase 3	0.68	0.68	-0.73	0.9					

IGF2BP2	10644	3	insulin-like growth factor 2 mRNA binding protein 2	3.13	2.60	2.66	2.34	2.69	6.42	9.8	2.6E-03	7.2E-01
IGFBP3	3486	7	insulin-like growth factor binding protein 3	2.46	2.04	4.06	3.08	1.18	-7.11	-1560.3	1.5E-03	7.0E-01
IKZF2	22807	2	IKAROS family zinc finger 2 (Helios)	4.70	3.92	3.77	2.93	3.93	2.32	-5.2	4.5E-02	5.9E-01
IKZF3	22806	17	IKAROS family zinc finger 3 (Aiolos)	4.94	4.86	5.15	4.06	3.89	1.70	-9.4	2.0E-03	7.3E-01
IL10RA	3587	11	interleukin 10 receptor, alpha	9.00	9.84	8.95	9.25	9.00	6.67	-5.0	1.6E-02	6.6E-01
IL11RA	3590	9	interleukin 11 receptor, alpha	6.04	5.63	5.11	6.26	6.14	7.97	3.8	3.6E-02	5.0E-01
IL17RE	132014	3	interleukin 17 receptor E	0.86	-0.11	-0.20	0.33	0.36	-7.11	-512.8	1.4E-02	5.2E-01
IL1B	3553	2	interleukin 1, beta	5.78	7.03	5.35	6.24	6.97	1.70	-17.0	3.8E-03	3.7E-01
IL20RB	53833	3	interleukin 20 receptor beta	-1.01	0.61	1.19	0.86	1.24	2.00	8.1	2.9E-02	1.2E-01
IL7	3574	8	interleukin 7	0.01	1.38	0.55	-0.67	0.13	-7.11	-284.0	4.1E-02	4.9E-01
INMT	11185	7	indolethylamine N-methyltransferase	0.60	0.79	0.09	0.84	0.36	4.13	11.6	6.4E-03	8.5E-01
INPP4B	8821	4	inositol polyphosphate-4-phosphatase, type II, 105kDa	5.13	4.73	2.80	4.40	3.56	1.58	-11.7	3.3E-03	2.5E-01
INTS5	80789	11	integrator complex subunit 5	5.91	3.95	4.21	3.84	4.12	3.04	-7.3	9.4E-03	8.5E-02
IREB2	3658	15	iron-responsive element binding protein 2	5.72	6.51	7.20	6.78	6.51	4.02	-3.2	4.6E-02	7.6E-02
IRF2BPL	64207	14	interferon regulatory factor 2 binding protein-like	5.90	6.90	5.04	6.69	6.86	3.35	-5.8	1.4E-02	5.1E-01
IRF6	3664	1	interferon regulatory factor 6	0.58	0.94	-0.03	1.02	0.06	-7.11	-422.4	1.8E-02	9.9E-01
IRS1	3667	2	insulin receptor substrate 1	3.57	2.57	2.59	2.03	2.33	1.00	-5.9	3.8E-02	3.1E-01
ITK	3702	5	IL2-inducible T-cell kinase	8.31	6.30	8.22	6.59	5.67	6.03	-4.9	3.7E-02	1.4E-01
ITLN1	55600	1	intelectin 1 (galactofuranose binding)	-0.73	-1.30	-4.87	-3.38	-2.19	5.16	59.6	2.3E-03	5.4E-01
ITPKB	3707	1	inositol-trisphosphate 3-kinase B	8.22	7.19	8.34	7.68	7.67	6.27	-3.9	2.8E-02	5.2E-01
ITPR1	3708	3	inositol 1,4,5-trisphosphate receptor, type 1	5.67	6.51	6.23	6.06	6.34	3.86	-3.5	2.5E-02	4.0E-01
ITPR2	3709	12	inositol 1,4,5-trisphosphate receptor, type 2	5.40	5.89	6.19	5.35	5.29	3.58	-3.5	3.5E-02	6.1E-01
ITPRIPL1	150771	2	inositol 1,4,5-trisphosphate receptor interacting protein-like 1	2.82	1.47	3.01	1.72	1.09	0.01	-7.0	4.8E-02	5.4E-01
ITSN1	6453	21	intersectin 1 (SH3 domain protein)	2.06	3.21	2.28	2.63	2.11	5.30	9.5	5.3E-03	4.4E-01
JAK1	3716	1	Janus kinase 1	8.94	8.24	8.82	8.09	8.03	7.14	-3.5	4.8E-02	5.4E-01
JAZF1	221895	7	JAZF zinc finger 1	4.14	5.02	4.39	4.53	4.43	6.64	5.7	1.5E-02	3.8E-01
KANK1	23189	9	KN motif and ankyrin repeat domains 1	3.50	2.96	2.25	1.60	1.14	0.33	-9.1	2.2E-02	2.1E-01
KANK2	25959	19	KN motif and ankyrin repeat domains 2	-0.37	0.01	0.35	-0.60	-0.27	6.11	89.6	9.4E-06	4.1E-01
KAT2B	8850	3	K(lysine) acetyltransferase 2B	5.61	6.39	6.31	6.19	5.77	8.26	6.3	1.4E-03	3.6E-01
KAT7	11143	17	K(lysine) acetyltransferase 7	5.84	7.70	6.45	7.01	6.87	3.93	-3.8	4.1E-02	1.6E-01
KBTBD12	166348	3	kelch repeat and BTB (POZ) domain containing 12	0.68	-0.31	-0.15	-0.21	0.13	3.35	6.4	3.6E-02	2.3E-01
KCNA3	3738	1	potassium voltage-gated channel, shaker-related subfamily, member 3	5.65	5.11	5.48	4.35	3.86	2.46	-9.2	2.8E-03	4.6E-01
KCNA6	3742	12	potassium voltage-gated channel, shaker-related subfamily, member 6	0.96	-1.30	-2.56	-2.68	-1.40	-7.11	-548.9	3.5E-02	1.8E-01
KCNK3	3748	19	potassium voltage-gated channel, Shaw-related subfamily, member 3	0.97	3.17	1.96	2.26	2.53	-7.11	-554.9	9.5E-03	1.2E-01
KCNQ1	3784	11	potassium voltage-gated channel, KQT-like subfamily, member 1	5.53	6.09	2.55	5.99	6.05	3.09	-5.4	4.5E-02	8.1E-01
KCNQ1OT1	10984	11	KCNQ1 opposite strand/antisense transcript 1 (non-protein coding)	0.96	1.38	0.35	0.71	0.53	3.83	7.3	2.2E-02	7.6E-01
KCTD7	154881	7	potassium channel tetramerisation domain containing 7	5.53	4.69	3.74	4.11	4.36	3.21	-5.0	2.3E-02	8.4E-02
KEL	3792	7	Kell blood group, metallo-endopeptidase	-2.33	-2.27	-4.03	-2.22	-1.13	6.60	497.0	2.9E-07	9.3E-01
KHDRBS1	10657	1	KH domain containing, RNA binding, signal transduction associated	7.30	7.35	6.91	7.30	7.24	5.68	-3.1	4.3E-02	9.1E-01
KIAA0101	9768	15	KIAA0101	0.94	-0.38	-1.02	0.49	0.16	4.41	11.1	1.1E-02	4.7E-01
KIAA0240	NA	NA	NA	5.98	4.92	5.26	4.61	4.42	4.00	-4.0	4.1E-02	2.4E-01
KIAA0494	NA	NA	NA	7.46	6.37	7.05	6.34	5.89	5.27	-4.6	2.0E-02	3.7E-01
KIAA1598	57698	10	KIAA1598	3.24	4.03	3.22	3.02	3.19	-0.98	-18.9	5.0E-03	5.9E-01
KIAA1656	85371	22	KIAA1656 protein	0.60	2.31	2.95	2.60	2.34	3.67	8.4	1.8E-02	1.2E-01
KIAA1875	340390	8	KIAA1875	2.45	2.74	2.95	2.50	2.33	4.78	5.0	3.6E-02	9.9E-01
KIF14	9928	1	kinesin family member 14	-0.33	-0.17	-0.87	-0.97	-0.52	3.49	14.2	3.9E-03	7.7E-01
KIF15	56992	3	kinesin family member 15	0.38	-1.04	0.44	-1.24	-1.62	3.58	9.2	2.3E-02	3.2E-01
KIF18A	81930	11	kinesin family member 18A	-0.30	0.65	0.29	0.95	0.92	3.46	13.5	4.2E-03	2.7E-01
KIF19	124602	17	kinesin family member 19	0.45	0.79	0.73	2.90	1.21	-7.11	-386.3	2.9E-02	3.7E-01
KIF21A	55605	12	kinesin family member 21A	3.35	2.71	3.72	3.33	2.59	-0.40	-13.6	5.9E-03	8.1E-01
KIF23	9493	15	kinesin family member 23	-0.05	0.17	-1.19	0.18	-0.27	4.08	17.7	4.8E-03	7.0E-01
KIF2C	11004	1	kinesin family member 2C	1.20	-0.54	0.12	0.36	0.20	4.84	12.5	3.3E-03	1.9E-01
KIF5C	3800	2	kinesin family member 5C	3.08	2.66	4.14	2.80	2.72	0.01	-8.5	2.4E-02	8.8E-01
KIR2DL3	3804	19	killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail	2.45	1.19	0.09	1.39	1.02	-0.98	-10.9	2.5E-02	2.1E-01
KIR3DL2	3812	19	killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail	3.25	1.64	1.17	2.25	1.61	-1.96	-37.6	1.3E-03	9.7E-02
KLC3	147700	19	kinesin light chain 3	-0.54	-1.61	-1.48	-1.87	-0.76	4.52	33.6	6.4E-04	3.6E-01
KLF1	10661	19	Kruppel-like factor 1 (erythroid)	-0.84	-3.52	-4.87	-3.91	-2.37	7.24	272.4	1.7E-05	1.2E-01
KLF12	11278	13	Kruppel-like factor 12	6.38	7.10	6.99	6.69	6.25	4.00	-9.2	3.8E-03	4.8E-01
KLF17	128209	1	Kruppel-like factor 17	-3.82	-3.52	-7.11	-7.11	-4.89	0.59	22.3	2.2E-02	2.6E-01
KLHDC7B	113730	22	kelch domain containing 7B	3.63	1.96	1.93	1.66	1.60	-0.98	-24.6	2.8E-03	1.2E-01
KLHDC8A	55220	1	kelch domain containing 8A	-4.33	-4.41	-4.03	-4.76	-4.05	0.33	27.2	3.6E-03	1.0E+00
KLHL25	64410	15	kelch-like family member 25	3.05	3.46	1.30	2.89	3.12	0.33	-6.6	5.0E-02	9.3E-01
KLHL32	114792	6	kelch-like family member 32	1.73	-0.63	-2.00	0.36	0.26	-7.11	-940.2	6.4E-03	6.2E-02
KLHL36	79786	16	kelch-like family member 36	5.59	5.73	5.84	5.76	5.54	3.86	-3.3	4.3E-02	9.2E-01
KLHL9	55958	9	kelch-like family member 9	5.62	5.20	4.67	4.48	4.13	3.17	-5.5	1.9E-02	5.4E-01
KLRB1	3820	12	killer cell lectin-like receptor subfamily B, member 1	7.18	5.99	6.37	6.77	6.58	3.97	-9.2	2.8E-04	3.2E-01
KLRG1	10219	12	killer cell lectin-like receptor subfamily G, member 1	5.13	1.38	0.60	1.89	1.55	1.32	-14.0	4.8E-02	8.0E-02
KREMEN1	83999	22	kringle containing transmembrane protein 1	1.00	1.09	2.72	0.93	1.90	4.66	12.6	7.1E-03	4.0E-01
KREMEN2	79412	16	kringle containing transmembrane protein 2	-1.44	-1.61	-1.38	-1.58	0.16	2.46	15.1	1.1E-02	5.5E-01
KRT1	3848	12	keratin 1	2.52	-3.52	-1.59	-4.76	0.20	7.60	33.9	1.5E-02	5.9E-02
KRT23	25984	17	keratin 23 (histone deacetylase inducible)	-1.72	-1.80	-7.11	-0.54	-0.90	2.64	20.7	3.8E-02	7.7E-01
KRT72	140807	12	keratin 72	2.60	2.68	1.52	4.71	2.22	5.67	8.4	2.9E-02	4.3E-01
KY	339855	3	kyphoscoliosis peptidase	-3.82	-2.27	-3.12	-1.87	0.06	0.01	14.9	4.9E-02	1.5E-01
KYNU	8942	2	kynureninase	3.24	4.61	5.08	3.92	3.73	-0.98	-18.8	1.2E-03	9.2E-02
LARGE	9215	22	like-glycosyltransferase	2.13	2.59	3.03	1.64	2.93	-1.96	-17.3	1.2E-02	6.9E-01
LATS1	9113	6	LATS, large tumor suppressor, homolog 1 (Drosophila)	4.51	6.34	5.75	5.69	5.44	2.39	-4.3	2.0E-02	7.2E-02
LAX1	54900	1	lymphocyte transmembrane adaptor 1	5.17	3.80	5.18	3.61	2.68	2.58	-6.0	2.9E-02	3.2E-01
LCN8	138307	9	lipocalin 8	-3.44	-4.41	-3.50	-2.43	-2.19	4.26	217.0	1.1E-04	8.6E-01
LDOC1L	84247	22	leucine zipper, down-regulated in cancer 1-like	5.02	5.00	5.37	4.71	4.55	1.46	-11.8	3.1E-04	9.2E-01
LEPREL1	55214	3	leprecan-like 1	0.86	-1.16	-3.50	-0.97	-1.13	-7.11	-512.8	2.2E-02	6.8E-02
LEPROTL1	23484	8	leptin receptor overlapping transcript-like 1	7.09	6.29	7.33	6.78	6.77	4.54	-5.9	1.2E-02	8.3E-01
LGALS1	3956	22	lectin, galactoside-binding, soluble, 1	7.11	7.05	5.98	7.61	7.66	3.64	-11.1	3.4E-04	7.9E-01
LGALS2	3957	22	lectin, galactoside-binding, soluble, 2	5.62	5.59	3.02	2.99	5.54	-1.96	-194.9	7.6E-05	6.3E-01
LGR6	59352	1	leucine-rich repeat containing G protein-coupled receptor 6	3.60	4.10	3.23	3.89	1.99	-0.98	-24.1	2.5E-03	7.6E-01
LILRB4	11006	19	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIMs)	4.54	4.66	3.88	4.49	4.97	-1.96	-91.7	3.0E-06	7.7E-01
LINC00202	NA	NA	NA	-1.72	-0.38	0.29	0.06	-0.10	1.81	11.6	3.9E-02	3.5E-01
LINC00260	84719	1	long intergenic non-protein coding RNA 260	-3.14	-3.52	-1.85	-3.91	-2.84	1.46	25.0	5.4E-03	6.2E-01
LINC00261	140828	20	long intergenic non-protein coding RNA 261	-7.11	-7.11	-7.11	-7.11	-7.11	2.90	2121.7	3.3E-05	1.0E+00
LINC00273	649159	16	long intergenic non-protein coding RNA 273	-1.62	-0.82	-4.03	-3.91	-3.14	5.69	160.3	1.7E-04	4.6E-01
LINC00311	197196	16	long intergenic non-protein coding RNA 311	-0.54	-1.30	-1.19	-1.58	-1.21	2.58	8.8	2.4E-02	2.9E-01
LIX1	167410	5	Lix1 homolog (chicken)	-2.33	-1.04	-1.02	-1.46	-0.47	1.00	10.3	2.2E-02	2.9E-01
LIX1L	128077	1	Lix1 homolog (mouse)-like	4.53	2.92	4.02	2.90	2.10	2.09	-5.5	4.2E-02	1.8E-01
LMOD1	25802	1	leiomodlin 1 (smooth muscle)	-5.13	-2.98	-3.12	-4.76	-3.14	1.91	151.2	5.2E-04	3.8E-01
LMOD3	56203	3	leiomodlin 3 (fetal)	0.43	0.75	0.47	1.27	1.07	3.64	9.3	1.2E-02	7.7E-01
LNPEP	4012	5	leucyl/cystinyl aminopeptidase	4.7								

LOC100190938	NA	NA	NA		0.54	-0.93	-0.11	-0.97	-0.70	3.25	6.5	4.2E-02	1.1E-01
LOC100216479	100216479	2	uncharacterized LOC100216479		-0.19	-1.16	-1.48	-2.22	-2.59	2.95	8.8	3.4E-02	2.3E-01
LOC100271722	100271722	22	uncharacterized LOC100271722		1.06	-0.38	-0.80	-0.06	-0.32	-7.11	-591.0	1.2E-02	2.9E-01
