

# Integrated analysis of lncRNA and mRNA repertoires in lung from Pneumocystis infected mice

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## Research Article

**Keywords:** Pneumocystis pneumonia, BAFF-R, lncRNA

**Posted Date:** February 25th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-236505/v1>

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**Integrated analysis of lncRNA and mRNA repertoires in lung from *Pneumocystis* infected mice**

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Running head: lncRNA sequencing in BAFF-R deficient *Pneumocystis* infected mice

Key words: *Pneumocystis* pneumonia; BAFF-R; lncRNA

## Abstract

**Background** According to the widespread use of highly active antiretroviral therapy (HAART) to HIV patients with *Pneumocystis pneumonia* (PCP), the incidence of PCP associated with HIV has fallen. There is an enhancement of population of HIV-negative patients with PCP, who receiving immunosuppressive therapies or genetic primary immune deficiency disorders. Most of the previous studies have concentrated on the immune cell responses in PCP individuals, the present study aimed to integrate lncRNA and mRNA expression profiles in PCP patients without HIV.

**Methods** The lung tissue of WT mice and BAFF-R<sup>-/-</sup> mice were harvested at 3 weeks after infected with pneumocystis. After total RNAs being extracted, transcriptome profiling was performed following the Illumina HiSeq 3000 protocol. The microarrays and quantitative RT-PCR analysis were conducted to evaluate the lncRNA and mRNA expression profiles in WT-PCP mice and BAFF-R<sup>-/-</sup> PCP mice.

**Results** Compared with the control group, 166 mRNAs were observed to be aberrantly expressed (Fold change value  $\geq 2$ ;  $P < 0.05$ ) in B cell-activating factor receptor deficient (BAFF-R<sup>-/-</sup>) PCP group, including 99 up-regulated and 67 down-regulated, while there were 39 lncRNAs differently expressed in BAFF-R<sup>-/-</sup> PCP group, including 24 up-regulated and 15 down-regulated genes. In addition, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed on all of the differently expressed genes and the data showed that BAFF-R deficiency played an important role in the primary and adaptive immune responses in PCP. Furthermore, the lncRNA and mRNA co-expression network was established. We noted that in the core network of lncRNA-TF (transcription factors) pairs could be classified into the categories including infection and immunity pathways.

**Conclusion** In summary, in this study we furtherly explored the role of mature B cells in the pathogenesis and disease progression of PCP and the data demonstrated that BAFF-R deficiency play a significant role in immune regulation in PCP population.

## Introduction

*Pneumocystis pneumonia* (PCP) is a kind of devastating fungal disease in immunocompromised patients. Nowadays according to the widespread use of effective antiviral

therapies have decreased the numbers of HIV positive patients with PCP, but there is a growing number of HIV negative PCP individuals who receiving immunosuppressive therapies <sup>(1)</sup>. While non-HIV-infected PCP population is characterized by a more rapid disease progression and higher mortality <sup>(2,3)</sup>. Recently, the susceptibility of HIV-negative individuals for PCP has drawn the attention of researchers, but the pathogenesis and development of PCP remains to be clarified. Currently, however, the infected mice model remains the main source of laboratory studies and investigators have made improvement in understanding the host-pathogen relationship with *Pneumocystis* in immunology <sup>(4)</sup>. Macrophages play a significant role as the first line of host defense against *Pneumocystis* in the innate immune of pathogen invasion <sup>(5,6)</sup>. In adaptive immunity, CD4<sup>+</sup> T cells are the central factor for the clearance of *Pneumocystis* by the recruitment of the other effector cells <sup>(7)</sup>. Furthermore, there are some researchers verified that T helper cells including Th1, Th2, Th9 and Th17 cells could have the immune regulatory function in *Pneumocystis* infected mice model. Additionally, the role of B cells in PCP also has been reported in a few studies and our previous study has indicated that IL-1beta is a likely determinant of the IL-10-producing B cell-mediated suppression of Th1/Th17-cell immune responses in B cell-activating factor receptor deficient (BAFF-R<sup>-/-</sup>) PCP mice. It suggested that B cells play an important role in the regulation of Th cells in response to *Pneumocystis* infection <sup>(8)</sup>.

Noncoding RNAs (NcRNAs) were characterized as the deficiency of clear potential to encode proteins or peptides, while they could affect the expression of other genes via a variety of mechanisms<sup>(9)</sup>. Researches over the last decades discovered many classes of ncRNA, including microRNA(miRNA), small nucleolar RNA (snoRNA), long ncRNAs (lncRNA), and circular RNA (circRNA). LncRNA are classified into ncRNA > 200bp and have been demonstrated to play critical roles in the modification of genes and post-gene regulation mechanisms<sup>(10)</sup>. In recent decades, studies of lncRNA suggest that lncRNA acting as the crucial and diverse roles in the development and progression of various diseases<sup>(11)</sup>. While the function of lncRNA in the pathogenesis of PCP has not been clearly clarified yet. Our previous study has verified that in *Pneumocystis* infected mice, there were a great quantity of differentially

expressed genes (DEGs) between WT mice and BAFF-R<sup>-/-</sup> mice and IL-1β related genes could play an immune regulatory role in Th1/Th17 cell immune responses<sup>(8)</sup>.

In the present study, RNA sequencing was performed to explore the lncRNA and mRNA expression profiles in lung tissue of WT PCP mice and BAFF-R<sup>-/-</sup> PCP mice. Additionally, to identify the underlying biological functions of differentially expressed RNAs, bioinformatics analysis including GO and KEGG pathway analysis and lncRNA-mRNA co-expression network construction was conducted.

## **Materials and Methods**

### **Mice**

Female 6- to 8-wk-old C57BL/6 mice (Vital River Laboratory Animal Co., Ltd, Beijing, China) and BAFF-R<sup>-/-</sup> mice (stock no. 007212, the Jackson Laboratory, Bar Harbor, ME) were used for this study. Mice were bred in our animal facility and kept in sterile isolated ventilated cages. All animal experiments were approved by the Capital Medical University Animal Care and Use Committee.

### **Infection with *Pneumocystis***

*Pneumocystis murina* was maintained in CB17 SCID mice as previously described with some modifications<sup>(8)</sup>. Briefly, mice were intratracheally inoculated with *Pneumocystis* cysts and control mice were administered with 100ul PBS. Mice survived at 3 weeks after infection and we obtained middle lobe from the right lung of each mouse.

### **Transcriptomics analysis of lung tissue**

Transcriptome profiling was performed by RiboBio following the Illumina HiSeq 3000 protocol (Guangzhou, China). Firstly, total RNA was extracted from mixed samples of mice using TRIzol reagent (Invitrogen, Carlsbad, CA). Subsequently, ribosomes kit was used to remove rRNA and then mRNA was purified and fragmented into small pieces with elute, prime and fragment mix. After end-repair and adapter-ligation, the products were amplified through PCR and purified to construct the cDNA library.

The original sequencing data should be filtered, adaptor removed and low-quality reads processed to acquire clean data. For the detected mRNA, the gene expression was further calculated and the differentially expressed genes (DEGs) among the samples were analyzed. After that, DEGs were performed for Gene Ontology (GO) analysis and Kyoto encyclopedia of

genes and genomes (KEGG) pathway analysis. The expression of lncRNA was calculated and the differentially expressed lncRNA was analyzed among the samples, and specific annotations were given for the identification of the corresponding ORF, protein domain and coding potential, as well as the prediction of secondary structure and family analysis.

### **LncRNA target gene prediction**

Target genes of lncRNA were predicted by establishing the relationship between lncRNA and mRNA and analyzing the mode of action of lncRNA. A large number of lncRNA play significant biological roles by regulating contiguous gene by integrating differentially expressed lncRNA with mRNA of their neighbors (10KB), thus obtained potential target genes of lncRNA. After the interaction between lncRNA and target genes was obtained, the network diagram of the interaction between lncRNA and target genes were drawn.

### **qPCR validation**

RNAs were purified and quantified as previously described. RT-PCR was executed by SYBR PreMix Ex Taq TM II ROX (Takara) and the ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA). Gene expression was calculated using the  $2^{-\Delta\Delta CT}$  method. GAPDH was used as a reference gene to analyze the gene quantitatively.

### **Statistical analysis**

Data of this study was expressed as mean  $\pm$  SE. Comparison of continuous data was performed by independent Student's *t*-test. The Prism 8 (GraphPad Software, San Diego, CA) was used for statistical analysis. *P*-value < 0.05 were considered statistically significant.

## **Results**

### **Identification of differentially expressed lncRNA and mRNA**

A total of 6 samples from lung tissue of pneumocystis infected mice were used for this study. Removing reads with non-canonical letters or with low quality, and discarding the sequences shorter than 18 nt, the mean numbers of clean reads were 117,007,330. In total, 27343 transcripts were detected in the present study, in which 16344 transcripts were identified as mRNA and 5328 transcripts as lncRNA. The top 10 differentially expressed genes (DEGs) are shown in Table 1 (lncRNAs) and Table 2 (mRNAs). Compared with RNA acquired from lung tissue in WT-PCP mice, we explored 166 mRNAs (99 up-regulated and 67 down-

regulated; Fig 1) and 39 lncRNAs (24 up-regulated and 15 down-regulated; Fig 2) in BAFF-R<sup>-</sup> PCP mice.

Furthermore, we also found 3 novel lncRNAs increased in BAFF-R<sup>-</sup> PCP mice (Fig.3A). On the analysis of the species on the basis of the known genetic model, using all the sample sequence data to forecast the new transcription area (FPKM > 1), and the expression level of new transcription area were analyzed (Fig.3B).

