

### Research Article

## **Comparison of Host Gene Expression Profiles in Spleen Tissues of Genetically Susceptible and Resistant Mice during ECTV Infection**

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Ectromelia virus (ECTV), the causative agent of mousepox, has emerged as a valuable model for investigating the host-*Orthopoxvirus* relationship as it relates to pathogenesis and the immune response. ECTV is a mouse-specific virus and causes high mortality in susceptible mice strains, including BALB/c and C3H, whereas C57BL/6 and 129 strains are resistant to the disease. To understand the host genetic factors in different mouse strains during the ECTV infection, we carried out a microarray analysis of spleen tissues derived from BALB/c and C57BL/6 mice, respectively, at 3 and 10 days after ECTV infection. Differential Expression of Genes (DEGs) analyses revealed distinct differences in the gene profiles of susceptible and resistant mice. The susceptible BALB/c mice generated more DEGs than the resistant C57BL/6 mice. Additionally, gene ontology and KEGG pathway analysis showed the DEGs of susceptible mice were involved in innate immunity, apoptosis, metabolism, and cancer-related pathways, while the DEGs of resistant mice were largely involved in MAPK signaling and leukocyte transendothelial migration. Furthermore, the BALB/c mice showed a strong induction of interferon-induced genes, which, however, were weaker in the C57BL/6 mice. Collectively, the differential transcriptome profiles of susceptible and resistant mouse strains with ECTV infection will be crucial for further uncovering the molecular mechanisms of the host-*Orthopoxvirus* interaction.

#### 1. Introduction

Poxviruses comprise a diverse family of double-stranded DNA viruses that remain a threat to the human and livestock, despite the fact that naturally circulating variola virus (VARV), the causative agent of smallpox, was eradicated decades ago [1–3]. The possibility that clandestine stocks are being held by rogue nations or terrorist groups, as well as an increase in the frequency of zoonotic poxvirus infections, including monkeypox virus (MPXV), has increased attention in recent years [3–7]. VARV has a restricted host range and is known to only infect humans. Closely related *Orthopoxviruses* such as ectromelia virus (ECTV) are the best surrogate for the study of VARV in small animal models, as it also has a restricted host range and, in mice, the resulting disease shares common features with VARV [7–10]. ECTV has a very narrow host range and infection in mice causes mousepox. All laboratory mouse strains can be infected with very low doses of infectious particles, but different mouse genotypes display different susceptibility to lethal infection with ECTV [9–11]. Strains such as BALB/c, DBA/2, DBA2/J, CBA/H, and A/J are considered susceptible to severe disease, while C57BL/6, C57BL/10, AKR, and 129 mice show very low morbidity and mortality and limited pathology and are classified as resistant [12–15]. In addition to virus strain and other factors, such as route of infection, age, sex, and immune status, host genetic background is a critical factor which governs resistance to mousepox [9, 11]. At present, at least four known genetic loci have been identified in resistant inbred and out-bred mice [9, 16, 17]. *Ly49H* (also called *resistance to mousepox-1, Rmp-1*) maps to the

natural killer gene complex (NKC) and activates NK cells to control early virus replication in C57BL/6 mice, but this is lacking in BALB/c mice [11, 18-20]. Other loci, such as the *Rmp-2* locus that maps near the complement component C5 gene, *Rmp-3* locus that is linked to the MHC and is also gonad-dependent, and the *Rmp-4* locus that maps near the selectin gene complex, are also responsible for resistance to ECTV infection [11, 16, 21]. In addition, the humoral and cell mediated immune responses to ECTV infection are very different between BALB/c and C57BL/6 mice [11, 22-26]. C57BL/6 mice can generate robust NK cell, cytotoxic T lymphocytes (CTLs), and IFN- $\gamma$  responses. However, these responses are suboptimal but high levels of IL-4 are produced in BALB/c mice [11, 12, 26–28]. A polarized type 1 cytokine response, in particular IFN-y, and a potent cell mediated immune response determine the genetic resistance of C57BL/6 mice to mousepox. In contrast, a polarized type 2 cytokine response is generated in susceptible mouse strains (BALB/c and A/J), which is associated with a weak or absent CTL response, resulting in uncontrolled virus replication and animal death [11, 12]. Additional factors involved in innate and adaptive immunity are also required for inherent resistance to mousepox [11]. Type I IFNs induced by viral proteins and nucleic acids through the recognition of pathogen recognition receptors (PRRs) are essential for inherent resistance to mousepox in C57BL/6 mice [28-30]. Deficiencies in TLR9-MyD88-IRF7 and STING-IRF7/NF- $\kappa$ B result in inefficient production of type I IFNs, higher mortality rates, and accelerated death in C57BL/6 mice [28]. Other components of innate immunity, such as phagocytes, antigen-presenting cells, granzymes A and B, nitric oxide synthase 2, IL-12, and IL-18, also play essential roles in inherent resistance to mousepox [31–37].

Innate immunity is required, but not sufficient, for inherent resistance to mousepox. Decades of work on adaptive immunity have shown that a number of factors, at the molecular and cellular level, are essential to control ECTV infection in resistant strains [11]. B lymphocytes produce antibodies that can directly neutralize virus particles to prevent infection, and cluster of differentiation 40 (CD40) is essential for efficient antibody production and isotype switching [11, 22]. T cells are also involved in conveying resistance to primary ECTV infection [11]. CD8<sup>+</sup> T cell responses to ECTV in C57BL/6 mice are extremely strong and bond with the cell surface major histocompatibility complex I (MHC I), which recognizes viral peptides, resulting in high susceptibility to mousepox [11, 22]. The function of CD4<sup>+</sup> T cells in resistance to mousepox is to produce anti-ECTV antibodies and kill the infected cells in a perforin-dependent manner [24, 38].

Despite the fact that decades of work have contributed to our knowledge of the pathogenesis and immunobiology of ECTV infection *in vivo*, the systemic differences between susceptible and resistant mouse strains during the ECTV infection have not yet been investigated. On the other hand, microarray methodology has been developed as a high throughput method to simultaneously analyze large datasets of gene expression patterns under various biological conditions [39]. Thus, to obtain a comprehensive view of the host responses to ECTV infection in different mouse strains at the mRNA level, we performed cDNA microarray analysis of mRNAs obtained from the spleens of two mouse strains, one susceptible and another one resistant to ECTV. This analysis revealed the shared and distinct expression profiles and strain-specific pathogenesis may be due to the differentially active pathways and differences of gene expression levels in the two different mouse strains.

#### 2. Materials and Methods

2.1. Ethics Statement. All animal work was conducted according to the Good Animal Practice Requirements of the Animal Ethics Procedures and Guidelines of the People's Republic of China. All experimental protocols were approved by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Science (permit number LVRIAEC2016-008).

2.2. Mice and Virus. Seven- to nine-week-old BALB/c and C57BL/6 SPF mice were purchased from the Experimental Animal Center of Lanzhou University, China. Upon arrival, animals were housed in a biosafety level 3 room and given free access to commercial mouse chow and water. After a one-week acclimatization period, the two strains of mice were randomly assigned to three experimental groups, with 5 mice per group.

The wild-type strain of ECTV was originally isolated from naturally infected laboratory mice and then propagated in Vero cells (unpublished data). Virus was confirmed by PCR using the specific primers (5'-ATGGACGGAACTCTT-TTC-3' and 5'-AACTTCATCGTTGCGTTTAC-3') and sequenced. The experimental infection of susceptible BALB/c mice was less virulent than the ECTV-Moscow strain (data unpublished). Plaque-purified ECTV was serially passaged in Vero cell for 21 generations, and virus titer was measured using a 50% tissue culture infective dose (TCID<sub>50</sub>) assay.

2.3. Virus Infection and Confirmation of Infection. Infection groups comprising 10 BALB/c and C57BL/6 mice were anesthetized and infected subcutaneously into the abdomen with 100 µL PBS containing 10<sup>4</sup> TCID<sub>50</sub> of ECTV. Ten uninfected age-matched mice (5 BALB/c and 5 C57BL/6) served as the control group and were euthanized by cervical dislocation before the spleen tissues were isolated. On days 3 and 10 after infection, 5 mice from each infection group were sacrificed and whole spleen tissues were harvested. All spleen tissues from each group were pooled into a cell culture plate and cut into pieces using surgical scissors. A total of 1.6 grams pooled spleen tissues were equally divided into 4 tubes, then snap frozen in liquid nitrogen, and stored at -70°C. For confirmation of infection, genomic DNA was extracted from the spleen tissues of infected or control groups. Then a PCR was performed to detect infection using the above-mentioned primers.

2.4. Virus Titration. To determine virus titers, 0.25 mL PBS was added to 0.25 g pooled spleen tissues and the tissue was homogenized using a disposable tissue-grinding pestle

(Sangon, Shanghai, China). The homogenized samples were frozen and thawed three times. Virus titration was assessed using a TCID<sub>50</sub> assay. Briefly, 100  $\mu$ L Vero cell suspension, containing 2 × 10<sup>4</sup> cells in DMEM with 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA), was seeded into each well of a 96-well plate and incubated at 37°C under 5% CO<sub>2</sub>. After 24 h incubation, 25  $\mu$ L of 10-fold serial dilutions was added to each well, with 8 replicates per dilution. Plates were incubated at 37°C for 10 days and checked daily for characteristic cytopathic effect (CPE). TCID<sub>50</sub> end-point titers were calculated using the Reed and Muench method [40].

2.5. Histopathology. Spleens were harvested and fixed with 10% neutral buffered formalin solution at 4°C for 4 h and then were embedded in paraffin. The paraffin-embedded specimens were cut into 5  $\mu$ m thick sections and stained with hematoxylin-eosin (H&E). Each slide of the samples was photographed with a digital optical microscope (Olympus, Tokyo, Japan).

2.6. Microarray Analysis. Frozen samples were sent to CapitalBio Co. (Beijing, China), who performed the microarray experiment according to protocols provided by Affymetrix. Briefly, total RNA was isolated from the samples and the quality and quantity of RNA were assessed using formaldehyde agarose gel electrophoresis and spectrophotometry. Biotin-labeled fragmented cRNA samples were subjected to hybridization with GeneChip Mouse Genome Arrays (GeneChip® Mouse Genome 430 2.0) (Affymetrix, Santa Clara, California, USA), which contained 39000 probes representing 34000 mouse genes. Hybridization was performed at 45°C in an Affymetrix GeneChip Hybridization Oven 640 (Affymetrix, Santa Clara, California, USA), with rotation, for 16 h. Arrays were scanned using a confocal scanner (LuxScan 10K-A, CapitalBio, Beijing, China) and images were analyzed using SpotData software (CapitalBio, Beijing, China). Scanned images were assessed first by visual inspection and then analyzed to generate raw data files that were saved as .cel files using the default settings of the Gene Chip operating software. All data were deposited into GEO (Gene Expression Omnibus) database at http://www.ncbi.nlm.nih.gov/geo/ info/linking.html under the accession number GSE100644. Microarray data were analyzed by using Bio MAS (molecule annotation system) 3.0 software (CapitalBio Corporation, Beijing, China). Using the criterion of cutoff limitation as a fold change  $\geq 2$  or  $\leq 0.5$ , differential expression genes were screened and clustered.

2.7. Validation of Microarray Data. Nine DEGs from each time point were selected and used to quantify gene expression levels using real-time quantitative PCR. A total of 26 DEGs were verified and expression levels were normalized against the housekeeping standard, glyceraldehyde-3phosphate dehydrogenase (GAPDH). Primers were designed and synthesized by Sangon Biotech Company, and sequences are listed in Table S1. Total RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) and reversetranscribed to single strand cDNA using a first strand cDNA synthesis kit (AMV, Roche, Germany) according the manufacturer's instructions. Real-time quantitative PCR was performed using SG Fast qPCR Master Mix Kit (BBI, Shanghai, China) in a final volume of 20  $\mu$ L, containing 10  $\mu$ L 2x SYBR Green qPCR Master Mix, 4  $\mu$ M each primer, 7.2  $\mu$ L nuclease-free water, and 2  $\mu$ L cDNA. PCR amplification was performed and run in triplicate under the following conditions: one cycle of 95°C for 3 min, followed by 40 cycles of 95°C for 7 s, 57°C for 30 s, and 72°C for 15 s. Gene expression was analyzed using the  $2^{-\Delta\Delta Ct}$  method.

#### 3. Results

3.1. Virus Infection and Viral Loads in the Spleen Tissues from ECTV-Infected Mice. Genetically susceptible BALB/c and resistant C57BL/6 mice were injected with 10<sup>4</sup> TCID<sub>50</sub> of ECTV (in 100  $\mu$ L PBS) into the abdominal subcutaneous tissue. After infection, the BALB/c mice began to exhibit disease symptoms at 7 days after infection (dpi) and one animal succumbed to the disease at 10 dpi. The C57BL/6 mice developed no significant symptoms during the course of the experiment and exhibited no mortality (Figure 1(b)). Spleen tissues were chosen because of the essential roles for the induction of protective antiviral immune responses to ECTV and the site for virus replication [11, 28]. Moreover, the viral loads and the level of responses to ECTV in the spleen are discrepant between susceptible BALB/c and resistant C57BL/6 mice [11]. Our preexperiment of pathological sections from ECTV-infected BALB/c and C57BL/6 mice displayed significantly higher pathology in the former mice at 10 dpi (Figure 1(a)). We next measured ECTV viral titers in pooled spleen tissues from the two mouse strains at 3, 7, and 10 dpi. Virus particles were detected in spleen tissues from both mouse strains. In BALB/c mice, a low virus titer was detected as early as 3 dpi and continued to increase with time (Figure 1(c)). In contrast, the virus was not detected in C57BL/6 mice at 3 dpi but reached up to  $10^{4.48} \mbox{ and } 10^{5.66}$  $TCID_{50}$ /gram tissue by 7 and 10 dpi, respectively (Figure 1(c)). Despite the undetected virus particles at 3 dpi, the presence of virus genomes was confirmed by PCR assay (data not shown). In general, compared to susceptible BALB/c mice, lower viral loads were detected in resistant C57BL/6 mice at all time points, suggesting that ECTV infects efficiently cells of susceptible BALB/c mice.

3.2. Changes in the Transcriptome Profile of Spleens from BALB/c and C57BL/6 Mice during ECTV Infection. The overriding aim of these studies is to elucidate host transcriptome profile changes caused by ECTV infection, by comparing results from genetically susceptible and resistant mice. Spleen tissues were isolated from BALB/c and C57BL/6 mice at 3 and 10 dpi and used for microarray analysis. Both profiles were compared to samples from mock-infected control mice. The differentially expressed genes (DEGs) were filtered using the criterion of cutoff limitation as a fold change  $\geq 2$  or  $\leq 0.5$ . After normalization, a total of 744 genes were expressed differentially (with 470 up- and 274 downregulated) in BALB/c mice and approximately half of the number of genes (361) were found to be altered (123 up- and 238 downregulated) in



FIGURE 1: (a) Spleen sections of the indicated mice at 10 dpi stained with H&E at 10 dpi. (b) The number of BALB/c and C57BL/6 mice exhibiting disease symptoms after ECTV infection. Five mice in each group were injected with  $10^4$  TCID<sub>50</sub> of ECTV into the abdominal subcutaneous tissue. The status of infected mice was checked daily. (c) ECTV titers in spleen tissues of susceptible and resistant mice. Groups of BALB/c and C57BL/6 mice infected with ECTV were killed on the days indicated (3, 7, and 10 dpi). Viral titers in pooled spleen of each group were determined with three replications. \* \* \* means P < 0.001.

