Supplementary Methods

Study population

All participants were enrolled at People's hospital affiliated with Ningbo University from March 2018 to March 2019. Clinical information from electronic medical records, morningness-eveningness questionnaire (MEQ) and blood samples were collected subsequently. A total of 5 healthy controls, 5 T2DM patients, 5 DR patients, 5 DPN patients and 5 DN patients were recruited for the RNA sequencing analysis.

Inclusion criteria

T2DM was diagnosed by one of the following criteria: fasting plasma glucose≥7mmol/L, 2h oral glucose tolerance test (OGTT) ≥11.1mmol/L, medication treatment or physician-diagnosed diabetes [1]. DR is defined as the presence of typical retinal microvascular signs in cases according to the diagnostic criteria established by Chinese Medical Association in 2014. Eye examinations by slit-lamp bio-microscopy with a condensing lens/ direct ophthalmoscopy was performed by ophthalmologists for every patient. DPN was diagnosed by abnormal nerve conduction studies (NCS) and/or electromyography, and the symptom or sign of neuropathy including abnormal clinical examination for pain, pressure, touch, pinprick, vibration, and ankle reflex, and absence of nondiabetic causes of neuropathy [2]. DN was clinically defined based on persistent macroalbuminuria >300 mg/g creatinine or presence of retinopathy with microalbuminuria 30-300 mg/g creatinine, and the absence of other possible renal or urinary tract disease in patients [3]. Healthy controls free of any of major chronic diseases were matched with cases in terms of age and gender. Patients with type 1 diabetes mellitus, coronary atherosclerotic heart disease (CAD), myocardial

infarction, stroke, autoimmune disorders, acute infectious disease, hepatic disease and other types of endocrine diseases were excluded.

Questionnaire Survey

Morningness-eveningness questionnaire (MEQ) was performed to assess the sleep chronotype in subjects. MEQ is a self-evaluation questionnaire and yields a sum score from 19 items. The sleep chronotype was determined as follows: 'definitely morning type', score 70-86; 'moderately morning type', score 59-69; 'intermediate (neither) type', score 42-58; 'moderately evening type', score 31-41; and 'definitely evening type', score 16-30.

RNA preparation, Library construction, and sequencing

Total RNA was isolated from peripheral blood mononuclear cells (PBMCs) using RNA Extraction Kit (TAKARA BIO INC, Japan) according to the manufacturer protocol and tested for purity using the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, CA, USA). RNA concentration was determined using the Qubit® RNA Assay Kit in the Qubit® 3.0 Fluorometer (Life Technologies, CA, USA). RNA integrity was further assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Finally, RNA samples with an RNA integrity number (RIN) of $\geq$7.0 and 28S/18S>1.5 were subjected to subsequent experiments.

Library construction and RNA sequencing were performed by Microanaly Co., Ltd. (Shanghai, China). The ribosomal RNA (rRNA) of samples was eliminated using Ribo-zero rRNA Removal kit (Illumina, San Diego, CA, USA). RNA-sequencing libraries were generated using NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs, MA, USA). The libraries were checked for quality by Agilent 2100 Bioanalyzer (Agilent

Technologies, Santa Clara, CA, USA), and accurately quantified by quantitative real-time polymerase chain reaction (Kapa Biosystems, Woburn, MA, USA). Lastly, the pooled libraries were sequenced on Illumina NovaSeq 6000 platform (Illumina Inc.)

Quality Control and Mapping

Raw data (raw reads) of fastq format were processed through in-house perl scripts/Trimmomatic-0.36 software, and clean data (clean reads) were obtained by removing reads containing adapter, contaminants, and low-quality reads. Moreover, the Q20, Q30 and GC content of the clean data were calculated. Then, the clean reads were mapped to the reference genome (GRCh38/hg19) using Hisat2 v2.0.5.

Quantitative Analysis of mRNAs and LncRNAs

Reads counts aligned to each gene were expressed as FPKM using RSEM v.1.2.8. FPKM refers to the number of fragments per kilobase of transcript sequence per millions base pairs sequenced, considering the effect of sequencing depth and gene length for the reads count at the same time. Differential expression analysis of two groups was performed using the edgeR R package (3.22.5). A threshold value of $|\log2 \text{ (fold change)}| \geq 1$with q value$<0.05$ was considered significantly differential expression.

