Identification and analysis of long non-coding RNAs that are involved in inflammatory process in response to transmissible gastroenteritis virus infection

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Abstract

Abstract Background: Transmissible gastroenteritis virus (TGEV) infection can activate the immune response and cause inflammation. Long noncoding RNAs (lncRNAs) play important roles in antiviral innate immune response. However, whether lncRNAs participate in TGEV-induced inflammation in porcine intestinal epithelial cells (IPECs) is largely unknown. Results: In this study, the next-generation sequencing (NGS) technology was used to analyze the profiles of lncRNAs in Mock and TGEV-infected porcine intestinal epithelial cell-jejunum 2 (IPEC-J2) cell line. A total of 106 lncRNAs were differentially expressed. Many differentially expressed lncRNAs act as elements to competitively attach miRNAs with mRNAs to mediate expression of genes that related to Toll-like receptor, NOD-like receptor, TNF, and RIG-I-like receptor pathways. Functional analysis of the binding proteins and the up/down-stream genes of the differentially expressed lncRNAs revealed that lncRNAs were principally related to immune response. Meanwhile, we found that the differentially expressed lncRNA TCONS_00058367 might lead to a reduction of p-p65 in TGEV-infected IPEC-J2 cells by negatively regulating its antisense gene PML. Conclusions: The data showed that differentially expressed lncRNAs might be involved in immune response induced by TGEV through acting as miRNA sponges, regulating their up/down-stream genes, or directly binding proteins.

Background

Virus can activate the immune response and cause inflammation by multiple ways, including NF-kappa B, Jak-STAT, Toll-like receptor, T cell receptor NOD-like receptor, TNF, RIG-I-like receptor signaling pathway, and so on [1-7]. Several previous studies have described that virulent TGEV caused significant inflammatory response in intestinal tissues, and animal death was mainly attributed to the severe unbalance of Na+ and K+ ions caused by the clinical manifestation of the infection. The pathogenesis of TGEV is strongly associated with the powerful induction of innate immune response during TGEV infection in host cells. A new study confirms that the RIG-I-like receptor, Toll-like receptor and NF-kappa B signaling pathway are involved in TGEV-induced inflammatory responses [8]. Intestinal epithelial cells (IPEC) are the targets for TGEV, and play an important role in the nutrition absorption and immune response against pathogens.

Non-coding Ribose Nucleic Acids (ncRNAs) typically do not encode proteins but functionally regulate protein expression that comprise a class of RNA molecules [9], including microRNAs (miRNAs) and circular RNAs (circRNAs), as well as long ncRNAs (lncRNAs). It has been speculated that many ncRNAs are involved in inflammatory and immune response in cells [2, 3, 10-14]. In our previous study, we determined that many mRNAs, miRNAs and circRNAs were significantly changed in the IPEC-J2 of TGEV infection by sequencing analysis. We also predicted potential functions of differentially expressed mRNAs, miRNAs and circRNAs that were closely related to inflammatory and immune response [15]. Recently, increasing studies have indicated that lncRNAs play important roles during antiviral innate immune response [16-19]. A recent study has shown that PEDV infection regulated lncRNAs expression patterns in the IPEC-J2 cell line, and lncRNAs may be involved in PEDV induced innate immune response

by their host genes. While, the regulatory mechanism of lncRNAs is diverse. The lncRNAs play roles not only in transcriptional regulation of their host genes, but also in post-transcriptional regulation [20-22], and show functions in protein localization [23, 24]. LncRNAs can regulate the processes of translation by binding with miRNA or act as precursors of miRNA [25-27]. For example, lncRNA SBF2-AS1 acts as a ceRNA to modulate cell proliferation via binding with miR-188-5p in acute myeloid leukemia [26]. LncRNA HOTAIR functions as a competing endogenous RNA to upregulate SIRT1 by sponging miR-34a in diabetic cardiomyopathy [28]. LncRNAs can serve as scaffold, and their specific domains can bind to different types of proteins or transcription factors to form a skeleton complex, thereby regulating the effector elements upstream or downstream of the genes to activate or inhibit gene transcription. LncRNA H19 decreases the transcriptional activity of p53 [29]. LncRNA SNHG10 facilitates hepatocarcinogenesis and metastasis by modulating its homolog SCARNA13 via a positive feedback loop [30]. LncRNAs can also directly or indirectly achieve the regulation of the expression of the target genes by recruiting some RNA-binding proteins. A novel lncRNA (Lnczc3h7a) binds to TRIM25 and promotes RIG-I-mediated antiviral innate immune responses [16]. LncRNA-MEG3 inhibits cell proliferation and invasion by modulating Bmi1/RNF2 [31].

This is the first study to demonstrate the expression profiles and regulatory mechanisms of lncRNAs during TGEV infection by high-throughput sequencing methods. This information will enable further research on the TGEV infection and facilitate the development of novel TGE therapeutics targeting lncRNAs.

Results

Overview of the Solexa high-throughput sequencing data

To investigate the lncRNA expression profiles of TGEV infected IPECs, IPECs were infected with TGEV strain (TGEV-infected group) and the normal IPEC line (Mock-infected group) was used as a control. The RNA-seq was performed with the total RNA extracted from IPECs infected with 1 MOI TGEV at 24 hpi. Among all mapped transcripts 24337 (66.22%) were classified as known mRNAs, 10367 (28.21%) were classified as new mRNAs, 26 (0.07%) were classified as other RNAs (including pseudogenes), and 2023 (5.50%) were classified as lncRNAs (including 62 known lncRNAs and 1961 new lncRNAs) (Figure 1a and Additional file 1: Table S1). Among them, 215 were antisense lncRNAs, 1427 lincRNAs, 220 other lncRNAs, 24 Promoter-associated lncRNAs, 115 sense overlapping lncRNAs, and 22 UTR lncRNAs (Figure 1b and Additional file 2: Table S2). The expression levels of 629 transcripts were changed remarkably (fold change > 1.5, and $p < 0.01$). Among all remarkably changed transcripts, 267 (42.45%) were classified as known mRNAs, 256 (40.70%) were classified as new mRNAs, and 106 (16.85%) were classified as lncRNA (Figure 1c). Among 106 lncRNAs, 16 were antisense lncRNAs, 79 lincRNAs, 5 other lncRNAs, 2 Promoter-associated lncRNAs, 3 sense overlapping lncRNAs, and 1 UTR lncRNAs (Figure 1d).

Feature comparison of lncRNA and mRNA

In the current study, 2023 lncRNAs and 34704 mRNAs transcripts were identified. The lncRNA and mRNA transcripts were compared for their total length, exon number, exon length, and expression level. We

found that known lncRNAs and novel lncRNAs, compared with mRNAs, had significantly shorter transcript length (Fig. 2a), and longer exons (Fig. 2b). These properties were consistent with the lower estimated number of exons for known lncRNAs and novel lncRNAs compared with mRNAs (Fig. 2c). The expression profiles of lncRNA and mRNA biotypes were presented as logarithmic distributions. The average mRNA expression level was higher than that of the known lncRNAs and novel lncRNAs (Figure 2d).

Profiling of lncRNAs

The differential expression of multiple lncRNAs in TGEV-infected group compared with mock-infected group was observed in Fig. 3. The expression levels of 106 lncRNAs were changed remarkably (fold change > 1.5, and $p < 0.01$). Among them 96 lncRNAs were up-regulated and 10 lncRNAs were down-regulated. (Additional file 3: Table S3)

LncRNAs act as miRNA precursors

LncRNAs can be spliced into multiple small RNAs which function as post-transcriptional regulators. To find potential miRNA precursors, lncRNAs were aligned to miRBase (version 21). Our result showed that there were 7 lncRNAs producing precursors of 13 miRNAs possibly (Additional file 4: Table S4). The secondary structures of these lncRNAs and miRNA precursors were predicted via the RNAfold web server (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi). Fig. 4 illustrates the secondary structure of TCONS_00013287, which might release the precursor sequence of miR-365 by an endonuclease cleaving, and form mature miR-365-3p and miR-365-5p finally. The same to their precursors, these 13 miRNAs have no differences between TGEV-infected group and Mock-infected group.

LncRNAs act as miRNA sponges

LncRNAs can rescue the translation levels of mRNA via pairing to miRNAs to prevent the binding of miRNAs and mRNA untranslated regions (UTR). In our study, we constructed a lncRNA-miRNA-mRNA expression interaction network combinated with the miRNA sequencing data [15]. A total of 61 differentially expressed lncRNAs and 55 differentially expressed mRNAs targeted 11 differentially expressed miRNAs in the network respectively (Fig 5a and Additional file 5: Table S5). To find the potential function of these significantly differentially expressed lncRNAs acting as miRNA sponges, KEGG analysis of the 55 differentially expressed mRNAs was performed and presented. The result showed that these mRNAs were participated in the Toll-like receptor signaling pathway, Herpes simplex infection, NOD-like receptor signaling pathway, TNF signaling pathway, and NF-kappa B signaling pathway primarily (Figure 5b).

