**YMTHE, Volume *30***

## Supplemental Information

# Microglia secrete miR-146a-5p-containing exosomes to regulate neurogenesis in depression

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**Figure. S1**

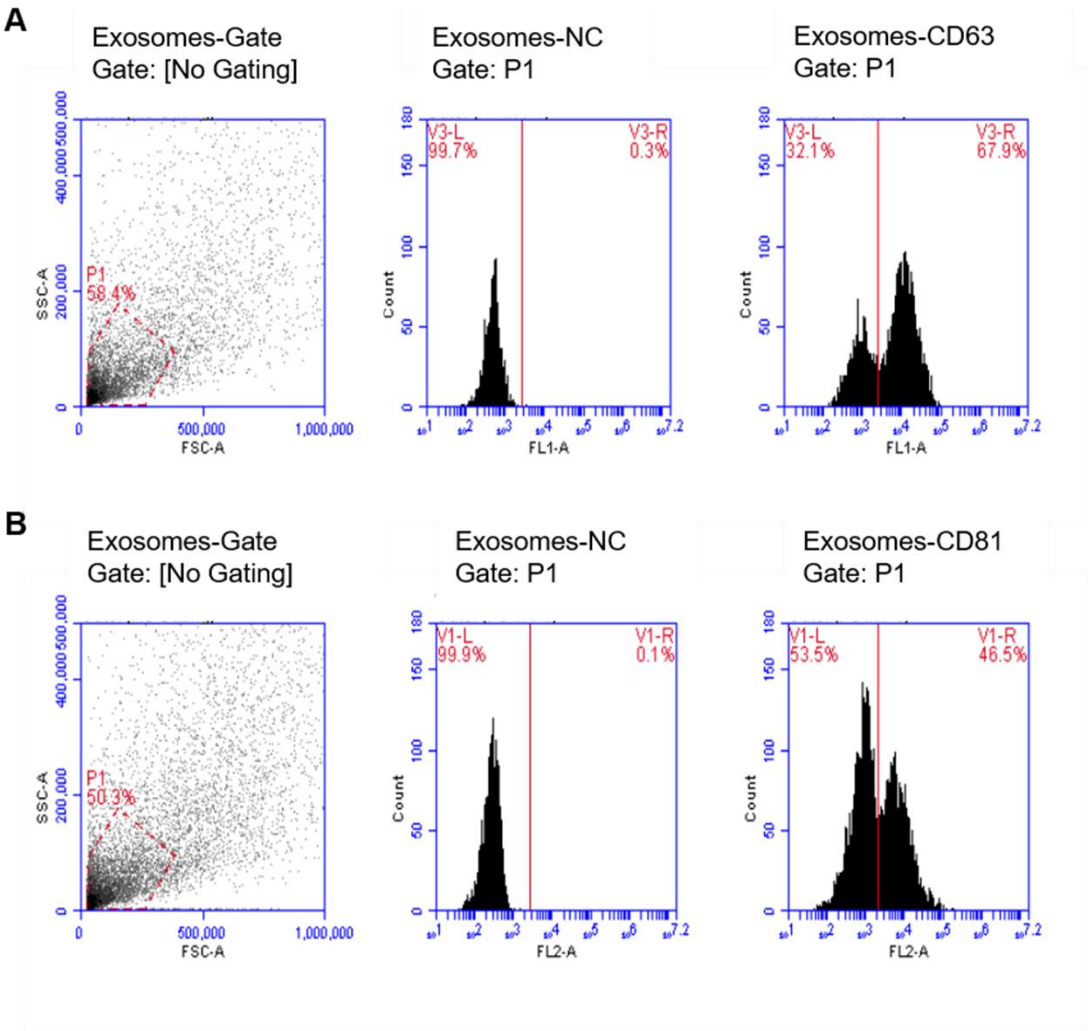



**Figure. S1. The expression levels of CD63 and CD81 in the exosomes derived from serum of rats.** (A) Representative flow cytometry data showing the expression levels of CD63 in the exosomes. (B) Representative flow cytometry data showing the expression levels of CD81 in the exosomes.

**Figure. S2**

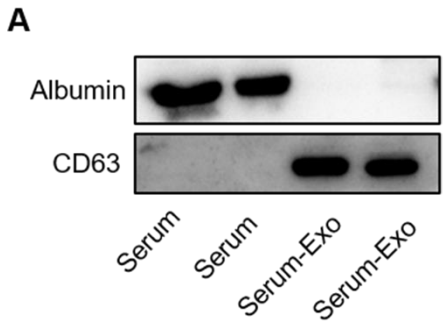



**Figure. S2. Identification of exosome from the serum of rats.** (A) Western blot analysis of the characteristic biomarker of exosomes, CD63 and Albumin.