### **GO and KEGG pathway analysis of DEGs**

GO analysis results demonstrated that changes in biological processes of DEGs were significantly enriched in anion binding, activin-activated receptor activity, sialic acid binding, hormone activity, fatty acid binding, cytokine activity, neuropeptide receptor binding, carboxylic acid binding, receptor binding and monocarboxylic acid binding. Changes in cellular component of DEGs were mainly enriched in intercellular canaliculus, mast cell granule, immunoglobulin complex, fibrinogen complex, spectrin-associated cytoskeleton, blood microparticle, side of membrane, cell surface, external side of plasma membrane and extracellular space. Changes in molecular function were mainly enriched in positive regulation of leukocyte proliferation, regulation of leukocyte proliferation, positive regulation of immune system process, leukocyte proliferation, leukocyte activation, cell activation, regulation of cell-cell adhesion, regulation of regulation of heterotypic cell-cell adhesion, immune system process and regulation of immune system process (Fig.4). KEGG pathway analysis showed that the DEGs were primarily enriched in cytokine-cytokine receptor interaction, complement and coagulation cascades, B cell receptor signaling pathway, malaria, primary immunodeficiency and hematopoietic cell lineage (Fig.5).

### **GO and pathway analysis of differentially expressed lncRNA-targeted mRNA**

Since most of the functional lncRNA are mainly executed in protein-coding target genes, we applied the co-expression patterns of mRNA and lncRNA to further study the biological function of these differentially expressed lncRNA. Using GO and KEGG pathway analysis, we aimed to identify the speculated functions about the differentially expressed lncRNAs' target genes between WT-PCP group and BAFF-R<sup>-</sup> PCP group.

GO analysis results demonstrated that changes in biological processes of DEGs were significantly enriched in telomerase RNA binding, actin binding, cytokine receptor activity,

ubiquitin-like protein transferase activity, ubiquitin-protein transferase activity, peptide antigen binding, endodeoxyribonuclease activity, drug binding, deoxyribonuclease activity and K63-linked polyubiquitin binding. Changes in cellular component of DEGs were mainly enriched in nuclear DNA-directed RNA polymerase complex, catalytic complex, spindle, RNA polymerase complex, nuclear lumen, nuclear part, histone pre-mRNA 3-end processing complex, mast cell granule, transferase complex and telomerase holoenzyme complex. Changes in molecular function of DEGs were significantly enriched in regulation of organelle organization, interstrand cross-link repair, DNA repair, regulation of vascular smooth muscle cell proliferation, vascular smooth muscle cell proliferation, organelle organization, L-amino acid import, regulation of telomere maintenance, telomere organization and telomere maintenance (Fig.6). KEGG pathway analysis demonstrated that the DEGs were mainly enriched in Chemokine signaling pathway, HTLV-I infection and endocytosis (Fig.7).

#### **Co-expression analysis of lncRNA and mRNA**

We performed a co-expression network of lncRNA and mRNA to infer the underlying regulatory function and potential target genes of lncRNA. LncRNA which locate within 300K windows downstream or upstream of the given mRNAs were subsequently further studied. Correlated protein coding genes and lncRNA were chosen to visualize the correlation of clusters (Fig.8a). Furthermore, we analyzed the functional regulatory relationship between the genes. Functional enrichment in each cluster indicated that lncRNA were functionally associated with mRNAs in particular biological processes, such as B cell receptor signaling pathway, Herpes simplex infection, Staphylococcus aureus infection, Choline metabolism in cancer, Measles, Ubiquitin mediated proteolysis, Fanconi anemia pathway and Endocytosis (Fig.8b).

#### **qRT-PCR validation**

To validate the credibility of the results of microarray analysis, we performed real-time PCR of the top 10 DEGs. Total RNAs from the lungs of WT C57BL/6, WT-PCP, or BAFF-R<sup>-/-</sup> PCP mice were extracted at 2 weeks after pneumocystis infection. For the lncRNA, the results showed that ENSMUSG00000097312.1, G530011006Rik, Gm41999 upregulated and ENSMUSG00000111593.1, ENSMUSG00000105646.1, ENSMUSG00000105646.1 downregulated(all  $P < 0.001$ , Fig.9A). For the mRNAs, the data demonstrated that Ocstamp,

Pou2af1, Pax5 downregulated and Nppa, Shisa8, Trim10 upregulated, Validation studies furtherly indicated that there were a majority of differently expressed genes between the BAFF-R<sup>-/-</sup> PCP group and the WT-PCP group, consistent with the RNA sequencing results (all  $P < 0.001$ , Fig.9B)

### **Discussion**

*Pneumocystis pneumonia* (PCP) is an opportunistic, continuously evolving disease challenging clinicians who have a life-threatening illness that inhibits their immune systems<sup>(12)</sup>. PCP is initially observed in acquired immunodeficiency syndrome (AIDS) patients, however, because of the new antiretroviral medications recently approved in HIV positive patients, pneumocystis has been prevalence declines in these individuals<sup>(13)</sup>. Accumulating evidence revealed that the increasing use of immunosuppressive agents in haematological malignancy, autoimmune conditions and transplant recipients has resulted in an increase in PCP in HIV-negative populations<sup>(12)</sup>. According to the previous study, HIV-negative patients with PCP are related to more severe outcomes and rapid disease deteriorate<sup>(3)</sup>. Multiple immune cells such as macrophages, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and B cells participate in the development of PCP. BAFF-R is early expressed in circulating B cells and play an important role in the maturation of B cells<sup>(14)</sup>. Using BAFF-R<sup>-/-</sup> mice, our previous study focused on the function of B cells and the results showed that mature B cells play a vital role in the regulation of Th cells in PCP. We also performed mRNA expression profile analysis of WT-PCP mice and BAFF-R<sup>-/-</sup> mice at 2wk post-infection and found that there were multiple differently expressed genes between the two groups. Also, GO pathway analysis indicated that a number of genes associated with immune modulatory pathways<sup>(8)</sup>.

To the best of our knowledge, there are few studies using microarray technology to reveal the immune regulatory function of cells in PCP. Advances in genetic characterization of pneumocystis indicates that specific polymorphisms are related to the epidemiology of this pathogen, containing the geographic distribution, medicine resistance, toxicity of several genotypes and genetics of this population<sup>(4)</sup>. To clarified the characteristics of the genetic diversity of PC, Magdalena et al used the sputum specimens of patients with PCP, and the results of the study showed that specific genes such as mtLSUrRNA, CYB, DHPS and SOD were correlated with certain patient characteristics<sup>(15)</sup>. Using RNA sequencing, some scholars

found that *Arp9*, *Sp* and *Gsc1* were differentially regulated in the predominant life forms in PCP population, providing a preliminary annotation of the *Pneumocystis murina* genome <sup>(16)</sup>. Also, a study focused on the antifungal agents of PCP individuals and noted that there were 80 genes up- or downregulated among uninfected, untreated mice and mice treated with anidulafungin to study the metabolism of persisting forms <sup>(17)</sup>.

Noncoding RNAs are defined as short noncoding RNAs or lncRNA based on the sequence-length cutoff of 200 nucleotides <sup>(9)</sup>. The biological roles of lncRNA are recently beginning to be investigated, and critical functions for lncRNA have been confirmed in almost every biological system studied. Up to now, the important function of lncRNA are mainly studied in malignant diseases and recent studies showed that lncRNA play important roles in different phases of cancer immunity via molecular mechanisms <sup>(18)</sup>. However, the significance of lncRNA in the pathogenesis and disease progression of PCP is still unclear. The present study performed RNA sequencing of PCP mice and aimed to clarify the important role of lncRNA and mRNA in pneumocystis infection. We built WT-PCP mice and BAFF-R PCP mice model and extracted RNAs for further analysis, including different genes comparison, GO and KEGG pathway analysis of DEGs, target gene analysis for regulated lncRNA and construction of the lncRNA and protein-coding genes co-expression network. The data showed that there were a number of different expressed genes between the two groups.

- The data in our study demonstrated that there were 39 lncRNA and 166 mRNA to be significantly different expressed ( $FC \geq 2.0$ ) in the BAFF-R PCP group, which including *Nppa*, *Shisa8*(Orf26) and *Trim10* up-regulated and *Ocstamp*, *POu2af1* and *Pax5* down-regulated, etc. The above genes are mostly related to immune regulatory systems: *Nppa* plays an important role in the control of extracellular fluid volume and electrolyte homeostasis and participates in the pathogenesis and disease progression of cardiac diseases <sup>(19)</sup>. *Shisa8* is a kind of the cysteine-knot AMPA receptor-modulating proteins, which could mediate most of the fast-excitatory transmission in the central nervous system of vertebrates <sup>(20)</sup>. *Trim10* is play a critical role in the globin gene transcription and its upregulation is a key factor required for terminal erythroid cell differentiation <sup>(21)</sup>. *Ocstamp* plays a key role in bone resorption and its partners *CD9* could promote periodontal bone destruction by up-regulation of OC-genesis <sup>(22)</sup>. *Pou2af1*, which

regulates B cell homeostasis and controls humoral immune, is proved to enhance IL-17A expression in Th17 cells via RORgamat<sup>(23)</sup>. Pax5 target genes are mostly expressed in leukemic cells and have been demonstrated as a tumor suppressor in lymphoblastic leukemia.<sup>(24)</sup> This suggests that BAFF-R deficiency has an effect on the expression of a number of protein coding genes. We also performed q-PCR experiment of the DEGs to further confirmed the results of gene sequencing and the data could support the microarray data. The different express of these genes could influence the modulation of immune cells and signaling pathways.