LncRNA-binding proteins

We determined lncRNA-protein interactions using the catRAPID omics algorithm [32]. The star rating system of catRAPID helped us rank the results. The score was the sum of three individual values: 1) catRAPID normalized propensity, 2) presence of RNA/DNA binding domains and disordered regions, and 3) presence of known RNA-binding motifs. 372 lncRNA-protein interactions were predicted for

differentially expressed lncRNAs (Fig 6a and Additional file 6: Table S6); the GO annotation of 26 proteins with a ranking score >2 were next explored using GO enrichment analysis. The result showed that 34 lncRNAs interacted with 4 proteins (protein names are C7, ID2, MYC, IRF1) which involve in immune system process (Fig 6b).

Up- and down-stream genes of differentially expressed lncRNAs

We predicted the up- and down-stream genes of differentially expressed lncRNAs (100 K). 443 genes were obtained, some of which are shown in Fig 7a, and the full results in the Additional file 7: Table S7. GO analysis was conducted to enrich up- and down-stream targets of DE lncRNAs (http://www.geneontology.org/). The results exhibited that the 34 up- and down-stream targets of differentially expressed lncRNAs were primarily enriched in immune system process (Fig. 7b).

Validation of lncRNAs by qRT-PCR

To validate the RNA-seq results of differentially expressed lncRNAs, we tested the expression levels of them using Real-time PCR. The fold changes of 8 lncRNAs in TGEV-infected cells were referred to that in mock-infected cells. The results indicated that our sequencing results were accurate. See Fig 8 and Additional file 3: Table S3.

Function analysis of the antisense lncRNA TCONS_00058367

The software RNAplex [3] (http://www.tbi.univie.ac.at/RNA/RNAplex.1.html) was used to predict the complementary correlation of antisense lncRNA and mRNA. The prediction of best base pairing was based on the calculation of minimum free energy (MFE) through thermodynamics structure. The result showed that lncRNA TCONS_00058367 was located in physical contiguity PML (MFE = -239.61) (Fig. 9a). The antisense lncRNA TCONS_00058367 was down-regulated in TGEV-infected group, and PML was up-regulated in TGEV-infected group. To further understand the regulatory relationship between TCONS_00058367 and PML, IPEC-J2 cells were transfected with siRNA of TCONS_00058367 (si-TCONS_00058367) (or negative control) subsequently. The TCONS_00058367 level was down-regulated by si-TCONS_00058367, while the PML level was up-regulated by si-TCONS_00058367 (Fig. 9b). The STRING database (version 10.0) was used to further understand the regulatory relationship between PML and other differentially expressed mRNAs related to immune system process (Fig 9c and Additional file 8: Table S8). To explore the function of PML in the process of TGEV induced NF-κB activation, The siRNA of PML (si-PML-1 and si-PML-2 or negative control) were transfected into IPEC-J2 cells respectively , then infected with TGEV at 1 MOI for 24 h. The PML level was down-regulated by si-PML-1 and not affected by si-PML-2 (Fig 9d). p-p65 was decreased by si-PML-1 and not affected by si-PML-2 (Fig 9e).The siRNA sequences were shown in Additional file 9: Table S9

Discussion

Recent studies have shown that lncRNAs play important roles during antiviral innate immune response [16-19]. In particular, lncRNAs have been reported to be involved in the coronavirus infections [19, 33], but the roles of lncRNAs during TGEV induced innate immune response have not yet been elucidated. In our study, high-throughput sequencing techniques were used to investigate the lncRNA expression profiles of TGEV infected IPEC-J2. Among the transcripts of IPEC-J2 obtained in our study, a total of 2023 lncRNAs across the entire genome were screened after sequencing and bioinformatics analysis. These lncRNAs were characterized by shorter transcript length, longer exons, lower estimated number of exons and lower expression levels. These properties were also observed in other reported lncRNAs within the genome [19, 34-36].

In a previous study, TGEV induced inflammatory and immune response via NF-kappa B signaling pathway, Toll-like receptor signaling pathway, NOD-like receptor signaling pathway, Jak-STAT signaling pathway, TNF signaling pathway and RIG-I-like receptor signaling pathway [15]. In our study, We identified 106 lncRNAs differential expression between TGEV-infected group and Mock-infected group, reminding us that lncRNAs may be involved in the regulatory process of TGEV infection. LncRNAs can rescue the translation levels of mRNA via pairing to miRNAs to prevent the binding of miRNAs and mRNA untranslated regions (UTR). In this study, we found mir-218, which we mentioned earlier, had three target genes, DDX58, IRF1 and STAT1 that might be involved in inflammatory and immune response. Additionally, ten lncRNAs TCONS_00002283, TCONS_00019226, TCONS_00019227, TCONS_00021915, TCONS_00037709, TCONS_00043977, TCONS_00052757, TCONS_00064461, TCONS_00067143 and TCONS_00067979, which were differentially expressed in TGEV-infected group, were predicted to be targeted by this miRNA, indicating that the lncRNAs may compete with DDX58, IRF1 and STAT1 to affect their expression levels and influence TGEV-induced inflammatory and immune response. Some lncRNAs can directly bind to proteins to regulate the functions of proteins [23, 24]. We determined lncRNA-protein interactions using the catRAPID omics algorithm, the result showed that 34 lncRNAs interacted with 4 proteins (protein names are C7, ID2, MYC, IRF1) which involve in immune system process. One of the important functions of lncRNA is to act as antisense transcripts of mRNAs or located adjacent to protein coding genes.

In our data, many of the neighbouring genes correspond to compartments of the immune system, such as PML (ENSSSCT00000002141), IFNB1(ENSSSCT00000005691), RSAD2 (ENSSSCT00000009461), and IFIT5(ENSSSCT00000011440). Previous studies have shown that NF-kappa B signaling pathway, one of the most important pathway, plays an important role during TGEV- induced inflammatory and immune response [8, 15, 37, 38]. Therefore, changes in the expression levels of genes, which related in NF-kappa B signaling pathway, might influence the TGEV-induced inflammatory and immune response. The differentially expressed lncRNAs may affect TGEV-induced inflammatory and immune response by affecting NF-kappa B signaling pathway. It has been proved that PML promotes TNFα-induced transcriptional responses by promoting NF-κB activity [39]. We further confirm that silencing PML gene expression rescued the TGEV-induced NF-κB activity. In our study, lncRNA TCONS_00058367 was identified as a potential antisense transcript of PML, which suppress transcription of PML. Our work

uncovered that lncRNAs might act as regulatory elements of the host immune response when TGEV infection. While, further efforts should be paied to confirm the present findings.

Materials And Methods

Research Material

The lncRNA expression profile of IPEC-J2 was compared between the IPEC-J2 infected with TGEV (n = 2) and Mock group (n = 2). To identify lncRNAs expressed in TGEV infected IPEC-J2, cDNA libraries were constructed and sequenced on the HiSeq 2500 Illumina platform (Illumina, San Diego, CA, USA).

Strand-specific library construction and sequencing

After total RNA was extracted, rRNAs were removed to retain mRNAs and ncRNAs. Following the purification, the enriched mRNAs and ncRNAs were iron-fragmented at 95℃. Then, reverse transcriptase and random primers were used to generate the first strand cDNA from the cleaved RNA fragments. The second strand DNA was amplified by PCR, QiaQuick PCR extraction kit was used to purify the cDNA fragments, then these fragments were end repaired, poly(A) added, and ligated to Illumina sequencing adapters. The second-strand cDNA was digested by UNG (Uracil-N-Glycosylase), the products were size selected by PCR amplified, agarose gel electrophoresis, and sequenced using Illumina HiSeqTM 2500 system (Illumina, USA).

Alignment with reference genome

Reads containing adapters, low quality reads, and rRNA reads were removed. The remaining reads of each sample were then mapped to Sus Scrofa reference genome (Sus Scrofa 10.2) by TopHat2(version 2.0.3.12), respectively.

Transcripts reconstruction

Cufflinks (V2.2.1), which preferring to the program reference annotation-based transcripts (RABT), was used to reconstruct the transcripts. The influence of low coverage sequencing was fixed through Cufflinks constructing faux reads based on reference. During the end of assembly, similar fragments were removed from all of the reassembled fragments by aligning with reference genes. Then we used Cuffmerge to merge transcripts from different replicates of a group into a comprehensive set of transcripts, and then the transcripts from multiple groups were merged into a finally comprehensive set of transcripts.

Identification and annotations for novel transcripts

To identify the novel transcripts, all of the reconstructed transcripts were aligned with reference genome and divided into twelve categories using Cuffcompare (V2.2.1). We used the following parameters to identify reliable novel transcripts: the length of transcript was longer than 200 bp and the exon number was more than 2.

Classification, Characterization, and Validation of lncRNAs

Two softwares Coding-Non-Coding Index (CNCI) (https://github.com/www-bioinfo-org/CNCI) [40] and Coding Potential Calculator (CPC) (http://cpc.cbi.pku.edu.cn/) [41] were used to assess the protein-coding potential of new transcripts by default parameters. The intersection of both results were chosen as long non-coding RNAs.