LOC100287846	100287846	8	patched 1 pseudogene		-1.93	-2.98	-2.56	-2.99	-2.19	1.46	10.6	2.6E-02	3.8E-01
LOC100288077	NA	NA	NA		-7.11	-7.11	-7.11	-7.11	-4.89	0.59	425.1	6.9E-04	4.2E-01
LOC100288778	100288778	12	WAS protein family homolog 1 pseudogene		3.30	4.09	4.60	5.83	4.53	6.04	6.7	1.1E-02	1.1E-01
LOC100505633	100505633	1	uncharacterized LOC100505633		-2.89	-7.11	-4.87	-3.91	-7.11	3.00	61.0	2.5E-03	9.4E-02
LOC100505676	100505676	8	uncharacterized LOC100505676		-1.44	-1.45	-1.85	-1.72	-2.84	2.00	11.0	1.8E-02	4.8E-01
LOC100505702	100505702	4	uncharacterized LOC100505702		-0.73	0.41	0.29	0.26	-0.32	2.17	7.5	3.6E-02	1.9E-01
LOC100505746	NA	NA	NA		5.92	5.83	2.44	6.07	5.66	8.51	6.0	3.1E-02	5.5E-01
LOC100506599	100506599	9	uncharacterized LOC100506599		-1.28	-1.61	-2.81	-2.99	-2.59	1.81	8.6	2.6E-02	2.0E-01
LOC100507178	NA	NA	NA		1.57	1.45	1.30	1.42	1.29	4.70	8.8	1.2E-02	6.1E-01
LOC100507537	100507537	3	uncharacterized LOC100507537		-2.89	-1.16	-0.80	-0.42	-0.64	3.00	61.0	1.3E-04	8.6E-02
LOC100509894	NA	NA	NA		-0.78	-1.80	-2.81	-1.46	-1.74	3.58	20.8	2.9E-03	1.6E-01
LOC145474	145474	14	uncharacterized LOC145474		-7.11	-2.58	-4.87	-3.38	-3.53	0.33	354.4	1.1E-03	7.1E-02
LOC145663	NA	NA	NA		-0.63	-0.17	-0.11	0.22	0.44	2.70	10.1	1.2E-02	5.9E-01
LOC145783	145783	15	uncharacterized LOC145783		1.58	0.01	-2.00	-0.42	-0.83	-7.11	-843.9	1.0E-02	2.0E-01
LOC147646	147646	19	uncharacterized LOC147646		-2.68	-1.16	-3.12	-2.03	-2.59	0.81	11.5	3.5E-02	4.3E-01
LOC148709	148709	1	actin pseudogene		-1.93	-0.11	-3.12	-1.58	-1.13	2.17	17.4	2.3E-02	7.7E-01
LOC149134	149134	1	uncharacterized LOC149134		-3.82	-2.02	-2.00	-1.87	-2.19	1.00	29.7	2.1E-03	2.1E-01
LOC151009	151009	2	uncharacterized LOC151009		-5.13	-3.52	-2.35	-3.91	-4.05	0.01	40.4	1.6E-02	4.0E-01
LOC152225	152225	3	uncharacterized LOC152225		0.56	-3.52	-1.59	-3.38	-2.37	-7.11	-416.4	4.0E-02	8.3E-02
LOC200261	NA	NA	NA		-3.44	-2.27	-2.81	-0.81	-0.52	0.59	16.9	2.0E-02	1.6E-01
LOC200772	200772	2	uncharacterized LOC200772		4.05	4.64	2.64	4.51	4.46	1.00	-8.3	1.1E-02	9.1E-01
LOC254559	254559	15	uncharacterized LOC254559		2.40	1.86	1.68	1.70	1.09	-0.98	-10.5	2.0E-02	4.8E-01
LOC255167	255167	5	uncharacterized LOC255167		-3.44	-3.52	-7.11	-7.11	-4.89	0.33	14.1	4.6E-02	1.4E-01
LOC283335	283335	12	uncharacterized LOC283335		-0.26	-1.61	-1.19	-1.87	-1.21	2.86	8.7	3.2E-02	9.9E-02
LOC284751	284751	20	uncharacterized LOC284751		-2.33	-2.02	-0.87	-0.67	-0.32	0.59	7.7	4.9E-02	2.2E-01
LOC284801	284801	20	uncharacterized LOC284801		0.60	-0.93	-4.03	-2.43	-2.59	6.44	57.3	9.6E-04	8.1E-02
LOC284837	284837	21	uncharacterized LOC284837		3.66	2.32	1.12	1.40	0.88	-7.11	-3577.2	1.5E-03	3.8E-01
LOC285758	285758	6	uncharacterized LOC285758		-2.89	-1.16	-2.35	-2.43	-1.88	1.58	22.9	3.6E-03	4.2E-01
LOC339505	339505	1	uncharacterized LOC339505		-4.33	-7.11	-4.03	-7.11	-4.05	0.59	32.6	5.8E-03	9.1E-01
LOC388387	NA	NA	NA		-0.30	0.61	-2.16	0.39	0.55	3.61	15.1	1.6E-02	9.1E-01
LOC388588	NA	NA	NA		-0.22	-2.58	-2.35	-1.72	-0.42	5.76	63.7	1.0E-04	1.5E-01
LOC392364	392364	9	nuclear pore associated protein 1 pseudogene		-7.11	-7.11	-7.11	-7.11	-7.11	0.33	354.4	1.6E-03	5.6E-01
LOC399753	399753	10	uncharacterized LOC399753		0.40	0.49	1.30	1.00	-0.97	-7.11	-374.3	3.0E-02	9.7E-01
LOC400084	400084	12	uncharacterized LOC400084		-2.68	-4.41	-7.11	-4.76	-3.53	1.46	18.0	3.1E-02	5.1E-02
LOC400685	400685	19	uncharacterized LOC400685		1.44	-0.38	-0.44	-1.05	-2.59	-7.11	-765.6	1.3E-02	1.4E-01
LOC401052	401052	3	uncharacterized LOC401052		-0.02	1.53	1.41	1.76	1.78	3.21	9.4	1.5E-02	1.9E-01
LOC440518	440518	19	golgin A2 pseudogene		-7.11	-7.11	-7.11	-7.11	-7.11	1.58	849.3	2.1E-04	5.6E-01
LOC441242	441242	7	uncharacterized LOC441242		0.23	-0.54	-1.38	-0.89	-0.90	-7.11	-332.1	3.7E-02	1.6E-01
LOC642846	642846	12	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11-like		0.64	0.27	0.68	4.53	1.45	4.46	14.1	3.2E-02	1.6E-01
LOC643723	643723	1	uncharacterized LOC643723		0.82	-2.58	-3.12	-0.97	-1.13	-7.11	-500.7	3.0E-02	8.1E-02
LOC727896	727896	18	cysteine and histidine-rich domain (CHORD) containing 1 pseudogene		-0.50	1.22	1.08	1.10	0.96	2.86	10.3	1.7E-02	2.5E-01
LOC728640	NA	NA	NA		-2.68	-2.98	-4.03	-4.76	-3.53	1.17	14.8	2.9E-02	3.4E-01
LOC729678	NA	NA	NA		4.49	3.58	4.35	2.91	3.01	1.81	-6.4	1.7E-02	3.3E-01
LOC731424	731424	4	uncharacterized LOC731424		-0.54	2.03	0.68	1.20	1.37	3.13	12.8	1.6E-02	9.8E-02
LOH12CR2	503693	12	loss of heterozygosity, 12, chromosomal region 2 (non-protein coding)		-1.01	-0.54	-0.34	-0.48	-0.18	2.00	8.1	2.3E-02	6.2E-01
LPAR2	9170	19	lysophosphatidic acid receptor 2		5.51	5.89	6.95	6.83	6.71	8.23	6.6	8.2E-03	2.9E-01
LPO	4025	17	lactoperoxidase		-4.33	-1.16	-1.85	-3.91	-3.14	2.64	135.6	2.1E-04	1.6E-01
LRAT	9227	4	lecithin retinol acyltransferase (phosphatidylcholine--retinol O-acyltransferase)		-1.28	-0.24	-0.11	-0.31	-0.10	2.46	13.5	6.5E-03	3.6E-01
LRBA	987	4	LPS-responsive vesicle trafficking, beach and anchor containing		6.30	6.22	6.08	5.70	5.64	4.54	-3.4	2.9E-02	6.5E-01
LRP3	4037	19	low density lipoprotein receptor-related protein 3		2.12	3.64	0.32	3.24	2.93	5.06	7.7	2.7E-02	3.4E-01
LRRC2	79442	3	leucine rich repeat containing 2		0.84	-1.30	0.58	-0.48	-0.18	4.02	9.1	3.0E-02	2.1E-01
LSM11	134353	5	LSM11, U7 small nuclear RNA associated		1.16	0.75	1.71	-1.58	-1.13	-7.11	-633.2	1.8E-02	8.8E-01
LTBR	56413	14	leukotriene B4 receptor 2		0.77	1.93	1.83	1.39	0.77	-7.11	-482.6	1.3E-02	4.6E-01
LTF	4055	12	lymphotoxin beta receptor (TNFR superfamily, member 3)		4.70	5.37	4.41	4.82	4.81	2.46	-4.7	2.9E-02	4.4E-01
LUC7L	55692	16	LUC7-like (S. cerevisiae)		5.53	6.25	6.48	6.75	7.12	7.54	4.0	3.1E-02	2.4E-01
LUC7L3	51747	17	LUC7-like 3 (S. cerevisiae)		7.10	7.55	7.42	7.17	7.03	8.85	3.4	2.6E-02	7.3E-01
LUZP1	7798	1	leucine zipper protein 1		4.89	7.47	5.52	6.04	5.69	2.17	-6.6	1.4E-02	1.1E-01
LY6E	4061	8	lymphocyte antigen 6 complex, locus E		7.56	6.22	4.61	6.86	6.96	5.55	-4.0	5.0E-02	2.0E-01
LY6G6D	58530	6	lymphocyte antigen 6 complex, locus G6D		-3.44	-3.52	-4.87	-2.68	-2.59	1.58	33.9	3.5E-03	9.5E-01
LYNX1	66004	8	Ly6/neurotoxin 1		3.25	1.45	0.16	1.56	2.11	-1.96	-37.7	3.2E-03	1.4E-01
LYPD2	137797	8	LY6/PLAUR domain containing 2		1.72	-1.45	-3.12	0.60	1.28	-7.11	-934.2	1.3E-02	3.8E-01
MACC1	346389	7	metastasis associated in colon cancer 1		0.58	1.86	2.02	1.51	1.91	3.46	7.4	2.0E-02	2.6E-01
MAF	4094	16	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)		5.52	4.45	5.01	5.15	4.44	2.32	-9.2	2.1E-03	6.8E-01
MALL	7851	2	mal, T-cell differentiation protein-like		-0.26	-2.02	-2.00	-0.97	-0.37	2.86	8.7	2.6E-02	2.9E-01
MAL1	10892	18	mucosa associated lymphoid tissue lymphoma translocation gene 1		6.24	6.42	6.93	6.55	6.42	4.41	-3.6	4.2E-02	3.3E-01
MAML1	9794	5	mastermind-like 1 (Drosophila)		6.40	5.83	6.40	5.13	4.79	4.04	-5.1	2.6E-02	7.1E-01
MAML2	84441	11	mastermind-like 2 (Drosophila)		5.97	5.32	4.13	4.69	4.34	3.04	-7.6	2.4E-03	6.5E-02
MAN1A2	10905	1	mannosidase, alpha, class 1A, member 2		4.83	5.34	4.64	4.20	3.71	2.25	-6.0	1.7E-02	8.5E-01
MAN2A1	4124	5	mannosidase, alpha, class 2A, member 1		5.28	6.29	6.06	6.04	5.87	3.55	-3.3	3.1E-02	2.0E-01
MAN2B2	23324	4	mannosidase, alpha, class 2B, member 2		6.69	5.84	5.56	5.49	5.23	4.80	-3.7	2.8E-02	1.2E-01
MAP1LC3A	84557	20	microtubule-associated protein 1 light chain 3 alpha		0.79	2.50	2.44	3.48	3.92	5.50	26.2	1.9E-03	7.9E-02
MAP2K3	5606	17	mitogen-activated protein kinase kinase 3		6.65	8.67	5.92	8.51	8.90	9.31	6.4	3.1E-02	1.5E-01
MAPK11	5600	22	mitogen-activated protein kinase 11		2.87	2.48	2.44	3.03	3.34	5.50	6.2	1.5E-02	8.7E-01
MAPK12	6300	22	mitogen-activated protein kinase 12		-2.19	-1.04	-1.59	-0.36	-0.58	1.58	13.9	1.4E-02	3.6E-01
MAPK13	5603	6	mitogen-activated protein kinase 13		5.46	4.65	5.11	4.24	4.69	3.64	-3.5	4.5E-02	1.8E-01
MARCH8	220972	10	membrane-associated ring finger (C3HC4) 8, E3 ubiquitin protein ligase		5.09	4.83	5.60	4.51	4.43	7.40	4.9	9.5E-03	9.6E-01
MARCO	8685	2	macrophage receptor with collagenous structure		3.13	3.06	1.35	1.20	1.45	-1.96	-34.7	9.2E-03	9.7E-01
MB21D1	115004	6	Mab-21 domain containing 1		3.17	4.49	2.88	4.01	3.72	0.81	-5.2	3.8E-02	3.3E-01
MBOAT2	129642	2	membrane bound O-acyltransferase domain containing 2		1.87	2.53	2.33	3.06	3.19	5.77	14.9	7.3E-04	3.7E-01
MBTPS1	8720	16	membrane-bound transcription factor peptidase, site 1		7.19	7.01	7.35	7.09	7.04	5.48	-3.3	2.6E-02	8.7E-01
MCM10	55388	10	minichromosome maintenance complex component 10		-0.78	-0.82	-0.20	-0.74	-1.40	3.04	14.3	5.5E-03	9.9E-01
MCOLN3	55283	1	mucopolip 3		-2.50	-1.30	-2.56	-1.58	-1.88	2.52	33.1	8.2E-04	5.7E-01
MDGA1	266727	6	MAM domain containing glycosylphosphatidylinositol anchor 1		3.87	3.33	4.46	4.35	3.35	0.33	-11.7	2.6E-02	7.7E-01
ME3	10873	11	malic enzyme 3, NADP(+)-dependent, mitochondrial		1.62	1.55	1.04	3.05	3.10	4.41	6.9	3.8E-02	5.7E-01
MEAF6	64769	1	MYST/Esa1-associated factor 6		5.93	4.94	5.24	4.96	4.83	3.97	-3.9	3.2E-02	3.6E-01
MECOM	2122	3	MDS1 and EVI1 complex locus		-1.93	-2.98	-2.56	-3.38	-1.88	2.86	28.0	4.9E-03	8.8E-01
MED29	55588	19	mediator complex subunit 29		6.12	5.40	6.10	5.25	5.32	4.32	-3.5		

MIR3687	100500815	21	microRNA 3687	-1.36	1.85	-1.85	-0.48	-1.88	7.11	358.7	3.1E-05	1.8E-01
MIR663A	724033	20	microRNA 663a	0.04	3.79	-0.87	0.93	0.09	7.05	129.2	8.0E-04	2.0E-01
MIR663B	100313824	2	microRNA 663b	-2.05	-0.72	-4.03	-3.38	-2.37	5.03	137.8	2.0E-04	7.9E-01
MIRLET7BHG	400931	22	MIRLET7B host gene (non-protein coding)	0.75	1.77	1.48	0.86	1.09	3.86	8.6	1.2E-02	6.1E-01
MKRN1	23608	7	makorin ring finger protein 1	7.41	8.77	7.60	8.50	8.28	9.86	5.5	1.1E-02	2.5E-01
MKRN2	23609	3	makorin ring finger protein 2	5.40	5.77	4.87	5.16	5.41	3.52	-3.7	3.8E-02	8.7E-01
MLF1IP	79682	4	MLF1 interacting protein	0.62	3.12	1.42	2.91	2.81	4.11	11.2	7.0E-03	5.0E-02
MLL	4297	11	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)	7.00	7.69	8.07	7.57	7.24	5.26	-3.3	4.1E-02	4.4E-01
MLLT4	4301	6	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)	3.09	5.10	6.01	5.15	4.95	0.33	-6.8	3.3E-02	5.6E-02
MLLT6	4302	17	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)	9.61	8.65	8.90	9.11	9.06	7.64	-3.9	3.1E-02	2.5E-01