- To further investigate the potential functions of different expressed protein encoding RNAs, we executed the GO and KEGG pathway enrichment analysis of the above genes. GO pathway analysis includes biological process, molecular function and cellular component and the results indicated that those differentially expressed mRNAs were mainly enriched in following biological processes (cytokine activity and chemokines receptor binding), molecular function (immunoglobulin complex, cell surface, external side of plasma membrane and extracellular space), and cellular component (leukocyte proliferation and activation, cell-cell adhesion). KEGG pathway analysis suggested that the biochemical and signaling pathways were mainly enriched in Chemokine signaling pathway, cytokine-cytokine receptor interaction, NF-kappa B signaling pathway and primary immunodeficiency.
- Furthermore, we performed GO pathway analysis of lncRNA targeted mRNA. The results showed that differentially expressed mRNAs were mainly involved in the molecular function terms of regulation of organelle organization, telomere organization and telomere maintenance and biological process terms of cytokine receptor activity and peptide antigen binding. Also KEGG pathway analysis results indicated that pathways were principally enriched in chemokine signaling pathway, AMPK signaling pathway, etc. According to these data, we found that the deficient of BAFF-R could have a significant influence on the immune microenvironment, thus might lead to the immunosuppressive patients' susceptible to PCP. Also, non-HIV PCP patients demonstrated the worsen disease progression, however, the treatment of non-HIV PCP has almost only concentrated on TMP-SMZ and caspofungin<sup>(25)</sup>. The other more efficient therapy is essentially required to be explored. The differentially

expressed pathways in the present study might provide the new target spot of the immune therapy of non-HIV PCP populations.

- To investigate the potential regulatory mechanism of the aberrantly expressed lncRNA, the lncRNA and mRNA the co-expression network of mRNA and lncRNA was built according to the correlation analysis. The network was based on the differentially expressed lncRNA and its neighboring protein-coding genes (distance < 300kb). Trans-regulatory lncRNA exert their functions via regulating the target genes expression at a long genomic distance away. The data demonstrated that in the core network of lncRNA-TF pairs, the lncRNA could be classified into categories of pathways such as Herpes simplex infection, Staphylococcus aureus infection, Choline metabolism in cancer, and Endocytosis, indicated that the function of trans-regulatory lncRNA was centralized on immune and infection pathways.
- In summary, this study focused on the non-coding and coding genes in PCP population. Using BAFF-R<sup>-/-</sup> mice, we furtherly explored the role of mature B cells in the pathogenesis and disease progression of PCP. The results demonstrated that BAFF-R deficiency play an important role in the regulation of immune regulatory related genes and pathways. Also, co-expression network analysis was performed and the data showed that lncRNA might provide as novel diagnostic biomarkers and therapeutic targets of PCP. However, in this study we have not performed the further gene functional experiments. In the future, gene functional studied in vivo and vitro are required and the blood and BALF samples from patients with PCP are essential for deliberate study.

## **Declarations**

Ethics approval and consent to participate

This study was approved by the ethics committee of Beijing Chao-Yang Hospital, Capital Medical University. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

The Author confirms:

- that the work described has not been published before (except in the form of an abstract or as part of a published lecture, review, or thesis);
- that it is not under consideration for publication elsewhere;
- that its publication has been approved by the responsible authorities at the institution where the work is carried out.

Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing non-financial/financial interests.

Funding: This work was funded by the National Natural Science Foundation of China (No. 81870004), Beijing Natural Science Foundation (KZ201910025031) and Beijing Municipal Administration of Hospital (No. DFL20150302).

Authors' contributions

Z.-H.T. conceived and designed research; H.-M.R and L.T performed experiments; H.-M.R. and Z.C analyzed data; D.W and X.-J.Q. interpreted results of experiments; Z.C prepared figures; H.-M.R drafted manuscript; All of the above authors approved final version of manuscript.

#### Acknowledgments

The authors are grateful to the National Natural Science Foundation of China for the support of this study.

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Table 1 Top 10 differentially expressed lncRNA

Top 10 up-regulated lncRNA		
Gene symbol	LogFC	<i>P</i> -Value
XR_869775.2	5.57612378	0.00169525
ENSMUST00000199065.1	5.33274839	0.00697579
XR_387599.3	5.1421598	0.00697579
XR_373476.1	5.01907903	0.01415054
ENSMUST00000187093.1	4.95844308	0.01415054
XR_868133.1	4.91818468	0.00697579
XR_868134.1	4.91818401	0.00697579
XR_871418.1	4.71967674	0.00020309
XR_868424.1	4.675006	0.00169525
XR_873200.1	4.66256844	0.00169525
Top 10 down-regulated lncRNA		
XR_876265.2	-5.3985048	0.00224578
XR_381821.2	-4.5093424	0.00442928
NR_026942.1	-4.4194587	0.00442928

XR_373980.2	-4.4192419	0.00224578
NR_077222.1	-4.2623705	0.00442928
XR_106110.3	-4.0407708	0.00873574
XR_001780647.1	-4.0043205	0.00117278
XR_874705.2	-3.982802	0.01722924
XR_001779198.1	-3.9165228	0.01722924
XR_386758.1	-3.8965689	0.01722924

Table 2 Top 10 differentially expressed mRNA

Top 10 up-regulated mRNA		
Gene symbol	LogFC	<i>P</i> -Value
Nppa	2.486418327	3.27E-06
Shisa8	2.083258651	1.93E-07
Trim10	2.029090596	2.34E-05
Havcr1	1.967959277	3.22E-14
Ngp	1.953260409	2.34E-05
Thrsp	1.758002901	2.97E-08
Cd163l1	1.584454345	1.93E-07
Stfa2l1	1.56832523	1.50E-11
Spta1	1.56110762	7.65E-07
Klb	1.554836132	1.16E-11
Top 10 down-regulated mRNA		
Rdm1	-2.283172926	0
Cr2	-1.862682812	3.25E-25
Cacna1i	-1.759016068	1.28E-09

Ocstamp	-1.678951188	4.04E-05
Gm41885	-1.645840227	5.06E-08
Cd19	-1.61610532	2.60E-26
Pou2af1	-1.591060029	9.96E-29
Fcrl1	-1.550715661	3.30E-11
Pax5	-1.483844438	7.04E-20
Bank1	-1.381420723	4.07E-21