**Figure. S3**

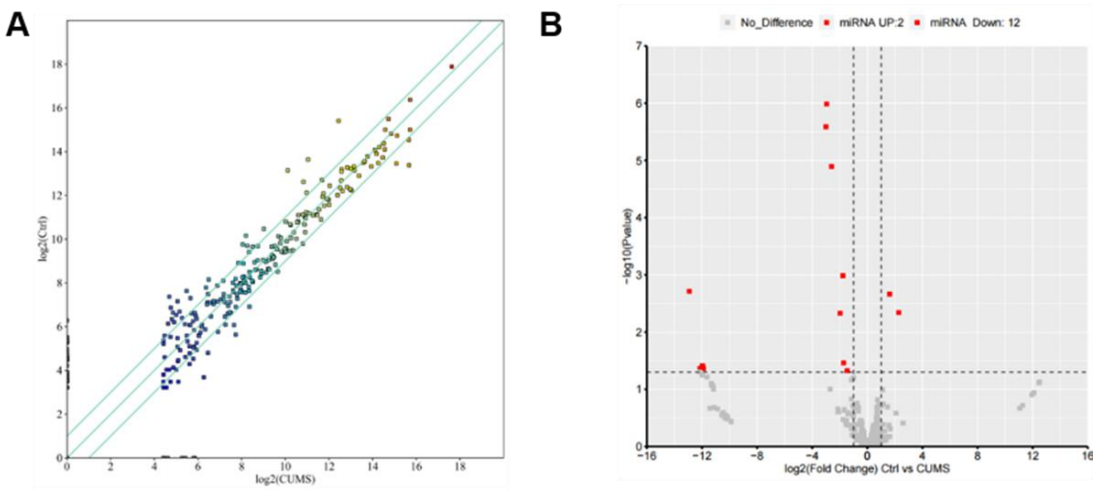



**Figure. S3. Differentially expressed miRNA profiles in the serum-derived exosomes from control and CUMS rats.** (A) Scatter plots are used to evaluate the difference in the expression of miRNAs between control and CUMS rat. The middle green line refers to no difference between the two groups. And the flank green lines represent 2.0fold changes. The miRNAs above the top green line and below the bottom green line indicate more than 2.0fold changes between two groups. (B) Differentially expressed miRNAs were displayed on a Volcano plots. The red represents the differentially expressed miRNAs with statistical significance, respectively ($P$<0.05). Gray indicates no significant difference.

**Figure. S4**

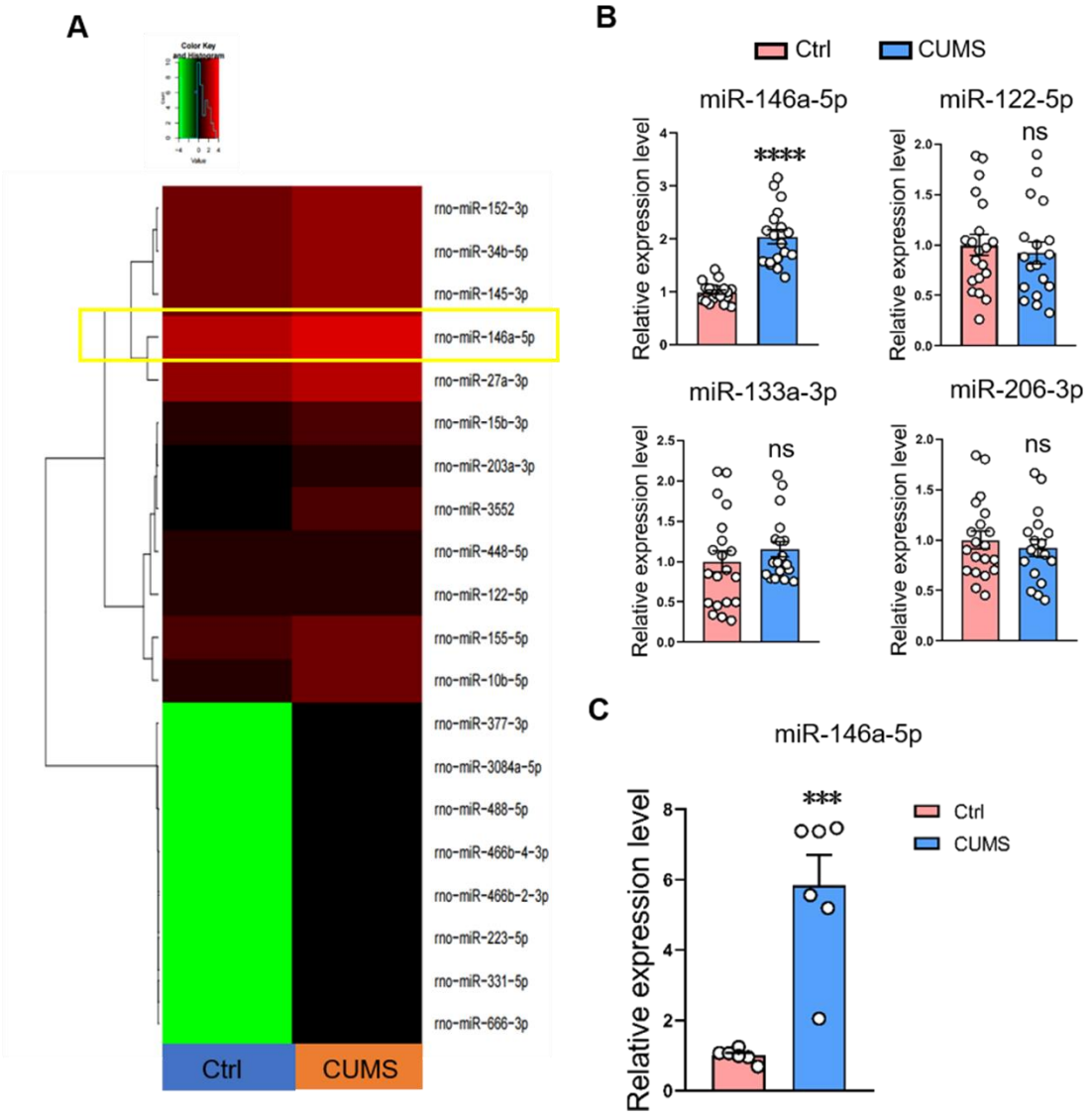



**Figure. S4. MiR-146a-5p is up-regulated in DG regions and Cerebrospinal fluid (CSF)of CUMS rats.** (A) Heat map of the top 10 up- and down-regulated expressed miRNAs in hippocampal DG regions of Ctrl and CUMS rats. (B) Relative expression levels of miR-146a-5p, miR-122-5p, miR-133a-3p and miR-206-3p in DG tissue. Control: n=20, CUMS: n=18. ****$P$<0.0001 CUMS vs. Ctrl. n,s, not significant. (C) Relative expression levels of miR-146a-5p in cerebrospinal fluid (CSF). n=6 per group. ***$P$<0.001 CUMS vs. Ctrl. Data represent means ± SEMs. Student t-tests for

comparisons between the two groups (B and C). Chronic unpredicted mild stress, CUMS. Control, Ctrl.

