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Identification and integrated analysis of neuropathic pain-related circular RNA signature

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Identification and integrated analysis of neuropathic pain-related circular RNA signature

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Abstract:

Background: More and more evidences show that non-coding RNAs are involved in neuropathic pain , however, there are few reports on the regulatory mechanism of competitive endogenous RNA (ceRNA) in neuropathic pain. The purpose of this study is to explore the possible molecular mechanisms of neuropathic pain.

Methods: We collected neuropathic pain-related microarray datasets providing expression profile of circular RNAs (circRNAs) and mRNAs from the Gene Expression Omnibus (GEO) and then performed bioinformatics analysis on them.

Results: The present study has identified that up-regulated circRNAs primarily regulate the activity of focal adhesion-associated biological processes and down-regulated primarily regulate the activity of metabolic-associated biological processes by means of ceRNAs.

Conclusions: Our data suggest that circRNAs may be candidates for pathogenesis in neuropathic pain and may be considered as promising therapeutic targets in the future.

Keywords: Neuropathic pain; circRNA; ceRNA; bioinformatics analysis

1. Background

Neuropathic pain is the most common symptom of lumbar disc herniation. The main pathogenesis is that spinal nerve roots produce a series of inflammatory reactions based on the compression of the protruding disc[1]. Currently, surgical treatments such as discectomy and lumbar interbody fusion are used, as well as conservative treatments such as drugs and massage acupuncture; however, the overall efficacy is not good, because the pathogenesis of neuropathic pain is still unclear. Therefore, exploring the underlying mechanism of the development of neuropathic pain, and then looking for early intervention methods and effective drug intervention targets, is a major clinical problem that needs to be solved urgently.

Circular RNAs (circRNAs) are a class of noncoding RNAs with closed continuous loop structure that are ubiquitously expressed in mammalian tissues. Due to the lack of terminal 5′ and 3′ ends, circRNAs are more stable than linear RNAs[2]. CircRNAs play pivotal roles in the pathogenesis of neuropathic pain. Cao et al first detected circRNA in the chronic constriction injury (CCI) model of the sciatic nerve. It was found that there were 469 circRNA differential expression between CCI and sham-operated rats[3]. Zhou et al detected the non-coding RNA expression profile involved in neuropathic pain after retention nerve injury by sequencing. It found that the expression of microRNA (miRNA) and 188 circRNA is significantly changed[4]. Wang et al. found that circHIPK3 and miRNA-124 bind to each other and regulate inflammatory factors IL-1β, IL-6, IL-12. And TNF-α expression, while intrathecal injection of circHIPK3 shRNA can be used to treat neuropathic pain in diabetic rats[5]. Although these studies highlighted the important role of circRNAs in neuropathic pain, the expression and function of most circRNAs in neuropathic pain are still

largely unknown. In addition, circRNAs are aberrantly expressed in various pathophysiological states. Through the construction of ceRNA networks, we could have a better understanding of the pathogenic mechanism of neuropathic pain.

In this study, we employed a combinative strategy of gene chip and computational biology to investigate novel circRNAs and their potential action mechanisms in neuropathic pain. We collected neuropathic pain-related microarray datasets providing expression profile of circRNAs and mRNAs from the GEO. And then we performed Bioinformatics analysis on these datasets. We aimed to find new molecular targets for therapy of neuropathic pain patients through microarray analysis and provide a solid foundation and effective tool for gene therapy of neuropathic pain.

2. Materials and methods

2.1 GEO datasets

Raw data was downloaded from NCBI SRA with accession number of PRJNA558403 and GSE30691。For identification and quantification of circRNAs, reads were mapped to the reference genome Rattus norvegicus（UCSC rn6）by STAR. CIRCexplprer2 were used to quantify and annotate circRNA. For the mRNA part, data were firstly normalized by 'RMA', and low expressed probes were filtered out. Only samples of Spinal Nerve Ligation group were used for the following analysis.

2.2 Differential expression analysis

Differential expression analysis of circRNA was performed using NOIseq with parameters, replicates = 'no' and lc=1[6]. CircRNA with a cutoff of q = 0.8 were considered as differential expressed. Considering circRNA with high expression is meaningful, circRNAs

with an absolute ranking value at top10 high and q>=0.6 were also considered differential expressed. Differential expression analysis of mRNA was performed using R 'limma' package. Probes with a cutoff of p-value<0.05 & absolute fold-changes>=1.5 were considered as differential expressed.

2.3 Functional annotation

Functional enrichment of circRNA host gene and differential expressed coding genes were performed by metascape online tools (http://metascape.org/gp/index.html#/main/step1). Plots were maded by R 'ggplot2' package.

2.4 CircRNA-miRNA-mRNA network

The potential binding sites of miRNA to all the differential expression circRNA were predicted by miRanda with the default parameters. The miRNA targets were obtained from targetscan (http://www.targetscan.org/vert_71/) and miRanda. Then only differential expressed targets were retained. We select circRNA acts as ceRNA on the basis of the ceRNA hypothesis. Any circRNA and mRNA pair with the same regulation direction (both up or down regulation between two group of samples) and bound by the same miRNA was considered as ceRNA interaction. From the ceRNA network, with target genes of selected pathway related were retained and played out by Cytoscape 3.6.1 software.

2.5 Animals and Spinal nerve ligation (SNL)

Male Sprague-Dawley rats, weighing 200-220g, from Southeast University Laboratory Animal Center. In rats under isoflurane (4%) anesthesia, the left L5 spinal nerve was isolated adjacent to the vertebral column and tightly ligated with 6–0 silk sutures distal to the dorsal root ganglion and proximal to the formation of the sciatic nerve. In sham-

operated rats, the L5 spinal nerves were identically exposed without ligation[7]. Animals were maintained under a 12-h light/dark cycle and allowed free activity in their cages. All experiments were reviewed and approved by the Institutional Animal Care and Use Committee in Southeast University School of Medicine, and were conducted according to the committees' guidelines.

2.6 Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

Total RNA was extracted from pooled Sham and SNL-treated group samples using Trizol (Thermo Fisher Scientific, Inc.), and 1 μg of total RNA was reverse transcribed into first-strand cDNA using a PrimeScript RT Reagent kit (Takara Bio, Inc., Otsu, Japan), according to the manufacturer's protocols. qPCR was performed with a SYBR-Green real-time PCR kit (Thermo Fisher Scientific, Inc.) using the ABI StepOnePlus Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). CircRNAs were analyzed with GAPDH as the internal standard. The reactions were prepared as follows: 7.5 μl SYBR Premixm Ex Taq II, 0.25 μl ROX Reference dye II, 0.125 μl forward primer, 0.125 μl reverse primer, 5 μl RNase-free water, and 2 μl cDNA. The thermocycling conditions were: one step at 95° C for 30 sec, followed by 40 cycles of 95° C for 5 sec and 60° C for 30 sec, and a final step of 95° C for 15 sec, 60° C for 15 sec and 95° C for 15 sec. The relative expression of circRNAs was quantified using the $2^{-\Delta\Delta cq}$ method[8]. Primer sequences are listed in Table 1.

Table 1 primer sequences for quantitative reverse transcription-polymerase chain reaction

Gene ID	Primer sequence	
	forward (5′-3′)	Reverse (5′-3′)

chr13:106507145-106507561+	TTCAAGAGGACAATTCAAGGCTTC	TTCCGTCTTGATGAAGGACGA
chr10:94292692-94314584+	ATCCCAAGACAGAAACCATATGAAC	TCTTCATGGTCTCATATCCAGACAC
chr6:57758533-57852070+	TTTCCATTGGCGTGAATGTG	TGTTCAGGCAGAGATTGCAGTAG
chr12:39564848-39567224+	GTGGAAATTGCTGGTTTTGGC	GCATTGGCTACCAATATAAGCAGA
GAPDH	ACAGCAACAGGGTGGTGGAC	TTTGAGGGTACAGCGAACTT

2.7 Statistical analysis

All data are expressed as mean ± standard deviation (SD). Statistical significance was determined using Student's t-test with a p value of <0.05.