Functional enrichment analyses

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed to predict the biological functions of differentially expressed mRNAs (DEmRNAs) and lncRNAs (DElncRNAs) by the Database for Annotation, Visualization, and Integrated Discovery (DAVID) 6.8 and KEGG Orthology Based Annotation System (KOBAS) 3.0. GO analyses consist of three categories in terms of

biological process (BP), cellular component (CC), and molecular function (MF). The adjusted *P* value<0.05 was considered to statistical significance. The results of the enrichment analysis were visualized using R software (version 4.1.0) and package ggplot2.

Protein-Protein Interactions (PPI) analysis

The PPI network of differentially expressed genes was conducted based on the STRING database (http://string-db.org/), and the hub genes of screened PPI were predicted using Cytohubba of Cytoscape3.8.0.

Prediction of lncRNA-mRNA interactions

The cis- and trans-acting target genes of lncRNAs were predicted. The cis-acting genes of lncRNA were determined if the adjacent genes located within 10 kb up- or down-stream of the lncRNA. The trans-acting genes were predicted with Pearson correlation coefficients (r^2>0.9, p value <0.01). Subsequently, the lncRNA-mRNA regulatory networks were conducted by Cytoscape3.8.0.

Statistical analysis

Continuous variables were presented as mean and standard deviation, and the differences between groups were evaluated with one-way analysis of variance (ANOVA). Categorical variables were presented as frequency and percentages, and proportions for categorical variables were compared using Fisher's exact test. Descriptive statistics were performed by IBM SPSS statistics version 24.0 (IBM, Armonk, New York, USA). A significant difference was considered to be indicated by *P* <0 05.

Reference

1. Luo, L., et al., *Gene Expression Profiling Identifies Downregulation of the Neurotrophin-MAPK Signaling Pathway in Female Diabetic Peripheral Neuropathy Patients.* J Diabetes Res, 2017. **2017**: p. 8103904.

2. Iqbal, Z., et al., *Diabetic Peripheral Neuropathy: Epidemiology, Diagnosis, and Pharmacotherapy.* Clin Ther, 2018. **40**(6): p. 828-849.
3. Tuttle, K.R., et al., *Diabetic kidney disease: a report from an ADA Consensus Conference.* Diabetes Care, 2014. **37**(10): p. 2864-83.

Table S1 Clinical and demographic characteristics of the participants.

racteristics		Controls	T2DM	DR	DPN	DN
mber		5	5	5	5	5
, years		56.2±9.01	58.8±3.96	51.4±9.32	62±7.35	54.8±10
der	Male	2	2	2	2	2
	Female	3	3	3	3	3
ting glucose, mmol/L		5.87±0.59	9.21±3.75	10.35±6.3	7.67±3.0	12.04±5
rningness-Eveningness Questionnaire [a]	Moderately morning type	1	4	2	5	3
	Intermediate type	4	1	0	0	2

T2DM, type 2 diabetes mellitus; DR, diabetic retinopathy; DPN, diabetic peripheral neuropathy; DN, diabetic nephropathy.

One-way analysis of variance (ANOVA) was used to compare age and fasting glucose between groups, and Bonferroni correction used to test the pairwise comparisons.

[a] Fisher's exact p value <0.05 after comparision between controls and T2DM as well as diabetic complications

Table S2. Number of differentially expressed genes in different groups

Group	Total	Up-regulate	Down-regulate
DE mRNAs			
T2DM vs. HC	2,776	1,815	961
DR vs. HC	2,697	987	1,710
DPN vs. HC	3641	2707	934
DN vs. HC	1606	837	769
DR vs. T2DM	2,926	589	2,337
DPN vs. T2DM	614	325	289
DN vs. T2DM	1,265	432	833
DE lncRNAs			
T2DM vs. HC	1,537	678	859
DR vs. HC	1,490	544	946
DPN vs. HC	1,925	1,076	849

DN vs. HC	882	428	454
DR vs. T2DM	1,840	714	1,126
DPN vs. T2DM	475	223	252
DN vs. T2DM	876	440	436

DE, differentially expressed; T2DM, type 2 diabetes mellitus; HC, health control; DR, diabetic retinopathy; DPN, diabetic peripheral neuropathy; DN, diabetic nephropathy.