**Figure. S5**

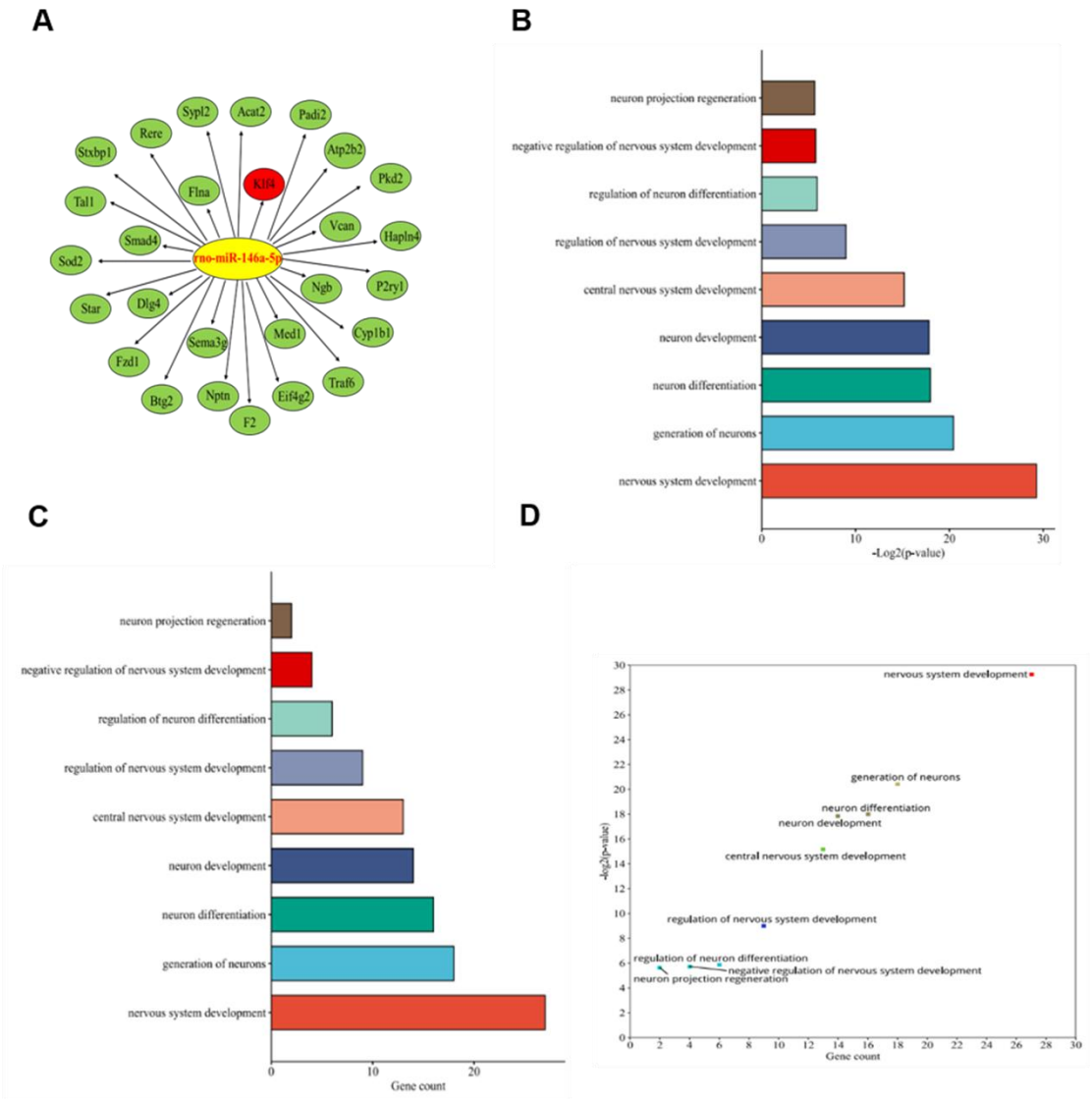



**Figure. S5. Function annotations for target genes mediated by miR-146a-5p.** (A) Bioinformatical prediction of miR-146a-5p target genes related to nervous system development. (B-C) Function annotation for miR-146a-5p targeted genes in pathways related to nervous system development. The vertical axis shows annotated functions of target genes and horizontal axes shows -log2 transformed P-values (B) and gene number of each cluster (C). (D) Scatter plots demonstrating all cluster features of P-values and gene counts.

