A Thesis

entitled

Beta-Defensin 3-Mediated Regulation of Transcriptional Changes During Oropharyngeal Candidiasis

by

Cole Jacob White

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Master of Science Degree in

Biomedical Science: Bioinformatics, Proteomics and Genomics

Dr. Heather Conti, Committee Chair

Dr. Alexei Fedorov, Committee Member

Dr. Sadik Khuder, Committee Member

Dr. Amanda Bryant-Friedrich, Dean
College of Graduate Studies

The University of Toledo

August 2018

An Abstract of

Beta-Defensin 3-Mediated Regulation of Transcriptional Changes During Oropharyngeal Candidiasis

by

Cole Jacob White

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the
Master of Science Degree in
Biomedical Science: Bioinformatics, Proteomics and Genomics

The University of Toledo

August 2018

Oropharyngeal candidiasis (OPC) is a fungal infection of the oral cavity caused by a human commensal fungus called *Candida albicans*. Even though *C. albicans* is a commensal, it can become pathogenic in immunocompromised patients. IL-17-mediated antifungal immunity has been implicated as the major pathway for fungal defense in the oral cavity. The IL-17 pathway has been found to regulate the release of antimicrobial peptides (AMPs) downstream, including murine β-defensin 3 (mBD3).

Murine β-defensin 3 is an antimicrobial peptide involved in the protection of the oral mucosa against pathogens. Murine BD3 can protect the oral mucosa through its direct antimicrobial activity, but it has also been found to have chemotactic properties as well. The human homolog of mBD3, human β-defensin 2 (hBD2), has been found to bind CCR6. CCR6 is found on the surface of cells like memory T-cells, dendritic cells (DCs), Th17 cells, and Treg cells. Studies have shown that hBD2 binding to CCR6 on the surface of memory T-cells and DC's can induce chemotaxis, and other studies have suggested the same could be true for Th17 cells and Treg cells.

The goal of this experiment was to study the chemotactic ability of mBD3 via RNA-sequencing to explore how the presence/absence of *Defb3* leads to changes in transcriptional regulation while in the presence/absence of an OPC infection. The RNA-sequencing results reveal that there are very few differences in transcript expression between wild-type mice and *Defb3* knockout mice. One difference in transcript expression between *Defb3* knockout mice and WT mice is found in *Sycp1*. *Sycp1* is expressed at higher levels in *Defb3* knockout infected mice when compared with WT infected mice and *Defb3* knockout sham mice when compared to WT sham mice. The DNA-binding ability of *Sycp1* makes it an interesting candidate for future studies.

To my family, thank you for your love and support throughout this journey. Without you, I wouldn’t be where I am today. May this be the first of many written theses…

And to my loving fiancé, thank you for all of your support.   I can’t wait to start the next leg of our journey in medical school together. Go Bobcats!

Acknowledgements

I would like to thank my advisor, Dr. Heather Conti for her guidance and support over the last few years. Without your willingness to accept me as an undergraduate researcher, I wouldn't be where I am today. Thank you for your guidance as I navigated my path from undergrad, to graduate school, and into medical school.

Thank you to the other members of my thesis committee, Dr. Alexei Fedorov and Dr. Sadik Khuder, for your commitment and your insight as a prepared for my defense.

I would like to thank the fellow graduate students/laboratory tech in the Conti Lab: Dylan Launder, Katie McKeone, Jackie Kratch, and Nathan Schmidt. Thank you for the stimulating conversations, the light-hearted laughs, and the fun times. Best of luck to all of you in your future endeavors.

Finally, to the rest of the Conti Lab, thank you for creating a welcoming environment. It's been fun…

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AMP	Antimicrobial Peptide
APC	Antigen Presenting Cell
BAM	Binary Alignment/Map
C. albicans	*Candida albicans*
Ccl20	Chemokine (C-C Motif) Ligand 20
CCR6	Chemokine (C-C Motif) Receptor 6
cDNA	Complementary DNA
CD4$^+$	Cluster of Differentiation 4
C/EBP	CCAAT-Enhancer Binding Protein
CFU	Colony-Forming Units
CLR	C-Type Lectin Receptor
cph1	Candida Pseudohyphal Regulator 1 Gene
dA	Deoxyadenosine Monophosphate
DC	Dendritic Cell
DNA	Deoxyribonucleic Acid
Defb1	Mouse β-Defensin 1 Gene
Defb2	Mouse β-Defensin 2 Gene
Defb3	Mouse β-Defensin 3 Gene
Defb4	Mouse β-Defensin 4 Gene
DEFB4A	Human β-Defensin 4A Gene
dPBS	Dulbecco’s Phosphate-Buffered Saline
dUTP	Deoxyuridine Triphosphate
efg1	Enhanced Filamentous Growth Protein 1 Gene
ER	Endoplasmic Reticulum
ERK1/2	Extracellular Signal-Regulated Protein Kinase 1/2
FC	Fold Change
FDR	False Discovery Rate
hBD2	Human β-Defensin 2
IACUC	Institutional Animal Care Unit Committee
IL-17	Interleukin-17
IL-17A	Interleukin-17
IL-17A/F	Interleukin-17A and Interleukin-17F Heterodimeric Complex
IL-17RA/RC	Interleukin-17 Receptor A and Interleukin-17 Receptor C Heterodimeric Receptor Complex

lncRNA	Long Non-Coding RNA
logFC	log(Fold Change)
mBD3	Murine β-Defensin 3
MAPK	Mitogen Activated Protein Kinase
MMRRC	Mutant Mouse Regional Resource Center
mRNA	Messenger RNA
NF-κB	Nuclear Factor κ-Light-Chain-Enhancer of Activated B cells
NGS	Next-Generation DNA Sequencing
OPC	Oropharyngeal Candidiasis
OEC	Oral Epithelial Cell
PAMP	Pathogen-Associated Molecular Pattern
PCR	Polymerase Chain Reaction
PRR	Pattern Recognition Receptor
poly(A)	polyadenylation
RIN	RNA Integrity Number
RNA	Ribonucleic Acid
RNA-seq	RNA-sequencing
rRNA	Ribosomal RNA
SAM	Sequence Alignment/Map
SC	Synaptonemal Complex
spp.	Several Species
Sycp1	Synaptonemal Complex Protein 1 Gene
Th17	T helper 17
TLR	Toll-Like Receptors
TMM	Trimmed Mean of M-Values
vs.	Versus
WT	Wild-Type
YPD	Yeast Extract Peptone Dextrose

List of Symbols

α	Alpha
β	Beta
γ	Gamma
δ	Delta
θ	Theta
κ	Kappa
μ	Mu
%	Percent
mL	Milliliter
μL	Microliter
pM	PicoMolar
g	Gram
C	Celsius
°	Degrees
<	less than
>	greater than
©	Copyright
®	Registered
™	Trademark

Chapter 1

Literature Review

1.1 *Candida albicans* and Oropharyngeal Infection

Candida albicans (*C. albicans*) is a commensal organism in healthy humans that can be found in the microbiota of the gastrointestinal tract (Neville, d'Enfert, & Bougnoux, 2015), the genitourinary tract (Achkar & Fries, 2010), the oral cavity (Ganguly & Mitchell, 2011), and the skin (Kashem & Kaplan, 2016). *C. albicans* is found in the oral cavity of 50-60% of the healthy population (Fidel, 2011). While *C. albicans* is not pathogenic in healthy individuals, those who are immunocompromised are at risk to develop a *Candida albicans* infection (Kiyoura & Tamai, 2015), including patients with HIV/AIDS (Fidel, 2011) and patients undergoing chemotherapy or radiotherapy (Pfaller & Diekema, 2007). This classifies *C. albicans* as an opportunistic pathogen (Conti et al., 2016).

Candida albicans infections can be superficial and limited to a specific tissue or mucosal surface, or they can become a serious, life-threatening, systemic infection (Mayer, Wilson, & Hube, 2013). Oropharyngeal candidiasis (OPC), also known as thrush, is a superficial *Candida albicans* infection in the oral cavity (Mayer et al., 2013). The location of the infection is important in determining the involvement of the innate and adaptive immunity in protection against *C. albicans* (Dongari-Bagtzoglou & Fidel, 2005). It was determined that adaptive immunity is important in the protection against OPC because of the prevalence of oropharyngeal candidiasis amongst HIV/AIDS patients (Rabeneck, Crane, Risser, Lacke, & Wray, 1993). The susceptibility of HIV/AIDS and other T-cell deficient patients to OPC is indicative of the importance of the adaptive immune response, specifically $CD4^+$ T-cells, in the protection of mucosal surfaces (Dongari-Bagtzoglou & Fidel, 2005). Further experimentation has determined that the most important factor in mucosal defense against *Candida albicans* is interleukin-17 (IL-17A or IL-17)-mediated fungal immunity. Both hematopoietic and non-hematopoietic cells have been implicated in this pathway. Neutrophils, monocytes/macrophages, adaptive T helper 17 cells (Th17) cells, natural Th17 cells, dendritic cells, and γδ T cells are all examples of hematopoietic cells involved in IL-17A-mediated antifungal immunity. Non-hematopoietic cells, like oral epithelial cells (OECs), are also involved (Conti et al., 2014).

1.2 Pattern Recognition of *Candida albicans*

The cell wall of *Candida albicans* is made up of 90% carbohydrates and only 10% proteins (Gow & Hube, 2012). Pattern Recognition Receptors (PRRs) in the host recognize Pathogen-Associated Molecular Patterns (PAMPs) on the cell wall of *C. albicans*. When these PRRs are bound to PAMPs, the innate immune response is activated and causes inflammation (Akira, Uematsu, & Takeuchi, 2006). The polysaccharide structures on the surface of *C. albicans*, including chitin, β-glucans, and mannoproteins, are recognized by Toll-like receptors (TLRs) and C-type lectin receptors

(CLRs). These are the two main PRRs involved in the recognition of *C. albicans* (Cheng, Joosten, Kullberg, & Netea, 2012). TLR2 and TLR4 in the host recognize phospholipomannan and O-linked mannan on *Candida albicans*, respectively (Jouault et al., 2003) (Netea et al., 2006). TLR2 expression has been found on myeloid cells like monocytes, macrophages, and dendritic cells (DCs). TLR4 has been found to be expressed on DCs, neutrophils, mast cells, and B cells (Bio-Rad, 2018). Dectin-1 and Dectin-2, which are examples of CLRs in the host, recognize β-glucan and α-mannan on *C. albicans*, respectively (Brown et al., 2002) (Saijo et al., 2010). Dectin-1 and Dectin-2 are commonly found on myeloid antigen presenting cells (APCs) like DCs and macrophages (Saijo & Iwakura, 2011).

The oral epithelial cells have long been thought of as a physical barrier to pathogen entry, but they are also involved in pattern recognition (Cheng et al., 2012). These OECs express all but two of the known TLRs (OECs express varying levels of TLR1, TLR2, TLR3, TLR4, TLR6, TLR8, TLR9, and TLR10) (Weindl et al., 2007). These TLRs on the surface of OECs can identify pathogens, including *C. albicans*. When bound to *Candida spp.*, these cells initiate a signaling cascade that leads to the secretion of antimicrobial peptides (AMPs) and cytokines. Beta-defensins were identified as one of the major AMPs produced and released by OECs (Cheng et al., 2012). After exposure to *Candida spp.*, OEC's upregulate the expression of TLRs on their extracellular surface and increase the expression of β-defensins and other AMPs (Bahri, Curt, Saidane-Mosbahi, & Rouabhia, 2010). The cytokines produced by OECs do not have direct antifungal affect, but they are able to initiate an antifungal and inflammatory response, which contributes to host protection against *Candida spp.* (Cheng et al., 2012).

1.3 Pathogenicity of *Candida albicans*

C. albicans is a dimorphic fungus, meaning that it exists in two forms: yeast and filamentous. The yeast form is unicellular and the filamentous, also known as hyphal, form is multicellular (Gow, van de Veerdonk, Brown, & Netea, 2011). The ability of *C. albicans* to switch between forms is thought to be one of the ways that it evades the human immune system (Lo et al., 1997). The transition of *C. albicans* from yeast to hyphal phase is one of the main virulence factors associated with the fungal infection. The transition of *C. albicans* between its morphological forms is carried out by various transcription factors such as Enhanced Filamentous Growth Protein 1 (*efg1)* and Candida Pseudohyphal Regulator 1 (*cph1)* (Cheng et al., 2012). Strains of *C. albicans* were found to be less infective in animal models when these dedicated transcription factors were defective (Lo et al., 1997). It has also been found that strains of *C. albicans* unable to induce the morphological switch between yeast form and hyphal form are less infectious, which supports the claim of this transition being an important virulence factor in the fungus (Cheng et al., 2012).

To invade host epithelium, *Candida albicans* go through three stages: epithelial attachment, tissue invasion, and tissue damage (Cheng et al., 2012). Attachment of *C. albicans* to epithelial tissue is important because it leads to the upregulation of genes associated with the formation of hyphae, which is considered the invasive form of the fungus (Ray & Payne, 1988). Because of this, the interaction between the epithelial cells and *C. albicans* is critical for the ability of the fungus to invade host tissue (Cheng et al., 2012).

Candida albicans has developed multiple ways to escape from host phagocytosis. One way is by shielding its PAMPs from PRRs on the host cells. If the PRRs are unable to bind to the PAMPs on the surface of *C. albicans*, there is no intracellular signaling in the host cells. Therefore, there would be no induction of the immune and inflammatory responses (Cheng et al., 2012). Some studies have shown that β-glucan on the cell wall of *C.albicans*, is shielded from coming in contact with dectin-1 on host cells, successfully protecting against recognition by the immune system (Gantner, Simmons, & Underhill, 2005). *C. albicans* can also avoid the host immune response via inhibition and degradation of C3b of the complement system, inhibition of phagolysosome formation, reactive oxygen species inhibition, and by modulating or inhibiting cytokines of the immune response. IL-17 is one of the cytokines that can be inhibited by *C. albicans*. By inhibiting tryptophan metabolism, the fungus can inhibit IL-17 production (Cheng et al., 2010).

1.4 IL-17 Mediated Antifungal Immunity

The IL-17 family is considered to be one of the most important regulators of antifungal immunity. This family is comprised of six cytokines (IL-17A-IL-17F) and five receptors (IL-17RA-IL-17E). IL-17A and IL-17F are the most studied and most understood of the IL-17 cytokines (Jin & Dong, 2013). IL-17A and IL-17F can form either homodimers or heterodimers (IL-17A/F) (Chang & Dong, 2007) that can bind to the IL-17RA and IL-17RC receptor complex (IL-17RA/RC) (Toy et al., 2006). IL-17RA can be found on the surface of both hematopoietic cells and non-hematopoietic cells, but IL-17RC is limited to non-hematopoietic tissues including epithelial and endothelial cells (Ho & Gaffen, 2010). This information implies the importance of IL-17 the IL-17 family in mucosal defense (Mengesha & Conti, 2017).

When an IL-17 dimer or an IL-17A/IL-17F heterodimer binds to IL-17RA/RC, intracellular signaling events are initiated. Downstream of these signaling events, the NF-κB, MAPK, and C/EBP pathways are activated. These signaling events are critical to the host antifungal defense, which is why IL-17RA knockout mice are more susceptible to OPC. These IL-17RA knockout mice were found to have less neutrophil recruitment to the site of infection and less human β-defensin 2 production from the epithelial cells (Kao et al., 2004). The decrease in neutrophil recruitment is likely due to the decrease in CXCL1 and CXCL5 chemokines in the oral mucosa of the IL-17RA knockout mice. CXCL1 and CXCL5 have been linked to neutrophil recruitment (Conti et al., 2009). Other than the impaired neutrophil recruitment in IL-17RA knockout mice, it is not understood how neutrophils are involved in antifungal defense at mucosal surfaces (Mengesha & Conti, 2017). Further studies have shown that neutrophil recruitment is less affected in IL-17RA tissue specific knockouts (IL-17RA knocked out in only the oral cavity and upper GI tract) than in IL-17RA complete knockouts, even though the susceptibility to OPC is comparable in both mouse strains. This likely means that the decrease in neutrophil recruitment is not the cause of OPC susceptibility (Conti et al., 2016).

IL-17 not only regulates neutrophil recruitment, but also regulates the production of AMPs (Mengesha & Conti, 2017). Out of these AMPs, murine β-defensin 3 (mBD3) is of the greatest interest. In previous bioinformatic studies, mBD3 was found to be differentially expressed between IL-17RA knockout mice and WT mice during an OPC infection (Conti et al., 2016). Studies have shown that β-defensins can bind to Chemokine (C-C Motif) Receptor 6 (CCR6), which could introduce some interesting chemotactic properties of mBD3. CCR6 is found on the surface of neutrophils, and β-defensins have been found to serve as chemoattractant for neutrophils through CCR6 binding (Niyonsaba, Ogawa, & Nagaoka, 2004).

1.5 Murine Beta-Defensin 3

Defensins are one subset of a diverse group of molecules found in organisms (Das et al., 2010) ranging from plants (Lacerda, Vasconcelos, Pelegrini, & Grossi de Sa, 2014) to vertebrates called antimicrobial peptides (Bals et al., 1999). There are two main subfamilies of defensins, α and β (Ganz, 2003), although there is a third family of θ-defensins (Das et al., 2010). These defensins are characterized by their six-cysteine pattern, which differs slightly between the subfamilies (Ganz, 2003) In α-defensins, the six cysteines form disulfide bridges between the first and sixth, second and fourth, and third and fifth cysteines (referred to as 1-6, 2-4, 3-5 pattern). In β-defensins, the six cysteines form disulfide bridges in a 1-5, 2-4, 3-6 pattern. The defensins are known for their prepropeptide structure. All α-defensins contain this structure, and all β-defensins contain a signal sequence with an optional pro sequence before the mature peptide sequence. This structure means that the defensin must be cleaved at least once, but normally two times before it is a fully-active peptide. The peptides are thought to be cleaved before they exit a host cell, meaning that they are secreted in an active form.

Because defensins are such small sequences, they can be transcribed and translated quickly, but they must go through proteolytic cleavage before exiting the cell. The process of cleaving these peptides can take many hours. (Zhao, Lin, Yang, Ye, & Li, 2015).

Murine β-defensin 3 has been identified as an important mediator of fungal immunity in the oral cavity (Conti et al., 2016). Murine BD3 is expressed in epithelial tissues throughout the body and is important for mucosal tissue defense against pathogenic bacteria and fungi (Jiang et al., 2010). The mBD3 protein is a 63 amino acid that contains a prepropeptide structure. The genes coding for mBD3 and human β-defensin 2 (hBD2) are homologous (Bals et al., 1999). *Defb3* is the mouse gene that codes for mBD3 (Conti et al., 2016). *DEFB4A* is the human gene that codes for hBD2 (Nuding et al., 2013). There is 36.7% DNA sequence homology between the *Defb3* and *DEFB4A* genes and 39.7% amino acid sequence identity between the mBD3 and hBD2 proteins (Bals et al., 1999).

Beta-defensin activity has been documented in the oral cavity against fungal microorganisms, including *C. albicans* (Joly, Maze, McCray, & Guthmiller, 2004). In the mouse OPC model, *Defb3* was expressed significantly less on days 1-3 in both the tissue-specific IL-17RA knockout and the complete IL-17RA knockout mice, which is comparable to the OPC susceptibility of these mice. *Defb1* was only significantly altered in the complete IL-17RA knockout mice, and *Defb2* and *Defb4* expression was not altered in the experiment. Further *in vitro* experiments in human oral epithelial cells found that OECs released hBD2 when stimulated with IL-17. Furthering the support that mBD3 is an important mediator of fungal immunity is that *Defb3* knockout mice and IL-

17RA knockout mice showed similar susceptibility to OPC (Conti et al., 2016). Other studies have also found hBD2 expression to be induced in buccal epithelial cells when exposed to *Candida spp.* (Sawaki, Mizukawa, Yamaai, Fukunaga, & Sugahara, 2002).

1.6 Mechanisms of BD3 Antimicrobial Activity

Beta-defensins function as antimicrobial agents in the oral cavity. They directly kill bacterial and fungal pathogens. Beta-defensins protect the oral mucosa against overgrowth of host commensals and prevent the growth of pathogens (Diamond & Ryan, 2011). There are many different proposed mechanisms for defensin antimicrobial activity in science literature (Brogden, 2005) (Ganz, 2003) (Silva, Goncalves, & Santos, 2014).

Before antimicrobial activity can take place, the defensins have to be produced and released. It is thought that defensins are produced in the rough endoplasmic reticulum (ER) as prepropeptides. The signal/pre sequence is then cleaved off as the defensins are packaged in the golgi. The final proteolytic cleavage of the pro sequence takes place after the defensins have been placed in vesicles. This means that the defensins are in their mature, active form before they are released from host cells (Ganz, 2003).

Murine BD3 is cationic (Bals et al., 1999) and hBD2 is recognized as amphipathic (Schroder & Harder, 1999), which allows these peptides to interact successfully with the fungal cell membrane. The positively-charged amino acids in the hBD2 and mBD3 molecules interact with the negatively-charged phosphate heads of the microbial cell membrane. These molecules also contain hydrophobic amino acids, which interact with the hydrophobic fatty acids. Both of these interactions allow for the insertion of the AMP into the microbial membrane (Silva et al., 2014).

Many of the models for antimicrobial peptide activity revolve around the interaction of the AMP with the pathogen cell wall/cell membrane. Membrane permeabilization of the pathogen can occur in three different ways. One model of pore formation is barrel-stave. In this model, after the AMPs have inserted into the cell membrane of the pathogen and reach a certain concentration (Silva et al., 2014), the antimicrobial peptides align forming a pore entirely lined by the peptides themselves. Another scenario of membrane permeabilization could happen via the toroidal model. This model is similar to the barrel-stave model in that once it reaches a specific AMP concentration in the microorganism's cell membrane, the AMPs form a complex that creates a pore. The difference is that cell membrane curves in this model, and the pore is lined by the AMPs and the phosphate heads of the phospholipid membrane. The final mode of membrane permeabilization is the carpet model. In this model, the AMPs only interact with the phosphate heads of the cell membrane. At a high enough AMP concentration in the plasma membrane, the antimicrobial peptides form micelles, which disrupts the membrane integrity (Brogden, 2005).

Other proposed mechanisms of defensin antimicrobial activity include intracellular targets leading to apoptosis of the fungal target. Because of the amphipathic nature of defensins, they are not only able to enter the cell membrane, but also pass through it via diffusion (Brogden, 2005). Defensins can also enter fungal cell via receptor-mediated endocytosis (Silva et al., 2014). Once these molecules enter the cell, they can affect a variety of intracellular processes. The AMPs have been found to inhibit the synthesis of the cell wall, nucleic acids, proteins, and enzymes in the target cells (Brogden, 2005). Defensins have even been found to create reactive oxygen species inside fungal cells, causing apoptosis (Silva et al., 2014).

A final, indirect manner for defensin antimicrobial activity is through cell chemotaxis. Human BD2 has been linked to memory T-cell and immature DC chemotaxis. It was determined in the same study that hBD2 was able to interact with CCR6 on these cells to produce chemotaxis (Yang et al., 1999). CCR6 has been found on the surface of both Th17 cells and Treg cells (Yamazaki et al., 2008). This experimental finding becomes more significant when compared to previous studies that show Th17 cells produce IL-17 and are important for fungal defense in the oral mucosa (Conti et al., 2014). The ability of hBD2 to bind to CCR6 and induce chemotaxis of different cell types could be an important way that β-defensins participate in adaptive immunity to an infection (Ganz, 2003) or a way that they recruit IL-17 producing cells for mucosal defense (Conti et al., 2014) (Yang et al., 1999) (Yamazaki et al., 2008).

1.7 Inbred Mouse Model

Age-matched, inbred mouse strains have many benefits, which makes mice an ideal mammalian model organism. Mice are physiologically similar to humans (National Human Genome Research Institute, 2012, May 23), which is why they are a particularly useful and accurate representation of the oropharyngeal candidiasis model. *C. albicans* is not a normal commensal in mice, which means that the mice form an acute immune response after they are exposed to the microorganism (Conti et al., 2014). In mice, the immune system response after exposure to *Candida albicans* can be measured. This type of study would be limited during *in vitro* studies. In addition to this, the mouse genome is very similar to the human genome and it can be easily manipulated for experiments (National Human Genome Research Institute, 2012, May 23). There are about 3.1 billion base pairs in both genomes, and the protein-coding genes present in both species are almost identical (National Human Genome Research Institute, 2010, July 23). In addition to this, the mice of an inbred strain are close to genetically identical. About 98.6% of the loci in an inbred mouse strain are homozygous after at least twenty generations (Beck et al., 2000). Because the inbred mice are almost completely identical at all other loci outside the gene of interest, mouse models have become very useful in array-based technology.

1.8 RNA-sequencing

RNA-sequencing (RNA-seq) is an analysis of RNA (ribonucleic acid) transcripts via high-throughput next-generation DNA sequencing (NGS) (Ozsolak & Milos, 2011). By identifying the RNA transcripts that are expressed in a given tissue, a gene expression profile can be created. This by itself can be a helpful tool because researchers can determine what genes are being expressed, which can explain what is happening at the molecular level in cells. But the true power of RNA-seq comes from the comparison of multiple samples, especially when these samples only differ by one experimental variable. This gives researchers the ability to compare the effect of one variable on the gene expression profile of a given cell/tissue. This type of study can be very powerful. An example of this is the comparison of RNA transcript expression in diseased tissues and healthy tissues to determine the pathology at a genetic level (Finotello & Di Camillo, 2015).

One of the advantages to RNA-seq is that the entire transcriptome can be explored. RNA-seq is not limited to only previously-discovered sequences, which means all transcript sequences can be mapped. This eliminates some of the bias that is recognized in other array-based technologies. RNA-seq has also been found to be more accurate and more sensitive to transcript expression changes, especially if those changes are very large or very small. This makes RNA-seq more accurate in many cases than microarray studies (Wang, Gerstein, & Snyder, 2009).

One of the challenges of RNA-seq is that approximately 90% of the RNA in a eukaryotic cell is ribosomal RNA (rRNA), which is not what researchers want to study in differential expression experiments. Messenger RNA (mRNA) is usually the greatest concern when dealing with differential expression, but mRNA only makes up 1-2% of the RNA in a typical eukaryotic cell. In order to avoid unwanted rRNA in RNA-seq studies, polyadenylation (poly(A)) selection can be used because mRNA has a poly(A) tail, while rRNA does not in eukaryotic cells. When using poly(A) RNA-seq, it is important to make sure that the RNA is high quality, which can be measured with a bioanalyzer by the RNA integrity number (RIN) (Conesa et al., 2016).

RNA-seq can be used in this project in combination with the inbred mouse model to create a comparison of transcripts between two experimental groups with only one variable. Depending on the comparison, the variable could be an infection, or the variable could be the presence of a functional gene like *Defb3*. In these comparisons, it is possible to observe the impact that one gene can have on the expression of every transcript inside cells from a given tissue. This type of study has the power to identify transcripts that are upstream or downstream of the affected gene and can also identify potential compensatory mechanisms for the transcript that is knocked out. There is a lot that can be learned from an RNA-seq project of this nature.

Chapter 2

Materials and Methods

2.1 Preparation of *Candida albicans* Culture

A single colony of *C. albicans* was cultured in YPD broth and incubated at 30°C overnight. The *C. albicans* strain used for these experiments is CAF 2-1. The morning of the infection, the broth is adjusted to OD600 = 1.2 and 1200 µL of the overnight culture was added to a separate 15 mL tube. This 15 mL tube was then centrifuged at 3200 rpm at 25°C for 5 minutes. After pouring off the culture, the pellet was resuspended in 4 mL of 1x dPBS. The *C. albicans* suspension was then transferred in 1 mL increments in four 1.5 mL microcentrifuge tubes.