3. Results

3.1 CircRNA expression profiling and enrichment analysis of maternal gene function.

We collected neuropathic pain-related microarray datasets from PRJNA558403 of the GEO and conducted data pre-processing (Fig S1). And then we performed bioinformatics analysis on them. As shown in the heatmaps, each circRNA is presented with the name of its corresponding maternal gene. Two methods were used to screen for significant DECs. Considering fold-change，the top 20 of the up-regulated and down-regulated sort results are shown separately (Fig 1A, 1C). Considering probability of statistical test, the top 20 of the up-regulated and down-regulated results are shown separately (Fig 1B, 1D). The scatter plot displayed a total of 111 up-regulated and 95 down-regulated DECs (Fig 1E). From the results of KEGG and GO enrichment, these DECs related maternal genes are mainly involved in neural projections, signal transduction, and adhesion (Fig 1F, 1G). This part of the results

indirectly suggests that DECs are likely involved in these biological functions.

3.2 mRNA expression profiling and enrichment analysis

Considering that circRNAs are likely to be involved in the transcriptional regulation and stability of disease-associated genes, we collected neuropathic pain-related microarray datasets from GSE30691 of the GEO and conducted data pre-processing (Fig S2). And then we performed bioinformatics analysis on them. First, the transcription factors differentially expressed with fold-changes >2.0 over the time course are shown in the heatmap (Fig 2A). Afterwards, from the results of GO enrichment and KEGG pathway analysis, part of differentially expressed mRNAs involved in axonal, signaling, metabolic and other biological functions (Fig 2B, 2C). Finally, since the samples had multiple time points, we used the GSVA method to analyze the function of differentially expressed genes at different time points. Results show that metabolism-related signaling pathways show a downward trend with time point, while inflammatory response, ECM-related increases with time point (Fig 2D).

3.3 ceRNA analysis of mRNA and circRNA

After the basic analysis of circRNAs in the above section, we then predicted a number of circRNAs that may play a role in disease-related biological functions by means of ceRNA analysis. As shown in the histograms, number of target genes corresponding to up- and down-regulated DECs. And the higher the number is, the more important these DECs are indirectly. (Fig 3A, 3B). We then performed pathway enrichment of target genes corresponding to DECs. The KEGG pathway analysis of up- and down-regulated target genes is shown in histograms (Fig 3C，3D). The results suggest that the two biological functions

of adhesion and metabolism are likely to be regulated by DECs via ceRNAs.

3.4 Construction of ceRNA network and qRT-PCR validation for the selected circRNAs

We screened TOP1 pathway genes to construct the ceRNA network, with DEGs for Focal adhesion selected in the up-regulation section and DEGs for Metabolic pathway selected in the down-regulation section (Fig S3A, S4A). We construct the ceRNA network with the miRNA part removed (Fig 4A, 4B). GO analysis of selected important up- and down-regulated circRNAs is shown in the results (Fig S3B, S3C, S4B, S4C). To validate the bioanalytical results, we collected spinal dorsal horn tissues from rats in the Sham and SNL-treated groups (Day 14), qRT-PCR was used to detect the predicted two up-regulated and two down-regulated circRNAs, which were selected based on the above criteria (Fig 4C, 4D).

4. Discussion

Numerous studies have been conducted to reveal the pathogenic mechanisms of neuropathic pain. However, the progression of neuropathic pain still remains elusive. CircRNAs are a kind of newly found functional noncoding RNA. In recent years, studies have emerged, showing the abnormal expression profiles of circRNAs in neuropathic pain[3][9]. CircRNAs have already emerged as important regulators of neuropathic pain, and up and down-regulated circRNAs are involved in different biological functions.

In the present study, a number of aberrantly expressed circRNAs in neuropathic pain were identified. Pathway enrichment results revealed that up-regulated circRNAs may regulate neuropathic pain progression through multiple signaling pathways, especially the

Focal adhesion pathways. Down-regulated circRNAs may regulate neuropathic pain progression through multiple signaling pathways, especially the Metabolic pathways. These results provided several potential biomarkers and therapeutic targets for neuropathic pain. The function of circRNAs predicted with KEGG analysis in the mechanisms of neuropathic pain should be studied more in-depth in future work.

Lines of evidence show that focal adhesion signaling modulates axon regeneration of peripheral neuron[10]. Advillin is involved in somatosensory neuron subtype-specific axonal regeneration and neuropathic pain, and has been shown to be highly correlated with neonatal focal adhesion proteins[11]. The present ceRNA network analysis demonstrated that a circRNA /miRNA axis may have important roles in focal adhesion-mediated neuropathic pain.

Yan et al revealed that most of the down-regulated genes are enriched in pathways such as phospholipid metabolic processes, positive regulation of protein kinase B signaling, and metabolism of xenobiotics by cytochrome P450 by functional enrichment analysis of bioinformatic studies on neuropathic pain[12]. Academic studies have shown that endocannabinoid metabolism and uptake are new targets for neuropathic pain[13]. The effects of endocannabinoids are terminated by the reuptake and metabolism of various enzymes, including fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MAGL), and cyclooxygenase type 2 (COX2), preventing the metabolism or uptake of endocannabinoids, elevating tissue levels of these lipid compounds, and producing behavioral analgesia in models of acute pain[14][15][16]. The present ceRNA network analysis demonstrated that a circRNA /miRNA axis may have important roles in metabolic-

mediated neuropathic pain. Competing endogenous RNAs (ceRNAs) are transcripts that act as miRNA sponges, modulating each other at post-transcriptional level via competitively binding to shared miRNAs[17]. Related circRNAs may affect the expression of downstream adhesion or metabolism-related genes through competitive combining to miRNAs.

Although only DEGs of the Top1 signaling pathway were selected to construct the ceRNA network, the top2 pathways are likely to be associated with pain. As a traditional Chinese Medicine, paeonia lactiflora pallas has been used to treat pain more than 1000 years in China, through PI3K-Akt signaling pathway[18]. Regulation of PI3K-Akt signaling can be an attractive alternative strategy for neuropathic pain[19][20]. Previously, epiregulin -mediated activation of EGFR activates dorsal root ganglion (DRG) neurons, producing pain behaviors through a mechanism that involves PI3K/AKT/mTOR signaling pathway[21]. Simultaneously，early HBO therapy could significantly improve symptoms of hyperalgesia of neuropathic pain in rats, possibly via activation of the NO-cGMP-PKG signaling transduction pathway[22]. Previous study has reported that the peripheral activation of A1R plays a role in the regulation of inflammatory hyperalgesia by a mechanism involving the NO/cGMP/PKG/KATP intracellular signaling pathway[23]. Huang et al discovered that cGMP-PKG signaling pathway can be activated by the in vivo chronic compression of DRG (CCD) or in vitro acute dissociation of DRG (ADD) treatment, and that continuing activation of cGMP-PKG pathway is necessary after these two dissimilar forms of injury-related stress[24].

At the end of this study, we selected several important predicted circRNAs and used qRT-PCR to validate the results of ceRNA analysis. Atp2a2, the down-regulated circRNA.

From the results of functional enrichment analysis of the target gene, the effect is mainly on steroid metabolism and also on signaling pathways. Esrrg, the up-regulated circRNA. From the results of functional enrichment analysis of the corresponding target genes, the main effects are cytoskeleton, ECM, cell adhesion, and so on.

Conclusions

In conclusions, by adopting a comprehensive strategy of the mining of circRNA and mRNA data in the GEO database and computational biology, we constructed a circRNA-miRNA-mRNA network and we found that circRNAs may function as ceRNAs to exert important roles in NP. Up-regulated circRNAs primarily regulate the activity of focal adhesion-associated and down-regulated circRNAs regulate the activity of metabolic-associated biological processes. In addition, qRT-PCR was performed to confirm the ceRNA network results. Our study provides new insights into the pathogenesis and treatment of neuropathic pain from a circRNA-miRNA-mRNA perspective.

Authors' contributions

WK and ZZm collected circRNA-and mRNA-related datasets; screened differently expressed circRNAs and mRNAs; conducted animal disease model experiment and qRT-PCR; and were the major contributors in writing the manuscript. BJP conducted KEGG and GO analyses. LD conducted ceRNA network. HYB checked all of the data used in the manuscript. WXT guided the design of all of the experiments and writing of the manuscript. All authors read and approved the final manuscript.