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Fig.1

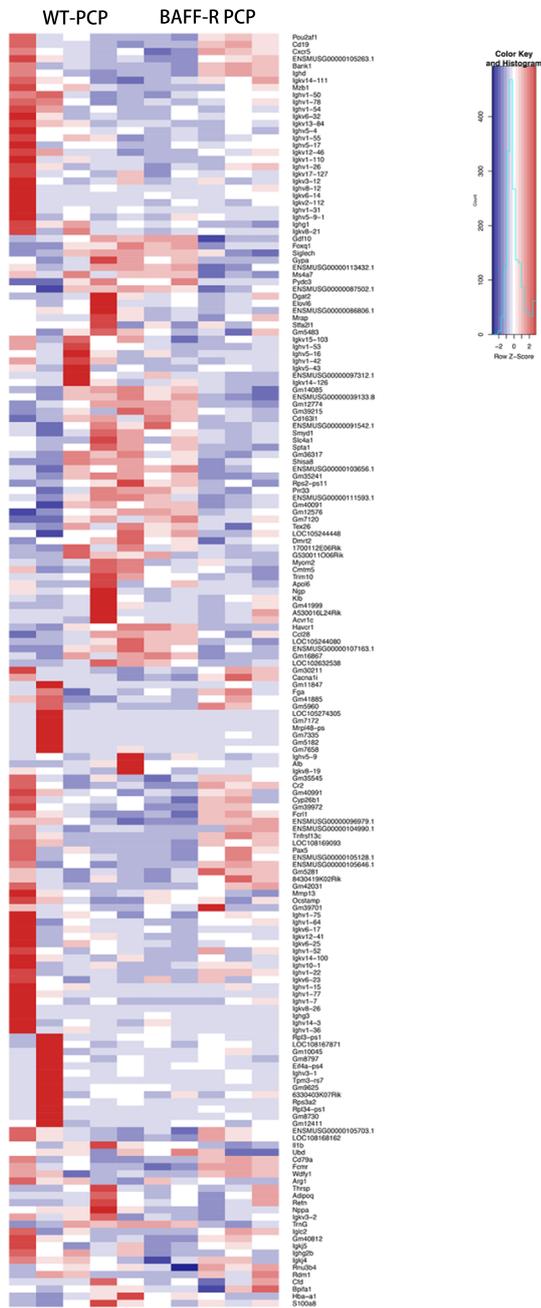


Fig.2

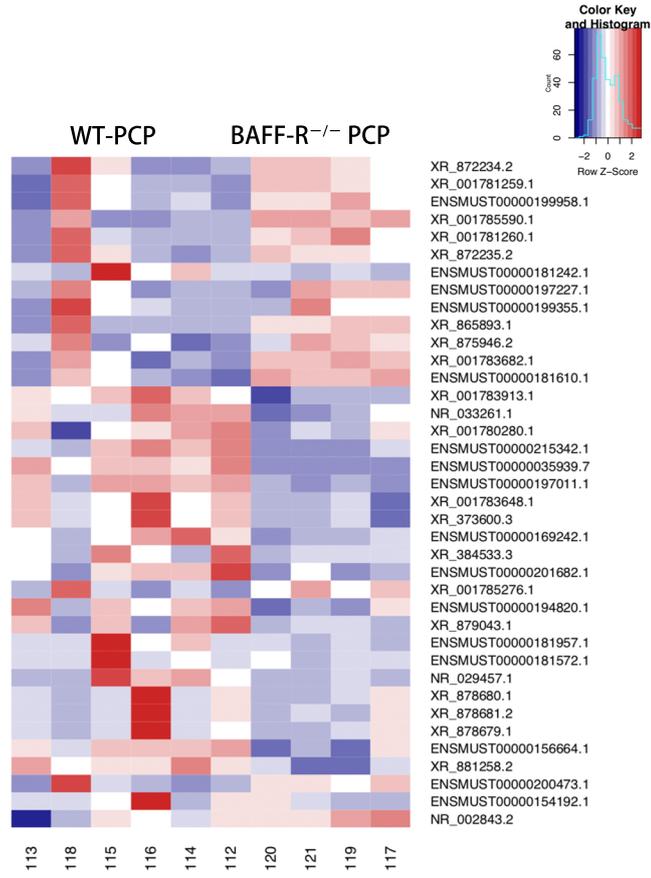


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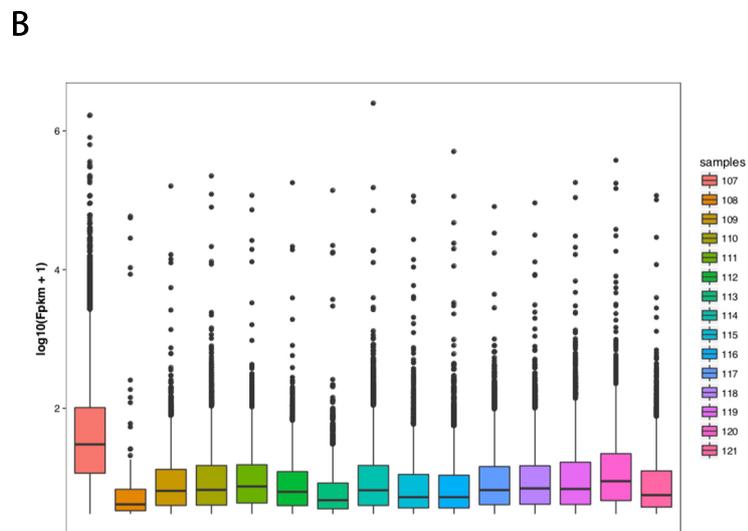
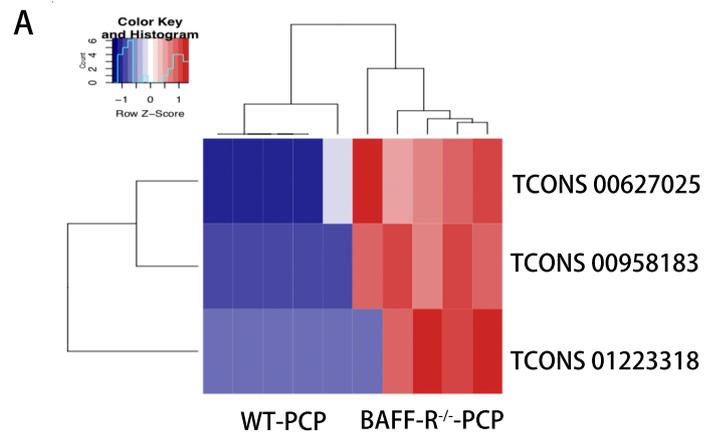


Fig.4

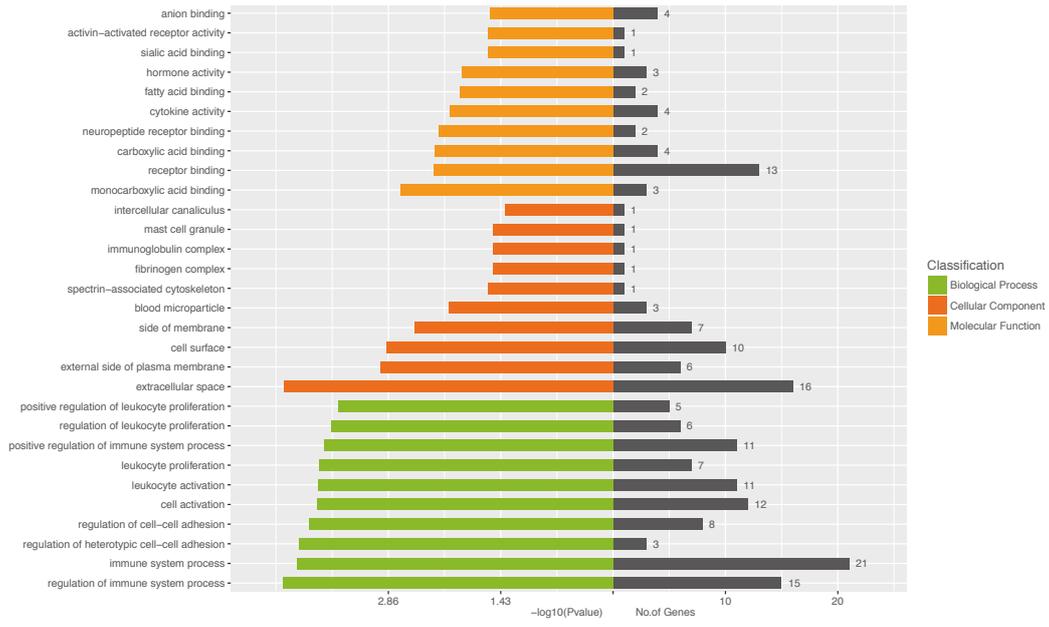


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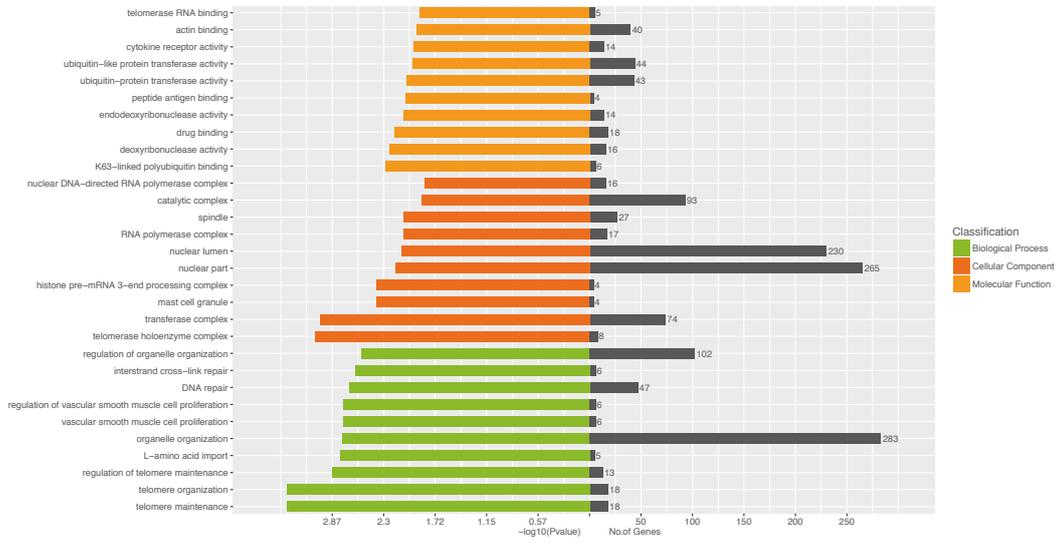


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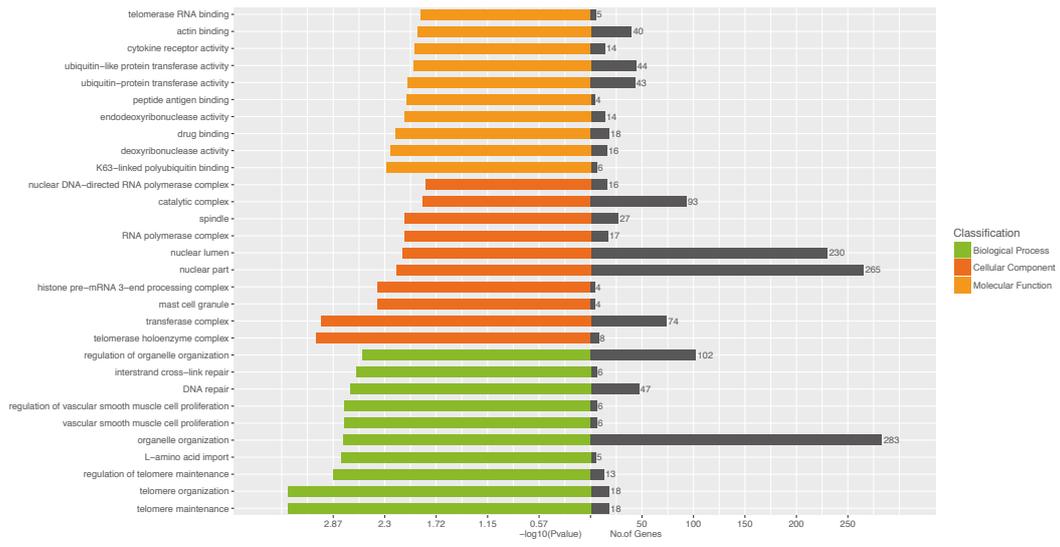