2.2 Induction of Oropharyngeal Candidiasis

To infect the mice with OPC, 0.0025g cotton balls were placed into the *C. albicans* suspension (preparation described in section 2.1). The cotton balls for the sham mice are placed in dPBS to act as a control for the experiment. During infection, a pre-infection swab of the oral cavity is done to test for the presence of fungi. A swab of every mouse oral cavity is taken using a cotton-tipped applicator, and then plated on a YPD plate. The YPD plate is then incubated for 48 hours at 37°C and then checked for the presence of fungi (there were no mice in this experiment that had fungal growth on the pre-infection swab plates). The mice also receive a subcutaneous 1 mL injection of 0.9% saline, the *Candida*-filled or dPBS-filled cotton balls are placed sublingually in the mouse oral cavity, and eye ointment is added to the mice to ensure that their eyes stay moisturized during the infection. The cotton balls are removed from the oral cavity after 75 minutes and each mouse received an addition 1 mL subcutaneous injection of 0.9% saline.

2.3 Harvesting Tongue Tissue

After 24 hours, the tongue tissue for our twelve experimental mice was harvested. This tissue was flash frozen in liquid Nitrogen and stored in a -80°C freezer. The tissue from the remaining two IL-17RA knockout mice was harvested 96 hours after the infection. The harvested tongue tissue from these mice was homogenized and plated on YPD agar plates. The plates were incubated at 30°C determine, and the colonies of *C. albicans* were counted to determine fungal burden. The IL-17RA knockout mice were used as a positive control for infection since it has been recognized in previous studies that they were susceptible to OPC. This was necessary because the tongue tissue from the twelve experimental mice was dedicated entirely to RNA extraction for RNA-sequencing.

2.4 Quantification of Fungal Burden

The harvested tongue tissue from the IL-17RA knockout mice was placed on ice and brought directly to the laboratory where it was placed in 500 μL of 1x dPBS and homogenized using the gentleMACS™ Octo Dissociator. After homogenization, 100 μL was placed on three YPD agar plates. The plates were then incubated at 30°C for 48 hours, and then the separate colonies were counted to determine the CFU/g of tongue tissue.

2.5 Experimental Mice

The mouse colony used to generate the twelve mice used in our experimental cohorts was originally acquired from the Mutant Mouse Regional Resource Center (MMRRC) at the University of California, Davis. The animal protocols were approved by the University of Toledo Institutional Animal Care Unit Committee. To generate the mice for this experiment, two mice heterozygous for *Defb3* were crossed, giving off wild-type, heterozygous, and *Defb3* knockout mice as offspring. The mice used in this study were born within 9 days of each other. Genotyping of the mice was performed by the Genotyping Center of America.

2.6 RNA-Sequencing

The tissue from the twelve experimental mice was sent to the Genomics, Epigenetics and Sequencing Core (GESC) at the University of Cincinnati. The RNA was extracted from each sample using the mirVana™ miRNA Isolation Kit using a total RNA isolation protocol. Every sample had a RIN greater than 7.0, which indicates the samples were of high enough quality for poly(A) RNA-seq (Appendix Figure C-1). The target RNA was enriched using NEBNext® Poly(A) mRNA Isolation Module from New England BioLabs. This is necessary to remove rRNA, which makes up the vast majority of the sample directly after extraction. The samples were then further prepared for RNA-seq with the SMARTer™ Apollo™ NGS Library Prep System from Takara. The RNA-seq library was prepared with the NEBNext® Ultra™ II Directional RNA Library Prep Kit from New England Bio Labs. The isolated poly(A) RNA was fragmented into segments of about 200 base pairs. These fragments were reverse transcribed into cDNA, and then a second strand of cDNA was transcribed and labelled with dUTP. The cDNA was then end repaired, underwent dA-tailing, and was ligated to the adapters. The cDNA labelled with dUTPs was enzymatically removed. Removing the dUTP-containing strand maintains the strand specificity. The cDNA was enriched via nine cycles of PCR, and the libraries were cleaned up. The quality of the purified libraries was checked on a bioanalyzer. Individually indexed and compatible libraries were proportionally pooled for clustering in an Illumina® cBot system. Libraries at a concentration of 15 pM were clustered onto a single read flow cell v3 using Illumina® TruSeq™ SR Cluster Kit v3 and sequenced to 51 base pairs using TruSeq™ SBS Kit v3 on the Illumina® HiSeq® system.

2.7 Processing the RNA-Sequencing Results

The RNA-seq results were sent back to the University of Toledo as FASTQ files. These generated FASTQ files were aligned to the Ensembl mouse reference genome, GRCm38.p6, by using STAR. The SAM files produced from STAR were processed using Picard to mark duplicates and index the files. Next, the files were processed using the Genome Analysis Toolkit (GATK) to clip regions prone to providing false variants. This entire process produces a BAM file.

These BAM files were then analyzed using R to determine which transcripts were differentially expressed between the four experimental conditions. The read counts for each sample were normalized by EdgeR using a method called Trimmed Mean of M-values (TMM). Essentially, the samples that have expression levels most similar to the mean across all samples are used as the reference. A normalization factor is determined for every sample using this method. This is performed to eliminate sample bias (Robinson & Oshlack, 2010). The samples were then tested for differential expression using EdgeR. The samples were compared by using the p-value, FDR, and logFC.

Chapter 3

Results

3.1 Experimental Design

My project used RNA-sequencing techniques to determine what RNA transcripts are differentially expressed between *Defb3* knockout mice and littermate control wild-type (WT) mice while infected with *C. albicans* or under sham conditions. This will help determine the role that murine β-defensin 3 plays in the protection of the oral mucosa against oropharyngeal candidiasis. This experiment consisted of four experimental groups: WT mice infected with *C. albicans, Defb3* knockout mice infected with *C. albicans*, WT sham mice, and *Defb3* knockout sham mice. Each experimental group was represented by three mice. There were also two IL-17RA knockout mice infected with *C. albicans* included in the experiment as a susceptibility control to show that our infection methods were successful. This was necessary because the tongue tissue from the main, four experimental groups was entirely dedicated to RNA-extraction for RNA-sequencing, meaning half of those tongues could not be used to determine fungal burden.

Before the infection took place, each mouse was injected with a ketamine working solution consisting of 0.9% saline (77.4% of the overall mixture), ketamine 100 mg/mL (15%), and xylazine 20 mg/mL (7.6 %), which acts as an anesthetic. During infection of the mice for the experiment, the mice received a 1 mL subcutaneous injection of 0.9% saline, a pre-infection swab of the mouse oral cavity was taken, and a cotton ball containing either a *Candida albicans* suspension or a solution of phosphate-buffered saline (for the sham mice) was placed sublingually in the oral cavity. After 75 minutes, the cotton ball was removed from the oral cavity of the mice and each mouse received another 1mL subcutaneous injection of 0.9% saline. After 24 hours, the tongue tissue for our twelve experimental mice was harvested. The tissue from the remaining IL-17RA knockout mice was harvested 96 hours after infection. The harvested tongue tissue from these mice was homogenized and plated on yeast extract peptone dextrose (YPD) agar plates. The plates were incubated at 30°C determine, and the colonies of *C. albicans* were counted after 48 hours to determine the fungal burden of the mice. The average fungal burden between the two IL-17RA knockout mice was 6,828 colony-forming units per gram (CFU/g) of tongue tissue (Figure 3-1). This confirms that the OPC infection model was successful at introducing *C. albicans* to the oral cavity of each mouse.

The tissue from the twelve experimental mice was flash frozen in liquid Nitrogen and stored in a -80°C freezer before being sent the Genomics, Epigenetics and Sequencing Core (GESC) at the University of Cincinnati. The RNA was extracted from each sample was checked for quality with a bioanalyzer. For poly(A) RNA-seq, it is important to have RNA samples with a RIN greater than 7.0. No sample received less than a RIN of 8.60, well above the necessary quality (Appendix Figure C-1). Single-end read, poly(A) RNA-sequencing was then performed on every sample. The RNA-seq results were sent back to the University of Toledo. This generated FASTQ files that were aligned to the mouse reference genome by using STAR. The SAM (Sequence

Alignment/Map) files produced from STAR were processed using Picard to mark duplicates and index the files. Next, the files were processed using the Genome Analysis Toolkit (GATK) to clip regions prone to providing false variants. This entire process produces a BAM (Binary Alignment/Map) file. These BAM files were then analyzed using R to determine which transcripts were differentially expressed between the four experimental conditions.

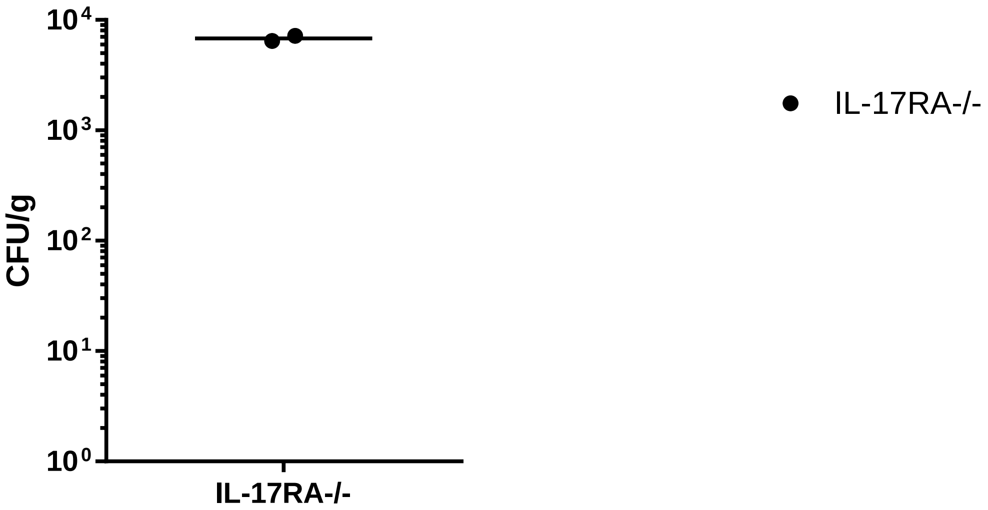


Figure 3-1 The CFU/g of tongue tissue of the positive control IL-17RA control mice is represented in the figure above. The average for the two samples is 6828 CFU/g and is represented by the horizontal line.

3.2 Wild-Type Infected versus Wild-Type Sham

The first comparison made from our experiment was between WT infected and WT sham mice. One thousand one hundred ninety-one different transcripts were identified as having a significant differential expression, meaning that the transcripts had a p-value less than 0.05 and an FDR less than 0.05. Table 3.1 represents the top thirty-five transcripts with the greatest (positive) logFC that also meet the previously-stated standards for significance. A positive logFC value represents a greater expression of the transcript in the WT infected cohort. Table 3.2 represents the top thirty-five transcripts with the lowest (negative) logFC that also meet the previously-stated standards for significance. A negative logFC value represents a greater expression in the WT sham experimental group.

3.3 *Defb3* Knockout Infected versus Wild-Type Infected

The second comparison made from our experiment was between *Defb3* knockout infected and WT infected mice. Table 3.3 represents the differentially expressed transcripts between the experimental groups. A positive logFC value represents a greater expression of the transcript in the *Defb3* knockout infected cohort, while a negative logFC value represents a greater expression in the WT infected experimental group. The transcripts represented in Table 3.3 have a p-value less than 0.05, an FDR less than 0.05, and the table is sorted from the largest to the smallest logFC. Only two transcripts were found to be differentially expressed between *Defb3* knockout infected mice and WT infected mice. The lone significant differentially expressed transcript that had greater expression in the *Defb3* knockout mouse is *Sycp1*. *Defb3* was the only transcript with a significantly greater expression in the WT cohort.

Genes	**logFC**	**P-Value**	**FDR**
Saa3	11.39303	2.28E-06	0.000768
Slc26a4	10.72678	7.04E-05	0.006962
Gm5483	10.72537	1.34E-05	0.002231
Il19	10.62021	6.72E-06	0.001419
AC110211.1	10.29827	6.77E-06	0.001419
Fpr1	10.1362	0.000333	0.021121
Cxcl5	9.8357	3.08E-07	0.000197
Cxcl2	9.71163	1.86E-05	0.00271
Trem1	9.629253	2.98E-06	0.000882
Cxcl3	9.606077	6.67E-06	0.001419
Il17a	9.307849	3.42E-06	0.000949
Fpr2	9.200458	1.62E-06	0.000596
Retnlg	9.14515	1.27E-05	0.002154
Il1b	8.941617	8.39E-06	0.001601
Cd300lf	8.869469	2.75E-08	3.44E-05
Gm9733	8.756683	2.85E-07	0.000187
Csf3	8.606316	1.72E-05	0.002545
Cd177	8.562038	2.93E-05	0.003664
Fgf23	8.428521	0.000564	0.030468
Osm	8.358338	1.72E-06	0.000624
Il12b	8.320581	8.26E-05	0.007733
Acod1	8.280447	4.40E-05	0.004878
Cxcl1	8.174724	2.70E-05	0.00342
Il22	8.161523	1.75E-07	0.00013
Gm14010	8.124756	1.29E-07	0.000101
Ccl20	8.107351	0.000993	0.04578
Saa2	8.066387	2.53E-06	0.000786
Olfm4	7.953813	0.000167	0.012722
Clec4d	7.939478	1.35E-05	0.002234
Uox	7.914814	0.00041	0.024837
Gm16556	7.875215	6.59E-07	0.000334
Il20	7.61286	6.52E-06	0.001419
Clec4e	7.546167	4.25E-06	0.001089
Nlrp12	7.503965	6.11E-07	0.000324
Ly6g	7.469695	2.50E-05	0.003256

Table 3.1 WT Infected vs. WT Sham. The transcripts in the table have a $p < 0.05$, FDR < 0.05 and are the 35 highest logFC values. Positive logFC values represent greater transcript expression in WT infected mice. The table is sorted by logFC from the largest value to the smallest.

Genes	**logFC**	**P-Value**	**FDR**
Ccnb1ip1	-8.45855	0.000497	0.02787
AC140393.1	-6.8363	0.000275	0.018374
Gm5828	-6.8309	3.89E-05	0.004478
Thegl	-5.53812	0.000638	0.033535
Gpr165	-5.53245	0.000634	0.033505
Gm7240	-5.52834	0.000951	0.044361
Kcnh6	-4.84258	0.000582	0.031288
Gm2199	-4.72075	2.57E-08	3.37E-05
Rgag1	-4.37314	0.000494	0.027852
S100a5	-4.35421	4.56E-05	0.005043
Sult1b1	-4.31325	3.29E-05	0.003987
Gm48550	-4.26375	0.00095	0.044361
Pla2g2d	-3.90829	8.69E-06	0.001647
A730046J19Rik	-3.79861	0.000402	0.024468
Pik3c2g	-3.75619	0.000828	0.04055
Gm16485	-3.64652	0.000273	0.018337
Gm28653	-3.46786	0.000438	0.02581
Lrrc31	-3.29848	0.000394	0.024078
Abhd12b	-3.25523	9.16E-06	0.001697
Hepacam2	-3.17438	1.52E-05	0.002391
Jakmip2	-3.12843	0.000406	0.024652
Rnf112	-3.03242	2.40E-05	0.003218
Prdm8	-3.00313	2.45E-06	0.000783
Rhcg	-2.96669	0.000497	0.02787
Diras2	-2.90141	0.000443	0.025975
Notumos	-2.88766	0.000436	0.025708
Gabra4	-2.79595	0.000338	0.021388
Gm11816	-2.7951	0.000539	0.029649
Ptprr	-2.74332	1.13E-05	0.001993
Slitrk2	-2.69947	0.000265	0.01786
P2rx2	-2.6982	0.001021	0.046854
Adamts19	-2.67393	0.000729	0.036837
Enpp6	-2.66693	6.93E-07	0.000349
Crtac1	-2.63911	0.000285	0.018958
Gm26888	-2.62038	0.000759	0.038008

Table 3.2 WT Infected vs. WT Sham. The transcripts in the table have a $p < 0.05$, FDR < 0.05 and are the 35 lowest logFC values. Negative logFC values represent greater transcript expression in WT sham mice. The table is sorted by logFC from the smallest value to the largest.

Genes	logFC	P-Value	FDR
Sycp1	7.139276	1.85E-07	0.004979
Defb3	-10.9139	4.78E-09	0.000257

Table 3.3 *Defb3* Knockout Infected vs. WT Infected. The transcripts in the table have a $p < 0.05$, FDR < 0.05, and a logFC > 2 or logFC < 2. Two different transcripts met these requirements and are considered to be differentially expressed and significant. Positive logFC values represent greater transcript expression in *Defb3* knockout infected mice. Negative logFC values represent greater transcript expression in WT infected mice. The table is sorted by logFC from the largest value to the smallest.

3.4 *Defb3* Knockout Sham versus Wild-Type Sham

The third comparison made from our experiment was between *Defb3* knockout sham and WT sham mice. Table 3.4 represents the differentially expressed transcripts between the experimental groups. A positive logFC value represents a greater expression of the transcript in the *Defb3* knockout sham cohort, while a negative logFC value represents a greater expression in the WT sham experimental group. The transcripts represented in Table 3.4 have a p-value less than 0.05, an FDR less than 0.05, and the table is sorted from the largest to the smallest logFC. Only six transcripts were found to be differentially expressed between *Defb3* knockout sham mice and WT sham mice. *Sycp1*, *Ankk1*, and *Atp13a4* are all transcripts with significantly greater expression in the *Defb3* knockout sham mice. The WT sham mice showed significantly greater expression in the *Defb3*, *Gm39154*, and *Gm2199* transcripts.

Genes	logFC	P-Value	FDR
Sycp1	5.663505	4.11E-07	0.005527
Ankk1	3.528387	6.92E-07	0.006209
Atp13a4	2.167586	6.57E-08	0.001178
Gm2199	-5.76768	1.35E-08	0.000725
Gm39154	-7.96418	6.84E-07	0.006209
Defb3	-10.0877	4.25E-08	0.001144

Table 3.4 *Defb3* Knockout Sham vs. WT Sham. The transcripts in the table have a $p < 0.05$, $FDR < 0.05$, and a $logFC > 2$ or $logFC < 2$. Six different transcripts met these requirements and are considered to be differentially expressed and and significant. Positive logFC values represent greater transcript expression in *Defb3* knockout sham mice. Negative logFC values represent greater transcript expression in WT sham mice. The table is sorted by logFC from the largest value to the smallest.

3.5 *Defb3* Knockout Infected versus *Defb3* Knockout Sham

The fourth comparison made from our experiment was between *Defb3* knockout infected and *Defb3* knockout sham mice. Five hundred eighty-eight different transcripts were identified as having a significant differential expression, meaning that the transcripts had a p-value less than 0.05 and an FDR less than 0.05. Table 3.5 represents the top thirty-five transcripts with the greatest (positive) logFC that also meet the previously-stated standards for significance. A positive logFC value represents a greater expression of the transcript in the *Defb3* knockout infected cohort. Table 3.6 represents the top thirty-five transcripts with the lowest (negative) logFC that also meet the previously-stated standards for significance. A negative logFC value represents a greater expression in the *Defb3* knockout sham experimental group.

Genes	**logFC**	**P-Value**	**FDR**
Gm5483	9.952193	2.90E-05	0.007719
Nlrp12	9.627635	1.09E-06	0.001007
Saa3	9.340649	4.41E-06	0.002372
Slc26a4	7.730627	0.000233	0.028186
Csf3	7.701927	4.94E-05	0.010661
Il19	7.696321	1.65E-05	0.005374
Cd177	7.643502	0.000138	0.020053
Retnlg	7.622437	4.43E-05	0.009931
Il22	7.576076	9.17E-07	0.000897
Trem1	7.488971	1.03E-05	0.004124
Clec4d	7.37194	3.30E-05	0.008399
Il6	7.333296	0.000274	0.031291
Osm	7.163162	7.12E-06	0.003247
AC110211.1	7.145829	4.39E-05	0.009922
Mir142b	6.991013	1.46E-05	0.005049
Csf3r	6.820779	9.95E-06	0.003998
Saa4	6.763833	2.16E-06	0.001613
Cd300lf	6.737686	1.24E-07	0.000222
Clec4e	6.622801	1.13E-05	0.004358
Il1b	6.592028	3.16E-05	0.008185
Cxcl1	6.588532	6.19E-05	0.012135
Cxcl5	6.510821	2.04E-06	0.001588
Acod1	6.456974	0.000138	0.020053
Mcemp1	6.452192	4.01E-05	0.00943
Il12b	6.44226	0.000108	0.017312
Fpr2	6.397273	8.69E-06	0.003681
Cxcr1	6.366694	0.000297	0.032894
Ly6g	6.322893	0.000365	0.038085
Cxcl2	6.284726	8.47E-05	0.014947
Ccl4	6.283194	0.000285	0.032091
Gm48671	6.280621	0.000145	0.020912
Gm16175	6.237508	3.53E-05	0.008794
Trem3	6.236911	2.58E-05	0.0072
Mcoln2	6.099284	2.50E-08	8.97E-05
A530032D15Rik	6.03783	0.000375	0.038683

Table 3.5 *Defb3* KO Infected vs. *Defb3* KO Sham. The transcripts in the table have a $p < 0.05$, FDR < 0.05, and are the 35 highest logFC values. Positive logFC values represent greater transcript expression in *Defb3* KO infected mice. The table is sorted by logFC from the smallest value to the largest.

Genes	logFC	P-Value	FDR
Gm26945	-6.68613	0.000123	0.018913
Gm34583	-5.72183	0.000249	0.029297
Gm28653	-4.25092	0.000125	0.018942
Gm15883	-4.1092	0.000261	0.030206
Diras2	-3.8994	1.41E-05	0.005005
Aass	-3.88805	6.35E-05	0.012337
Hcar1	-3.39813	2.52E-05	0.007154
Rnf112	-3.33643	5.89E-05	0.011877
Astn1	-3.12215	0.000328	0.035359
Hepacam2	-3.09096	1.65E-05	0.005374
Gm32926	-3.06423	0.000518	0.047829
Cldn3	-3.01995	4.77E-05	0.010464
4930511M06Rik	-2.8861	0.000213	0.026319
Gm43197	-2.73251	2.63E-05	0.007232
Gm15663	-2.73137	0.000391	0.039732
Gm45226	-2.70892	0.00046	0.04416
Celf5	-2.55417	5.38E-05	0.01118
Siglech	-2.34465	9.62E-05	0.016106
9230112J17Rik	-2.3399	0.00011	0.017482
Cxxc4	-2.26439	0.000391	0.039732
Abca9	-2.19426	2.57E-06	0.00182
Cubn	-2.18113	0.000171	0.023091
Pkhd1l1	-2.16286	2.67E-05	0.007232
Ttc25	-2.14457	0.000487	0.045751
Gm15935	-2.12519	0.000132	0.019788
Gpr34	-2.11385	3.39E-05	0.008519
Zcchc5	-2.0958	0.000324	0.035122
Fam198b	-2.09469	5.23E-06	0.002605
Tmem119	-2.09274	2.49E-05	0.007131
Capn6	-2.0567	9.01E-05	0.015464
Zdbf2	-2.05246	1.69E-06	0.001369
Lgi1	-2.05095	0.000221	0.026992
Smoc1	-2.05066	5.69E-05	0.011728
Slfn3	-2.04145	0.000248	0.029297
Nynrin	-2.01941	7.76E-05	0.013956

Table 3.6 *Defb3* KO Infected vs. *Defb3* KO Sham. The transcripts in the table have a $p < 0.05$, FDR < 0.05, and are the 35 lowest logFC values. Negative logFC values represent greater transcript expression in *Defb3* KO sham mice. The table is sorted by logFC from the smallest value to the largest.

Chapter 4

Discussion

4.1 The Wild-Type Infected versus Wild-Type Sham Results

One thousand one hundred ninety-one unique transcripts were found to have significant differential expression results between the WT infected and WT sham experimental groups (Appendix Table B.1). Appendix Table B.1 was broken down into Table 3.1 and Table 3.2. Table 3.1 represents the top thirty-five logFC values for the differentially expressed transcripts identified in the WT infected and WT sham comparison. Table 3.2 represents the thirty-five lowest logFC values from Appendix Table B.1. The results represented in these three tables are comparable to the results in previously-published experiments.

For example, *Cxcl1*, *Cxcl5*, *Ccl20*, *Csf3*, *Il23a*, *Defb3*, and *Lcn2* transcripts were identified as differentially expressed in an RNA-Seq study comparing WT infected and WT sham mice under the same OPC infection conditions. All of the previously listed transcript names had greater expression in WT infected mice (Conti et al., 2016). *Cxcl1*, *Cxcl5*, *Ccl20*, and *Csf3* can all be found in Table 3.1, which indicates that these results were among the top 35 differentially expressed transcripts with a positive logFC value. *Il23a*, *Defb3*, and *Lcn2* can be found in Appendix Table B.1, and all have positive logFC values. The positive logFC values indicate that there is greater transcript expression in WT infected mice, which is consistent with the findings in Conti et al., 2016.

A comparison of the supplemental materials from Conti et al., 2016 reveals similar information as the findings in Table 3.1, Table 3.2, and Appendix Table B.1. *Saa3, Slc26a4*, *Gm5483*, *Il19*, *Fpr1*, and *Cxcl2* are all examples of transcripts found in Table 3.1 that were also identified as differentially expressed with positive logFC values in Conti et al., 2016. *Gpr165, Ptprr*, and *A730046J19Rik* are examples of transcripts found in Table 3.2 that were also identified as differentially expressed with negative logFC values in Conti et al., 2016.

A microarray study was done in 2009 between WT infected and WT sham mice under the same experimental conditions that are being used in this thesis (Conti et al., 2009). The results in that study are very similar to what was found in Table 3.1 and the Appendix Table B.1. The microarray in Conti et al., 2009 revealed large, positive fold change values for the transcripts of *Cxcl1*, *Cxcl5, Il1b, Saa3, Cxcl2* (all of these transcripts can be found in Table 3.1), *Lcn2, Saa1*, *Clec4e*, *Mmp3, Il1rn*, *Il6,* and *Timp1* (which can all be found in Appendix Table B.1).

The significant differential expression results from the comparison of WT infected and WT sham mice shows that the information in this thesis is consistent with previous findings. This is important because small differences between the protocols of these studies could cause drastic changes in the NGS results. Conti et al., 2009, Conti et al., 2016, and this thesis all followed the same protocols for infection and tissue harvest. The comparison of the results between these three separate studies supports the idea that these studies followed the same protocols in sample preparation.

4.2 Increased *Sycp1* expression in *Defb3* Knockout Mice

Only two significant results were obtained from the differential expression analysis between *Defb3* infected and WT infected mice (Table 3.3). The *Defb3* result was expected, since the gene is not expressed in the *Defb3* knockout infected mice. The surprising result is that *Sycp1* is differentially expressed in this comparison, with a greater expression found in *Defb3* knockout mice. What made this result even more surprising is that *Sycp1* transcript expression was also upregulated in *Defb3* knockout sham mice when compared with WT sham mice (Table 3.4).

This finding is unusual considering that *Sycp1* has only been linked to forming synaptonemal complexes (SCs) in meiosis (UniProt, 2018, July 20). Making this discovery more interesting is the fact that the protein produced by the *Sycp1* has non-specific, DNA-binding ability (Dobson, Pearlman, Karaiskakis, Spyropoulos, & Moens, 1994). The *Sycp1* protein has a coiled-coil domain, a leucine zipper in the same region, and many S/T-P-X-X motifs. This combination of features gives the *Sycp1* protein the ability to bind to DNA (Meuwissen et al., 1992). Since meiosis does not take place in the oral cavity, it is possible that the DNA-binding ability of the *Sycp1* protein is being used in an additional way in the *Defb3* knockout mice. While there are no previous publications to support this, future studies exploring the possibility of the *Sycp1* protein functioning as a transcription factor could be justified.