C57BL/6 mice at 3 dpi. At 10 dpi, more genes were perturbed in both BALB/c and C57BL/6 mice. Scrutiny of the data showed that 2184 genes (with 1453 up- and 731 downregulated) were altered in susceptible BALB/c mice, while only 1619 DEGs (540 up- and 1079 downregulated) were perturbed by ECTV infection in C57BL/6 mice (Table 1 and Table S2). Of note, more genes were upregulated over the time course in BALB/c mice than they were in C57BL/6 mice.

To further understand the transcriptome profile changes in genetically susceptible and resistant mice during ECTV infection, we listed the genes that were most significantly up- or downregulated (fold change in expression) in the two mice strains at 3 and 10 dpi. As shown in Table 2, different genes were altered in each of the two strains during ECTV infection. At 3 dpi, interferon-stimulated genes (ISGs), including *Gbp1* (guanylate-binding protein 1), *Gbp2*, and *Iigp1* (interferon inducible GTPase 1), were upregulated in the susceptible mice, whereas only *Ifn-* $\zeta$  (interferon zeta) was

TABLE 1: Total number of differentially expressed genes (DEGs)<sup>a</sup>.

Mouse strain	BA	LB/c	C57	BL/6
After infection	3 dpi	10 dpi	3 dpi	10 dpi
Number of upregulated genes	470	1453	123	540
Number of downregulated genes	273	731	238	1079
Total number	743	2184	361	1619

<sup>a</sup>Microarray data of infected groups were normalized with uninfected group of each mouse strain. The DEGs were filtered using the criterion of cutoff limitation as a fold change  $\ge 2$  or  $\le 0.5$ .

slightly upregulated in the C57BL/6 mice at this time point. Among the genes that were upregulated at 10 dpi, *GzmB* (granzyme B) showed the greatest fold change in both strains. Other granzymes, such as *GzmD* and *GzmK*, were also upregulated in both strains. *Ifn*- $\gamma$  (interferon gamma) was activated during the later stage of infection, with a 24.2-fold increase in

TABLE 2: List of 10 DEGs that were most up	- or downregulated in BALB/c and	C57BL/6 during ECTV infection <sup>b</sup> .
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Mouse strain		BA	LB/c			C57	7BL/6	
After infection	3 dpi vers	us uninfected	10 dpi vei	rsus uninfected	3 dpi vers	sus uninfected	10 dpi ve	rsus uninfected
Gene category	Gene	Fold change	Gene	Fold change	Gene	Fold change	Gene	Fold change
	Кар	5.65	Spp1	46.2	Xist	27.28	Xist	50.37
	Hspa1b	4.39	Gzmb	39.4	Ttr	4.49	Hspa1b	26.23
	Gbp1	4.32	Ifng	24.2	Tsks	3.13	Gzmb	22.26
	Gdi2	3.99	Vcan	20.7	Mettl11a	2.93	Gbp1	17.84
Upregulated	Apol7c	3.95	Mmp3	17.9	Hspa1b	2.81	Hspala	15.25
opregulated	Slfn4	3.60	Nts	16.5	Ptger1	2.68	Ctsg	14.02
	Gbp2	3.57	Timp1	14.0	Alb	2.59	Mcpt8	10.06
	Ddx6	3.47	Gzmd	13.6	Ifnz	2.34	Rgs1	8.74
	Iigp1	3.42	Saa3	13.6	Dppa5a	2.32	Gzmk	7.71
	Acaa2	3.41	Cxcl5	12.6	Cml3	2.29	Prtn3	7.03
	Vmn1r148	0.33	Ctrb1	0.08	Psap	0.29	Bpgm	0.17
	Phxr5	0.33	Try4	0.02	Cyp4f16	0.28	Dbp	0.17
	Reg2	0.32	Cpb1	0.03	Foxp1	0.28	Igfbp5	0.17
	Psg28	0.29	Cela3b	0.03	Epsti1	0.28	Ccr9	0.16
Downregulated	Mup10	0.28	Marco	0.03	Abhd12	0.27	Apol7c	0.16
Downregulated	Astx	0.25	Clps	0.04	Кар	0.27	Kdm5d	0.16
	Adipoq	0.25	Zg16	0.05	Трт3	0.26	Cyr61	0.15
	Cfd	0.16	Emr4	0.06	Ddx3y	0.25	Ddx3y	0.14
	Car3	0.08	Pnlip	0.02	Sh3bgrl	0.25	Eif2s3y	0.13
	Mettl11a	0.06	Amy2a1	0.006	Hmgcs1	0.22	Igfbp3	0.12

<sup>b</sup>The DEGs were ranked by fold change and those were the most (fold change) up- or downregulated in expression at 3 and 10 dpi in BALB/c and C57BL/6 mice.

BALB/c mice (BALB/c 10 dpi versus BALB/c uninfected) and a 5.2-fold increase in C57BL/6 mice (C57BL/6 10 dpi versus C57BL/6 uninfected). Interestingly, *Gbp1*, *Gbp2*, and *Iigp1* which are induced by IFN- $\gamma$  were significantly upregulated at 3 dpi while IFN- $\gamma$  was upregulated late (10 dpi), suggesting that basic expression of IFN- $\gamma$  produced by CD8<sup>+</sup> cells may be able to induce the upregulation of *Gbp1*, *Gbp2*, and *Iigp1* at 3 dpi and then the expression of IFN- $\gamma$  was upregulated through feedback at 10 dpi. As the previous study showed, IFN- $\gamma$ -producing cells were detected as early as 2 dpi in the spleen and the peak IFN- $\gamma$  production by MHC class Irestricted CD8<sup>+</sup> T cells was presented at 8 dpi [12].

3.3. Pathway Analysis of ECTV Infection in Two Mouse Strains at Different Time Points. We constructed Venn diagrams to gain insight into the DEGs that were either unique or shared at the different time points and/or in the different mouse strains. As shown in Figures 2(a) and 2(b), 313 genes in BALB/c mice and 108 genes in C57BL/6 mice were common to all time points. KEGG pathway analysis of the 313 common genes revealed that the most statistically significant (P <0.05) canonical pathways (ranked by P value) were the T cell receptor signaling pathway, spliceosomes, antigen processing and presentation, prostate cancer, natural killer cell mediated cytotoxicity, the cytosolic DNA-sensing pathway, the Toll-like receptor (TLR) signaling pathway, and the NODlike receptor signaling pathway. Only the B cell receptor signaling pathway, leukocyte transendothelial migration, and MAPK signaling pathway were included in the 108 DEGs in C57BL/6 mice. For the cytosolic DNA-sensing pathway and TLR signaling pathway, DEGs including *Zbp1* (Z-DNA binding protein 1), *Cxcl10* (chemokine (C-X-C motif) ligand 10), *Chuk* (conserved helix-loop-helix ubiquitous kinase), *MAP2K4* (mitogen-activated protein kinase kinase 4), and *STAT1* (signal transducer and activator of transcription 1) showed increased expression in the susceptible mice.

The number of unique genes at 3 and 10 dpi in BALB/c mice was 430 and 1871, respectively. Of the 430 unique genes were those that could be classified under canonical pathways including natural killer cell mediated cytotoxicity, Wnt signaling, and allograft rejection. At 10 dpi, the canonical pathways associated with the 1871 unique transcripts included cytokine-cytokine receptor interaction, chemokine signaling pathway, complement and coagulation cascades, and Jak-STAT signaling pathway. All of these pathways are important in metabolism and host response, and the majority of genes involved showed increased expression in the susceptible mice.

In the C57BL/6 mice, 253 and 1511 unique transcripts were altered at 3 and 10 dpi, respectively. At the early challenge (3 dpi), fewer unique transcripts were differentially expressed that were involved in Fc gamma R-mediated phagocytosis, lysosome, and leukocyte transendothelial migration. Of the 1511 unique genes differentially expressed at 10 dpi, the most statistically significant canonical pathways were colorectal cancer, intestinal immune network for IgA production, DNA



FIGURE 2: Analysis of common and unique gene expression differentials in two mouse strains at different times of postinfection. Venn diagrams of differentially expressed genes (DEGs) in BALB/c (a) and C57BL/6 (b) mice at 3 days and 10 days after infection (dpi). Venn diagrams of differentially expressed genes (DEGs) at 3 dpi (c) and 10 dpi (d) in two mouse strains.

replication, and Fc Epsilon Receptor 1 signaling pathway. The genes involved in these pathways were mainly downregulated in the resistant mice.

Venn diagrams relating the same time points in different mouse strains showed that only 89 and 479 common DEGs were altered at 3 and 10 dpi, respectively (Figures 2(c) and 2(d)). KEGG pathway analysis revealed that no canonical pathways were associated with those 89 common genes at 3 dpi, while the 479 common altered genes at 10 dpi could be classified into canonical pathways that included natural killer cell mediated cytotoxicity and apoptosis. For natural killer cell mediated cytotoxicity, *Fasl* (Fas ligand), *Fcgr4* (Fc receptor, IgG, low affinity IV), *GzmB* (granzyme B), *Ifn*- $\gamma$  (interferon gamma), *Klrc1* (killer cell lectin-like receptor subfamily C, member 1), and *Klrk1* (killer cell lectin-like receptor subfamily K, member 1) showed increased expression, whereas only *Cd244* (CD244 natural killer cell receptor 2B4) showed decreased expression.

The numbers of genes unique at 3 and 10 dpi time points were 654 and 1705 in BALB/c mice, which were 272 and 1140 in C57BL/6 mice, respectively. At 3 dpi, the 654 unique genes in BALB/c mice were aligned with the canonical pathways associated with ubiquitin mediated-proteolysis, toxoplasmosis, and T cell receptor signaling pathways, while the 272 genes in C57BL/6 mice were only involved in leukocyte transendothelial migration, HIF-1 signaling pathway, and Fc

gamma R-mediated phagocytosis. All these pathways were important in regulation of immune response process. At 10 dpi, the pathways associated with 1705 unique transcripts in BALB/c mice encompassed protein digestion and absorption, biosynthesis of amino acids, transcriptional misregulation in cancer, glycerolipid metabolism, and Jak-STAT signaling pathway. Of the 1140 unique genes differentially expressed in C57BL/6 mice, the most statistically significant canonical pathways were HTLV-I infection, protein processing in endoplasmic reticulum, MAPK signaling pathway, intestinal immune network for IgA production, and T cell receptor signaling pathway. A higher proportion of the genes involved in adaptive immune response of C57BL/6 mice suggested a robust antiviral response against ECTV infection.

Taken together, these results suggest that ECTV infection affects the expression of genes involved in molecular and cellular functions. More pathways involved in the host metabolism and innate immune response to infection were induced in the susceptible BALB/c mice, such as metabolism of amino acids and innate immune signaling in nucleic acid recognition. The C57BL/6 mice showed resistance to the infection and more adaptive immune-related pathways were therefore affected during the infection, suggesting the different genetic factors and adaptive immune response are the most important to control the infection.



FIGURE 3: Verification of the gene expression by qRT-PCR. Nine differentially expressed genes (DEGs) containing up- or downregulated genes in microarray analysis at each time point (3 and 10 dpi) were selected randomly for validation of the RNA-seq data. Gene expression in ECTV-infected C57BL/6 (a) and BALB/c (b) mice using qRT-PCR was analyzed using the  $2^{-\Delta\Delta Ct}$  method.

3.4. Differential Expression Levels of Innate Immune Genes in Two Mouse Strains during Infection. The innate immune system represents the first line of host defense against pathogen infection. Various elements of the innate immune response have been implicated in the cellular reaction to, and restriction of, viral infection, including type I and type II IFNs, ISGs, chemokines, interleukins, granzymes, and innate immune cells (including dendritic cells, macrophages, and NK cells). To obtain transcriptomic information about these genes and innate immune cells related genes, we assessed differences in the expression level of selected genes involved in the innate antiviral immune response in the two mice strains during ECTV infection.

We performed a DEGs analysis of ISGs stimulated by ECTV infection in C57BL/6 and BALB/c mice at 3 and 10 dpi (Table 3). The results revealed that more ISGs were upregulated at 10 dpi than at 3 dpi in both C57BL/6 and BALB/c mice. Furthermore, all of these genes were more strongly upregulated at 10 dpi, suggesting a reinforcement of differential gene expression over time. In addition, almost all of these genes were more strongly upregulated in BALB/c mice than in C57BL/6 mice, and some of these genes were upregulated only in BALB/c mice, suggesting they are more sensitive to ECTV infection. Of note, some members of the interferoninduced GTPase family, including GBP1, 2, 3, 7, and 8, were found to follow a similar upward trend and were more strongly upregulated than other genes in both BALB/c and C57BL/6 mice, suggesting the importance of GBPs in the response to ECTV infection.

Finally, differences in chemokines, interleukins, and granzymes were examined in the two mouse strains (Table 4). IFN- $\zeta$  and IFN- $\alpha$ 2 were upregulated in C57BL/6 mice but not in BALB/c mice, and this was true for *GzmA* and *IL1F9* 

expression. GzmB was strongly upregulated in both mouse strains at the late stage of viral infection, while other members (GzmC, GzmD, GzmE, and GzmF) were upregulated only in BALB/c mice at 10 dpi. Some chemokines, such as Cxcl1, Cxcl5, Cxcl9, Cxcl10, Cxcl11, and Ccl3, were upregulated in BALB/c mice at 3 and 10 dpi but were only slightly upregulated in C57BL/6 at 10 dpi. As might be expected, interleukins, complement, and some immunoregulatory molecules were upregulated in BALB/c mice but were only slightly changed, or not at all, in C57BL/6 mice. Members of the killer cell lectin-like receptor subfamily were also affected by ECTV infection. KLRC1 and KLRK1 were upregulated in both C57BL/6 and BALB/c mice at 10 dpi, but KLRG1 was upregulated only in C57BL/6 mice. Taken together, these results suggest that a more enhanced innate immune response to ECTV infection occurred in BALB/c mice than in C57BL/6 mice, which may reflect fundamental differences in the genetic background of the host.