Quantification of lncRNA abundance

LncRNA abundance was quantified by RSEM (V1.2.8) and normalized to FPKM (Fragments Per Kilobase of transcript per Million mapped reads). The formula is shown as follow:

[Due to technical limitations this formula could not be inserted. It can be found in the supplemental files.]

C, the number of fragments that are mapped to transcripts; N, the total number of fragments that are mapped to reference genes; L, the number of base pairs of transcript.

Significance analysis of lncRNAs

The edgeR package (http://www.r-project.org/) was used to identify differentially expressed lncRNAs. A fold change ≥ 2 and ≤ 0.5, plus a false discovery rate (FDR) <0.05, were identified as significant differentially expressed lncRNAs.

miRNA precursor Prediction

LncRNAs can be spliced into multiple small RNAs which function as post-transcriptional regulators. To find potential miRNA precursors, lncRNAs were aligned to miRBase (version 21). Those with identity more than 90% were selected.

LncRNA-miRNA interaction

Based on the sequences of lncRNAs, three softwares RNAhybrid (v2.1.2) + svm_light (v6.01), Miranda (v3.3a) and TargetScan (Version:7.0) were used to the candidate target genes. The interaction networks among lncRNA and miRNA were built and visualized using Cytoscape (v3.5.1) (http://www.cytoscape.org/).

LncRNA cis-regulation Analysis

One of the functions of lncRNAs is cis-regulation of their neighboring genes on the same allele. The up-stream lncRNAs which have intersection of promoter or other cis-elements may regulate gene expression in transcriptional or post-transcriptional level. The downstream or 3'UTR region lncRNAs may have other regulatory functions. LncRNAs, which are classified as located in an "unknown region" in Cuffcompare (V2.2.1) were annotated as up-or downstream of a gene. LncRNAs in up/down stream of a gene were likely to be cis-regulators. The interaction networks among lncRNA and up-or downstream genes were built and visualized using Cytoscape (v3.5.1) (http://www.cytoscape.org/).

Antisense lncRNA Analysis

In order to reveal the interaction between antisense lncRNA and mRNA, the software RNAplex [42] (http://www.tbi.univie.ac.at/RNA/RNAplex.1.html) was used to predict the complementary correlation of antisense lncRNA and mRNA.

GO and KEGG analysis of differentially expressed lncRNAs

GO database (http://www.geneontology.org/) and KEGG database (http://www.genome.jp/kegg/) were used to annotate the pathways. The calculating formula is the same as the previous study [15].The interaction networks among lncRNAs, miRNAs, mRNAs or proteins were built and visualized using Cytoscape (v3.5.1) (http://www.cytoscape.org/).

Quantification of lncRNAs, miRNAs, and mRNAs using qRT-PCR

According to the manufacturer's instructions, TRIzol reagent was used to extract the total RNA of IPEC-J2 cells, then reverse transcription was carried out using M-MLV reverse transcriptase (Invitrogen, US). qRT-PCR was performed on iQ5 real-time PCR System (Bio-Rad, US). The primers are shown in additional file 10:Table S10.

Western blot analysis

RIPA lysis buffer containing phenylmethylsulfonyl fluoride (PMSF) was used to treat samples to extract the protein, then using BCA Protein Assay Reagent (Pierce, US) to measure the protein concentration. Proteins were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, US) subsequently. Block the PVDF membrane with 5% non-fat milk for 2 h at room temperature and then incubate the PVDF membrane with primary antibodies overnight at 4 ℃ and HRP-conjugated secondary antibody at room temperature for 1 h subsequently. In the last step, the membrane was developed with enhanced chemiluminescence (ECL) (Promega, US).

Statistical analysis

The data are presented as the means ± SEM. Statistical significance was analyzed by unpaired Student's t-test. $p < 0.05$ was defined as statistical significance.

List Of Abbreviations

TGEV, Transmissible gastroenteritis virus; NGS, Next-generation sequencing; ncRNAs, Non-coding Ribose Nucleic Acids; lncRNAs, Long noncoding RNAs; IPECs, porcine intestinal epithelial cells; IPEC-J2, porcine intestinal epithelial cells-jejunum 2; miRNAs, microRNAs; KEGG, Kyoto Encyclopedia of Genes and Genomes; circRNA, circular RNAs; DF-12, Dulbecco's Modified Eagle Medium (DMEM)/F-12/HAM; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride; ECL,

enhanced chemiluminescence; MOI, multiplicity of infection; RPKM, reads per kilobase of transcript per million mapped reads.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and materials

The raw data were submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) (https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?view=run_browser). The accession numbers of the TGEV-infected group (T1, T2) and the Mock-infected group (M1, M2) are No.SRR6447591 and No.SRR6447590.

Competing interests

The authors declare that they have no competing interests.

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Authors contributions

XM performed the experiments, analyzed the data, wrote the paper, prepared figures and tables. XZ conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and tables, reviewed drafts of the paper. KW and XT involved in drafting and revising the manuscript. JG and MM performed the experiments and analyzed the data. YQ and LC involved in drafting the manuscript and prepared figures. YH involved in revising the manuscript. DT conceived and designed the experiments, contributed reagents/materials/analysis tools, reviewed drafts of the paper. All authors have read and approved the manuscript.

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References

1. Sumner RP, Maluquer de Motes C, Veyer DL, Smith GL: Vaccinia virus inhibits NF-kappaB-dependent gene expression downstream of p65 translocation. *Journal of virology* 2014, 88(6):3092-3102.

2. Zhang E, Lu M: Toll-like receptor (TLR)-mediated innate immune responses in the control of hepatitis B virus (HBV) infection. *Medical microbiology and immunology* 2015, 204(1):11-20.

3. Yang L, Zhang YJ: Antagonizing cytokine-mediated JAK-STAT signaling by porcine reproductive and respiratory syndrome virus. *Veterinary microbiology* 2016.

4. Ando Y, Yasuoka C, Mishima T, Ikematsu T, Uede T, Matsunaga T, Inobe M: Concanavalin A-mediated T cell proliferation is regulated by herpes virus entry mediator costimulatory molecule. *In vitro cellular & developmental biology Animal* 2014, 50(4):313-320.

5. Coutermarsh-Ott S, Eden K, Allen IC: Beyond the inflammasome: regulatory NOD-like receptor modulation of the host immune response following virus exposure. *The Journal of general virology* 2016, 97(4):825-838.

6. Collins AS, Ahmed S, Napoletano S, Schroeder M, Johnston JA, Hegarty JE, O'Farrelly C, Stevenson NJ: Hepatitis C virus (HCV)-induced suppressor of cytokine signaling (SOCS) 3 regulates proinflammatory TNF-alpha responses. *Journal of leukocyte biology* 2014, 96(2):255-263.

7. Chan YK, Gack MU: RIG-I-like receptor regulation in virus infection and immunity. *Current opinion in virology* 2015, 12:7-14.

8. Ding Z, An K, Xie L, Wu W, Zhang R, Wang D, Fang Y, Chen H, Xiao S, Fang L: Transmissible gastroenteritis virus infection induces NF-kappaB activation through RLR-mediated signaling. *Virology* 2017, 507:170-178.

9. Mattick JS, Makunin IV: Non-coding RNA. *Human molecular genetics* 2006, 15 Spec No 1:R17-29.

10. Ratnik K, Viru L, Merits A: Control of the rescue and replication of Semliki Forest virus recombinants by the insertion of miRNA target sequences. *PloS one* 2013, 8(9):e75802.

11. Bianchessi V, Badi I, Bertolotti M, Nigro P, D'Alessandra Y, Capogrossi MC, Zanobini M, Pompilio G, Raucci A, Lauri A: The mitochondrial lncRNA ASncmtRNA-2 is induced in aging and replicative senescence in Endothelial Cells. *Journal of molecular and cellular cardiology* 2015, 81:62-70.

12. Dasgupta N, Peng Y, Tan Z, Ciraolo G, Wang D, Li R: miRNAs in mtDNA-less cell mitochondria. *Cell death discovery* 2015, 1:15004.

13. Ji J, Qin Y, Ren J, Lu C, Wang R, Dai X, Zhou R, Huang Z, Xu M, Chen M *et al*: Mitochondria-related miR-141-3p contributes to mitochondrial dysfunction in HFD-induced obesity by inhibiting PTEN.

Scientific reports 2015, 5:16262.

14. Zhou R, Wang R, Qin Y, Ji J, Xu M, Wu W, Chen M, Wu D, Song L, Shen H *et al*: Mitochondria-related miR-151a-5p reduces cellular ATP production by targeting CYTB in asthenozoospermia. *Scientific reports* 2015, 5:17743.

15. Ma X, Zhao X, Zhang Z, Guo J, Guan L, Li J, Mi M, Huang Y, Tong D: Differentially expressed non-coding RNAs induced by transmissible gastroenteritis virus potentially regulate inflammation and NF-kappaB pathway in porcine intestinal epithelial cell line. *BMC genomics* 2018, 19(1):747.