4.3 *Defb3* Knockout Sham versus Wild-Type Sham

Outside of the *Sycp1* and the expected *Defb3* results, the *Defb3* knockout sham and WT sham comparison produced four other significant results: *Ankk1*, *Atp13a4*, *Gm2199*, and *Gm39154*. *Ankk1* codes for a protein that binds ATP and catalyzes a reaction that can add a phosphate group to a protein (UniProt, 2018, February 28). *Ankk1* has been linked to addiction and is downstream of dopamine receptors (Ponce et al., 2016). *Atp13a4* codes for an ATPase (National Center for Biotechnology Information, 2018, May 23) and defects in this gene have been associated with autism (Vallipuram, Grenville, & Crawford, 2010). *Gm2199* is a long non-coding RNA (lncRNA) that has recently been related to the protection and rescue of hepatocytes when exposed to liver tissue damage. It operates by inducing extracellular signal-regulated kinase-1/2 (ERK1/2) upregulation (Gao et al., 2018). *Gm39154* is a lncRNA on chromosome 8 for which not much is known (Ensembl, 2018, April). Although it is unclear what role these four transcripts are fulfilling in the context of the OPC infection model, they could serve as a valuable starting point for future experiments.

4.4 ***Defb3*** Knockout Infected versus ***Defb3*** Knockout Sham Results

The results from the *Defb3* knockout infected and *Defb3* knockout sham comparison are very similar to those of the WT infected vs WT sham comparison (simplified to *Defb3* comparison and WT comparison, respectively, in this section only for simplicity). The results between these two comparisons are so similar that Table 3.1 (WT comparison) and Table 3.5 (*Defb3* comparison) share 23 out of the 35 top results listed between the two.

The *Defb3* comparison results in Table 3.5 and Table 3.6 that the findings of previous experiments. While these other experiments were in wild-type mice, the results are similar. In Conti et al., 2009, a microarray comparison found that the transcripts for

Cxcl1, *Cxcl5*, *Il1b*, *Saa3*, *Cxcl2*, *Clec4e*, *Il6*, and *Timp1* were all found to be upregulated in an OPC infection. All of these transcripts were found to be upregulated in the *Defb3* knockout mice in an OPC infection (Table 3.5 and Appendix Table B.2). In Conti et al., 2016, an RNA-seq study found the *Cxcl1*, *Cxcl5*, *Csf3*, *Saa3*, *Slc26a4*, *Gm5483*, *Il19*, and *Cxcl2* transcripts to be differentially expressed and upregulated during OPC infection in WT mice. All of these transcripts were also found to be upregulated during OPC infection in *Defb3* knockout mice. All of this supports the idea that there are not many differences between transcript expression in WT and *Defb3* knockout mice.

4.5 Conclusion

There are very few transcriptional differences between *Defb3* knockout mice and WT mice. Only eight significant results were found between the *Defb3* knockout infected/WT infected and *Defb3* knockout sham/WT sham comparisons, combined (Table 3.3 and Table 3.4). Because of the chemotactic ability of *Defb3*, this result is disappointing. What this could mean is that mBD3/hBD2 are the furthest downstream effect of IL-17 signaling. While hBD2 can induce chemotaxis in memory T-cells, immature DCs (Yang et al., 1999), and possibly Th17 cells (Conti et al., 2014), it is possible that this effect is only an indirect mechanism of β-defensin killing. This could mean that the chemotactic abilities of mBD3/hBD2 are a small, insignificant portion of their function. Murine BD3/hBD2 bind to CCR6 (Ganz, 2003), but CCR6 is also bound by CCL20 (Yamazaki et al., 2008). If CCL20 is involved in the majority of CCR6 binding, the small amount of missing β-defensin 3 signaling would go relatively unnoticed in an RNA-seq study like this one. It is important to note that Ccl20 was a transcript found to be highly upregulated in WT infected mice when compared to WT sham mice (Table 3.1). If this is the case, the observed *Defb3* susceptibility to OPC (Conti et al., 2016) could strictly be due to the loss of the direct antimicrobial properties of β-defensin 3, rather than its indirect chemotactic and signaling abilities.

One promising result from this experiment is that the *Sycp1* transcript was differentially expressed in the both the *Defb3* knockout infection/WT infection and *Defb3* knockout sham/WT sham comparisons. While the *Sycp1* protein has only been linked to meiosis in previous papers, its ability to bind to DNA provides an interesting foundation for future studies. If *Sycp1* is acting as a transcription factor under certain conditions, it would be interesting to explore what conditions cause this change in function, the genes that are affected by *Sycp1* acting as a transcription factor, and the specific DNA sequences that *Sycp1* identifies and binds to in promoter regions. It should also be noted that the loss of mBD3 could be contributing to a signaling deficit within the epithelial cells of the *Defb3* knockout mice. Without mBD3, it is possible that the intracellular signaling events that lead to the formation of β-defensin 3 are never downregulated because mBD3 is never formed in the *Defb3* knockout mice. It is possible that under infection conditions, the versatile DNA-binding ability of *Sycp1* is exploited as a transcription factor because the cell needs the antimicrobial activity of β-defensin 3.

The results of this thesis provide interesting areas to explore in future studies. This is one of the greatest advantages of next-generation sequencing. Under normal circumstances, the amount of information can be overwhelming. In this study, the differences between the WT mice and the *Defb3* mice is limited enough that every result can be explored.

References

Achkar, J. M., & Fries, B. C. (2010). Candida infections of the genitourinary tract. *Clin Microbiol Rev, 23*(2), 253-273. doi:10.1128/CMR.00076-09

Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen recognition and innate immunity. *Cell, 124*(4), 783-801. doi:10.1016/j.cell.2006.02.015

Bahri, R., Curt, S., Saidane-Mosbahi, D., & Rouabhia, M. (2010). Normal human gingival epithelial cells sense C. parapsilosis by toll-like receptors and module its pathogenesis through antimicrobial peptides and proinflammatory cytokines. *Mediators Inflamm, 2010*, 940383. doi:10.1155/2010/940383

Bals, R., Wang, X., Meegalla, R. L., Wattler, S., Weiner, D. J., Nehls, M. C., & Wilson, J. M. (1999). Mouse beta-defensin 3 is an inducible antimicrobial peptide expressed in the epithelia of multiple organs. *Infect Immun, 67*(7), 3542-3547.

Beck, J. A., Lloyd, S., Hafezparast, M., Lennon-Pierce, M., Eppig, J. T., Festing, M. F., & Fisher, E. M. (2000). Genealogies of mouse inbred strains. *Nat Genet, 24*(1), 23-25. doi:10.1038/71641

Bio-Rad. (2018). Innate immunity: Tlr2 and tlr4. Retrieved from https://www.bio-rad-antibodies.com/tlr2-tlr4-antibodies.html

Brogden, K. A. (2005). Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat Rev Microbiol, 3*(3), 238-250. doi:10.1038/nrmicro1098

Brown, G. D., Taylor, P. R., Reid, D. M., Willment, J. A., Williams, D. L., Martinez-Pomares, L., . . . Gordon, S. (2002). Dectin-1 is a major beta-glucan receptor on macrophages. *J Exp Med, 196*(3), 407-412.

Chang, S. H., & Dong, C. (2007). A novel heterodimeric cytokine consisting of IL-17 and IL-17F regulates inflammatory responses. *Cell Res, 17*(5), 435-440. doi:10.1038/cr.2007.35

Cheng, S. C., Joosten, L. A., Kullberg, B. J., & Netea, M. G. (2012). Interplay between Candida albicans and the mammalian innate host defense. *Infect Immun, 80*(4), 1304-1313. doi:10.1128/IAI.06146-11

Cheng, S. C., van de Veerdonk, F., Smeekens, S., Joosten, L. A., van der Meer, J. W., Kullberg, B. J., & Netea, M. G. (2010). Candida albicans dampens host defense by downregulating IL-17 production. *J Immunol, 185*(4), 2450-2457. doi:10.4049/jimmunol.1000756

Conesa, A., Madrigal, P., Tarazona, S., Gomez-Cabrero, D., Cervera, A., McPherson, A., . . . Mortazavi, A. (2016). A survey of best practices for RNA-seq data analysis. *Genome Biol, 17*, 13. doi:10.1186/s13059-016-0881-8

Conti, H. R., Bruno, V. M., Childs, E. E., Daugherty, S., Hunter, J. P., Mengesha, B. G., . . . Gaffen, S. L. (2016). IL-17 Receptor Signaling in Oral Epithelial Cells Is Critical for Protection against Oropharyngeal Candidiasis. *Cell Host Microbe, 20*(5), 606-617. doi:10.1016/j.chom.2016.10.001

Conti, H. R., Peterson, A. C., Brane, L., Huppler, A. R., Hernandez-Santos, N., Whibley, N., . . . Gaffen, S. L. (2014). Oral-resident natural Th17 cells and gammadelta T cells control opportunistic Candida albicans infections. *J Exp Med, 211*(10), 2075-2084. doi:10.1084/jem.20130877

Conti, H. R., Shen, F., Nayyar, N., Stocum, E., Sun, J. N., Lindemann, M. J., . . . Gaffen, S. L. (2009). Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. *J Exp Med, 206*(2), 299-311. doi:10.1084/jem.20081463

Das, S., Nikolaidis, N., Goto, H., McCallister, C., Li, J., Hirano, M., & Cooper, M. D. (2010). Comparative genomics and evolution of the alpha-defensin multigene family in primates. *Mol Biol Evol, 27*(10), 2333-2343. doi:10.1093/molbev/msq118

Diamond, G., & Ryan, L. (2011). Beta-defensins: what are they really doing in the oral cavity? *Oral Dis, 17*(7), 628-635. doi:10.1111/j.1601-0825.2011.01799.x

Dobson, M. J., Pearlman, R. E., Karaiskakis, A., Spyropoulos, B., & Moens, P. B. (1994). Synaptonemal complex proteins: occurrence, epitope mapping and chromosome disjunction. *J Cell Sci, 107 (Pt 10)*, 2749-2760.

Dongari-Bagtzoglou, A., & Fidel, P. L., Jr. (2005). The host cytokine responses and protective immunity in oropharyngeal candidiasis. *J Dent Res, 84*(11), 966-977. doi:10.1177/154405910508401101

Ensembl. (2018, April). Gene: Gm39154. Retrieved from http://useast.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG00000109666;r=8:30112310-30121214;t=ENSMUST00000210257

Fidel, P. L., Jr. (2011). Candida-host interactions in HIV disease: implications for oropharyngeal candidiasis. *Adv Dent Res, 23*(1), 45-49. doi:10.1177/0022034511399284

Finotello, F., & Di Camillo, B. (2015). Measuring differential gene expression with RNA-seq: challenges and strategies for data analysis. *Brief Funct Genomics, 14*(2), 130-142. doi:10.1093/bfgp/elu035

Ganguly, S., & Mitchell, A. P. (2011). Mucosal biofilms of Candida albicans. *Curr Opin Microbiol, 14*(4), 380-385. doi:10.1016/j.mib.2011.06.001

Gantner, B. N., Simmons, R. M., & Underhill, D. M. (2005). Dectin-1 mediates macrophage recognition of Candida albicans yeast but not filaments. *EMBO J, 24*(6), 1277-1286. doi:10.1038/sj.emboj.7600594

Ganz, T. (2003). Defensins: antimicrobial peptides of innate immunity. *Nat Rev Immunol, 3*(9), 710-720. doi:10.1038/nri1180

Gao, Q., Gu, Y., Jiang, Y., Fan, L., Wei, Z., Jin, H., . . . Liu, Y. (2018). Long non-coding RNA Gm2199 rescues liver injury and promotes hepatocyte proliferation through the upregulation of ERK1/2. *Cell Death Dis, 9*(6), 602. doi:10.1038/s41419-018-0595-9

Gow, N. A., & Hube, B. (2012). Importance of the Candida albicans cell wall during commensalism and infection. *Curr Opin Microbiol, 15*(4), 406-412. doi:10.1016/j.mib.2012.04.005

Gow, N. A., van de Veerdonk, F. L., Brown, A. J., & Netea, M. G. (2011). Candida albicans morphogenesis and host defence: discriminating invasion from colonization. *Nat Rev Microbiol, 10*(2), 112-122. doi:10.1038/nrmicro2711

Ho, A. W., & Gaffen, S. L. (2010). IL-17RC: a partner in IL-17 signaling and beyond. *Semin Immunopathol, 32*(1), 33-42. doi:10.1007/s00281-009-0185-0

Jiang, Y., Wang, Y., Wang, B., Yang, D., Yu, K., Yang, X., . . . Li, M. (2010). Antifungal activity of recombinant mouse beta-defensin 3. *Lett Appl Microbiol, 50*(5), 468-473. doi:10.1111/j.1472-765X.2010.02824.x

Jin, W., & Dong, C. (2013). IL-17 cytokines in immunity and inflammation. *Emerg Microbes Infect, 2*(9), e60. doi:10.1038/emi.2013.58

Joly, S., Maze, C., McCray, P. B., Jr., & Guthmiller, J. M. (2004). Human beta-defensins 2 and 3 demonstrate strain-selective activity against oral microorganisms. *J Clin Microbiol, 42*(3), 1024-1029.

Jouault, T., Ibata-Ombetta, S., Takeuchi, O., Trinel, P. A., Sacchetti, P., Lefebvre, P., . . . Poulain, D. (2003). Candida albicans phospholipomannan is sensed through toll-like receptors. *J Infect Dis, 188*(1), 165-172. doi:10.1086/375784

Kao, C. Y., Chen, Y., Thai, P., Wachi, S., Huang, F., Kim, C., . . . Wu, R. (2004). IL-17 markedly up-regulates beta-defensin-2 expression in human airway epithelium via JAK and NF-kappaB signaling pathways. *J Immunol, 173*(5), 3482-3491.

Kashem, S. W., & Kaplan, D. H. (2016). Skin Immunity to Candida albicans. *Trends Immunol, 37*(7), 440-450. doi:10.1016/j.it.2016.04.007

Kiyoura, Y., & Tamai, R. (2015). Innate immunity to Candida albicans. *Japanese Dental Science Review, 51*(3), 59-64. doi:https://doi.org/10.1016/j.jdsr.2014.12.001

Lacerda, A. F., Vasconcelos, E. A., Pelegrini, P. B., & Grossi de Sa, M. F. (2014). Antifungal defensins and their role in plant defense. *Front Microbiol, 5*, 116. doi:10.3389/fmicb.2014.00116

Lo, H. J., Kohler, J. R., DiDomenico, B., Loebenberg, D., Cacciapuoti, A., & Fink, G. R. (1997). Nonfilamentous C. albicans mutants are avirulent. *Cell, 90*(5), 939-949.

Mayer, F. L., Wilson, D., & Hube, B. (2013). Candida albicans pathogenicity mechanisms. *Virulence, 4*(2), 119-128. doi:10.4161/viru.22913

Mengesha, B. G., & Conti, H. R. (2017). The Role of IL-17 in Protection against Mucosal Candida Infections. *J Fungi (Basel), 3*(4). doi:10.3390/jof3040052

Meuwissen, R. L., Offenberg, H. H., Dietrich, A. J., Riesewijk, A., van Iersel, M., & Heyting, C. (1992). A coiled-coil related protein specific for synapsed regions of meiotic prophase chromosomes. *EMBO J, 11*(13), 5091-5100.

National Center for Biotechnology Information. (2018, May 23). Atp13a4 atpase type 13a4 [mus musculus (house mouse)]. Retrieved from https://www.ncbi.nlm.nih.gov/gene/224079

National Human Genome Research Institute. (2010, July 23). Why mouse matters. Retrieved from https://www.genome.gov/10001345/importance-of-mouse-genome/

National Human Genome Research Institute. (2012, May 23). Background on mouse as a model organism. Retrieved from https://www.genome.gov/10005834/background-on-mouse-as-a-model-organism/

Netea, M. G., Gow, N. A., Munro, C. A., Bates, S., Collins, C., Ferwerda, G., . . . Kullberg, B. J. (2006). Immune sensing of Candida albicans requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors. *J Clin Invest, 116*(6), 1642-1650. doi:10.1172/JCI27114

Neville, B. A., d'Enfert, C., & Bougnoux, M. E. (2015). Candida albicans commensalism in the gastrointestinal tract. *FEMS Yeast Res, 15*(7). doi:10.1093/femsyr/fov081

Niyonsaba, F., Ogawa, H., & Nagaoka, I. (2004). Human beta-defensin-2 functions as a chemotactic agent for tumour necrosis factor-alpha-treated human neutrophils. *Immunology, 111*(3), 273-281.

Nuding, S., Gersemann, M., Hosaka, Y., Konietzny, S., Schaefer, C., Beisner, J., . . . Wehkamp, J. (2013). Gastric antimicrobial peptides fail to eradicate Helicobacter pylori infection due to selective induction and resistance. *PLoS One, 8*(9), e73867. doi:10.1371/journal.pone.0073867

Ozsolak, F., & Milos, P. M. (2011). RNA sequencing: advances, challenges and opportunities. *Nat Rev Genet, 12*(2), 87-98. doi:10.1038/nrg2934

Pfaller, M. A., & Diekema, D. J. (2007). Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev, 20*(1), 133-163. doi:10.1128/CMR.00029-06

Ponce, G., Quinones-Lombrana, A., Martin-Palanco, N. G., Rubio-Solsona, E., Jimenez-Arriero, M. A., Palomo, T., & Hoenicka, J. (2016). The Addiction-Related Gene Ankk1 is Oppositely Regulated by D1R- and D2R-Like Dopamine Receptors. *Neurotox Res, 29*(3), 345-350. doi:10.1007/s12640-015-9545-9

Rabeneck, L., Crane, M. M., Risser, J. M., Lacke, C. E., & Wray, N. P. (1993). A simple clinical staging system that predicts progression to AIDS using CD4 count, oral thrush, and night sweats. *J Gen Intern Med, 8*(1), 5-9.

Ray, T. L., & Payne, C. D. (1988). Scanning electron microscopy of epidermal adherence and cavitation in murine candidiasis: a role for Candida acid proteinase. *Infect Immun, 56*(8), 1942-1949.

Robinson, M. D., & Oshlack, A. (2010). A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol, 11*(3), R25. doi:10.1186/gb-2010-11-3-r25

Saijo, S., Ikeda, S., Yamabe, K., Kakuta, S., Ishigame, H., Akitsu, A., . . . Iwakura, Y. (2010). Dectin-2 recognition of alpha-mannans and induction of Th17 cell differentiation is essential for host defense against Candida albicans. *Immunity, 32*(5), 681-691. doi:10.1016/j.immuni.2010.05.001

Saijo, S., & Iwakura, Y. (2011). Dectin-1 and Dectin-2 in innate immunity against fungi. *Int Immunol, 23*(8), 467-472. doi:10.1093/intimm/dxr046

Sawaki, K., Mizukawa, N., Yamaai, T., Fukunaga, J., & Sugahara, T. (2002). Immunohistochemical study on expression of alpha-defensin and beta-defensin-2 in human buccal epithelia with candidiasis. *Oral Dis, 8*(1), 37-41.

Schroder, J. M., & Harder, J. (1999). Human beta-defensin-2. *Int J Biochem Cell Biol, 31*(6), 645-651.

Silva, P. M., Goncalves, S., & Santos, N. C. (2014). Defensins: antifungal lessons from eukaryotes. *Front Microbiol, 5,* 97. doi:10.3389/fmicb.2014.00097

Toy, D., Kugler, D., Wolfson, M., Vanden Bos, T., Gurgel, J., Derry, J., . . . Peschon, J. (2006). Cutting edge: interleukin 17 signals through a heteromeric receptor complex. *J Immunol, 177*(1), 36-39.

UniProt. (2018, February 28). Q8bz25 (ankk1_mouse). Retrieved from https://www.uniprot.org/uniprot/Q8BZ25

UniProt. (2018, July 20). Uniprotkb - q62209 (sycp1_mouse). Retrieved from https://www.uniprot.org/uniprot/Q62209

Vallipuram, J., Grenville, J., & Crawford, D. A. (2010). The E646D-ATP13A4 mutation associated with autism reveals a defect in calcium regulation. *Cell Mol Neurobiol, 30*(2), 233-246. doi:10.1007/s10571-009-9445-8

Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet, 10*(1), 57-63. doi:10.1038/nrg2484

Weindl, G., Naglik, J. R., Kaesler, S., Biedermann, T., Hube, B., Korting, H. C., & Schaller, M. (2007). Human epithelial cells establish direct antifungal defense through TLR4-mediated signaling. *J Clin Invest, 117*(12), 3664-3672. doi:10.1172/JCI28115

Yamazaki, T., Yang, X. O., Chung, Y., Fukunaga, A., Nurieva, R., Pappu, B., . . . Dong, C. (2008). CCR6 regulates the migration of inflammatory and regulatory T cells. *J Immunol, 181*(12), 8391-8401.

Yang, D., Chertov, O., Bykovskaia, S. N., Chen, Q., Buffo, M. J., Shogan, J., . . . Oppenheim, J. J. (1999). Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. *Science, 286*(5439), 525-528.

Zhao, B. C., Lin, H. C., Yang, D., Ye, X., & Li, Z. G. (2015). Disulfide Bridges in Defensins. *Curr Top Med Chem, 16*(2), 206-219.