3.5. Validation of Microarray Data. To validate the microarray data, we used the same RNA samples to perform qRT-PCR. We measured the expression of 26 upregulated and downregulated genes at each postinfection time point for the two mouse strains. The selected genes are mostly involved in innate immune response which are interested in our future work. As shown in Figure 3, the qRT-PCR results were largely consistent with the microarray analysis. For some genes, however, the fold change values were lower in the qRT-PCR data than in the microarray results. These included *IFNZ* (0.16-fold versus 0.44-fold), *OASL2* (2.25-fold versus 3.01-fold), *ZBP1* (2.77-fold versus 5.29-fold), *IFI205* (1.27-fold versus 2.27-fold) in BALB/c mice, and *METTL11A* (1.65-fold versus 2.91-fold) and *IFI44* (1.49-fold versus 2.42-fold) in

		Genes	3 d	pi*	10 d	pi*
Gene symbol	RefSeq	Gene name	$B^i/B^u$	C <sup>i</sup> /C <sup>u</sup>	$B^i/B^u$	C <sup>i</sup> /C <sup>u</sup>
OASL1	AB067533	2′-5′-Oligoadenylate synthetase-like 1	1.91	0.82	3.34	1.21
OAS2	AB067535	2′-5′-Oligoadenylate synthetase 2	1.90	1.13	4.15	1.23
IGTP	NM_018738	Interferon gamma induced GTPase	2.03	0.88	2.48	2.39
IFI47	NM_008330	Interferon gamma inducible protein 47	2.07	0.81	2.60	1.66
IFIT2	NM_008332	Interferon-induced protein with tetratricopeptide repeats 2	1.54	0.80	3.69	1.29
IIGPI	BM239828	Interferon inducible GTPase I	3.42	0.96	5.32	3.64
IFI204	NM_008329	Interferon activated gene 204	1.52	0.85	6.10	2.16
IFI202B	NM_011940	Interferon activated gene 202B	1.49	0.90	4.00	1.15
IFI44	BB329808	Interferon-induced protein 44	2.49	0.89	4.93	2.42
IFITM1	BC027285	Interferon induced transmembrane protein 1	1.03	0.95	2.10	1.36
IFITM3	BC010291	Interferon induced transmembrane protein 3	1.09	1.13	1.73	1.49
IFITM6	BB193024	Interferon induced transmembrane protein 6	1.18	1.15	1.92	1.86
IFIHI	AY075132	Interferon induced with helicase C domain 1	1.57	1.17	1.82	1.42
ISG15	AK019325	Interferon-stimulated protein (15 kda)	1.73	1.22	1.93	0.92
GBPI	NM_010259	Guanylate binding protein 1	4.32	0.86	6.60	17.84
GBP2	NM_010260	Guanylate binding protein 2	3.60	0.82	7.15	3.28
GBP3	NM_018734	Guanylate binding protein 3	1.82	0.98	3.27	2.10
GBP7	BC010229	Guanylate binding protein 7	2.39	1.04	3.61	2.44
GBP8	NM_029509	Guanylate binding protein 8	1.69	1.21	2.24	3.20
MXI	M21039	Myxovirus resistance 1	1.29	1.16	3.30	1.05
$EIF2\alpha K2$	AV328340	Eukaryotic translation initiation factor 2- $\alpha$ kinase 2	2.17	0.97	2.49	0.91
CH25H	NM_009890	Cholesterol 25-hydroxylase	0.97	0.84	2.68	0.84
<sup>c</sup> The interferon-stimulat Superscripts "i" and "u"	ted genes (ISGs) differentia represent infected and unir	lly expressed in BALB/c and/or C57BL/6 were selected for analysis at 3 and 10 d nfected mice, respectively.	dpi. *The capital "B" aı	nd "C" represent BAL	B/c and C57BL/6 mic	e, respectively.

TABLE 3: The interferon-stimulated genes (ISGs) that were upregulated in BALB/c and C57BL/6 during ECTV infection<sup>c</sup>.