**Figure. S6**

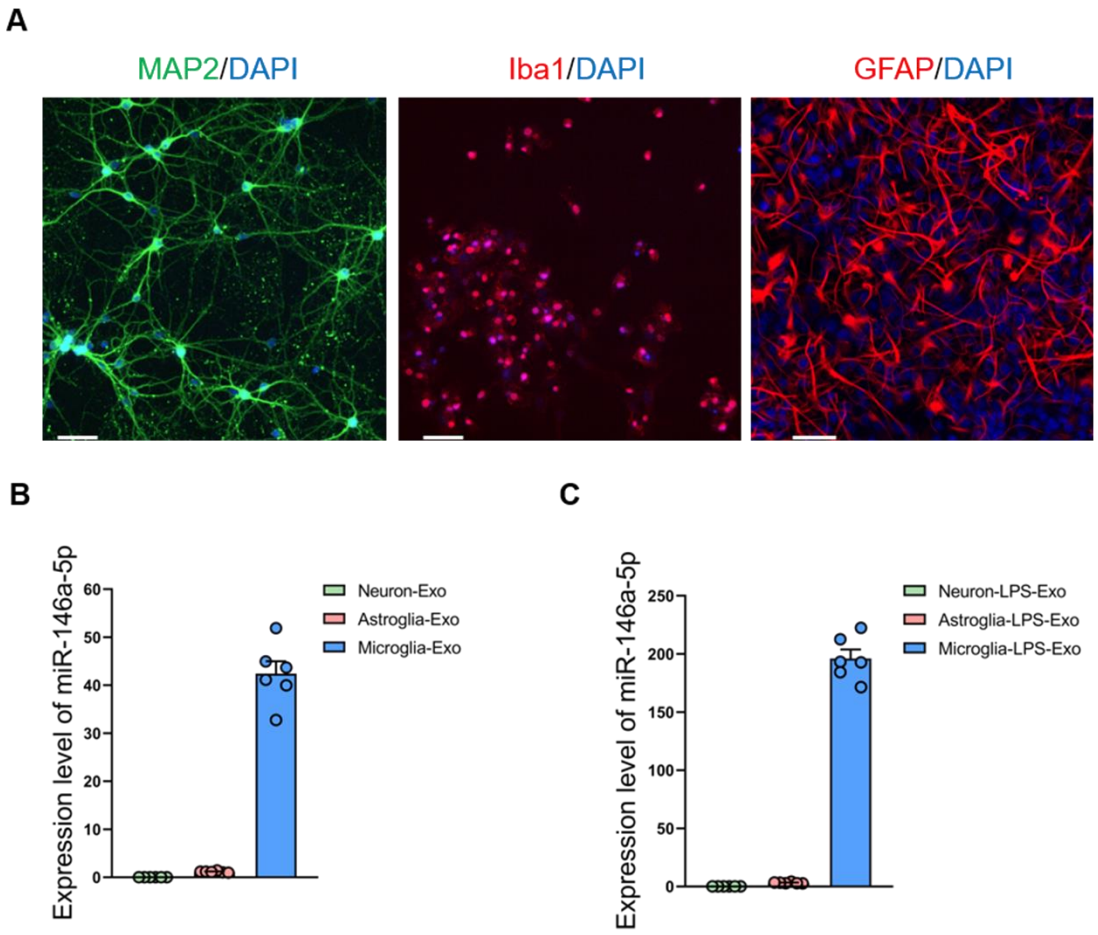



**Figure. S6. MiR-146a-5p was enriched in microglia-derived exosomes but not in that from neurons.** (A) Immunofluorescence staining of MAP2, Iba-1 and GFAP for identifying primary hippocampal neurons, microglia and astroglial revealed a pure culture. (B) Quantitative PCR data of miR-146a-5p in exosomes derived from cultured primary neuron, astroglia and microglia. n=6 per group. (C) Quantitative PCR data of miR-146a-5p in exosomes derived from cultured primary neuron, astroglial and microglia with LPS treatment. n=6 per group. Data represent means ± SEMs. Exosome, Exo.

**Figure. S7**

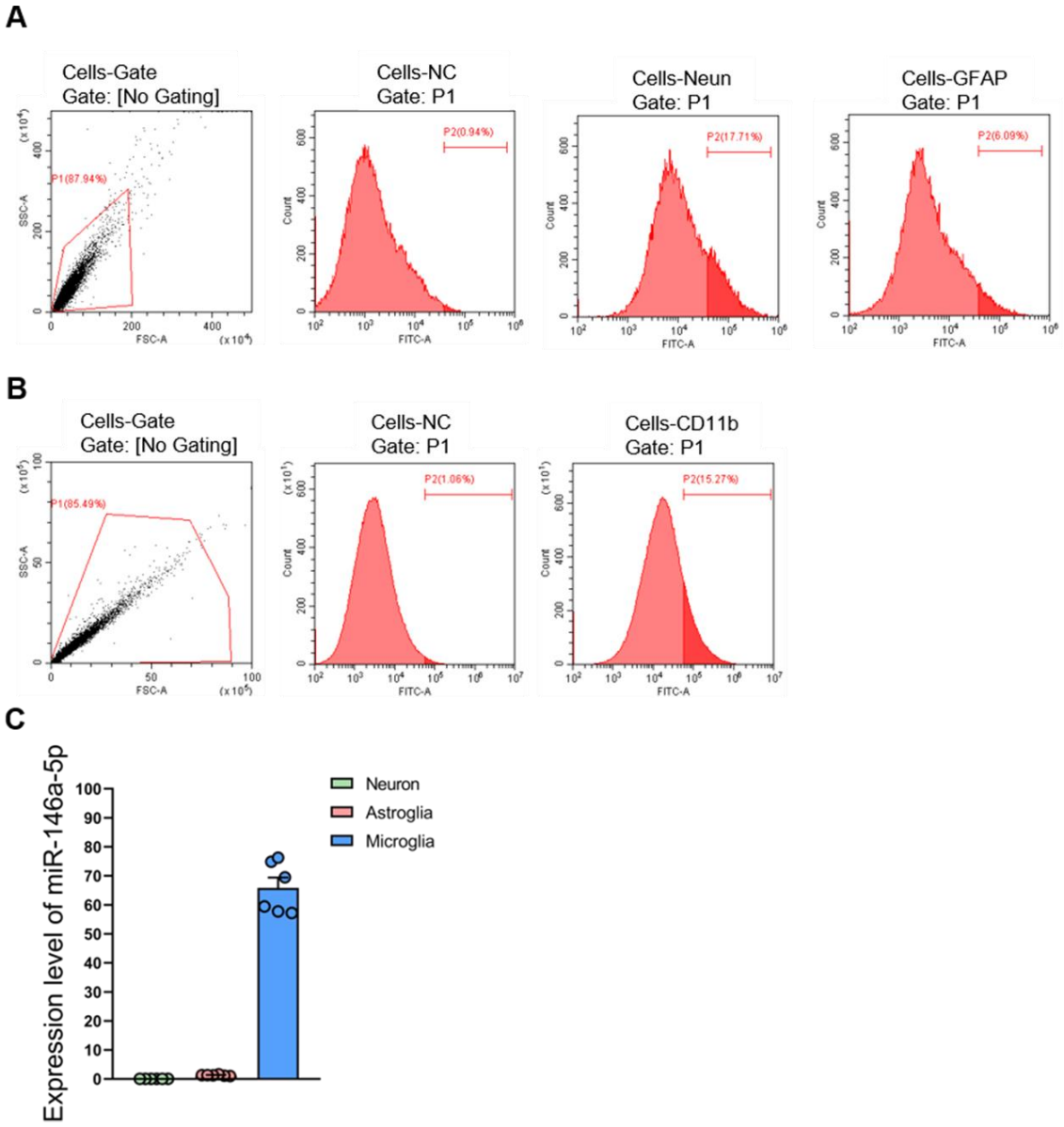



**Figure. S7. MiR-146a-5p was enriched in hippocampal microglia but not in neurons.** (A-B) Representative flow cytometry plots of all Neun$^{+}$, GFAP$^{+}$ (A) and CD11b$^{+}$ (B) cells from brain tissues of the hippocampus, accompanied by the quantification of the percentages of neuron, astroglial and microglia. (C) Quantitative PCR data of miR-146a-5p in neuron, astroglial and microglia taken from dissociated brain tissue. n=6 per group.