Figure S1 Venn diagram showing the shared and distinct gene expression changes of DR, DPN and DN compared with T2DM. (A) The overlap of genes with up- and downregulated expression between the DR and T2DM groups. (B) The overlap of genes with up- and downregulated expression between the DPN and T2DM groups. (C) The overlap of genes with up- and downregulated expression between the DN and T2DM groups. T2DM, type 2 diabetes mellitus; HC, healthy control; DR, diabetic retinopathy; DPN, diabetic peripheral neuropathy; DN, diabetic nephropathy.

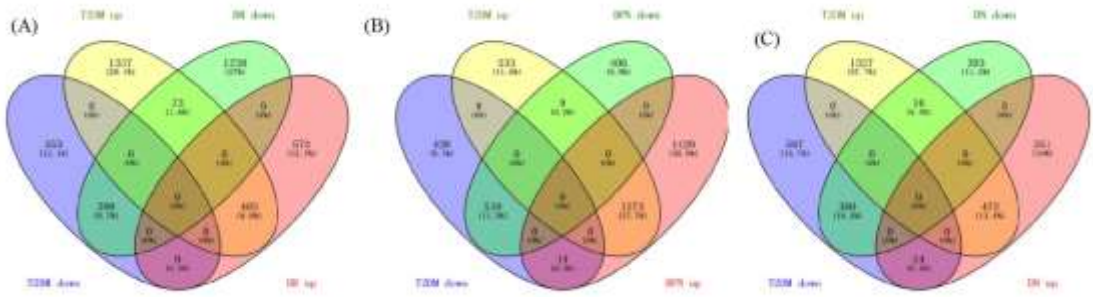

Figure S2 Venn diagram showing the shared and distinct lncRNA changes of DR, DPN and DN compared with T2DM. (A) The overlap of genes with up- and downregulated expression between the DR and T2DM groups. (B) The overlap of genes with up- and downregulated expression between the DPN and T2DM groups. (C) The overlap of genes with up- and downregulated expression between the DN and T2DM groups. T2DM, type 2 diabetes mellitus; HC, healthy control; DR, diabetic retinopathy; DPN, diabetic peripheral neuropathy; DN, diabetic nephropathy.

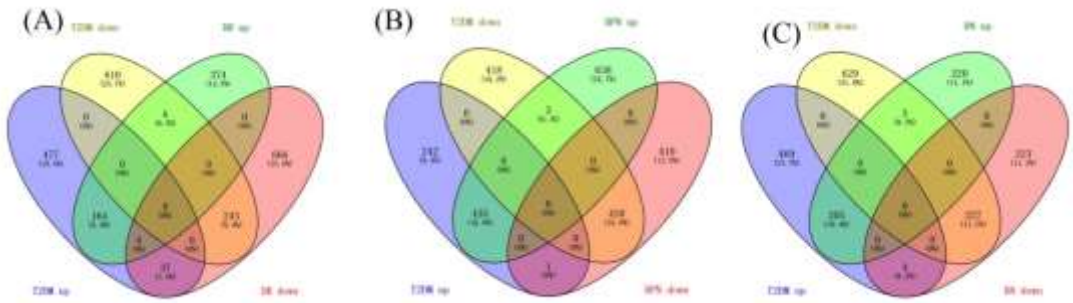

Figure S3 GO analysis of DEmRNAs in T2DM and diabetic complications compared with health control. Scatterplots of different colors presented different comparison groups. The horizontal axis was the negative $\log_{10}$ (*P* value) of GO analysis. GO, Gene Ontology; DEmRNAs, differentially expressed mRNAs; T2DM, type 2 diabetes mellitus; DR, diabetic retinopathy; DPN, diabetic peripheral neuropathy; DN, diabetic nephropathy; HC, health control; BP, biological processes.