IEA         Gene mute $y_1^{(10)}$ $C_1^{(10)}$	Res         Gene symbol         Releq         Gene symbol         Releq         Gene symbol         Releq         Circle         Bips         Circle			Genes	3 d	pi*	10 d	pi*
IN-y         N0063         Interferon gamma         0.45         0.99         0.417         5.21           FN-d2         N10.0030         Interferon alpha 2         0.41         0.89         0.41         0.83         1.93           FN-d2         N10.0032         Interferon alpha 2         0.64         0.89         0.89         1.93         5.33           Gamb         N10.0172         Gamsyme B         0.73         0.88         0.89         0.89         0.93         5.94         2.23           Gamb         NN 0.0072         Gamsyme B         0.73         0.88         0.89         0.89         0.93         0.73         2.23         0.73         2.23         0.73         2.23         0.73         0.74         0.74         0.73         2.23         0.73         0.74         0.74         0.73         0.73         0.73         0.74         0.74         0.74         0.74         0.74         0.74         0.74         0.74         0.74         0.74         0.74         0.75         0.75         0.75         0.75         0.75         0.75         0.75         0.75         0.75         0.75         0.75         0.75         0.75         0.75         0.75         0.75         0.75 </th <th>IPA         Notosis         Interferon garma         0.95         0.89         3.17           FFN-d2         NM 00030         Interferon alpha 2         0.74         2.34         0.89           FFN-d2         NM 00137         Genrayne B         112         0.89         9.36           Gamb         NM 00137         Genrayne B         112         0.89         9.36           Gamb         NM 00172         Genrayne B         112         0.89         9.36           Gamb         NM 00172         Genrayne B         112         0.89         9.36           Gamb         NM 00172         Genrayne B         0.33         0.89         9.36           Gamb         NM 00172         Genrayne B         0.81         1.3         0.93         9.36           Gamb         MM 0017         Genrayne B         0.81         1.3         1.1         1.1         1.1         1.1         1.1         1.1         1.1         1.1         1.1         1.1         1.1         1.2         2.5         5           Gamb         MM 0014         Cennotine (C-X Contol) ligned 1.1         1.1         1.1         1.1         2.4         1.1         2.4         1.1         2.6</th> <th>Gene symbol</th> <th>RefSeq</th> <th>Gene name</th> <th><math>B^i/B^u</math></th> <th>C<sup>i</sup>/C<sup>u</sup></th> <th><math>B^i/B^u</math></th> <th>C<sup>i</sup>/C<sup>u</sup></th>	IPA         Notosis         Interferon garma         0.95         0.89         3.17           FFN-d2         NM 00030         Interferon alpha 2         0.74         2.34         0.89           FFN-d2         NM 00137         Genrayne B         112         0.89         9.36           Gamb         NM 00137         Genrayne B         112         0.89         9.36           Gamb         NM 00172         Genrayne B         112         0.89         9.36           Gamb         NM 00172         Genrayne B         112         0.89         9.36           Gamb         NM 00172         Genrayne B         0.33         0.89         9.36           Gamb         NM 00172         Genrayne B         0.81         1.3         0.93         9.36           Gamb         MM 0017         Genrayne B         0.81         1.3         1.1         1.1         1.1         1.1         1.1         1.1         1.1         1.1         1.1         1.1         1.1         1.2         2.5         5           Gamb         MM 0014         Cennotine (C-X Contol) ligned 1.1         1.1         1.1         1.1         2.4         1.1         2.4         1.1         2.6	Gene symbol	RefSeq	Gene name	$B^i/B^u$	C <sup>i</sup> /C <sup>u</sup>	$B^i/B^u$	C <sup>i</sup> /C <sup>u</sup>
IBV         BI03227         Interform adda         0.44         2.14         0.89         100           RN-43         NU00070         Granyme A         0.93         1.93         1.13         2.93         2.93           Gam         NU 00070         Granyme A         0.93         1.13         0.87         3.93         3.93           Gam         NU 00073         Granyme A         0.93         0.87         3.93         3.93           Gam         NU 00073         Granyme A         0.93         0.87         3.93         3.93           Gam         NU 00073         Granyme A         0.93         0.87         3.93         3.93           Gam         NU 00073         Granyme A         0.93         0.93         3.23         3.93           Gam         MU 00793         Granyme A         0.63         0.93         3.23         3.93           Gam         MU 00793         Granyme A         0.63         0.93         3.23         3.23           Gam         MU 00793         Killer cell certu-like receptor subianty G, member 1         1.13         1.13         3.22         3.23           Killer cell certu-like receptor subianty G, member 1         1.13         1.13         3.13 <th>IFN-C         BRO-202 BRO-2017         Interferen acta         0.44         2.34         0.09           Gam         NM 000570         Gamzyne B         0.29         1.26         0.29           Gam         NM 00077         Gamzyne B         0.29         1.26         0.29           Gam         NM 0072         Gamzyne B         0.29         0.87         9.39           Gam         NM 0072         Gamzyne B         0.27         0.87         9.39           Gam         NM 00723         Gamzyne B         0.27         0.87         9.39           Gamzyne B         Gamzyne B         0.38         0.39         0.36         9.36           Gamzyne B         Gamzyne B         0.38         0.39         0.35         5.36           Gamzyne B         Gamzyne B         0.38         0.39         0.35         5.36           Gamzyne B         Gamzyne B         0.36         0.37         5.36         5.36           KIRG         MM 00470         Killer cell tech 4/k menber 1         1.13         0.07         5.26           Gamzyne B         Gamzyne B         Gamzyne B         0.36         0.37         5.36           CKCI         MM 00470         Killer cell tech</th> <th>IFN-y</th> <th>K00083</th> <th>Interferon gamma</th> <th>0.95</th> <th>0.89</th> <th>24.17</th> <th>5.21</th>	IFN-C         BRO-202 BRO-2017         Interferen acta         0.44         2.34         0.09           Gam         NM 000570         Gamzyne B         0.29         1.26         0.29           Gam         NM 00077         Gamzyne B         0.29         1.26         0.29           Gam         NM 0072         Gamzyne B         0.29         0.87         9.39           Gam         NM 0072         Gamzyne B         0.27         0.87         9.39           Gam         NM 00723         Gamzyne B         0.27         0.87         9.39           Gamzyne B         Gamzyne B         0.38         0.39         0.36         9.36           Gamzyne B         Gamzyne B         0.38         0.39         0.35         5.36           Gamzyne B         Gamzyne B         0.38         0.39         0.35         5.36           Gamzyne B         Gamzyne B         0.36         0.37         5.36         5.36           KIRG         MM 00470         Killer cell tech 4/k menber 1         1.13         0.07         5.26           Gamzyne B         Gamzyne B         Gamzyne B         0.36         0.37         5.36           CKCI         MM 00470         Killer cell tech	IFN-y	K00083	Interferon gamma	0.95	0.89	24.17	5.21
FN-act         NM 00050         Interference (april 2)         0.79         1.76         0.82         1.35           Gamb         NM 01957         Granzyme 5         Granzyme 7         0.83         0.93         0.83         0.93         0.83         0.93 </td <td>FN-42         NM.00503         Interfeon alpha 2         0.23         1.76         0.83           Gam         NM.01537         Ganzyme A         0.33         1.26         0.83         3.93           Gam         NM.01537         Ganzyme A         0.83         1.13         0.89         5.93           Gam         NM.01037         Ganzyme C         0.82         0.83         3.93           Gam         NM.01037         Ganzyme C         0.82         0.83         1.03         3.93           Gam         NM.01037         Ganzyme C         0.83         0.93         5.93         5.93           Ganzyme C         Ganzyme C         0.84         0.83         0.93         5.26           KIMG         NM.01087         Granzyme K         0.84         0.93         5.26           KIMA         Af99308         Killer cell letrin like respons subfamily G, member 1         1.13         2.72           KIMA         Af99308         Killer cell letrin like respons subfamily G, member 1         1.13         2.72           KIMA         Af99308         Killer cell letrin like respons subfamily G, member 1         1.13         2.72           KIMA         Af9308         Killer cell letrin like respons subfamily G, member 1</td> <td>IFN-Ç</td> <td>BF022827</td> <td>Interferon zeta</td> <td>0.44</td> <td>2.34</td> <td>0.89</td> <td>1.00</td>	FN-42         NM.00503         Interfeon alpha 2         0.23         1.76         0.83           Gam         NM.01537         Ganzyme A         0.33         1.26         0.83         3.93           Gam         NM.01537         Ganzyme A         0.83         1.13         0.89         5.93           Gam         NM.01037         Ganzyme C         0.82         0.83         3.93           Gam         NM.01037         Ganzyme C         0.82         0.83         1.03         3.93           Gam         NM.01037         Ganzyme C         0.83         0.93         5.93         5.93           Ganzyme C         Ganzyme C         0.84         0.83         0.93         5.26           KIMG         NM.01087         Granzyme K         0.84         0.93         5.26           KIMA         Af99308         Killer cell letrin like respons subfamily G, member 1         1.13         2.72           KIMA         Af99308         Killer cell letrin like respons subfamily G, member 1         1.13         2.72           KIMA         Af99308         Killer cell letrin like respons subfamily G, member 1         1.13         2.72           KIMA         Af9308         Killer cell letrin like respons subfamily G, member 1	IFN-Ç	BF022827	Interferon zeta	0.44	2.34	0.89	1.00
Gam         NM.0020         Granzyne K         Granzyne K         Ganzyne K         Ganz	Grant         NM.0020         Granzyme A         0.68         1.83         1.13           Grant         NM.01037         Granzyme F         Granzyme F         0.83         0.93         3.93           Grant         NM.01037         Granzyme F         Granzyme F         Granzyme F         0.83         0.93         3.93           Grant         NM.01037         Granzyme F         Granzyme F         Granzyme F         0.83         0.93         2.53           Granzyme F         Granzyme F         Granzyme F         Granzyme F         0.84         0.93         2.53         0.93         2.53         0.93         2.53           Granzyme F         Granzyme F         Granzyme F         0.84         0.93         2.53         2.53         2.53           KIJKC         NM.01037         Granzyme F         Granzyme F         0.84         0.93         2.53         2.53           KIJKC         NM.01087         Killer cell lettri-like receptor subfamily K, member 1         1.13         1.13         2.73         2.73           KIJKC         NM.01087         Killer cell lettri-like receptor subfamily K, member 1         1.13         1.13         2.73         2.73           KIJKC         NM.01087         Killer cell lettri-li	IFN- $\alpha 2$	NM_010503	Interferon alpha 2	0.79	1.76	0.82	1.54
Gamb         NM 01343         Canayme B         112         0.87         33.93         35.95         0.323           Gamb         NM 01073         Gamyme C         Gamyme B         112         0.87         33.95         0.103           Gamb         NM 01073         Gamyme B         Gamyme B         113         0.98         5.96         0.103           Gamb         NM 01073         Gamyme B         Gamyme B         0.01         0.93         2.03         0.103           Gamb         NM 01074         Gamyme B         Gamyme B         0.03         0.03         2.03         0.03         2.03         0.03           KLKR         M 010570         Kliller cell letrih lite receitor submity G, member 1         1.13         1.13         1.13         2.74         2.74         2.74         2.74	Gzmb         NM 01343         Granzyme B         112         0.05         39.3           Gzmb         NM 010373         Granzyme D         Granzyme D         Granzyme D         0.39         39.3           Gzmb         NM 01073         Granzyme D         Granzyme D         Granzyme D         0.39         39.3           Gzmb         NM 01073         Granzyme D         Granzyme D         0.39         39.3         39.3           Gzmb         NM 01073         Granzyme D         Granzyme D         0.38         0.39         3.26           Gzmb         NM 01073         Granzyme D         Granzyme D         0.38         0.39         3.26           Gzmb         NM 010814         Lille cell lectri-like receptor subfamily G, member 1         1.19         0.07         3.26           KLKC         NM 010914         Chemolate CX C contril) igand 1         2.43         0.09         3.26           CXCL10         NM 00914         Chemolate CX C contril) igand 1         2.41         1.11         5.56           CXCL11         NM 00934         Chemolate CX C contril) igand 1         2.43         0.30         3.36           CXCL11         NM 00934         Chemolate CX C contril) igand 1         1.14         1.11         3.36 </td <td>GzmA</td> <td>NM_010370</td> <td>Granzyme A</td> <td>0.68</td> <td>1.83</td> <td>1.13</td> <td>5.51</td>	GzmA	NM_010370	Granzyme A	0.68	1.83	1.13	5.51
Game         NM 00071         Granyme C         Granyme T         Granym T <thgranyme t<="" th=""> <thgranyme< td=""><td>Game         NM 00071         Granzyme F         Granzym F         Granzyme F         Granzym F</td><td>GzmB</td><td>NM_013542</td><td>Granzyme B</td><td>1.12</td><td>0.87</td><td>39.39</td><td>22.26</td></thgranyme<></thgranyme>	Game         NM 00071         Granzyme F         Granzym F         Granzyme F         Granzym F	GzmB	NM_013542	Granzyme B	1.12	0.87	39.39	22.26
Gamb         NMJ.0072         Granyme D         Gauyme D         Gauyme D         Gauyme D         MMJ.0073         Gauyme D         MMJ.0073         Gauyme D         MMJ.0074         MMJ.0074         MMJ.0074         MMJ.0074         MMJ.0074         MMJ.0074         Gauyme D         MMJ.0074         MJJ.0074         MMJ.0074 <t< td=""><td>Gamb         NM 00032         Granzyme E         Gamzyme F         0.95         1.65           Gamf         NM 00033         Granzyme K         1.97         0.95         1.65           Gams         NM 00033         Granzyme K         1.97         0.95         1.65           Gams         NM 00033         Granzyme K         0.67         1.17         1.06         2.66           Gams         NM 00035         Killer cell letrin-like receptor subfamily C, member 1         0.67         1.17         1.06         2.67           KLRK1         NM 00830         Killer cell letrin-like receptor subfamily C, member 1         1.13         1.13         2.75           KLRK1         NM 00840         Chenokine (C-X c motif) ligand 1         2.43         1.16         2.66           CXC12         NM 01934         Chenokine (C-X c motif) ligand 1         1.47         1.11         2.33           CXC12         NM 01934         Chenokine (C-X c motif) ligand 1         1.47         1.11         2.34           CXC13         NM 01934         Chenokine (C-X c motif) ligand 1         1.47         1.11         2.34           CXC14         NM 01934         Chenokine (C-X c motif) ligand 1         1.47         1.11         2.66           <td< td=""><td>GzmC</td><td>NM_010371</td><td>Granzyme C</td><td>0.92</td><td>0.89</td><td>5.96</td><td>0.96</td></td<></td></t<>	Gamb         NM 00032         Granzyme E         Gamzyme F         0.95         1.65           Gamf         NM 00033         Granzyme K         1.97         0.95         1.65           Gams         NM 00033         Granzyme K         1.97         0.95         1.65           Gams         NM 00033         Granzyme K         0.67         1.17         1.06         2.66           Gams         NM 00035         Killer cell letrin-like receptor subfamily C, member 1         0.67         1.17         1.06         2.67           KLRK1         NM 00830         Killer cell letrin-like receptor subfamily C, member 1         1.13         1.13         2.75           KLRK1         NM 00840         Chenokine (C-X c motif) ligand 1         2.43         1.16         2.66           CXC12         NM 01934         Chenokine (C-X c motif) ligand 1         1.47         1.11         2.33           CXC12         NM 01934         Chenokine (C-X c motif) ligand 1         1.47         1.11         2.34           CXC13         NM 01934         Chenokine (C-X c motif) ligand 1         1.47         1.11         2.34           CXC14         NM 01934         Chenokine (C-X c motif) ligand 1         1.47         1.11         2.66 <td< td=""><td>GzmC</td><td>NM_010371</td><td>Granzyme C</td><td>0.92</td><td>0.89</td><td>5.96</td><td>0.96</td></td<>	GzmC	NM_010371	Granzyme C	0.92	0.89	5.96	0.96
Grant         NMJ.00233         Granzyme E         L33         0.03         10.1         10.1           KLIKGI         NMJ.00734         Granzyme K         0.03         0.02         1.02         1.01           KLIKGI         NMJ.00730         KILler cell tectin-like receptor subfamily G, member 1         1.19         0.67         1.17         1.06         355           KLIKGI         AT00005         Killer cell tectin-like receptor subfamily G, member 1         1.13         1.13         2.23         2.24         1.19         2.25         2.25         2.26<	Game         NM 00373         Gransyme E         133         0.98         0.01           Gam         NM 00374         Gransyme K         6         9.9         268           Gam         NM 00374         Gransyme K         6         9.9         268           KLRd1         NM 00370         Killer cell hertin-like receptor subfamily C, member 1         11.9         0.07         11.7         11.6           KLRd1         NM 00375         Killer cell hertin-like receptor subfamily C, member 1         11.9         0.67         5.57           CXCL3         NM 00376         Killer cell hertin-like receptor subfamily C, member 1         11.9         0.67         5.57           CXCL3         NM 00374         Chemokine (C, XC motif) ligand 1         2.43         0.99         2.66           CXCL1         NM 00374         Chemokine (C, XC motif) ligand 3         2.14         0.97         3.93           CXCL1         NM 00374         Chemokine (C, XC motif) ligand 3         2.14         0.97         3.93           CXCL1         NM 0137         Chemokine (C, XC motif) ligand 3         1.14         1.11         5.66           CXCL3         NM 0137         Chemokine (C, XC motif) ligand 3         1.14         1.11         5.46	GzmD	NM_010372	Granzyme D	1.07	0.95	13.63	1.02
Gam         Nu 0034         Gramyme F         0.88         0.99         2.68         1.23           KLRG         NM .00570         Killer cell lectri-like receptor subfamily G, member 1         0.11         0.61         0.99         2.68         1.23           KLRG         NM .00870         Killer cell lectri-like receptor subfamily G, member 1         0.11         0.61         0.99         2.68         1.23           KLRG         AP09303         Killer cell lectri-like receptor subfamily G, member 1         1.13         1.13         1.13         2.75         2.19           CXCL1         NM .00879         Chemokine (C.X.C motf) lignal 1         2.43         0.16         2.22         2.26           CXCL1         NM .00879         Chemokine (C.X.C motf) lignal 1         1.47         1.11         2.23         2.26         2.26           CXCL1         NM .00879         Chemokine (C.X.C motf) lignal 1         2.41         0.03         2.66         2.21           CXCL1         NM .01874         Chemokine (C.X.C motf) lignal 1         1.14         1.11         5.36         2.26           CXL1         NM .01874         Chemokine (C.X.C motf) lignal 1         1.47         1.11         5.34         1.24           CXL1         NO8999         <	Gzmif         NM.003/4         Gransyme F         Gamsyme K         0.38         0.99         258           KIRCI         NM.016970         Killer cell lextin-like receptor subfamily G, member 1         113         0.67         117         105         577           KIRCI         NM.016970         Killer cell lextin-like receptor subfamily G, member 1         113         0.67         117         105         577           KIRCI         NM.008176         Killer cell lextin-like receptor subfamily G, member 1         113         0.67         113         2.24         116         2.756           CXCL15         NM.009149         Chemokine (C.X. cmotif) lignad 3         1.47         1.19         0.09         2.56           CXCL10         NM.009149         Chemokine (C.X. cmotif) lignad 4         1.47         1.11         5.36           CXCL10         NM.009494         Chemokine (C.X. cmotif) lignad 4         1.14         1.11         5.36           CXCL11         NM.019494         Chemokine (C.X. cmotif) lignad 4         0.33         1.24         0.93         5.46           CXCL12         NM.01949         Chemokine (C.X. cmotif) lignad 4         1.14         1.11         5.56           CXCL2         AV084994         Chemokine (C.X. cmotif) lignad 4 <t< td=""><td>GzmE</td><td>NM_010373</td><td>Granzyme E</td><td>1.33</td><td>0.98</td><td>10.21</td><td>1.07</td></t<>	GzmE	NM_010373	Granzyme E	1.33	0.98	10.21	1.07