Competing of interest

All authors declare no conflicts of interest

Availability of data and materials

The data set used and/or analyzed during this study can be reasonably requested from the corresponding author

Consent for publication

Not applicable.

Acknowledgements

Not applicable.

Ethics approval and consent to participate

The Animal Care & Welfare Committee of Southeast University approved the present study.

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References

Figure legend

Figure 1. Screening of DECs and enrichment analysis of maternal gene function. (A, B, C, D) Heat maps of significantly DECs. Each circRNA is represented by the corresponding maternal gene name of the circRNA. Red represents a higher expression and blue represents

a lower expression. (E) Differential expression scatter plot, a total of 111 up-regulation and 95 down-regulation. Each dot represents each circRNA, with red dots representing up-regulation, blue dots representing down-regulation, and gray dots representing non-differentially expressed portions. Significantly DECs are simultaneously labeled with names. (F) GO analysis. p-value is graded from red to blue from smallest to largest. Circle size is graded from large to small by the number of different parent genes. (G) KEGG pathway analysis. The horizontal axis represents $-log10$(p-value), and the colors are gradient-filled with $-log10$(p-value). The number on the right represents the number of differences

Figure 2. mRNA expression profiling and enrichment analysis. (A) Heatmap of differentially expressed transcription factors (fold-changes>2.0). (B) GO enrichment. P value is graded from small to large from red to blue. Circle size is graded from largest to smallest by number of differential genes. BP, CC, MF are represented by three different point shapes. (C) KEGG pathway analysis. Relatively different genes are enriched in the top ranked pathways. (D) Heatmap of KEGG pathways. The color blue to red represents a gradient from low to high expression

Figure 3. ceRNA analysis of mRNA and circRNA. (A) (B) Number of target genes corresponding to up- and down-regulated circRNAs. (C) (D) KEGG pathway results of target gene of up- and down-regulated circRNAs

Figure 4. CircRNAs-mRNAs network and validation results for the selected circRNAs. (A) (B) Up- and down-regulated circRNAs-mRNAs network. Red pattern is circRNA, purple pattern is mRNA, the size of the node is graded according to the number of connected edges, the larger the number of connected edges. (C) (D) Quantitative reverse transcription-

polymerase chain reaction analysis. 2 up- and 2 down-regulated circRNAs were demonstrated to be consistent with the ceRNA network results (n=3)

Figure S1. CircRNAs data pre-processing. (A) Distribution of circRNA on chromosomes, horizontal axis is chromosomes, vertical axis is circRNA numbers. (B) Principal Component Analysis (PCA). (C) Sample Cluster Analysis. Sham is more similar to D14, but different from Day7

Figure S2. mRNA data pre-processing. (A) PCA. (B) Sample Cluster Analysis. (C) Differentially expressed genes Venn diagram. The cci group was not considered in subsequent analyses due to few DEGs in this group

Figure S3. ceRNA network and GO analysis of selected up-regulated cricRNAs. (A) ceRNA network. Red pattern represents circRNA, purple pattern represents mRNA, and triangle represents miRNA. (B) (C) GO analysis of important selected 2 up-regulated circRNAs.

Figure S4. ceRNA network and GO analysis of selected down-regulated cricRNAs. (A) ceRNA network. (B) (C) GO analysis of important selected 2 down-regulated circRNAs.

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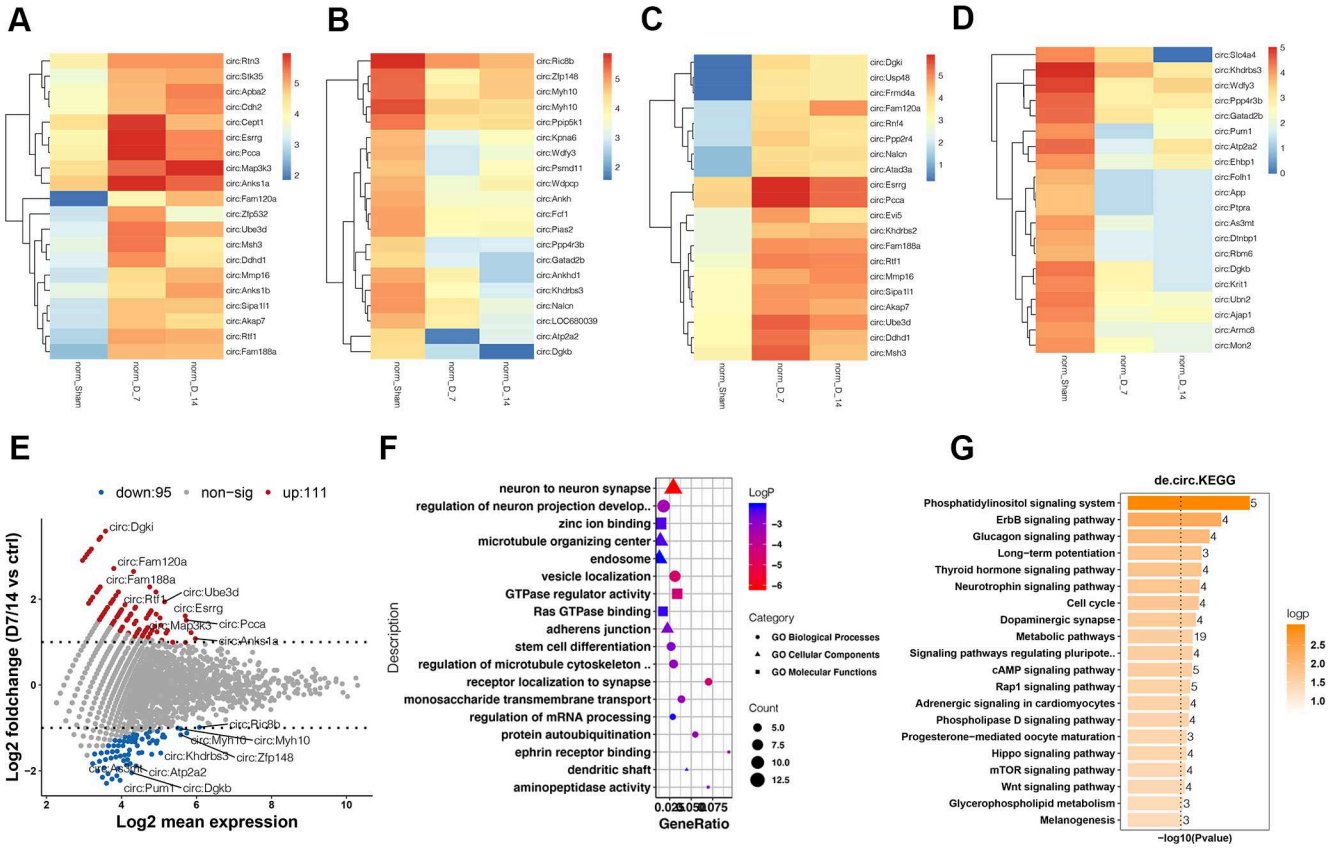
Fig 1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Long−term potentiation
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Rap1 signaling pathway
Adrenergic signaling in cardiomyocytes
Phospholipase D signaling pathway
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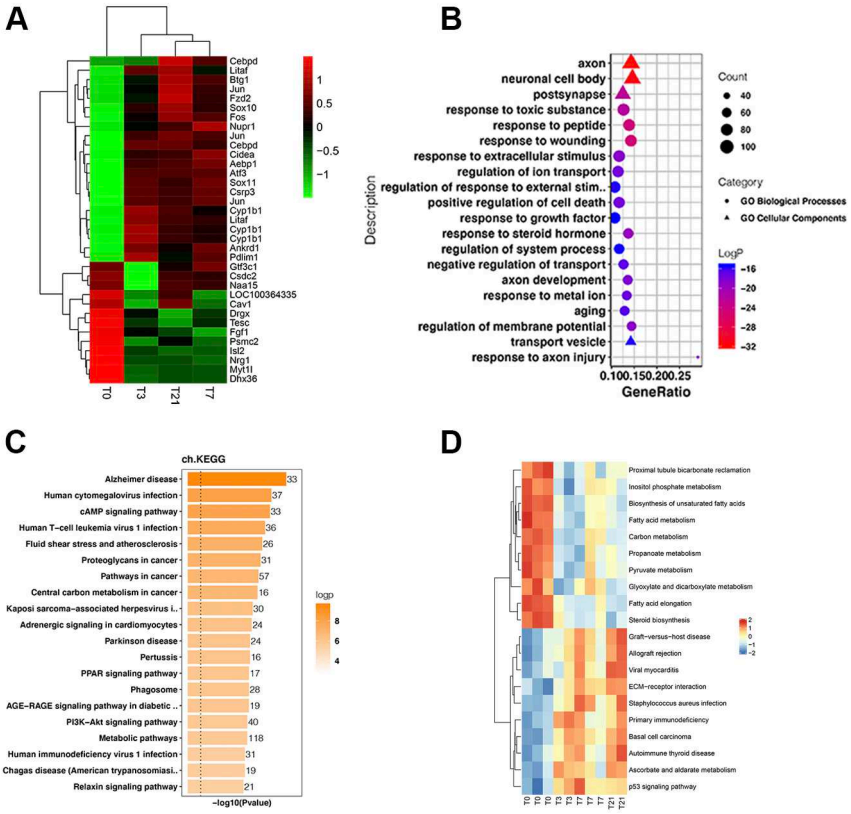
Fig 2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Fgf1
Psmc2
Isl2
Nrg1
Myt1l
Dhx36
T0
T3
T21
T7
1
0.5
0
−0.5
−1
B
axon
neuronal cell body
postsynapse
response to toxic substance
response to peptide
response to wounding
response to extracellular stimulus
regulation of ion transport
regulation of response to external stim..
positive regulation of cell death
response to growth factor
response to steroid hormone
regulation of system process
negative regulation of transport
axon development
response to metal ion
aging
regulation of membrane potential
transport vesicle
response to axon injury
Description
0.10
0.15
0.20
0.25
GeneRatio
Count
40
60
80
100
Category
GO Biological Processes
GO Cellular Components
LogP
−16
−20
−24
−28
−32
C
ch.KEGG
Alzheimer disease
Human cytomegalovirus infection
cAMP signaling pathway
Human T−cell leukemia virus 1 infection
Fluid shear stress and atherosclerosis
Proteoglycans in cancer
Pathways in cancer
Central carbon metabolism in cancer
Kaposi sarcoma−associated herpesvirus i..
Adrenergic signaling in cardiomyocytes
Parkinson disease
Pertussis
PPAR signaling pathway
Phagosome
AGE−RAGE signaling pathway in diabetic ..
PI3K−Akt signaling pathway
Metabolic pathways
Human immunodeficiency virus 1 infection
Chagas disease (American trypanosomiasi..
Relaxin signaling pathway
33
37
33
36
26
31
57
16
30
24
24
16
17
28
19
40
118
31
19
21
−log10(Pvalue)
logp
8
6
4
D
Proximal tubule bicarbonate reclamation
Inositol phosphate metabolism
Biosynthesis of unsaturated fatty acids
Fatty acid metabolism
Carbon metabolism
Propanoate metabolism
Pyruvate metabolism
Glyoxylate and dicarboxylate metabolism
Fatty acid elongation
Steroid biosynthesis
Graft−versus−host disease
Allograft rejection
Viral myocarditis
ECM−receptor interaction
Staphylococcus aureus infection
Primary immunodeficiency
Basal cell carcinoma
Autoimmune thyroid disease
Ascorbate and aldarate metabolism
p53 signaling pathway
T0
T0
T0
T3
T3
T7
T7
T21
T21
2
1
0
−1
−2