MMP1	4312	11	matrix metalloproteinase 1 (interstitial collagenase)	-2.89	-4.41	-4.87	-4.76	-4.89	0.81	13.4	2.0E-02	2.5E-01
MMP25	64386	16	matrix metalloproteinase 25	3.53	4.02	2.89	8.94	6.65	8.29	27.2	3.4E-02	9.0E-02
MMP8	4317	11	matrix metalloproteinase 8 (neutrophil collagenase)	-3.82	-2.58	-7.11	-7.11	-2.59	7.99	3767.8	3.4E-12	5.4E-01
MPO	4353	17	myeloperoxidase	1.05	2.38	1.25	2.03	2.40	10.81	871.6	1.6E-14	2.0E-01
MRAS	22808	3	muscle RAS oncogene homolog	2.42	3.63	2.76	3.45	4.51	-0.98	-10.7	1.5E-02	1.4E-01
MRC2	9902	17	mannose receptor, C type 2	3.23	2.77	3.90	2.09	3.37	5.81	6.0	3.5E-02	6.5E-01
MRFAP1L1	114932	4	Morf4 family associated protein 1-like 1	6.54	5.08	6.23	5.05	4.67	4.54	-4.0	4.6E-02	2.9E-01
MRPL49	740	11	mitochondrial ribosomal protein L49	5.82	5.18	5.61	5.64	5.45	4.11	-3.3	4.0E-02	6.9E-01
MRS2P2	729633	12	MRS2 magnesium homeostasis factor homolog (S. cerevisiae) pseudogene	-1.28	2.14	1.42	1.06	1.86	3.75	33.1	8.9E-03	9.6E-02
MS4A1	931	11	membrane-spanning 4-domains, subfamily A, member 1	6.63	8.06	7.43	7.23	8.09	9.02	5.2	1.2E-02	2.6E-01
MS4A10	341116	11	membrane-spanning 4-domains, subfamily A, member 10	-0.22	-0.38	-0.73	-0.48	-0.27	3.49	13.2	1.2E-02	5.4E-01
MS4A3	932	11	membrane-spanning 4-domains, subfamily A, member 3 (hematopoietic)	-2.19	-3.52	-2.81	-2.68	-0.76	7.26	711.5	5.6E-08	6.8E-01
MS4A4A	51338	11	membrane-spanning 4-domains, subfamily A, member 4A	1.98	0.01	3.16	-0.74	-0.22	-7.11	-1114.8	9.6E-03	1.0E+00
MS4A6A	64231	11	membrane-spanning 4-domains, subfamily A, member 6A	6.07	5.33	3.10	5.03	4.62	1.17	-29.9	8.4E-03	9.0E-01
MS4A7	58475	11	membrane-spanning 4-domains, subfamily A, member 7	4.76	5.07	5.28	3.93	3.78	2.00	-6.8	4.5E-02	4.0E-01
MSC	9242	8	musculin	1.65	2.11	1.42	2.58	4.25	-7.11	-886.0	1.2E-02	3.1E-01
MSL2	55167	3	male-specific lethal 2 homolog (Drosophila)	6.54	7.93	6.99	7.84	7.75	4.84	-3.3	4.7E-02	1.4E-01
MST1	4485	3	macrophage stimulating 1 (hepatocyte growth factor-like)	1.48	2.12	2.67	2.73	2.89	4.32	7.2	1.5E-02	2.9E-01
MSX1	4487	4	msh homeobox 1	-3.14	-1.80	-2.35	-1.72	-1.74	2.52	52.3	6.8E-04	4.0E-01
MT1E	4493	16	metallothionein 1E	0.21	0.12	-1.85	1.59	0.39	-7.11	-326.1	4.0E-02	7.8E-01
MT2A	4502	16	metallothionein 2A	4.59	3.99	3.12	5.78	4.47	1.91	-6.4	2.9E-02	7.5E-01
MTMR11	10903	1	myotubularin related protein 11	3.24	4.40	2.83	4.35	4.30	-1.96	-37.3	5.7E-04	1.7E-01
MTRNR2L8	100463486	11	MT-RNR2-like 8	8.03	7.31	6.29	6.64	6.78	13.90	58.1	2.3E-05	6.1E-02
MTRR	4552	5	5-methyltetrahydrofolate-homocysteine methyltransferase reductase	4.97	5.76	5.54	5.59	5.13	2.64	-5.0	7.6E-03	3.4E-01
MUC1	4582	1	mucin 1, cell surface associated	0.45	1.93	4.02	2.61	2.91	4.02	11.9	2.2E-02	8.4E-02
MUC6	4588	11	mucin 6, oligomeric mucus/gel-forming	-0.54	-0.31	-0.24	-0.11	0.47	6.58	140.2	1.3E-04	9.9E-01
MXD3	83463	5	MAX dimerization protein 3	3.21	3.01	3.86	3.45	3.25	5.87	6.4	7.2E-03	8.7E-01
MXI1	4601	10	MAX interactor 1, dimerization protein	5.24	6.24	6.10	6.50	6.34	7.88	6.2	3.7E-03	1.1E-01
MYBL2	4605	20	v-myb myeloblastosis viral oncogene homolog (avian)-like 2	2.70	1.94	1.33	5.02	5.98	6.53	14.3	3.3E-02	2.5E-01
MYC	4609	8	v-myc myelocytomatosis viral oncogene homolog (avian)	7.91	7.46	6.12	7.22	8.09	5.97	-3.8	4.2E-02	2.9E-01
MYCL1	4610	1	v-myc myelocytomatosis viral oncogene homolog 1, lung carcinoma	4.14	3.60	2.46	1.98	0.88	-7.11	-4992.1	5.8E-04	7.7E-01
MYL4	4635	17	myosin, light chain 4, alkali; atrial, embryonic	-4.33	-7.11	-4.87	-3.38	0.70	8.07	5854.2	1.1E-04	1.7E-01
MYO9A	4649	15	myosin IXA	3.96	4.39	3.20	3.53	3.30	1.70	-4.8	4.5E-02	8.2E-01
MYOF	26509	10	myoferlin	3.59	3.72	3.82	2.40	3.28	-7.11	-3414.7	1.8E-04	8.5E-01
NAAA	27163	4	N-acyl ethanolamine acid amidase	6.39	6.54	4.90	6.22	6.20	3.73	-6.4	7.3E-03	9.7E-01
NANOG	79923	12	Nanog homeobox	-0.68	-0.82	-1.72	-2.03	-1.30	2.81	11.3	2.2E-02	3.2E-01
NANOGNB	360030	12	NANOG neighbor homeobox	-4.33	-4.41	-7.11	-7.11	-4.89	1.58	65.1	1.4E-03	6.3E-01
NARF	26502	17	nuclear prelamin A recognition factor	5.78	5.29	6.70	6.17	6.13	7.65	3.6	3.4E-02	7.5E-01
NBPF15	284565	1	neuroblastoma breakpoint family, member 15	3.42	3.37	3.36	3.07	2.74	0.59	-7.2	1.6E-02	6.8E-01
NBPF9	400818	1	neuroblastoma breakpoint family, member 9	3.97	4.89	5.29	5.75	5.29	1.91	-4.2	2.9E-02	9.6E-02
NCAPG	64151	4	non-SMC condensin I complex, subunit G	0.52	0.36	0.32	0.81	0.26	4.66	17.7	1.1E-03	9.5E-01
NCF4	4689	22	neutrophil cytosolic factor 4, 40kDa	5.03	5.21	4.50	5.85	5.62	7.51	5.6	7.0E-03	5.3E-01
NCK1	4690	3	NCK adaptor protein 1	5.05	5.63	4.67	5.62	5.36	2.32	-6.6	3.3E-03	5.3E-01
NCOA2	10499	8	nuclear receptor coactivator 2	5.11	6.17	6.24	5.17	4.64	3.04	-4.2	3.0E-02	4.2E-01
NCOA4	8031	10	nuclear receptor coactivator 4	8.37	7.94	7.33	7.54	7.13	10.81	5.4	3.1E-02	7.9E-01
NCOR2	9612	12	nuclear receptor corepressor 2	7.40	7.94	7.99	7.79	7.93	5.76	-3.1	4.1E-02	5.1E-01
NCRUPAR	100302746	5	non-protein coding RNA, upstream of F2R/PAR1	-1.21	-1.30	-0.80	-0.60	-0.42	1.70	7.6	3.2E-02	7.5E-01
NDC80	10403	18	NDC80 kinetochore complex component	1.89	1.06	1.94	1.63	1.24	4.34	5.5	3.9E-02	8.3E-01
NDFIP1	80762	5	Nedd4 family interacting protein 1	7.09	6.71	7.66	7.42	6.77	5.41	-3.2	4.5E-02	7.1E-01
NDNL2	56160	15	neodin-like 2	5.43	6.15	5.70	6.54	6.42	3.04	-5.2	9.8E-03	3.1E-01
NDRG2	57447	14	NDRG family member 2	4.63	2.93	2.76	3.66	3.50	2.17	-5.5	2.3E-02	5.9E-02
NECAB2	54550	16	N-terminal EF-hand calcium binding protein 2	-4.33	-2.27	-1.85	-1.46	-0.10	0.01	21.8	2.3E-02	6.2E-02
NEDD4L	23327	18	neural precursor cell expressed, developmentally down-regulated 4	1.83	3.67	3.78	3.33	3.66	4.70	7.3	1.0E-02	7.7E-02
NEDD9	4739	6	neural precursor cell expressed, developmentally down-regulated 9	5.53	5.91	5.18	6.41	6.06	3.21	-5.0	1.3E-02	3.6E-01
NEFL	4747	8	neurofilament, light polypeptide	2.25	-0.82	0.01	-1.58	0.60	-7.11	-1343.6	7.1E-03	1.9E-01
NEIL3	55247	4	nei endonuclease VIII-like 3 (E. coli)	-1.28	-2.02	-3.50	-3.91	-4.05	2.09	10.4	2.6E-02	8.2E-02
NEK2	4751	1	NIMA-related kinase 2	0.31	0.01	-0.73	-0.81	0.09	4.50	18.4	4.1E-03	6.8E-01
NELL2	4753	12	NEL-like 2 (chicken)	7.37	6.09	7.32	6.18	6.21	4.21	-8.9	5.1E-04	1.6E-01
NEO1	4756	15	neogenin 1	3.83	2.73	2.91	3.42	2.89	0.81	-8.1	1.0E-02	2.9E-01
NEU3	10825	11	sialidase 3 (membrane sialidase)	2.94	4.64	4.33	3.71	3.36	0.01	-7.6	1.8E-02	2.5E-01
NEURL	9148	10	neuralized homolog (Drosophila)	3.40	2.83	1.15	3.20	3.72	0.01	-10.5	1.8E-02	8.9E-01
NEUROD2	4761	17	neuronal differentiation 2	-5.13	-7.11	-2.56	-2.43	-1.51	1.00	80.7	2.8E-03	1.2E-01
NFATC1	4772	18	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	6.47	5.12	5.96	5.08	5.27	4.52	-3.9	2.6E-02	1.5E-01
NFATC2	4773	20	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2	5.31	3.50	4.84	3.29	2.76	1.46	-14.5	2.8E-03	1.3E-01
NFATC4	4776	14	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4	-3.82	-1.80	-1.72	-2.22	-0.90	0.33	18.6	1.0E-02	1.0E-01
NFE2L1	4779	17	nuclear factor (erythroid-derived 2)-like 1	6.74	5.69	6.52	5.59	5.56	4.58	-4.5	1.9E-02	4.4E-01
NFIB	4781	9	nuclear factor I/B	-3.82	-3.52	-3.50	-3.91	-4.05	0.01	14.9	2.6E-02	8.0E-01
NFIX	4784	19	nuclear factor I/X (CCAAT-binding transcription factor)	2.05	2.59	1.38	1.87	1.96	6.69	24.9	1.0E-04	6.7E-01
NFXL1	152518	4	nuclear transcription factor, X-box binding-like 1	3.28	3.71	4.80	3.62	3.57	1.00	-4.9	4.6E-02	4.6E-01
NMU	10874	4	neuromedin U	-7.11	-7.11	-4.87	-7.11	-7.11	0.33	354.4	5.5E-03	2.4E-01
NOA1	84273	4	nitric oxide associated 1	5.60	4.80	4.18	4.75	4.58	3.58	-4.0	2.9E-02	2.7E-01
NOB1	28987	16	NIN1/RPN12 binding protein 1 homolog (S. cerevisiae)	5.86	5.35	4.52	5.55	5.56	3.97	-3.7	3.0E-02	4.3E-01
NOMO3	408050	16	NODAL modulator 3	-0.02	2.58	1.70	1.74	1.00	-7.11	-277.9	3.8E-02	5.5E-02
NOV	4856	8	nephroblastoma overexpressed	-3.44	-1.45	-3.50	-1.05	0.79	0.81	19.8	2.5E-02	8.5E-02
NOVA1	4857	14	neuro-oncological ventral antigen 1	-3.14	-3.52	-7.11	-2.99	-2.84	0.33	11.4	4.8E-02	8.0E-01
NOX4	50507	11	NADPH oxidase 4	-0.63	0.32	0.38	0.55	0.58	3.32	15.6	5.9E-03	4.4E-01
NPAT	4863	11	nuclear protein, ataxia-telangiectasia locus	5.53	5.34	6.85	5.16	5.03	2.75	-6.9	7.1E-03	6.7E-01
NPC2	10577	14	Niemann-Pick disease, type C2	5.91	6.70	5.92	6.38	6.73	4.06	-3.6	2.9E-02	2.6E-01
NPDC1	56654	9	neural proliferation, differentiation and control, 1	4.42	3.43	2.37	3.67	4.96	8.53	17.3	2.8E-04	5.3E-01
NPFF	8620	12	neuropeptide FF-amide peptide precursor	0.54	1.09	1.95	1.06	0.81	3.88	10.2	1.7E-02	6.9E-01
NR1D1	9572	17	nuclear receptor subfamily 1, group D, member 1	4.51	4.52	4.37	4.74	5.12	1.17	-10.1	2.5E-03	9.6E-01
NR3C2	4306	4	nuclear receptor subfamily 3, group C, member 2	4.95	4.35	4.05	3.90	3.70	0.33	-24.7	9.5E-05	1.4E-01
NR6A1	2649	9	nuclear receptor subfamily 6, group A, member 1	-3.82	0.01	-2.16	-1.15	-0.97	0.01	14.9	2.7E-02	5.2E-02
NRCAM	4897	7	neuronal cell adhesion molecule	2.63	1.34	2.00	1.98	2.30	-7.11	-1759.0	5.4E-04	

NUDT12	83594	5	nudix (nucleoside diphosphate linked moiety X)-type motif 12	0.81	-1.30	-0.44	-0.48	-1.51	-7.11	-494.7	2.3E-02	3.9E-01
NUF2	83540	1	NUF2, NDC80 kinetochore complex component, homolog (S. cerevi	0.31	0.57	0.63	0.81	0.58	4.30	15.9	1.8E-03	5.9E-01
NUSAP1	51203	15	nucleolar and spindle associated protein 1	2.00	3.06	2.89	2.85	2.68	5.81	14.0	5.3E-04	3.4E-01
OAS2	4939	12	2'-5'-oligoadenylate synthetase 2, 69/71kDa	7.00	4.56	5.29	5.39	4.56	3.97	-8.1	1.4E-02	1.3E-01
OBSL1	23363	2	obscurin-like 1	0.23	0.12	-1.28	-0.89	0.13	3.83	12.1	4.8E-02	6.8E-01
OIP5	11339	15	Opa interacting protein 5	-0.30	-0.46	-0.80	-1.46	-1.88	3.13	10.8	1.6E-02	5.4E-01
OLFM1	10439	9	olfactomedin 1	0.58	3.06	3.22	-0.21	-0.18	-7.11	-422.4	3.4E-02	2.0E-01
OLFM4	10562	13	olfactomedin 4	-7.11	-3.52	-4.03	-7.11	-3.53	2.90	2121.7	7.7E-05	1.6E-01
OLIG1	116448	21	oligodendrocyte transcription factor 1	2.75	2.74	1.99	2.96	1.84	-0.98	-13.4	3.1E-02	5.7E-01
OR2T8	343172	1	olfactory receptor, family 2, subfamily T, member 8	-7.11	-7.11	-7.11	-7.11	-1.88	1.46	778.6	3.7E-03	1.1E-01
ORC1	4998	1	origin recognition complex, subunit 1	1.19	3.14	3.89	3.60	3.61	4.34	8.9	3.3E-02	1.0E-01
OSBPL6	114880	2	oxysterol binding protein-like 6	-2.68	-1.16	-2.00	-1.72	-2.59	2.81	45.9	4.7E-04	5.4E-01
OXNAD1	92106	3	oxidoreductase NAD-binding domain containing 1	5.97	5.76	5.73	5.21	4.66	3.52	-5.5	8.6E-03	6.1E-01