16. Lin H, Jiang M, Liu L, Yang Z, Ma Z, Liu S, Ma Y, Zhang L, Cao X: The long noncoding RNA Lnczc3h7a promotes a TRIM25-mediated RIG-I antiviral innate immune response. *Nature immunology* 2019.

17. Liu S, Liu X, Li J, Zhou H, Carr MJ, Zhang Z, Shi W: Long noncoding RNAs: Novel regulators of virus-host interactions. *Reviews in medical virology* 2019:e2046.

18. Maarouf M, Chen B, Chen Y, Wang X, Rai KR, Zhao Z, Liu S, Li Y, Xiao M, Chen JL: Identification of lncRNA-155 encoded by MIR155HG as a novel regulator of innate immunity against influenza A virus infection. *Cellular microbiology* 2019:e13036.

19. Chen J, Zhang C, Zhang N, Liu G: Porcine endemic diarrhea virus infection regulates long noncoding RNA expression. *Virology* 2019, 527:89-97.

20. D'Angelo D, Mussnich P, Sepe R, Raia M, Del Vecchio L, Cappabianca P, Pellecchia S, Petrosino S, Saggio S, Solari D *et al*: RPSAP52 lncRNA is overexpressed in pituitary tumors and promotes cell proliferation by acting as miRNA sponge for HMGA proteins. *Journal of molecular medicine (Berlin, Germany)* 2019.

21. Li C, Tan F, Pei Q, Zhou Z, Zhou Y, Zhang L, Wang D, Pei H: Non-coding RNA MFI2-AS1 promotes colorectal cancer cell proliferation, migration and invasion through miR-574-5p/MYCBP axis. *Cell proliferation* 2019:e12632.

22. Liao YW, Ho BC, Chen MH, Yu SL: Host relieves lnc-IRAK3-3-sequestered miR-891b to attenuate apoptosis in Enterovirus 71 infection. *Cellular microbiology* 2019:e13043.

23. Yu S, Yang D, Ye Y, Liu P, Chen Z, Lei T, Pu J, Liu L, Wang Z: lncRNA AFAP1-AS1 promotes malignant phenotype through binding with LSD1 and repressing HBP1 in non-small cell lung cancer. *Cancer science* 2019.

24. Zhou X, Rao Y, Sun Q, Liu Y, Chen J, Bu W: Long noncoding RNA CPS1-IT1 suppresses melanoma cell metastasis through inhibiting Cyr61 via competitively binding to BRG1. *Journal of cellular physiology* 2019.

25. Tam C, Wong JH, Tsui SKW, Zuo T, Chan TF, Ng TB: LncRNAs with miRNAs in regulation of gastric, liver, and colorectal cancers: updates in recent years. *Applied microbiology and biotechnology* 2019.

26. Tian YJ, Wang YH, Xiao AJ, Li PL, Guo J, Wang TJ, Zhao DJ: Long noncoding RNA SBF2-AS1 act as a ceRNA to modulate cell proliferation via binding with miR-188-5p in acute myeloid leukemia. *Artificial cells, nanomedicine, and biotechnology* 2019, 47(1):1730-1737.

27. Wang W, Lou W, Ding B, Yang B, Lu H, Kong Q, Fan W: A novel mRNA-miRNA-lncRNA competing endogenous RNA triple sub-network associated with prognosis of pancreatic cancer. *Aging* 2019, 11(9):2610-2627.

28. Lopez-Jimenez E, Rojas AM, Andres-Leon E: RNA sequencing and Prediction Tools for Circular RNAs Analysis. *Advances in experimental medicine and biology* 2018, 1087:17-33.

29. Wang J, Cao B, Zhao H, Gao Y, Luo Y, Chen Y, Feng J: Long Noncoding RNA H19 Prevents Neurogenesis in Ischemic Stroke through p53/Notch1 pathway. *Brain research bulletin* 2019.

30. Lan T, Yuan K, Yan X, Xu L, Liao H, Hao X, Wang J, Liu H, Xie K, Li J *et al*: LncRNA SNHG10 facilitates hepatocarcinogenesis and metastasis by modulating its homolog SCARNA13 via a positive feedback loop. *Cancer research* 2019.

31. Li J, Jiang X, Li C, Liu Y, Kang P, Zhong X, Cui Y: LncRNA-MEG3 inhibits cell proliferation and invasion by modulating Bmi1/RNF2 in cholangiocarcinoma. *Journal of cellular physiology* 2019.

32. Agostini F, Zanzoni A, Klus P, Marchese D, Cirillo D, Tartaglia GG: catRAPID omics: a web server for large-scale prediction of protein-RNA interactions. *Bioinformatics (Oxford, England)* 2013, 29(22):2928-2930.

33. An H, Cai Z, Yang Y, Wang Z, Liu DX, Fang S: Identification and formation mechanism of a novel noncoding RNA produced by avian infectious bronchitis virus. *Virology* 2019, 528:176-180.

34. He Y, Ding Y, Zhan F, Zhang H, Han B, Hu G, Zhao K, Yang N, Yu Y, Mao L *et al*: The conservation and signatures of lincRNAs in Marek's disease of chicken. *Scientific reports* 2015, 5:15184.

35. Liu H, Wang R, Mao B, Zhao B, Wang J: Identification of lncRNAs involved in rice ovule development and female gametophyte abortion by genome-wide screening and functional analysis. *BMC genomics* 2019, 20(1):90.

36. Majewska M, Lipka A, Paukszto L, Jastrzebski JP, Gowkielewicz M, Jozwik M, Majewski MK: Preliminary RNA-Seq Analysis of Long Non-Coding RNAs Expressed in Human Term Placenta. *International journal of molecular sciences* 2018, 19(7).

37. Guo J, Li F, Qian S, Bi D, He Q, Jin H, Luo R, Li S, Meng X, Li Z: TGEV infection up-regulates FcRn expression via activation of NF-kappaB signaling. *Scientific reports* 2016, 6:32154.

38. Wang L, Qiao X, Zhang S, Qin Y, Guo T, Hao Z, Sun L, Wang X, Wang Y, Jiang Y *et al*: Porcine transmissible gastroenteritis virus nonstructural protein 2 contributes to inflammation via NF-kappaB activation. *Virulence* 2018, 9(1):1685-1698.

39. Ahmed A, Wan X, Mitxitorena I, Lindsay AJ, Paolo Pandolfi P, McCaffrey MW, Keeshan K, Chen YH, Carmody RJ: Regulation of NF-kappaB by PML and PML-RARalpha. *Scientific reports* 2017, 7:44539.

40. Sun L, Luo H, Bu D, Zhao G, Yu K, Zhang C, Liu Y, Chen R, Zhao Y: Utilizing sequence intrinsic composition to classify protein-coding and long non-coding transcripts. *Nucleic acids research* 2013, 41(17):e166.

41. Kong L, Zhang Y, Ye ZQ, Liu XQ, Zhao SQ, Wei L, Gao G: CPC: assess the protein-coding potential of transcripts using sequence features and support vector machine. *Nucleic acids research* 2007, 35(Web Server issue):W345-349.

42. Tafer H, Hofacker IL: RNAplex: a fast tool for RNA-RNA interaction search. *Bioinformatics (Oxford, England)* 2008, 24(22):2657-2663.

Additional File Legend

Additional file 1: Table S1. Classification of the assembled transcripts of IPEC-J2.

Additional file 2: Table S2. Classification of the lncRNAs of IPEC-J2.

Additional file 3: Table S3. The detailed information of differentially expressed lncRNAs.

Additional file 4: Table S4. Prediction of miRNA precursor of lncRNA.

Additional file 5: Table S5. The lncRNA-miRNA-mRNA regulation network.

Additional file 6: Table S6. The lncRNA-proteins regulation network.

Additional file 7: Table S7. The lncRNA-up and down genes network.

Additional file 8: Table S8. The interactions of differential expression mRNAs.

Additional file 9: Table S9. The sequences of TCONS_00058367 shRNA and PML siRNA.

Additional file 10: Table S10. Primers designed for qRT-PCR.