Appendix A

Code

```
#Download the RNAseq fasta files
wget --user=genomicsdata --password='results373'
http://eh3.uc.edu/genomics/results/fastq/180413_SNL140_0186_ACANE6ACXX/Data/I
ntensities/Unaligned_Bcl/Project_HeatherConti_ColeWhite_NEB_RNA/rawData/HC5_S
5_L001_R1_001.fastq.gz
wget --user=genomicsdata --password='results373'
http://eh3.uc.edu/genomics/results/fastq/180413_SNL140_0186_ACANE6ACXX/Data/I
ntensities/Unaligned_Bcl/Project_HeatherConti_ColeWhite_NEB_RNA/rawData/HC4_S
4_L001_R1_001.fastq.gz
wget --user=genomicsdata --password='results373'
http://eh3.uc.edu/genomics/results/fastq/180413_SNL140_0186_ACANE6ACXX/Data/I
ntensities/Unaligned_Bcl/Project_HeatherConti_ColeWhite_NEB_RNA/rawData/HC3_S
3_L001_R1_001.fastq.gz
wget --user=genomicsdata --password='results373'
http://eh3.uc.edu/genomics/results/fastq/180413_SNL140_0186_ACANE6ACXX/Data/I
ntensities/Unaligned_Bcl/Project_HeatherConti_ColeWhite_NEB_RNA/rawData/HC2_S
2_L001_R1_001.fastq.gz
wget --user=genomicsdata --password='results373'
http://eh3.uc.edu/genomics/results/fastq/180413_SNL140_0186_ACANE6ACXX/Data/I
ntensities/Unaligned_Bcl/Project_HeatherConti_ColeWhite_NEB_RNA/rawData/HC1_S
1_L001_R1_001.fastq.gz
wget --user=genomicsdata --password='results373'
http://eh3.uc.edu/genomics/results/fastq/180413_SNL140_0186_ACANE6ACXX/Data/I
ntensities/Unaligned_Bcl/Project_HeatherConti_ColeWhite_NEB_RNA/rawData/HC12_
S12_L002_R1_001.fastq.gz
wget --user=genomicsdata --password='results373'
http://eh3.uc.edu/genomics/results/fastq/180413_SNL140_0186_ACANE6ACXX/Data/I
```

```
ntensities/Unaligned_Bcl/Project_HeatherConti_ColeWhite_NEB_RNA/rawData/HC11_
S11_L002_R1_001.fastq.gz
wget --user=genomicsdata --password='results373'
http://eh3.uc.edu/genomics/results/fastq/180413_SNL140_0186_ACANE6ACXX/Data/I
ntensities/Unaligned_Bcl/Project_HeatherConti_ColeWhite_NEB_RNA/rawData/HC10_
S10_L002_R1_001.fastq.gz
wget --user=genomicsdata --password='results373'
http://eh3.uc.edu/genomics/results/fastq/180413_SNL140_0186_ACANE6ACXX/Data/I
ntensities/Unaligned_Bcl/Project_HeatherConti_ColeWhite_NEB_RNA/rawData/HC9_S
9_L002_R1_001.fastq.gz
wget --user=genomicsdata --password='results373'
http://eh3.uc.edu/genomics/results/fastq/180413_SNL140_0186_ACANE6ACXX/Data/I
ntensities/Unaligned_Bcl/Project_HeatherConti_ColeWhite_NEB_RNA/rawData/HC8_S
8_L001_R1_001.fastq.gz
wget --user=genomicsdata --password='results373'
http://eh3.uc.edu/genomics/results/fastq/180413_SNL140_0186_ACANE6ACXX/Data/I
ntensities/Unaligned_Bcl/Project_HeatherConti_ColeWhite_NEB_RNA/rawData/HC7_S
7_L001_R1_001.fastq.gz
wget --user=genomicsdata --password='results373'
http://eh3.uc.edu/genomics/results/fastq/180413_SNL140_0186_ACANE6ACXX/Data/I
ntensities/Unaligned_Bcl/Project_HeatherConti_ColeWhite_NEB_RNA/rawData/HC6_S
6_L001_R1_001.fastq.gz

#Decompress the fasta files
gunzip HC10_S10_L002_R1_001.fastq.gz
gunzip HC11_S11_L002_R1_001.fastq.gz
gunzip HC10_S12_L002_R1_001.fastq.gz
gunzip HC1_S1_L001_R1_001.fastq.gz
gunzip HC2_S2_L001_R1_001.fastq.gz
gunzip HC3_S3_L001_R1_001.fastq.gz
gunzip HC4_S4_L001_R1_001.fastq.gz
gunzip HC5_S5_L001_R1_001.fastq.gz
gunzip HC6_S6_L001_R1_001.fastq.gz
gunzip HC7_S7_L001_R1_001.fastq.gz
gunzip HC8_S8_L001_R1_001.fastq.gz
gunzip HC9_S9_L002_R1_001.fastq.gz

#Download the Reference Genome
```

```
wget ftp.ensembl.org/pub/release-92/fasta/mus_musculus/dna/Mus_musculus.GRCm38.dna.primary_assembly.fa.gz
gunzip Mus_musculus.GRCm38.dna.primary_assembly.fa.gz
mv Mus_musculus.GRCm38.dna.primary_assembly.fa mm38.fa
mkdir mm38
mv mm38.fa /home/cwhite18/RNAseq/mm38

#Make Individual Folders for each sample to organize the results
mkdir Sample1
mkdir Sample2
mkdir Sample3
mkdir Sample4
mkdir Sample5
mkdir Sample6
mkdir Sample7
mkdir Sample8
mkdir Sample9
mkdir Sample10
mkdir Sample11
mkdir Sample12



#Move each sample file to its respective folder
mv /home/cwhite18/RNAseq/HC1_S1_L001_R1_001.fastq /home/cwhite18/RNAseq/Sample1/
mv /home/cwhite18/RNAseq/HC2_S2_L001_R1_001.fastq /home/cwhite18/RNAseq/Sample2/
mv /home/cwhite18/RNAseq/HC3_S3_L001_R1_001.fastq /home/cwhite18/RNAseq/Sample3/
mv /home/cwhite18/RNAseq/HC4_S4_L001_R1_001.fastq /home/cwhite18/RNAseq/Sample4/
mv /home/cwhite18/RNAseq/HC5_S5_L001_R1_001.fastq /home/cwhite18/RNAseq/Sample5/
mv /home/cwhite18/RNAseq/HC6_S6_L001_R1_001.fastq /home/cwhite18/RNAseq/Sample6/
mv /home/cwhite18/RNAseq/HC7_S7_L001_R1_001.fastq /home/cwhite18/RNAseq/Sample7/
```

```
mv /home/cwhite18/RNAseq/HC8_S8_L001_R1_001.fastq
/home/cwhite18/RNAseq/Sample8/
mv /home/cwhite18/RNAseq/HC9_S9_L002_R1_001.fastq
/home/cwhite18/RNAseq/Sample9/
mv /home/cwhite18/RNAseq/HC10_S10_L002_R1_001.fastq
/home/cwhite18/RNAseq/Sample10/
mv /home/cwhite18/RNAseq/HC11_S11_L002_R1_001.fastq
/home/cwhite18/RNAseq/Sample11/
mv /home/cwhite18/RNAseq/HC12_S12_L002_R1_001.fastq
/home/cwhite18/RNAseq/Sample12/

#Add 1pass and 2pass folders to each Sample folder
mkdir 1pass \ 2pass

#Make mm38 index file
java -jar /home/tobi/phillip/picard.jar CreateSequenceDictionary R= mm38.fa O=
mm38.dict
##net.sf.picard.sam.CreateSequenceDictionary R= mm38.fa O= mm38.dict

#/home/basil/RSEM/RSEM-1.3.0/samtools-1.3/samtools faidx mm38.fa
samtools faidx mm38.fa

########################################################################
##################
#Sample 1 - Step 1
genomeDir=/home/cwhite18/RNAseq/mm38
mkdir $genomeDir
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
mm38.fa \ --runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample1/1pass
mkdir $runDir
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample1/HC1_S1_L001_R1_001.fastq --runThreadN 30

genomeDir=/home/cwhite18/RNAseq/mm38_2pass
mkdir $genomeDir
cd $genomeDir
```

```
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--sjdbFileChrStartEnd /home/cwhite18/RNAseq/Sample1/1pass/SJ.out.tab --
sjdbOverhang 74 --runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample1/2pass
mkdir $runDir
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample1/HC1_S1_L001_R1_001.fastq --runThreadN 30

#I would like to keep the mm38_2pass folder with each sample as well.  This is to fix the
first Sample.
mv mm38_2pass /home/cwhite18/RNAseq/Sample1/

#Sample 1 - Step 2
#Make sure you are in the sample 2pass folder
java -jar /home/tobi/phillip/picard.jar AddOrReplaceReadGroups I=Aligned.out.sam
O=rg_added_sorted.bam SO=coordinate RGID=CANE:1 RGLB=HiSeq RGPL=Illumina
RGPU=CANE6ACXX:1 RGSM=Mouse_Tongue_HC912

java -jar /home/tobi/phillip/picard.jar MarkDuplicates I=rg_added_sorted.bam
O=dedupped.bam  CREATE_INDEX=true VALIDATION_STRINGENCY=SILENT
M=output.metrics

#Sample 1 - Step 3
#Make sure you are in the sample 2pass folder
java -jar /home/basil/IndiaProject/GenomeAnalysisTK.jar -T SplitNCigarReads -R
/home/cwhite18/RNAseq/mm38.fa -I dedupped.bam -o split.bam -rf
ReassignOneMappingQuality -RMQF 255 -RMQT 60 -U ALLOW_N_CIGAR_READS


##########################################################################
##################
#Sample 2 - Step 1
genomeDir=/home/cwhite18/RNAseq/mm38
mkdir $genomeDir
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
```

```
--runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample2/1pass
mkdir $runDir
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample2/HC2_S2_L001_R1_001.fastq --runThreadN 30

genomeDir=/home/cwhite18/RNAseq/Sample2/mm38_2pass
mkdir $genomeDir
cd $genomeDir
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--sjdbFileChrStartEnd /home/cwhite18/RNAseq/Sample2/1pass/SJ.out.tab --
sjdbOverhang 74 --runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample2/2pass
mkdir $runDir
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample2/HC2_S2_L001_R1_001.fastq --runThreadN 30

#Adjust commands
java -jar /home/tobi/phillip/picard.jar AddOrReplaceReadGroups I=Aligned.out.sam
O=rg_added_sorted.bam SO=coordinate RGID=CANE:1 RGLB=HiSeq RGPL=Illumina
RGPU=CANE6ACXX:1 RGSM=Mouse_Tongue_HC915

java -jar /home/tobi/phillip/picard.jar MarkDuplicates I=rg_added_sorted.bam
O=dedupped.bam  CREATE_INDEX=true VALIDATION_STRINGENCY=SILENT
M=output.metrics

java -jar /home/basil/IndiaProject/GenomeAnalysisTK.jar -T SplitNCigarReads -R
/home/cwhite18/RNAseq/mm38.fa -I dedupped.bam -o split.bam -rf
ReassignOneMappingQuality -RMQF 255 -RMQT 60 -U ALLOW_N_CIGAR_READS

##########################################################################
#################
#Sample 3 - Step 1
genomeDir=/home/cwhite18/RNAseq/mm38
```

```
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample3/1pass
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample3/HC3_S3_L001_R1_001.fastq --runThreadN 30

genomeDir=/home/cwhite18/RNAseq/Sample3/mm38_2pass
mkdir $genomeDir
cd $genomeDir
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--sjdbFileChrStartEnd /home/cwhite18/RNAseq/Sample3/1pass/SJ.out.tab --
sjdbOverhang 74 --runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample3/2pass
mkdir $runDir
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample3/HC3_S3_L001_R1_001.fastq --runThreadN 30

java -jar /home/tobi/phillip/picard.jar AddOrReplaceReadGroups I=Aligned.out.sam
O=rg_added_sorted.bam SO=coordinate RGID=CANE:1 RGLB=HiSeq RGPL=Illumina
RGPU=CANE6ACXX:1 RGSM=Mouse_Tongue_HC917

java -jar /home/tobi/phillip/picard.jar MarkDuplicates I=rg_added_sorted.bam
O=dedupped.bam  CREATE_INDEX=true VALIDATION_STRINGENCY=SILENT
M=output.metrics

java -jar /home/basil/IndiaProject/GenomeAnalysisTK.jar -T SplitNCigarReads -R
/home/cwhite18/RNAseq/mm38.fa -I dedupped.bam -o split.bam -rf
ReassignOneMappingQuality -RMQF 255 -RMQT 60 -U ALLOW_N_CIGAR_READS


################################################################################
#################
#Sample 4 - Step 1
```

```
genomeDir=/home/cwhite18/RNAseq/mm38
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample4/1pass
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample4/HC4_S4_L001_R1_001.fastq --runThreadN 30

genomeDir=/home/cwhite18/RNAseq/Sample4/mm38_2pass
mkdir $genomeDir
cd $genomeDir
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--sjdbFileChrStartEnd /home/cwhite18/RNAseq/Sample4/1pass/SJ.out.tab --
sjdbOverhang 74 --runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample4/2pass
mkdir $runDir
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample4/HC4_S4_L001_R1_001.fastq --runThreadN 30

java -jar /home/tobi/phillip/picard.jar AddOrReplaceReadGroups I=Aligned.out.sam
O=rg_added_sorted.bam SO=coordinate RGID=CANE:1 RGLB=HiSeq RGPL=Illumina
RGPU=CANE6ACXX:1 RGSM=Mouse_Tongue_HC919

java -jar /home/tobi/phillip/picard.jar MarkDuplicates I=rg_added_sorted.bam
O=dedupped.bam  CREATE_INDEX=true VALIDATION_STRINGENCY=SILENT
M=output.metrics

java -jar /home/basil/IndiaProject/GenomeAnalysisTK.jar -T SplitNCigarReads -R
/home/cwhite18/RNAseq/mm38.fa -I dedupped.bam -o split.bam -rf
ReassignOneMappingQuality -RMQF 255 -RMQT 60 -U ALLOW_N_CIGAR_READS


################################################################################
#################
```

```
#Sample 5 - Step 1
genomeDir=/home/cwhite18/RNAseq/mm38
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample5/1pass
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample5/HC5_S5_L001_R1_001.fastq --runThreadN 30

genomeDir=/home/cwhite18/RNAseq/Sample5/mm38_2pass
mkdir $genomeDir
cd $genomeDir
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--sjdbFileChrStartEnd /home/cwhite18/RNAseq/Sample5/1pass/SJ.out.tab --
sjdbOverhang 74 --runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample5/2pass
mkdir $runDir
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample5/HC5_S5_L001_R1_001.fastq --runThreadN 30

java -jar /home/tobi/phillip/picard.jar AddOrReplaceReadGroups I=Aligned.out.sam
O=rg_added_sorted.bam SO=coordinate RGID=CANE:1 RGLB=HiSeq RGPL=Illumina
RGPU=CANE6ACXX:1 RGSM=Mouse_Tongue_HC920

java -jar /home/tobi/phillip/picard.jar MarkDuplicates I=rg_added_sorted.bam
O=dedupped.bam  CREATE_INDEX=true VALIDATION_STRINGENCY=SILENT
M=output.metrics

java -jar /home/basil/IndiaProject/GenomeAnalysisTK.jar -T SplitNCigarReads -R
/home/cwhite18/RNAseq/mm38.fa -I dedupped.bam -o split.bam -rf
ReassignOneMappingQuality -RMQF 255 -RMQT 60 -U ALLOW_N_CIGAR_READS
```

```
########################################################################
#################
#Sample 6 - Step 1
genomeDir=/home/cwhite18/RNAseq/mm38
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample6/1pass
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample6/HC6_S6_L001_R1_001.fastq --runThreadN 30

genomeDir=/home/cwhite18/RNAseq/Sample6/mm38_2pass
mkdir $genomeDir
cd $genomeDir
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--sjdbFileChrStartEnd /home/cwhite18/RNAseq/Sample6/1pass/SJ.out.tab --
sjdbOverhang 74 --runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample6/2pass
mkdir $runDir
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample6/HC6_S6_L001_R1_001.fastq --runThreadN 30

java -jar /home/tobi/phillip/picard.jar AddOrReplaceReadGroups I=Aligned.out.sam
O=rg_added_sorted.bam SO=coordinate RGID=CANE:1 RGLB=HiSeq RGPL=Illumina
RGPU=CANE6ACXX:1 RGSM=Mouse_Tongue_HC923

java -jar /home/tobi/phillip/picard.jar MarkDuplicates I=rg_added_sorted.bam
O=dedupped.bam  CREATE_INDEX=true VALIDATION_STRINGENCY=SILENT
M=output.metrics

java -jar /home/basil/IndiaProject/GenomeAnalysisTK.jar -T SplitNCigarReads -R
/home/cwhite18/RNAseq/mm38.fa -I dedupped.bam -o split.bam -rf
ReassignOneMappingQuality -RMQF 255 -RMQT 60 -U ALLOW_N_CIGAR_READS
```

```
############################################################################
#################
#Sample 7 - Step 1
genomeDir=/home/cwhite18/RNAseq/mm38
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample7/1pass
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample7/HC7_S7_L001_R1_001.fastq --runThreadN 30

genomeDir=/home/cwhite18/RNAseq/Sample7/mm38_2pass
mkdir $genomeDir
cd $genomeDir
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--sjdbFileChrStartEnd /home/cwhite18/RNAseq/Sample7/1pass/SJ.out.tab --
sjdbOverhang 74 --runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample7/2pass
mkdir $runDir
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample7/HC7_S7_L001_R1_001.fastq --runThreadN 30

java -jar /home/tobi/phillip/picard.jar AddOrReplaceReadGroups I=Aligned.out.sam
O=rg_added_sorted.bam SO=coordinate RGID=CANE:1 RGLB=HiSeq RGPL=Illumina
RGPU=CANE6ACXX:1 RGSM=Mouse_Tongue_HC925

java -jar /home/tobi/phillip/picard.jar MarkDuplicates I=rg_added_sorted.bam
O=dedupped.bam  CREATE_INDEX=true VALIDATION_STRINGENCY=SILENT
M=output.metrics

java -jar /home/basil/IndiaProject/GenomeAnalysisTK.jar -T SplitNCigarReads -R
/home/cwhite18/RNAseq/mm38.fa -I dedupped.bam -o split.bam -rf
ReassignOneMappingQuality -RMQF 255 -RMQT 60 -U ALLOW_N_CIGAR_READS
```

```
################################################################################
#################
#Sample 8 - Step 1
genomeDir=/home/cwhite18/RNAseq/mm38
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample8/1pass
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample8/HC8_S8_L001_R1_001.fastq --runThreadN 30

genomeDir=/home/cwhite18/RNAseq/Sample8/mm38_2pass
mkdir $genomeDir
cd $genomeDir
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--sjdbFileChrStartEnd /home/cwhite18/RNAseq/Sample8/1pass/SJ.out.tab --
sjdbOverhang 74 --runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample8/2pass
mkdir $runDir
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample8/HC8_S8_L001_R1_001.fastq --runThreadN 30

java -jar /home/tobi/phillip/picard.jar AddOrReplaceReadGroups I=Aligned.out.sam
O=rg_added_sorted.bam SO=coordinate RGID=CANE:1 RGLB=HiSeq RGPL=Illumina
RGPU=CANE6ACXX:1 RGSM=Mouse_Tongue_HC926

java -jar /home/tobi/phillip/picard.jar MarkDuplicates I=rg_added_sorted.bam
O=dedupped.bam  CREATE_INDEX=true VALIDATION_STRINGENCY=SILENT
M=output.metrics
```

```
java -jar /home/basil/IndiaProject/GenomeAnalysisTK.jar -T SplitNCigarReads -R
/home/cwhite18/RNAseq/mm38.fa -I dedupped.bam -o split.bam -rf
ReassignOneMappingQuality -RMQF 255 -RMQT 60 -U ALLOW_N_CIGAR_READS


########################################################################
#################
#Sample 9 - Step 1
genomeDir=/home/cwhite18/RNAseq/mm38
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample9/1pass
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample9/HC9_S9_L002_R1_001.fastq --runThreadN 30

genomeDir=/home/cwhite18/RNAseq/Sample9/mm38_2pass
mkdir $genomeDir
cd $genomeDir
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--sjdbFileChrStartEnd /home/cwhite18/RNAseq/Sample9/1pass/SJ.out.tab --
sjdbOverhang 74 --runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample9/2pass
mkdir $runDir
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample9/HC9_S9_L002_R1_001.fastq --runThreadN 30

java -jar /home/tobi/phillip/picard.jar AddOrReplaceReadGroups I=Aligned.out.sam
O=rg_added_sorted.bam SO=coordinate RGID=CANE:1 RGLB=HiSeq RGPL=Illumina
RGPU=CANE6ACXX:1 RGSM=Mouse_Tongue_HC927

java -jar /home/tobi/phillip/picard.jar MarkDuplicates I=rg_added_sorted.bam
O=dedupped.bam  CREATE_INDEX=true VALIDATION_STRINGENCY=SILENT
M=output.metrics
```

```
java -jar /home/basil/IndiaProject/GenomeAnalysisTK.jar -T SplitNCigarReads -R
/home/cwhite18/RNAseq/mm38.fa -I dedupped.bam -o split.bam -rf
ReassignOneMappingQuality -RMQF 255 -RMQT 60 -U ALLOW_N_CIGAR_READS


##########################################################################
#################
#Sample 10 - Step 1
genomeDir=/home/cwhite18/RNAseq/mm38
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample10/1pass
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample10/HC10_S10_L002_R1_001.fastq --runThreadN 30

genomeDir=/home/cwhite18/RNAseq/Sample10/mm38_2pass
mkdir $genomeDir
cd $genomeDir
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--sjdbFileChrStartEnd /home/cwhite18/RNAseq/Sample10/1pass/SJ.out.tab --
sjdbOverhang 74 --runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample10/2pass
mkdir $runDir
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample10/HC10_S10_L002_R1_001.fastq --runThreadN 30

java -jar /home/tobi/phillip/picard.jar AddOrReplaceReadGroups I=Aligned.out.sam
O=rg_added_sorted.bam SO=coordinate RGID=CANE:1 RGLB=HiSeq RGPL=Illumina
RGPU=CANE6ACXX:1 RGSM=Mouse_Tongue_HC928
```

```
java -jar /home/tobi/phillip/picard.jar MarkDuplicates I=rg_added_sorted.bam
O=dedupped.bam  CREATE_INDEX=true VALIDATION_STRINGENCY=SILENT
M=output.metrics

java -jar /home/basil/IndiaProject/GenomeAnalysisTK.jar -T SplitNCigarReads -R
/home/cwhite18/RNAseq/mm38.fa -I dedupped.bam -o split.bam -rf
ReassignOneMappingQuality -RMQF 255 -RMQT 60 -U ALLOW_N_CIGAR_READS

########################################################################
#################
#Sample 11 - Step 1
genomeDir=/home/cwhite18/RNAseq/mm38
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample11/1pass
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample11/HC11_S11_L002_R1_001.fastq --runThreadN 30

genomeDir=/home/cwhite18/RNAseq/Sample11/mm38_2pass
mkdir $genomeDir
cd $genomeDir
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--sjdbFileChrStartEnd /home/cwhite18/RNAseq/Sample11/1pass/SJ.out.tab --
sjdbOverhang 74 --runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample11/2pass
mkdir $runDir
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample11/HC11_S11_L002_R1_001.fastq --runThreadN 30

java -jar /home/tobi/phillip/picard.jar AddOrReplaceReadGroups I=Aligned.out.sam
O=rg_added_sorted.bam SO=coordinate RGID=CANE:1 RGLB=HiSeq RGPL=Illumina
RGPU=CANE6ACXX:1 RGSM=Mouse_Tongue_HC930
```

```
java -jar /home/tobi/phillip/picard.jar MarkDuplicates I=rg_added_sorted.bam
O=dedupped.bam  CREATE_INDEX=true VALIDATION_STRINGENCY=SILENT
M=output.metrics

java -jar /home/basil/IndiaProject/GenomeAnalysisTK.jar -T SplitNCigarReads -R
/home/cwhite18/RNAseq/mm38.fa -I dedupped.bam -o split.bam -rf
ReassignOneMappingQuality -RMQF 255 -RMQT 60 -U ALLOW_N_CIGAR_READS

#########################################################################
#################
#Sample 12 - Step 1
genomeDir=/home/cwhite18/RNAseq/mm38
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample12/1pass
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample12/HC12_S12_L002_R1_001.fastq --runThreadN 30

genomeDir=/home/cwhite18/RNAseq/Sample12/mm38_2pass
mkdir $genomeDir
cd $genomeDir
STAR --runMode genomeGenerate --genomeDir $genomeDir --genomeFastaFiles
/home/cwhite18/RNAseq/mm38.fa \
--sjdbFileChrStartEnd /home/cwhite18/RNAseq/Sample12/1pass/SJ.out.tab --
sjdbOverhang 74 --runThreadN 30

runDir=/home/cwhite18/RNAseq/Sample12/2pass
mkdir $runDir
cd $runDir
STAR --genomeDir $genomeDir --readFilesIn
/home/cwhite18/RNAseq/Sample12/HC12_S12_L002_R1_001.fastq --runThreadN 30

java -jar /home/tobi/phillip/picard.jar AddOrReplaceReadGroups I=Aligned.out.sam
O=rg_added_sorted.bam SO=coordinate RGID=CANE:1 RGLB=HiSeq RGPL=Illumina
RGPU=CANE6ACXX:1 RGSM=Mouse_Tongue_HC932
```

```
java -jar /home/tobi/phillip/picard.jar MarkDuplicates I=rg_added_sorted.bam
O=dedupped.bam  CREATE_INDEX=true VALIDATION_STRINGENCY=SILENT
M=output.metrics

java -jar /home/basil/IndiaProject/GenomeAnalysisTK.jar -T SplitNCigarReads -R
/home/cwhite18/RNAseq/mm38.fa -I dedupped.bam -o split.bam -rf
ReassignOneMappingQuality -RMQF 255 -RMQT 60 -U ALLOW_N_CIGAR_READS

##########################################################################
#################
#Move all resulting bam files to the same directory/rename from split.bam to
sample_name_split.bam, move and rename 1 index file

mkdir /home/cwhite18/RNAseq/BamFiles

cp /home/cwhite18/RNAseq/Sample1/2pass/split.bam
/home/cwhite18/RNAseq/BamFiles/
mv /home/cwhite18/RNAseq/BamFiles/split.bam
/home/cwhite18/RNAseq/BamFiles/HC1_split.bam
cp /home/cwhite18/RNAseq/Sample2/2pass/split.bam
/home/cwhite18/RNAseq/BamFiles/
mv /home/cwhite18/RNAseq/BamFiles/split.bam
/home/cwhite18/RNAseq/BamFiles/HC2_split.bam
cp /home/cwhite18/RNAseq/Sample3/2pass/split.bam
/home/cwhite18/RNAseq/BamFiles/
mv /home/cwhite18/RNAseq/BamFiles/split.bam
/home/cwhite18/RNAseq/BamFiles/HC3_split.bam
cp /home/cwhite18/RNAseq/Sample4/2pass/split.bam
/home/cwhite18/RNAseq/BamFiles/
mv /home/cwhite18/RNAseq/BamFiles/split.bam
/home/cwhite18/RNAseq/BamFiles/HC4_split.bam
cp /home/cwhite18/RNAseq/Sample5/2pass/split.bam
/home/cwhite18/RNAseq/BamFiles/
mv /home/cwhite18/RNAseq/BamFiles/split.bam
/home/cwhite18/RNAseq/BamFiles/HC5_split.bam
cp /home/cwhite18/RNAseq/Sample6/2pass/split.bam
/home/cwhite18/RNAseq/BamFiles/
mv /home/cwhite18/RNAseq/BamFiles/split.bam
/home/cwhite18/RNAseq/BamFiles/HC6_split.bam
```

```
cp /home/cwhite18/RNAseq/Sample7/2pass/split.bam
/home/cwhite18/RNAseq/BamFiles/
mv /home/cwhite18/RNAseq/BamFiles/split.bam
/home/cwhite18/RNAseq/BamFiles/HC7_split.bam
cp /home/cwhite18/RNAseq/Sample8/2pass/split.bam
/home/cwhite18/RNAseq/BamFiles/
mv /home/cwhite18/RNAseq/BamFiles/split.bam
/home/cwhite18/RNAseq/BamFiles/HC8_split.bam
cp /home/cwhite18/RNAseq/Sample9/2pass/split.bam
/home/cwhite18/RNAseq/BamFiles/
mv /home/cwhite18/RNAseq/BamFiles/split.bam
/home/cwhite18/RNAseq/BamFiles/HC9_split.bam
cp /home/cwhite18/RNAseq/Sample10/2pass/split.bam
/home/cwhite18/RNAseq/BamFiles/
mv /home/cwhite18/RNAseq/BamFiles/split.bam
/home/cwhite18/RNAseq/BamFiles/HC10_split.bam
cp /home/cwhite18/RNAseq/Sample11/2pass/split.bam
/home/cwhite18/RNAseq/BamFiles/
mv /home/cwhite18/RNAseq/BamFiles/split.bam
/home/cwhite18/RNAseq/BamFiles/HC11_split.bam
cp /home/cwhite18/RNAseq/Sample12/2pass/split.bam
/home/cwhite18/RNAseq/BamFiles/
mv /home/cwhite18/RNAseq/BamFiles/split.bam
/home/cwhite18/RNAseq/BamFiles/HC12_split.bam
cp /home/cwhite18/RNAseq/Sample1/2pass/split.bai /home/cwhite18/RNAseq/BamFiles/
mv /home/cwhite18/RNAseq/BamFiles/split.bai
/home/cwhite18/RNAseq/BamFiles/HC1_split.bai

#decompress mouse gene annotation file
gunzip Mus_musculus.GRCm38.92.gtf.gz

##########################################################################
#################
#Beginning of RNAseq Expression Analysis using R
R

setwd("/home/cwhite18/RNAseq")
dir <- "/home/cwhite18/RNAseq/BamFiles"
```

```
source("http://bioconductor.org/biocLite.R")
biocLite(c('airway','Rsamtools','GenomicFeatures','GenomicAlignments','DESeq2','rtrackl
ayer',   'pheatmap','BSgenome.Hsapiens.UCSC.hg19','org.Hs.eg.db','AnnotationDbi'))

list.files(dir)

csvfile <- file.path(dir,"sample_table.csv")
(sampleTable <- read.csv(csvfile,row.names=1))

filenames <- file.path(dir, paste0(sampleTable$Run, "_split.bam"))

library("Rsamtools")
bamfiles <- BamFileList(filenames, yieldSize=2000000)

seqinfo(bamfiles[1])

library("GenomicFeatures")
gtffile <- file.path(dir,"Mus_musculus.GRCm38.92.gtf")
(txdb <- makeTxDbFromGFF(gtffile, format="gtf"))

(exonsByGene <- exonsBy(txdb, by="gene"))

library("GenomicAlignments")
library("BiocParallel")
register(SerialParam())

se <- summarizeOverlaps(features=exonsByGene, reads=bamfiles,
                    mode="Union",
                    singleEnd=FALSE,
                    ignore.strand=TRUE,
                    fragments=TRUE )

head(assay(se))
colSums(assay(se))

(colData(se) <- DataFrame(sampleTable))

round( colSums(assay(se)) / 1e6, 1 )
colData(se)
```

```
write.csv (assay(se), file = "Cole.csv")

##############################################################################
#################
#Comparison of Experimental Groups using R/Calculation of differential expression

setwd("C:\\Users\\Cole\\Desktop\\Thesis")
library(edgeR)
library(limma)
library(Glimma)
library(gplots)
library(org.Mm.eg.db)
library(RColorBrewer)

targets <- read.table("cw3.txt", stringsAsFactors= FALSE)
targets

group <- paste(targets$Genotype, targets$Status, sep=".")
group <- factor(group)
table(group)


d1 <- read.csv("Cole.csv")
GenewiseCounts = d1[,-1]
row.names(GenewiseCounts) = d1[,1]
dim(GenewiseCounts)

head(GenewiseCounts)

convertIDs <- function( ids, from, to, db, ifMultiple=c("putNA", "useFirst")) {
  stopifnot( inherits( db, "AnnotationDb" ) )
  ifMultiple <- match.arg( ifMultiple )
  suppressWarnings( selRes <- AnnotationDbi::select(
    db, keys=ids, keytype=from, columns=c(from,to) ) )
  if ( ifMultiple == "putNA" ) {
    duplicatedIds <- selRes[ duplicated( selRes[,1] ), 1 ]
    selRes <- selRes[ ! selRes[,1] %in% duplicatedIds, ]
  }
```

```
  return( selRes[ match( ids, selRes[,1] ), 2 ] )
}

y2 =convertIDs(row.names(GenewiseCounts), "ENSEMBL", "SYMBOL",
org.Mm.eg.db)

y <- DGEList(GenewiseCounts, group=group,
                genes=y2)

head(y$genes)

dim(y)

y <- calcNormFactors(y)
options(digits=3)
y$samples

plotMD(y, column=1)
abline(h=0, col="red", lty=2, lwd=2)

pch <- c(0,1,2,15,16,17)
colors <- rep(c("darkgreen", "red", "blue"), 2)
plotMDS(y, col=colors[group], pch=pch[group])
legend("topleft", legend=levels(group), pch=pch, col=colors, ncol=2)

design <- model.matrix(~0+group)
colnames(design) <- levels(group)
design

library(statmod)

y <- estimateDisp(y, design, robust=TRUE)

plotBCV(y)

fit <- glmQLFit(y, design, robust=TRUE)
head(fit$coefficients)

plotQLDisp(fit)
```

```
summary(fit$df.prior)

#################### Home.infected vs Hom.sham

HI.HS <- makeContrasts(Hom.Infected - Hom.Sham, levels=design)

res1 <- glmQLFTest(fit, contrast=HI.HS)

topTags(res1)

is.de1 <- decideTestsDGE(res1)
summary(is.de1)

plotMD(res1, status=is.de1, values=c(1,-1), col=c("red","blue"),
       legend="topright")

#Differential expression above a fold-change threshold
tr1 <- glmTreat(fit, contrast=HI.HS, lfc=log2(1.5))
topTags(tr1)

is.de1 <- decideTestsDGE(tr1)
summary(is.de1)

plotMD(tr1, status=is.de1, values=c(1,-1), col=c("red","blue"),
       legend="topright")

logCPM <- cpm(y, prior.count=2, log=TRUE)
rownames(logCPM) <- y$genes$Symbol
colnames(logCPM) <- paste(y$samples$group, 1:2, sep="-")

o <- order(tr1$table$PValue)
logCPM <- logCPM[o[1:30],]

logCPM <- t(scale(t(logCPM)))

library(gplots)
col.pan <- colorpanel(100, "blue", "white", "red")
heatmap.2(logCPM, col=col.pan, Rowv=TRUE, scale="none",
```

```
    trace="none", dendrogram="both", cexRow=1, cexCol=1.4,
    margin=c(10,9), lhei=c(2,10), lwid=c(2,6))

tab1 <- topTags(tr1, n=Inf)
write.csv(tab1, file = "Hom.Infected_vs_Hom.Sham.csv")

################### Hom.infected vs WT.infected

HI.WI <- makeContrasts(Hom.Infected - WT.Infected, levels=design)

res2 <- glmQLFTest(fit, contrast=HI.WI)

topTags(res2)

is.de2 <- decideTestsDGE(res2)
summary(is.de2)

plotMD(res2, status=is.de2, values=c(1,-1), col=c("red","blue"),
        legend="topright")

#Differential expression above a fold-change threshold
tr2 <- glmTreat(fit, contrast=HI.WI, lfc=log2(1.5))
topTags(tr2)

is.de2 <- decideTestsDGE(tr2)
summary(is.de2)

plotMD(tr2, status=is.de2, values=c(1,-1), col=c("red","blue"),
        legend="topright")

logCPM <- cpm(y, prior.count=2, log=TRUE)
rownames(logCPM) <- y$genes$Symbol
colnames(logCPM) <- paste(y$samples$group, 1:2, sep="-")

o <- order(tr1$table$PValue)
logCPM <- logCPM[o[1:30],]

logCPM <- t(scale(t(logCPM)))
```

```
library(gplots)
col.pan <- colorpanel(100, "blue", "white", "red")
heatmap.2(logCPM, col=col.pan, Rowv=TRUE, scale="none",
    trace="none", dendrogram="both", cexRow=1, cexCol=1.4,
    margin=c(10,9), lhei=c(2,10), lwid=c(2,6))

tab2 <- topTags(tr2, n=Inf)
write.csv(tab2, file = "Hom.Infected_vs_WT.Infected.csv")

#################### Hom.infected vs WT.sham

HI.WS <- makeContrasts(Hom.Infected - WT.Sham, levels=design)

res3 <- glmQLFTest(fit, contrast=HI.WS)

topTags(res3)

is.de3 <- decideTestsDGE(res3)
summary(is.de3)

plotMD(res3, status=is.de3, values=c(1,-1), col=c("red","blue"),
        legend="topright")

#Differential expression above a fold-change threshold
tr3 <- glmTreat(fit, contrast=HI.WS, lfc=log2(1.5))
topTags(tr3)

is.de3 <- decideTestsDGE(tr3)
summary(is.de3)

plotMD(tr3, status=is.de3, values=c(1,-1), col=c("red","blue"),
        legend="topright")

logCPM <- cpm(y, prior.count=2, log=TRUE)
rownames(logCPM) <- y$genes$Symbol
colnames(logCPM) <- paste(y$samples$group, 1:2, sep="-")

o <- order(tr1$table$PValue)
logCPM <- logCPM[o[1:30],]
```

```
logCPM <- t(scale(t(logCPM)))

library(gplots)
col.pan <- colorpanel(100, "blue", "white", "red")
heatmap.2(logCPM, col=col.pan, Rowv=TRUE, scale="none",
    trace="none", dendrogram="both", cexRow=1, cexCol=1.4,
    margin=c(10,9), lhei=c(2,10), lwid=c(2,6))

tab3 <- topTags(tr3, n=Inf)
write.csv(tab3, file = "Hom.Infected_vs_WT.Sham.csv")

#################### Hom.sham vs WT.infected

HS.WI <- makeContrasts(Hom.Sham - WT.Infected, levels=design)

res4 <- glmQLFTest(fit, contrast=HS.WI)

topTags(res4)

is.de4 <- decideTestsDGE(res4)
summary(is.de4)

plotMD(res4, status=is.de4, values=c(1,-1), col=c("red","blue"),
        legend="topright")

#Differential expression above a fold-change threshold
tr4 <- glmTreat(fit, contrast=HS.WI, lfc=log2(1.5))
topTags(tr4)

is.de4 <- decideTestsDGE(tr4)
summary(is.de4)

plotMD(tr4, status=is.de4, values=c(1,-1), col=c("red","blue"),
        legend="topright")

logCPM <- cpm(y, prior.count=2, log=TRUE)
rownames(logCPM) <- y$genes$Symbol
colnames(logCPM) <- paste(y$samples$group, 1:2, sep="-")
```

```
o <- order(tr1$table$PValue)
logCPM <- logCPM[o[1:30],]

logCPM <- t(scale(t(logCPM)))

library(gplots)
col.pan <- colorpanel(100, "blue", "white", "red")
heatmap.2(logCPM, col=col.pan, Rowv=TRUE, scale="none",
    trace="none", dendrogram="both", cexRow=1, cexCol=1.4,
    margin=c(10,9), lhei=c(2,10), lwid=c(2,6))

tab4 <- topTags(tr4, n=Inf)
write.csv(tab4, file = "Hom.Sham_vs_WT.Infected.csv")

#################### Hom.sham vs WT.sham

HS.WS <- makeContrasts(Hom.Sham - WT.Sham, levels=design)

res5 <- glmQLFTest(fit, contrast=HS.WS)

topTags(res5)

is.de5 <- decideTestsDGE(res5)
summary(is.de5)

plotMD(res5, status=is.de5, values=c(1,-1), col=c("red","blue"),
        legend="topright")

#Differential expression above a fold-change threshold
tr5 <- glmTreat(fit, contrast=HS.WS, lfc=log2(1.5))
topTags(tr5)

is.de5 <- decideTestsDGE(tr5)
summary(is.de5)

plotMD(tr5, status=is.de5, values=c(1,-1), col=c("red","blue"),
        legend="topright")
```

```
logCPM <- cpm(y, prior.count=2, log=TRUE)
rownames(logCPM) <- y$genes$Symbol
colnames(logCPM) <- paste(y$samples$group, 1:2, sep="-")

o <- order(tr1$table$PValue)
logCPM <- logCPM[o[1:30],]

logCPM <- t(scale(t(logCPM)))

library(gplots)
col.pan <- colorpanel(100, "blue", "white", "red")
heatmap.2(logCPM, col=col.pan, Rowv=TRUE, scale="none",
    trace="none", dendrogram="both", cexRow=1, cexCol=1.4,
    margin=c(10,9), lhei=c(2,10), lwid=c(2,6))

tab5 <- topTags(tr5, n=Inf)
write.csv(tab5, file = "Hom.Sham_vs_WT.Sham.csv")

#################### WT.infected vs WT.sham

WI.WS <- makeContrasts(WT.Infected - WT.Sham, levels=design)

res6 <- glmQLFTest(fit, contrast=WI.WS)

topTags(res6)

is.de6 <- decideTestsDGE(res6)
summary(is.de6)

plotMD(res6, status=is.de6, values=c(1,-1), col=c("red","blue"),
        legend="topright")

#Differential expression above a fold-change threshold
tr6 <- glmTreat(fit, contrast=WI.WS, lfc=log2(1.5))
topTags(tr6)

is.de6 <- decideTestsDGE(tr6)
summary(is.de6)
```

```
plotMD(tr6, status=is.de6, values=c(1,-1), col=c("red","blue"),
        legend="topright")

logCPM <- cpm(y, prior.count=2, log=TRUE)
rownames(logCPM) <- y$genes$Symbol
colnames(logCPM) <- paste(y$samples$group, 1:2, sep="-")

o <- order(tr1$table$PValue)
logCPM <- logCPM[o[1:30],]

logCPM <- t(scale(t(logCPM)))

library(gplots)
col.pan <- colorpanel(100, "blue", "white", "red")
heatmap.2(logCPM, col=col.pan, Rowv=TRUE, scale="none",
    trace="none", dendrogram="both", cexRow=1, cexCol=1.4,
    margin=c(10,9), lhei=c(2,10), lwid=c(2,6))

tab6 <- topTags(tr6, n=Inf)
write.csv(tab6, file = "WT.Infected_vs_WT.Sham.csv")
```