Fig.7

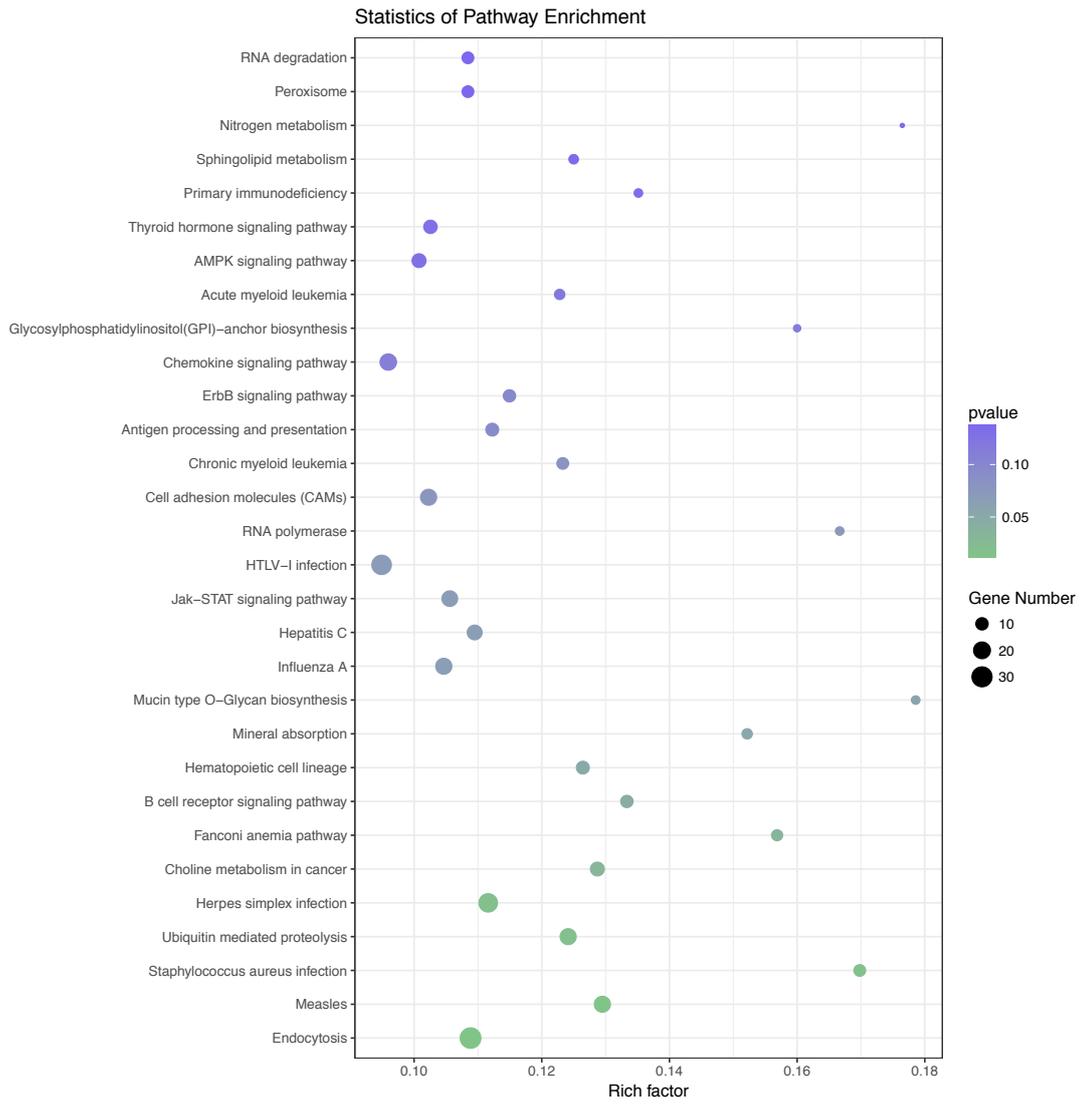


Fig.8

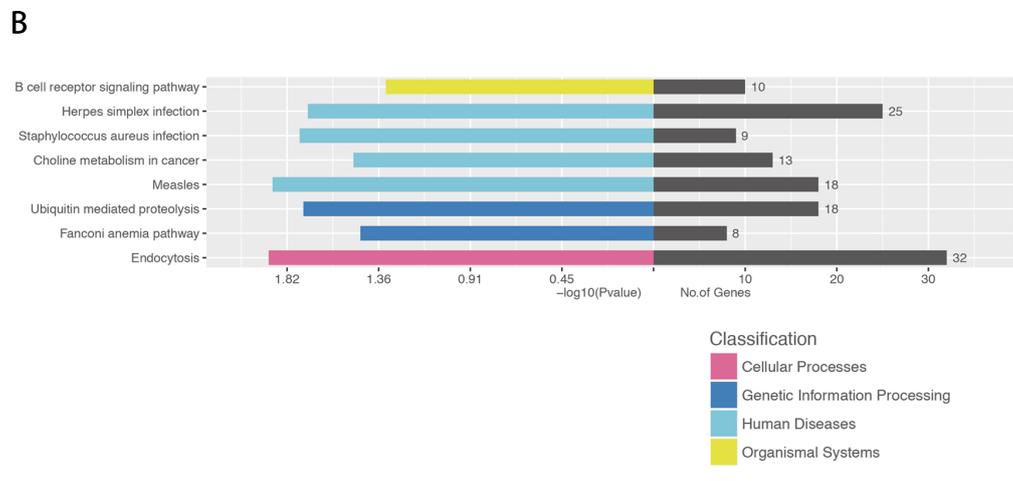
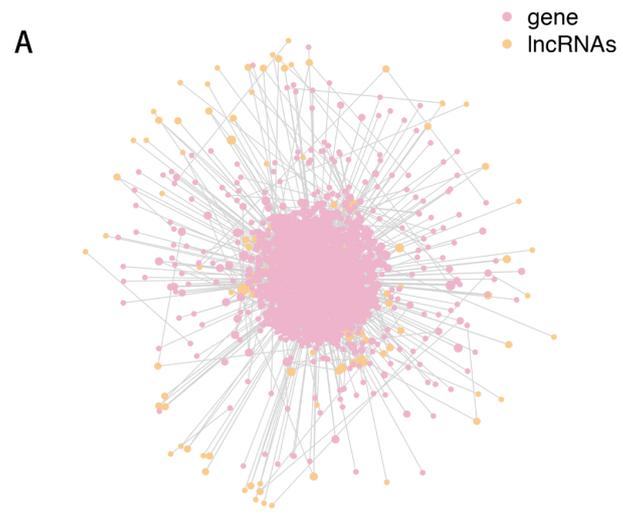


Fig.9

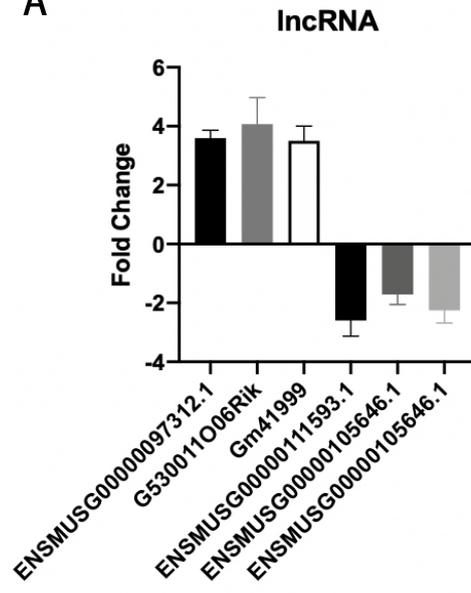
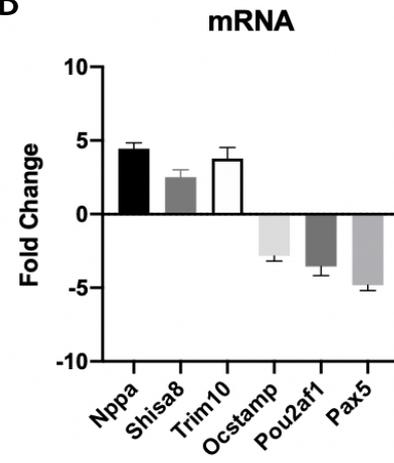
**A****B**

Figure 1 Analysis of differentially expressed genes (DEGs) in WT Pneumocystis infection and BAFF-R<sup>-/-</sup> Pneumocystis infection. A) Heat map shows the differentially expressed mRNAs between the WT PCP group and BAFF-R<sup>-/-</sup> PCP group. B) Volcano plots indicate the respective upregulated and downregulated mRNAs in mice from the BAFF-R<sup>-/-</sup> PCP group compared with those from the control group. In the clustering analysis, upregulated and downregulated genes are colored in red and green, respectively.

Figure 2 Analysis of differentially expressed genes (DEGs) in WT Pneumocystis infection and BAFF-R<sup>-/-</sup> Pneumocystis infection. A) Heat map shows the differentially expressed lncRNA between the WT PCP group and BAFF-R<sup>-/-</sup> PCP group. B) Volcano plots indicate the respective upregulated and downregulated mRNAs in mice from the BAFF-R<sup>-/-</sup> PCP group compared with those from the control group. In the clustering analysis, upregulated and downregulated genes are colored in red and green, respectively.

Figure 3 Novel lncRNA and new transcription area in BAFF-R<sup>-/-</sup> PCP mice. A) Heat map demonstrated that compared with WT PCP group, there were 3 novel lncRNA increased in BAFF-R<sup>-/-</sup> PCP group. B) Analysis of new transcription area in WT group, WT PCP group and BAFF-R<sup>-/-</sup> PCP group.

Figure 4 Gene ontology (GO) analysis of DEGs in WT PCP mice and BAFF-R<sup>-/-</sup> PCP mice. A) Changes in biological processes of DEGs; B) Changes in cellular component of DEGs; C) Changes in molecular function of DEGs.

Figure 5 KEGG pathway analysis of DEGs in WT PCP mice and BAFF-R<sup>-/-</sup> PCP mice.