GzmK         M0.06700         Granyme K         0.61         0.93         5.22         7.21           K1RC1         AF006006         Killer cell lectri-like receptor subfamily G, member 1         0.61         0.93         5.52         2.15           K1RC1         AF006006         Killer cell lectri-like receptor subfamily G, member 1         1.13         1.13         2.16         1.19           CXCL3         NM.008140         Chemokine (C-X-C motif) ligand 5         2.43         1.06         2.54         2.16         1.18           CXCL10         NM.003174         Chemokine (C-X-C motif) ligand 5         2.43         1.07         2.54         2.24           CXCL11         NM.003174         Chemokine (C-X-C motif) ligand 4         2.41         0.93         5.46         2.27           CXCL11         NM.01377         Chemokine (C-X-C motif) ligand 4         2.41         0.93         5.46         2.27           CXCL11         NM.01377         Chemokine (C-X-C motif) ligand 4         2.41         0.93         5.46         2.27           CXCL11         NM.01377         Chemokine (C-X-C motif) ligand 4         2.41         1.11         5.11         2.47         2.47           CXCL12         NM.01377         Chemokine (C-C motif) ligand 4 <td< td=""><td>Gamk         M032200         Granzyne K         O.61         0.93         723           KIRG         NM 00570         Killer cell lectin-like receptor subfamily G, member 1         0.61         0.93         723           KIRG         AP00005         Killer cell lectin-like receptor subfamily G, member 1         0.61         0.93         527           KIRG         AP00005         Killer cell lectin-like receptor subfamily G, member 1         1.97         1.13         2.72           CXCL1         NM 00914         Chemokine (C.X.C motif) ligand 3         2.44         0.99         2.64           CXCL1         NM 00914         Chemokine (C.X.C motif) ligand 3         2.44         0.93         5.46           CXCL1         NM 00914         Chemokine (C.X.C motif) ligand 3         2.44         0.93         5.46           CXCL1         NM 00914         Chemokine (C.X.C motif) ligand 3         1.17         1.11         5.39           CXCL1         NM 00914         Chemokine (C.X.C motif) ligand 3         1.13         1.11         5.46           CXCL1         NM 00914         Chemokine (C.X.C motif) ligand 3         1.12         1.11         5.46           CXCL2         NM 00125         Chemokine (C.X.C motif) ligand 3         1.13         1.11         1.11&lt;</td><td>GzmF</td><td>NM_010374</td><td>Granzyme F</td><td>0.88</td><td>0.99</td><td>2.68</td><td>1.22</td></td<>	Gamk         M032200         Granzyne K         O.61         0.93         723           KIRG         NM 00570         Killer cell lectin-like receptor subfamily G, member 1         0.61         0.93         723           KIRG         AP00005         Killer cell lectin-like receptor subfamily G, member 1         0.61         0.93         527           KIRG         AP00005         Killer cell lectin-like receptor subfamily G, member 1         1.97         1.13         2.72           CXCL1         NM 00914         Chemokine (C.X.C motif) ligand 3         2.44         0.99         2.64           CXCL1         NM 00914         Chemokine (C.X.C motif) ligand 3         2.44         0.93         5.46           CXCL1         NM 00914         Chemokine (C.X.C motif) ligand 3         2.44         0.93         5.46           CXCL1         NM 00914         Chemokine (C.X.C motif) ligand 3         1.17         1.11         5.39           CXCL1         NM 00914         Chemokine (C.X.C motif) ligand 3         1.13         1.11         5.46           CXCL1         NM 00914         Chemokine (C.X.C motif) ligand 3         1.12         1.11         5.46           CXCL2         NM 00125         Chemokine (C.X.C motif) ligand 3         1.13         1.11         1.11<	GzmF	NM_010374	Granzyme F	0.88	0.99	2.68	1.22
KLRCI         NM 00670         Klaile receptor subfamily G, member 1 $0.67$ $117$ $106$ $355$ KLRCI         AF100008         Killer cell lectri-like receptor subfamily G, member 1 $119$ $0.67$ $112$ $110$ $273$ $119$ $275$ $118$ $273$ $119$ $275$ $119$ $275$ $119$ $275$ $119$ $275$ $119$ $275$ $119$ $275$ $218$ $226$	KLRG1         M.006700         Killer cell lectin-like receptor subfamity G, member 1         0.67         117         116           KLRG1         AF00000         Killer cell lectin-like receptor subfamity G, member 1         0.97         57         57           KLRK1         AF00003         Killer cell lectin-like receptor subfamity G, member 1         113         113         2.13         2.76           CXCL10         NM.00849         Chemokine (C-X-C motif) ligand 10         2.43         116         2.33           CXCL10         NM.00849         Chemokine (C-X-C motif) ligand 10         2.43         111         5.19         2.76           CXCL10         NM.00849         Chemokine (C-X-C motif) ligand 10         2.43         111         5.19         2.76           CXCL10         NM.01843         Chemokine (C-X-C motif) ligand 10         2.41         111         5.19         2.46           CXCL2         NM.01843         Chemokine (C-X-C motif) ligand 10         2.41         111         5.19         2.46           CXCL3         NM.01843         Chemokine (C-X-C motif) ligand 10         2.43         111         5.19         2.46           CXCL3         NM.01843         Chemokine (C-X-C motif) ligand 10         2.41         111         2.41         1.46	GzmK	AB032200	Granzyme K	0.61	0.93	7.52	7.71
KLRC         AF10008         Killer cell terin ike receptor subfamily C, member 1         11.9         0.67         557         11.8           CXCL3         NM.00914         Chernokine (C, X-C motf) ligand 5         2.43         0.90         1.16         7.16         1.85           CXCL1         NM.00914         Chernokine (C, X-C motf) ligand 5         2.43         0.90         2.261         1.86           CXCL10         NM.00137         Chernokine (C, X-C motf) ligand 1         2.41         0.93         5.46         2.21           CXCL11         NM.00137         Chernokine (C, X-C motf) ligand 1         1.47         1.11         5.86         1.07           CXCL13         NM.00137         Chernokine (C, X-C motf) ligand 1         1.47         1.11         5.86         1.15           CXCL2         APR65933         Chernokine (C, C motf) ligand 1         1.47         1.11         5.86         1.17           CXCL2         APR69333         Chernokine (C, C motf) ligand 1         1.47         1.11         5.86         1.17           CXCL3         NM.01343         Chernokine (C, C motf) ligand 1         1.47         1.11         5.86         1.75           CXCL3         NM.01343         Chernokine (C, C motf) ligand 1         1.47         1.1	KLRCI         AF09008         Killer cell letrin-like receptor subfamily K, member 1         11.9         0.67         557           KLRKI         NF00008         Killer cell letrin-like receptor subfamily K, member 1         11.3         11.3         27.2           CXCL1         NM.00914         Chemokine (C.X.C motif) ligand 5         2.43         11.0         2.39           CXCL1         NM.00944         Chemokine (C.X.C motif) ligand 3         2.41         0.99         2.61           CXCL1         NM.00949         Chemokine (C.X.C motif) ligand 4         0.91         1.47         1.11         5.86           CXCL1         NM.01337         Chemokine (C.X.C motif) ligand 4         0.93         5.46         5.95         5.46           CXCL1         NM.01137         Chemokine (C.X.C motif) ligand 4         0.85         1.11         5.86         5.46           CXCL2         NM.01137         Chemokine (C.C motif) ligand 4         0.85         0.23         4.45           CXCL2         NM.01137         Chemokine (C.C motif) ligand 4         0.85         0.23         4.45           CXCL2         NM.01137         Chemokine (C.C motif) ligand 4         0.85         0.23         4.45           CXCL2         NM.01137         Chemokine (C.C motif) ligand 7 </td <td>KLRG1</td> <td>NM_016970</td> <td>Killer cell lectin-like receptor subfamily G, member l</td> <td>0.67</td> <td>1.17</td> <td>1.06</td> <td>3.56</td>	KLRG1	NM_016970	Killer cell lectin-like receptor subfamily G, member l	0.67	1.17	1.06	3.56
KLRK1         AF03005         Kliller ccll letti-like receptor subfamily K member 1         113         113         272         273         213           CXCL1         NM.00876         Chemokine (C.X. C motf) ligand 1         2.43         116         7.66         119           CXCL3         NM.00879         Chemokine (C.X. C motf) ligand 1         2.43         116         7.66         129           CXCL10         NM.008793         Chemokine (C.X. C motf) ligand 1         1.47         1.11         5.39         2.261           CXCL10         NM.01949         Chemokine (C.X. C motf) ligand 1         1.47         1.11         5.39         2.261           CXCL10         NM.01949         Chemokine (C.X. C motf) ligand 1         1.47         1.11         5.39         2.271           CXCL2         AP065933         Chemokine (C.X. C motf) ligand 2         1.14         1.11         5.36         1.15           CCL2         AP083091         Chemokine (C.X. C motf) ligand 4         0.05         2.46         2.27           CCL2         AP083091         Chemokine (C.X. C motf) ligand 4         0.85         2.24         1.11           CCL3         AP083091         Chemokine (C.X. C motf) ligand 4         0.85         2.46         2.27	KLRKI         AF09005         Killer cell letrih like receptor subfanity K, member 1         113         113         222           CXCL1         NM 00815         Chemokine (C-X-C motif) ligand 1         2.43         116         7.16           CXCL13         NM 00815         Chemokine (C-X-C motif) ligand 1         1.47         1.07         3.39           CXCL13         NM 00849         Chemokine (C-X-C motif) ligand 2         1.14         1.11         5.19           CXCL13         NM 009494         Chemokine (C-X-C motif) ligand 2         1.14         1.11         5.19           CXCL13         NM 009494         Chemokine (C-X-C motif) ligand 2         1.14         1.11         5.19           CXCL2         AP06533         Chemokine (C-X contf) ligand 4         0.35         1.24         5.6           CXCL2         AP05393         Chemokine (C-C motif) ligand 5         1.13         1.11         5.15         5.6           CXCL2         AP138193         Chemokine (C-C motif) ligand 6         1.68         0.37         3.04           CXCL2         AP138193         Chemokine (C-C motif) ligand 1         1.13         1.11         1.11         1.11         1.11         1.11         1.11         1.11         1.11         1.11         1.11	KLRC1	AF106008	Killer cell lectin-like receptor subfamily C, member 1	1.19	0.67	5.57	1.89
CXCLI         NM 00876         Chemokine $(C, XC motif)$ ligand 1         2.43         116         716         129           CXCLID         NM 00814         Chemokine $(C, XC motif)$ ligand 5         2.43         0.90         12.61         1.86           CXCLID         NM 001244         Chemokine $(C, XC motif)$ ligand 10         2.41         0.93         5.46         2.21           CXCLID         NM 01337         Chemokine $(C, XC motif)$ ligand 11         1.14         1.11         5.86         1.15           CXCLIA         AP065933         Chemokine $(C, XC motif)$ ligand 1         1.14         1.11         5.86         1.15           CXCLA         AP065933         Chemokine $(C, C, motif)$ ligand 3         1.17         0.97         2.246         4.25           CXCLA         AP084904         Chemokine $(C, C, motif)$ ligand 3         1.14         1.11         5.86         1.17           CXCLA         AP084904         Chemokine $(C, C, motif)$ ligand 4         0.83         1.23         2.746         4.75           CXCLA         AP084904         Chemokine $(C, C, motif)$ ligand 4         0.85         0.97         2.86         1.77           CXCLA         AP084904         Chemokine $(C, C, motif)$ ligand 4         0.85         0.97 <t< td=""><td>CXCLI         NM 008176         Chemokine (C-X-C motif) ligand 1         2.43         116         716           CXCLI         NM 00914         Chemokine (C-X-C motif) ligand 5         2.83         0.09         1261           CXCLI0         NM 00839         Chemokine (C-X-C motif) ligand 10         1.47         1.11         5.95           CXCL10         NM 01337         Chemokine (C-X-C motif) ligand 10         1.47         1.11         5.19           CXCL11         NM 01337         Chemokine (C-X-C motif) ligand 10         1.47         1.11         5.19           CXCL13         AN084904         Chemokine (C-X-C motif) ligand 1         1.14         1.11         5.86           CXCL3         NM 01337         Chemokine (C-X-C motif) ligand 3         1.13         1.13         5.86           CXC13         NM 01347         Obset (C-C motif) ligand 4         0.85         1.23         4.65           CXC14         AT13218         Chemokine (C-C motif) ligand 1         1.16         1.11         5.86           CXC12         NM 01347         Obset (C-C motif) ligand 1         1.66         1.26         1.24           CXC12         M 00347         AT13218         Chemokine (C-C motif) ligand 1         1.66         1.26         1.13      <tr< td=""><td>KLRK1</td><td>AF039026</td><td>Killer cell lectin-like receptor subfamily K, member 1</td><td>1.13</td><td>1.13</td><td>2.72</td><td>2.15</td></tr<></td></t<>	CXCLI         NM 008176         Chemokine (C-X-C motif) ligand 1         2.43         116         716           CXCLI         NM 00914         Chemokine (C-X-C motif) ligand 5         2.83         0.09         1261           CXCLI0         NM 00839         Chemokine (C-X-C motif) ligand 10         1.47         1.11         5.95           CXCL10         NM 01337         Chemokine (C-X-C motif) ligand 10         1.47         1.11         5.19           CXCL11         NM 01337         Chemokine (C-X-C motif) ligand 10         1.47         1.11         5.19           CXCL13         AN084904         Chemokine (C-X-C motif) ligand 1         1.14         1.11         5.86           CXCL3         NM 01337         Chemokine (C-X-C motif) ligand 3         1.13         1.13         5.86           CXC13         NM 01347         Obset (C-C motif) ligand 4         0.85         1.23         4.65           CXC14         AT13218         Chemokine (C-C motif) ligand 1         1.16         1.11         5.86           CXC12         NM 01347         Obset (C-C motif) ligand 1         1.66         1.26         1.24           CXC12         M 00347         AT13218         Chemokine (C-C motif) ligand 1         1.66         1.26         1.13 <tr< td=""><td>KLRK1</td><td>AF039026</td><td>Killer cell lectin-like receptor subfamily K, member 1</td><td>1.13</td><td>1.13</td><td>2.72</td><td>2.15</td></tr<>	KLRK1	AF039026	Killer cell lectin-like receptor subfamily K, member 1	1.13	1.13	2.72	2.15
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	<b>CXCL1</b>	NM_008176	Chemokine (C-X-C motif) ligand 1	2.43	1.16	7.16	1.99
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	CXCLJ9         NM.008599         Chemokine (C.X-C motif) ligand J         1.45         1.07         3.39           CXCL10         NM.001274         Chemokine (C.X-C motif) ligand J         1.14         1.11         5.16           CXCL11         NM.091274         Chemokine (C.X-C motif) ligand J         1.14         1.11         5.86           CXCL12         AP065933         Chemokine (C.X-C motif) ligand J         1.14         1.11         5.86           CXCL3         NM.01337         Chemokine (C.X-C motif) ligand J         0.97         2.24         5.95           CXL3         NM.01337         Chemokine (C.X-C motif) ligand J         1.14         1.11         5.86           CXL2         AP128193         Chemokine (C.X-C motif) ligand J         0.97         2.94         5.86           CXL2         AP128193         Chemokine (C.X-C motif) ligand J         0.97         2.94         5.86           CXL2         AP1782183         Chemokine (C.X-C motif) ligand J         0.95         2.46         5.86           CXL2         AP1782183         Chemokine (C.X-C motif) ligand J         0.95         2.96         1.33           CXL2         D150712         D1601611         1.24         1.11         1.36         1.33 <t< td=""><td>CXCL5</td><td>NM_009141</td><td>Chemokine (C-X-C motif) ligand 5</td><td>2.83</td><td>0.90</td><td>12.61</td><td>1.86</td></t<>	CXCL5	NM_009141	Chemokine (C-X-C motif) ligand 5	2.83	0.90	12.61	1.86
CXCL10         NM.021274         Chemokine (C-X-C motf) ligand 10         2.41         0.93         5.46         2.21           CXCL11         NM.093494         Chemokine (C-X-C motf) ligand 1         1.47         1.11         5.19         1.07           CXCL13         NM.093494         Chemokine (C-X-C motf) ligand 2         1.14         1.11         5.86         1.12           CXCL3         NM.01337         Chemokine (C-C motf) ligand 2         1.13         0.97         12.36         4.65         2.77           CXCL4         AN12803         Chemokine (C-C motf) ligand 4         0.85         1.23         4.65         2.73           CXCL5         AN034904         Chemokine (C-C motf) ligand 12         1.68         0.73         3.04         0.99           CXCL2         NM.021443         Chemokine (C-C motf) ligand 12         1.68         0.73         3.04         0.99           CXCL2         NM.021443         Chemokine (C-C motf) ligand 12         1.68         0.73         3.04         0.99           CXCL2         NM.021443         Chemokine (C-C motf) ligand 12         1.68         0.73         3.04         0.99           CXL12         NO7323         Interleukin 1 arpha         1.11         1.45         0.98         0.9	CXCL10         NM.02124         Chemokine (C.X. C motif) ligand 10         2.41         0.93         5.46           CXCL11         NM.09394         Chemokine (C.X. C motif) ligand 11         1.47         1.11         5.19           CXCL13         NM.01137         Chemokine (C.X. C motif) ligand 12         1.14         1.11         5.19           CXCL3         NM.01137         Chemokine (C.X. C motif) ligand 12         1.14         1.11         5.86           CXCL4         APD6593         Chemokine (C.C. contf) ligand 12         1.14         1.11         5.89           CXCL4         APD8494         Chemokine (C.C. contf) ligand 12         1.14         1.11         5.46           CXCL3         NN.01343         Chemokine (C.C. contf) ligand 12         1.86         0.73         3.04           CXCL2         APD8494         Chemokine (C.C. contf) ligand 12         1.08         1.35         3.04           CXL1         APD84443         Chemokine (C.C. contf) ligand 12         1.08         1.35         3.04           CXL1         APD8445         Chemokine (C.C. contf) ligand 12         1.08         1.35         3.04           CXL1         DV03732         Interleukin 1 receptor storestorestorestorestorestorestorestore	CXCL9	NM_008599	Chemokine (C-X-C motif) ligand 9	1.45	1.07	3.99	2.86
CXCLII         NM.019494         Chemokine $(C-X-c motif)$ ligand 1         1.47         1.11         5.19         1.07           CXC12         NM.01337         Chemokine $(C-c motif)$ ligand 2         1.14         1.11         5.86         1.15           CXC13         NM.01337         Chemokine $(C-c motif)$ ligand 3         1.23         4.65         2.72           CXL14         AF128218         Chemokine $(C-c motif)$ ligand 4         0.85         1.23         4.65         2.72           CXL17         AN084904         Chemokine $(C-c motif)$ ligand 5         0.85         1.23         4.65         2.72           CXL17         AN124393         Chemokine $(C-c motif)$ ligand 4         0.85         1.26         4.15           CXL12         NM.01343         Chemokine $(C-c motif)$ ligand 5         0.86         0.73         3.04         1.79           CXL12         NM.01349         Chemokine $(C-c motif)$ ligand 12         1.08         1.35         4.46         4.25           CXL12         NM.013410         Chemokine $(C-c motif)$ ligand 12         1.08         1.35         1.74           US0712         ILLP         NM.013410         1.11         1.15         1.25         1.25           CXL12         NM.013410         <	CXCLII         NM.00944         Chemokine (C. Cmotif) ligand I         1.47         1.11         5.19           CCI2         NM.01333         Chemokine (C. Cmotif) ligand 2         1.14         1.11         5.46           CCI2         NM.01333         Chemokine (C. Cmotif) ligand 3         1.23         9.97         1.246           CCI2         AP138218         Chemokine (C. Cmotif) ligand 4         0.85         1.23         9.67         3.04           CCL7         AP138193         Chemokine (C. Cmotif) ligand 4         0.85         1.23         9.74         5.64           CCL12         NM.01343         Chemokine (C. Cmotif) ligand 12         0.85         1.35         4.81           L1         N.02143         Chemokine (C. Cmotif) ligand 12         0.93         0.97         3.04           CCL12         US0712         Us0712         Interleukin 1         1.11         1.45         0.93         3.04           CCL12         W.01343         Interleukin 1         Interleukin 1         1.11         1.12         0.93         3.04           II.RA         NM.071843         Interleukin 1         Interleukin 1         1.12         0.75         2.65         1.16         1.17         0.75         2.65	CXCL10	NM_021274	Chemokine (C-X-C motif) ligand 10	2.41	0.93	5.46	2.21