Fig 3

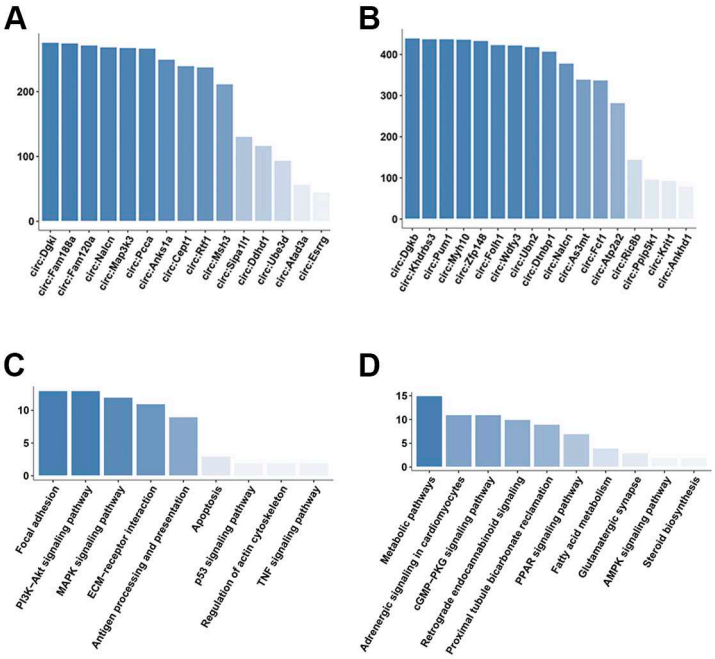

A
200
100
0
circ:Dgkl
circ:Fam188a
circ:Fam120a
circ:Nalcn
circ:Map3k3
circ:Pcca
circ:Ankfs1a
circ:Capl1
circ:Rfl1
circ:Msh3
circ:Slpa1l1
circ:Ddhd1
circ:Ube2d
circ:Atad5a
circ:Esrrg
B
400
300
200
100
0
circ:Dgkb
circ:Khdrbs3
circ:Pum1
circ:Myh10
circ:Zfp148
circ:Folh1
circ:Wdfy3
circ:Ubn2
circ:Dhdpl1
circ:Malcn
circ:As3mt
circ:Fcf1
circ:Atp2a2
circ:Rictb
circ:Ppp5k1
circ:Krit1
circ:Ankhd1
C
10
5
0
Focal adhesion
PI3K−Akt signaling pathway
MAPK signaling pathway
ECM−receptor interaction
Antigen processing and presentation
Apoptosis
p53 signaling pathway
Regulation of actin cytoskeleton
TNF signaling pathway
D
15
10
5
0
Metabolic pathways
Adrenergic signaling in cardiomyocytes
cGMP−PKG signaling pathway
Retrograde endocannabinoid signaling
Proximal tubule bicarbonate reclamation
PPAR signaling pathway
Fatty acid metabolism
Glutamatergic synapse
AMPK signaling pathway
Steroid biosynthesis

Fig 4

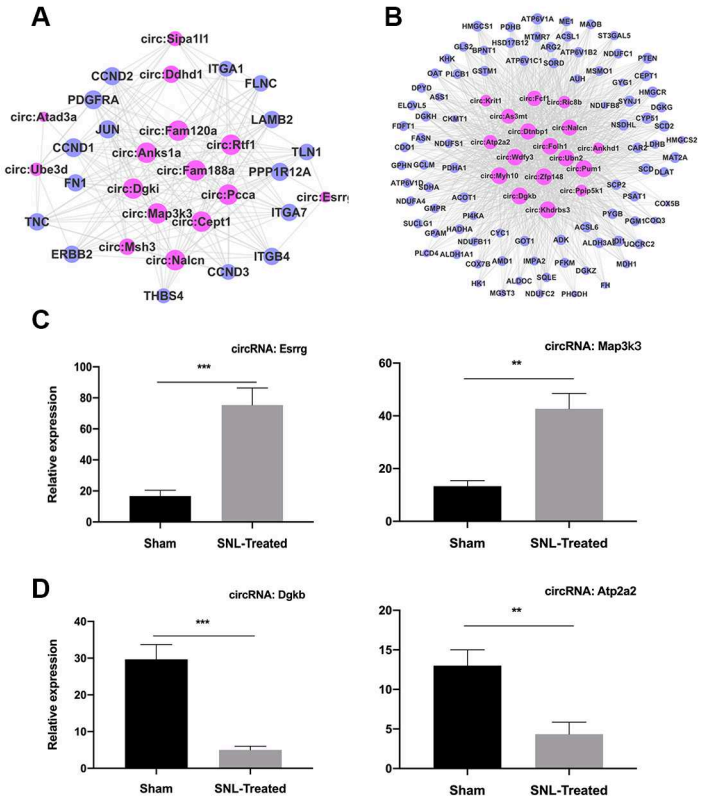

A
circ:Sipa1l1
CCND2
circ:Ddhd1
ITGA1
FLNC
PDGFRA
circ:Atad3a
LAMB2
JUN
circ:Fam120a
CCND1
circ:Anks1a
circ:Rtf1
TLN1
circ:Ube3d
circ:Fam188a
PPP1R12A
FN1
circ:Dgki
circ:Pcca
circ:Esrrg
TNC
circ:Map3k3
circ:Cept1
ITGA7
ERBB2
circ:Msh3
circ:Nalcn
ITGB4
CCND3
THBS4
B
C
circRNA: Esrrg
Relative expression
***
Sham
SNL-Treated
circRNA: Map3k3
**
Sham
SNL-Treated
D
circRNA: Dgkb
Relative expression
***
Sham
SNL-Treated
circRNA: Atp2a2
**
Sham
SNL-Treated