OXR1	55074	8	oxidation resistance 1	4.86	4.49	4.41	4.48	4.06	2.75	-4.3	4.6E-02	9.5E-01
P4HA1	5033	10	prolyl 4-hydroxylase, alpha polypeptide I	4.15	5.04	6.31	5.57	5.47	2.25	-3.7	4.8E-02	7.2E-02
PACS1	55690	11	phosphofurin acidic cluster sorting protein 1	8.48	7.38	7.27	7.59	7.54	6.84	-3.1	3.6E-02	5.4E-02
PADI2	11240	1	peptidyl arginine deiminase, type II	3.25	3.89	3.24	2.92	3.96	5.82	5.9	2.3E-02	6.2E-01
PADI4	23569	1	peptidyl arginine deiminase, type IV	3.66	4.88	1.61	5.11	4.00	7.63	15.7	8.6E-03	4.1E-01
PAQR5	54852	15	progesterin and adipoQ receptor family member V	-3.44	-4.41	-3.50	-4.76	-3.14	1.00	22.6	5.1E-03	6.3E-01
PAQR9	344838	3	progesterin and adipoQ receptor family member IX	-7.11	-7.11	-7.11	-7.11	-7.11	4.35	5797.5	1.6E-06	1.0E+00
PARD3B	117583	2	par-3 partitioning defective 3 homolog B (C. elegans)	1.22	-1.45	-2.81	-2.22	-0.97	-7.11	-657.2	2.5E-02	9.3E-02
PARD6B	84612	20	par-6 partitioning defective 6 homolog beta (C. elegans)	0.81	2.33	2.91	3.00	2.79	3.61	7.0	2.7E-02	9.7E-02
PARPBP	55010	12	PARP1 binding protein	-0.95	0.75	0.38	-0.11	0.20	2.00	7.8	3.1E-02	1.4E-01
PAX5	5079	9	paired box 5	4.28	4.66	4.97	3.09	4.25	6.40	4.4	3.7E-02	9.3E-01
PBK	55872	8	PDZ binding kinase	-2.89	-1.04	-2.35	-0.54	-0.47	1.70	24.8	6.9E-03	1.8E-01
PBRM1	55193	3	polybromo 1	5.92	5.64	5.92	5.41	5.13	4.00	-3.8	3.5E-02	8.0E-01
PCBP3	54039	21	poly(rC) binding protein 3	0.33	-0.31	2.81	0.58	0.68	4.55	18.7	1.2E-02	5.2E-01
PCNX	22990	14	pecanex homolog (Drosophila)	6.49	7.07	8.00	6.85	6.54	4.66	-3.6	4.2E-02	3.7E-01
PCLCE2	26577	3	procollagen C-endopeptidase enhancer 2	-7.11	-7.11	-7.11	-4.76	-7.11	1.00	566.5	5.7E-04	5.5E-01
PDCD7	10081	15	programmed cell death 7	5.40	3.88	4.51	3.99	3.65	3.28	-4.3	3.8E-02	1.8E-01
PDE11A	50940	2	phosphodiesterase 11A	-0.33	-0.82	-1.19	-1.46	-1.30	3.61	15.5	6.1E-03	2.1E-01
PDE3B	5140	11	phosphodiesterase 3B, cGMP-inhibited	7.01	6.43	7.51	6.04	5.58	4.00	-8.0	2.9E-03	7.1E-01
PDE4A	5141	19	phosphodiesterase 4A, cAMP-specific	4.30	5.72	5.83	5.55	5.33	2.09	-4.6	2.4E-02	6.8E-02
PDE6A	5145	5	phosphodiesterase 6A, cGMP-specific, rod, alpha	0.36	0.22	-0.15	0.10	-0.14	3.35	8.0	2.2E-02	5.0E-01
PDIA3	2923	15	protein disulfide isomerase family A, member 3	7.77	8.63	7.82	8.38	8.45	6.08	-3.2	4.8E-02	3.4E-01
PDIA6	10130	2	protein disulfide isomerase family A, member 6	6.04	5.05	4.53	4.94	5.43	4.06	-3.9	4.8E-02	4.4E-01
PK4	5166	7	pyruvate dehydrogenase kinase, isozyme 4	2.91	5.48	4.46	3.53	2.56	-1.96	-29.7	4.5E-03	1.3E-01
PDPK1	5170	16	3-phosphoinositide dependent protein kinase-1	6.58	7.02	7.50	6.84	6.83	4.75	-3.6	2.3E-02	4.5E-01
PDPR	55066	16	pyruvate dehydrogenase phosphatase regulatory subunit	5.46	6.83	6.59	6.03	5.57	3.73	-3.3	4.8E-02	1.7E-01
PEA15	8682	1	phosphoprotein enriched in astrocytes 15	6.21	6.77	6.23	6.30	6.29	3.55	-6.3	1.4E-03	6.6E-01
PEL13	246330	11	pellino E3 ubiquitin protein ligase family member 3	3.44	2.82	2.18	2.06	2.17	0.33	-8.7	2.0E-02	3.7E-01
PER3	8863	1	period circadian clock 3	3.49	3.38	3.25	3.83	3.48	1.00	-5.6	2.5E-02	9.9E-01
PEX19	5824	1	peroxisomal biogenesis factor 19	5.86	5.06	5.14	4.97	4.93	4.17	-3.2	5.0E-02	3.3E-01
PF4V1	5197	4	platelet factor 4 variant 1	2.13	0.72	0.97	0.93	1.44	5.18	8.3	3.5E-02	3.5E-01
PFN1P2	767846	1	profilin 1 pseudogene 2	1.46	2.57	2.94	1.59	1.41	-1.96	-10.8	4.3E-02	5.3E-01
PGF	5228	14	placental growth factor	-2.05	-1.80	-0.87	-2.43	-1.62	2.75	28.4	1.6E-03	7.4E-01
PGLYRP1	8993	19	peptidoglycan recognition protein 1	-2.68	-2.27	-1.85	-2.03	0.39	8.55	2467.5	1.1E-07	3.1E-01
PGM5	5239	9	phosphoglucomutase 5	-2.05	-1.04	-1.59	-0.89	-2.02	1.00	8.4	4.7E-02	4.2E-01
PGM5P2	595135	9	phosphoglucomutase 5 pseudogene 2	1.09	1.96	1.91	1.64	1.94	6.61	46.1	5.6E-05	6.5E-01
PGS1	9489	17	phosphatidylglycerophosphate synthase 1	5.64	5.58	5.65	5.52	5.72	3.95	-3.2	4.7E-02	8.2E-01
PHC1	1911	12	polyhomeotic homolog 1 (Drosophila)	5.97	5.56	4.61	5.50	5.38	4.08	-3.7	3.1E-02	4.3E-01
PHF15	23338	5	PHD finger protein 15	8.15	6.60	7.48	6.86	6.58	5.05	-8.6	4.1E-04	5.9E-02
PHLDB2	90102	3	pleckstrin homology-like domain, family B, member 2	2.30	2.58	2.93	3.83	3.62	-1.96	-19.5	6.2E-03	2.9E-01
PHOSPHO1	162466	17	phosphatase, orphan 1	1.64	1.47	1.75	5.10	5.68	8.46	113.7	2.3E-03	1.4E-01
PIF1	80119	15	PIF1 5'-to-3' DNA helicase homolog (S. cerevisiae)	0.68	0.49	1.79	1.14	0.65	3.61	7.7	2.9E-02	8.8E-01
PIGQ	9091	16	phosphatidylinositol glycan anchor biosynthesis, class Q	5.04	4.55	5.52	4.60	4.84	7.92	7.4	1.3E-03	6.9E-01
PIK3CG	5294	7	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gar	5.42	6.88	5.36	5.66	5.96	3.35	-4.2	3.8E-02	2.3E-01
PIK3IP1	113791	22	phosphoinositide-3-kinase interacting protein 1	9.09	8.83	10.26	9.34	8.96	6.30	-6.9	2.0E-02	7.2E-01
PIP5K1B	8395	9	phosphatidylinositol-4-phosphate 5-kinase, type I, beta	1.34	2.68	0.95	1.29	1.83	4.83	11.2	1.0E-02	4.8E-01
PITHD1	57095	1	PITH (C-terminal proteasome-interacting domain of thioredoxin-like)	5.64	6.10	5.62	6.55	6.68	7.38	3.4	4.2E-02	4.0E-01
PIWIL4	143689	11	piwi-like RNA-mediated gene silencing 4	1.42	3.48	2.74	3.74	3.58	3.67	4.7	4.7E-02	6.0E-02
PKDCC	91461	2	protein kinase domain containing, cytoplasmic	-1.53	-2.98	-2.81	-3.91	-1.88	2.32	14.5	1.7E-02	2.0E-01
PKLR	5313	1	pyruvate kinase, liver and RBC	-7.11	-3.52	-7.11	-4.76	-7.11	3.42	3040.6	6.3E-06	2.1E-01
PKMYT1	9088	16	protein kinase, membrane associated tyrosine/threonine 1	-0.15	-0.82	-2.81	-1.05	-0.42	3.67	14.2	8.1E-03	3.7E-01
PLA2G16	11145	11	phospholipase A2, group XVI	3.69	2.00	2.14	2.70	1.81	-0.40	-17.2	3.0E-03	8.5E-02
PLCL1	5334	2	phospholipase C-like 1	4.22	5.09	4.43	4.33	3.83	2.09	-4.4	3.7E-02	8.3E-01
PLCXD3	345557	5	phosphatidylinositol-specific phospholipase C, X domain containing	-4.33	-7.11	-7.11	-2.99	-4.89	4.15	384.9	2.2E-04	6.0E-01
PLEK2	26499	14	pleckstrin 2	-0.37	-2.27	-1.72	-2.68	-1.40	3.73	17.2	3.5E-03	9.9E-02
PLEKHA1	59338	10	pleckstrin homology domain containing, family A (phosphoinositide b	5.55	5.56	6.25	5.73	5.55	3.39	-4.5	1.1E-02	6.4E-01
PLEKHF1	79156	19	pleckstrin homology domain containing, family F (with FYVE domain	5.27	2.61	4.55	3.75	3.44	1.70	-11.9	2.6E-03	7.3E-02
PLIN5	440503	19	perilipin 5	-0.54	1.11	2.26	2.82	2.30	2.95	11.3	2.3E-02	6.4E-02
PLK1	5347	16	polo-like kinase 1	1.93	1.31	1.59	1.50	1.94	4.84	7.5	1.4E-02	5.1E-01
PLVAP	83483	19	plasmalemma vesicle associated protein	0.45	-1.45	-2.56	-0.97	-1.21	4.54	17.0	4.5E-03	6.5E-02
PLXNB2	23654	22	plexin B2	7.17	8.51	8.13	8.47	8.86	5.02	-4.5	1.6E-02	9.1E-02
PLXND1	23129	3	plexin D1	6.32	7.34	7.51	7.65	7.12	4.60	-3.3	3.0E-02	1.0E-01
PMCHL2	5370	5	pro-melanin-concentrating hormone-like 2, pseudogene	-3.14	-1.80	-4.03	-3.91	-2.19	1.91	34.1	4.5E-03	8.5E-01
POLE2	5427	14	polymerase (DNA directed), epsilon 2, accessory subunit	-1.36	-0.31	-0.61	-0.97	-1.05	2.39	13.6	7.3E-03	4.2E-01
POLQ	10721	3	polymerase (DNA directed), theta	-0.02	-0.46	0.19	-1.15	-0.70	2.58	6.1	4.6E-02	7.0E-01
POLR1E	64425	9	polymerase (RNA) I polypeptide E, 53kDa	5.19	4.18	4.55	4.46	4.20	3.00	-4.6	1.6E-02	2.1E-01
POLR3D	661	8	polymerase (RNA) III (DNA directed) polypeptide D, 44kDa	4.16	3.91	2.67	3.38	3.17	2.00	-4.5	4.6E-02	3.6E-01
POM121C	100101267	7	POM121 transmembrane nucleoporin C	6.08	6.98	6.96	6.60	6.56	4.34	-3.4	3.9E-02	3.2E-01
POM121L8P	29797	22	POM121 transmembrane nucleoporin-like 8 pseudogene	-2.33	-1.30	-1.38	-1.05	-2.37	1.58	15.4	1.1E-02	6.0E-01
POMT1	10585	9	protein-O-mannosyltransferase 1	4.91	5.19	6.31	5.74	5.54	6.88	3.9	4.4E-02	4.8E-01
POU5F1	5460	6	POU class 5 homeobox 1	1.32	0.32	1.04	0.49	0.41	3.95	6.2	4.5E-02	2.4E-01
PPIAL4G	644591	1	peptidylprolyl isomerase A (cyclophilin A)-like 4G	-2.89	-7.11	-4.87	-7.11	-4.89	0.33	9.5	4.9E-02	6.1E-02
PPIB	5479	15	peptidylprolyl isomerase B (cyclophilin B)	6.07	5.20	5.05	5.43	5.51	3.95	-4.3	1.5E-02	3.8E-01
PPII6	285755	6	peptidylprolyl isomerase (cyclophilin)-like 6	0.38	0.32	0.38	-0.16	0.55	3.49	8.6	1.4E-02	8.4E-01
PPM1J	333926	1	protein phosphatase, Mg2+/Mn2+ dependent, 1J	1.62	1.51	-0.61	1.40	2.10	-1.96	-12.1	4.1E-02	7.5E-01
PPM1L	151742	3	protein phosphatase, Mg2+/Mn2+ dependent, 1L	2.05	3.07	2.23	2.16	2.35	-1.96	-16.3	1.3E-02	4.7E-01
PPOX	5498	1	protoporphyrinogen oxidase	4.61	3.20	3.21	3.76	3.82	6.71	4.3	2.9E-02	9.5E-02
PPP1R11	6992	6	protein phosphatase 1, regulatory (inhibitor) subunit 11	6.08	4.48	5.05	4.50	4.31	4.02	-4.2	3.7E-02	1.3E-01
PPP1R14C	81706	6	protein phosphatase 1, regulatory (inhibitor) subunit 14C	-3.44	-3.52	-4.87	-0.54	-1.74	1.00	22.6	2.2E-02	2.3E-01
PPP1R17	10842	7	protein phosphatase 1, regulatory subunit 17	1.45	-2.02	-1.85	-3.91	-2.02	-7.11	-771		

PRKACB	5567	1	protein kinase, cAMP-dependent, catalytic, beta	7.25	5.31	5.82	5.23	5.19	4.99	-4.8	3.8E-02	1.0E-01
PRKAG2	51422	7	protein kinase, AMP-activated, gamma 2 non-catalytic subunit	4.90	6.12	6.24	6.12	6.15	3.25	-3.1	4.8E-02	6.3E-02
PRKAR2A	5576	3	protein kinase, cAMP-dependent, regulatory, type II, alpha	4.19	5.16	4.55	5.18	4.97	1.58	-6.1	8.8E-03	2.0E-01
PRKCA	5578	17	protein kinase C, alpha	6.30	5.55	5.85	5.66	5.34	4.32	-4.0	1.5E-02	2.4E-01
PRKCH	5583	14	protein kinase C, eta	8.13	7.04	7.33	7.57	7.21	5.41	-6.5	1.1E-03	2.1E-01
PRKG2	5593	4	protein kinase, cGMP-dependent, type II	0.62	-1.30	0.29	-2.99	-0.76	-7.11	-434.5	2.8E-02	1.5E-01
PRKRIR	5612	11	protein-kinase, interferon-inducible double stranded RNA dependent	6.47	7.29	6.68	7.00	6.98	4.61	-3.6	2.2E-02	3.1E-01
PROCR	10544	20	protein C receptor, endothelial	1.09	-0.24	-0.24	-0.11	-0.42	-7.11	-603.1	1.0E-02	3.1E-01
PROM1	8842	4	prominin 1	-1.21	-1.80	-2.16	-1.87	-0.52	3.09	19.8	8.2E-03	6.1E-01
PRR12	57479	19	proline rich 12	6.78	5.19	6.87	4.79	4.49	4.15	-6.2	1.9E-02	1.3E-01
PRRC1	133619	5	proline-rich coiled-coil 1	5.26	5.70	5.32	4.96	5.16	3.42	-3.6	4.2E-02	6.1E-01
PRRG4	79056	11	proline rich Gla (G-carboxyglutamic acid) 4 (transmembrane)	1.29	2.55	1.59	1.32	1.02	4.41	8.7	4.4E-02	2.1E-01
PRRT4	401399	7	proline-rich transmembrane protein 4	-0.84	-0.17	-0.94	-0.02	0.26	4.11	31.0	4.7E-04	6.0E-01
PRSS23	11098	11	protease, serine, 23	3.80	3.17	1.60	2.15	1.34	0.01	-13.9	2.4E-02	5.3E-01
PRTN3	5657	19	proteinase 3	-5.13	-4.41	-7.11	-3.38	-3.53	7.52	7390.7	3.3E-12	7.4E-01