**Figure. S8**

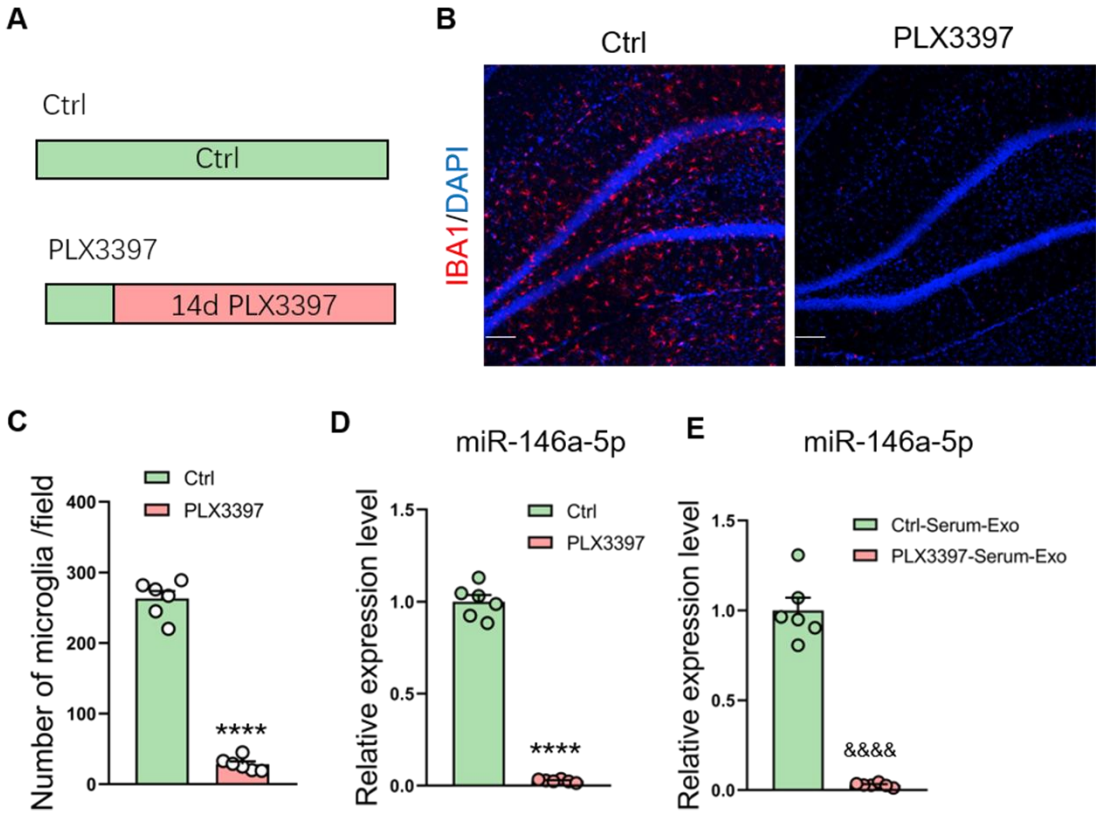



**Figure. S8. Microglia are eliminated with CSF1R inhibitor and presented reduced levels of miR-146a-5p.** (A) Schematic of the experimental design: Wister wild-type rats were treated with PLX3397 for 14 days to deplete microglia. (B) Representative images from brain sections stained with anti- Iba-1 from the hippocampal region of No-PLX3397 (Left) and PLX3397 treated rat. Scale bar, 100μm. (C) Quantification of the number of Iba-1$^{+}$ cells in the hippocampus shows >90% elimination of microglia with PLX3397 treatment. n=6 per group. (D) Quantitative PCR analysis shows robust reductions in miR-146a-5p in hippocampal DG of rat treated with PLX3397. n=6 per group. (E) Quantitative PCR analysis shows significant reductions in miR-146a-5p in exosomes derived from serum of rat treated with PLX3397. n=6 per group. ****$P$<0.0001 PLX3397 vs. Ctrl. $^{\&\&\&\&}P$<0.0001

PLX3397-Serum-Exo vs. Ctrl-Serum-Exo. Data represent means ± SEMs. One-way ANOVA with Tukey's post-hoc test for multiple comparisons involving >2 groups (C-E). Control, Ctrl. Exosome, Exo.