CCL2         AF065933         Chemokine (C- c motif) ligand 2         114         111         5.86         115           CCL3         NM.011337         Chemokine (C- c motif) ligand 3         1.73         0.97         12.46         4.52           CCL4         AF128193         Chemokine (C- c motif) ligand 4         0.85         1.23         4.65         2.72           CCL2         AF128193         Chemokine (C- c motif) ligand 5         0.98         0.73         3.04         0.97           CCL2         AF128193         Chemokine (C- c motif) ligand 5         0.98         0.73         3.04         0.73           CCL2         NM.021443         Chemokine (C- c motif) ligand 12         1.08         1.25         4.16         4.25           CL112         U50712         Chemokine (C- c motif) ligand 12         1.08         1.35         1.74           ILLR2         NM.010555         Interleukin 1 amly, member 9         1.23         1.24         4.29         1.74           ILLR2         NM.010555         Interleukin 1 receptor sccssory protein         1.17         0.75         2.73         1.06           ILLR2         NM.010555         Interleukin 1 receptor sccssory protein         1.17         0.75         2.73         1.06      <	CCL2         AF065933         Chemokine (C- cmotf) ligaid 2         1.14         1.11         5.86           CCL3         NM.01337         Chemokine (C- cmotf) ligaid 3         1.73         0.97         12.46           CCL3         NM.01337         Chemokine (C- cmotf) ligaid 4         0.85         1.23         3.04           CCL6         AY084904         Chemokine (C- cmotf) ligaid 7         0.98         0.97         12.46           CCL12         V0302443         Chemokine (C- cmotf) ligaid 7         0.98         0.95         3.34           CCL12         V0302443         Chemokine (C- cmotf) ligaid 8         0.85         1.26         4.16           V030241         Chemokine (C- cmotf) ligaid 8         0.85         1.26         4.16           CL12         V030242         Chemokine (C- cmotf) ligaid 8         0.85         1.26         4.16           US0012         Chemokine (C- cmotf) ligaid 8         0.85         1.26         4.16         1.33           LLh-a         BC00372         Interleukin 1 receptor accost         1.17         0.73         1.99         0.77           LLh-a         BC0372         Interleukin 1 receptor accosory protein         1.17         0.73         2.64           LLh-a         M13033 <td>CXCL11</td> <td>NM_019494</td> <td>Chemokine (C-X-C motif) ligand 11</td> <td>1.47</td> <td>1.11</td> <td>5.19</td> <td>1.07</td>	CXCL11	NM_019494	Chemokine (C-X-C motif) ligand 11	1.47	1.11	5.19	1.07
CCI3         NM.01137         Chemokine (C-C motif) ligand 3         1.73         0.97         12.46         4.52           CCI4         AF128218         Chemokine (C-C motif) ligand 4         0.85         1.23         4.65         2.72           CCL5         AF128193         Chemokine (C-C motif) ligand 6         0.85         1.23         4.65         2.72           CCL5         AF128193         Chemokine (C-C motif) ligand 6         0.85         1.25         4.46         2.73           CCL12         AF128193         Chemokine (C-C motif) ligand 7         0.85         1.26         4.16         4.29           CCL12         NM.021443         Chemokine (C-C motif) ligand 12         1.08         1.35         1.41           CL12         U56772         Chemokine (C-C motif) ligand 12         1.08         1.35         1.74           U56772         Chemokine (C-C motif) ligand 12         1.08         1.35         1.74         1.29           CL112         U56772         Chemokine (C-C motif) ligand 12         1.08         1.33         1.74           L118         M.079355         Interleukin 1 receptor, type I1         1.15         1.25         4.46         2.75           L1118         M.0709555         Interleukin 1 receptor acc	CCI3         NM.01137         Chemokine (C-C motif) ligand 4         0.97         12.46           CCL4         AF128218         Chemokine (C-C motif) ligand 4         0.85         1.23         4.65           CCL5         AF128218         Chemokine (C-C motif) ligand 6         0.85         1.23         4.65           CCL6         AF128218         Chemokine (C-C motif) ligand 6         0.85         1.23         4.65           CCL12         NM.021443         Chemokine (C-C motif) ligand 12         1.08         1.33         4.16           CCL12         U50712         Chemokine (C-C motif) ligand 12         1.08         1.33         4.16           CCL12         U50712         Chemokine (C-C motif) ligand 12         1.08         1.33         4.16           ILLR         BC00372         Chemokine (C-C motif) ligand 12         1.08         1.33         4.81           ILLR         NM.010555         Interlevin 1 receptor, type II         1.45         0.89         8.99           ILLR         NM.01343         Interlevin 1 receptor antagonist         1.17         0.75         2.73           ILLR         MA00353         Interlevin 1 receptor, d chain         1.17         0.75         2.64           ILLR         MA0353         I	CCL2	AF065933	Chemokine (C-C motif) ligand 2	1.14	1.11	5.86	1.15
CCL4         AFI28218         Chemokine (C-C motif) ligand 4         0.85         1.23         4.65         2.72           CCL6         AN084904         Chemokine (C-C motif) ligand 6         1.68         0.73         3.04         0.90           CCL7         AF128193         Chemokine (C-C motif) ligand 5         0.85         1.26         4.16         4.29           CCL12         U50712         U50712         Chemokine (C-C motif) ligand 12         1.08         1.35         4.81         1.141           L11.e         BC003727         Interleukin Lapha         1.06         1.08         1.35         4.81         1.41           L11.e         BC003727         Interleukin Lecptor scressory protein         1.06         1.08         1.26         4.29           CL11.2         U50752         Interleukin Leceptor scressory protein         1.17         0.90         0.77         1.17           L11.RAP         NM.134103         Interleukin Leceptor scressory protein         1.17         0.90         0.75         1.65           L11.RAP         NM.134103         Interleukin Leceptor scressory protein         1.17         0.75         2.64         0.75           L11.RAP         NM.133775         Interleukin Leceptor scressory protein         1.17	CCL4         AF128218         Chemokine (C- C motif) ligand 4         0.85         1.23         4.65           CCL6         AY084904         Chemokine (C- c motif) ligand 6         1.68         0.73         3.04           CCL6         AY084904         Chemokine (C- c motif) ligand 6         1.68         0.73         3.04           CCL7         AY084904         Chemokine (C- c motif) ligand 6         1.68         0.73         3.04           CCL12         U50712         Chemokine (C- c motif) ligand 12         1.08         1.35         4.41           L11.e         U50712         Chemokine (C- c motif) ligand 12         1.08         1.35         4.41           L11.e         U50712         Chemokine (C- c motif) ligand 12         1.08         1.35         4.41           L11.e         U50712         Chemokine (C- c motif) ligand 12         1.08         1.35         4.416           L11.e         NM.010555         Interleukin 1 apla         1.16         1.18         1.13           L1.IRN         M57525         Interleukin 1 receptor accesory protein         1.17         0.75         2.73           L1.IRN         M77353         Interleukin 1 receptor accesory protein         1.17         0.76         2.64           L1.I.R.A	CCL3	NM_011337	Chemokine (C-C motif) ligand 3	1.73	0.97	12.46	4.52
CCL6         AV084904         Chemokine (C-C motif) ligand 6         L68         0.73         3.04         0.90           CCL7         AF128193         Chemokine (C-C motif) ligand 7         0.98         0.73         3.34         1.79           CCL18         NM.021443         Chemokine (C-C motif) ligand 7         0.98         0.95         3.34         1.74           CCL12         U50712         Chemokine (C-C motif) ligand 12         1.08         1.35         4.416         4.29           CCL12         U50712         Chemokine (C-C motif) ligand 12         1.08         1.35         4.416         4.29           CCL118         W.010555         Interleukin 1 receptor, type II         1.45         0.89         1.99         1.99           ILLRAP         NM.010555         Interleukin 1 receptor antagonist         1.17         0.75         2.75         1.65           ILLRAP         NM.13475         Interleukin 1 receptor antagonist         1.11         0.77         1.65           ILLRAP         NM.008353         Interleukin 1 receptor antagonist         1.11         0.75         2.73         1.06           ILLRAP         M26054581         Interleukin 1 receptor antagonist         1.11         0.77         2.65         1.65 <t< td=""><td>CCL6         AV084904         Chemokine (C- motif) ligand 5         Less         0.73         3.04           CL12         V084904         Chemokine (C- motif) ligand 5         0.98         0.75         3.34           CCL13         U30143         Chemokine (C- motif) ligand 5         0.98         0.95         3.34           CCL13         U30143         Chemokine (C- motif) ligand 12         1.08         1.35         3.416           CUL12         US0712         Chemokine (C- motif) ligand 12         1.08         1.35         3.416           CUL12         US0712         Chemokine (C- motif) ligand 12         1.08         1.35         3.416           LILR2         NM 010555         Interleukin 1 alpta         1.06         1.08         1.35           ILLRA         NM 3375         Interleukin 1 receptor atcessory protein         1.17         0.56         2.64           ILLRA         M38052         Interleukin 1 receptor atcessory protein         1.117         0.56         2.64           ILLRA         M38052         Interleukin 1 receptor atcessory protein         1.117         0.56         2.64           ILLRA         M33052         Interleukin 1 receptor atcessory         1.117         0.56         2.64           ILLRA</td><td>CCL4</td><td>AF128218</td><td>Chemokine (C-C motif) ligand 4</td><td>0.85</td><td>1.23</td><td>4.65</td><td>2.72</td></t<>	CCL6         AV084904         Chemokine (C- motif) ligand 5         Less         0.73         3.04           CL12         V084904         Chemokine (C- motif) ligand 5         0.98         0.75         3.34           CCL13         U30143         Chemokine (C- motif) ligand 5         0.98         0.95         3.34           CCL13         U30143         Chemokine (C- motif) ligand 12         1.08         1.35         3.416           CUL12         US0712         Chemokine (C- motif) ligand 12         1.08         1.35         3.416           CUL12         US0712         Chemokine (C- motif) ligand 12         1.08         1.35         3.416           LILR2         NM 010555         Interleukin 1 alpta         1.06         1.08         1.35           ILLRA         NM 3375         Interleukin 1 receptor atcessory protein         1.17         0.56         2.64           ILLRA         M38052         Interleukin 1 receptor atcessory protein         1.117         0.56         2.64           ILLRA         M38052         Interleukin 1 receptor atcessory protein         1.117         0.56         2.64           ILLRA         M33052         Interleukin 1 receptor atcessory         1.117         0.56         2.64           ILLRA	CCL4	AF128218	Chemokine (C-C motif) ligand 4	0.85	1.23	4.65	2.72
CCL7         AF128193         Chemokine (C- C motif) ligand 7         0.98         0.95         3.34         1.79           CCL12         NM.021443         Chemokine (C- C motif) ligand 8         0.85         1.26         4.16         4.29           CCL12         U50712         Chemokine (C- C motif) ligand 12         1.08         1.35         4.81         1.41           IL1-\alpha         BC003727         Interleukin 1 receptor, type II         1.06         1.08         1.33         1.74           IL1-a         BC003727         Interleukin 1 family, member 9         1.23         4.81         1.41           IL1-a         NM.010555         Interleukin 1 receptor, type II         1.23         0.89         8.99         1.99           IL1RAP         NM.133403         Interleukin 1 receptor antagonist         1.17         0.77         1.71           IL1RAP         NM.2952         Interleukin 1 receptor antagonist         1.17         0.75         2.64         0.95           IL12R-β         M20553         Interleukin 1 receptor antagonist         1.17         0.77         2.65         1.65           IL2R-β         M20553         Interleukin 1 receptor sciencin β chain         1.17         0.55         2.64         0.95           IL	CCL7         AF128193         Chemokine (C- Cmotif) ligand 7         0.98         0.95         3.34           CCL12         UM0.021443         Chemokine (C- motif) ligand 8         0.85         1.26         4.16           CCL12         UM0.021443         Chemokine (C- motif) ligand 12         1.08         1.35         4.81           CL12         U05727         Chemokine (C- motif) ligand 12         1.06         1.08         1.33           IL1R         BC03727         Interleukin 1 apkin         1.06         1.08         1.33           IL1R         BC03727         Interleukin 1 teceptor, type II         1.46         0.89         8.99           IL1R         NM.010555         Interleukin 1 teceptor accessory protein         1.17         1.90         0.77           IL1RN         NM.134103         Interleukin 1 receptor accessory protein         1.17         0.97         2.64           IL1RN         M57525         Interleukin 1 receptor accessory protein         1.11         0.75         2.64           IL1RA         M28052         Interleukin 1 receptor accessory protein         1.11         0.56         2.64           IL1RN         M57725         Interleukin 1 receptor accessory protein         1.11         0.56         2.64	CCL6	AV084904	Chemokine (C-C motif) ligand 6	1.68	0.73	3.04	06.0
CCL8         NM.021443         Chemokine (C-C motif) ligand 8         0.85         1.26         4.16         4.29           CCL12         U50712         U50712         Chemokine (C-C motif) ligand 12         1.08         1.33         1.41           IL1-\alpha         BC003727         Interleukin I alpha         1.06         1.08         1.33         1.74           IL1-R         NM.010555         Interleukin I receptor, type II         1.45         0.89         1.99         1.71           IL1R2         NM.010555         Interleukin I receptor, type II         1.45         0.89         1.99         1.71           IL1R2         NM.134103         Interleukin I receptor accessory protein         1.17         0.75         2.73         1.06           IL1RN         M57525         Interleukin 1 receptor accessory protein         1.17         0.75         2.73         1.06           IL1RN         M57525         Interleukin 1 receptor accessory protein         1.17         0.75         2.73         1.06           IL1RN         M57525         Interleukin 1 receptor accessory protein         1.17         0.75         2.64         0.95           IL2R- $\beta$ M28052         Interleukin 1 receptor, $\beta$ chain         1.11         0.56         2.64	CCL8         NM.021443         Chemokine (C-C motif) ligand 12         0.85         1.26         4.16           CCL12         U56712         U56712         Chemokine (C-C motif) ligand 12         1.08         1.35         4.16           IL1-a         BC003727         Interleukin 1 apha         1.06         1.08         1.33           IL1.p         NM.010555         Interleukin 1 receptor, type II         1.45         0.89         8.99           IL1.R2         NM.010555         Interleukin 1 receptor, type II         1.45         0.89         0.37           IL1.R4         M57525         Interleukin 1 receptor accessory protein         1.17         1.95         0.77           IL1.RA         M57525         Interleukin 1 receptor, $\phi$ chain         1.17         0.75         2.73           IL1.RA         M28052         Interleukin 1 receptor, $\phi$ chain         1.11         0.56         2.64           IL1.RA         M28053         Interleukin 1 receptor, $\phi$ chain         1.11         0.56         2.64           IL1.RA         M28053         Interleukin 1 receptor, $\phi$ chain         1.11         0.56         2.64           IL1.RA         M28053         Interleukin 1 receptor, $\phi$ chain         1.11         0.56         2.64      <	CCL7	AF128193	Chemokine (C-C motif) ligand 7	0.98	0.95	3.34	1.79
CCL12         U50712         U50712         Chemokine (C-C motif) ligand 12         1.08         1.35         4.81         1.41           IL1-a         BC003727         Interleukin 1 alpha         106         1.08         1.33         1.74           IL1.a         BC003727         Interleukin 1 receptor, type II         1.45         0.89         8.99         1.99           IL1.R2         NM.010555         Interleukin 1 receptor, type II         1.45         0.89         8.99         1.91           IL1.R9         MY071843         Interleukin 1 receptor accessory protein         1.23         1.90         0.77         1.65           IL1.RN         M57525         Interleukin 1 receptor accessory protein         1.17         0.56         2.64         0.95           IL1.RN         M57525         Interleukin 1 receptor accessory protein         1.11         0.56         2.64         0.95           IL2.R-β         MM.03353         Interleukin 1 receptor, a chain         1.11         0.56         2.64         0.95           IL2.R-β         NM.03353         Interleukin 1 receptor, a chain         1.11         0.55         2.64         0.95           IL2.R-β         NM.133775         Interleukin 12 receptor, a chain         1.03         0.77	CCL12         U50712         Chemokine (C-C motif) ligand 12         1.08         1.35         4.81           IL1.a         BC003727         Interleukin 1 alpha         1.06         1.08         1.33           IL1.R2         NM.010555         Interleukin 1 receptor, type II         1.45         0.89         8.99           IL1.R2         NM.010555         Interleukin 1 family, member 9         1.23         1.90         0.77           IL1.RAP         NM.134103         Interleukin 1 family, member 9         1.23         1.90         0.77           IL1.RAP         NM.134103         Interleukin 1 receptor accessory protein         1.17         0.75         2.64           IL1.RA         M57525         Interleukin 1 receptor actasory protein         1.11         0.76         5.48           IL1.R.A         M28052         Interleukin 1 receptor, a chain         1.11         0.75         2.64           IL1.R.A         M28053         Interleukin 1 receptor, a chain         1.11         0.77         2.64           IL1.R.A         M28053         Interleukin 1 receptor, a chain         1.11         0.77         2.65           IL1.R.A         M28053         Signal transducer and activator of transcription 2         0.77         2.65           IL	CCL8	NM_021443	Chemokine (C-C motif) ligand 8	0.85	1.26	4.16	4.29
ILL-a         BC003727         Interleukin I apha         1.06         1.08         1.33         1.74           ILLR2         NM.010555         Interleukin I receptor, type II         1.45         0.89         8.99         1.99           ILLR2         NM.010555         Interleukin I receptor, type II         1.45         0.89         8.99         1.91           ILLRAP         NM.134103         Interleukin I receptor accessory protein         1.23         1.90         0.77         1.71           ILLRAP         NM.134103         Interleukin I receptor accessory protein         1.30         1.17         1.95         1.65           ILLRAP         M57525         Interleukin I receptor accessory protein         1.30         1.17         1.95         1.65           ILLRAP         M57525         Interleukin I receptor accessory protein         1.17         0.75         2.73         1.06           ILLRAP         M57525         Interleukin 1 receptor accessory protein         1.11         0.75         2.64         0.95           ILLRAP         NM.030353         Interleukin 2 receptor, chain         1.13         0.77         0.75         2.64         0.75           ILL2R-a         NM.030353         Interleukin 12 receptor, chain         1.15 <t< td=""><td>II.1-<math>\alpha</math>         BC003727         Interleukin I apha         106         108         1.33           ILIR2         NM-010555         Interleukin I receptor, type II         1.45         0.89         899           ILIR2         NM-010555         Interleukin I receptor accessory protein         1.23         1.90         0.77           ILIRAP         NM-134103         Interleukin I receptor accessory protein         1.30         1.17         1.95           ILIRAP         NM-138052         Interleukin I receptor accessory protein         1.30         1.17         0.75         2.54           ILI2R-<math>\beta</math>         NM-038353         Interleukin 1 receptor, <math>\beta</math> chain         1.11         0.56         2.64           ILI2R-<math>\beta</math>         NM-038353         Interleukin 1 receptor, <math>\beta</math> chain         1.11         0.70         5.48           ILI2R-<math>\beta</math>         NM-038353         Interleukin 1 receptor, <math>\beta</math> chain         1.11         0.56         2.64           ILI2R-<math>\beta</math>         NM-038353         Interleukin 1 receptor, <math>\beta</math> chain         1.11         0.70         5.48           ILI2R-<math>\beta</math>         NM-038353         Interleukin 1 receptor, <math>\beta</math> chain         1.13         0.97         2.65           ILI2R-<math>\beta</math>         NM-038353         Interleukin 1 receptor, <math>\beta</math> chain         1.13</td><td>CCL12</td><td>U50712</td><td>Chemokine (C-C motif) ligand 12</td><td>1.08</td><td>1.35</td><td>4.81</td><td>1.41</td></t<>	II.1- $\alpha$ BC003727         Interleukin I apha         106         108         1.33           ILIR2         NM-010555         Interleukin I receptor, type II         1.45         0.89         899           ILIR2         NM-010555         Interleukin I receptor accessory protein         1.23         1.90         0.77           ILIRAP         NM-134103         Interleukin I receptor accessory protein         1.30         1.17         1.95           ILIRAP         NM-138052         Interleukin I receptor accessory protein         1.30         1.17         0.75         2.54           ILI2R- $\beta$ NM-038353         Interleukin 1 receptor, $\beta$ chain         1.11         0.56         2.64           ILI2R- $\beta$ NM-038353         Interleukin 1 receptor, $\beta$ chain         1.11         0.70         5.48           ILI2R- $\beta$ NM-038353         Interleukin 1 receptor, $\beta$ chain         1.11         0.56         2.64           ILI2R- $\beta$ NM-038353         Interleukin 1 receptor, $\beta$ chain         1.11         0.70         5.48           ILI2R- $\beta$ NM-038353         Interleukin 1 receptor, $\beta$ chain         1.13         0.97         2.65           ILI2R- $\beta$ NM-038353         Interleukin 1 receptor, $\beta$ chain         1.13	CCL12	U50712	Chemokine (C-C motif) ligand 12	1.08	1.35	4.81	1.41