PTCH1	5727	9	patched 1	4.81	3.18	3.41	3.60	3.07	0.01	-28.0	2.5E-04	1.5E-01
PTCHD2	57540	1	patched domain containing 2	-0.68	-2.02	-1.10	-1.58	-1.05	3.42	17.3	4.3E-03	4.7E-01
PTCHD4	442213	6	patched domain containing 4	-2.89	-0.05	0.05	-1.72	-0.52	2.39	40.0	9.5E-04	6.8E-02
PTGFRN	5738	1	prostaglandin F2 receptor inhibitor	1.71	0.36	2.02	-0.97	-0.76	-7.11	-928.2	8.1E-03	5.4E-01
PTMS	5763	12	parathyrosin	3.09	2.65	0.84	2.98	3.08	6.30	9.3	6.1E-03	7.5E-01
PTPN13	5783	4	protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (F	2.53	2.26	2.94	2.73	2.24	-7.11	-1632.6	6.6E-04	9.8E-01
PTPN14	5784	1	protein tyrosine phosphatase, non-receptor type 14	1.20	1.80	1.79	2.17	1.66	4.67	11.1	5.0E-03	6.1E-01
PTPRC	5788	1	protein tyrosine phosphatase, receptor type, C	10.16	9.27	9.83	9.00	8.96	7.85	-5.0	4.5E-02	5.7E-01
PTTG1	9232	5	pituitary tumor-transforming 1	2.10	2.63	2.43	2.96	2.99	5.47	10.3	3.6E-03	5.3E-01
PUM2	23369	2	pumilio homolog 2 (Drosophila)	7.46	6.73	7.89	7.06	7.05	5.73	-3.3	4.5E-02	8.8E-01
PXDN	7837	2	peroxidasin homolog (Drosophila)	-2.89	-7.11	-2.81	-4.76	-1.62	2.00	30.5	2.5E-02	4.4E-01
PYHIN1	149628	1	pyrin and HIN domain family, member 1	5.21	3.94	4.12	4.08	3.30	2.81	-5.3	3.7E-02	3.0E-01
PYY2	23615	17	peptide YY, 2 (pseudogene)	-3.44	-1.16	-0.67	-1.24	-0.58	0.81	19.8	1.6E-02	1.3E-01
QTRT1	81890	19	queuine tRNA-ribosyltransferase 1	4.92	4.46	4.76	5.18	5.47	6.80	3.7	3.9E-02	8.5E-01
R3HDM4	91300	19	R3H domain containing 4	7.88	6.96	7.98	7.47	8.06	9.84	3.9	3.0E-02	6.4E-01
RAB11FIP5	26056	2	RAB11 family interacting protein 5 (class I)	4.32	2.89	3.47	3.87	3.88	0.01	-20.0	4.7E-04	3.9E-01
RAB13	5872	1	RAB13, member RAS oncogene family	2.11	2.58	2.35	2.19	1.91	6.44	20.1	6.0E-05	6.6E-01
RAB3GAP1	22930	2	RAB3 GTPase activating protein subunit 1 (catalytic)	5.84	5.37	5.99	5.76	5.81	4.11	-3.3	3.5E-02	1.0E+00
RAB3IL1	5866	11	RAB3A interacting protein (rabn3)-like 1	-3.82	-2.98	-2.56	-2.99	-3.14	5.50	671.3	1.6E-05	3.0E-01
RAC3	5881	17	ras-related C3 botulinum toxin substrate 3 (rho family, small GTP bir	-0.41	0.41	2.20	1.14	0.79	2.90	10.0	2.6E-02	2.0E-01
RAD23A	5886	19	RAD23 homolog A (S. cerevisiae)	6.84	6.23	6.86	6.61	6.72	8.66	3.5	2.0E-02	6.1E-01
RAD51	5888	15	RAD51 homolog (S. cerevisiae)	0.49	0.57	0.23	0.79	1.00	3.28	6.9	2.8E-02	9.9E-01
RANBP10	57610	16	RAN binding protein 10	5.59	5.25	5.32	5.51	5.63	7.54	3.8	1.3E-02	8.1E-01
RAPGEF3	10411	12	Rap guanine nucleotide exchange factor (GEF) 3	0.23	-0.11	-0.80	-0.06	-0.32	3.09	7.2	3.2E-02	6.0E-01
RAPGEF6	51735	5	Rap guanine nucleotide exchange factor (GEF) 6	6.72	6.19	6.95	5.77	5.83	3.86	-7.3	2.3E-03	7.6E-01
RASA2	5922	3	RAS p21 protein activator 2	5.02	5.55	5.48	4.98	4.27	2.81	-4.6	1.9E-02	7.3E-01
RASGRP1	10125	15	RAS guanyl releasing protein 1 (calcium and DAG-regulated)	6.85	6.42	7.46	6.05	5.83	4.75	-4.3	2.2E-02	7.4E-01
RBM15B	29890	3	RNA binding motif protein 15B	6.61	6.21	6.14	6.29	6.26	4.71	-3.7	2.0E-02	7.6E-01
RBM27	54439	5	RNA binding motif protein 27	5.41	6.77	6.52	6.76	6.52	3.64	-3.4	4.1E-02	6.1E-02
RCAN3	11123	1	RCAN family member 3	6.72	5.87	6.83	5.99	5.67	4.83	-3.7	3.0E-02	5.1E-01
RECK	8434	9	reversion-inducing-cysteine-rich protein with kazal motifs	4.31	2.31	3.43	2.29	2.01	0.81	-11.3	1.5E-02	1.6E-01
RECQL4	9401	8	RecQ protein-like 4	3.18	2.67	1.47	2.84	2.96	5.62	5.4	2.5E-02	4.0E-01
RELN	5649	7	reelin	-7.11	-7.11	-7.11	-7.11	-7.11	0.81	495.8	7.4E-04	5.5E-01
REST	5978	4	RE1-silencing transcription factor	5.45	7.35	6.26	6.52	6.20	3.42	-4.1	2.5E-02	1.1E-01
RETN	56729	19	resistin	1.70	1.96	2.03	2.07	2.20	10.23	368.3	8.4E-16	5.5E-01
RFESD	317671	5	Rieske (Fe-S) domain containing	1.02	-1.30	-0.34	-1.35	-1.30	4.00	7.9	4.1E-02	2.0E-01
RFX8	731220	2	RFX family member 8, lacking RFX DNA binding domain	-3.14	-2.58	-0.29	-1.46	-1.13	0.81	15.9	3.3E-02	2.1E-01
RGL1	23179	1	ral guanine nucleotide dissociation stimulator-like 1	1.98	3.21	1.89	1.74	2.46	-1.96	-15.6	2.0E-02	4.8E-01
RGMB	285704	5	RGM domain family, member B	3.49	4.73	4.12	4.27	4.29	1.17	-5.0	4.1E-02	4.5E-01
RHAG	6005	6	Rh-associated glycoprotein	-5.13	-4.41	-7.11	-7.11	-4.05	7.00	5165.5	3.8E-11	1.0E+00
RHCE	6006	1	Rh blood group, CcEe antigens	-2.50	-2.27	-1.38	-1.46	-1.88	5.93	352.3	3.2E-07	5.4E-01
RHD	6007	1	Rh blood group, D antigen	0.10	1.22	1.39	-1.24	-0.14	5.24	35.6	7.0E-04	5.0E-01
RIBC2	26150	22	RIB43A domain with coiled-coils 2	-4.33	-2.98	-1.10	-7.11	-2.84	1.17	48.9	1.8E-02	3.8E-01
RILP	83547	17	Rab interacting lysosomal protein	2.60	3.64	1.99	3.77	4.48	5.67	8.4	8.2E-03	2.6E-01
RM12	116028	16	RM12, RecQ mediated genome instability 2, homolog (S. cerevisiae)	0.87	-0.93	-0.49	-1.87	-1.05	4.02	8.9	4.6E-02	4.5E-01
RNASE2	6036	14	ribonuclease, RNase A family, 2 (liver, eosinophil-derived neurotoxin	2.18	0.85	-1.48	2.30	1.51	6.32	17.6	4.5E-02	4.9E-01
RNASE3	6037	14	ribonuclease, RNase A family, 3	-2.33	-2.58	-4.87	-3.38	-2.19	6.71	539.3	7.4E-05	5.0E-01
RNASE6	6039	14	ribonuclease, RNase A family, k6	4.99	4.74	3.07	4.26	4.31	1.58	-10.6	1.4E-02	9.8E-01
RND2	8153	17	Rho family GTPase 2	-0.41	-2.27	-0.67	-0.97	-1.05	2.81	9.4	2.4E-02	3.1E-01
RNF10	9921	12	ring finger protein 10	7.46	8.14	7.79	8.54	8.58	10.08	6.1	6.9E-03	3.5E-01
RNF182	221687	6	ring finger protein 182	-4.33	-2.27	-2.81	-2.22	-1.88	0.81	38.0	3.4E-03	1.6E-01
RNF4	6047	4	ring finger protein 4	6.79	6.83	7.21	7.29	7.27	4.94	-3.6	3.8E-02	4.7E-01
ROGDI	79641	16	roqdi homolog (Drosophila)	4.59	5.04	3.73	4.68	4.62	6.57	3.9	3.9E-02	8.0E-01
RPH3A	22895	12	rabphilin 3A homolog (mouse)	3.08	3.69	1.39	2.24	0.50	-0.98	-16.8	2.0E-02	8.2E-01
RPL21P44	402176	4	ribosomal protein L21 pseudogene 44	-2.50	-1.61	-0.87	-2.22	-2.19	0.59	8.6	4.3E-02	4.7E-01
RPPH1	85495	14	ribonuclease P RNA component H1	-0.50	5.96	-0.49	0.71	-0.14	5.97	88.9	3.2E-02	1.1E-01
RPRD2	23248	1	regulation of nuclear pre-mRNA domain containing 2	6.41	5.97	6.49	6.13	5.94	4.00	-5.3	3.9E-03	8.0E-01
RPS16P5	647190	6	ribosomal protein S16 pseudogene 5	-2.05	1.11	-1.48	0.46	0.72	2.64	26.3	1.3E-02	1.7E-01
RPTOR	57521	17	regulatory associated protein of MTOR, complex 1	5.85	5.27	5.57	5.06	5.10	4.19	-3.2	4.3E-02	3.6E-01
RRM2	6241	2	ribonucleotide reductase M2	2.97	1.71	0.05	1.74	2.09	6.20	9.4	5.9E-03	8.7E-02
RRP1B	23076	21	ribosomal RNA processing 1 homolog B (S. cerevisiae)	6.32	5.98	6.00	6.05	5.91	4.50	-3.5	2.5E-02	7.3E-01
RSAD2	91543	2	radical S-adenosyl methionine domain containing 2	2.46	1.61	3.22	3.25	2.05	5.63	9.1	1.2E-02	3.9E-01
RTEL1	51750	20	regulator of telomere elongation helicase 1	1.19	1.03	0.63	1.51	2.12	3.93	6.7	3.8E-02	9.6E-01
RTN1	6252	14	reticulin 1	3.62	3.19	-1.72	1.59	1.52	-1.96	-48.5	2.5E-02	8.5E-01
RUND3A	10900	17	RUN domain containing 3A	-2.68	-0.31	-0.34	-0.16	0.84	6.06	439.1	3.4E-06	6.4E-02
RUNX3	864	1	run1-related transcription factor 3	8.52	8.95	9.19	9.59	9.08	6.34	-4.6	2.5E-02	3.7E-01
S100A10	6281	1	S100 calcium binding protein A10	7.55	7.11	6.86	7.09	7.93	5.52	-4.1	2.0E-02	9.6E-01
S100A13	6284	1	S100 calcium binding protein A13	1.76	2.39	2.43	2.31	2.76	4.04	4.9	4.3E-02	5.2E-01
S100A8	6279	1	S100 calcium binding protein A8	7.36	8.21	4.18	8.11	8.42	11.98	24.6	7.7E-03	3.3E-01
S100A9	6280	1	S100 calcium binding protein A9	8.92	9.77	4.17	9.78	10.08	12.61	12.9	4.6E-02	4.0E-01
S100P	6286	4	S100 calcium binding protein P	-0.59	-1.16	-0.44	1.61	3.56	7.52	276.9	5.0E-05	7.4E-02
S1PR1	1901	1	sphingosine-1-phosphate receptor 1	7.95	7.37	7.63	6.74	7.03	4.49	-11.0	8.0E-04	5.9E-01
S1PR5	53637	19	sphingosine-1-phosphate receptor 5	6.21	4.18	3.68	4.50	3.68	2.39	-14.1	2.3E-03	6.8E-02
SACS	26278	13	spastic ataxia of Charlevoix-Saguenay (sacsin)	4.86	5.96	5.38	4.70	4.75	2.25	-6.1	8.3E-03	4.0E-01
SAMHD1	25939	20	SAM domain and HD domain 1	8.05	5.89	6.88	6.05	5.31	4.83	-9.3	2.4E-02	3.1E-01
SAP25	100316904	7	Sin3A-associated protein, 25kDa	5.93	5.41	6.35	6.31	6.30	8.12	4.6	2.2E-02	9.0E-01
SART3	9733	12	squamous cell carcinoma antigen recognized by T cells 3	6.16	6.16	6.47	6.14	6.06	4.50	-3.1	4.6E-02	7.7E-01
SCAMP5	192683	15	secretory carrier membrane protein 5	2.84	2.58	1.86	1.54	2.05	-0.98	-14.2	8.1E-03	4.2E-01
SCARNA16	677781	17	small Cajal body-specific RNA 16	-3.14	0.57	-1.28	-0.74	-0.18	1.58	27.3	6.1E-03	7.0E-02
SCARNA17	677769	18	small Cajal body-specific RNA 17	0.97	3.06	0.77						

SCN9A	6335	2	sodium channel, voltage-gated, type IX, alpha subunit	0.49	0.65	-3.12	-1.24	-0.27	-7.11	-398.4	3.6E-02	5.1E-01
SCRN1	9805	7	secernin 1	5.40	5.38	5.43	4.88	4.90	2.90	-5.6	5.7E-03	8.7E-01
SCT	6343	11	secretin	0.07	-1.04	-2.81	-1.15	-0.64	-7.11	-296.0	4.6E-02	3.9E-01
SDK2	54549	17	sidekick cell adhesion molecule 2	3.50	4.09	3.76	3.93	4.32	0.59	-7.5	2.2E-02	6.8E-01
SDR42E1	93517	16	short chain dehydrogenase/reductase family 42E, member 1	1.89	1.00	1.63	1.02	0.70	-1.96	-14.6	2.0E-02	5.5E-01
SEC14L3	266629	22	SEC14-like 3 (S. cerevisiae)	-7.11	-7.11	0.32	-7.11	-4.89	0.59	425.1	3.0E-02	7.8E-02
SEC23A	10484	14	Sec23 homolog A (S. cerevisiae)	5.05	5.24	5.86	5.61	5.22	3.00	-4.1	1.7E-02	3.7E-01
SEC24D	9871	4	SEC24 family, member D (S. cerevisiae)	4.46	5.14	4.56	4.99	4.80	2.25	-4.6	1.8E-02	3.4E-01
SEC62	7095	3	SEC62 homolog (S. cerevisiae)	6.36	7.11	7.05	7.24	7.26	8.36	4.0	2.0E-02	1.6E-01
SECTM1	6398	17	secreted and transmembrane 1	5.88	5.83	4.26	5.16	5.59	3.04	-7.1	8.7E-03	8.8E-01
SEL1L	6400	14	sel-1 suppressor of lin-12-like (C. elegans)	5.48	6.51	7.63	6.47	6.23	3.61	-3.6	3.7E-02	5.3E-02
SEL1L3	23231	4	sel-1 suppressor of lin-12-like 3 (C. elegans)	6.90	6.10	6.67	6.12	6.45	5.06	-3.6	2.3E-02	2.9E-01
SELENBP1	8991	1	selenium binding protein 1	-2.89	-4.41	-4.87	-1.58	2.14	9.71	6415.1	1.0E-04	1.6E-01
SELM	140606	22	selenoprotein M	4.09	3.67	3.95	3.82	3.99	0.81	-9.7	5.1E-03	5.7E-01
SELO	83642	22	selenoprotein O	5.37	6.78	7.65	6.53	7.12	7.33	3.9	4.7E-02	7.7E-02
SEMA3E	9723	7	sema domain, immunoglobulin domain (Ig), short basic domain, sec	-2.50	-0.05	-0.20	0.02	-0.22	1.81	20.1	3.5E-03	6.4E-02
SEPP1	6414	5	selenoprotein P, plasma, 1	-2.19	-1.80	-1.28	-2.68	-2.84	1.00	9.3	3.9E-02	8.4E-01
SEPT9	10801	17	septin 9	10.13	8.88	9.04	9.12	9.05	8.19	-3.9	3.9E-02	9.5E-02
SESN1	27244	6	sestrin 1	5.98	6.71	6.03	7.21	6.60	4.00	-4.0	2.9E-02	4.4E-01