**Figure. S9**

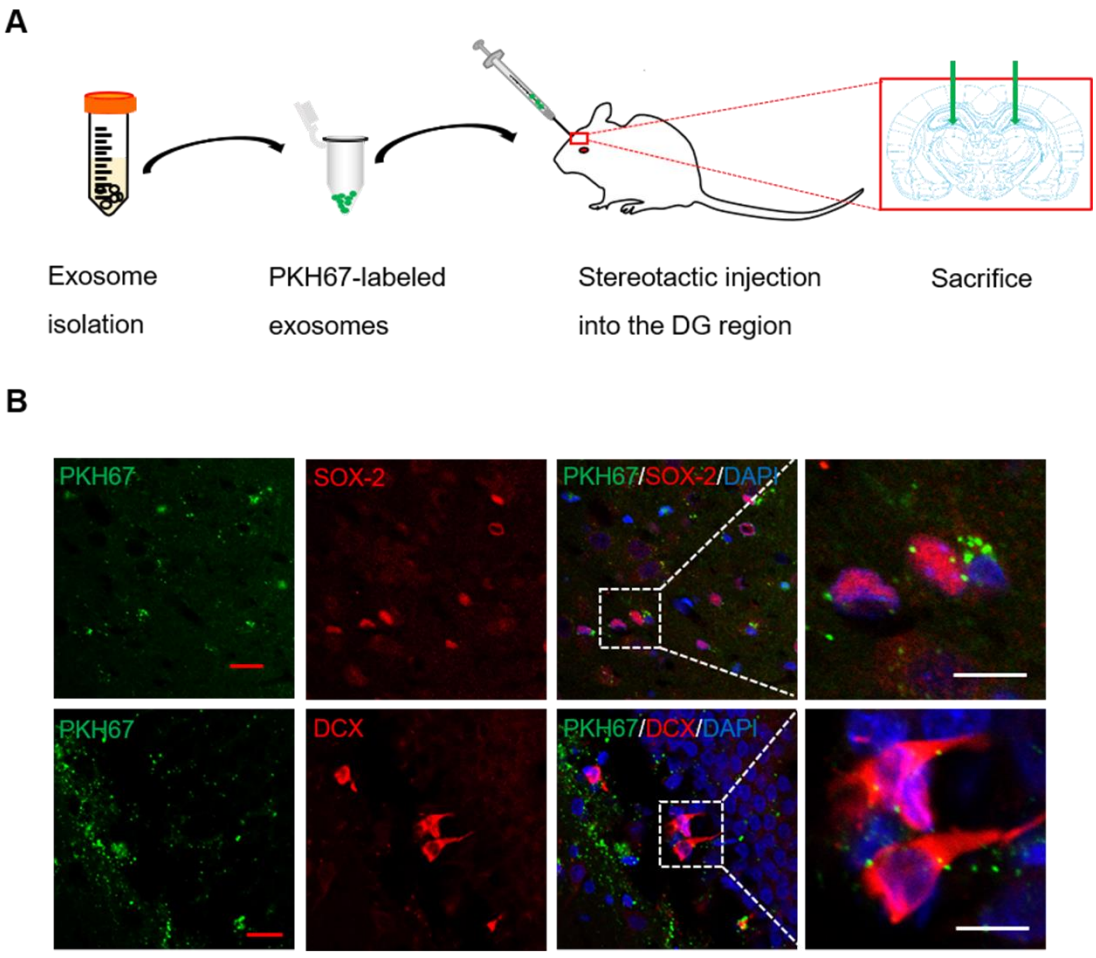
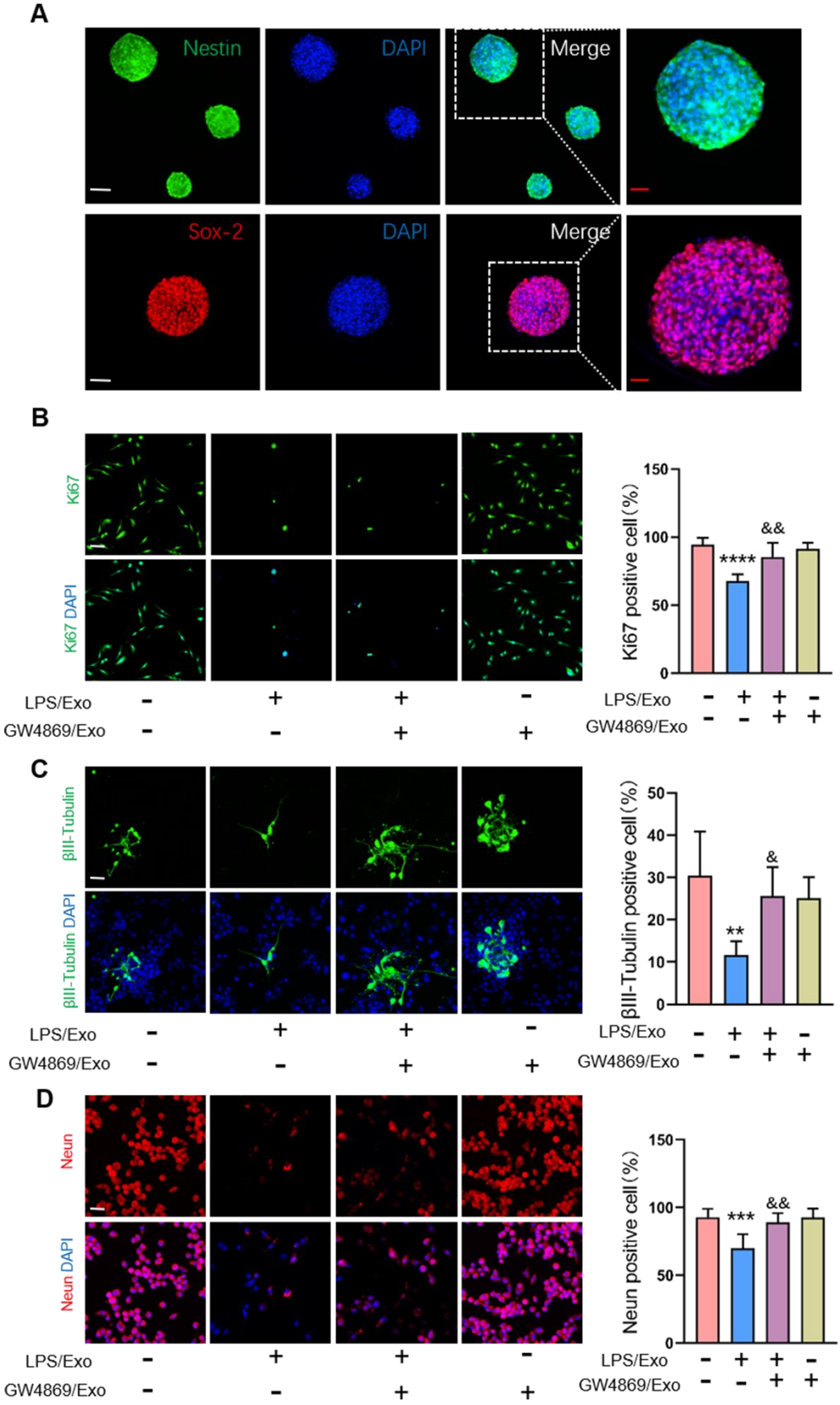



**Figure. S9. Internalization of microglia-derived exosomes in DG regions.** (A) Experimental paradigm for determining exosome uptake in DG neurons. (B) Confocal microscopy images showing internalization of exosomes in DG regions of SOX-2 cells and DCX cells. Scale bar: White, 10μm, Red,10μm. n=6 per group.