Appendix B

Appendix Tables

Wild-Type Infected versus Wild-Type Sham

Genes	logFC	P-Value	FDR
Saa3	11.39303	2.28E-06	0.000768
Slc26a4	10.72678	7.04E-05	0.006962
Gm5483	10.72537	1.34E-05	0.002231
Il19	10.62021	6.72E-06	0.001419
AC110211.1	10.29827	6.77E-06	0.001419
Fpr1	10.1362	0.000333	0.021121
Cxcl5	9.8357	3.08E-07	0.000197
Cxcl2	9.71163	1.86E-05	0.00271
Trem1	9.629253	2.98E-06	0.000882
Cxcl3	9.606077	6.67E-06	0.001419
Il17a	9.307849	3.42E-06	0.000949
Fpr2	9.200458	1.62E-06	0.000596
Retnlg	9.14515	1.27E-05	0.002154
Il1b	8.941617	8.39E-06	0.001601
Cd300lf	8.869469	2.75E-08	3.44E-05
Gm9733	8.756683	2.85E-07	0.000187
Csf3	8.606316	1.72E-05	0.002545
Cd177	8.562038	2.93E-05	0.003664
Fgf23	8.428521	0.000564	0.030468
Osm	8.358338	1.72E-06	0.000624
Il12b	8.320581	8.26E-05	0.007733
Acod1	8.280447	4.40E-05	0.004878
Cxcl1	8.174724	2.70E-05	0.00342
Il22	8.161523	1.75E-07	0.00013

Gm14010	8.124756	1.29E-07	0.000101
Ccl20	8.107351	0.000993	0.04578
Saa2	8.066387	2.53E-06	0.000786
Olfm4	7.953813	0.000167	0.012722
Clec4d	7.939478	1.35E-05	0.002234
Uox	7.914814	0.00041	0.024837
Gm16556	7.875215	6.59E-07	0.000334
Il20	7.61286	6.52E-06	0.001419
Clec4e	7.546167	4.25E-06	0.001089
Nlrp12	7.503965	6.11E-07	0.000324
Ly6g	7.469695	2.50E-05	0.003256
Cxcr2	7.436262	6.65E-06	0.001419
Trem3	7.397419	2.07E-06	0.000713
LOC100038947	7.270487	6.04E-06	0.001353
Csf3r	7.252168	5.25E-06	0.001238
Tarm1	7.201193	6.23E-12	1.43E-07
Mefv	7.187343	1.72E-07	0.00013
Ccl4	7.145361	9.12E-05	0.008296
Fcgr4	7.101728	1.18E-08	1.87E-05
Gm39321	7.085192	4.37E-05	0.004878
Gm30873	7.034464	4.17E-05	0.004736
Gm5150	6.98023	4.50E-07	0.00026
Mrgpra2b	6.885724	0.00017	0.012916
Mcemp1	6.854824	1.46E-05	0.002325
Siglecf	6.837425	1.52E-05	0.002391
Cxcr1	6.788825	0.00011	0.009403
Il6	6.763108	7.53E-05	0.00726
Gm44428	6.753539	0.000239	0.016646
Sell	6.693503	8.40E-07	0.000386
Rrad	6.659048	1.92E-07	0.000137
Trpm2	6.639622	3.19E-06	0.000912
Slfn4	6.592387	3.44E-06	0.000949
Wfdc21	6.54293	0.00048	0.027349
Sh2d5	6.533468	0.000193	0.014277
Slfn1	6.519998	7.25E-09	1.44E-05
Csn1s1	6.464894	0.000199	0.014571
Hsh2d	6.447707	0.000105	0.009134
Il17f	6.436547	7.41E-05	0.007231
Gm5547	6.383853	4.30E-05	0.004834
Ccl3	6.338256	0.00015	0.011962

Ccl7	6.308331	0.000126	0.010531
Rnase13	6.30025	0.000293	0.019356
Lcn2	6.220745	2.28E-05	0.003119
Chil3	6.213794	0.000157	0.012222
Gm20744	6.213794	0.000244	0.016877
Sirpb1a	6.188323	4.18E-06	0.001075
Gm26877	6.167526	0.000164	0.012571
Spatc1	6.148476	0.000912	0.04296
Timp1	6.141493	6.75E-06	0.001419
Nlrp3	6.108607	1.17E-05	0.002048
Gm2511	6.103603	0.000345	0.021684
Csf2	6.102708	0.000203	0.014747
Trim30b	5.988455	1.80E-07	0.000131
Ambp	5.964374	0.000488	0.027668
6430710C18Rik	5.959414	8.70E-05	0.00804
Gfap	5.957624	0.000163	0.012553
Mmp3	5.957485	0.000603	0.032275
B430306N03Rik	5.947428	7.21E-11	4.85E-07
1200007C13Rik	5.896757	1.71E-05	0.00253
AC133156.2	5.85756	0.000311	0.020239
B230303A05Rik	5.835105	5.06E-07	0.000284
Tnfsf14	5.822948	1.08E-09	4.62E-06
9830107B12Rik	5.787958	4.99E-06	0.001209
Ceacam18	5.747481	0.001055	0.048116
Ccr3	5.73225	0.000378	0.023206
Hdc	5.632951	2.48E-05	0.003256
Rtn4rl2	5.613289	1.07E-07	9.01E-05
Gm42676	5.60749	0.000274	0.018337
Il17c	5.604826	0.000876	0.042087
Cd14	5.561121	2.48E-05	0.003256
Mmp8	5.552605	7.17E-07	0.000357
Cyp4f18	5.531854	0.000106	0.009222
AA467197	5.506367	8.39E-12	1.43E-07
CR974586.5	5.493869	6.30E-07	0.000326
CT009530.1	5.475108	1.36E-08	2.00E-05
Gm16556	5.451586	7.44E-07	0.000357
A130071D04Rik	5.439948	3.31E-07	0.000205
AC139671.1	5.43855	3.27E-06	0.000931
Ccr1	5.393471	7.27E-06	0.001474
Gad1-ps	5.369834	1.19E-06	0.000484

Plet1	5.34512	0.000295	0.01944
Bpifb5	5.338488	0.000169	0.01279
Adamts4	5.329325	4.39E-06	0.001097
Nppb	5.288327	0.000648	0.033895
Mcoln2	5.278228	5.54E-08	5.42E-05
Clec4n	5.260439	4.35E-06	0.001095
H2-Q10	5.243591	0.000246	0.016973
Gm17980	5.233019	0.000866	0.041718
Gm36259	5.227593	0.000878	0.042087
Mir142b	5.224055	3.62E-06	0.000969
Tmprss11g	5.222136	7.05E-06	0.001453
I830127L07Rik	5.221728	1.87E-05	0.002714
Cdh16	5.174975	7.61E-10	3.72E-06
Spdef	5.133452	0.000817	0.040269
Samd7	5.111487	1.07E-07	9.01E-05
Sirpb1b	5.081931	3.73E-07	0.000226
Lilr4b	5.069253	6.15E-06	0.001368
Ptgs2	5.045688	0.000165	0.01263
C530050E15Rik	5.043506	2.39E-08	3.29E-05
Ccno	5.038612	8.25E-07	0.000383
Saa1	5.030269	0.000559	0.030288
Plac8	5.027784	5.91E-06	0.001336
Ankk1	4.985134	2.40E-09	7.59E-06
Mgam	4.97128	2.46E-05	0.003256
Saa4	4.9293	5.40E-06	0.001252
Gm35279	4.927155	0.000518	0.028828
Gm48671	4.923573	8.14E-05	0.007683
Ccl2	4.905066	2.39E-05	0.003212
Urah	4.880594	2.36E-05	0.003177
Ms4a4c	4.866442	5.43E-08	5.41E-05
Gm17619	4.857362	7.30E-05	0.00714
Klra17	4.856382	3.35E-06	0.000945
Samsn1	4.854544	4.29E-06	0.001093
Mir142hg	4.823649	1.49E-06	0.00056
Olr1	4.820858	1.43E-05	0.002286
Ifitm1	4.767876	3.41E-05	0.004081
BC048679	4.724001	0.000424	0.025287
Il23a	4.705179	0.001102	0.049786
Gm48786	4.694084	2.99E-06	0.000882
Treml2	4.692154	2.69E-09	8.05E-06

Wfdc17	4.690702	5.13E-05	0.005509
A530040E14Rik	4.687007	0.000166	0.012675
Klra2	4.684785	6.05E-08	5.71E-05
Cd300ld	4.66939	6.46E-05	0.006546
Fgr	4.654105	5.40E-06	0.001252
Lilrb4	4.645758	1.24E-06	0.000493
AC102815.1	4.631099	0.000356	0.022255
Nfe2	4.623357	5.45E-05	0.005756
Ptx3	4.607018	0.000515	0.028749
Ccl12	4.602554	0.000559	0.030288
Plaur	4.555571	4.88E-06	0.001192
Angptl4	4.549125	0.000654	0.034115
Clec7a	4.486443	1.18E-07	9.61E-05
Gm36546	4.443754	6.15E-07	0.000324
F10	4.440371	2.19E-09	7.37E-06
Adm2	4.428812	4.23E-07	0.000247
Sycp2	4.422094	3.04E-05	0.003755
Gm6524	4.380861	6.65E-06	0.001419
Ifitm6	4.374256	3.89E-06	0.001019
Gm21859	4.370304	1.81E-05	0.002658
Pilra	4.347231	5.56E-07	0.000299
Slc51b	4.338156	0.000154	0.012096
Treml4	4.33694	9.87E-05	0.008762
Gzmb	4.326699	6.34E-05	0.00647
Gm46224	4.324971	2.63E-05	0.003368
Il18rap	4.317196	4.36E-05	0.004878
A530064D06Rik	4.301182	1.55E-05	0.002404
Thbs1	4.269114	9.83E-05	0.008739
Gm13889	4.260761	2.31E-05	0.003133
Slc6a14	4.257711	5.00E-05	0.0054
4930546K05Rik	4.238159	8.93E-05	0.008202
Cd244	4.214406	2.44E-06	0.000783
Tlr13	4.208492	8.13E-05	0.007683
Plek	4.185805	2.42E-05	0.003237
Gm13567	4.170531	0.000154	0.012096
4833407H14Rik	4.140898	9.92E-06	0.001785
Gm16194	4.133294	0.000412	0.024905
Socs3	4.115883	3.98E-08	4.62E-05
Padi4	4.108145	4.04E-08	4.62E-05
Gm14548	4.097705	1.08E-08	1.82E-05

Ipcef1	4.092424	0.000101	0.008921
Ccl19-ps1	4.085098	1.53E-05	0.002398
AC113595.1	4.07145	8.49E-09	1.52E-05
Adam8	4.070637	6.81E-06	0.00142
Ankrd66	4.064877	1.21E-05	0.002107
Itgal	4.063249	2.72E-06	0.000827
Aldh1a3	4.061593	0.000377	0.023187
Selp	4.057302	1.18E-07	9.61E-05
Tmem156	4.053916	7.37E-07	0.000357
Fcgr1	4.042608	3.30E-09	8.17E-06
E230032D23Rik	4.022054	0.000422	0.025287
Siglece	4.016306	1.56E-09	5.61E-06
Il21r	4.007793	4.33E-08	4.75E-05
Mirt1	3.95899	3.58E-07	0.000219
Gm16175	3.9588	0.000462	0.026817
Slc15a3	3.949145	1.46E-06	0.00056
Slc5a8	3.93641	1.48E-05	0.00235
Bcl3	3.935889	2.39E-06	0.000779
Mab21l3	3.923347	0.000372	0.022948
Inhbb	3.91787	4.72E-05	0.00519
Ms4a6d	3.915945	3.47E-06	0.000953
BC023105	3.884213	5.63E-05	0.005896
Fcrlb	3.878488	0.000894	0.042651
Gm38275	3.869317	0.00057	0.030739
5930438M14Rik	3.867662	0.000912	0.04296
Ltb	3.863877	7.03E-05	0.006962
Alox8	3.863139	8.87E-05	0.008153
Nlrp1a	3.853634	0.001001	0.046061
Mmp9	3.853618	9.08E-05	0.008296
1600010M07Rik	3.837769	2.63E-06	0.000809
Upp1	3.834916	0.000491	0.027763
Slc25a48	3.811989	0.000227	0.016069
Smim3	3.802416	6.70E-06	0.001419
Prss22	3.798348	0.000126	0.010547
Tfec	3.796878	8.11E-07	0.000383
A630023A22Rik	3.791538	0.000636	0.033507
Ccl19	3.762804	0.000643	0.033689
Stat4	3.761982	2.53E-06	0.000786
8430408G22Rik	3.755395	3.76E-05	0.004393
Nfkbid	3.753245	2.22E-05	0.003079

Ncf4	3.750509	3.60E-06	0.000969
C5ar1	3.73092	2.91E-05	0.003651
Cebpd	3.721497	1.22E-07	9.82E-05
Mybpc3	3.704477	0.00037	0.022888
A530032D15Rik	3.693865	0.001069	0.048556
Rnd1	3.680832	0.000168	0.012753
Snx20	3.673776	1.88E-06	0.000662
Msr1	3.666344	6.03E-05	0.006186
Il4ra	3.661758	1.40E-05	0.002271
Gm14767	3.661691	0.000435	0.025664
Rnf183	3.654978	1.29E-05	0.002166
Tnfrsf9	3.654236	4.73E-06	0.001163
Tlr1	3.645669	1.20E-06	0.000484
Ncf2	3.605134	1.17E-05	0.002048
Pdk4	3.591645	0.000434	0.025657
Il1rn	3.58384	0.000324	0.020729
Srgn	3.581812	5.16E-05	0.005527
4833421G17Rik	3.563698	0.000195	0.014389
Serpina3i	3.557296	1.41E-05	0.002271
Gm37648	3.551836	0.00018	0.013618
Hbegf	3.532835	7.01E-05	0.006962
Halr1	3.526805	1.02E-06	0.000454
4732490B19Rik	3.518606	0.000441	0.02591
Styk1	3.515078	1.22E-05	0.002117
Gm26857	3.480746	1.09E-06	0.000471
F630028O10Rik	3.480738	0.000156	0.012199
Gm10388	3.467995	5.69E-05	0.005948
Rab20	3.455742	1.12E-09	4.62E-06
Lilra6	3.449318	3.83E-06	0.001011
Amer2	3.441314	0.000681	0.035092
1700025N21Rik	3.426915	1.24E-05	0.002129
Plscr1	3.413761	3.03E-12	1.43E-07
Socs1	3.405286	1.02E-05	0.001828
Slc2a6	3.39415	3.08E-05	0.003789
Cntn2	3.39061	1.58E-05	0.002435
Itih4	3.387973	2.31E-07	0.000157
Cyp7b1	3.385524	3.55E-05	0.004202
Lrrc25	3.377889	1.57E-05	0.002426
Rasd1	3.376468	3.16E-06	0.000908
Trim30c	3.371666	0.000228	0.016075

Gm44369	3.371129	7.48E-05	0.007254
Dnase2b	3.369564	0.00025	0.017164
Hck	3.360782	9.05E-07	0.000409
LOC102639543	3.359131	0.000366	0.022702
Krt90	3.358559	9.17E-06	0.001697
Artn	3.350404	1.96E-05	0.002807
Tnfsf11	3.343952	0.000384	0.023495
Cd80	3.343027	5.40E-07	0.000296
Bcl2a1a	3.341333	0.000638	0.033535
Gm11346	3.333526	7.93E-06	0.001569
Gm45479	3.321435	8.26E-06	0.001591
Krt75	3.317673	0.000542	0.029769
Gm6377	3.300527	0.000192	0.014194
Atp13a4	3.296091	1.11E-11	1.43E-07
Gm42809	3.295291	2.16E-05	0.00302
Gstp3	3.29038	0.0002	0.014618
Sele	3.275557	3.19E-08	3.90E-05
Gm38118	3.27272	8.79E-05	0.0081
Gm32250	3.272045	0.000742	0.037266
Zbp1	3.263082	4.06E-05	0.004627
Rcan1	3.261456	4.20E-05	0.004756
Ms4a4b	3.253738	6.95E-05	0.006962
Csf2rb	3.253655	2.31E-06	0.000773
Rgs1	3.247676	1.69E-05	0.002507
Scrg1	3.238496	0.000215	0.015344
Cxcr4	3.235012	4.89E-05	0.005327
Cd52	3.223507	4.00E-05	0.004573
Gm45153	3.208918	0.000618	0.032862
Pirb	3.208157	2.18E-05	0.003039
BC037039	3.197276	3.60E-06	0.000969
Lst1	3.194158	4.84E-09	1.04E-05
Vmn1r181	3.19239	0.000543	0.029769
Slc11a1	3.191709	0.000214	0.015323
Hk3	3.184946	2.28E-05	0.003119
Steap1	3.164945	2.33E-05	0.003144
Casp4	3.159031	9.37E-05	0.008443
Fyb	3.15602	9.83E-06	0.001778
Gbp8	3.144993	0.000259	0.017638
Gk	3.139625	1.76E-06	0.000627
Zc3h12a	3.112077	7.55E-05	0.007269

Cd300lb	3.107756	3.61E-06	0.000969
Anxa1	3.107053	0.000647	0.033854
Tyrobp	3.103442	9.52E-05	0.008509
Slc5a1	3.081269	4.32E-06	0.001095
Fam65b	3.076557	7.63E-07	0.000363
Ceacam1	3.07143	2.79E-06	0.000843
Slc1a1	3.056532	4.55E-08	4.80E-05
Scimp	3.052272	7.89E-06	0.001566
Rhoh	3.040544	1.77E-07	0.00013
Acat3	3.034113	0.000103	0.009027
Lcp1	3.031648	0.000107	0.00926
Lcp2	3.028052	6.66E-06	0.001419
Cd53	3.026415	0.000103	0.009074
AB124611	3.019787	5.86E-06	0.001336
Fcer1g	3.017074	0.000239	0.016628
Vav1	3.000055	2.52E-08	3.37E-05
Pmaip1	2.997276	6.99E-06	0.001445
Ccr5	2.990484	2.10E-05	0.002962
Tac1	2.989037	0.000443	0.025982
Ccl9	2.979091	4.86E-05	0.005302
Aif1	2.975995	5.25E-06	0.001238
Cd33	2.973056	0.000225	0.015902
C130050O18Rik	2.964338	3.55E-05	0.004202
Cd300a	2.954419	3.93E-07	0.000235
Fam26f	2.950315	0.000621	0.03295
9430025C20Rik	2.94305	1.94E-06	0.000677
Pglyrp4	2.935939	4.67E-05	0.005149
Gzma	2.929094	0.000492	0.027787
Ptprc	2.926844	0.000159	0.01235
Ifi209	2.922047	1.15E-06	0.000481
C430019N0Rik	2.921355	0.000101	0.008911
Zfp831	2.92121	0.000853	0.041325
Ifi202b	2.920743	0.00071	0.036081
Itgax	2.920006	9.32E-05	0.008431
Htr1b	2.919809	0.000163	0.012553
Rgs16	2.917955	2.66E-07	0.000177
Gm44292	2.91746	0.000108	0.009283
Gpr65	2.907736	1.74E-06	0.000625
Cytip	2.901737	8.28E-06	0.001591
Slc7a11	2.900439	0.000723	0.036597