SEZ6L	23544	22	seizure related 6 homolog (mouse)-like	1.81	1.61	-1.85	-0.21	0.30	-7.11	-994.4	7.9E-03	4.2E-01
SFRP2	6423	4	secreted frizzled-related protein 2	-7.11	-7.11	-7.11	-7.11	-2.19	2.32	1414.8	6.2E-03	2.0E-01
SFTPD	6441	10	surfactant protein D	0.56	-0.63	-4.87	-1.58	-0.76	-7.11	-416.4	3.7E-02	2.6E-01
SGIP1	84251	1	SH3-domain GRB2-like (endophilin) interacting protein 1	-3.82	-2.58	-3.50	-2.99	-3.53	1.00	29.7	1.6E-02	5.7E-01
SGK196	84197	8	protein kinase-like protein SgK196	1.42	2.58	1.33	0.18	0.16	-7.11	-759.6	7.9E-03	9.8E-01
SGOL1	151648	3	shugoshin-like 1 (S. pombe)	1.03	1.03	0.35	0.52	0.55	3.78	6.7	2.3E-02	5.8E-01
SGPL1	8879	10	sphingosine-1-phosphate lyase 1	5.59	5.71	6.44	5.61	5.44	3.64	-3.9	2.3E-02	5.9E-01
SGPP1	81537	14	sphingosine-1-phosphate phosphatase 1	4.04	4.88	4.53	4.38	4.64	1.91	-4.4	2.6E-02	2.6E-01
SH2D2A	9047	1	SH2 domain containing 2A	5.55	4.25	4.68	3.78	2.80	3.04	-5.7	2.0E-02	8.9E-02
SHANK3	85358	22	SH3 and multiple ankyrin repeat domains 3	-1.21	-1.80	-1.48	-1.87	0.20	3.73	30.9	2.7E-03	9.9E-01
SHCBP1	79801	16	SHC SH2-domain binding protein 1	1.02	3.31	1.10	2.44	2.39	3.93	7.5	3.2E-02	2.5E-01
SHD	56961	19	Src homology 2 domain containing transforming protein D	0.40	1.24	-0.61	0.36	1.21	-7.11	-374.3	2.7E-02	9.3E-01
SHPRH	257218	6	SNF2 histone linker PHD RING helicase, E3 ubiquitin protein ligase	3.93	3.91	5.19	3.20	3.16	1.32	-6.1	3.0E-02	8.0E-01
SIAH2	6478	3	siah E3 ubiquitin protein ligase 2	5.47	6.00	5.73	6.59	6.53	7.82	5.1	7.6E-03	3.3E-01
SIN3A	25942	15	SIN3 transcription regulator homolog A (yeast)	6.96	7.66	7.74	7.16	7.36	4.84	-4.3	1.1E-02	5.1E-01
SIPA1L3	23094	19	signal-induced proliferation-associated 1 like 3	5.49	5.80	5.26	5.00	5.50	3.17	-5.0	9.7E-03	7.5E-01
SIX4	51804	14	SIX homeobox 4	0.84	0.12	-0.15	0.29	0.16	3.67	7.1	3.6E-02	2.3E-01
SKA1	220134	18	spindle and kinetochore associated complex subunit 1	1.37	1.17	0.99	1.72	1.74	4.34	7.9	2.1E-02	8.3E-01
SKA3	221150	13	spindle and kinetochore associated complex subunit 3	-1.53	-3.52	-3.12	-1.87	-1.88	2.58	17.4	7.8E-03	6.3E-01
SLAMF1	6504	1	signaling lymphocytic activation molecule family member 1	4.91	4.22	4.64	4.12	3.67	2.81	-4.3	2.6E-02	3.3E-01
SLAMF7	57823	1	SLAM family member 7	6.07	4.46	4.48	4.49	4.11	3.67	-5.3	3.5E-02	1.7E-01
SLC14A1	6563	18	solute carrier family 14 (urea transporter), member 1 (Kidd blood gro	2.54	2.56	-0.20	2.62	2.14	7.12	23.9	1.3E-03	5.7E-01
SLC15A1	6564	13	solute carrier family 15 (oligopeptide transporter), member 1	-0.37	-1.04	-1.10	-0.97	-1.05	2.58	7.8	3.0E-02	3.2E-01
SLC17A9	63910	20	solute carrier family 17, member 9	3.92	4.48	2.66	4.44	5.28	6.57	6.3	1.6E-02	6.0E-01
SLC1A2	6506	11	solute carrier family 1 (glial high affinity glutamate transporter), mem	-0.46	0.94	-0.03	0.42	0.65	3.97	21.7	3.7E-03	5.7E-01
SLC22A16	85413	6	solute carrier family 22 (organic cation/carnitine transporter), membe	-1.62	0.36	-0.80	-1.58	-1.21	4.04	51.1	3.6E-04	2.4E-01
SLC22A31	146429	16	solute carrier family 22, member 31	-0.50	0.06	0.55	-0.42	0.96	2.90	10.6	1.5E-02	5.3E-01
SLC25A21	89874	14	solute carrier family 25 (mitochondrial oxoanipate carrier), member 2	-7.11	-7.11	-4.87	-7.11	-4.89	1.58	849.3	1.4E-04	1.3E-01
SLC25A39	51629	17	solute carrier family 25, member 39	6.40	6.49	6.01	6.85	7.23	10.78	20.8	2.6E-05	8.7E-01
SLC26A4	5172	7	solute carrier family 26, member 4	-1.08	0.36	0.12	0.18	0.23	3.09	18.0	2.2E-03	2.4E-01
SLC26A8	116369	6	solute carrier family 26, member 8	-3.14	-0.93	-1.85	-0.42	-1.74	1.81	31.9	2.5E-03	1.4E-01
SLC28A3	64078	9	solute carrier family 28 (sodium-coupled nucleoside transporter), me	-2.33	-0.38	-3.50	-1.24	-1.74	2.39	26.9	5.0E-03	3.6E-01
SLC2A1	6513	1	solute carrier family 2 (facilitated glucose transporter), member 1	5.58	6.25	6.54	6.32	6.95	8.70	8.6	9.5E-04	3.1E-01
SLC2A13	114134	12	solute carrier family 2 (facilitated glucose transporter), member 13	1.29	2.39	1.86	1.70	1.62	-7.11	-693.4	6.2E-03	3.1E-01
SLC2A5	6518	1	solute carrier family 2 (facilitated glucose/fructose transporter), mem	0.94	1.57	4.28	1.18	1.80	5.39	21.9	1.1E-02	2.4E-01
SLC2A9	56606	4	solute carrier family 2 (facilitated glucose transporter), member 9	1.12	2.21	-1.38	1.71	1.62	-7.11	-615.1	1.8E-02	4.2E-01
SLC35D1	23169	1	solute carrier family 35 (UDP-glucuronic acid/UDP-N-acetylgalactosi	4.77	5.62	4.73	5.01	4.86	2.25	-5.8	1.4E-02	4.4E-01
SLC35F1	222553	6	solute carrier family 35, member F1	0.21	0.82	0.65	0.95	1.11	4.13	15.2	3.2E-03	6.5E-01
SLC35G5	83650	8	solute carrier family 35, member G5	-3.44	-7.11	-7.11	-7.11	-4.05	1.17	25.4	1.5E-02	2.3E-01
SLC35G6	643664	17	solute carrier family 35, member G6	-7.11	-3.52	-3.50	-4.76	-7.11	0.59	425.1	1.4E-03	1.1E-01
SLC38A1	81539	12	solute carrier family 38, member 1	8.31	8.39	8.85	8.76	8.52	6.05	-4.8	1.2E-02	6.1E-01
SLC39A6	25800	18	solute carrier family 39 (zinc transporter), member 6	4.95	5.81	5.38	5.21	5.43	2.64	-4.9	8.7E-03	3.4E-01
SLC39A9	55334	14	solute carrier family 39 (zinc transporter), member 9	5.18	5.50	6.05	5.81	5.57	3.49	-3.2	4.4E-02	3.0E-01
SLC41A1	254428	1	solute carrier family 41, member 1	5.13	5.42	5.57	5.48	5.26	3.32	-3.5	4.2E-02	8.0E-01
SLC41A2	84102	12	solute carrier family 41, member 2	1.24	2.40	2.70	2.16	2.37	3.70	5.5	4.1E-02	3.1E-01
SLC45A1	50651	1	solute carrier family 45, member 1	0.45	-0.31	-1.02	-0.06	-0.18	-7.11	-386.3	2.2E-02	4.7E-01
SLC46A2	57864	9	solute carrier family 46, member 2	2.25	1.80	1.12	1.27	0.60	-7.11	-1343.6	1.0E-02	6.7E-01
SLC4A1	6521	17	solute carrier family 4, anion exchanger, member 1 (erythrocyte me	0.10	1.29	0.86	1.59	2.81	11.56	2838.5	7.6E-11	1.6E-01
SLC4A10	57282	2	solute carrier family 4, sodium bicarbonate transporter, member 10	3.72	0.94	0.35	0.39	0.30	-0.40	-17.5	5.0E-02	2.2E-01
SLC4A11	83959	20	solute carrier family 4, sodium borate transporter, member 11	-0.84	-1.16	0.16	-0.74	-0.42	2.32	9.0	3.1E-02	9.5E-01
SLC4A3	6508	2	solute carrier family 4, anion exchanger, member 3	1.88	1.80	-1.59	1.61	2.28	-7.11	-1042.6	5.7E-03	6.3E-01
SLC6A10P	386757	16	solute carrier family 6 (neurotransmitter transporter, creatine), mem	-5.13	-2.58	-1.59	-2.03	-3.14	0.59	60.6	5.8E-03	1.4E-01
SLC6A12	6539	12	solute carrier family 6 (neurotransmitter transporter, betaine/GABA),	1.09	-0.05	-2.35	-0.97	-2.19	-7.11	-603.1	3.4E-02	6.8E-01
SLC6A19	340024	5	solute carrier family 6 (neutral amino acid transporter), member 19	-7.11	-7.11	-7.11	-7.11	-7.11	5.74	15128.5	1.4E-08	1.0E+00
SLC6A9	6536	1	solute carrier family 6 (neurotransmitter transporter, glycine), mem	-2.89	-2.98	-2.35	-3.91	-3.53	5.76	413.2	3.6E-07	7.3E-01
SLC7A5P2	387254	16	solute carrier family 7 (amino acid transporter light chain, L system),	1.08	3.34	1.88	2.32	2.41	3.70	6.2	4.9E-02	2.3E-01
SLC7A6OS	84138	16	solute carrier family 7, member 6 opposite strand	3.18	2.89	2.73	2.07	1.47	0.33	-7.3	2.9E-02	4.6E-01
SLC7A7	9056	14	solute carrier family 7 (amino acid transporter light chain, y+L system	6.16	7.68	5.36	6.92	6.76	3.35	-7.0	1.0E-02	1.9E-01
SLFN14	342618	17	schlafen family member 14	0.52	-0.31	0.65	0.58	-0.18	3.75	9.4	1.6E-02	8.4E-01
SLFN5	162394	17	schlafen family member 5	7.12	5.20	6.78	5.12	3.23	3.32	-13.9	9.3E-03	4.2E-01
SLK	9748	10	STE20-like kinase	5.64	6.01	6.51	6.03	5.66	3.80	-3.6	3.8E-02	2.9E-01
SMARCA2	6595	9	SWI/SNF related, matrix associated, actin dependent regulator of ch	7.30	6.84	6.99	6.56	6.43	5.25	-4.1	1.1E-02	3.8E-01
SMARCA5	8467	4	SWI/SNF related, matrix associated, actin dependent regulator of ch	6.78	7.93	7.05	7.71	7.44	5.13	-3.1	4.5E-02	2.3E-01
SMC1B	27127	22	structural maintenance of chromosomes 1B	-3.44	-0.46	0.44	-0.89	-1.21	0.59	16.9	2.4E-02	7.0E-02
SMCR7L	54471	22	Smith-Magenis syndrome chromosome region, candidate 7-like	5.94	6.31	6.24	6.18	5.90	4.00	-3.8	2.3E-02	5.8E-01
SMCR8	140775	17	Smith-Magenis syndrome chromosome region, candidate 8	6.70	7.83	7.80	7.49	7.26	4.30	-5.3	5.9E-03	1.8E-01
SMYD3	64754	1	SET and MYND domain containing 3	3.41	2.41	1.72	3.09	2.68	0.81	-6.1	3.6E-02	3.5E-01
SNED1	25992	2	sushi, nidogen and EGF-like domains 1	4.03	4.86	6.05	4.90	4.34	1.17	-7.3	2.0E-02	3.5E-01
SNORA12	677800	10	small nucleolar RNA, H/ACA box 12	-1.93	0.61	-0.94	0.66	-0.06	2.25	18.4	1.8E-02	2.3E-01
SNORA11	619569	2	small nucleolar RNA, H/ACA box 41	-2.89	-0.24	0.01	0.10	1.16	0.81	13.4	3.4E-02	5.2E-02
SNORA57	692158	11	small nucleolar RNA, H/ACA box 57	-1.01	-0.17	0.26	-0.42	-0.06	2.46	11.2	1.8E-02	5.9E-01
SNORD104	692227	17	small nucleolar RNA, C/D box 104	2.02	2.25	2.86	3.18	3.64	4.78	6.8	3.5E-02	5.0E-01
SNORD17	692086	20	small nucleolar RNA, C/D box 17	-2.33	1.70	-2.81	-2.68	-2.02	3.70	66.6	8.7E-03	

SNX30	401548	9	sorting nexin family member 30	5.06	6.46	6.27	6.15	5.77	2.70	-5.2	7.0E-03	6.5E-02
SNX32	254122	11	sorting nexin 32	-1.53	-1.30	-2.56	-1.72	-0.70	2.64	18.2	4.9E-03	9.9E-01
SORBS1	10580	10	sorbin and SH3 domain containing 1	-0.12	1.11	0.16	1.29	0.68	3.13	9.5	1.2E-02	3.0E-01
SORL1	6653	11	sortilin-related receptor, L(DLR class) A repeats containing	8.61	8.91	9.67	8.64	7.91	6.60	-4.0	4.4E-02	7.0E-01
SOX11	6664	2	SRY (sex determining region Y)-box 11	-4.33	-2.27	-1.85	-2.68	-2.59	0.01	21.8	1.2E-02	1.5E-01
SOX2-OT	347689	3	SOX2 overlapping transcript (non-protein coding)	-3.14	-1.80	-1.72	-1.72	-1.30	0.59	13.7	2.2E-02	3.5E-01
SOX6	55553	11	SRY (sex determining region Y)-box 6	-5.13	-4.41	-7.11	-2.68	-4.05	4.93	1228.5	2.4E-06	5.5E-01
SP4	6671	7	Sp4 transcription factor	4.92	5.05	4.83	4.33	4.39	2.81	-4.3	3.5E-02	7.9E-01
SPAG6	9576	10	sperm associated antigen 6	-2.68	-2.98	-4.03	-2.68	-3.14	1.00	13.1	1.8E-02	4.5E-01
SPATA13	221178	13	spermatogenesis associated 13	7.01	8.08	7.77	8.15	7.72	5.22	-3.5	3.7E-02	1.8E-01
SPATC1	375686	8	spermatogenesis and centriole associated 1	-1.62	-0.72	-0.39	-0.67	-1.13	2.00	12.4	1.2E-02	4.5E-01
SPC24	147841	19	SPC24, NDC80 kinetochore complex component, homolog (S. cerev	-1.01	-4.41	-2.35	-2.43	-4.05	2.09	8.6	4.7E-02	1.6E-01
SPDYC	387778	11	speedy homolog C (Xenopus laevis)	-3.82	-2.58	-2.35	-3.91	-3.14	1.91	55.7	6.9E-03	1.9E-01
SPG7	6687	16	spastic paraplegia 7 (pure and complicated autosomal recessive)	6.34	6.68	7.74	7.18	7.02	8.29	3.9	4.4E-02	4.6E-01