**Figure. S10**


A
Nestin
DAPI
Merge
Sox-2
DAPI
Merge
B
Ki67
Ki67 DAPI
LPS/Exo
GW4869/Exo
Ki67 positive cell (%)
****
&&
C
βIII-Tubulin
βIII-Tubulin DAPI
LPS/Exo
GW4869/Exo
βIII-Tubulin positive cell (%)
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&
D
Neun
Neun DAPI
LPS/Exo
GW4869/Exo
Neun positive cell (%)
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&&

**Figure. S10. MiR-146a-5p-containing exosomes derived from BV-2 cells inhibited the proliferation and differentiation of NSCs.** (A) Confocal microscopy images showing the neural stem cells. (B-D) Exosomes derived from LPS-treated BV-2 cells (LPS/Exo) or GW4869-treated BV-2 cells (GW4869/Exo) were added into the medium. Proliferation(B) and differentiation(C-D) of NSCs in vitro detected by immunofluorescence. Scale bar, 30 μm. n=5 per group (C), n=4 per group (B, D). **P<0.01, ***P<0.001, ****P<0.0001 LPS/Exo group (exosomes from LPS treated BV-2 cells) vs. naive group (exosomes from non-LPS and non-GW4869 treated BV-2 cells), $^{\&}P<0.05$, $^{\&\&}P<0.01$ LPS/GW4869/Exo group (exosomes from LPS and GW4869 treated BV-2 cells) vs. LPS/Exo group (exosomes from LPS treated BV-2 cells). Data represent means ± SEMs. One-way ANOVA with Tukey's post-hoc test for multiple comparisons involving >2 groups (B-C). Control, Ctrl. Exosome, Exo.

**Figure. S11**

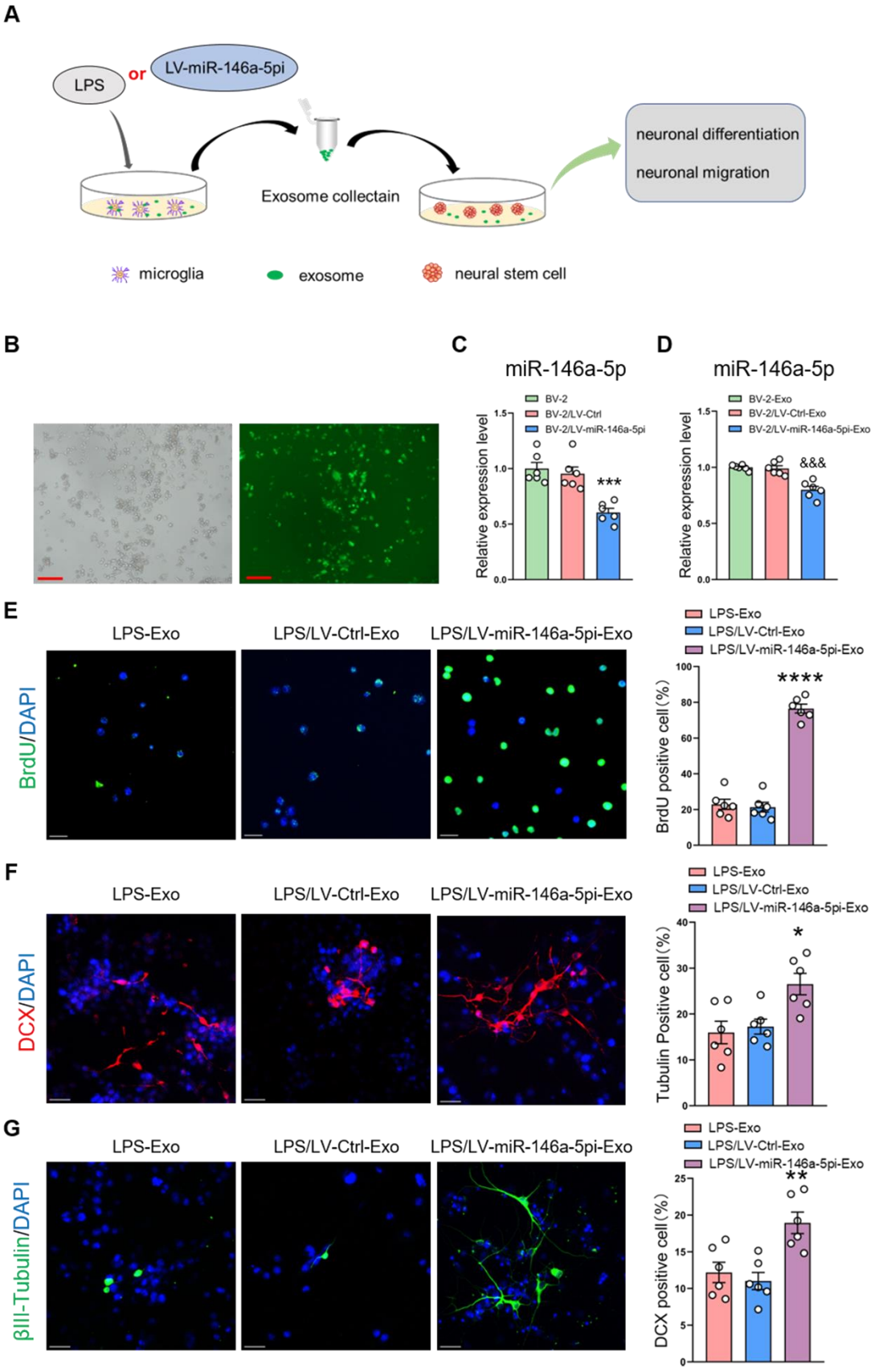
A
LPS
or
LV-miR-146a-5pi
Exosome collectain
neuronal differentiation
neuronal migration
microglia
exosome
neural stem cell
B
C
miR-146a-5p
BV-2
BV-2/LV-Ctrl
BV-2/LV-miR-146a-5pi
Relative expression level
***
D
miR-146a-5p
BV-2-Exo
BV-2/LV-Ctrl-Exo
BV-2/LV-miR-146a-5pi-Exo
&&&
E
LPS-Exo
LPS/LV-Ctrl-Exo
LPS/LV-miR-146a-5pi-Exo
BrdU/DAPI
BrdU positive cell (%)
****
F
DCX/DAPI
Tubulin Positive cell (%)
*
G
βIII-Tubulin/DAPI
DCX positive cell (%)
**