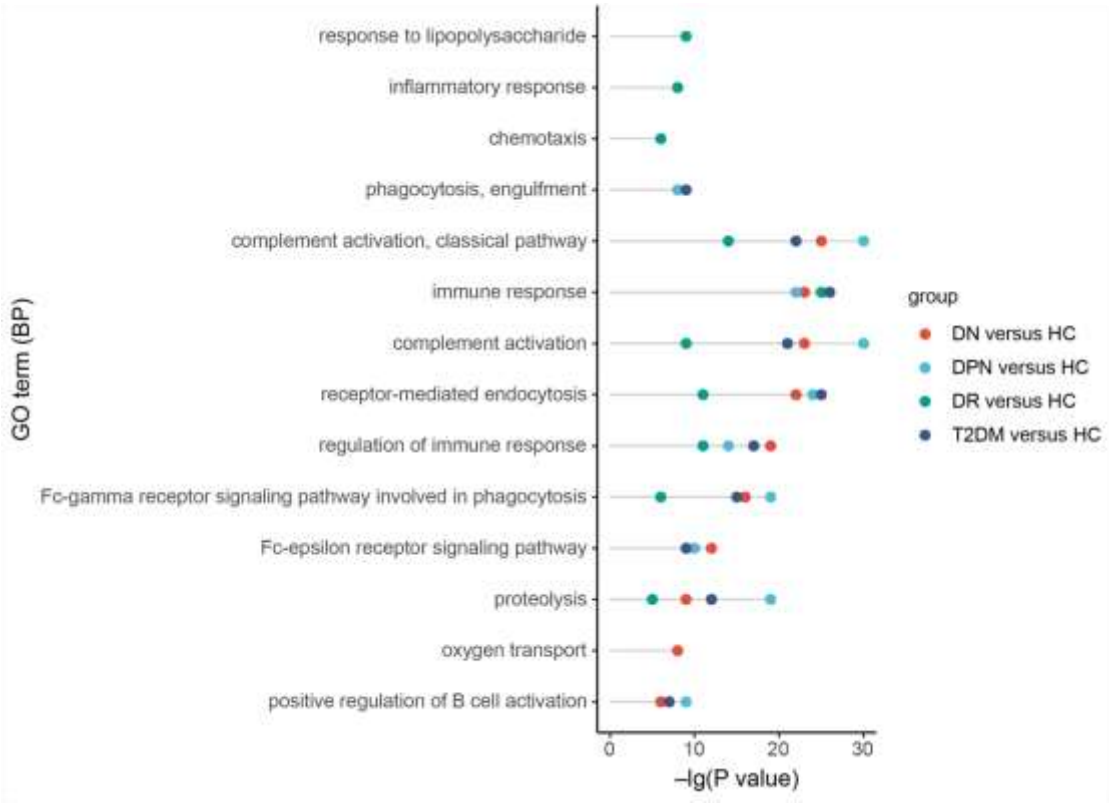

Figure S4 GO analysis of DEmRNAs in diabetic complications compared with T2DM. Scatterplots of different colors presented different comparison groups. The horizontal axis was the negative $\log_{10}$ (*P* value) of GO analysis. GO, Gene Ontology; DEmRNAs, differentially expressed mRNAs; T2DM, type 2 diabetes mellitus; DR, diabetic retinopathy; DPN, diabetic peripheral neuropathy; DN, diabetic nephropathy; BP, biological processes.