SPINK8	646424	3	serine peptidase inhibitor, Kazal type 8 (putative)	-7.11	-2.98	-7.11	-2.22	-4.05	0.81	495.8	3.3E-03	9.7E-02
SPIRE2	84501	16	spire homolog 2 (Drosophila)	0.62	0.27	-0.49	0.76	-0.52	3.80	9.1	2.1E-02	4.4E-01
SPNS2	124976	17	spinster homolog 2 (Drosophila)	0.79	1.11	-1.10	0.74	0.98	3.46	6.4	4.9E-02	7.3E-01
SPOCK2	9806	10	sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testic	10.11	9.43	10.82	10.73	10.32	7.47	-6.2	2.5E-02	8.4E-01
SPSB3	90864	16	splA/ryanodine receptor domain and SOCS box containing 3	6.82	6.64	7.16	7.19	7.12	8.41	3.0	4.8E-02	9.1E-01
SPTA1	6708	1	spectrin, alpha, erythrocytic 1 (elliptocytosis 2)	-2.50	-2.98	-4.87	-3.38	-1.88	8.29	1804.5	1.5E-11	6.8E-01
SRGAP1	57522	12	SLIT-ROBO Rho GTPase activating protein 1	-0.78	1.09	2.37	0.69	1.16	2.46	9.5	2.8E-02	1.1E-01
SRRM4	84530	12	serine/arginine repetitive matrix 4	-2.89	-2.27	-3.50	-2.68	-3.53	1.32	19.1	6.2E-03	7.7E-01
SSR2	6746	1	signal sequence receptor, beta (translocon-associated protein beta)	8.39	7.46	7.26	7.94	8.05	6.45	-3.8	2.0E-02	2.7E-01
ST14	6768	11	suppression of tumorigenicity 14 (colon carcinoma)	5.26	5.55	7.19	5.07	4.96	2.00	-9.6	7.5E-03	5.2E-01
ST6GAL2	84620	2	ST6 beta-galactosamide alpha-2,6-sialyltransferase 2	-0.41	-0.46	-1.19	0.06	0.06	2.64	8.4	3.7E-02	8.3E-01
ST6GALNAC4	27090	9	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgl	4.21	3.87	4.42	3.54	4.46	6.43	4.7	1.4E-02	9.6E-01
STEAP2	261729	7	STEAP family member 2, metalloredutase	-0.54	1.03	0.47	0.71	0.58	2.70	9.5	2.0E-02	3.8E-01
STK4	6789	20	serine/threonine kinase 4	7.96	8.05	8.67	8.09	7.96	6.27	-3.2	4.5E-02	6.7E-01
STOM	2040	9	stomatin	6.57	6.08	5.56	5.92	5.71	8.42	3.6	4.1E-02	6.1E-01
STRADB	55437	2	STE20-related kinase adaptor beta	4.18	2.99	2.77	2.96	3.50	8.68	22.6	1.2E-05	1.7E-01
SUCLG2	8801	3	succinate-CoA ligase, GDP-forming, beta subunit	5.99	4.80	4.38	4.93	4.58	3.70	-4.9	1.8E-02	1.2E-01
SUFU	51684	10	suppressor of fused homolog (Drosophila)	5.10	3.48	4.53	3.76	3.59	2.39	-6.5	1.2E-02	1.5E-01
SULT1A2	6799	16	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 2	-3.14	-1.04	-1.28	0.42	-0.76	0.59	13.7	2.5E-02	6.2E-02
SULT1E1	6783	4	sulfotransferase family 1E, estrogen-preferring, member 1	-3.82	-2.02	-2.81	-2.22	-2.84	0.01	14.9	3.4E-02	4.8E-01
SUN3	256979	7	Sad1 and UNC84 domain containing 3	-3.44	-2.58	-3.50	-3.91	-3.53	0.01	11.3	2.9E-02	9.1E-01
SYNE2	23224	14	spectrin repeat containing, nuclear envelope 2	7.02	7.25	7.44	7.13	6.53	4.89	-4.4	1.0E-02	7.8E-01
SYNRG	11276	17	synerglin, gamma	7.38	5.80	6.89	5.47	5.20	5.00	-5.2	2.4E-02	1.1E-01
SYS1	90196	20	SYS1 Golgi-localized integral membrane protein homolog (S. cerevi	5.38	6.53	5.23	6.26	6.24	3.39	-4.0	3.5E-02	3.7E-01
SYT11	23208	1	synaptotagmin XI	4.77	4.75	5.05	5.11	5.02	3.00	-3.4	4.2E-02	5.4E-01
SYT17	51760	16	synaptotagmin XVII	1.03	0.12	-0.29	0.74	0.96	-7.11	-579.0	9.6E-03	5.9E-01
TAF7	6879	5	TAF7 RNA polymerase II, TATA box binding protein (TBP)-associate	7.40	7.06	6.87	6.93	6.97	5.66	-3.3	2.6E-02	5.8E-01
TAGAP	117289	6	T-cell activation RhoGTPase activating protein	8.54	8.66	7.58	7.74	8.36	6.34	-4.6	1.6E-02	4.7E-01
TAP1	6890	6	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	7.64	7.00	7.58	7.10	7.02	5.59	-4.2	1.0E-02	4.8E-01
TARM1	441864	19	T cell-interacting, activating receptor on myeloid cells 1	-4.33	-4.41	-7.11	-3.91	-4.89	2.81	151.8	2.9E-04	7.5E-01
TBC1D14	57533	4	TBC1 domain family, member 14	5.44	4.53	4.58	3.96	3.51	2.70	-6.7	1.7E-02	4.4E-01
TBC1D26	353149	17	TBC1 domain family, member 26	-1.08	-1.80	-1.10	-0.74	-1.51	2.00	8.5	4.8E-02	5.9E-01
TBC1D2B	23102	15	TBC1 domain family, member 2B	6.68	6.19	7.31	6.29	5.98	4.80	-3.7	3.2E-02	7.5E-01
TBC1D8	11138	2	TBC1 domain family, member 8 (with GRAM domain)	4.53	5.66	6.48	5.22	5.46	1.81	-6.6	5.5E-03	1.1E-01
TBX21	30009	17	T-box 21	6.04	4.61	4.80	5.69	4.84	3.97	-4.2	3.2E-02	2.2E-01
TC2N	123036	14	tandem C2 domains, nuclear	7.03	6.16	7.16	6.66	6.26	5.01	-4.1	1.8E-02	5.8E-01
TCN1	6947	11	transcobalamin I (vitamin B12 binding protein, R binder family)	-5.13	-7.11	-7.11	-3.38	-3.14	5.35	1641.4	3.1E-07	4.0E-01
TDP1	55775	14	tyrosyl-DNA phosphodiesterase 1	5.21	4.26	5.13	4.63	4.66	3.25	-3.9	2.8E-02	4.8E-01
TEAD3	7005	6	TEA domain family member 3	-3.44	-2.58	-2.56	-3.38	-3.53	0.01	11.3	2.9E-02	5.6E-01
TECPR1	25851	7	tectonin beta-propeller repeat containing 1	6.80	5.87	5.80	5.78	5.63	5.19	-3.1	4.1E-02	7.6E-02
TERF2IP	54386	16	telomeric repeat binding factor 2, interacting protein	6.88	7.15	7.01	7.55	7.34	8.66	3.4	2.9E-02	5.7E-01
TES	26136	7	testis derived transcript (3 LIM domains)	6.71	7.67	6.96	7.04	7.13	4.89	-3.5	2.3E-02	3.5E-01
TESC	54997	12	tescalcin	3.84	4.47	2.93	4.57	4.32	7.23	10.5	5.9E-04	5.3E-01
TFDP1	7027	13	transcription factor Dp-1	5.50	4.69	5.07	4.64	4.53	8.05	5.9	3.7E-03	4.8E-01
TFR2	7036	7	transferrin receptor 2	1.79	1.73	2.24	2.29	1.81	6.07	19.4	2.9E-04	9.4E-01
TGFB1	7045	5	transforming growth factor, beta-induced, 68kDa	6.49	8.25	6.45	7.08	7.30	-0.98	-178.4	1.7E-07	1.1E-01
TGFBR2	7048	3	transforming growth factor, beta receptor II (70/80kDa)	8.29	7.96	8.64	7.95	7.60	5.47	-7.1	4.9E-03	9.2E-01
TGFBR3	7049	1	transforming growth factor, beta receptor III	5.55	5.31	5.86	5.60	4.61	3.09	-5.5	8.7E-03	8.7E-01
TGM2	7052	20	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutar	1.23	1.94	3.19	2.01	2.42	5.43	18.4	6.9E-04	1.6E-01
TGOLN2	10618	2	trans-golgi network protein 2	8.44	8.87	8.45	8.71	8.33	6.62	-3.5	3.8E-02	6.8E-01
THEM5	284486	1	thioesterase superfamily member 5	2.31	-0.38	0.84	-0.16	-0.22	5.87	11.8	1.7E-02	6.1E-02
TIAM1	7074	21	T-cell lymphoma invasion and metastasis 1	5.82	5.65	5.27	4.69	4.90	4.00	-3.5	4.3E-02	4.3E-01
TICAM1	148022	19	tolil-like receptor adaptor molecule 1	5.22	5.95	3.69	5.55	5.86	2.32	-7.5	8.3E-03	7.8E-01
TIGD5	84948	8	tigger transposable element derived 5	3.78	1.06	1.63	0.33	1.02	0.33	-11.0	2.8E-02	5.5E-02
TIGIT	201633	3	T cell immunoreceptor with Ig and ITIM domains	5.42	2.97	3.93	2.91	2.55	2.32	-8.5	2.2E-02	1.1E-01
TIPARP-AS1	100287227	3	TIPARP antisense RNA 1	0.04	0.49	0.38	-0.02	0.44	-7.11	-290.0	3.3E-02	7.6E-01
TK1	7083	17	thymidine kinase 1, soluble	2.12	2.48	2.81	1.83	2.27	5.90	13.7	1.4E-03	9.4E-01
TLN2	83660	15	talin 2	-0.09	0.12	-0.61	0.02	0.30	2.75	7.2	2.6E-02	8.5E-01
TLR3	7098	4	tolil-like receptor 3	-0.02	0.27	-0.87	-0.02	-0.42	-7.11	-277.9	4.5E-02	7.3E-01
TMC1	117531	9	transmembrane channel-like 1	-2.05	-7.11	-3.50	-7.11	-4.05	1.81	14.7	2.2E-02	7.6E-02
TMC5	79838	16	transmembrane channel-like 5	-0.02	-2.27	-2.81	-1.15	-0.02	4.57	24.2	4.1E-03	1.6E-01
TMED10	10972	14	transmembrane emp24-like trafficking protein 10 (yeast)	7.19	6.37	6.96	6.79	6.73	5.38	-3.5	4.0E-02	9.1E-01
TMEM106B	54664	7	transmembrane protein 106B	4.67	4.54	5.50	4.19	3.73	2.17	-5.7	2.6E-02	5.9E-01
TMEM130	222865	7	transmembrane protein 130	-0.05	-1.45	-0.61	-1.15	-1.13	2.75	7.0	4.2E-02	1.8E-01
TMEM136	219902	11	transmembrane protein 136	-1.08	-2.27	-2.81	-2.99	-2.84	2.09	9.0	2.7E-02	6.2E-02
TMEM150B	284417	19	transmembrane protein 150B	1.42	2.15	-1.28	1.47	0.72	-7.11	-759.6	1.8E-02	6.6E-01
TMEM176A	55365	7	transmembrane protein 176A	4.10	5.37	2.73	5.51	3.05	-7.11	-4853.6	3.0E-04	6.3E-01
TMEM176B	28959	7	transmembrane protein 176B	5.52	5.53	3.25	5.43	2.57	-0.40	-61.0	1.0E-03	4.8E-01
TMEM217	221468	6	transmembrane protein 217	-2.50	-1.61	-1.85	-0.89	-0.97	1.58	17.3	1.4E-02	1.9E-01
TMEM233	387890	12	transmembrane protein 233	-7.11	-7.11	-7.11	-4.76	-7.11	1.00	566.5	3.8E-04	4.2E-01
TMEM56	148534	1	transmembrane protein 56	0.89	1.11	-0.07	-0.74	-0.02	4.90	16.2	7.0E-03	2.7E-01
TMEM65	157378	8	transmembrane protein 65	3.56	4.07	3.06	2.89	3.67	1.00	-5.9	3.6E-02	5.9E-01
TMEM66	51669	8	transmembrane protein 66	9.65	9.11	10.19	9.56	9.45	7.35	-4.9	3.8E-02	8.0E-01
TMEM86B	255043	19	transmembrane protein 86B	3.90	3.69	4.51	4.11	4.50	6.66	6.8	4.4E-03	8.7E-01
TMEM87B	84910	2	transmembrane protein 87B	4.98	4.34	5.28	4.90	4.18	3.00	-3.9	5.0E-02	9.6E-01
TMEM92	162461	17	transmembrane protein 92	-5.13	-4.41	-7.11	-4.76	-3.14	1.91	151.2	4.1E-04	4.1E-01
TMOD1	7111	9	tropomodulin 1	0.38	-1.80	-0.94	-1.87	-0.37	6.37	63.7	1.2E-04	1.7E-01
TMPRSS4	56649	11	transmembrane protease, serine 4	-7.11	-4.41	-2.16	-7.11	-7.11	2.00	1132.0	2.6E-03	1.5E-01
TNFAIP1	7126	17	tumor necrosis factor, alpha-induced protein 1 (endothelial)	4.33	4.28	4.71	4.54	4.31	2.17	-4.5	2.1E-02	7.9E-01
TNFAIP8	25816	5	tumor necrosis factor, alpha-induced protein 8	6.78	6.55	6.51	5					

TNS1	7145	2	tensin 1	3.49	2.81	7.81	1.96	2.97	8.36	29.3	3.4E-02	2.7E-01
TOB2	10766	22	transducer of ERBB2, 2	6.25	5.04	4.71	4.81	4.84	3.93	-5.0	1.1E-02	6.8E-02
TOMM7	54543	7	translocase of outer mitochondrial membrane 7 homolog (yeast)	8.63	7.62	7.69	8.27	8.13	6.54	-4.3	1.3E-02	1.9E-01
TOMM70A	9868	3	translocase of outer mitochondrial membrane 70 homolog A (S. cere	5.60	5.62	5.85	5.79	5.43	3.49	-4.3	1.5E-02	6.6E-01
TOP2A	7153	17	topoisomerase (DNA) II alpha 170kDa	2.33	1.96	2.57	1.75	2.12	5.24	7.5	9.3E-03	8.7E-01
TOX	9760	8	thymocyte selection-associated high mobility group box	4.27	3.17	2.81	2.80	2.34	1.00	-9.6	9.9E-03	1.5E-01
TP53I13	90313	17	tumor protein p53 inducible protein 13	5.72	5.33	5.85	5.83	5.81	7.32	3.0	4.5E-02	8.1E-01
TP73	7161	1	tumor protein p73	-0.95	-1.80	-0.80	-1.72	-0.83	1.81	6.8	4.5E-02	6.9E-01
TPM2	7169	9	tropomyosin 2 (beta)	4.92	4.84	5.15	5.57	5.11	7.20	4.8	4.6E-02	8.9E-01
TPX2	22974	20	TPX2, microtubule-associated, homolog (Xenopus laevis)	2.02	1.14	0.70	1.37	1.26	5.94	15.2	7.4E-04	2.9E-01
TRANK1	9881	3	tetratricopeptide repeat and ankyrin repeat containing 1	6.75	5.97	7.12	5.87	5.38	4.90	-3.6	4.9E-02	5.3E-01
TRERF1	55809	6	transcriptional regulating factor 1	5.94	5.96	5.46	5.82	5.35	3.86	-4.2	1.5E-02	6.0E-01
TRIM10	10107	6	tripartite motif containing 10	-0.73	-2.98	-3.12	0.42	-2.02	6.64	166.7	2.9E-04	6.6E-01
TRIM15	89870	6	tripartite motif containing 15	-5.13	-3.52	-7.11	-2.68	-7.11	2.58	241.8	8.2E-04	4.2E-01
TRIM4	89122	7	tripartite motif containing 4	5.66	4.12	5.01	4.34	4.15	3.28	-5.2	1.8E-02	2.3E-01
TRIM45	80263	1	tripartite motif containing 45	0.33	0.32	-0.07	0.36	0.36	3.09	6.7	4.3E-02	6.5E-01