Figures

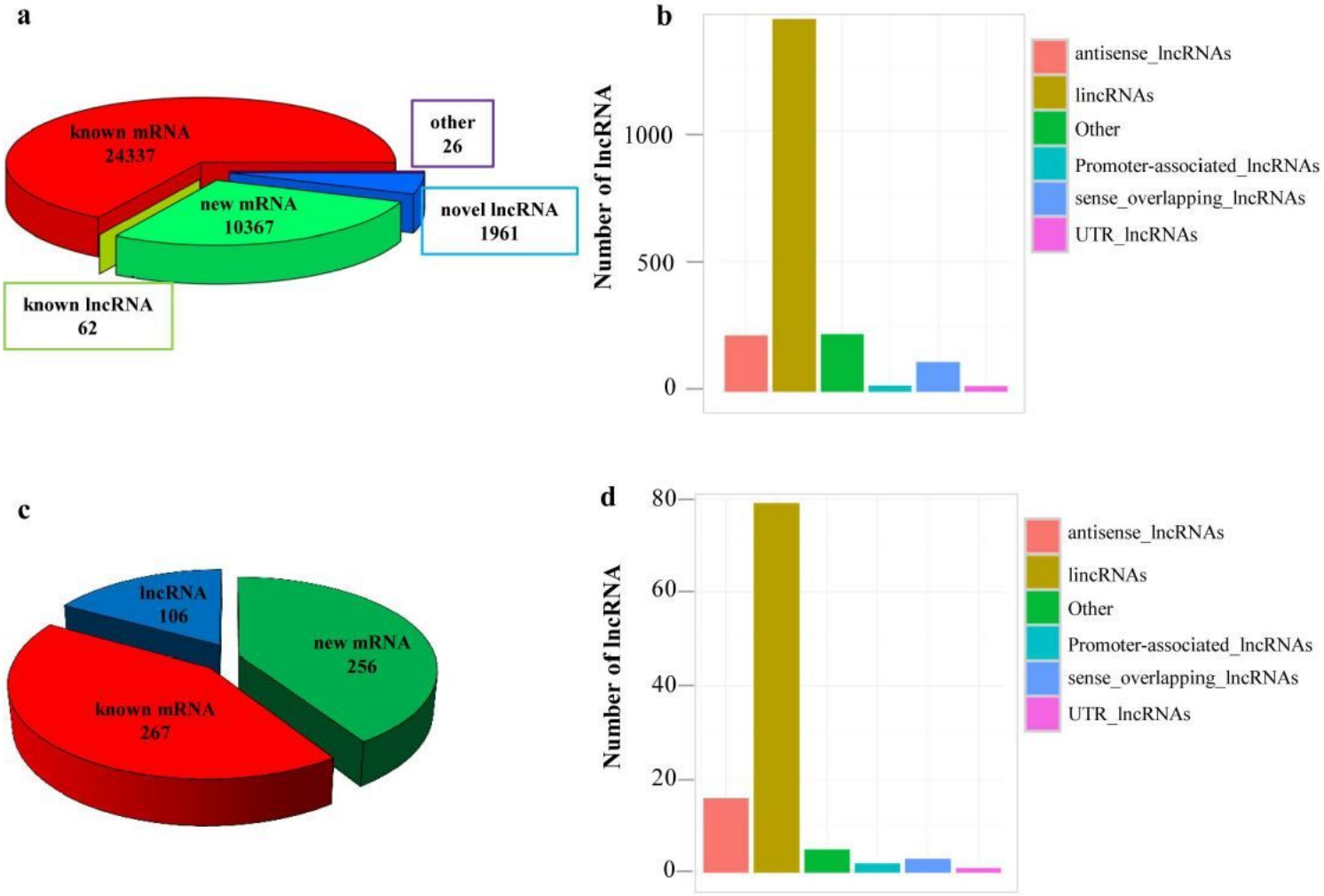

Figure 1

Classification of the assembled transcripts of IPEC-J2 according to their Ensembl code class (pie graphs) detailing lncRNA distribution (bar graphs) of: (a) and (b) all expressed transcripts; (c) and (d) transcripts were changed remarkably (fold change > 1.5, and $p < 0.01$).

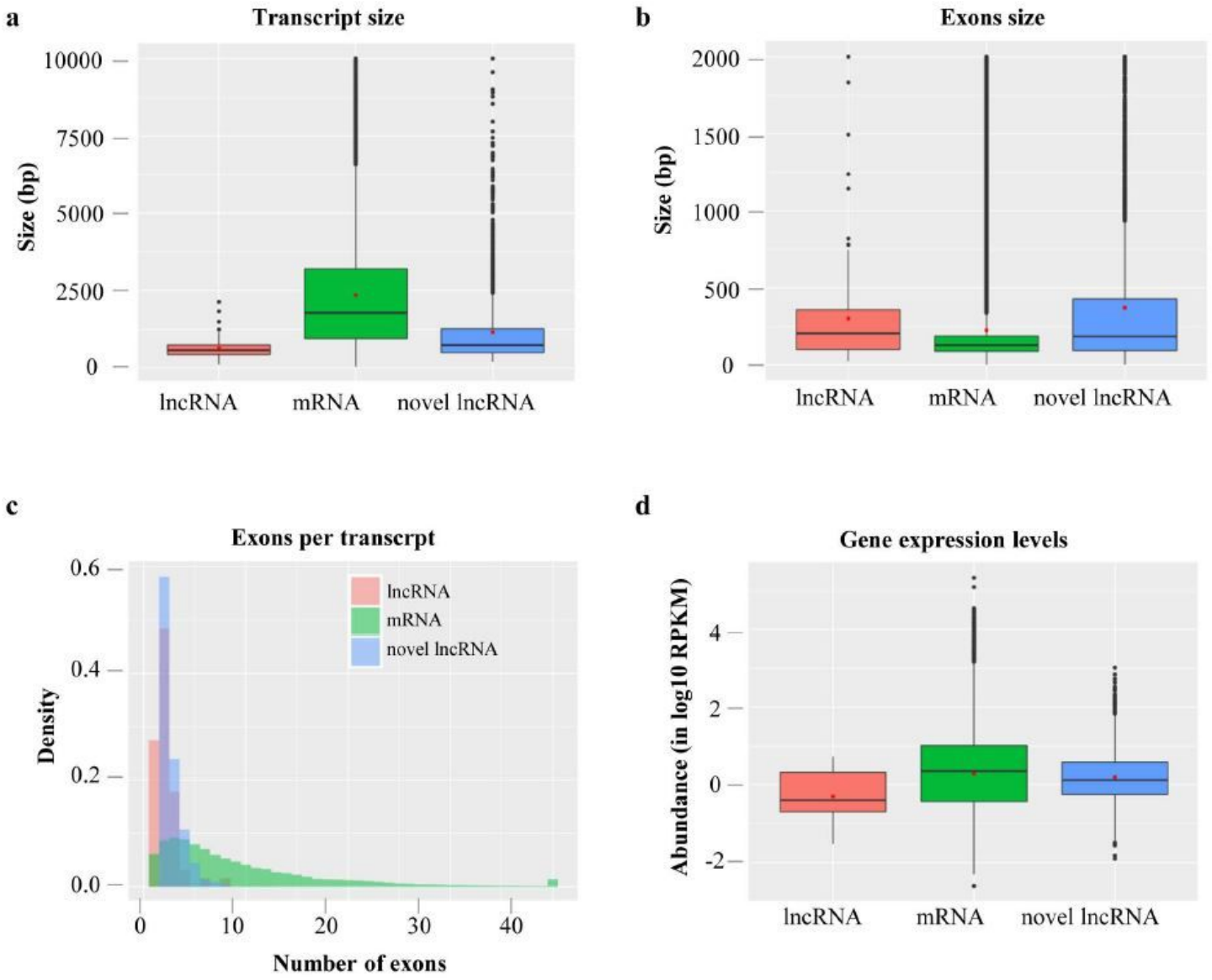

Figure 2

Genomic features of lncRNAs. (a) Transcript sizes of lncRNAs, novel lncRNAs, and mRNAs. (b) Exon sizes of lncRNAs, novel lncRNAs, and mRNAs. (c) Numbers of exons per lncRNAs, novel lncRNAs, and mRNAs. (d) Expression levels (FPKM values) of known lncRNAs, novel lncRNAs, and mRNAs. (a), (b), (d) are standard boxplots, which display the distribution of data by presenting the inner fence (the whisker, taken to 1.5× the Inter Quartile range, or IQR, from the quartile), first quartile, median, third quartile and outliers. The means are marked as tan diamonds.