ILIR2         NM.010555         Interleukin 1 receptor, type II         1.45         0.89         8.99         1.91           ILIR2         AY071843         Interleukin 1 family, member 9         1.23         1.90         0.77         1.71           ILIRAP         NM.134103         Interleukin 1 receptor accessory protein         1.30         1.17         1.95         1.65           ILIRAP         NM.134103         Interleukin 1 receptor accessory protein         1.30         1.17         1.95         1.65           ILIRN         M.57525         Interleukin 1 receptor accessory protein         1.30         1.17         1.95         1.06           ILIRN         M.57525         Interleukin 1 receptor accessory protein         1.17         0.75         2.73         1.06           ILIRN         M.57525         Interleukin 1 receptor accessory protein         1.11         0.56         2.64         0.95           ILI2R- $\beta$ M200353         Interleukin 12 receptor, $\beta$ chain         1.11         0.77         2.64         0.95           ILI2R- $\beta$ NM_003333         Interleukin 12 receptor, $\beta$ chain         1.16         0.97         0.64         3.83         0.81           IL2R- $\beta$ NM_00333375         Signal transducer and activator of trans	II.IR2         NM.010555         Interleukin I receptor, type II         1.45         0.89         8.99           II.IF9         AY071843         Interleukin I family, member 9         1.23         1.90         0.77           II.IRAP         NM.134103         Interleukin I family, member 9         1.23         1.90         0.77           II.IRAP         NM.134103         Interleukin I receptor accessory protein         1.30         1.17         0.75         2.73           II.I.RN         M57525         Interleukin I receptor, accasory protein         1.17         0.75         2.64           II.I.RN         M28052         Interleukin 2 receptor, $\beta$ chain         1.11         0.56         2.64           II.I.2R- $\beta$ NM.03375         Interleukin 12 receptor, $\beta$ chain         1.11         0.75         0.77         2.64           II.I.2R- $\beta$ NM.03375         Interleukin 12 receptor, $\beta$ chain         1.12         0.75         0.79         5.64           II.I.2R- $\beta$ NM.03375         Interleukin 12 receptor, $\beta$ chain         1.13         0.75         0.77         2.64           II.1.3         NM.03375         Interleukin 12 receptor, $\beta$ in 1.12         0.75         0.79         0.65         3.65           III.38 <t< td=""><td>IL1-<math>\alpha</math></td><td>BC003727</td><td>Interleukin 1 alpha</td><td>1.06</td><td>1.08</td><td>1.33</td><td>1.74</td></t<>	IL1- $\alpha$	BC003727	Interleukin 1 alpha	1.06	1.08	1.33	1.74
II.JF9         AY071843         Interleukin I family, member 9         1.23         1.90         0.77         1.71           II.IRAP         NM.134103         Interleukin I receptor accessory protein         1.30         1.17         1.95         1.65           II.IRAP         NM.134103         Interleukin I receptor accessory protein         1.30         1.17         1.95         1.65           II.IRN         M57525         Interleukin 1 receptor accessory protein         1.30         1.17         0.55         2.73         1.06           II.I.RN         M57525         Interleukin 1 receptor accessory protein         1.11         0.56         2.64         0.95           II.2R- $\beta$ M2008353         Interleukin 12 receptor, $\beta$ chain         1.11         0.56         2.64         0.63           II.2R- $\beta$ I         NM.008353         Interleukin 12 receptor, $\alpha$ chain         1.16         0.56         2.64         0.63           II.2R- $\beta$ I         NM.008353         Interleukin 12 receptor, $\alpha$ chain         1.16         0.77         0.67         2.64         0.61           II.2R- $\beta$ I         NM.008353         Interleukin 12 receptor, $\alpha$ chain         1.15         0.97         0.63         1.59           II.2R- $\beta$ I         NM.008353         Sig	ILIF9         AY071843         Interleukin I family, member 9         1.23         1.90         0.77           ILIRAP         NM.134103         Interleukin 1 receptor accessory protein         1.30         1.17         1.95           ILIRN         M57525         Interleukin 1 receptor accessory protein         1.30         1.17         0.75         2.73           ILIRN         M57525         Interleukin 1 receptor accessory protein         1.11         0.56         2.64           ILIRN         M28052         Interleukin 1 receptor, $\beta$ chain         1.11         0.56         2.64           ILIZR- $\beta$ M28052         Interleukin 12 receptor, $\alpha$ chain         1.11         0.56         2.64           ILIZR- $\beta$ NM.008353         Interleukin 12 receptor, $\alpha$ chain         1.16         0.57         2.65           ILIZR- $\beta$ NM.133775         Interleukin 12 receptor, $\beta$ chain         1.15         0.97         2.65           ILI2R- $\beta$ NM.133775         Interleukin 12 receptor, $\beta$ chain         1.15         0.97         2.65           IL2R- $\beta$ NM.133775         II.08         0.70         0.84         3.83           IL33         NM.133775         Signal transducer and activator of transcription 2         1.77	IL1R2	NM_010555	Interleukin 1 receptor, type II	1.45	0.89	8.99	1.99
ILIRAP         NM.134103         Interleukin I receptor accessory protein         1.30         1.17         1.95         1.65           ILIRN         M57525         Interleukin I receptor antagonist         1.17         0.75         2.73         1.06           ILIRN         M57525         Interleukin 1 receptor antagonist         1.17         0.75         2.73         1.06           ILIRN         M57525         Interleukin 2 receptor, $\beta$ chain         1.11         0.56         2.64         0.95           ILI.2R- $\beta$ M20353         Interleukin 2 receptor, $\beta$ chain         1.11         0.70         5.48         0.54           ILI.2R- $\beta$ NM.008353         Interleukin 1 receptor, $\beta$ chain         1.16         0.70         5.48         0.54           ILI.2R- $\beta$ NM.008353         Interleukin 1 receptor, $\beta$ chain         1.16         0.70         5.48         0.54           ILI.2R- $\beta$ NM.008353         Interleukin 1 receptor, $\beta$ 1.16         0.70         5.48         0.81           ILI.2R- $\beta$ NM.008353         Interleukin 12 receptor, $\beta$ 1.15         0.97         5.65         1.56           ILL2R- $\beta$ NM.133775         Signal transducer and activator of transcription 1         2.97	ILIRAP         NM.134103         Interleukin I receptor accessory protein         1.30         1.17         1.95           ILIRN         M57525         Interleukin I receptor accessory protein         1.17         0.75         2.73           ILIRN         M57525         Interleukin I receptor accessory for an agonist         1.17         0.75         2.73           ILIRN         M57525         Interleukin 2 receptor, $\beta$ chain         1.11         0.56         2.64           ILI.2R- $\beta$ M200353         Interleukin 2 receptor, $\alpha$ chain         1.11         0.70         5.48           ILI.2R- $\beta$ I         NM.008353         Interleukin 12 receptor, $\alpha$ chain         1.16         0.56         2.64           IL12R- $\beta$ I         NM.008353         Interleukin 12 receptor, $\alpha$ chain         1.16         0.70         5.48           IL12R- $\beta$ I         NM.008353         Interleukin 12 receptor, $\alpha$ chain         1.15         0.97         2.64           IL12R- $\beta$ I         NM.008353         Interleukin 12 receptor, $\alpha$ chain         1.16         0.70         5.48           IL12R- $\beta$ I         NM.008353         Signal transducer and activator of transcription 1         1.05         0.80         5.34           STAT2         BB2298862         Signal transducer and activator of transcr	IL1F9	AY071843	Interleukin 1 family, member 9	1.23	1.90	0.77	1.71
ILJRN         M57525         Interleukin 1 receptor antagonist         1.17         0.75         2.73         1.06           IL2R- $\beta$ M28052         Interleukin 2 receptor, $\beta$ chain         1.11         0.56         2.64         0.95           IL2R- $\beta$ M28052         Interleukin 2 receptor, $\beta$ chain         1.11         0.56         2.64         0.95           IL2R- $\beta$ M20353         Interleukin 2 receptor, $\beta$ chain         1.11         0.70         5.48         0.54           IL2R- $\beta$ I         NM.008353         Interleukin 12 receptor, $\beta$ I         1.11         0.70         5.48         0.54           IL12R- $\beta$ I         NM.008353         Interleukin 12 receptor, $\beta$ I         1.15         0.97         2.65         1.59           IL12R- $\beta$ I         NM.133775         Interleukin 12 receptor, $\beta$ I         1.16         0.70         5.48         0.81           IL12R- $\beta$ I         NM.133775         Interleukin 12 receptor, $\beta$ I         1.15         0.97         2.65         1.16           STAT1         BB229853         Signal transducer and activator of transcription 1         2.97         0.80         6.34         1.16           STAT2         BG069527         Signal transducer and activator of transcription 2         1.77	ILJRNM57525Interleukin 1 receptor antagonist1.170.752.73IL2R- $\beta$ M28052Interleukin 2 receptor, $\beta$ chain1.110.562.64IL2R- $\alpha$ AF054581Interleukin 2 receptor, $\alpha$ chain1.110.562.64IL2R- $\beta$ NM_008353Interleukin 2 receptor, $\beta$ chain1.130.705.48IL2R- $\beta$ 1NM_008353Interleukin 12 receptor, $\beta$ 11.150.972.65IL33NM_133775Interleukin 331.050.843.83STAT1BB229853Signal transducer and activator of transcription 12.970.806.34STAT2AF088862Signal transducer and activator of transcription 21.771.012.04STAT3BG069527Signal transducer and activator of transcription 31.501.382.73TNFRSPBC028507Tumor necrosis factor receptor superfamily, member 91.030.976.23A <sup>T</sup> Te cytokines differentially expressed in BALB/c and/or C57BL/6 were selected for analysis at 3 and 10 qbi. "The capital "B" and "C" represent BALB/c and c57BL/6 wice, respectively. Superscripts "I"	ILIRAP	NM_134103	Interleukin 1 receptor accessory protein	1.30	1.17	1.95	1.65
IL2R- $\beta$ M28052         Interleukin 2 receptor, $\beta$ chain         1.11         0.56         2.64         0.95           IL2R- $\alpha$ AF054581         Interleukin 2 receptor, $\alpha$ chain         1.03         0.70         5.48         0.54           IL2R- $\alpha$ AF054581         Interleukin 2 receptor, $\alpha$ chain         1.03         0.70         5.48         0.54           IL2R- $\beta$ 1         NM.108353         Interleukin 12 receptor, $\beta$ 1         1.15         0.97         2.65         1.59           IL12R- $\beta$ 1         NM.133775         Interleukin 12 receptor, $\beta$ 1         1.15         0.97         2.65         1.50           IL33         NM.133775         Interleukin 33         Interleukin 33         0.84         3.83         0.81           STA71         BB229853         Signal transducer and activator of transcription 1         2.97         0.80         6.34         1.10           STA72         AF088862         Signal transducer and activator of transcription 2         1.77         1.01         2.04         1.60           STA73         BG069527         Signal transducer and activator of transcription 3         1.50         1.38         2.73         0.93           TNFRSF9         BC028507         Tumor necrosis factor receptor superfamily, member 9 <td>IL2R-<math>\beta</math>M28052Interleukin 2 receptor, <math>\beta</math> chain1.110.562.64IL2R-<math>\alpha</math>AF054581Interleukin 2 receptor, <math>\alpha</math> chain1.030.705.48IL2R-<math>\beta</math>1NM-008353Interleukin 12 receptor, <math>\beta</math>11.150.972.65IL33NM-133775Interleukin 131.050.843.83STAT1BB229853Signal transducer and activator of transcription 12.970.806.34STAT2AF08862Signal transducer and activator of transcription 21.771.012.04STAT3BG069527Signal transducer and activator of transcription 31.501.382.73TNFRSF9BC028507Tumor necrosis factor receptor superfamily, member 91.030.976.23Artne cytokines differentially expressed in BALB/c and/or C57BL/6 were selected for analysis at 3 and 10 qbi. "The capital "B" and "C" represent BALB/c and C57BL/6 mice, respectively. Superscripts "I"</td> <td>ILIRN</td> <td>M57525</td> <td>Interleukin 1 receptor antagonist</td> <td>1.17</td> <td>0.75</td> <td>2.73</td> <td>1.06</td>	IL2R- $\beta$ M28052Interleukin 2 receptor, $\beta$ chain1.110.562.64IL2R- $\alpha$ AF054581Interleukin 2 receptor, $\alpha$ chain1.030.705.48IL2R- $\beta$ 1NM-008353Interleukin 12 receptor, $\beta$ 11.150.972.65IL33NM-133775Interleukin 131.050.843.83STAT1BB229853Signal transducer and activator of transcription 12.970.806.34STAT2AF08862Signal transducer and activator of transcription 21.771.012.04STAT3BG069527Signal transducer and activator of transcription 31.501.382.73TNFRSF9BC028507Tumor necrosis factor receptor superfamily, member 91.030.976.23Artne cytokines differentially expressed in BALB/c and/or C57BL/6 were selected for analysis at 3 and 10 qbi. "The capital "B" and "C" represent BALB/c and C57BL/6 mice, respectively. Superscripts "I"	ILIRN	M57525	Interleukin 1 receptor antagonist	1.17	0.75	2.73	1.06
IL2R- $\alpha$ AF054581         Interleukin 2 receptor, $\alpha$ chain         1.03         0.70         5.48         0.54           IL2R- $\beta$ I         NM-008353         Interleukin 12 receptor, $\beta$ I         1.15         0.97         2.65         1.59           IL12R- $\beta$ I         NM-108353         Interleukin 12 receptor, $\beta$ I         1.15         0.97         2.65         1.59           IL33         NM-103533         Interleukin 12 receptor, $\beta$ I         1.15         0.97         2.65         1.50           IL33         NM-1033775         Interleukin 33         I.165         0.84         3.83         0.81           STATI         BB229853         Signal transducer and activator of transcription 1         2.97         0.80         6.34         1.11           STAT2         AF088862         Signal transducer and activator of transcription 2         1.77         1.01         2.04         1.60           STAT3         BG069527         Signal transducer and activator of transcription 3         1.50         1.38         2.73         0.93           TNFRSF9         BC028507         Tumor necrosis factor receptor superfamily, member 9         1.03         0.97         6.23         1.06	IL2R- $\alpha$ AF054581Interleukin 2 receptor, $\alpha$ chain1.030.705.48IL2R- $\beta$ 1NM.008353Interleukin 12 receptor, $\beta$ 11.150.972.65IL33NM.133775Interleukin 331.050.843.83STAT1BB229853Signal transducer and activator of transcription 12.970.806.34STAT2AF08862Signal transducer and activator of transcription 21.771.012.04STAT3BG069527Signal transducer and activator of transcription 31.501.382.73TNFRSF9BC028507Tumor necrosis factor receptor superfamily, member 91.030.976.23d <sup>-T</sup> Te cytokines differentially expressed in BALB/c and/or C57BL/6 were selected for analysis at 3 and 10 dpi. "The capital "B" and "C" represent BALB/c and C57BL/6 mice, respectively. Superscripts "i"	IL2R- $\beta$	M28052	Interleukin 2 receptor, $\beta$ chain	1.11	0.56	2.64	0.95
ILl2R- $\beta$ l         NM-008353         Interleukin 12 receptor, $\beta$ l         1.15         0.97         2.65         1.59           ILl2R- $\beta$ l         NM-133775         Interleukin 12 receptor, $\beta$ l         Interleukin 33         0.84         3.83         0.81           ILl33         NM-133775         Signal transducer and activator of transcription 1         2.97         0.80         6.34         1.11           STAT2         AF088862         Signal transducer and activator of transcription 2         1.77         1.01         2.04         1.60           STAT2         BG069527         Signal transducer and activator of transcription 3         1.50         1.38         2.73         0.93           TNFRSF9         BC028507         Tumor necrosis factor receptor superfamily, member 9         1.03         0.97         6.23         1.06	ILl2R- $\beta$ lNM.008353Interleukin 12 receptor, $\beta$ l1.150.972.65IL.33NM.133775Interleukin 331.050.843.83STAT1BB229853Signal transducer and activator of transcription 12.970.806.34STAT2AF08862Signal transducer and activator of transcription 21.771.012.04STAT3BG069527Signal transducer and activator of transcription 31.501.382.73TNFRSF9BC028507Tumor necrosis factor receptor superfamily, member 91.030.976.23 <sup>d</sup> The cytokines differentially expressed in BALB/c and/or C57BL/6 were selected for analysis at 3 and 10 qbi. "The capital "B" and "C" represent BALB/c and C57BL/6 mice, respectively. Superscripts "i"	IL2R-a	AF054581	Interleukin 2 receptor, $\alpha$ chain	1.03	0.70	5.48	0.54
IL33         NM.133775         Interleukin 33         Interleukin 33         I.05         0.84         3.83         0.81           STAT1         BB229853         Signal transducer and activator of transcription 1         2.97         0.80         6.34         1.11           STAT2         AF088862         Signal transducer and activator of transcription 2         1.77         1.01         2.04         1.60           STAT2         BG069527         Signal transducer and activator of transcription 3         1.50         1.38         2.73         0.93           TNFRSF9         BC028507         Tumor necrosis factor receptor superfamily, member 9         1.03         0.97         6.23         1.06	IL33NM.133775Interleukin 33I.050.843.83STAT1BB229853Signal transducer and activator of transcription 12.970.806.34STAT2AF088862Signal transducer and activator of transcription 21.771.012.04STAT3BG069527Signal transducer and activator of transcription 31.501.382.73TNFRSF9BC028507Tumor necrosis factor receptor superfamily, member 91.030.976.23 <sup>d</sup> The cytokines differentially expressed in BALB/c and/or C57BL/6 were selected for analysis at 3 and 10 qbi. "The capital "B" and "C" represent BALB/c and C57BL/6 mice, respectively. Superscripts "i"	IL12R- $\beta$ 1	NM_008353	Interleukin 12 receptor, $\beta 1$	1.15	0.97	2.65	1.59
STAT1BB229853Signal transducer and activator of transcription 12.970.806.341.11STAT2AF08862Signal transducer and activator of transcription 21.771.012.041.60STAT3BG069527Signal transducer and activator of transcription 31.501.382.730.93TNFRSF9BC028507Tumor necrosis factor receptor superfamily, member 91.030.976.231.06	STAT1BB229853Signal transducer and activator of transcription 12.970.806.34STAT2AF088862Signal transducer and activator of transcription 21.771.012.04STAT3BG069527Signal transducer and activator of transcription 31.501.382.73TNFRSF9BC028507Tumor necrosis factor receptor superfamily, member 91.030.976.23 <sup>d</sup> The cytokines differentially expressed in BALB/c and/or C57BL/6 were selected for analysis at 3 and 10 dpi. "The capital "B" and "C" represent BALB/c and C57BL/6 mice, respectively. Superscripts "i"	IL33	NM_133775	Interleukin 33	1.05	0.84	3.83	0.81
STAT2AF088862Signal transducer and activator of transcription 21.771.012.041.60STAT3BG069527Signal transducer and activator of transcription 31.501.382.730.93TNFRSF9BC028507Tumor necrosis factor receptor superfamily, member 91.030.976.231.06	STAT2AF088862Signal transducer and activator of transcription 21.771.012.04STAT3BG069527Signal transducer and activator of transcription 31.501.382.73TNFRSF9BC028507Tumor necrosis factor receptor superfamily, member 91.030.976.23dThe cytokines differentially expressed in BALB/c and/or C57BL/6 were selected for analysis at 3 and 10 dpi. *The capital "B" and "C" represent BALB/c and C57BL/6 mice, respectively. Superscripts "i"	<b>STAT1</b>	BB229853	Signal transducer and activator of transcription 1	2.97	0.80	6.34	1.11
STAT3BG069527Signal transducer and activator of transcription 31.501.382.730.93TNFRSF9BC028507Tumor necrosis factor receptor superfamily, member 91.030.976.231.06	STAT3BG069527Signal transducer and activator of transcription 31.501.382.73TNFRSF9BC028507Tumor necrosis factor receptor superfamily, member 91.030.976.23dThe cytokines differentially expressed in BALB/c and/or C57BL/6 were selected for analysis at 3 and 10 dpi. *The capital "B" and "C" represent BALB/c and C57BL/6 mice, respectively. Superscripts "i"	STAT2	AF088862	Signal transducer and activator of transcription 2	1.77	1.01	2.04	1.60
TNFRSF9 BC028507 Tumor necrosis factor receptor superfamily, member 9 1.03 0.97 6.23 1.06	TNFRSF9       BC028507       Tumor necrosis factor receptor superfamily, member 9       1.03       0.97       6.23 <sup>d</sup> The cytokines differentially expressed in BALB/c and/or C37BL/6 were selected for analysis at 3 and 10 dpi. *The capital "B" and "C" represent BALB/c and C37BL/6 mice, respectively. Superscripts "i"	STAT3	BG069527	Signal transducer and activator of transcription 3	1.50	1.38	2.73	0.93
	<sup>d</sup> The cytokines differentially expressed in BALB/c and/or C57BL/6 were selected for analysis at 3 and 10 dpi. *The capital "B" and "C" represent BALB/c and C57BL/6 mice, respectively. Superscripts "i"	TNFRSF9	BC028507	Tumor necrosis factor receptor superfamily, member 9	1.03	0.97	6.23	1.06