Ly6i	2.898622	0.000485	0.027573
Themis2	2.898125	2.49E-05	0.003256
Gmfg	2.894338	4.78E-08	4.95E-05
Cd84	2.889419	0.00019	0.014125
Tmem173	2.886772	1.99E-11	1.79E-07
Tnfaip6	2.883408	4.18E-07	0.000247
Gpr132	2.883364	8.28E-06	0.001591
Gm16174	2.875479	1.25E-05	0.002138
Ccl6	2.872481	2.48E-05	0.003256
Selplg	2.87233	0.000494	0.027852
Tlr6	2.866637	3.88E-05	0.004477
Slfn2	2.862341	0.000319	0.020563
Spi1	2.861103	0.00015	0.011983
Sprr1a	2.854082	2.51E-05	0.003256
Batf	2.844745	5.13E-07	0.000285
Il1r2	2.843969	0.000497	0.02787
Bnc1	2.841981	0.000472	0.027212
Ms4a6c	2.840399	0.000447	0.026143
Cdh3	2.836501	0.000538	0.029639
Gm5530	2.822411	2.70E-05	0.003424
Itgam	2.812788	0.000311	0.020239
Runx3	2.808219	6.24E-07	0.000326
Junb	2.798323	3.49E-09	8.17E-06
Pde4b	2.790804	3.80E-05	0.00443
Sp140	2.789436	1.54E-05	0.002404
Ffar2	2.787774	8.73E-07	0.000398
Ptgs2os2	2.783762	0.000294	0.019414
Sbno2	2.773859	1.33E-11	1.43E-07
Gm44851	2.771125	0.000863	0.041698
Elf3	2.767674	5.12E-06	0.00122
I830077J02Rik	2.765564	3.47E-05	0.00413
Ms4a4a	2.751839	0.00063	0.033322
Mid1ip1	2.748666	3.00E-09	8.17E-06
Arid5a	2.741366	5.46E-07	0.000297
Ier3	2.734896	1.35E-05	0.002234
Nxpe5	2.733189	0.000543	0.029769
Il1a	2.731564	0.00025	0.017132
Ceacam16	2.730094	0.000197	0.014502
Rac2	2.726116	0.000311	0.020239
Gm1045	2.709918	0.000824	0.040485

Slc43a1	2.700257	8.22E-06	0.001591
Tuba1c	2.691092	5.80E-05	0.006041
Ptges	2.690041	2.37E-06	0.000778
Snora78	2.68947	0.000551	0.029956
Pik3r5	2.68658	3.30E-05	0.003993
Gm17334	2.68379	0.00085	0.041264
Mmp25	2.680266	0.000695	0.035584
Mpeg1	2.676721	0.000229	0.016131
Tnfaip2	2.671566	2.54E-05	0.003293
AC122397.1	2.666742	0.000709	0.036053
Stom	2.665873	2.49E-06	0.000786
Pik3ap1	2.662122	1.08E-06	0.00047
Gadd45a	2.651981	1.28E-07	0.000101
Nfam1	2.648902	2.08E-05	0.002943
Gm15832	2.644485	5.72E-08	5.50E-05
Nfkbiz	2.642178	8.97E-06	0.001682
Mt1	2.641314	6.78E-06	0.001419
Gm15987	2.637493	4.16E-08	4.67E-05
Defb1	2.636822	3.15E-07	0.000199
Atp1a3	2.621045	5.09E-06	0.001219
Itgb2	2.613199	0.000149	0.011959
Ankrd2	2.606055	0.000215	0.015362
Lrg1	2.601604	2.91E-05	0.003651
Scgb3a1	2.592363	3.60E-05	0.004244
1700071M16Rik	2.590034	0.000611	0.03261
Tnfrsf26	2.589825	0.000219	0.015587
Hcls1	2.585499	7.46E-05	0.007254
Gm11714	2.570787	0.000224	0.015896
Coro1a	2.570544	0.000152	0.012041
AI662270	2.567455	7.30E-07	0.000357
Ppp1r3b	2.566058	0.000474	0.027246
Gm26742	2.559588	3.38E-05	0.004055
Nod2	2.555998	1.26E-05	0.002147
Gm13091	2.54861	0.000191	0.014177
Clec5a	2.545382	1.04E-05	0.001855
Tmprss11b	2.53982	0.000489	0.027673
A430010J10Rik	2.536522	0.000287	0.019001
Clec4a2	2.536233	0.000184	0.013793
Galnt6	2.531035	0.000324	0.020729
Sla	2.530479	0.000184	0.013775

5430427O19Rik	2.523221	1.36E-05	0.002245
Gm19345	2.518085	0.000127	0.010564
Ly6c2	2.511083	0.000329	0.020945
Mt2	2.510164	0.000104	0.009134
Cdk5r1	2.495504	0.000474	0.027246
Gch1	2.490554	6.21E-06	0.001372
Odc1	2.489178	7.23E-05	0.007113
2210407C18Rik	2.482535	7.01E-05	0.006962
Myd88	2.455596	2.03E-10	1.15E-06
Ccr2	2.453987	0.000311	0.020239
Fam169b	2.450539	4.40E-05	0.004878
Prss27	2.44853	2.13E-07	0.000149
Gm45774	2.446642	0.000642	0.033689
Gm31718	2.445495	8.18E-06	0.001591
Skap2	2.441894	7.22E-09	1.44E-05
Neurl3	2.432487	8.03E-05	0.007629
Adamts9	2.431217	0.000575	0.030957
Gm21975	2.429257	0.000832	0.040689
Fcgr2b	2.429159	7.74E-05	0.007412
Cd101	2.427418	0.000193	0.014277
Ms4a6b	2.426947	7.51E-05	0.00726
Zfp36	2.416164	3.88E-05	0.004477
Pilrb2	2.414999	6.97E-05	0.006962
Bst1	2.413271	0.000229	0.016093
Stfa2	2.40512	0.000348	0.021819
Spp1	2.403277	0.000741	0.037266
Bdkrb1	2.39982	1.20E-05	0.002082
Milr1	2.398767	6.00E-05	0.006169
Bin2	2.398266	1.49E-05	0.002352
4833427F10Rik	2.393714	0.000456	0.026537
Slfn10-ps	2.392142	5.34E-05	0.005679
Nfkbia	2.391197	1.65E-05	0.002485
Hcst	2.3895	9.94E-05	0.008806
Pdpn	2.380834	0.0002	0.014618
Alox5ap	2.360178	8.73E-05	0.008054
Cass4	2.354209	6.58E-06	0.001419
Ccdc88b	2.337973	0.000422	0.025287
Ptafr	2.332931	0.000524	0.029089
Dgat1	2.332047	4.38E-05	0.004878
Smox	2.331125	6.22E-06	0.001372

Ptger2	2.319403	0.000311	0.020239
Tnfrsf1b	2.317792	1.53E-05	0.002399
Adora3	2.316421	0.000223	0.015884
Litaf	2.3117	2.13E-10	1.15E-06
Il1bos	2.303301	0.000203	0.014747
Myo1f	2.302607	0.000242	0.016785
Ifitm5	2.302408	0.000167	0.012722
Gapt	2.301747	0.000843	0.041041
Csrnp1	2.299284	4.45E-08	4.79E-05
BE692007	2.296404	0.000314	0.020354
Dmp1	2.291916	0.000508	0.028414
Nek6	2.291207	5.63E-05	0.005896
Nfkbie	2.283707	1.05E-06	0.000462
Gm47615	2.282547	0.000537	0.029639
0610043K17Rik	2.279542	5.31E-06	0.001247
Cd200r1	2.276683	2.31E-05	0.003133
Lbp	2.269113	0.000216	0.015403
Gmfg-ps	2.266116	4.22E-05	0.004771
Havcr2	2.263781	3.26E-05	0.003964
Adam28	2.260078	2.90E-07	0.000188
Slamf8	2.257533	0.000711	0.036087
Runx1	2.25658	3.32E-11	2.55E-07
Ubash3b	2.252009	3.02E-05	0.003746
Napsa	2.24222	2.40E-05	0.003218
Arhgap30	2.231616	9.18E-06	0.001697
Ccl5	2.228493	0.000864	0.041698
A630001G21Rik	2.221664	2.11E-05	0.002962
Csf2rb2	2.22075	2.66E-08	3.40E-05
Rnasel	2.216802	3.86E-08	4.62E-05
Atp8b4	2.213824	2.31E-05	0.003133
Ch25h	2.211326	0.000149	0.011962
Gm31683	2.195025	0.000886	0.042458
Defb14	2.193125	2.44E-06	0.000783
Apobr	2.189034	3.43E-06	0.000949
Trbc2	2.184785	0.000678	0.035061
Cyth4	2.182651	0.000228	0.016075
Steap4	2.179924	0.000437	0.025757
4933423p22Rik	2.1794	5.62E-05	0.005896
Phlda1	2.174871	0.000237	0.016528
Fas	2.17366	1.55E-09	5.61E-06

Igsf6	2.166728	0.000358	0.022349
Gm21188	2.149324	0.00041	0.024837
Il10ra	2.147419	4.93E-05	0.005356
Fkbp11	2.146288	2.72E-05	0.003435
Fcgr3	2.143684	0.000541	0.029732
Rab44	2.139215	0.000486	0.027591
Jaml	2.135191	3.20E-05	0.003905
Dennd1c	2.130782	1.83E-05	0.002679
Trim63	2.130749	0.00048	0.027349
Prr13	2.124441	0.000319	0.020563
1700066B19Rik	2.122568	0.000737	0.037172
Sh3bp2	2.122517	3.24E-09	8.17E-06
Sox9	2.107062	0.000462	0.026817
BC100530	2.106932	0.000253	0.017261
Irgm2	2.102733	0.000128	0.010592
Was	2.10017	3.69E-05	0.004317
Gm43775	2.100085	0.000702	0.035781
Efhd2	2.097011	9.18E-05	0.008331
AI839979	2.091266	3.11E-06	0.000908
Il13ra1	2.088593	0.000142	0.011511
Gm43430	2.087667	0.000697	0.035627
Gm47316	2.08133	0.000113	0.009592
Slc2a3	2.07359	3.01E-05	0.003744
Tifab	2.071485	5.44E-05	0.005756
AC133868.1	2.070099	5.82E-05	0.006045
Npl	2.062514	0.000317	0.020478
D6Ertd527e	2.058016	0.000157	0.012222
Gm12840	2.055672	0.000892	0.042651
Rasgrp4	2.049176	0.000115	0.009748
Il17ra	2.047831	5.34E-08	5.41E-05
Gucy2c	2.042203	0.000335	0.021245
Hmox1	2.042116	0.000107	0.009252
Gpr35	2.041405	2.85E-06	0.000857
Bfsp1	2.028546	0.000544	0.029782
Ppp1r3d	2.024579	7.21E-06	0.001469
Marcksl1	2.024387	0.000547	0.02983
Cd68	2.019861	0.00027	0.018127
Muc1	2.019406	1.40E-05	0.002271
Tnfsf9	2.017117	8.46E-05	0.007876
Nrros	2.003274	2.14E-07	0.000149

Glipr2	2.002334	0.000516	0.028749
Acpp	1.998937	1.11E-05	0.001963
B3gnt5	1.991948	0.000521	0.028954
Rab32	1.985121	0.000738	0.037184
Nfil3	1.984185	0.000969	0.045061
Dsg3	1.961435	9.03E-05	0.008263
Mir7688	1.958223	0.000998	0.045952
Pygl	1.955097	0.000682	0.035092
Ifitm3	1.940819	1.48E-06	0.00056
Gm16045	1.938517	0.000476	0.027277
5330406M23Rik	1.935338	6.42E-05	0.006518
Trim30a	1.93005	2.08E-06	0.000713
Cldn5	1.929801	1.20E-06	0.000484
Tlr2	1.922795	0.000205	0.014812
Ltb4r1	1.920153	1.15E-08	1.87E-05
Defb3	1.917219	0.000338	0.021388
Dram1	1.916737	0.000106	0.009222
Btk	1.912295	8.58E-05	0.007962
Birc3	1.911504	9.35E-08	8.53E-05
Inpp5d	1.910102	0.000214	0.015323
Gm4262	1.903457	0.000853	0.041325
Laptm5	1.903408	0.000699	0.03564
Ptk6	1.900263	1.73E-06	0.000625
Ifi211	1.898019	0.000431	0.025525
Shf	1.893831	0.000429	0.025503
Gm44916	1.889899	0.00046	0.026753
Ier5l	1.888078	3.81E-09	8.54E-06
Trim15	1.878843	0.000182	0.01372
Hspa2	1.867514	0.000298	0.0196
Cpne8	1.867392	1.47E-06	0.00056
Gm26586	1.865814	0.000108	0.009303
E230014E18Rik	1.865049	0.000353	0.022109
Cnr2	1.862725	0.000155	0.01218
Slc23a4	1.861422	0.00026	0.017644
Epha2	1.857198	1.31E-05	0.002193
Basp1	1.851852	7.52E-06	0.001509
Sema6b	1.85141	4.63E-07	0.000265
Stat3	1.842151	1.27E-08	1.95E-05
C920009B18Rik	1.841912	0.000424	0.025287
Grrp1	1.837536	5.54E-06	0.00128

Syt12	1.836801	6.23E-05	0.006372
Cebpb	1.835364	7.49E-09	1.44E-05
Apold1	1.832095	5.91E-06	0.001336
Ankrd10	1.825499	3.49E-09	8.17E-06
Naip2	1.821662	0.00036	0.022364
Dhrs13	1.813789	1.97E-05	0.002817
Osmr	1.810343	7.80E-09	1.45E-05
Snhg15	1.807759	1.30E-06	0.000513
Btg2	1.804415	0.000138	0.01129
Cdk6	1.802138	0.001047	0.047804
Sowahb	1.801974	0.000143	0.011564
2310034G01Rik	1.801319	0.000244	0.016877
Irf8	1.79194	1.39E-05	0.002271
Gm45266	1.791614	0.000506	0.028352
Gsap	1.790399	0.000422	0.025287
Tmem125	1.786161	0.000118	0.009964
Crispld2	1.784936	0.000237	0.016525
Zfp456	1.783146	0.000809	0.040057
Cyba	1.777808	2.37E-06	0.000778
Rab31	1.775438	0.001009	0.046377
Slfn8	1.771881	0.0002	0.014615
Gprc5c	1.770074	9.02E-06	0.001685
Icam1	1.770003	1.76E-08	2.50E-05
2010204K13Rik	1.767491	0.000974	0.045155
Mapk11	1.764466	1.19E-06	0.000484
Mir7678	1.763412	0.000108	0.009272
Gm45222	1.759401	0.000477	0.027286
Ifitm2	1.75674	2.50E-06	0.000786
Srm	1.755954	8.08E-05	0.007663
Gjb2	1.748749	3.33E-05	0.004014
Cfp	1.748156	0.000777	0.03881
Gpsm3	1.745586	4.40E-05	0.004878
Klf2	1.744179	9.32E-09	1.62E-05
Gm47644	1.742743	0.000584	0.03137
Serpinb1a	1.741328	4.84E-05	0.005296
Card9	1.735951	4.47E-05	0.004944
Gm38312	1.734045	0.000612	0.032633
Abcg4	1.732657	0.000142	0.011544
Syk	1.728621	0.000974	0.045155
Mustn1	1.727884	0.000792	0.039416

Catsper4	1.719711	0.000171	0.012974
P2ry6	1.71961	0.000537	0.029639
Irf9	1.715152	1.41E-06	0.000545
Pde1b	1.713742	5.05E-05	0.005441
Gm10320	1.707808	0.000609	0.032522
Slc35f2	1.706836	0.000653	0.034097
Fam179a	1.700116	0.001025	0.047022
Adgrg3	1.6986	0.000138	0.01129
Acap1	1.69595	0.00016	0.0124
E430021H15Rik	1.694987	0.00073	0.036873
Oit1	1.688141	0.000315	0.020407
Rinl	1.683041	0.000201	0.014639
Ncf1	1.67875	2.56E-05	0.003315
Gm43420	1.67702	0.00056	0.030295
Nme1	1.669794	1.08E-05	0.001916
Nr2c2ap	1.665229	1.24E-05	0.002129
Hif1a	1.66488	0.000342	0.021573
Dok2	1.66402	1.41E-05	0.002271
Tmem51	1.657318	5.10E-05	0.005492
Dok3	1.650771	7.53E-05	0.00726
Baz1a	1.647494	1.22E-06	0.000491
Fes	1.641752	2.47E-06	0.000785
Apobec3	1.6382	3.70E-06	0.000984
A930012L18Rik	1.635211	0.000538	0.029639
Adamts1	1.627616	0.000852	0.041325
Rgs14	1.624222	0.000348	0.021819
Relb	1.623791	1.04E-07	9.00E-05
Lyn	1.6232	0.000864	0.041698
Adora2b	1.621218	1.77E-05	0.002613
Trim8	1.617903	6.50E-07	0.000333
B4galt5	1.617775	0.000207	0.015002
Pop1	1.615608	9.75E-06	0.001778
Slc6a20a	1.613379	0.000306	0.020073
Eif1a	1.61319	7.45E-08	6.91E-05
Fzd4	1.602191	5.02E-06	0.00121
Fosl2	1.601101	1.54E-06	0.000574
Gm45151	1.599737	0.000833	0.040689
Traf1	1.599415	0.000345	0.021694
Art5	1.599318	8.62E-05	0.007978
Gm37452	1.597595	0.000968	0.045046

Ripk3	1.59329	0.000159	0.01235
Cxadr	1.58703	4.97E-05	0.005385
Il2rg	1.585574	0.000249	0.017126
Ly6a	1.574455	0.000164	0.012553
Trem2	1.573169	0.000513	0.028698
Exoc3l4	1.57222	0.00013	0.010691
Trpv2	1.564329	0.000106	0.009235
Fmnl1	1.56425	0.000904	0.042839
Ptpn6	1.563621	0.000591	0.031679
Tmsb10	1.559095	0.000428	0.025496
Ip6k3	1.554997	3.30E-07	0.000205
Pdzk1ip1	1.551562	5.98E-05	0.006164
Il1r1	1.550255	8.19E-06	0.001591
Comtd1	1.545879	0.00011	0.009434
Gm26917	1.545694	3.39E-06	0.000949
Tnfrsf1a	1.536218	7.35E-07	0.000357
Rdh10	1.531353	1.12E-06	0.000473
Gm42659	1.527531	0.000931	0.043742
Snai1	1.520937	0.000752	0.037742
Irf1	1.518465	0.000814	0.040214
Tmem140	1.511397	0.000146	0.011759
Arpc1b	1.495648	0.000188	0.014017
Mxd1	1.490624	0.000399	0.024275
Gm15943	1.489281	0.000287	0.019005
Slc29a2	1.485529	5.27E-05	0.005619
Lce3b	1.48324	2.69E-05	0.00342
Marcks	1.480986	1.67E-05	0.002493
Camkk2	1.480967	8.31E-06	0.001591
Smpdl3b	1.473836	4.75E-05	0.005213
Nop58	1.469851	1.12E-06	0.000473
Tnfrsf18	1.467336	0.000452	0.026355
Sept9	1.466332	5.88E-06	0.001336
Liph	1.460729	0.000683	0.035153
Chrna10	1.459454	0.000106	0.009222
Arhgdib	1.458606	0.001076	0.048829
Trmt61a	1.457592	1.36E-05	0.002245
Asns	1.450164	2.50E-05	0.003256
Npm1	1.446123	3.97E-05	0.004544
Endou	1.445783	7.10E-06	0.001458
Rhbdf2	1.442825	2.58E-06	0.000796

Tgm1	1.439586	2.27E-05	0.003119
Crlf2	1.436726	5.90E-05	0.006101
Fbxl22	1.434488	0.000252	0.017258
Grwd1	1.431434	3.93E-06	0.001022
Ppan	1.42817	2.18E-07	0.00015
Trib1	1.4197	7.82E-06	0.001558
Vasp	1.418958	0.00048	0.027352
Tnfrsf23	1.416059	0.000937	0.043931
Sfn	1.414833	9.56E-05	0.008511
Samhd1	1.413283	2.37E-06	0.000778
Hilpda	1.41132	0.000895	0.042669
Hpn	1.410711	7.82E-06	0.001558
Gpr146	1.409681	1.56E-05	0.002419
Rnf122	1.409018	4.40E-06	0.001097
Creld2	1.407458	0.000189	0.014062
Eif4ebp1	1.406321	1.84E-06	0.000653
Tiparp	1.401593	5.10E-06	0.001219
Tmprss4	1.400516	9.40E-05	0.008443
Sdf2l1	1.399798	0.000186	0.013847
Csf1	1.397845	1.56E-06	0.00058
Cstb	1.392424	5.57E-05	0.005864
Snhg4	1.391941	8.00E-06	0.001576
Fabp5	1.391247	3.45E-05	0.004117
Plk2	1.38955	3.90E-06	0.001019
Ptpn2	1.381527	1.85E-05	0.002707
Pgam1	1.380958	1.41E-05	0.002271
Gnl3	1.37975	1.15E-06	0.000481
Armc7	1.37974	0.000128	0.010577
Rab26os	1.378123	0.000618	0.032862
Vmp1	1.376289	1.48E-05	0.002352
Ier2	1.37519	0.000974	0.045155
Ltbr	1.374327	9.85E-07	0.000442
1110038B12Rik	1.373254	1.88E-05	0.002725
Wdr74	1.371087	8.21E-05	0.00771
Itpk1	1.369693	6.26E-06	0.001375
Flot1	1.360383	4.35E-06	0.001095
Ctps	1.354791	7.29E-06	0.001474
Sec61b	1.350812	2.24E-05	0.003092
Actg1	1.346712	3.59E-05	0.004232
Snhg12	1.341729	2.10E-06	0.000714

Sphk1	1.340076	6.90E-05	0.006941
Mcc	1.336971	1.01E-05	0.001812
Tnfrsf10b	1.334624	0.000125	0.010478
Efemp2	1.33269	0.000988	0.04558
Hsp90aa1	1.332293	3.18E-05	0.003892
Pik3r1	1.32601	3.71E-06	0.000984
D1Ertd622e	1.325468	3.83E-05	0.004453
Tdh	1.32361	0.000211	0.015151
Tuba1b	1.32291	2.00E-05	0.002848
Clu	1.318863	4.43E-06	0.001097
Ampd2	1.315887	2.90E-06	0.000867
Manf	1.315121	9.13E-05	0.008296
Bhlhe40	1.315032	1.08E-06	0.00047
Hcar2	1.31388	0.000678	0.035061
Ctla2a	1.313459	0.000894	0.042651
Ranbp1	1.312219	0.000158	0.01229
Shisa5	1.311123	7.83E-05	0.007487
1810037I17Rik	1.308816	1.96E-06	0.00068
Nfkb2	1.30349	5.37E-06	0.001252
Nsmce1	1.303361	1.59E-05	0.002438
Arrb2	1.301631	0.000161	0.012445
Mettl1	1.298224	3.14E-06	0.000908
Zfp36l1	1.295258	8.77E-06	0.001649
Arl4d	1.294851	0.000796	0.039518
Midn	1.292836	9.24E-06	0.001703
Ckap4	1.285886	8.74E-06	0.001649
Mir17hg	1.285751	7.15E-05	0.007061
Galnt3	1.282539	0.000909	0.042908
Shb	1.279805	2.18E-05	0.003039
Fbl	1.27847	7.03E-05	0.006962
Nop56	1.277144	1.67E-05	0.002495
Fam217b	1.271014	0.00015	0.011962
Nolc1	1.269415	1.61E-05	0.00245
Rnf149	1.269069	0.000682	0.035092
Rel	1.267273	5.97E-06	0.001345
Slc39a1	1.264253	1.28E-05	0.002166
Csta1	1.262022	0.000329	0.020945
Klk9	1.260445	0.000233	0.016308
Snhg1	1.259879	2.05E-05	0.002915
Mrto4	1.259818	9.33E-06	0.001712

Rpain	1.257817	0.000153	0.012092
Ak2	1.255769	2.49E-05	0.003256
Arhgef16	1.255205	0.000606	0.032395
Tacc2	1.255028	8.18E-05	0.007696
Heatr1	1.252224	1.30E-05	0.002186
1700017B05Rik	1.251641	8.35E-05	0.007795
Actb	1.249531	0.000248	0.017083
Ctsz	1.249453	0.000278	0.018574
Zfas1	1.24203	1.28E-05	0.002162
Klf7	1.240887	0.000937	0.043931
Lyar	1.240866	1.66E-05	0.00249
Cd3eap	1.240768	6.74E-06	0.001419
Arntl2	1.239279	0.000143	0.011564
Nhp2	1.236402	2.61E-05	0.003362
Rpl36al	1.235765	5.93E-05	0.006124
Aprt	1.231948	6.97E-05	0.006962
Chka	1.231422	0.000449	0.026211
Ppp4r1	1.231111	3.69E-05	0.004317
Zfp281	1.229689	4.96E-05	0.005377
Wdr43	1.228975	9.85E-06	0.001778
Ifnar2	1.224768	0.000158	0.012282
Trim11	1.224456	6.93E-06	0.001439
Polr1e	1.223808	0.000156	0.012222
Alg8	1.222491	0.000878	0.042087
Cadm4	1.216071	2.19E-05	0.003039
Xbp1	1.214652	2.74E-05	0.003448
Gar1	1.214284	2.66E-05	0.00339
Hivep3	1.212097	0.000248	0.017083
Pfkfb4	1.207969	2.08E-05	0.002938
Piga	1.201388	2.24E-05	0.003092
Rab8b	1.200607	0.000699	0.03564
Snrpa1	1.200337	4.82E-05	0.005283
Sh2d3c	1.196307	0.000375	0.023099
Rrp12	1.194727	9.78E-06	0.001778
Nip7	1.191243	2.98E-05	0.003714
Klk13	1.185705	0.000476	0.027281
Rhou	1.185128	2.61E-05	0.003362
Fchsd1	1.184774	0.00014	0.011368
Igf1r	1.18376	0.000132	0.010805
Gm26880	1.18281	0.0009	0.042748

Jdp2	1.182583	0.000566	0.030524
Snhg5	1.179639	7.29E-05	0.00714
Ext1	1.176632	0.000172	0.012987
Riox1	1.172669	0.000153	0.012092
2810474O19Rik	1.171812	3.44E-05	0.004107
Ulk1	1.171397	0.000812	0.040168
Wdr77	1.170462	1.37E-05	0.002247
Cdkn2d	1.169084	2.28E-05	0.003119
Rnf144b	1.168033	6.49E-05	0.006567
Rrp15	1.163608	9.54E-05	0.008509
Art1	1.162379	0.000144	0.011607
Tpd52	1.161547	0.000849	0.041248
Sat1	1.161359	0.000258	0.017565
B4galt1	1.160026	1.60E-05	0.002438
Snrpd1	1.15861	0.000125	0.010478
Mydgf	1.15573	0.000205	0.014812
Susd6	1.153764	8.16E-05	0.007691
Rrs1	1.15069	0.000107	0.009235
Cyb561	1.150683	0.000127	0.010564
Rgs19	1.1492	0.000903	0.042827
Tcof1	1.148407	6.39E-05	0.006503
Rhog	1.147997	0.000297	0.019513
Noc4l	1.144188	3.54E-05	0.0042
Nop16	1.143017	6.16E-05	0.006316
Ggt5	1.141795	0.000759	0.038008
Llph	1.138919	7.30E-05	0.00714
Arrdc4	1.138673	7.61E-05	0.007308
Gpatch4	1.137765	7.17E-05	0.007063
Glul	1.13757	0.000988	0.04558
Nop2	1.136462	5.25E-05	0.005617
Ddx39	1.135645	0.000677	0.035061
Gcnt2	1.134495	0.000414	0.02499
Qsox1	1.133907	0.000332	0.021082
Ggct	1.133334	0.000529	0.029299
Yrdc	1.129152	0.000224	0.015894
Pdia6	1.128999	0.000359	0.022364
Rrp1b	1.128809	8.10E-05	0.007673
Mthfd2	1.127848	0.000269	0.018127
Txn1	1.12645	0.000953	0.044382
Tgm2	1.126417	0.000253	0.017261