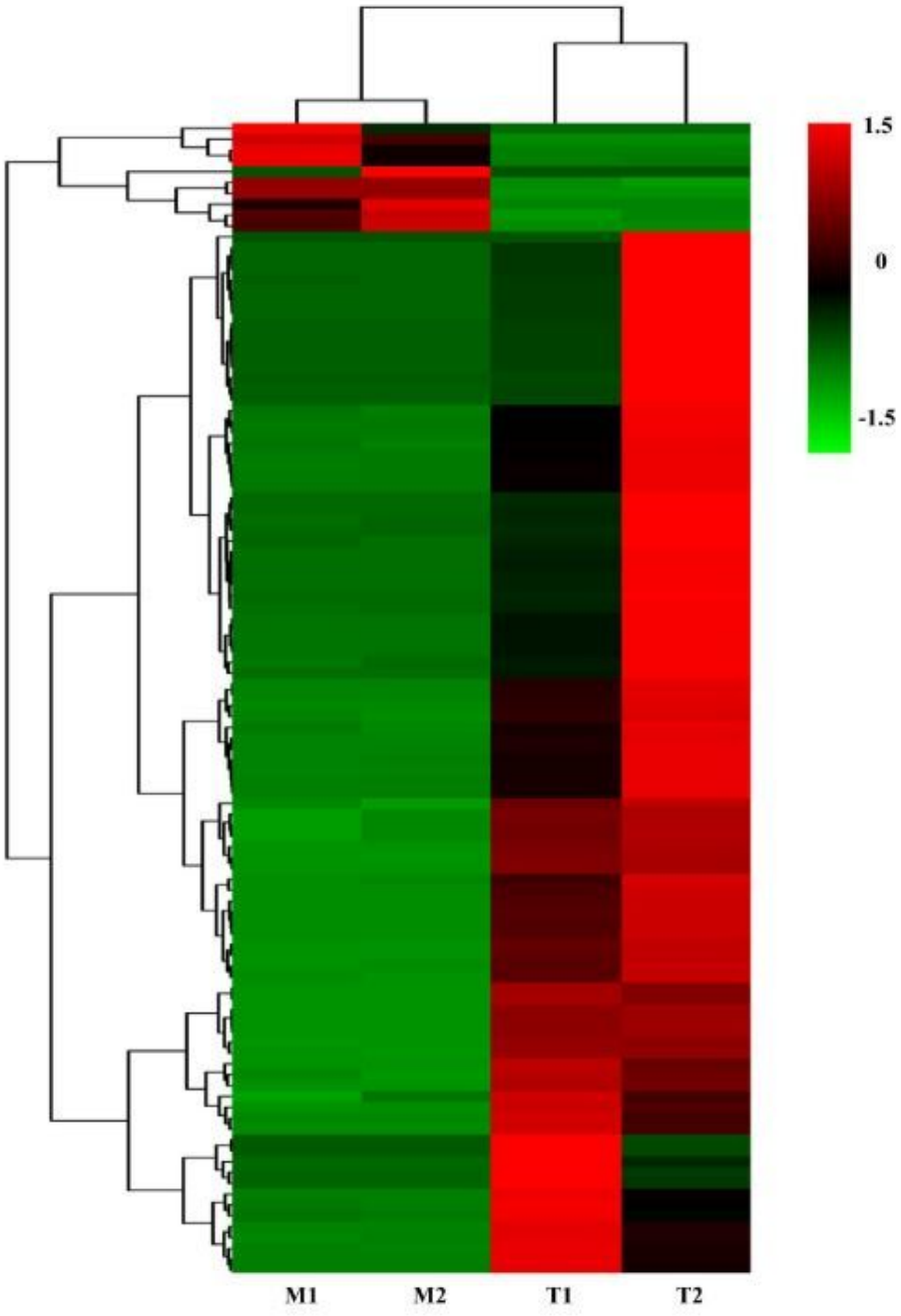

Figure 3

Clustering and Heatmap analysis of differentially expressed lncRNAs (FPKM) across TGEV infection (T1, T2) and Mock infection (M1, M2). Among them 96 lncRNAs were up-regulated and 10 lncRNAs were down-regulated (fold change > 1.5, and $p < 0.01$).

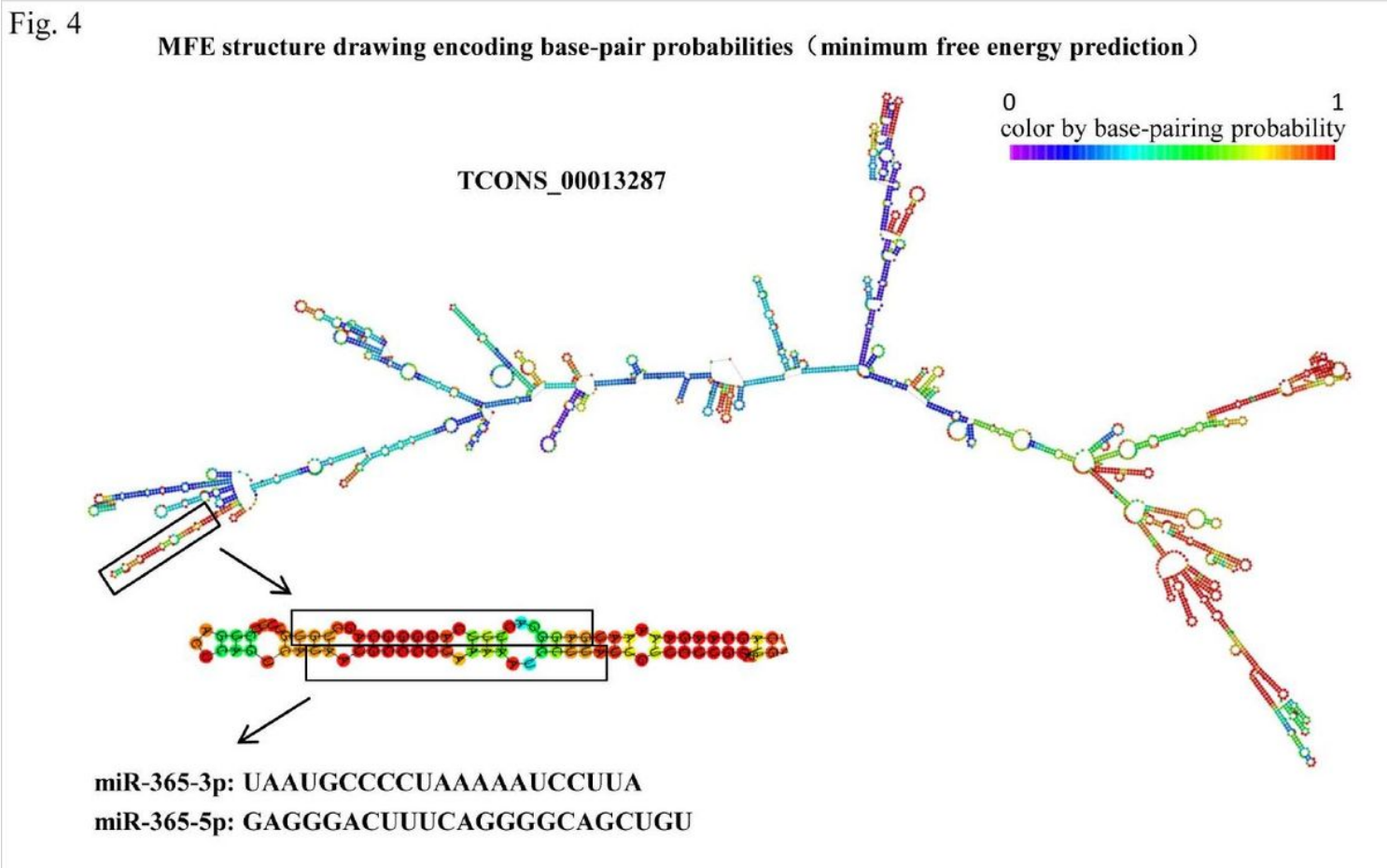

Figure 4

Prediction of miRNA Precursor of lncRNA (take TCONS_00013287 for example).