Fig S1

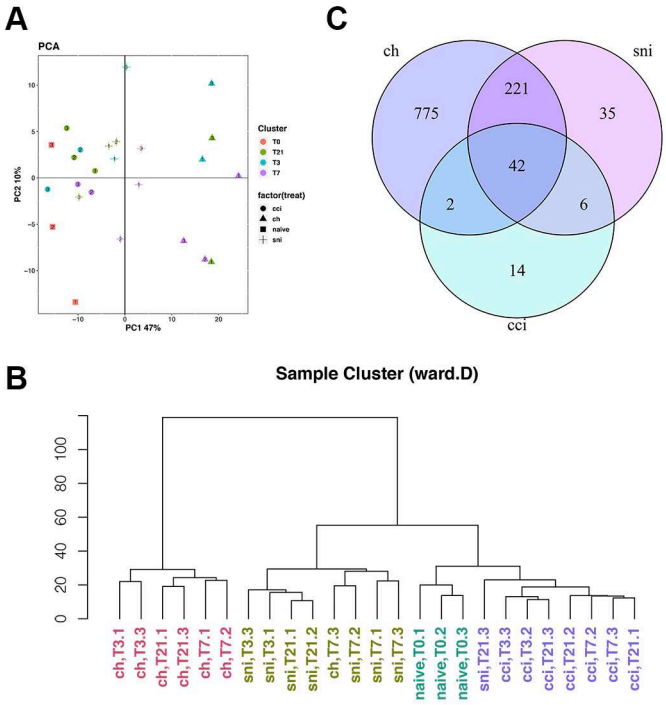
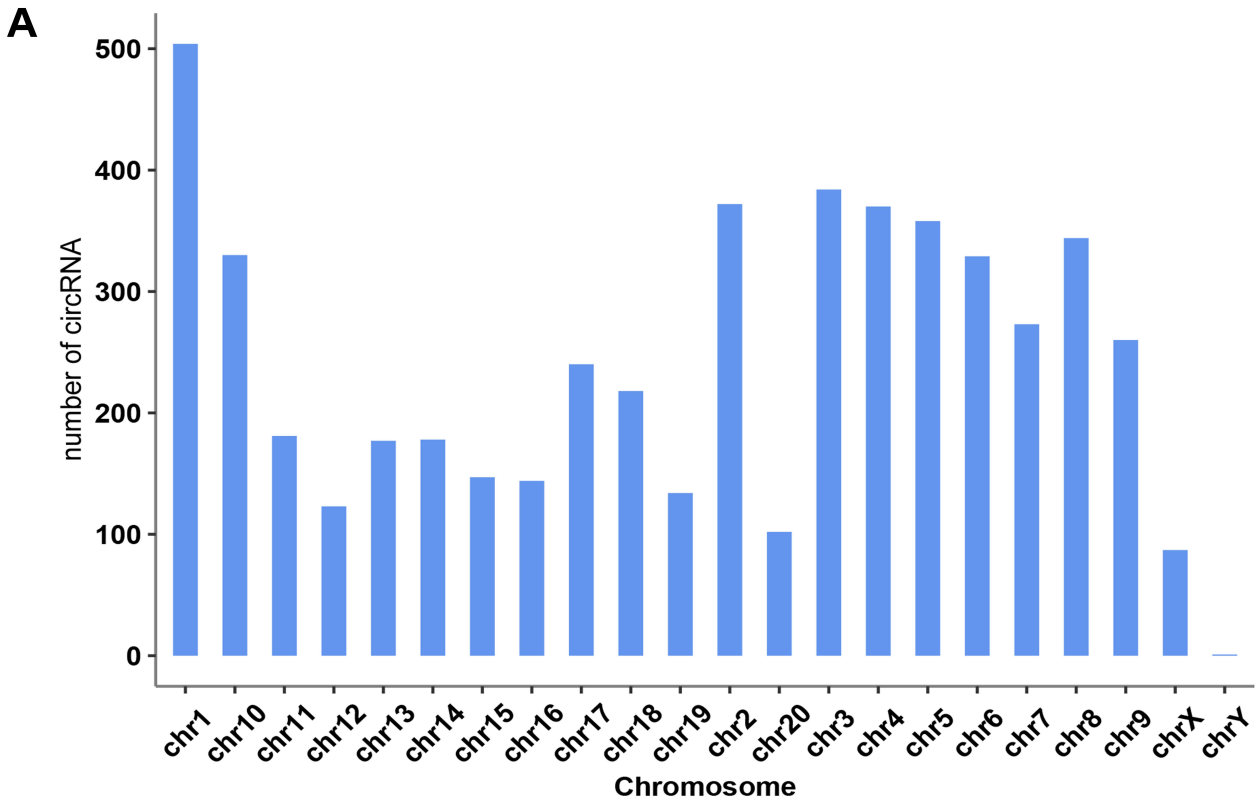
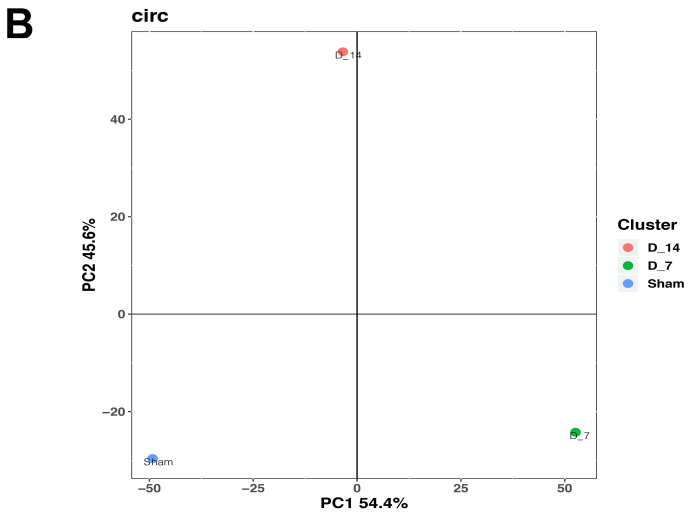

A
PCA
PC1 47%
PC2 10%
Cluster
T0
T21
T3
T7
factor(treat)
cci
ch
naive
sni
C
ch
sni
cci
775
221
35
42
2
6
14
B
Sample Cluster (ward.D)
ch,T3.1
ch,T3.3
ch,T21.1
ch,T21.3
ch,T7.1
ch,T7.2
sni,T3.3
sni,T3.1
sni,T21.1
sni,T21.2
ch,T7.3
sni,T7.2
sni,T7.1
sni,T7.3
naive,T0.1
naive,T0.2
naive,T0.3
sni,T21.3
cci,T3.3
cci,T3.2
cci,T21.3
cci,T21.2
cci,T7.2
cci,T7.3
cci,T21.1

Fig S2


A
number of circRNA
500
400
300
200
100
0
chr1
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chr2
chr20
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chrX
chrY
Chromosome