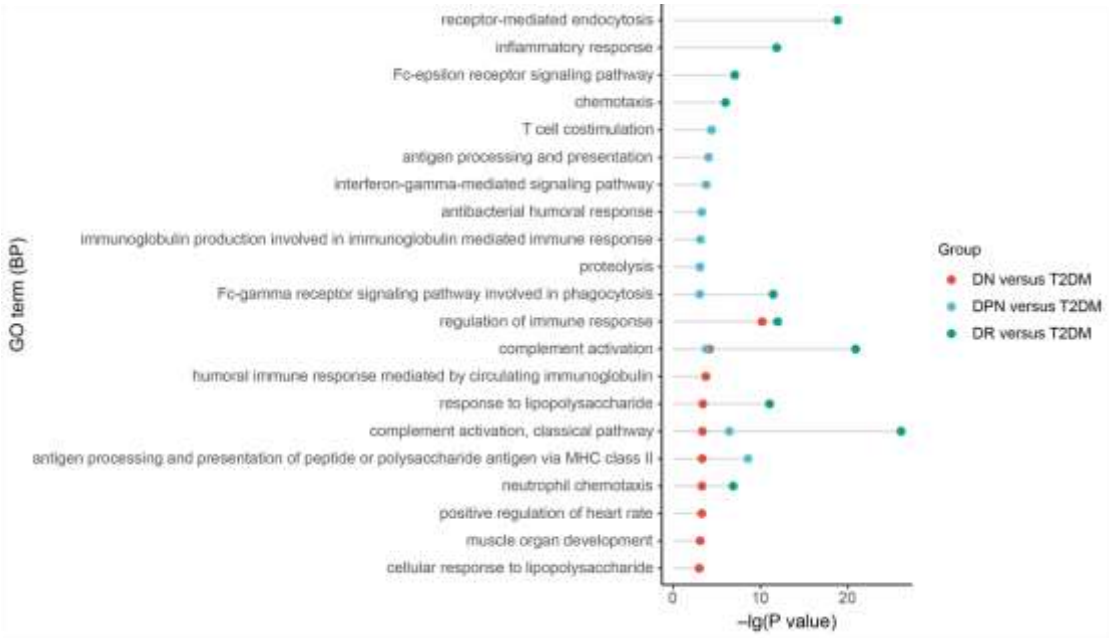

Figure S5 KEGG analysis of DEmRNAs. (A) Top 10 KEGG pathways of DEmRNAs between DR and T2DM. (B) Top 10 KEGG pathways of DEmRNAs between DPN and T2DM. (C) Top 10 KEGG pathways of DEmRNAs between DN and T2DM. The size of the spot indicated the gene numbers enriched in the pathway.KEGG, Kyoto Encyclopedia of Genes and Genomes; DEmRNAs differentially expressed mRNAs; T2DM, type 2 diabetes mellitus; DR, diabetic retinopathy; DPN, diabetic peripheral neuropathy; DN, diabetic nephropathy. The length of the bars in Fig. 1B and Fig. 1D indicated the number of targeted genes enriched in the pathway.