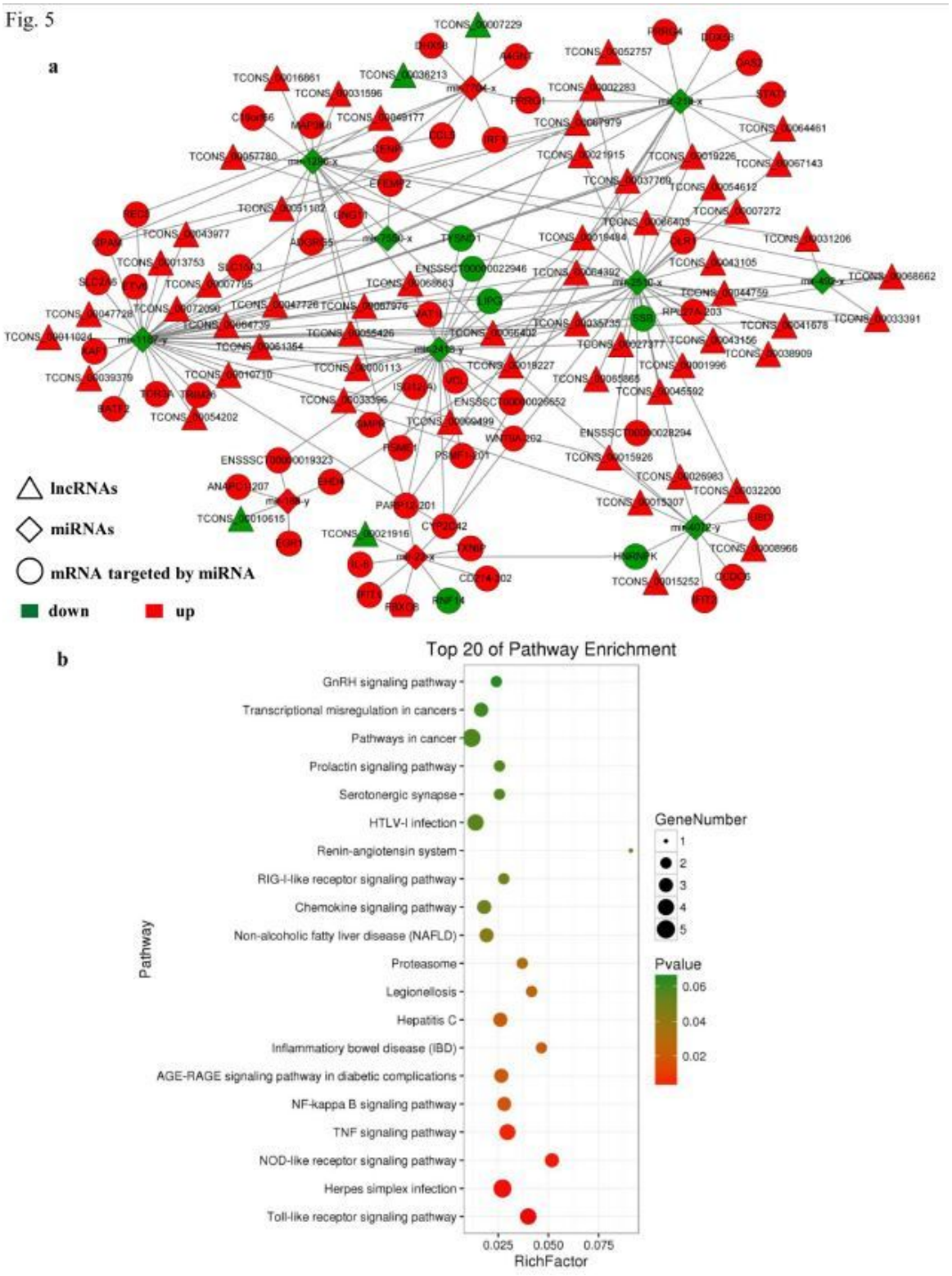

Figure 5

Regulatory network analysis of lncRNA-miRNA-mRNA. (a) The interaction network of lncRNA-miRNA-mRNA. Red and green respectively represent up- and down-regulated genes. roundness, triangle, and rhombus respectively indicate mRNAs, lncRNAs, and miRNAs. (b) KEGG enrichment analysis of lncRNA-miRNA-mRNA. In this graphic, the degree of KEGG enrichment is assessed by the Rich Factor, P-value, and

Gene Number. The closer the P-value is to zero, the greater the Rich factor is. The greater the Gene Number is, the more significant the enrichment is.

Fig. 6

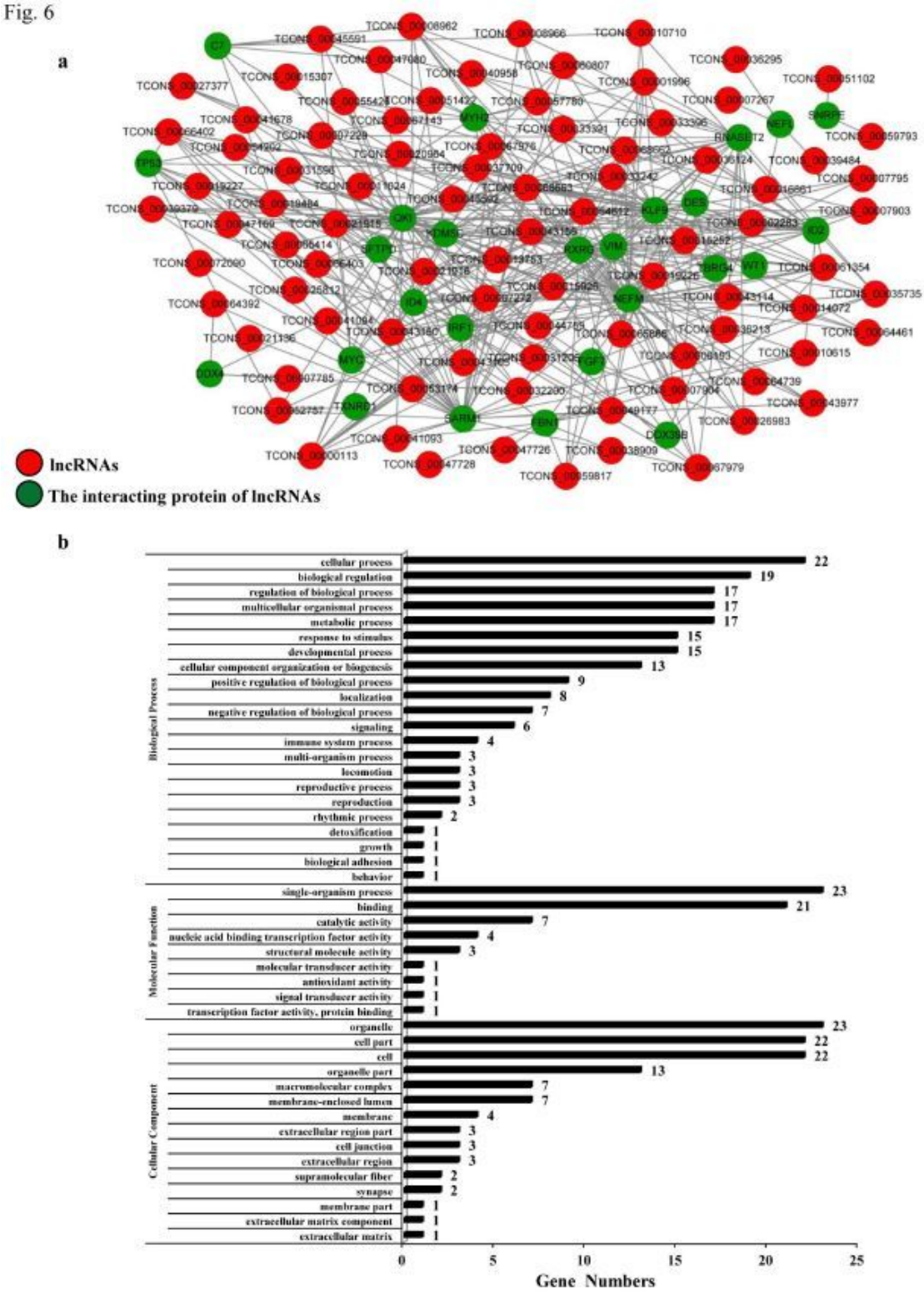

Figure 6

Regulatory network analysis of lncRNA-proteins. (a) The interaction network of lncRNA-proteins. Red and green respectively represent lncRNAs and the interacting proteins of lncRNAs. (b) GO enrichment analysis of the interacting proteins of lncRNAs.

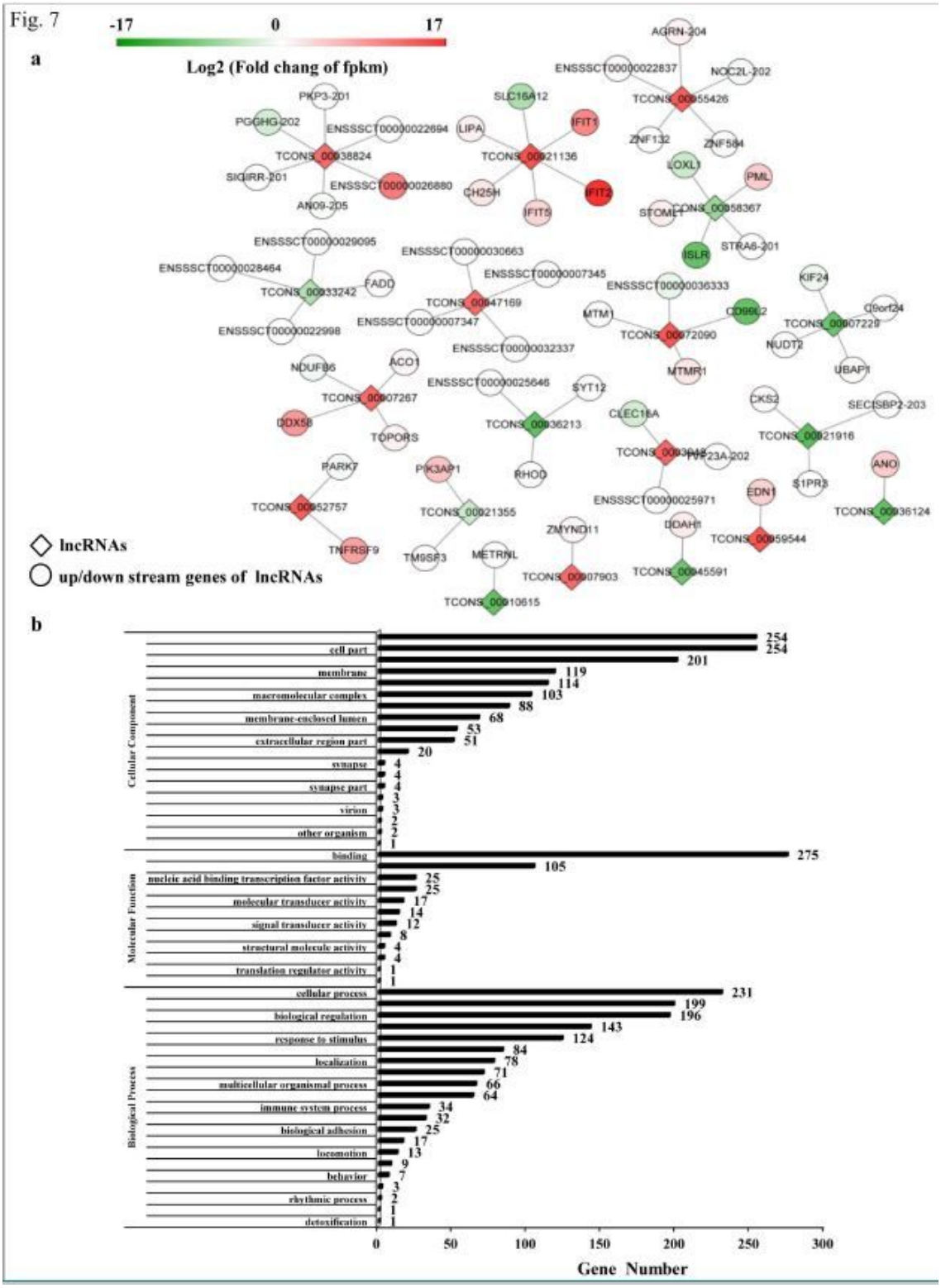

Figure 7

Regulatory network analysis of up- and down-stream genes of lncRNAs (a) The interaction network of lncRNAs and their up- and down-stream genes. (b) GO enrichment analysis of up- and down-stream genes of lncRNAs.