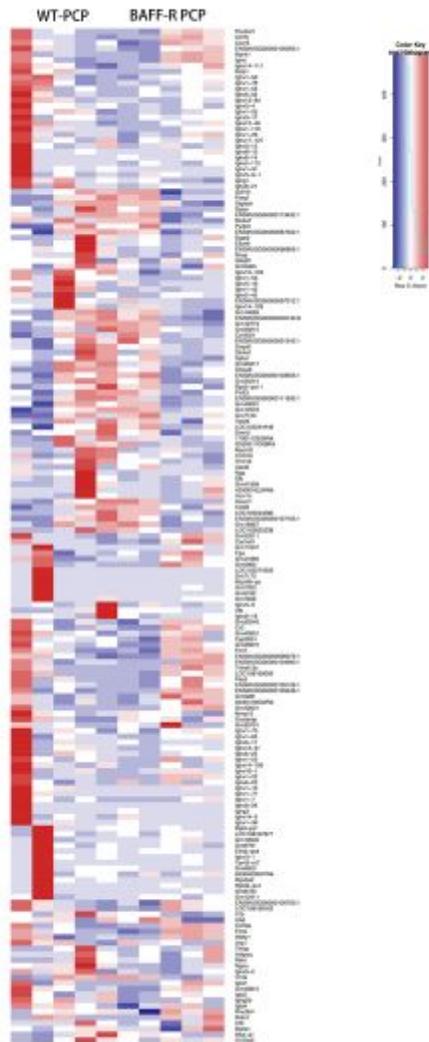
Figure 6 Gene ontology (GO) analysis of differentially expressed lncRNA-targeted mRNA in WT PCP mice and BAFF-R<sup>-/-</sup> PCP mice. A) Changes in biological processes of DEGs; B) Changes in cellular component of DEGs; C) Changes in molecular function of DEGs.

Figure 7 KEGG pathway analysis of differentially expressed lncRNA-targeted mRNA in WT PCP mice and BAFF-R<sup>-/-</sup> PCP mice.

Figure 8 Construction of the lncRNA and protein-coding genes co-expression network. A) LncRNA-TF core network consisting of the top 200 pairs of lncRNA and TF with the most relevance. TF transcript factor. B) Functional enrichment in each cluster indicated that lncRNA were functionally associated with mRNAs in particular biological processes.

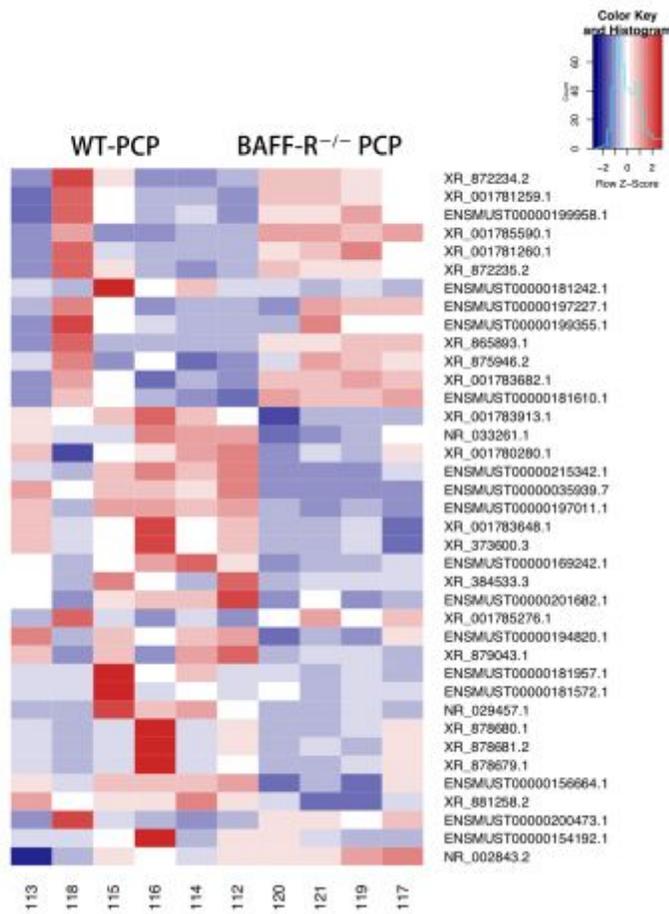
Figure 9 RT-PCR validation of differentially expressed lncRNA and mRNA. Quantitative reverse transcription PCR was performed to confirm the expression of the 6 select lncRNA (A) and 6 mRNAs (B).

# Figures



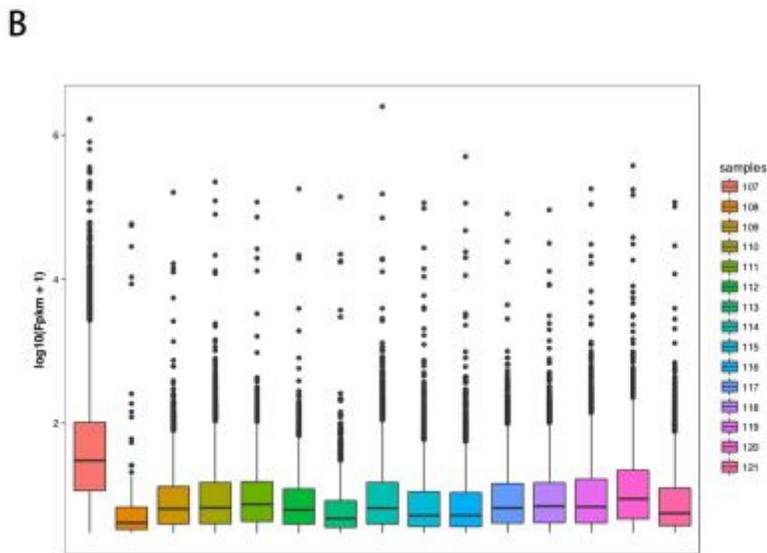
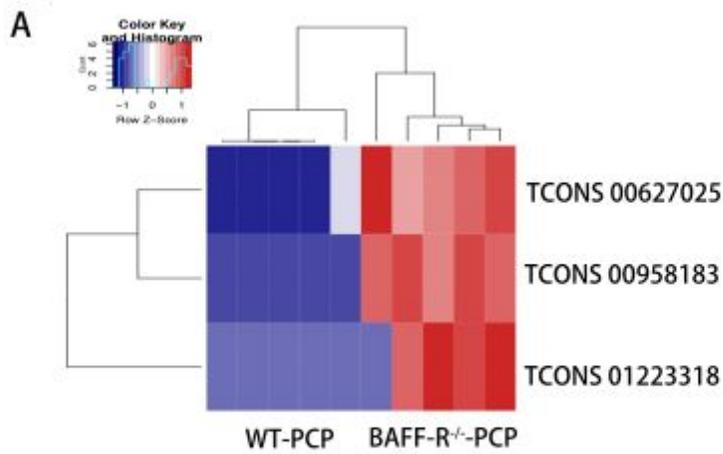
**Figure 1**

Analysis of differentially expressed genes (DEGs) in WT Pneumocystis infection and BAFF-R<sup>-/-</sup> Pneumocystis infection. A) Heat map shows the differentially expressed mRNAs between the WT PCP group and BAFF-R<sup>-/-</sup> PCP group. B) Volcano plots indicate the respective upregulated and downregulated mRNAs in mice from the BAFF-R<sup>-/-</sup> PCP group compared with those from the control group. In the clustering analysis, upregulated and downregulated genes are colored in red and green, respectively.