B
circ
PC2 45.6%
PC1 54.4%
Cluster
D_14
D_7
Sham


C
Sample Cluster(average)
0
20
40
60
80
D_7
Sham
D_14

Fig S3

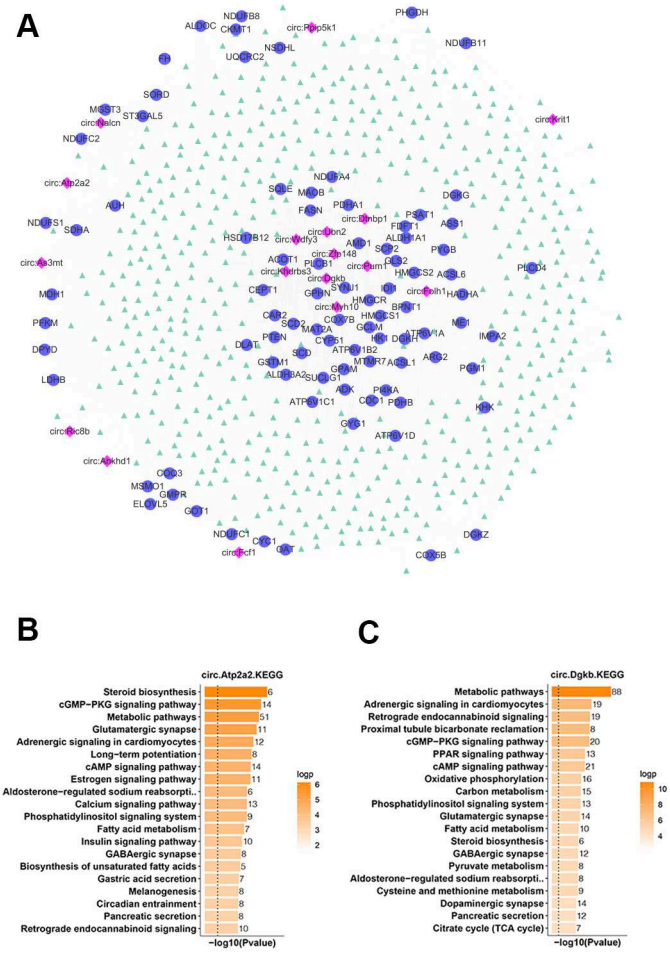
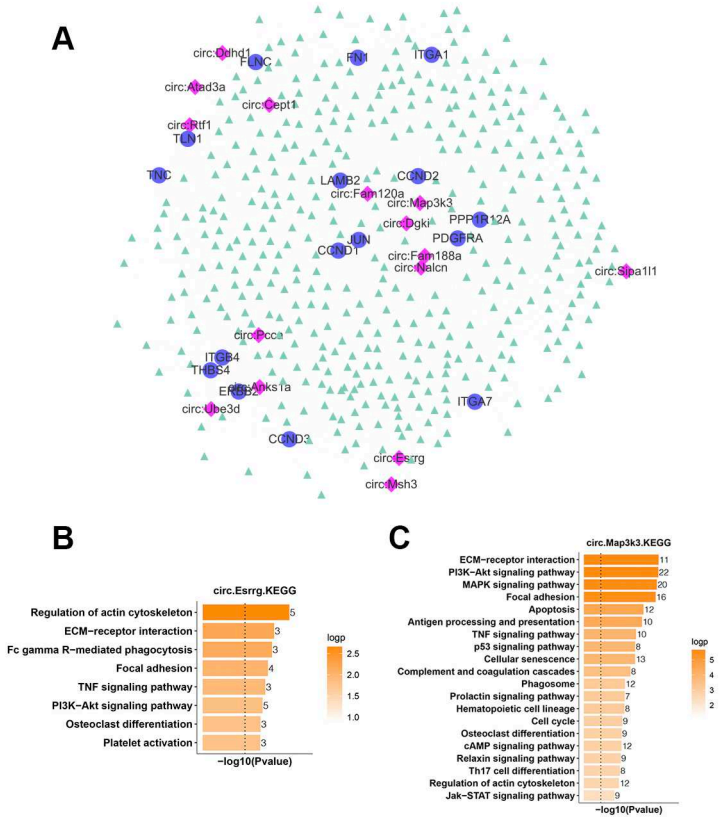

A
B
C
circ.Atp2a2.KEGG
Steroid biosynthesis 6
cGMP−PKG signaling pathway 14
Metabolic pathways 51
Glutamatergic synapse 11
Adrenergic signaling in cardiomyocytes 12
Long−term potentiation 8
cAMP signaling pathway 14
Estrogen signaling pathway 11
Aldosterone−regulated sodium reabsorpti.. 6
Calcium signaling pathway 13
Phosphatidylinositol signaling system 9
Fatty acid metabolism 7
Insulin signaling pathway 10
GABAergic synapse 8
Biosynthesis of unsaturated fatty acids 5
Gastric acid secretion 7
Melanogenesis 8
Circadian entrainment 8
Pancreatic secretion 8
Retrograde endocannabinoid signaling 10
−log10(Pvalue)
logp
circ.Dgkb.KEGG
Metabolic pathways 88
Adrenergic signaling in cardiomyocytes 19
Retrograde endocannabinoid signaling 19
Proximal tubule bicarbonate reclamation 8
cGMP−PKG signaling pathway 20
PPAR signaling pathway 13
cAMP signaling pathway 21
Oxidative phosphorylation 16
Carbon metabolism 15
Phosphatidylinositol signaling system 13
Glutamatergic synapse 14
Fatty acid metabolism 10
Steroid biosynthesis 6
GABAergic synapse 12
Pyruvate metabolism 8
Aldosterone−regulated sodium reabsorpti.. 8
Cysteine and methionine metabolism 9
Dopaminergic synapse 14
Pancreatic secretion 12
Citrate cycle (TCA cycle) 7
−log10(Pvalue)
logp

Fig S4


A
circ:Ddhd1
FLNC
FN1
ITGA1
circ:Atad3a
circ:Cept1
circ:Rtf1
TCF7
TNC
LAMB2
CCND2
circ:Fam120a
circ:Map3k3
circ:Pgki
PPP1R12A
JUN
PDGFRA
CCND1
circ:Fam188a
circ:Nalcn
circ:Slpa1l1
circ:Pcca
ITGB4
THBS4
ERBB2
circ:Ube3d
ITGA7
CCND3
circ:Esrrg
circ:Msh3
B
circ.Esrrg.KEGG
Regulation of actin cytoskeleton 5
ECM−receptor interaction 3
Fc gamma R−mediated phagocytosis 3
Focal adhesion 4
TNF signaling pathway 3
PI3K−Akt signaling pathway 5
Osteoclast differentiation 3
Platelet activation 3
−log10(Pvalue)
logp
2.5
2.0
1.5
1.0
C
circ.Map3k3.KEGG
ECM−receptor interaction 11
PI3K−Akt signaling pathway 22
MAPK signaling pathway 20
Focal adhesion 16
Apoptosis 12
Antigen processing and presentation 10
TNF signaling pathway 10
p53 signaling pathway 8
Cellular senescence 13
Complement and coagulation cascades 8
Phagosome 12
Prolactin signaling pathway 7
Hematopoietic cell lineage 8
Cell cycle 9
Osteoclast differentiation 9
cAMP signaling pathway 12
Relaxin signaling pathway 9
Th17 cell differentiation 8
Regulation of actin cytoskeleton 12
Jak−STAT signaling pathway 9
−log10(Pvalue)
logp
5
4
3
2