Fig. 8

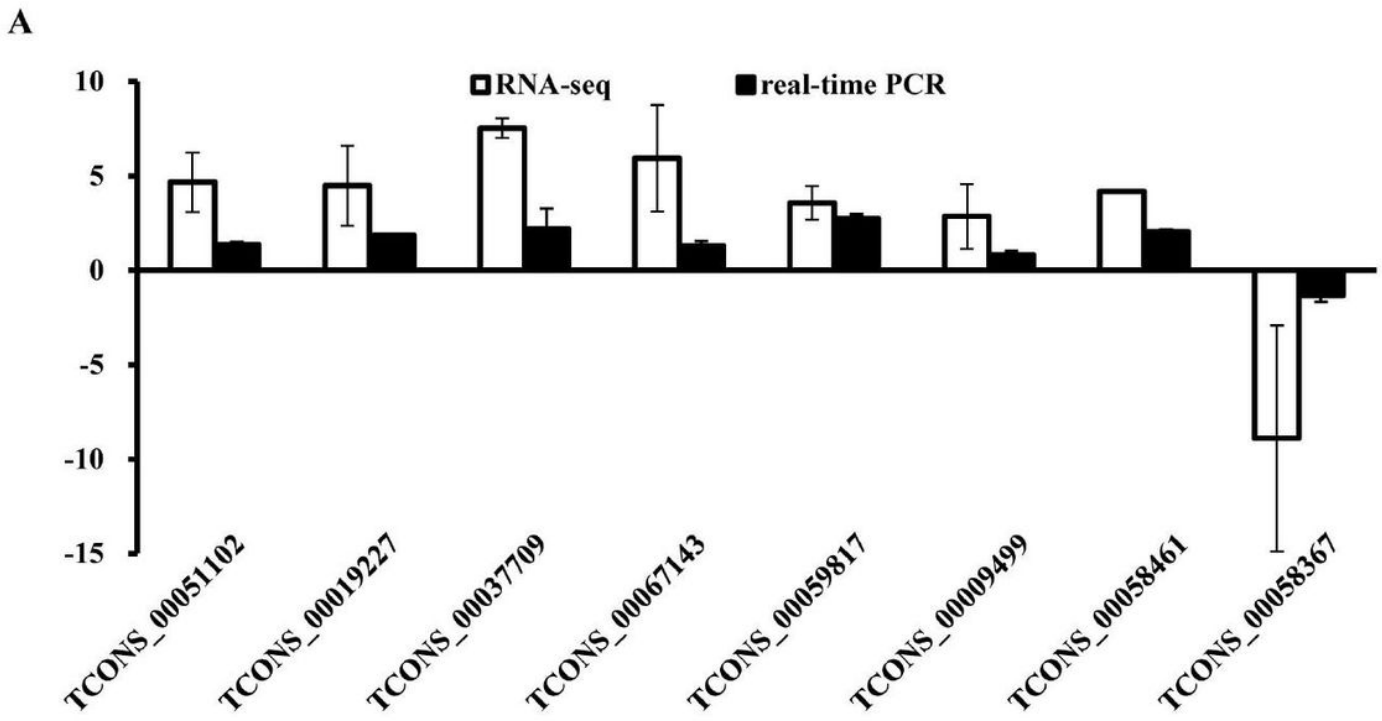

Figure 8

qRT-PCR validation of lncRNAs. The fold change was determined normalized toβ-actin using the 2-ΔΔCt method. The data from real-time PCR are shown as mean ± standard deviation (S.D.).

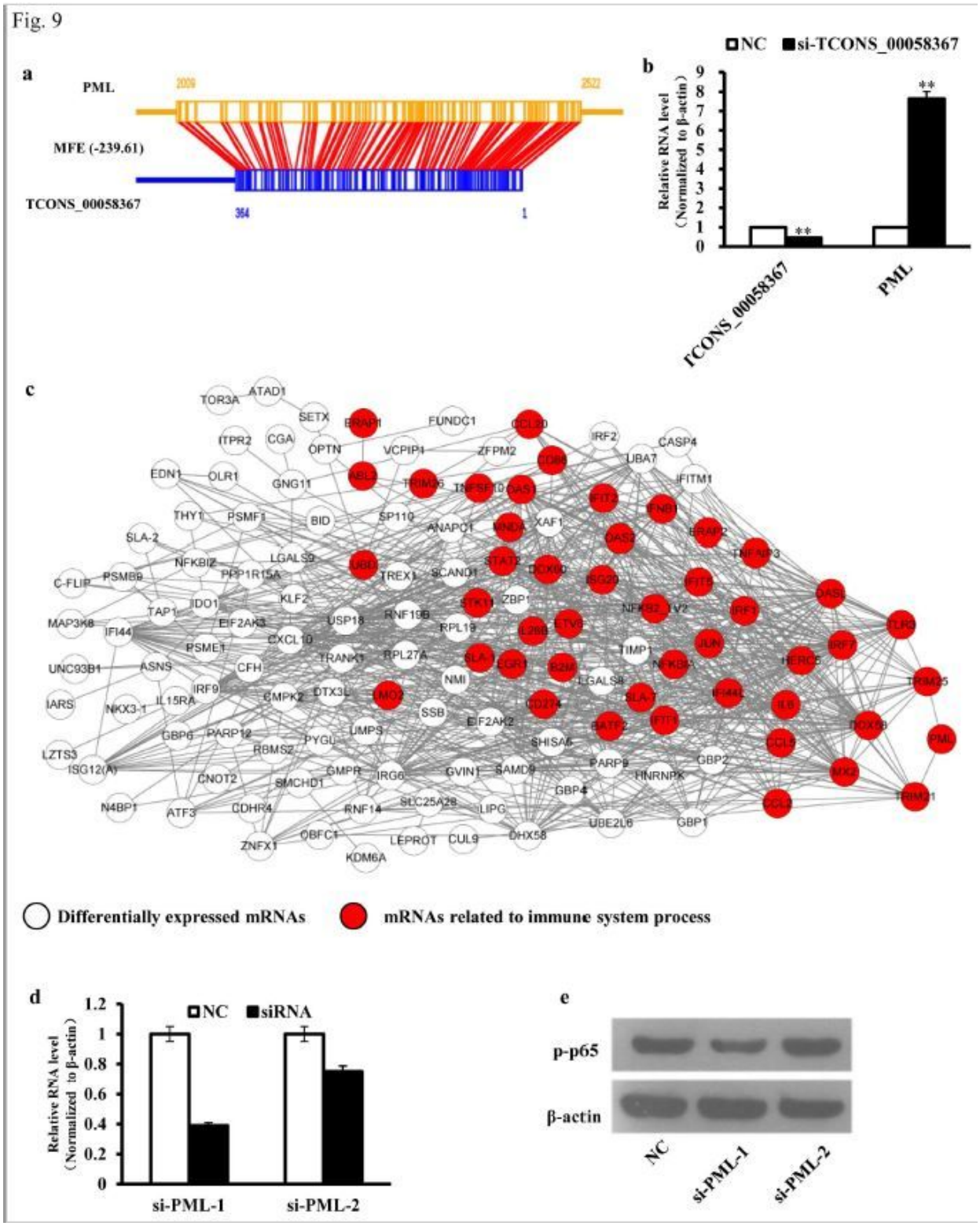

Figure 9

Function analysis of the antisense lncRNA TCONS_00058367. (a) The pattern diagrams showed that lncRNA TCONS_00058367 was located in physical contiguity PML (MFE = -239.61). (b) Knockdown effect of si-TCONS_00058367 on TCONS_00058367. The relative levels of TCONS_00058367 were measured by real-time PCR (normalized toβ-actin and in reference to the control). (c) The regulatory relationship between PML and other differentially expressed mRNAs related to immune system process

(red). (d) Knockdown effect of si-PML-1 and si-PML-2 on PML. The relative levels of PML were measured by real-time PCR (normalized toβ-actin and in reference to the control). (e) The effects of si-PML-1 and si-PML-2 on p-p65. **p < 0.01 in comparison with the control.

Supplementary Files

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