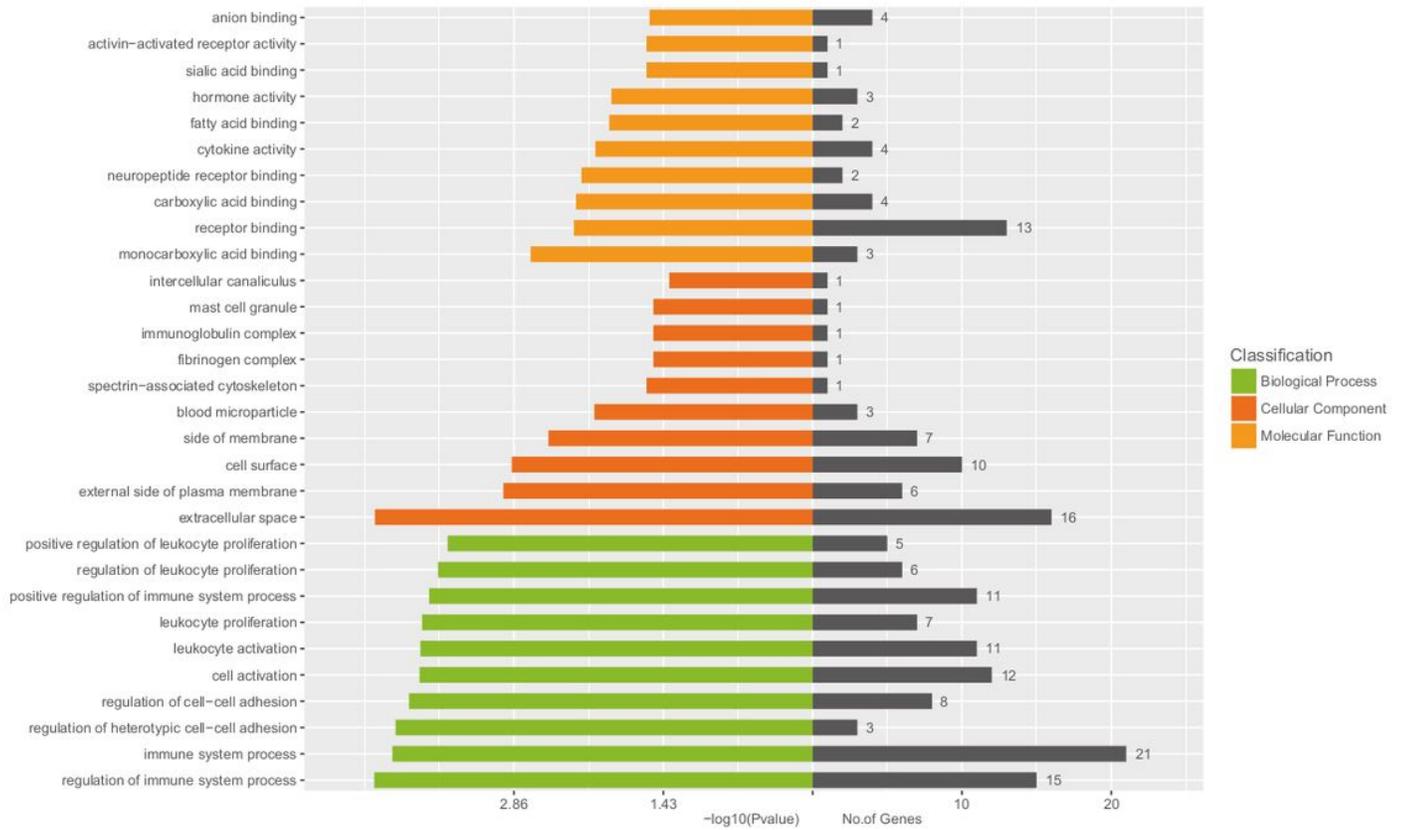
**Figure 2**

Analysis of differentially expressed genes (DEGs) in WT Pneumocystis infection and BAFF-R<sup>-/-</sup> Pneumocystis infection. A) Heat map shows the differentially expressed lncRNA between the WT PCP group and BAFF-R<sup>-/-</sup> PCP group. B) Volcano plots indicate the respective upregulated and downregulated mRNAs in mice from the BAFF-R<sup>-/-</sup> PCP group compared with those from the control group. In the clustering analysis, upregulated and downregulated genes are colored in red and green, respectively.



**Figure 3**

Novel lncRNA and new transcription area in BAFF-R<sup>-/-</sup> PCP mice. A) Heat map demonstrated that compared with WT PCP group, there were 3 novel lncRNA increased in BAFF-R<sup>-/-</sup> PCP group. B) Analysis of new transcription area in WT group, WT PCP group and BAFF-R<sup>-/-</sup> PCP group.



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Gene ontology (GO) analysis of DEGs in WT PCP mice and BAFF-R<sup>-/-</sup> PCP mice. A) Changes in biological processes of DEGs; B) Changes in cellular component of DEGs; C) Changes in molecular function of DEGs.

Statistics of Pathway Enrichment

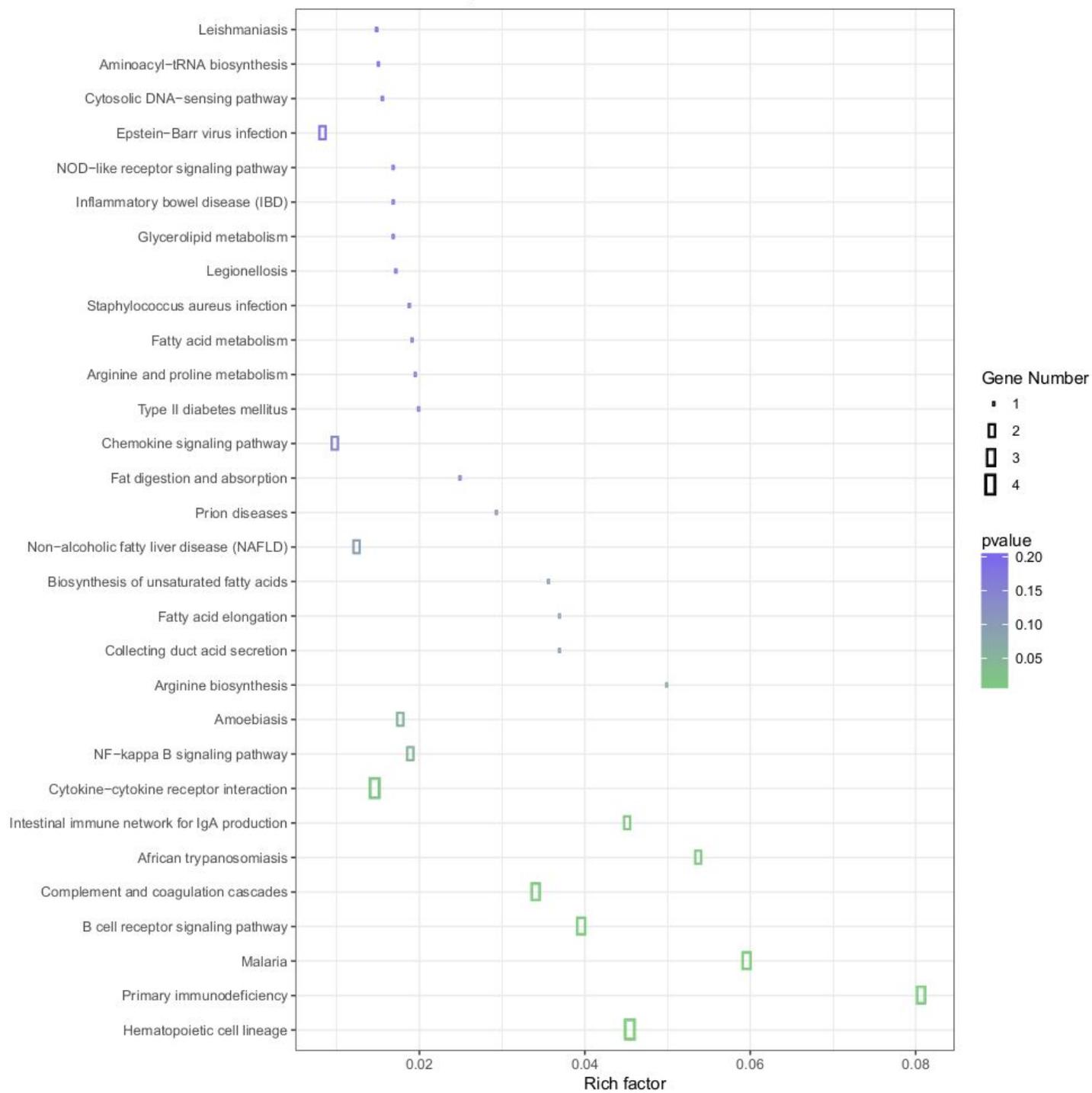
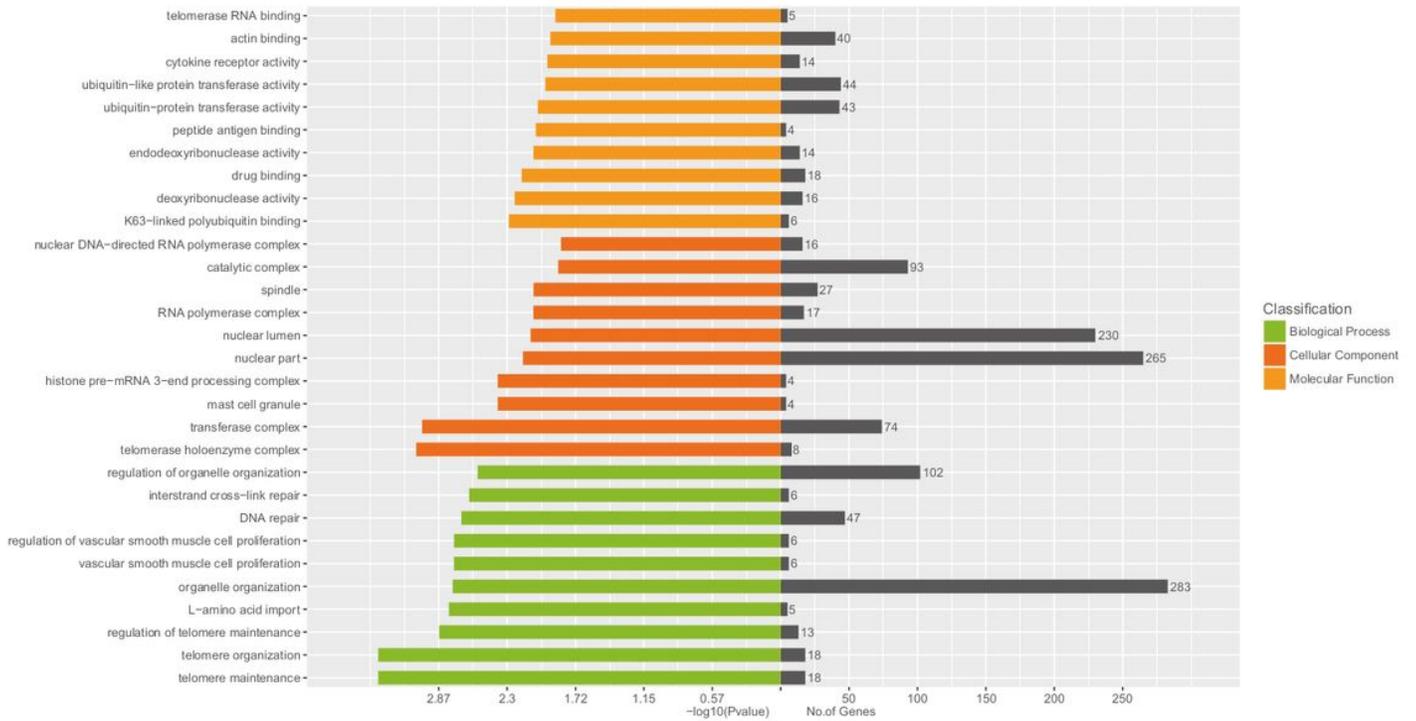