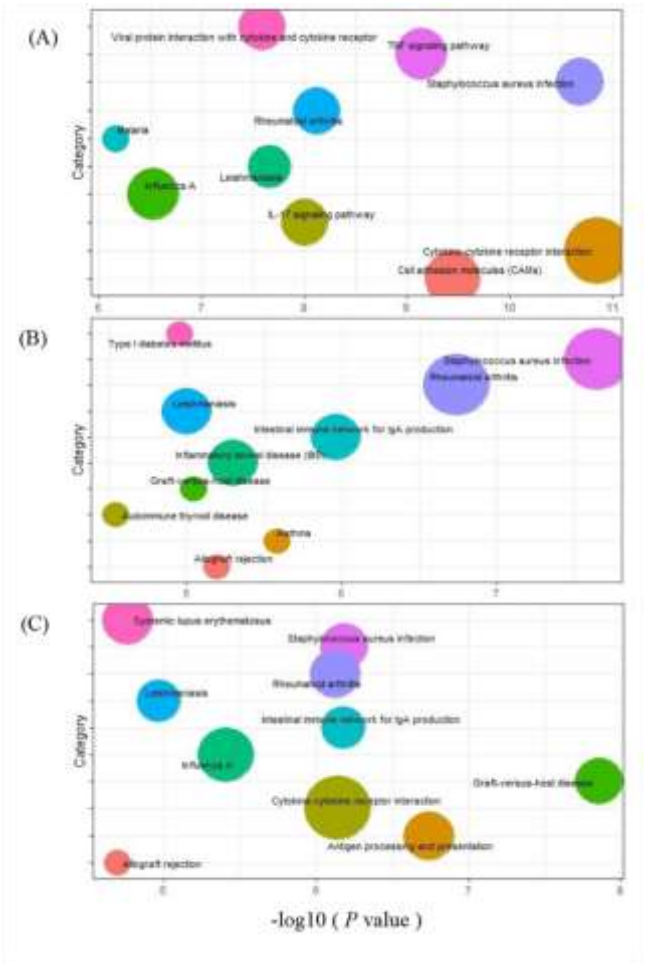

Figure S6 Top 20 hub genes of PPI analysis. The green, red and blue bars displayed hub genes related to DR, DPN and DN compared with T2DM.The size of the bars represented the interaction nodes for each gene in PPI network. PPI, protein-protein interaction; T2DM, type 2 diabetes mellitus; DR, diabetic retinopathy; DPN, diabetic peripheral neuropathy; DN, diabetic nephropathy.

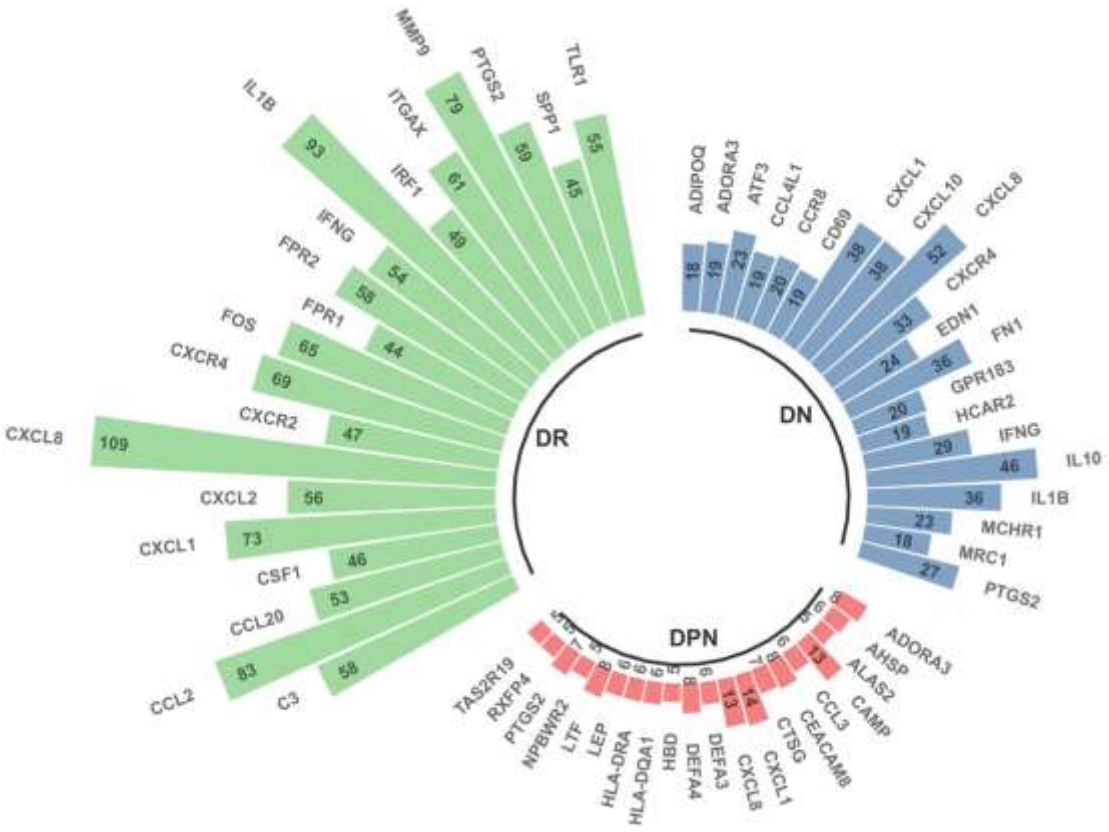

Figure S7 Hub genes in shared pathways of diabetic complications. (A) Top 10 hub genes in the shared pathway of T2DM, DR, DPN and DN compared with HC. (B) Top 10 hub genes in shared pathways of DR, DPN and DN compared with T2DM. The colour of nodes changed gradually from yellow to red in ascending order according to the predicted interaction score by cytoHubba.