TRIP4	9325	15	thyroid hormone receptor interactor 4	4.77	3.87	4.06	4.12	3.91	2.75	-4.0	3.7E-02	4.7E-01
TROAP	10024	12	trophinin associated protein	0.04	-2.02	-1.02	-1.05	-0.83	5.24	37.1	1.6E-04	1.6E-01
TRPS1	7227	8	trichorhinophalangeal syndrome I	3.64	3.98	4.96	3.70	3.65	1.17	-5.5	3.3E-02	2.6E-01
TSC22D2	9819	3	TSC22 domain family, member 2	5.01	6.89	5.48	6.53	6.62	3.00	-4.0	3.8E-02	8.8E-02
TSKS	60385	19	testis-specific serine kinase substrate	-0.95	0.32	-0.29	-1.05	1.90	2.95	15.1	2.0E-02	3.0E-01
TSNARE1	203062	8	t-SNARE domain containing 1	3.60	2.94	3.46	2.60	2.57	1.32	-4.9	4.8E-02	3.8E-01
TSPO2	222642	6	translocator protein 2	-2.33	-0.82	1.36	-1.72	-2.19	5.25	196.0	6.4E-04	2.4E-01
TSSK6	83983	19	testis-specific serine kinase 6	1.55	1.36	2.09	1.59	1.99	-1.96	-11.6	3.1E-02	8.2E-01
TTC21A	199223	3	tetratricopeptide repeat domain 21A	2.44	3.37	2.70	3.36	3.86	5.02	6.0	2.9E-02	4.8E-01
TTC30B	150737	2	tetratricopeptide repeat domain 30B	0.75	-0.38	0.50	-1.46	-0.70	-7.11	-476.6	2.6E-02	9.1E-01
TTC39A	22996	1	tetratricopeptide repeat domain 39A	-0.63	0.12	-0.15	0.49	1.35	2.17	7.0	4.9E-02	4.0E-01
TTK	7272	6	TTK protein kinase	-0.63	0.01	-1.28	0.06	-0.32	2.75	10.5	1.4E-02	6.2E-01
TTIL10	254173	1	tubulin tyrosine ligase-like family, member 10	-2.33	-2.58	-2.81	-2.43	-1.74	2.17	23.1	2.6E-03	9.5E-01
TTYH2	94015	17	tweety homolog 2 (Drosophila)	4.75	4.55	3.01	4.45	4.37	2.09	-6.3	1.6E-02	8.2E-01
TUBBP5	643224	9	tubulin, beta pseudogene 5	0.15	-0.63	-0.73	0.29	-0.58	-7.11	-314.1	4.2E-02	6.8E-01
TUBG1	7283	17	tubulin, gamma 1	2.48	2.50	3.16	2.89	2.46	5.13	6.2	1.3E-02	7.2E-01
TXNIP	10628	1	thioredoxin interacting protein	11.85	11.96	10.78	11.28	10.21	8.98	-7.3	2.4E-02	5.9E-01
UBA2	10054	19	ubiquitin-like modifier activating enzyme 2	6.53	6.26	6.55	6.27	6.20	4.87	-3.2	3.5E-02	9.0E-01
UBAC1	10422	9	UBA domain containing 1	5.26	5.49	4.94	5.45	5.58	6.91	3.1	3.9E-02	8.4E-01
UBB	7314	17	ubiquitin B	8.95	8.25	7.60	8.52	8.69	11.85	7.5	6.6E-03	5.7E-01
UBE2C	11065	20	ubiquitin-conjugating enzyme E2C	0.94	-0.24	-1.10	0.97	0.13	4.04	8.6	2.3E-02	2.6E-01
UBE2H	7328	7	ubiquitin-conjugating enzyme E2H	6.77	5.71	6.55	6.13	6.25	8.53	3.4	4.8E-02	7.1E-01
UBE2O	63893	17	ubiquitin-conjugating enzyme E2O	6.11	5.27	6.24	5.52	5.59	8.19	4.2	1.1E-02	4.4E-01
UBE2Z	65264	17	ubiquitin-conjugating enzyme E2Z	7.02	6.60	6.86	6.53	7.22	5.30	-3.3	3.0E-02	8.1E-01
UBL3	5412	13	ubiquitin-like 3	6.10	6.57	6.46	6.33	6.07	3.42	-6.4	4.6E-03	3.4E-01
UBXN2A	165324	2	UBX domain protein 2A	3.27	4.07	3.44	4.69	4.48	1.00	-4.8	5.0E-02	2.2E-01
UBXN6	80700	19	UBX domain protein 6	6.61	6.61	7.02	7.06	7.22	8.77	4.5	9.4E-03	6.4E-01
UMPS	7372	3	uridine monophosphate synthetase	4.77	4.11	4.35	4.09	3.92	2.46	-5.0	1.5E-02	4.4E-01
UNC119B	84747	12	unc-119 homolog B (C. elegans)	5.49	4.16	4.78	4.47	4.15	3.55	-3.8	3.4E-02	1.6E-01
UNC5A	90249	5	unc-5 homolog A (C. elegans)	-1.93	-1.80	-2.56	-1.87	-2.19	1.32	9.7	2.2E-02	9.3E-01
UPF2	26019	10	UPF2 regulator of nonsense transcripts homolog (yeast)	5.83	5.84	6.14	5.62	5.38	7.74	3.8	1.8E-02	8.6E-01
UPK1A	11045	19	uroplakin 1A	-3.14	-7.11	-4.87	-3.38	-4.05	0.33	11.4	3.5E-02	4.7E-01
UROD	7389	1	uroporphyrinogen decarboxylase	5.33	4.28	5.31	4.62	4.52	8.04	6.5	1.5E-03	3.1E-01
USHBP1	83878	19	Usher syndrome 1C binding protein 1	-5.13	-4.41	-2.35	-3.91	-4.05	1.00	80.7	7.1E-04	2.7E-01
USP25	29761	21	ubiquitin specific peptidase 25	5.87	6.36	6.81	5.68	5.69	4.13	-3.3	4.4E-02	4.9E-01
USP53	54532	4	ubiquitin specific peptidase 53	4.36	3.81	4.90	3.99	4.44	2.00	-5.1	2.1E-02	8.9E-01
USP6	9098	17	ubiquitin specific peptidase 6 (Tre-2 oncogene)	-0.95	-0.11	-0.44	0.10	0.23	1.91	7.3	3.7E-02	4.6E-01
VEZF1	7716	17	vascular endothelial zinc finger 1	6.08	7.40	6.46	7.06	6.84	3.95	-4.4	1.1E-02	1.8E-01
VGLL3	389136	3	vestigial like 3 (Drosophila)	-3.82	-3.52	-4.87	-3.38	-4.89	0.59	22.3	1.5E-02	6.0E-01
VGLL4	9686	3	vestigial like 4 (Drosophila)	4.75	3.76	4.67	3.73	3.29	2.58	-4.5	3.6E-02	3.9E-01
VNN3	55350	6	vanin 3	0.38	1.31	0.90	1.99	2.18	3.39	8.1	1.5E-02	1.4E-01
VPRBP	9730	3	Vpr (HIV-1) binding protein	5.34	5.63	5.76	5.69	5.53	3.52	-3.5	2.9E-02	7.1E-01
VPS13C	54832	15	vacuolar protein sorting 13 homolog C (S. cerevisiae)	6.88	7.16	7.44	5.98	5.82	4.67	-4.6	3.5E-02	8.6E-01
VPS13D	55187	1	vacuolar protein sorting 13 homolog D (S. cerevisiae)	6.34	6.65	7.52	6.27	6.07	4.42	-3.8	3.4E-02	6.5E-01
VPS4B	9525	18	vacuolar protein sorting 4 homolog B (S. cerevisiae)	5.86	6.70	6.16	6.38	6.35	3.95	-3.7	2.4E-02	2.5E-01
VSIG10	54621	12	V-set and immunoglobulin domain containing 10	-0.30	-0.17	-0.03	0.18	0.06	3.00	9.8	1.4E-02	6.6E-01
VSTM1	284415	19	V-set and transmembrane domain containing 1	2.07	3.47	-0.15	2.19	3.40	5.13	8.3	2.9E-02	5.3E-01
VSTM4	196740	10	V-set and transmembrane domain containing 4	1.27	0.57	-0.29	-0.16	1.04	4.08	7.1	3.5E-02	1.9E-01
VWA2	340706	10	von Willebrand factor A domain containing 2	-2.19	-3.52	-3.50	-4.76	-3.53	1.32	11.6	2.3E-02	1.5E-01
WASH1	100287171	9	WAS protein family homolog 1	3.70	3.93	4.52	4.35	2.78	6.94	9.5	1.1E-03	5.9E-01
WDR34	98981	9	WD repeat domain 34	3.03	2.06	2.53	2.22	2.85	5.79	6.8	1.1E-02	3.5E-01
WDR82	80335	3	WD repeat domain 82	8.42	8.21	7.93	8.04	7.93	5.63	-6.9	2.2E-03	8.0E-01
WFDC1	58189	16	WAP four-disulfide core domain 1	-7.11	-4.41	-7.11	-7.11	-7.11	0.81	495.8	8.1E-04	3.3E-01
WFDC8	90199	20	WAP four-disulfide core domain 8	-3.44	-4.41	-7.11	-4.76	-7.11	0.33	14.1	3.6E-02	7.7E-02
WWP1	11059	8	WW domain containing E3 ubiquitin protein ligase 1	6.22	6.39	6.90	6.56	6.00	3.70	-5.8	3.4E-03	7.5E-01
XBP1	7494	22	X-box binding protein 1	7.60	7.06	7.00	7.44	7.24	5.63	-3.9	1.3E-02	5.7E-01
XPO7	23039	8	exportin 7	6.14	5.63	6.60	5.47	5.27	8.12	4.0	2.5E-02	8.2E-01
XYLT1	64131	16	xylosyltransferase I	5.48	6.73	5.65	5.71	5.81	3.46	-4.1	2.3E-02	4.7E-01
YJEFN3	734887	19	YjeF N-terminal domain containing 3	1.90	2.53	2.10	2.73	3.15	4.42	5.8	4.2E-02	6.3E-01
YPEL2	388403	17	yippee-like 2 (Drosophila)	5.79	7.55	7.24	7.14	6.62	3.80	-4.0	2.5E-02	8.1E-02
YPEL4	219539	11	yippee-like 4 (Drosophila)	0.04	1.17	3.25	1.45	1.16	5.87	57.3	4.9E-04	1.3E-01
ZADH2	284273	18	zinc binding alcohol dehydrogenase domain containing 2	5.70	4.94	4.68	4.69	4.81	3.80	-3.7	4.0E-02	3.9E-01
ZBED3	84327	5	zinc finger, BED-type containing 3	0.70	0.53	0.53	-0.36	-0.47	-7.11	-458.6	2.1E-02	9.7E-01
ZBED4	9889	22	zinc finger, BED-type containing 4	5.51	5.42	5.72	5.78	4.70	3.73	-3.4	4.6E-02	8.7E-01
ZBTB16	7704	11	zinc finger and BTB domain containing 16	3.24	3.10	3.04	3.05	1.77	0.59	-6.3	3.4E-02	7.9E-01
ZBTB2	57621	6	zinc finger and BTB domain containing 2	5.23	6.35	5.14	6.13	5.85	2.17	-8.4	1.1E-03	3.4E-01
ZBTB38	253461	3	zinc finger and BTB domain containing 38	6.11	5.46	5.56	4.65	4.06	2.25	-14.5	8.2E-04	4.4E-01
ZBTB4	57659	17	zinc finger and BTB domain containing 4	7.61	5.88	7.44	5.92	5.73	4.28	-10.0	1.1E-03	1.3E-01
ZBTB8A	653121	1	zinc finger and BTB domain containing 8A	2.48	2.07	1.70	1.67	1.44	5.42	7.7	1.4E-02	2.5E-01
ZC3H14	79882	14	zinc finger CCCH-type containing 14	5.29	5.20	5.50	5.16	5.06	3.49	-3.5	3.3E-02	9.6E-01
ZFAND1	79752	8	zinc finger, AN1-type domain 1	4.87	4.73	4.67	4.97	5.04	3.13	-3.3	4.4E-02	8.5E-01
ZFP106	64397	15	zinc finger protein 106 homolog (mouse)	6.43	7.26	6.61	7.08	6.49	4.74	-3.2	4.0E-02	3.2E-01
ZFP37	7539	9	ZFP37 zinc finger protein	1.54	0.85	1.24	-0.97	0.20	-7.11	-825.8	5.5E-03	3.0E-01
ZNF132	7691	19	zinc finger protein 132	1.59	-1.04	-0.11	-2.43	-2.84	-7.11	-849.9	1.8E-02	2.3E-01
ZNF134	7693	19	zinc finger protein 134	4.26	5.31	4.76	4.58	4.66	2.09	-4.5	2.8E-02	3.8E-01
ZNF146	7705	19	zinc finger protein 146	6.26	4.21	5.92	4.52	4.31	3.93	-5.0	4.7E-02	4.1E-01
ZNF217	7764	20	zinc finger protein 217	6.75	7.08	7.04	6.49	6.13	3.83	-7.5	1.2E-03	7.0E-01
ZNF280B	140883	22	zinc finger protein 280B	1.64	1.59	2.54	1.64	0.98	-1.96	-12.3	3.0E-02	8.0E-01
ZNF281	23528	1	zinc finger protein 281	4.22	6.58	4.98	6.04	5.37	2.00	-4.7	3.0E-02	

ZNF500	26048	16	zinc finger protein 500	4.16	3.11	4.11	2.64	2.40	1.81	-5.1	4.5E-02	2.6E-01
ZNF518B	85460	4	zinc finger protein 518B	4.67	5.33	5.41	4.46	4.79	2.86	-3.5	4.7E-02	4.8E-01
ZNF540	163255	19	zinc finger protein 540	2.25	2.06	2.62	1.21	1.34	-0.98	-9.4	3.0E-02	8.4E-01
ZNF541	84215	19	zinc finger protein 541	0.47	-0.11	0.26	0.63	-0.42	-7.11	-392.3	2.2E-02	5.9E-01
ZNF550	162972	19	zinc finger protein 550	3.48	2.11	3.79	1.86	1.29	0.59	-7.5	4.0E-02	4.0E-01
ZNF571	51276	19	zinc finger protein 571	1.62	2.86	3.10	2.55	2.51	-7.11	-868.0	3.6E-03	2.0E-01
ZNF572	137209	8	zinc finger protein 572	0.26	-0.54	-0.61	-2.03	-1.62	-7.11	-338.2	3.9E-02	5.6E-01
ZNF615	284370	19	zinc finger protein 615	3.24	4.31	4.41	2.89	3.18	0.01	-9.4	1.2E-02	3.7E-01
ZNF627	199692	19	zinc finger protein 627	3.45	3.04	4.06	3.16	2.97	0.01	-10.9	6.7E-03	8.5E-01
ZNF643	NA	NA	NA	1.60	0.36	1.29	-0.42	-0.27	-7.11	-855.9	5.9E-03	4.2E-01
ZNF662	389114	3	zinc finger protein 662	1.67	1.09	0.80	1.45	1.28	4.30	6.2	2.9E-02	4.4E-01
ZNF682	91120	19	zinc finger protein 682	0.99	2.59	3.20	2.54	2.98	4.47	11.2	8.8E-03	1.2E-01
ZNF689	115509	16	zinc finger protein 689	3.70	4.27	4.00	4.26	3.88	0.81	-7.4	9.3E-03	5.2E-01
ZNF700	90592	19	zinc finger protein 700	3.79	4.92	5.47	5.16	5.25	5.74	3.9	4.6E-02	1.6E-01
ZNF710	374655	15	zinc finger protein 710	4.83	5.06	5.03	4.67	4.70	3.04	-3.5	4.6E-02	6.7E-01
ZNF770	54989	15	zinc finger protein 770	5.10	6.22	6.32	5.76	5.62	3.35	-3.3	4.1E-02	1.1E-01
ZNF776	284309	19	zinc finger protein 776	4.88	5.84	5.86	5.51	5.36	3.00	-3.7	3.1E-02	2.6E-01
ZNF791	163049	19	zinc finger protein 791	3.47	5.72	5.20	4.76	4.48	0.81	-6.3	1.7E-02	5.1E-02
ZNF830	91603	17	zinc finger protein 830	4.62	4.27	3.77	4.25	3.95	2.64	-4.0	3.9E-02	6.0E-01
ZNF831	128611	20	zinc finger protein 831	5.86	4.09	4.98	5.05	4.18	3.75	-4.3	3.0E-02	1.2E-01
ZNF845	91664	19	zinc finger protein 845	3.87	4.60	4.36	3.78	3.91	1.00	-7.3	7.7E-03	4.9E-01
ZNF853	54753	7	zinc finger protein 853	4.30	2.58	3.62	2.87	2.91	1.70	-6.1	2.1E-02	1.0E-01
ZNRF2	223082	7	zinc and ring finger 2	3.82	5.14	4.81	4.42	4.26	1.70	-4.3	3.1E-02	1.4E-01
ZZZ3	26009	1	zinc finger, ZZ-type containing 3	5.09	5.29	5.38	5.22	4.89	3.04	-4.1	1.9E-02	7.4E-01