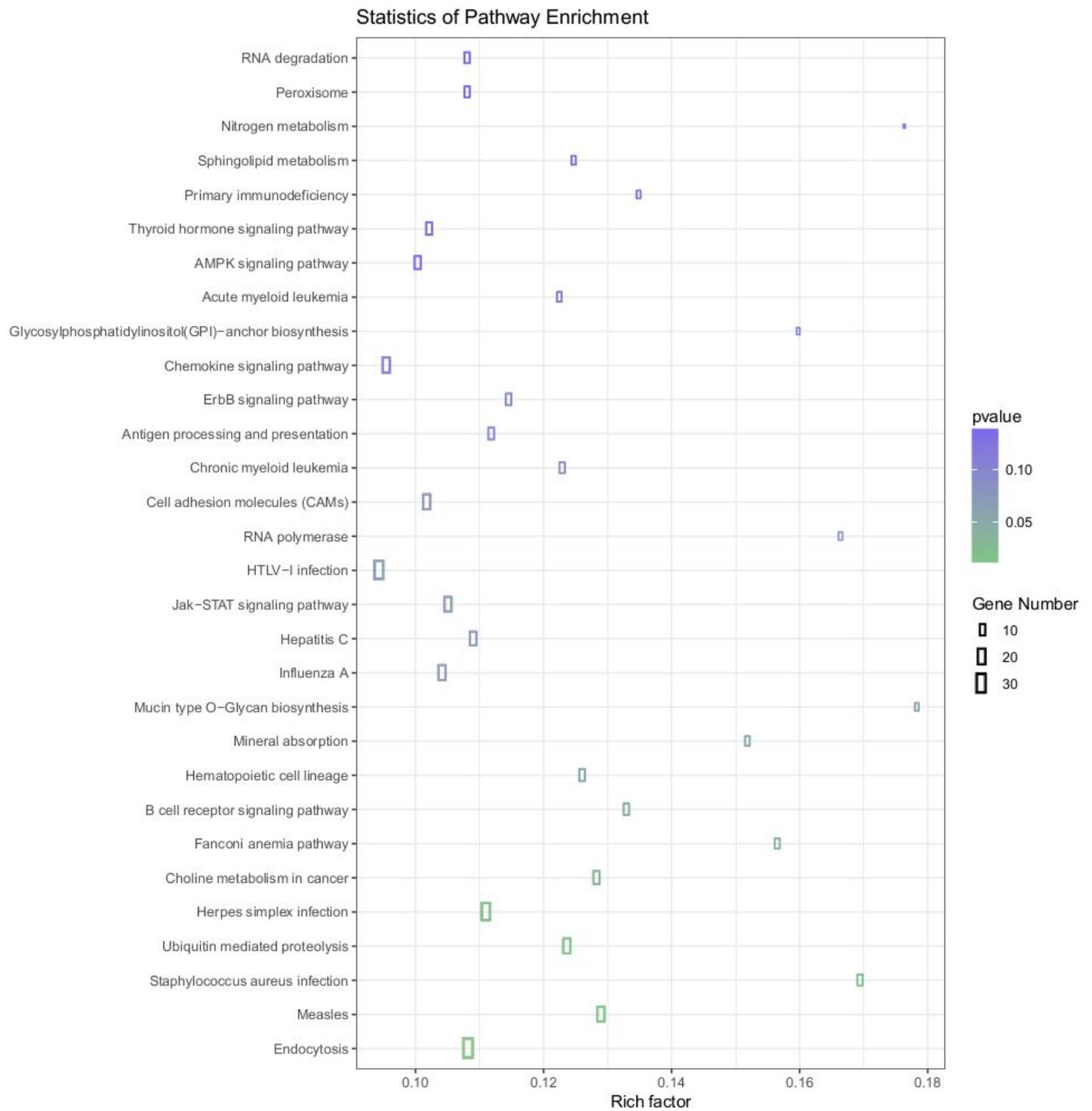
Figure 5

KEGG pathway analysis of DEGs in WT PCP mice and BAFF-R<sup>-/-</sup> PCP mice.



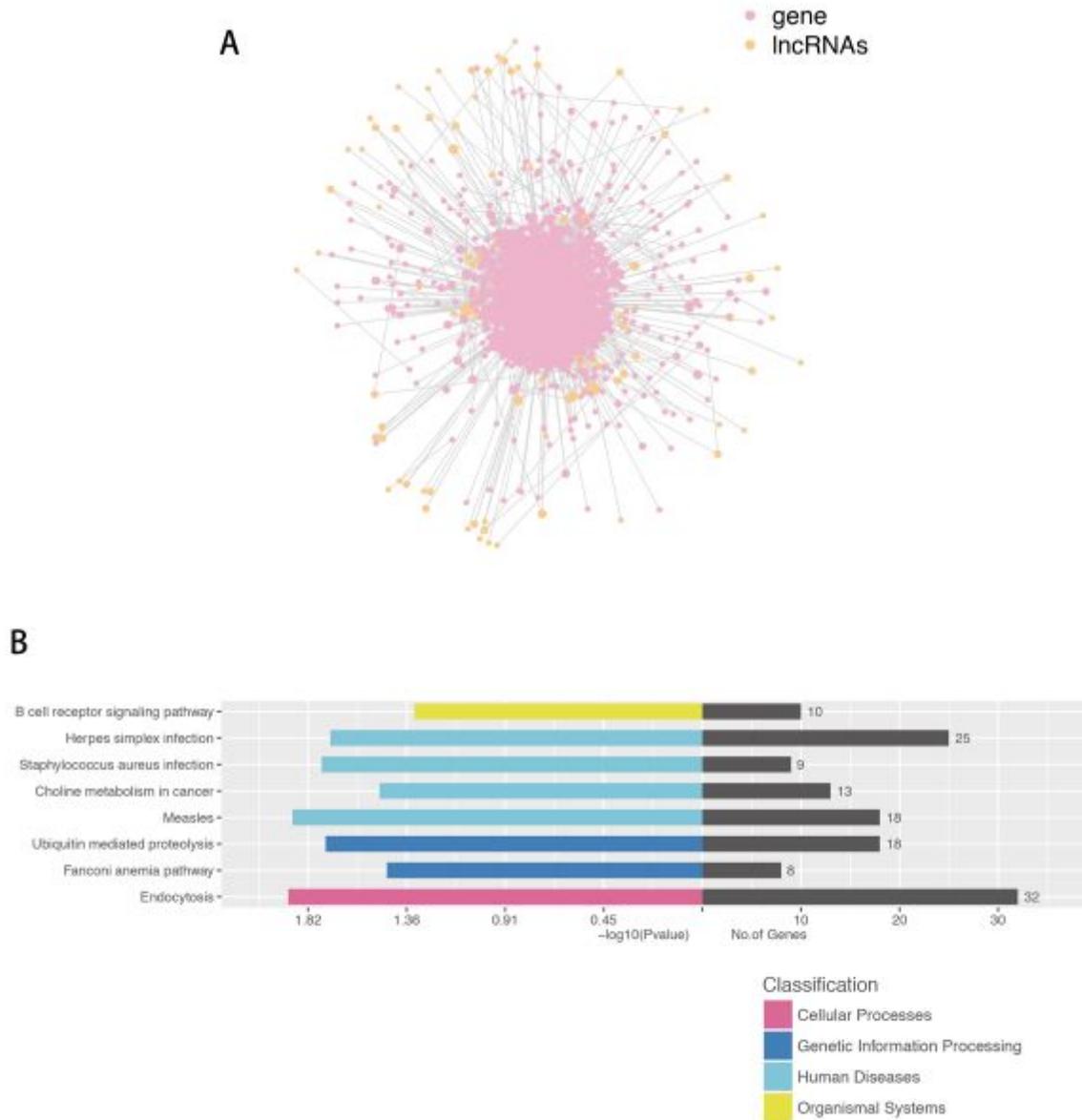
**Figure 6**

Gene ontology (GO) analysis of differentially expressed lncRNA-targeted mRNA in WT PCP mice and BAFF-R<sup>-/-</sup> PCP mice. A) Changes in biological processes of DEGs; B) Changes in cellular component of DEGs; C) Changes in molecular function of DEGs.



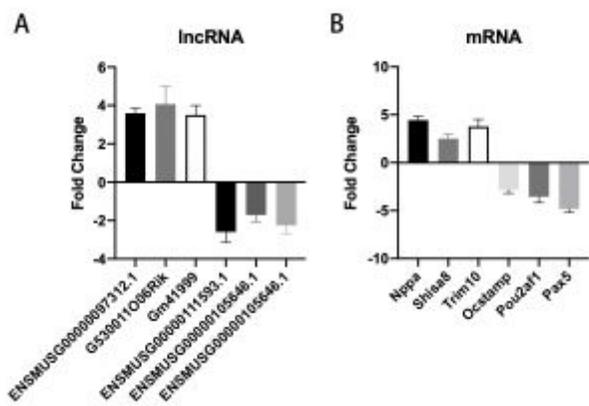
**Figure 7**

KEGG pathway analysis of differentially expressed lncRNA-targeted mRNA in WT PCP mice and BAFF-R<sup>-/-</sup> PCP mice.



**Figure 8**

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**Figure 9**

RT-PCR validation of differentially expressed lncRNA and mRNA. Quantitative reverse transcription PCR was performed to confirm the expression of the 6 select lncRNA (A) and 6 mRNAs (B).

## Supplementary Files

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- [Table1.pdf](#)
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