Psmb10	1.122074	0.000285	0.018956
D16Ertd472e	1.11942	0.000548	0.029866
Chd7	1.119294	0.000127	0.010572
Slc20a1	1.118847	0.000682	0.035092
Grina	1.11728	0.000313	0.02034
Frrs1	1.115152	0.000495	0.02787
Ldlr	1.108205	0.000984	0.045496
Rpl12	1.105258	0.000552	0.030001
Tpm3	1.10412	0.000431	0.025525
Slc16a6	1.10365	0.000658	0.034284
Kti12	1.100228	0.000152	0.012092
Slc3a2	1.097779	0.001045	0.047789
Tbc1d15	1.096105	0.000946	0.044235
Tnip2	1.093203	0.000139	0.011333
Ero1l	1.092296	0.000875	0.042058
Pgs1	1.092028	8.53E-05	0.007922
Ets2	1.091926	0.000203	0.014747
Xdh	1.091908	0.000185	0.013844
Cd63	1.090386	0.000153	0.012092
Slc26a6	1.089897	0.000814	0.040214
Irak4	1.088989	0.001069	0.048556
Ugcg	1.086843	0.000316	0.020437
Eif4a1	1.086102	0.000394	0.024082
Lpin2	1.084918	0.000907	0.042897
Efna5	1.083335	0.000674	0.034933
Mcrs1	1.082164	9.53E-05	0.008509
Mif	1.082107	0.000199	0.014571
Panx1	1.079432	0.000384	0.023495
Mogs	1.072547	0.00026	0.017644
Set	1.072408	0.000344	0.021655
Ndrg3	1.067317	0.000196	0.014454
Trp53i11	1.0646	0.000892	0.042651
Phgdh	1.061193	0.000754	0.037787
Pqlc1	1.059284	0.000395	0.024082
Tnfrsf21	1.056963	0.000898	0.042703
Pde12	1.054185	0.000548	0.029859
6430548M08Rik	1.053895	0.000905	0.04287
Ssr2	1.047692	0.000358	0.022349
Pum3	1.046676	0.000791	0.039416
Taf1d	1.044675	0.000324	0.020729

Sema7a	1.040565	0.000866	0.041718
Pdia4	1.039622	0.00065	0.033978
Igsf3	1.038819	0.00038	0.023327
Dkc1	1.034572	0.000265	0.017881
Ccdc86	1.034343	0.000625	0.033117
Pwp2	1.033393	0.000441	0.025915
Tssc4	1.032822	0.000741	0.037266
H2afz	1.02008	0.001092	0.049469
Cblc	1.01839	0.000927	0.043584
Polr2h	1.016499	0.001056	0.048116
Trim27	1.015695	0.000427	0.025435
Anp32b	1.013791	0.001047	0.047804
Dnajc2	1.01301	0.000421	0.025287
Ddx18	1.012427	0.000939	0.043969
Pla2g4e	1.012289	0.000816	0.040269
Ebna1bp2	1.001709	0.000825	0.040485
Pprc1	0.997964	0.000486	0.027591
Rsl24d1	0.991058	0.000695	0.035584
Nifk	0.987484	0.000713	0.036143
Ptp4a1	0.983148	0.00064	0.033606
Ddi2	0.981707	0.000898	0.042703
Rfng	0.970652	0.00052	0.028925
Ftsj3	0.969181	0.000843	0.041041
Crip1	-1.0232	0.000982	0.045435
Mical2	-1.03021	0.000792	0.039416
Kitl	-1.03662	0.000312	0.020294
Dst	-1.07119	0.000546	0.02983
Cd93	-1.07253	0.001036	0.047466
Colec12	-1.07944	0.000795	0.039505
Olfml2b	-1.07958	0.000827	0.04055
Plpp3	-1.07972	0.000274	0.018337
Fzd7	-1.08331	0.000624	0.03309
Ramp2	-1.09159	0.000211	0.015147
Ltbp4	-1.09308	0.000408	0.024756
Arhgef10	-1.09997	0.000695	0.035584
Pitx2	-1.11791	0.000423	0.025287
Itga9	-1.12143	0.000464	0.026817
Hsf2	-1.12336	0.000835	0.040747
Mmrn1	-1.13263	0.000793	0.039416
Sptbn1	-1.13741	9.03E-05	0.008263

Marveld1	-1.14863	1.59E-05	0.002438
Itpkb	-1.15092	7.92E-05	0.007555
Tek	-1.15522	0.000128	0.010593
Sulf2	-1.15538	0.000294	0.019414
Mfap5	-1.15904	0.000363	0.02256
Nid1	-1.15968	0.000108	0.009283
Timp2	-1.16568	9.27E-05	0.008394
F2r	-1.17557	0.000324	0.020729
Zfp395	-1.18377	0.000287	0.019001
Adamts2	-1.18759	0.000479	0.027349
Col4a5	-1.18845	0.000398	0.024254
Sema3c	-1.19087	0.000233	0.016316
Jam2	-1.19343	0.000662	0.034436
Col1a2	-1.20036	0.000259	0.017634
Sncaip	-1.20194	0.000557	0.030233
Pkd1	-1.2258	2.65E-05	0.003381
Ptpn14	-1.23068	0.000423	0.025287
Hspa12a	-1.24733	0.00053	0.029321
Thsd4	-1.26091	0.00069	0.035466
Bcar3	-1.27985	0.000526	0.029168
Kif26a	-1.28662	0.000635	0.033507
Hecw2	-1.28717	0.000978	0.045283
4931406P16Rik	-1.28783	2.48E-05	0.003256
Slc24a3	-1.2909	0.000845	0.041105
Lpar1	-1.29582	0.001056	0.048116
Fzd10	-1.29595	0.000475	0.027246
Nlrc3	-1.31153	0.000667	0.034605
Palm	-1.32058	5.85E-05	0.006065
Podxl	-1.32284	3.20E-05	0.003905
Ptprb	-1.32637	0.000131	0.010796
Axin2	-1.32909	0.000364	0.022565
Lurap1l	-1.33423	0.000643	0.033689
Amigo2	-1.33528	0.00082	0.040391
Mmp2	-1.33588	3.00E-06	0.000882
Esrrg	-1.33721	0.000713	0.036143
Thbs3	-1.33749	1.65E-05	0.002485
Col5a2	-1.34012	5.69E-06	0.001308
Lrp4	-1.34134	6.38E-05	0.0065
Kcnj12	-1.34192	2.15E-06	0.000726
Cdon	-1.34473	3.97E-05	0.004544

Zfp385c	-1.34894	0.000617	0.032862
Atp2b4	-1.35045	3.05E-05	0.003756
Dach1	-1.35496	0.000473	0.027233
Ndrg4	-1.36073	3.85E-05	0.004465
Fsd1l	-1.36447	2.63E-05	0.003368
Gucy1b3	-1.39117	9.42E-05	0.008445
Klhl4	-1.39778	0.000419	0.025241
Rp1	-1.39839	0.000871	0.04192
Olfml1	-1.40249	0.000893	0.042651
Map1a	-1.40414	0.000832	0.040689
Lrrc52	-1.40718	0.000434	0.025657
Fat4	-1.40772	9.11E-05	0.008296
Tmod2	-1.40842	4.63E-06	0.001143
Slc6a4	-1.41225	0.001063	0.048388
Xirp1	-1.41796	0.000261	0.017675
Adgrl4	-1.42715	0.001038	0.04751
Prickle1	-1.43129	0.000636	0.033507
Npr3	-1.43413	7.00E-05	0.006962
Daam2	-1.4379	0.000138	0.011273
Zfp467	-1.43893	0.000323	0.020729
Adam33	-1.44064	0.000113	0.009592
1810010H24Rik	-1.44115	0.001009	0.046377
Fam20a	-1.44234	0.000952	0.044361
Smim1	-1.44235	0.000695	0.035584
Sec16b	-1.45165	0.00021	0.015136
Pcdh18	-1.45189	0.000909	0.042908
Bmp6	-1.45371	0.000578	0.03109
Dusp18	-1.45645	4.95E-07	0.00028
Ankrd24	-1.45681	0.000101	0.008917
Igsf10	-1.46214	0.000122	0.010255
Uph	-1.4673	0.00079	0.039416
Tmem200b	-1.47063	0.000491	0.027763
Reps2	-1.47194	0.000732	0.036941
Ncam1	-1.47454	7.71E-05	0.007397
Nov	-1.47492	0.000148	0.011897
Slitrk6	-1.48464	0.000479	0.027349
Radil	-1.48546	0.000285	0.018958
Hic1	-1.48978	2.50E-05	0.003256
Lhx6	-1.49677	0.001089	0.049356
Reck	-1.51208	8.22E-07	0.000383

C1qtnf1	-1.51284	0.000329	0.020945
Rtn4rl1	-1.51565	0.000413	0.024926
Pdgfd	-1.51639	0.000167	0.012722
Insl6	-1.52659	9.40E-05	0.008443
Cav1	-1.52804	2.66E-06	0.000814
Col1a1	-1.53024	1.97E-05	0.002817
Ints6l	-1.542	3.22E-05	0.00392
Plxdc1	-1.54205	0.000323	0.020729
Rasgrf2	-1.55354	0.000181	0.013663
Il17d	-1.55429	0.000233	0.016308
Col3a1	-1.55459	0.000209	0.015098
Gucy1a3	-1.55668	3.35E-05	0.004028
Zfp273	-1.57059	0.0011	0.049717
Ptch2	-1.5755	0.000163	0.012553
Stard9	-1.57862	0.000125	0.010478
Kcnc1	-1.58347	1.03E-07	9.00E-05
Mansc1	-1.59019	0.000619	0.032882
Fam81a	-1.59282	0.000153	0.012092
Mamdc2	-1.59285	1.66E-05	0.00249
Dzip1	-1.59437	0.000126	0.010531
Actg2	-1.59671	0.000666	0.034566
Zc2hc1c	-1.5989	0.000897	0.042703
Coro2b	-1.59934	0.000125	0.010478
G6b	-1.60439	0.000698	0.03564
Bean1	-1.60615	0.000465	0.026886
Timp3	-1.60809	0.00043	0.025525
Slc9a3r2	-1.60918	5.27E-05	0.005619
Islr2	-1.62129	0.000329	0.020954
Arhgap28	-1.62495	0.000262	0.017678
Dnm1	-1.63707	1.31E-06	0.000514
Lrrc3b	-1.64118	0.000659	0.034297
Ntf3	-1.64833	0.000348	0.021819
Lsamp	-1.65457	0.000131	0.010793
Foxp2	-1.66276	0.000523	0.029012
Gli1	-1.66405	8.50E-06	0.001616
Gm16185	-1.66935	0.00047	0.027105
Fam221a	-1.67171	0.000116	0.00982
Dtx1	-1.67544	0.000136	0.011134
Ankrd29	-1.677	7.45E-05	0.007254
Sdpr	-1.68476	0.000306	0.020072

Tppp3	-1.69596	1.59E-07	0.000122
Gm45805	-1.69642	0.000183	0.013768
Gm26788	-1.70029	0.000232	0.016298
Cd248	-1.70714	2.66E-07	0.000177
Papln	-1.72152	0.000369	0.022834
Tmem35a	-1.72626	0.000449	0.026205
Gabrg2	-1.73434	0.000241	0.016747
Mybl1	-1.73619	0.00086	0.041599
Nynrin	-1.74552	0.000378	0.023206
Prlr	-1.75329	5.56E-05	0.005864
Col5a1	-1.75844	0.000308	0.020158
Rgs9bp	-1.76143	0.000933	0.043812
Fndc1	-1.76271	1.62E-05	0.002466
Cers1	-1.76403	0.000839	0.040928
Adamts12	-1.76592	1.93E-05	0.00279
Tppp	-1.77388	5.73E-05	0.005972
Ppp2r2b	-1.77463	0.000372	0.022952
Cubn	-1.77534	0.001096	0.049592
Higd1b	-1.78389	0.000147	0.011827
Cacna1h	-1.79467	0.000113	0.009618
1700019D03Rik	-1.7987	0.000151	0.012018
Gal3st1	-1.80556	1.95E-05	0.002802
Tagln3	-1.80819	0.000463	0.026817
Olfr1372-ps1	-1.81522	0.000112	0.00955
Gm16299	-1.81537	0.000825	0.040485
Pltp	-1.81655	1.01E-07	9.00E-05
Myct1	-1.8228	0.000197	0.014502
Crip3	-1.831	7.41E-06	0.001493
Dpep1	-1.83304	8.45E-05	0.007876
Rarb	-1.83485	0.000156	0.012199
Chp2	-1.83605	9.44E-06	0.001728
Hand2	-1.85617	0.000145	0.01172
Capn6	-1.85982	0.00028	0.018671
Chst3	-1.86972	0.000822	0.04046
Gm33103	-1.88224	0.000463	0.026817
Nkd1	-1.88517	1.24E-05	0.002133
Omd	-1.88617	0.000769	0.038461
Lgi1	-1.91377	0.000446	0.026122
Cyp46a1	-1.91636	0.00056	0.030295
Ascl4	-1.92322	0.000602	0.032267

Myrip	-1.92483	8.26E-05	0.007733
Dchs2	-1.93104	0.00097	0.045077
Heph	-1.93502	0.000236	0.016469
Fam171b	-1.93575	4.30E-05	0.004834
Gpr34	-1.97878	5.43E-05	0.005756
Tril	-1.99928	3.90E-05	0.004478
Snurf	-2.00023	2.97E-05	0.00371
Abca9	-2.03795	7.14E-06	0.001461
Gm38910	-2.0558	0.000104	0.009082
Mme	-2.06363	3.31E-06	0.000937
Zp2	-2.07018	8.01E-05	0.007626
Olfml2a	-2.0791	0.001071	0.048608
Rasl10a	-2.09505	0.000261	0.017675
Gdf10	-2.09872	0.000185	0.013833
D830013O20Rik	-2.11764	0.000118	0.009964
Fam131b	-2.14797	6.75E-05	0.00681
Cdh22	-2.15814	4.93E-06	0.001199
Dkk3	-2.1746	3.68E-05	0.004317
1700080G11Rik	-2.2032	0.000798	0.03956
Zcchc5	-2.21166	0.000247	0.017045
Trim66	-2.21233	7.48E-05	0.007254
Col8a2	-2.22757	0.000514	0.028712
Kctd12b	-2.23853	1.65E-05	0.002485
Nat8f3	-2.23945	0.000908	0.042908
Cercam	-2.25021	1.94E-05	0.002802
Sec14l4	-2.26435	0.000103	0.00903
Kcnc2	-2.26653	0.000339	0.021393
Fam198b	-2.29152	1.65E-06	0.000603
2310015K22Rik	-2.31764	6.10E-06	0.001362
Wnt10b	-2.32706	0.000278	0.018569
Smad9	-2.33318	1.09E-05	0.001921
Stmnd1	-2.33683	3.96E-06	0.001024
Id4	-2.36373	1.41E-06	0.000545
Gm12002	-2.37028	0.000546	0.02983
Sez6l	-2.37042	0.000945	0.044224
Cacna1g	-2.39093	1.38E-05	0.002251
Ackr4	-2.39191	8.21E-06	0.001591
Glt8d2	-2.40938	0.00021	0.015147
Tmem119	-2.42953	3.61E-06	0.000969
Best2	-2.43163	0.000214	0.015323

Capsl	-2.44475	4.24E-05	0.004788
Slc6a2	-2.4822	1.37E-08	2.00E-05
Gng4	-2.49187	9.38E-05	0.008443
Pkhd1l1	-2.52572	3.14E-06	0.000908
Gm43389	-2.54006	0.000416	0.025056
Mapk15	-2.54455	4.16E-05	0.004736
Acss3	-2.55382	0.000915	0.043084
Doc2b	-2.55879	3.03E-05	0.003753
Gm45226	-2.56968	0.000706	0.035941
Gm43272	-2.58654	0.00016	0.012359
Spon2	-2.59654	6.76E-05	0.00681
Spo11	-2.61838	0.000328	0.020945
Gm26888	-2.62038	0.000759	0.038008
Crtac1	-2.63911	0.000285	0.018958
Enpp6	-2.66693	6.93E-07	0.000349
Adamts19	-2.67393	0.000729	0.036837
P2rx2	-2.6982	0.001021	0.046854
Slitrk2	-2.69947	0.000265	0.01786
Ptprr	-2.74332	1.13E-05	0.001993
Gm11816	-2.7951	0.000539	0.029649
Gabra4	-2.79595	0.000338	0.021388
Notumos	-2.88766	0.000436	0.025708
Diras2	-2.90141	0.000443	0.025975
Rhcg	-2.96669	0.000497	0.02787
Prdm8	-3.00313	2.45E-06	0.000783
Rnf112	-3.03242	2.40E-05	0.003218
Jakmip2	-3.12843	0.000406	0.024652
Hepacam2	-3.17438	1.52E-05	0.002391
Abhd12b	-3.25523	9.16E-06	0.001697
Lrrc31	-3.29848	0.000394	0.024078
Gm28653	-3.46786	0.000438	0.02581
Gm16485	-3.64652	0.000273	0.018337
Pik3c2g	-3.75619	0.000828	0.04055
A730046J19Rik	-3.79861	0.000402	0.024468
Pla2g2d	-3.90829	8.69E-06	0.001647
Gm48550	-4.26375	0.00095	0.044361
Sult1b1	-4.31325	3.29E-05	0.003987
S100a5	-4.35421	4.56E-05	0.005043
Rgag1	-4.37314	0.000494	0.027852
Gm2199	-4.72075	2.57E-08	3.37E-05

Kcnh6	-4.84258	0.000582	0.031288
Gm7240	-5.52834	0.000951	0.044361
Gpr165	-5.53245	0.000634	0.033505
Thegl	-5.53812	0.000638	0.033535
Gm5828	-6.8309	3.89E-05	0.004478
AC140393.1	-6.8363	0.000275	0.018374
Ccnb1ip1	-8.45855	0.000497	0.02787

Appendix Table B.1 WT Infected vs. WT Sham. This table represents all of the differentially expressed transcripts that are considered significant results (p-value < 0.05, FDR < 0.05). The table is sorted by logFC from the largest value to the smallest. 1191 transcripts were identified.

Defb3 Knockout Infected versus *Defb3* Knockout Sham

Genes	**logFC**	**P-Value**	**FDR**
Gm5483	9.952193	2.90E-05	0.007719
Nlrp12	9.627635	1.09E-06	0.001007
Saa3	9.340649	4.41E-06	0.002372
Slc26a4	7.730627	0.000233	0.028186
Csf3	7.701927	4.94E-05	0.010661
Il19	7.696321	1.65E-05	0.005374
Cd177	7.643502	0.000138	0.020053
Retnlg	7.622437	4.43E-05	0.009931
Il22	7.576076	9.17E-07	0.000897
Trem1	7.488971	1.03E-05	0.004124
Clec4d	7.37194	3.30E-05	0.008399
Il6	7.333296	0.000274	0.031291
Osm	7.163162	7.12E-06	0.003247
AC110211.1	7.145829	4.39E-05	0.009922
Mir142b	6.991013	1.46E-05	0.005049
Csf3r	6.820779	9.95E-06	0.003998
Saa4	6.763833	2.16E-06	0.001613
Cd300lf	6.737686	1.24E-07	0.000222
Clec4e	6.622801	1.13E-05	0.004358
Il1b	6.592028	3.16E-05	0.008185
Cxcl1	6.588532	6.19E-05	0.012135
Cxcl5	6.510821	2.04E-06	0.001588
Acod1	6.456974	0.000138	0.020053
Mcemp1	6.452192	4.01E-05	0.00943

Il12b	6.44226	0.000108	0.017312
Fpr2	6.397273	8.69E-06	0.003681
Cxcr1	6.366694	0.000297	0.032894
Ly6g	6.322893	0.000365	0.038085
Cxcl2	6.284726	8.47E-05	0.014947
Ccl4	6.283194	0.000285	0.032091
Gm48671	6.280621	0.000145	0.020912
Gm16175	6.237508	3.53E-05	0.008794
Trem3	6.236911	2.58E-05	0.0072
Mcoln2	6.099284	2.50E-08	8.97E-05
A530032D15Rik	6.03783	0.000375	0.038683
Rrad	6.01907	3.70E-07	0.000497
Gm9733	5.977747	4.60E-06	0.002404
Sirpb1b	5.971056	4.62E-07	0.000564
Gm39321	5.918422	0.000374	0.038683
Gm32250	5.831577	0.000256	0.029856
Lcn2	5.695191	3.89E-05	0.00927
Trim30b	5.62543	5.20E-07	0.000613
Sell	5.584597	3.19E-06	0.001971
Il17a	5.528481	5.07E-05	0.010767
Gm5150	5.509076	2.97E-06	0.001971
Fcgr4	5.492667	1.72E-07	0.000289
Cxcl3	5.46907	7.25E-05	0.013396
Sh2d5	5.331875	0.000493	0.046085
LOC100038947	5.302743	4.58E-05	0.01014
Saa2	5.274793	1.17E-05	0.004411
Mefv	5.236395	1.49E-06	0.001294
Gm16556	5.139063	2.09E-05	0.006341
Ms4a4c	5.128389	6.46E-08	0.000164
Tnfsf14	5.089841	5.59E-09	4.30E-05
Slfn1	4.984457	9.76E-08	0.000202
Cxcr2	4.957257	4.56E-05	0.010138
Gm44165	4.861189	7.80E-06	0.003438
Treml2	4.845248	8.99E-09	4.84E-05
Timp1	4.843583	3.13E-05	0.008129
CR974586.5	4.837449	1.53E-06	0.0013
A630023A22Rik	4.798235	0.000132	0.019788
B230303A05Rik	4.775795	2.62E-05	0.007232
Hdc	4.743348	7.57E-05	0.01372
Fam26f	4.697423	1.54E-05	0.005237

1700025N21Rik	4.633196	3.52E-06	0.002058
Sirpb1a	4.624105	7.66E-05	0.01383
I830127L07Rik	4.525643	0.000204	0.025759
Ccr1	4.504187	2.49E-05	0.007131
Cd14	4.463507	9.32E-05	0.015823
Klra2	4.455591	3.19E-07	0.00044
C530050E15RIk	4.451339	2.54E-07	0.000391
Slfn4	4.446047	3.06E-05	0.007994
Trpm2	4.394382	4.54E-05	0.010127
Nlrp3	4.379352	0.000102	0.016627
Samd7	4.378306	8.51E-07	0.000847
Gm14010	4.304882	8.37E-06	0.003632
Gm36546	4.268691	5.35E-07	0.000613
1200007C13Rik	4.218578	7.22E-05	0.013396
Tmem156	4.214717	2.08E-06	0.001602
Urah	4.208144	6.01E-05	0.011974
Ifitm6	4.207614	9.80E-06	0.003998
Lilr4b	4.156771	2.53E-05	0.007154
Gm6524	4.117885	2.02E-05	0.006285
Selp	4.084195	1.21E-07	0.000222
Fgr	4.022986	1.83E-05	0.005904
Treml4	4.013967	0.000471	0.044691
Mmp8	4.002651	1.44E-05	0.005005
Wfdc17	3.99547	0.000138	0.020053
CT009530.1	3.988529	1.43E-05	0.005005
Serpina3i	3.975649	4.13E-06	0.002317
Ccl2	3.971067	9.73E-05	0.016149
Sycp2	3.964468	0.000154	0.021592
Nfe2	3.882468	0.000252	0.029634
Mir142hg	3.876744	1.16E-05	0.004397
Clec4n	3.871771	4.33E-05	0.009922
Clec7a	3.848276	5.32E-07	0.000613
Adamts4	3.841022	5.75E-05	0.011728
Socs3	3.816164	8.19E-08	0.000192
A130071D04Rik	3.786261	8.20E-06	0.003587
AC154270.2	3.768714	0.000244	0.029047
4833407H14Rik	3.766919	4.15E-05	0.009663
Gad1-ps	3.761574	5.96E-05	0.011914
Gm20768	3.695043	0.000364	0.037976
Plaur	3.667934	2.69E-05	0.007232

Ifitm1	3.660705	0.000176	0.023412
Tlr1	3.651171	2.32E-06	0.001688
Ipcef1	3.650551	0.000355	0.037492
Olr1	3.595528	0.000149	0.021196
Tfec	3.573523	4.79E-06	0.002457
Bdkrb1	3.512286	9.74E-08	0.000202
Pdk4	3.504102	0.000506	0.046944
Lilrb4	3.485887	1.39E-05	0.005005
F10	3.483567	5.29E-08	0.000142
Bcl3	3.474878	5.96E-06	0.002835
Gm46224	3.466496	0.000241	0.028854
Ms4a6d	3.463075	9.53E-06	0.003944
Il18rap	3.462771	0.000228	0.027738
6720475M21Rik	3.441637	1.88E-05	0.005977
Mmp9	3.405423	0.000254	0.029742
Siglece	3.403073	2.67E-08	8.98E-05
Gm13889	3.394035	0.000113	0.017853
Cd244	3.315534	2.56E-05	0.007167
Tmprss11g	3.305216	0.00011	0.017482
Rnd1	3.294909	0.000401	0.040374
1600010M07Rik	3.2858	6.54E-05	0.012559
Samsn1	3.273773	8.88E-05	0.015419
Plek	3.273311	0.00013	0.019615
Il20	3.227179	0.000428	0.042144
Ccno	3.19813	0.000106	0.017174
Msr1	3.195792	0.000177	0.023503
Ch25h	3.186688	2.85E-06	0.001971
Nfkbid	3.185989	0.000109	0.017427
Dmp1	3.185821	2.48E-05	0.007131
Gm14548	3.173762	4.00E-07	0.000524
Ankrd66	3.12669	0.000219	0.026759
Scgb3a1	3.104199	4.04E-06	0.002311
Adam8	3.080572	5.76E-05	0.011728
Csf2rb	3.06883	4.20E-06	0.002322
Smim3	3.059226	4.22E-05	0.009754
Cyp7b1	3.016408	9.04E-05	0.015464
Halr1	3.012981	1.36E-05	0.004932
Fcgr1	3.010332	1.96E-07	0.00032
Gm45479	2.998469	7.83E-05	0.014044
AC113595.1	2.997086	4.39E-07	0.000549