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C57BL/6 mice. This discrepancy is likely due to the different detection methods. Overall, these results validate our microarray data and they can therefore be used to infer biological relevance.

#### 4. Discussion

ECTV infection can lead to different outcomes in inbred mouse strains. Some strains are susceptible to severe disease and have a high mortality rate, while others, such as C57BL/6, C57BL/10, AKR, and some sublines of 129 mice, show resistance to the virus [12-15]. This is not only due to virus strain, virus immune evasion strategies, dose, and route of infection, but also due to the genetic background of the host [9, 11]. Over the past decades, a number of works have shown that the numerous host factors associated with innate and adaptive immune responses are essential for resistance to mousepox [11]. However, details regarding the host immune response to ECTV infection in genetically susceptible and resistant mice remain to be elucidated. To address this, we used a well-defined mousepox model, with BALB/c as the susceptible strain and C57BL/6 as the resistant strain, which were challenged with ECTV. The susceptible strain reflected a significantly higher virus titer in spleen tissues and one animal death at 10 dpi, but the resistant C57BL/6 strain showed no significant symptoms and no animal deaths. Of note, the virus was detectable in the spleen of BALB/c mice at 3 dpi, but not in C57BL/6 mice, suggesting the importance of genetic background. So far, at least 4 genetic loci in the mouse genome are known to confer resistance to mousepox [16]. The susceptible BALB/c mice were found to be lacking these resistance alleles and the lack of these immunity related genes leads to weak and delayed immune response against ECTV infection [15-18].

Transcriptomic studies provide useful information about underlying pathogenic mechanisms of different genetic backgrounds and interactions following a course of virus infection [41, 42]. In the current study, we utilized microarray technology to examine the host gene expression profiles of susceptible and resistant mice in response to ECTV infection. Our analysis showed that ECTV strongly altered gene expression in both mouse strains. In particular, gene expression was greatly altered at the late stage of infection, and more genes were altered in the susceptible mice than in the resistant mice during the course of the infection. In addition, more upregulated genes than downregulated genes were observed in the BALB/c mice which was the opposite result to that for the C57BL/6 mice. These observations may be the result of a higher viral loads in the spleen of BALB/c mice that would in turn affect the expression of more host genes. We showed that a number of genes were upregulated during infection in the susceptible BALB/c mice, but these were unchanged or downregulated in the resistant C57BL/6 mice, suggesting different mechanisms exist in the two mouse strains in response to ECTV infection. These DEGs could potentially be the key to understanding the different pathologies associated with the two mouse strains. Of note, Hspalb was found to be upregulated in both mouse strains during infection. Previous studies on the transcriptome of host cells during VACV infection also showed *Hspalb* upregulation, and data from RNAi screens identified a necessary role for Hspalb in *Orthopoxvirus* infection [43, 44].

We performed pathway analyses of shared DEGs at different time points after ECTV infection in two different mouse strains. Pathways involved in the innate and adaptive immune systems in the control of ECTV infection were found in the susceptible BALB/c mice. These include nucleic acid recognition pathways, natural killer cell mediated cytotoxicity, and the APC-TCR signaling pathway. Nucleic acid recognition pathways are important components of the innate immune system, which serves as the first line of defense and directs subsequent events to activate the host's adaptive immune system [45, 46]. PRRs, including TLR9, STING, and their relevant adaptor Myd88 and nuclear transcription factors, IRF3 and IRF7, are essential for resistance to mousepox [28, 29]. And also, the importance of these molecules in response to the infection has been addressed in VACV, CPXV, and MPXV [30, 41]. Other PRRs, such as cGAS, a critical cytosolic DNA sensor, were speculated that it plays an essential role in inherent resistance to mousepox [28]. In the present study, the expression of these genes was unchanged or slightly upregulated in the two mouse strains which may be due to their expression in certain cell types and/or tissues [47-49]. Despite less genes induced in resistant C57BL/6 mice, the commonly affected genes during the infection were highly enriched in leukocyte transendothelial migration and MAPK signaling pathway, which were also affected by CPXV and MPXV [41].

NK cells are part of the first line of defense to viral infection. The importance of NK cells in defense against poxviruses has emerged over several decades, and they have been shown to play an essential role in inherent resistance to mousepox [50-52]. A number of previous studies have found increased numbers of NK cells in popliteal lymph nodes, spleen, and liver after infection, with peak NK activity occurring at 5 days after infection in both susceptible and resistant mouse strains [52, 53]. Depletion studies have shown that severe infection occurs in resistant C57BL/6 mice, and the NK response is required for resistance during the first few days, so that by day 5 the depletion does not have a major impact on recovery [52, 54]. Our analyses showed that NK cells and NK cell mediated cytotoxicity were stimulated in both mouse strains at 10 dpi, indicating the importance of NK cells [10]. In addition, the delayed induction of NK response presented in our study might be explained by the different routes of infection and the less virulence of the virus strain used in our work. And also, the upregulation of NK cells related genes were observed late in the spleens, where maybe they act earlier than our detection time or are secreted from other tissues. Granzymes (Gzm) are serine proteases expressed by cytotoxic T cells and NK cells and are important for the destruction of virally infected cells [55]. C57BL/6 mice deficient in both GzmA and GzmB are susceptible to mousepox, while moderate susceptibility to the virus is seen in mice that are deficient in only one, demonstrating some overlap between these two effectors [11, 56]. In the present study, GzmB was strongly upregulated in the two mouse strains at 10 dpi, suggesting an adaptive immune response and more specifically that cytotoxic T lymphocytes may have taken over the response. Other granzymes were also increased mainly in the BALB/c mice, suggesting a stronger immune response in the susceptible strain.

Type I and II IFNs are among the first cytokines to be produced during viral infection and are essential for inherent resistance to mousepox. Both types of IFN induce the expression of ISGs, which have a variety of functions ranging from direct inhibition of viral components to activation of other immune cell types. C57BL/6 mice deficient in IFN- $\alpha$ and IFN- $\beta$  showed increased mortality and enhanced viral loads following ECTV infection [11]. In addition, resistant mice (C57BL/6 and 129) with a targeted deletion of the IFN- $\alpha/\beta$  receptor are highly susceptible to mousepox [11, 27]. Results from the current study also showed increased expression of IFN- $\zeta$  and IFN- $\alpha$ 2, as well as ISGs, in resistant mice, suggesting that they play an important role in the control of ECTV infection. IFN- $\gamma$  is produced by NK cells and CD8<sup>+</sup> T cells. The essential role of IFN-y in the control of ECTV infection was confirmed in IFN-y-deficient C57BL/6 mice, which are highly susceptible to mousepox and promote ECTV spread in vivo [11, 12]. However, ECTV encodes an IFN- $\gamma$  decoy receptor, which binds directly to the host IFN- $\gamma$ with high affinity and blocks cytokine action extracellularly, prior to receptor engagement [57]. ECTV deficient in this molecular is mildly attenuated, suggesting other ECTVencoded factors may modify the function of IFN- $\gamma$  [58].

In summary, we characterized global gene expression patterns that are shared and distinct between the spleen tissues from ECTV-susceptible and ECTV-resistant mouse strains. The susceptible mice showed a stronger response to the infection with higher viral loads than the resistant strain. Our results highlight differences in the response to ECTV between ECTV-susceptible and ECTV-resistant mice. Although a global overview of some events occurring in ECTV-susceptible and ECTV-resistant mice was observed by using microarray analysis, the complicated mechanisms of host responses in different mouse strains were not clearly elucidated. Therefore, considering the data in the present study, more detection time points and target tissues, such as skin, blood, liver, and regional lymph nodes, should be performed in the future.

#### **Conflicts of Interest**

The authors declare that they have no conflicts of interest.

#### **Authors' Contributions**

Wen-Yu Cheng, Huai-Jie Jia, and Zhi-Zhong Jing conceived and designed the study and critically revised the manuscript. Wen-Yu Cheng, Huai-Jie Jia, Xiao-Bing He, Yuan Feng, and Xiao-Xia Wang performed the experiments and drafted the manuscript. Wen-Yu Cheng, Guo-Hua Chen, and Chun-Yan Wang performed the bioinformatics analyses of the data. All authors read and approved the final manuscript.

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#### **Supplementary Materials**

Supplementary 1. Table S1: genes and primers used in qRT-PCR validation.

*Supplementary 2.* Table S2: the list of the differentially expressed genes in BALB/c and C57BL/6 mice infected with ECTV at 3 and 10 dpi.

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