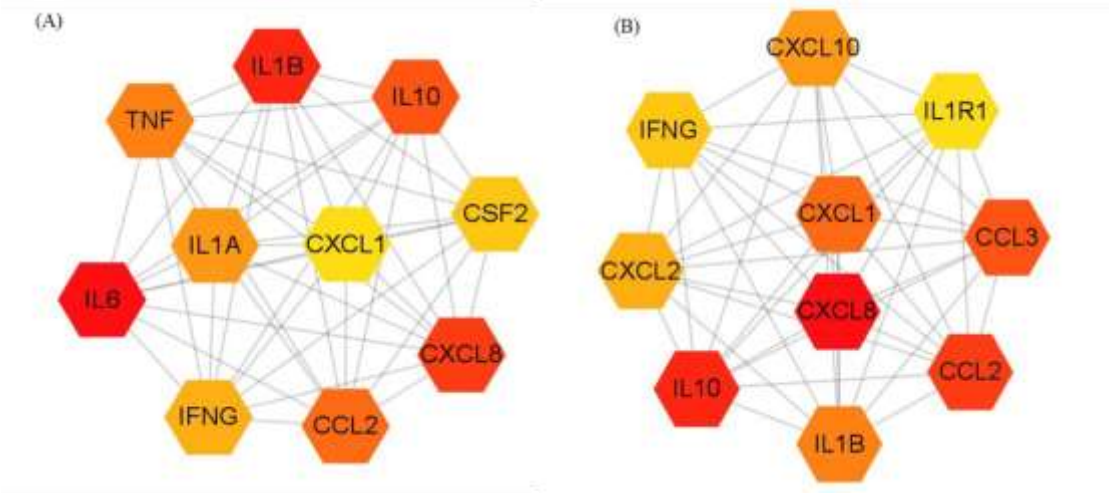

Figure S8 Networks of DElncRNAs and targeted genes in cytokine-cytokine receptor interaction and chemokine signalling pathway. The blue rings indicated DElncRNAs, and the red hexagon indicated target genes. DElncRNAs, differentially expressed lncRNAs.

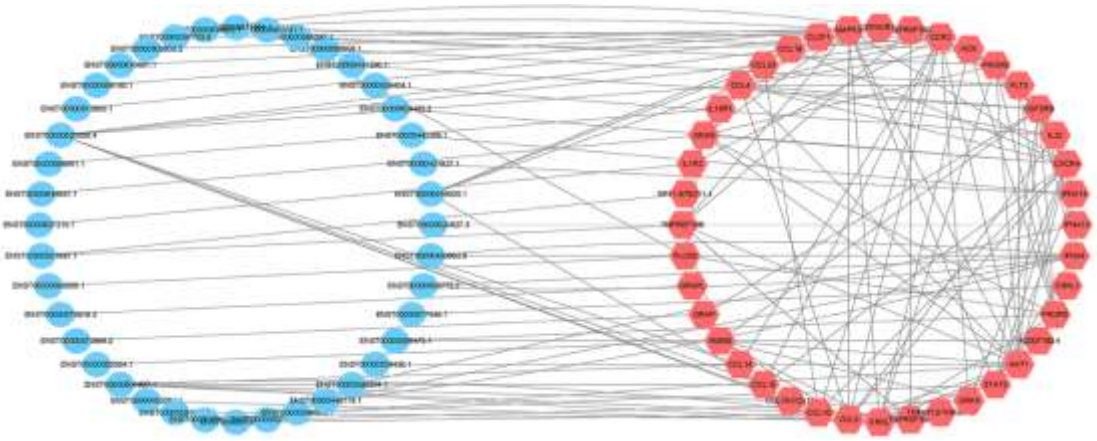

Figure S9 Networks of DElncRNAs and targeted genes in neuroactive ligand-receptor interaction pathway. The blue rings indicated DElncRNAs, and the red hexagon indicated targeted genes. DElncRNAs, differentially expressed lncRNAs.