Rtn4rl2	2.994209	2.65E-05	0.007232
Cebpd	2.982958	1.02E-06	0.000976
Ankrd2	2.928988	9.53E-05	0.016106
Slc15a3	2.902562	2.67E-05	0.007232
Rasd1	2.897815	1.27E-05	0.004672
Il4ra	2.893556	8.03E-05	0.014307
Il21r	2.873112	3.04E-06	0.001971
C5ar1	2.855767	0.000216	0.02654
Ceacam16	2.834051	6.68E-05	0.012621
Steap1	2.83043	0.000266	0.030679
AA467197	2.825759	3.72E-08	0.000111
Pilra	2.805852	4.39E-05	0.009922
Gm45774	2.803714	0.000275	0.031291
Zc3h12a	2.779204	0.000168	0.022794
Ccr5	2.778692	4.97E-05	0.010661
Mt1	2.776905	4.10E-06	0.002317
BC037039	2.770779	2.05E-05	0.006305
Srgn	2.760421	0.000319	0.034695
Rcan1	2.759028	0.000148	0.021088
E030026E10Rik	2.74415	0.000229	0.027738
Lst1	2.734266	9.46E-08	0.000202
Lcp2	2.72069	2.23E-05	0.006711
Ncf4	2.713245	7.06E-05	0.01324
Lrrc25	2.705072	0.000141	0.020351
Itgal	2.700951	9.68E-05	0.016126
Mt2	2.699726	5.72E-05	0.011728
Gm48717	2.653877	0.000257	0.029929
Ncf2	2.653687	0.000146	0.020912
Gm26742	2.62338	2.47E-05	0.007131
Zbp1	2.616413	0.000211	0.026157
AB124611	2.613832	3.21E-05	0.008261
Trpc5	2.604558	0.000156	0.021774
Cd53	2.590778	0.000339	0.036119
Rhoh	2.589674	3.35E-06	0.002024
Sele	2.578514	1.21E-06	0.001087
Gm21188	2.550636	4.75E-05	0.010464
Ptges	2.539639	4.32E-06	0.00235
Plac8	2.529418	0.000538	0.049183
Ccl9	2.529094	0.000168	0.022794
Pik3r5	2.51024	7.10E-05	0.013257

Gm12098	2.508841	2.28E-05	0.006748
Gm42809	2.507481	0.000428	0.042144
Artn	2.501937	0.000327	0.035273
Fyb	2.500819	0.000101	0.016507
2210407C18Rik	2.483928	7.96E-05	0.014229
Gm5530	2.468977	0.000151	0.021284
Cd52	2.46763	0.000319	0.034695
Cd300lb	2.463499	6.63E-05	0.012621
Socs1	2.457373	0.000168	0.022794
Lrg1	2.450409	5.03E-05	0.010733
Tyrobp	2.449183	0.000546	0.049633
0610043K17Rik	2.43959	3.21E-06	0.001971
Ffar2	2.433878	6.41E-06	0.002962
Aif1	2.430398	5.10E-05	0.010767
Snx20	2.425291	0.000139	0.020146
Rgs16	2.418774	3.79E-06	0.002194
Padi4	2.418354	6.17E-05	0.012135
Nod2	2.403242	2.38E-05	0.006996
Gm15844	2.385178	0.000168	0.022794
Itih4	2.379474	2.69E-05	0.007232
Ly6c2	2.377502	0.000466	0.04447
Il1bos	2.375409	0.00026	0.03014
Stom	2.359067	9.12E-06	0.003834
AI662270	2.352562	3.13E-06	0.001971
Pirb	2.348971	0.000286	0.032091
Gm13091	2.341961	0.000392	0.039767
Gm16045	2.339938	0.000195	0.025028
Cd300a	2.329763	1.26E-05	0.004672
Gadd45a	2.328642	6.92E-07	0.000741
Ccr2	2.324207	0.000486	0.045751
Hck	2.320335	6.44E-05	0.012461
Rab20	2.316565	1.09E-06	0.001007
Junb	2.31439	6.69E-08	0.000164
Syt12	2.300389	2.93E-06	0.001971
Ccl6	2.292134	0.00016	0.022256
Gm15987	2.291314	2.97E-07	0.000432
Lilra6	2.290779	0.000302	0.033191
Cd80	2.288272	8.74E-05	0.015274
Pdpn	2.282605	0.000284	0.032058
Pilrb2	2.281694	0.000206	0.025858

Arid5a	2.272371	4.54E-06	0.002394
Scimp	2.270107	0.000366	0.038111
Gmfg	2.268258	3.19E-06	0.001971
Cass4	2.252464	1.56E-05	0.005277
Nfkbiz	2.243437	4.20E-05	0.009733
Bin2	2.242951	5.15E-05	0.010822
Rab44	2.235048	0.000383	0.039146
Adamts5	2.232957	0.000338	0.036111
Cytip	2.218123	0.00015	0.021284
Tuba1c	2.211519	0.000287	0.032091
Slc5a1	2.202848	8.92E-05	0.015437
Gch1	2.193718	2.55E-05	0.007167
Npl	2.19206	0.000208	0.026063
Adm2	2.191319	0.000362	0.037855
Ackr1	2.181561	0.000125	0.018942
Atp1a3	2.166644	7.46E-05	0.013619
Clec5a	2.164432	0.000101	0.016507
Kmo	2.162926	0.000163	0.022415
Glt1d1	2.161875	6.06E-06	0.002835
Gk	2.16011	5.85E-05	0.01184
Nfam1	2.149151	0.000155	0.02172
Grrp1	2.113634	8.30E-07	0.000843
Gm44916	2.112267	0.000195	0.025028
Gpr132	2.109756	0.000373	0.038676
Vav1	2.10275	5.90E-06	0.002835
Gpr65	2.081459	0.000161	0.022256
Ier3	2.053188	0.00018	0.023699
Mid1ip1	2.050834	3.15E-07	0.00044
Csf2rb2	2.047092	1.10E-07	0.000219
Fam65b	2.041853	0.00012	0.018582
Mirt1	2.038975	0.000352	0.037384
9430002A10Rik	2.024678	0.000504	0.046864
Runx3	2.021801	6.23E-05	0.012135
Odc1	2.009436	0.000442	0.043001
AC133868.1	2.00851	9.97E-05	0.016397
Alox5ap	2.007447	0.000395	0.039821
Batf	2.002294	6.69E-05	0.012621
Il13ra1	1.999044	0.000209	0.02609
Slc43a1	1.988818	0.000184	0.024046
Myd88	1.986964	1.22E-08	5.95E-05

Il2rb	1.985154	0.000549	0.049783
Napsa	1.980179	0.000118	0.018441
Cpne8	1.968221	7.02E-07	0.000741
Ltb4r1	1.967032	8.11E-09	4.84E-05
Fcgr2b	1.963542	0.000474	0.044891
Gm17907	1.963092	0.000434	0.042418
Sh3bp2	1.962156	1.34E-08	5.99E-05
Tnfaip2	1.94806	0.000409	0.040793
Nfkbia	1.93845	0.000135	0.019898
A930012L18Rik	1.935446	8.78E-05	0.015283
Ubash3b	1.9253	0.000167	0.022794
D6Ertd527e	1.917604	0.000406	0.04061
Adam28	1.908895	3.20E-06	0.001971
Ceacam1	1.898718	0.00024	0.028709
Ifi209	1.896273	0.000287	0.032091
Elf3	1.886015	0.000208	0.026063
Nek6	1.875102	0.000378	0.038893
Defb1	1.86921	1.93E-05	0.006078
Fas	1.868781	3.10E-08	9.80E-05
Jaml	1.866494	0.000182	0.02391
Dhrs13	1.863488	1.31E-05	0.004805
Gm31718	1.859857	0.000306	0.033503
Gm37452	1.858179	0.000201	0.02556
Gm47316	1.835472	0.000394	0.039821
Arhgdig	1.829406	0.000382	0.039101
Atp8b4	1.824095	0.00038	0.039028
Nfkbie	1.806087	3.34E-05	0.008434
Ifitm2	1.769873	2.29E-06	0.001688
Prss27	1.766312	1.57E-05	0.005285
Tnfrsf18	1.766072	4.06E-05	0.009497
Litaf	1.765486	4.70E-08	0.000133
Hspa2	1.755174	0.000531	0.048596
Icam1	1.754223	2.20E-08	8.46E-05
Abcg4	1.746812	0.000103	0.016627
Runx1	1.746355	7.72E-09	4.84E-05
Gm26917	1.746351	2.97E-07	0.000432
Cldn5	1.743998	5.05E-06	0.002564
Snhg15	1.733235	2.88E-06	0.001971
Slc6a20a	1.728845	0.000111	0.017594
Gm47644	1.723888	0.000501	0.046742

Trim30a	1.721394	1.14E-05	0.004384
Skap2	1.719777	2.46E-06	0.001768
Ifitm3	1.714254	7.74E-06	0.003438
Gm43420	1.704836	0.000454	0.04381
Tnfrsf1b	1.690773	0.00041	0.040869
Gm26586	1.69036	0.000451	0.04362
Rnasel	1.689301	4.54E-06	0.002394
Slc27a3	1.666857	1.96E-06	0.001554
Il17ra	1.664868	1.55E-06	0.0013
CT010505.3	1.663317	0.000507	0.046944
Slc1a1	1.658447	0.000302	0.033191
Gm26804	1.655971	0.000147	0.020918
Apobr	1.633514	0.000197	0.025133
Gm15943	1.624391	0.000118	0.018398
Tmprss4	1.61052	1.26E-05	0.004672
Pgpep1l	1.606773	0.000424	0.041886
Smox	1.604911	0.000322	0.034944
Irf1	1.602983	0.000517	0.047749
Gm10277	1.6006	0.00043	0.042181
Ppan	1.589095	1.70E-08	7.02E-05
AI839979	1.586106	0.000239	0.028709
Zfp593	1.585876	0.000393	0.039821
Ly6a	1.57616	0.000162	0.022347
Snhg4	1.572066	6.92E-07	0.000741
Srm	1.567803	0.000249	0.029297
Stat3	1.565837	4.28E-07	0.000549
Micall2	1.562507	4.41E-05	0.009922
Birc3	1.562506	3.45E-06	0.002039
Osmr	1.560255	2.19E-07	0.000346
Nr2c2ap	1.545682	4.84E-05	0.010547
Defb14	1.538814	0.000162	0.022347
Nrros	1.534954	2.04E-05	0.006305
Vdr	1.521242	0.000385	0.039336
Grwd1	1.506349	1.39E-06	0.001229
Nop58	1.503107	6.92E-07	0.000741
Arl4d	1.499606	0.000132	0.019788
Gm15832	1.497759	0.000354	0.037398
Ripk3	1.489135	0.000424	0.041886
Sept9	1.482962	4.80E-06	0.002457
Hivep3	1.474325	7.66E-06	0.003436

Ydjc	1.472795	0.000237	0.028546
Snhg1	1.468645	7.80E-07	0.000808
Camkk2	1.468381	9.96E-06	0.003998
Epb41l4aos	1.468049	4.96E-05	0.010661
1110038B12Rik	1.467358	5.68E-06	0.002755
Fbl	1.466534	5.44E-06	0.002684
BC064078	1.465072	9.96E-05	0.016397
Ier5l	1.459533	1.13E-06	0.001033
Trim11	1.456326	1.15E-07	0.000221
Mapk11	1.451039	3.69E-05	0.009014
Tuba1b	1.449726	3.22E-06	0.001971
Sphk1	1.448005	1.91E-05	0.006032
Rpl28	1.440872	0.000269	0.03091
Gins1	1.440784	0.000224	0.027211
Ctps	1.436355	2.16E-06	0.001613
Trmt61a	1.433973	1.86E-05	0.00597
Wdr74	1.431407	4.34E-05	0.009922
Npm3	1.431206	1.11E-05	0.004354
Npm1	1.429152	4.80E-05	0.010505
Ctla2a	1.420274	0.000358	0.03759
Sema6b	1.417684	5.93E-05	0.011904
Tdh	1.413747	6.63E-05	0.012621
Irf9	1.410279	3.44E-05	0.008607
Gpr35	1.398581	0.000348	0.037026
Nme1	1.396203	0.000124	0.018942
Ankrd10	1.391748	1.69E-06	0.001369
Cyba	1.39143	9.23E-05	0.015716
Gprc5c	1.364253	0.000242	0.028899
Tnfrsf1a	1.361073	9.38E-06	0.003912
Tnfrsf10b	1.35941	9.61E-05	0.016106
Plk2	1.348074	7.32E-06	0.003308
Adgrb2	1.331179	0.000331	0.035563
Ptk6	1.33044	0.000214	0.026372
Dok2	1.324372	0.000476	0.044975
Shisa5	1.316317	7.39E-05	0.013619
Fzd4	1.313553	0.000135	0.019898
Zfas1	1.308153	4.23E-06	0.002322
Mettl1	1.306224	2.89E-06	0.001971
Nhp2	1.304751	8.50E-06	0.003636
Ranbp1	1.304203	0.000175	0.023389

Aprt	1.302916	2.45E-05	0.007131
Txn1	1.302716	9.64E-05	0.016106
Eva1c	1.298308	0.000447	0.043321
Eif1a	1.294903	9.85E-06	0.003998
Ppih	1.293795	0.000521	0.047957
Phgdh	1.292434	2.08E-05	0.006341
Rpl12	1.288237	3.87E-05	0.00927
Nop56	1.284361	1.51E-05	0.005163
Ltbr	1.279407	5.12E-06	0.002574
Snhg12	1.278336	6.44E-06	0.002962
Tmem238	1.266387	0.000135	0.01991
Hgh1	1.26567	0.000153	0.021592
Trim8	1.261789	7.54E-05	0.013699
Ip6k3	1.258863	2.96E-05	0.007819
Relb	1.255427	2.84E-05	0.007614
Marcks	1.250353	0.000259	0.030117
Gar1	1.246866	1.58E-05	0.005285
Polr1e	1.242174	0.000122	0.018873
Noc4l	1.241106	6.05E-06	0.002835
Snhg5	1.237778	2.96E-05	0.007819
Nip7	1.234448	1.43E-05	0.005005
Gm17066	1.233831	0.000308	0.03371
Gnl3	1.224975	1.63E-05	0.005374
Apobec3	1.224427	0.000492	0.046029
Nradd	1.223302	0.000248	0.029297
Rhov	1.221906	0.000354	0.037398
Cebpb	1.21717	5.24E-05	0.010959
Wdr77	1.217005	5.68E-06	0.002755
Ccdc86	1.213796	3.01E-05	0.007904
Mrto4	1.21045	2.27E-05	0.006748
Ptpn2	1.207614	0.000201	0.02556
Rrp12	1.202836	8.52E-06	0.003636
Cd3eap	1.201008	1.42E-05	0.005005
D16Ertd472e	1.200419	0.000172	0.023091
Nop16	1.199888	2.28E-05	0.006748
Nolc1	1.199853	5.09E-05	0.010767
Slc39a1	1.198958	3.89E-05	0.00927
Mst1r	1.19326	3.93E-05	0.009319
Baz1a	1.192993	0.000316	0.034495
Efna5	1.19124	0.000133	0.01981

Sat1	1.189019	0.000173	0.023101
Mir17hg	1.184316	0.000302	0.033191
Rpl10a	1.182958	4.35E-05	0.009922
Tnfrsf21	1.182115	0.000134	0.01988
Lyar	1.173594	5.38E-05	0.01118
Igsf3	1.167289	3.78E-05	0.009149
Mzt2	1.159071	0.000205	0.025858
Rel	1.158269	3.96E-05	0.009343
Arntl2	1.157207	0.000444	0.043092
Cdkn2d	1.151987	3.29E-05	0.008399
Nsmce1	1.151367	0.000178	0.02351
1700017B05Rik	1.149926	0.000377	0.038855
Pgam1	1.145061	0.000413	0.041082
Ext1	1.142103	0.000295	0.032741
Llph	1.141203	7.04E-05	0.01324
Mif	1.137846	7.47E-05	0.013619
Hpn	1.1377	0.000418	0.04146
Snrpd1	1.13762	0.000176	0.023446
2410006H16Rik	1.136313	7.23E-05	0.013396
Hmgn1	1.136022	0.000125	0.018942
Bola2	1.125426	9.90E-05	0.016393
Cyb561	1.12507	0.000192	0.024836
Tnip2	1.123136	8.10E-05	0.014391
Flot1	1.120429	0.000213	0.026319
Rdh10	1.119229	0.000441	0.042987
Ssbp4	1.11613	0.000109	0.017479
Rps12	1.114932	0.000487	0.045751
Eif4a1	1.109529	0.000271	0.03106
Art1	1.109098	0.000334	0.035704
Pak6	1.108558	0.000236	0.028518
Polr2h	1.105898	0.000253	0.029654
Ckap4	1.105098	0.000187	0.024401
Pum3	1.080736	0.000466	0.04447
Nop2	1.065503	0.000196	0.02503
Rrp1b	1.059752	0.000282	0.031893
Ampd2	1.059735	0.000266	0.030679
Pprc1	1.052469	0.000172	0.023101
Wdr43	1.047244	0.000276	0.031399
Xbp1	1.046452	0.000487	0.045751
Gpatch4	1.045512	0.000373	0.038676

Heatr1	1.042119	0.000492	0.046029
Rfng	1.034298	0.000145	0.020848
Col5a2	-1.08675	0.000358	0.03759
Kitl	-1.09477	0.000107	0.017174
Rassf8	-1.0952	0.000464	0.044383
Sptbn1	-1.10151	0.000169	0.022814
Fzd7	-1.10386	0.000455	0.043814
Ntn1	-1.1039	0.000146	0.020912
Usf3	-1.11062	0.000124	0.018942
Colec12	-1.11325	0.00047	0.044635
Dnaja4	-1.15113	0.000331	0.035563
Sema3c	-1.15256	0.000401	0.040374
Podxl	-1.16877	0.000281	0.031893
Fgd4	-1.17564	0.000404	0.040519
Dusp18	-1.18233	4.97E-05	0.010661
Pltp	-1.18746	0.000332	0.035588
Cdon	-1.19768	0.000301	0.033191
Tmod2	-1.20924	9.05E-05	0.015464
Pkd1	-1.2434	1.96E-05	0.006115
Pdpr	-1.24391	0.000522	0.047972
Kcnj12	-1.24546	1.15E-05	0.004384
Itpkb	-1.25096	1.41E-05	0.005005
Pm20d2	-1.25333	0.00042	0.0416
Itga9	-1.25405	6.65E-05	0.012621
Kcnc1	-1.26006	1.65E-05	0.005374
Shroom4	-1.26984	8.55E-05	0.015019
Cav1	-1.27056	8.46E-05	0.014947
Jam2	-1.27174	0.000271	0.03106
Tiam2	-1.27998	0.000209	0.02609
Ptprb	-1.29204	0.000195	0.025028
Slc6a2	-1.30576	0.000542	0.0494
Ncam1	-1.30833	0.000469	0.044635
Crybg3	-1.36183	0.000113	0.017847
Npr3	-1.37474	0.000124	0.018942
Stard9	-1.40081	0.000504	0.046864
Igsf10	-1.41166	0.000194	0.025028
Asah2	-1.41803	9.03E-05	0.015464
4931406P16Rik	-1.42037	3.39E-06	0.002024
Adamts12	-1.42057	0.000433	0.042418
Myh10	-1.42168	3.64E-05	0.008985

Dach1	-1.45987	0.000179	0.023645
Nov	-1.46118	0.000166	0.022794
Il17d	-1.47116	0.000452	0.04362
Gucy1b3	-1.48272	3.31E-05	0.008399
Mme	-1.48365	0.000215	0.026492
Gm45884	-1.49795	0.00029	0.032322
Map1a	-1.5013	0.000441	0.042987
Adamtsl3	-1.50572	0.000137	0.020053
Nkd1	-1.51443	0.000245	0.029047
Fat4	-1.53105	2.23E-05	0.006711
Sema3d	-1.54022	0.000187	0.024401
Btc	-1.54725	0.000404	0.040519
Dact3	-1.55552	0.000204	0.025759
Adcy5	-1.5596	9.59E-05	0.016106
Slc9a3r2	-1.5887	6.22E-05	0.012135
Lrrc52	-1.59848	7.43E-05	0.013619
Tril	-1.61402	0.000523	0.047988
Sec16b	-1.61524	3.75E-05	0.009127
Chp2	-1.63306	5.85E-05	0.01184
Fam221a	-1.63736	0.000134	0.01988
Timp3	-1.64101	0.000362	0.037855
Myrip	-1.64821	0.000546	0.049633
Rarb	-1.65136	0.000529	0.048471
Stmnd1	-1.66294	0.000293	0.032655
Esrrg	-1.66346	6.49E-05	0.012509
Gucy1a3	-1.67853	1.07E-05	0.004218
Xirp1	-1.68833	3.56E-05	0.008834
Strip2	-1.70331	0.000285	0.032091
Tppp3	-1.70836	1.38E-07	0.000239
Snurf	-1.72682	0.000296	0.03279
Hecw2	-1.72918	1.12E-05	0.004354
Peg10	-1.73027	0.000122	0.018873
4932422M17Rik	-1.75385	0.000551	0.049905
Mest	-1.7812	3.66E-05	0.008985
Nt5c1a	-1.78796	0.00019	0.024748
Ackr4	-1.80778	0.000219	0.026759
Mamdc2	-1.8276	1.71E-06	0.001369
Kctd12b	-1.8368	0.000159	0.022114
Fam171b	-1.83855	8.57E-05	0.015019
Cnn1	-1.8626	0.000457	0.043889

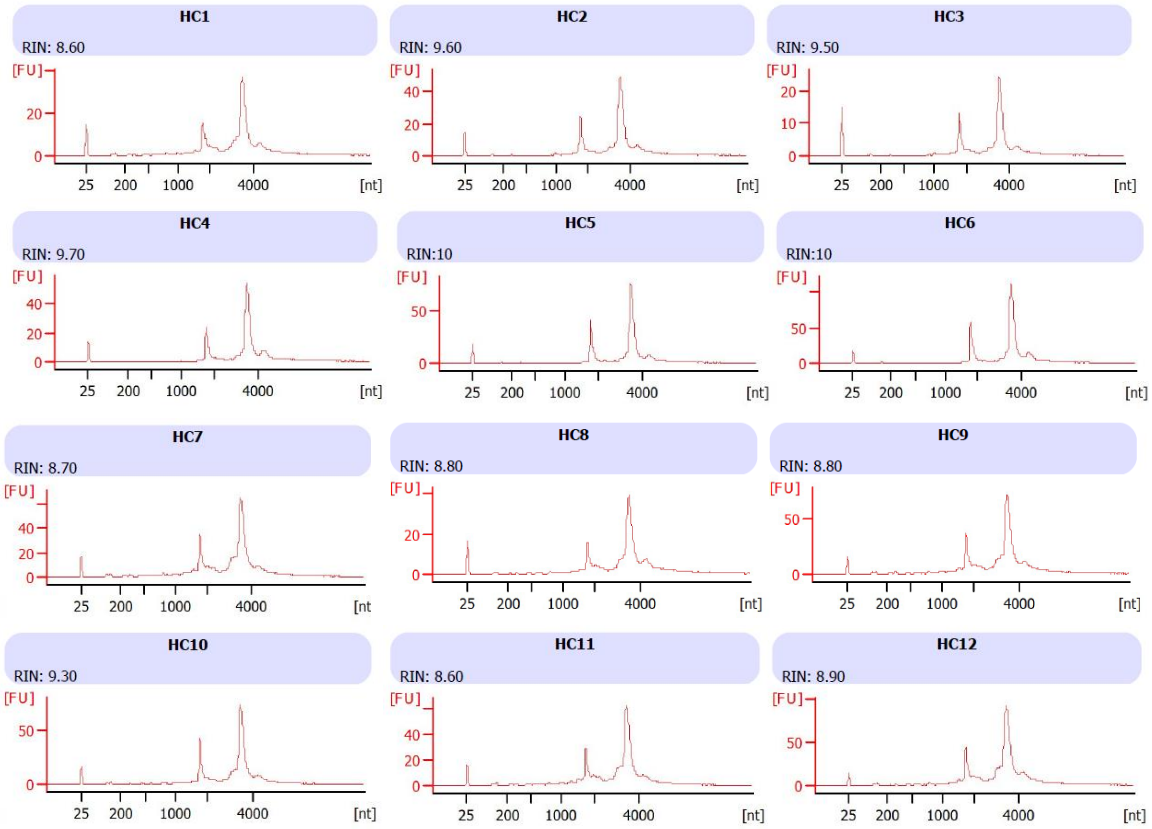
Tmem204	-1.88436	3.84E-05	0.00927
Cacna1h	-1.89845	6.06E-05	0.011991
Gabrg2	-1.9203	0.000103	0.016627
Nrk	-1.92572	0.000195	0.025028
2310015K22Rik	-1.93777	0.000115	0.018064
Tppp	-1.94341	1.82E-05	0.005904
Ikzf5	-1.9458	0.000119	0.018542
Gm16185	-1.99477	6.05E-05	0.011991
Phex	-1.99814	5.58E-05	0.011546
Nynrin	-2.01941	7.76E-05	0.013956
Slfn3	-2.04145	0.000248	0.029297
Smoc1	-2.05066	5.69E-05	0.011728
Lgi1	-2.05095	0.000221	0.026992
Zdbf2	-2.05246	1.69E-06	0.001369
Capn6	-2.0567	9.01E-05	0.015464
Tmem119	-2.09274	2.49E-05	0.007131
Fam198b	-2.09469	5.23E-06	0.002605
Zcchc5	-2.0958	0.000324	0.035122
Gpr34	-2.11385	3.39E-05	0.008519
Gm15935	-2.12519	0.000132	0.019788
Ttc25	-2.14457	0.000487	0.045751
Pkhd1l1	-2.16286	2.67E-05	0.007232
Cubn	-2.18113	0.000171	0.023091
Abca9	-2.19426	2.57E-06	0.00182
Cxxc4	-2.26439	0.000391	0.039732
9230112J17Rik	-2.3399	0.00011	0.017482
Siglech	-2.34465	9.62E-05	0.016106
Celf5	-2.55417	5.38E-05	0.01118
Gm45226	-2.70892	0.00046	0.04416
Gm15663	-2.73137	0.000391	0.039732
Gm43197	-2.73251	2.63E-05	0.007232
4930511M06Rik	-2.8861	0.000213	0.026319
Cldn3	-3.01995	4.77E-05	0.010464
Gm32926	-3.06423	0.000518	0.047829
Hepacam2	-3.09096	1.65E-05	0.005374
Astn1	-3.12215	0.000328	0.035359
Rnf112	-3.33643	5.89E-05	0.011877
Hcar1	-3.39813	2.52E-05	0.007154
Aass	-3.88805	6.35E-05	0.012337
Diras2	-3.8994	1.41E-05	0.005005

Gm15883	-4.1092	0.000261	0.030206
Gm28653	-4.25092	0.000125	0.018942
Gm34583	-5.72183	0.000249	0.029297
Gm26945	-6.68613	0.000123	0.018913

Appendix Table B.2 *Defb3* Knockout Infected vs. *Defb3* Knockout Sham. This table represents all of the differentially expressed transcripts that are considered significant results (p-value < 0.05, FDR < 0.05). The table is sorted by logFC from the largest value to the smallest. 588 transcripts were identified.

Appendix C

Bioanalyzer Results



Appendix Table C-1 These are the bioanalyzer results from the University of Cincinnati. A RIN > 7 is high enough quality RNA for poly(A) RNA-seq. The key for the sample names can be found in Appendix Table C.3 (below).

RNA-Seq Name	Sample Name	Genotype	Status
HC1	912	Hom	Sham
HC2	915	WT	Sham
HC3	917	WT	Sham
HC4	919	WT	Sham
HC5	920	Hom	Sham
HC6	923	Hom	Sham
HC7	925	Hom	Infected
HC8	926	WT	Infected
HC9	927	Hom	Infected
HC10	928	Hom	Infected
HC11	930	WT	Infected
HC12	932	WT	Infected

Appendix Table C.3 This is the key for the name information used for the bioanalyzer results. 'Hom' stands for homozygous mutant, meaning a *Defb3* knockout mouse.