Figures

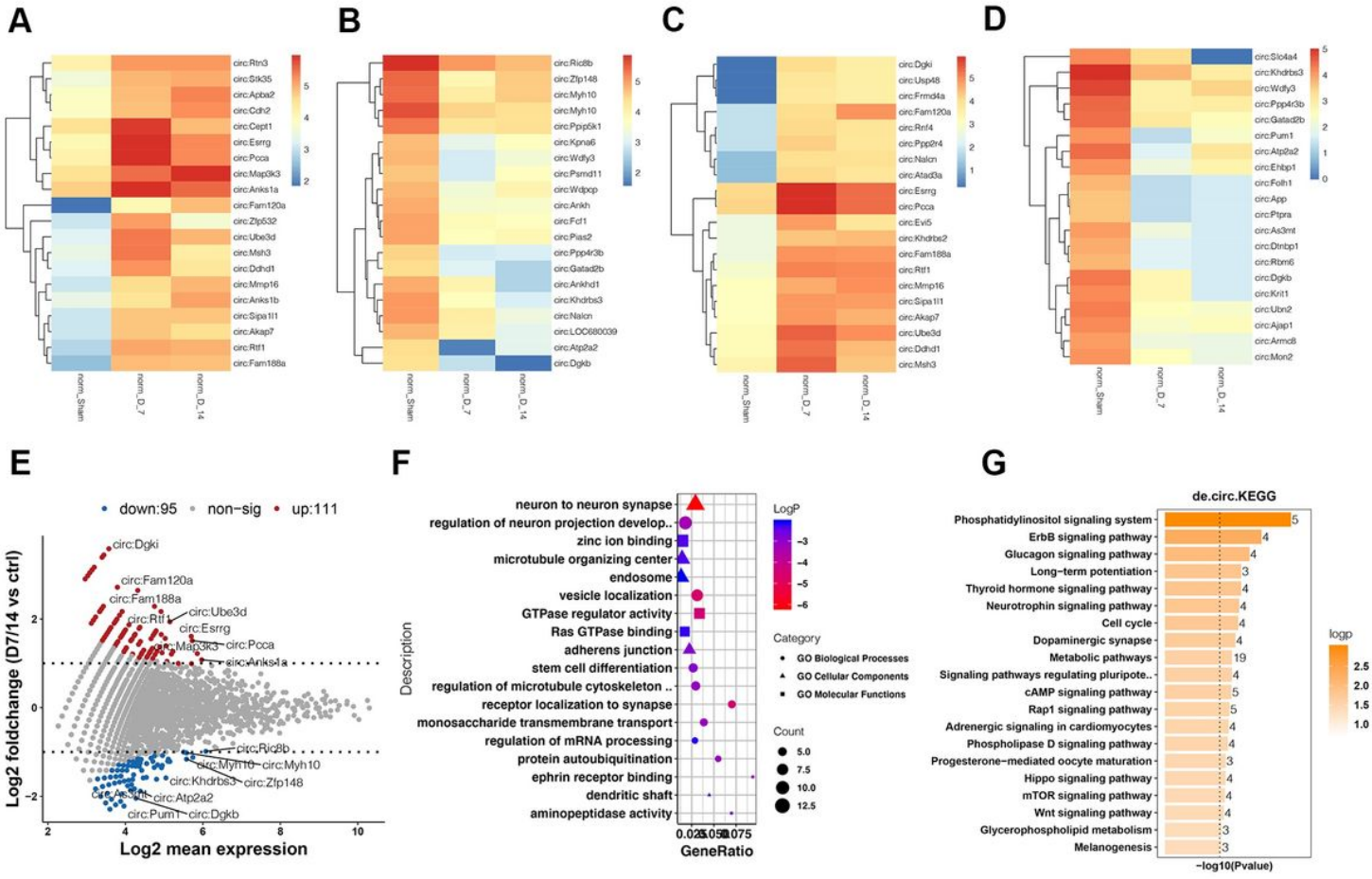


Figure 1

Screening of DECs and enrichment analysis of maternal gene function. (A, B, C, D) Heat maps of significantly DECs. Each circRNA is represented by the corresponding maternal gene name of the circRNA. Red represents a higher expression and blue represents a lower expression. (E) Differential expression scatter plot, a total of 111 up-regulation and 95 down-regulation. Each dot represents each circRNA, with red dots representing upregulation, blue dots representing down-regulation, and gray dots representing nondifferentially expressed portions. Significantly DECs are simultaneously labeled with names. (F) GO analysis. p-value is graded from red to blue from smallest to largest. Circle size is graded from large to small by the number of different parent genes. (G) KEGG pathway analysis. The horizontal axis represents –"#$10(p-value), and the colors are gradient-filled with –"#$10(p-value). The number on the right represents the number of differences

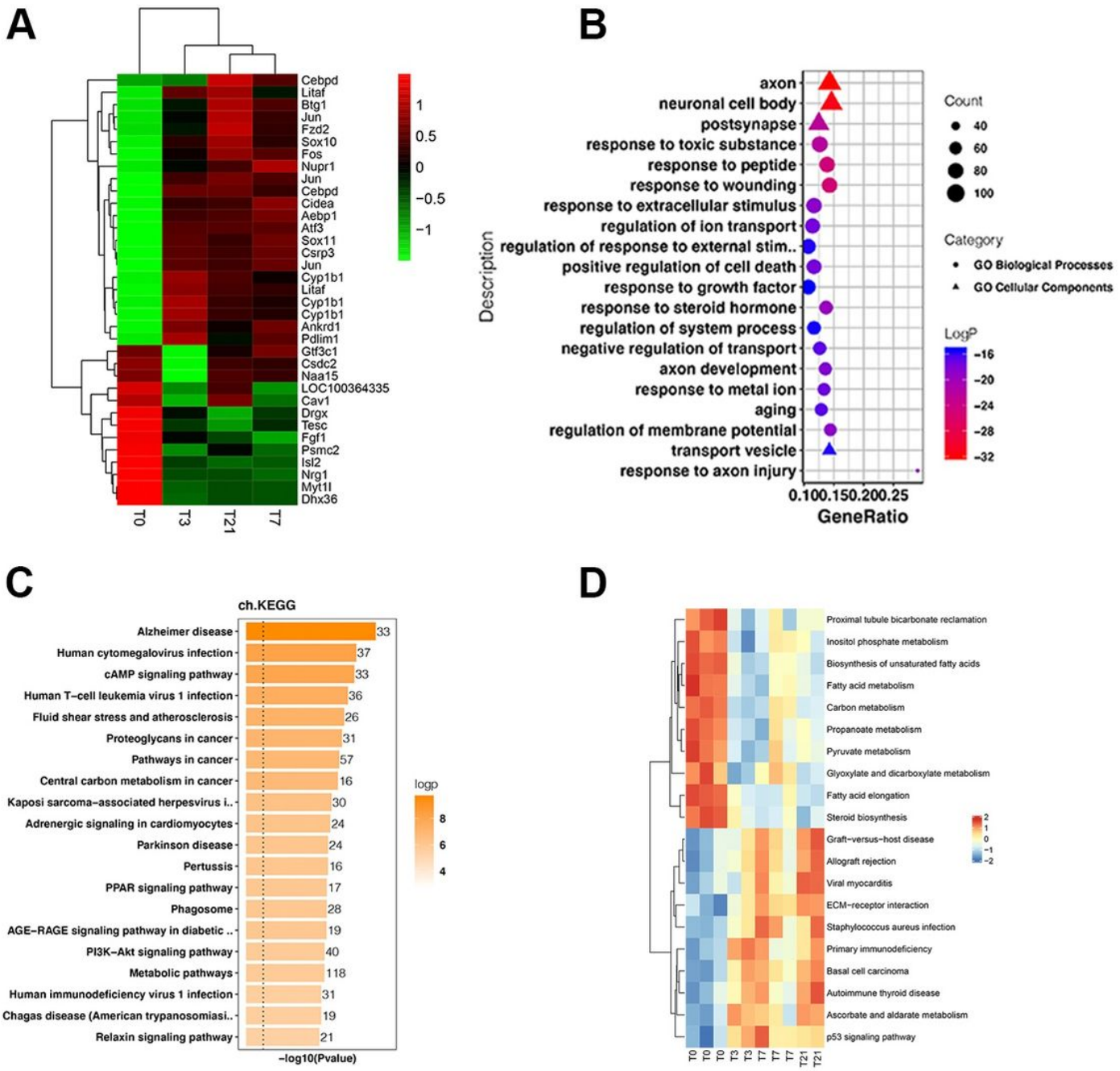


Figure 2

mRNA expression profiling and enrichment analysis. (A) Heatmap of differentially expressed transcription factors (fold-changes>2.0). (B) GO enrichment. P value is graded from small to large from red to blue. Circle size is graded from largest to smallest by number of differential genes. BP, CC, MF are represented by three different point shapes. (C) KEGG pathway analysis. Relatively different genes are enriched in the top ranked pathways. (D) Heatmap of KEGG pathways. The color blue to red represents a gradient from low to high expression

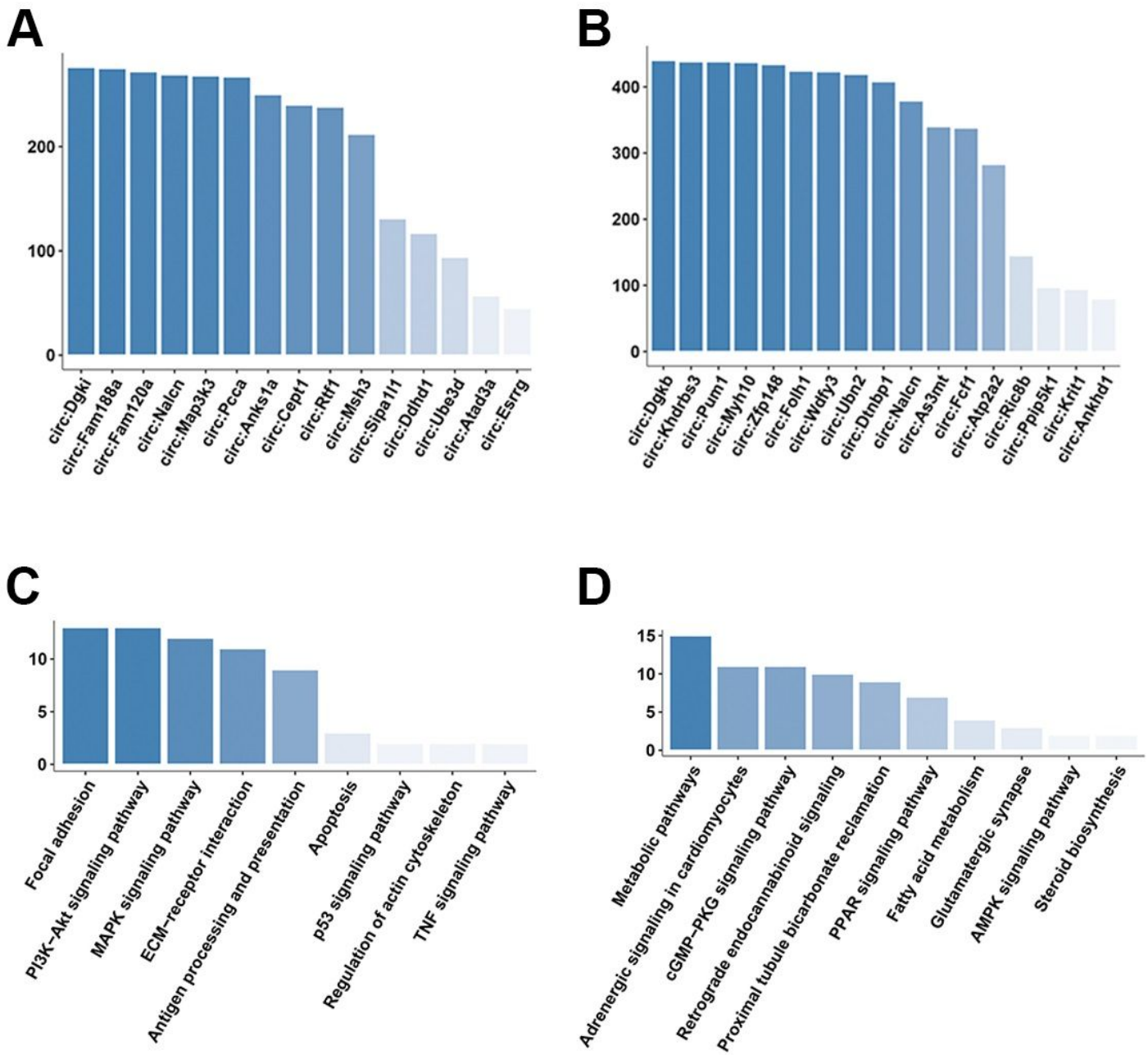


Figure 3

ceRNA analysis of mRNA and circRNA. (A) (B) Number of target genes corresponding to up- and down-regulated circRNAs. (C) (D) KEGG pathway results of target gene of up- and down-regulated circRNAs

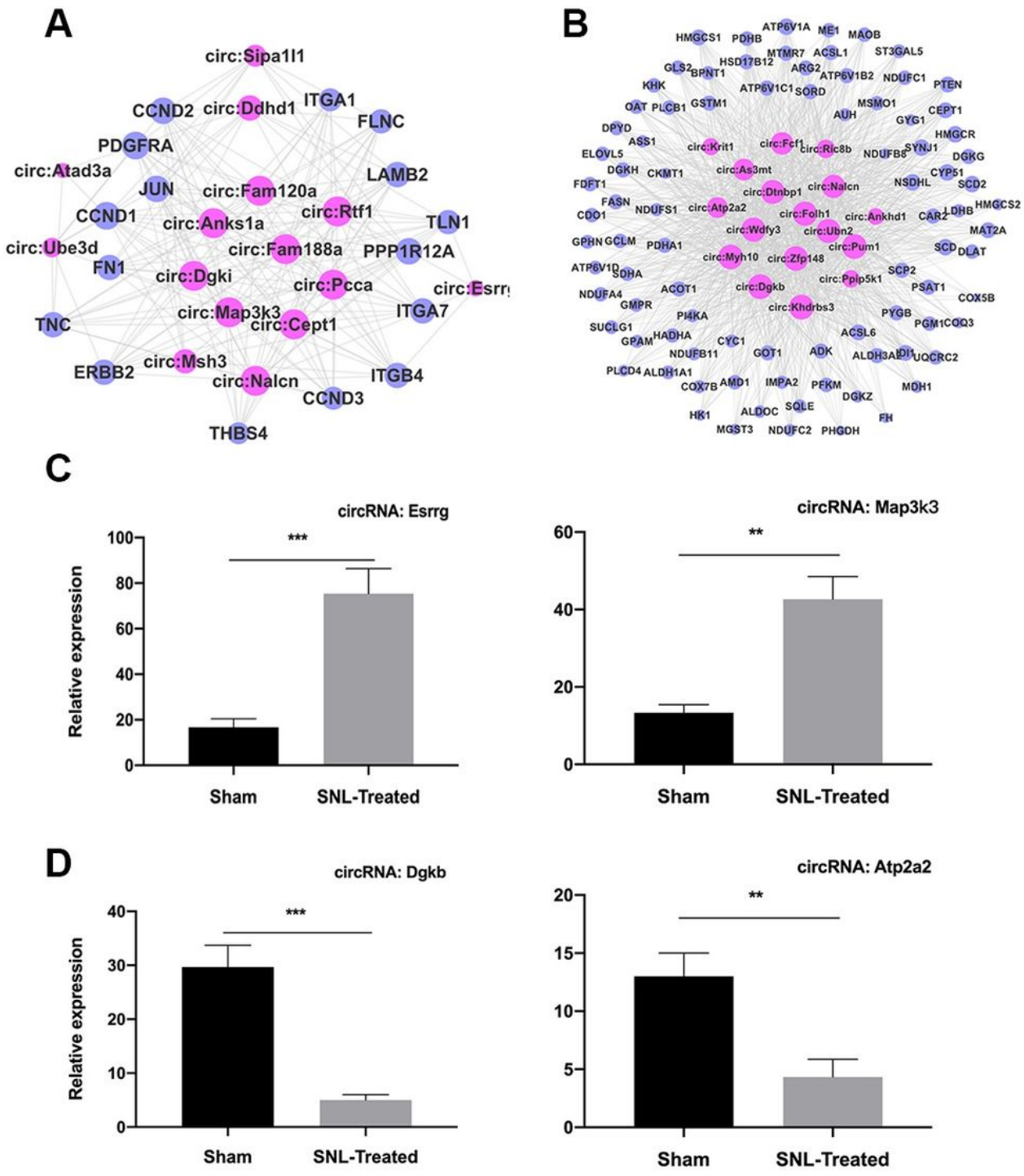


Figure 4

CircRNAs-mRNAs network and validation results for the selected circRNAs. (A) (B) Up- and down-regulated circRNAs-mRNAs network. Red pattern is circRNA, purple pattern is mRNA, the size of the node is graded according to the number of connected edges, the larger the number of connected edges. (C) (D) Quantitative reverse transcriptionpolymerase chain reaction analysis. 2 up- and 2 down-regulated circRNAs were demonstrated to be consistent with the ceRNA network results (n=3)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.jpg
- FigureS2.jpg
- FigureS3.jpg
- FigureS4.jpg