**Figure. S11. Exosomes derived from the BV-2 cells which knocking-out miR-146a-5p have no inhibitory effects on the neurogenesis.** (A) Schematic representation of NSCs co-cultured with BV-2-derived exosomes. (B) Representative images of BV-2 cells transfected with Lentivirus. Scale bar, 150 μm. (C) The altered miR-146a-5p level in BV-2cells after LV-146a-5pi transfection. n=6 per group. (D) The altered miR-146a-5p level in exosomes derived from BV-2 cells transfected with LV-146a-5pi. n=6 per group. (E-G) Proliferation (E) and differentiation (F, G) of neuronal stem cells (NSCs) as determined in vitro using immunofluorescence. Scale bar, 30 μm. n=6 per group. ***$P$<0.001 BV-2/LV-miR-146a-5pi vs. BV-2/LV, $^{\&\&\&}P$<0.001 BV-2/LV-miR-146a-5pi-Exo vs. BV-2/LV-Exo. Data represent means ± SEMs. One-way ANOVA with Tukey's post-hoc test for multiple comparisons involving >2 groups (C-G). Exosome, Exo.

**Figure. S12**

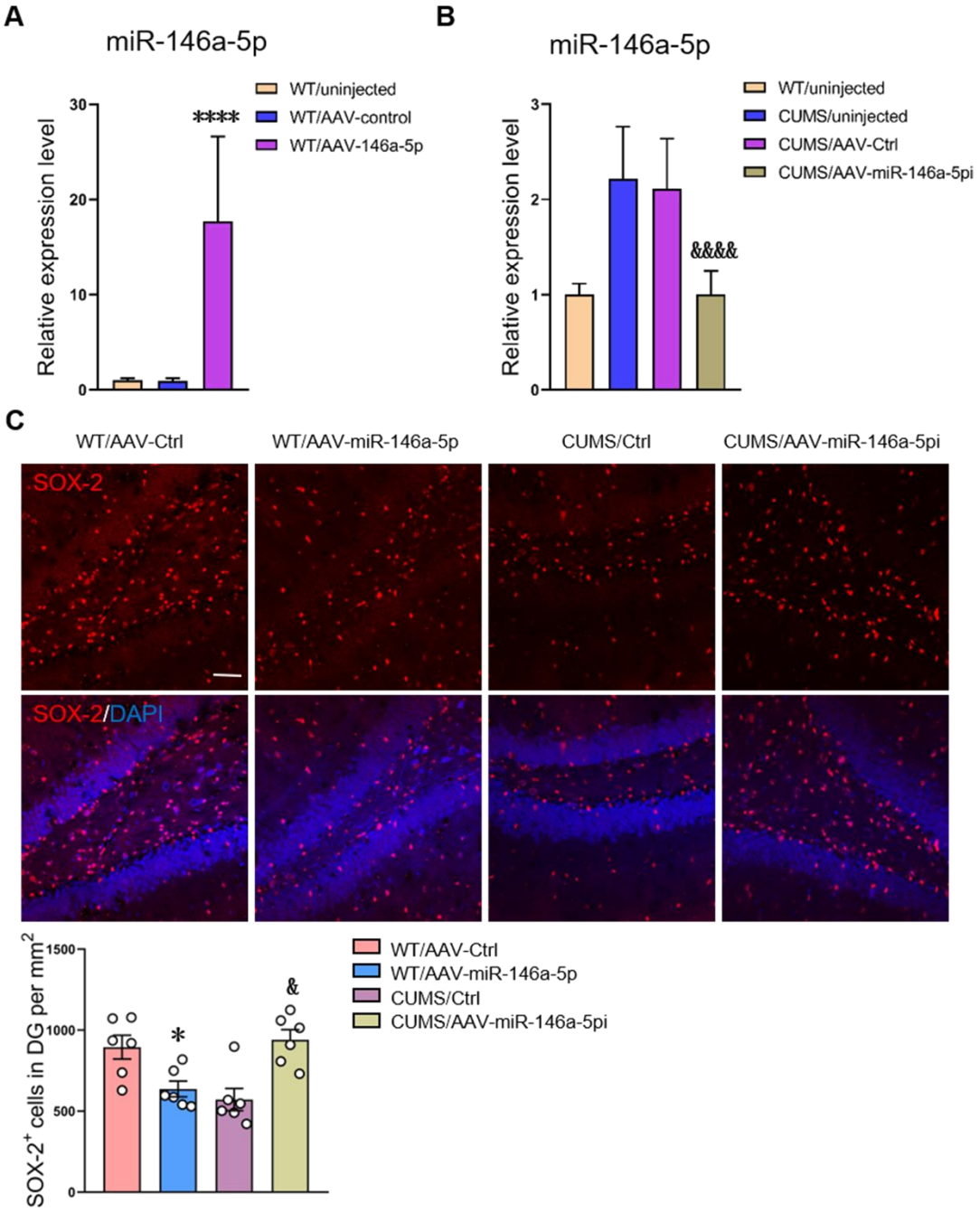


**Figure. S12. Estimation of expression level of viral constructs and neurogenesis through Stereotactic injection.** (A) The expression level of miR-146a-5p in DG region infected with AAV-146a-5p. n=10 per group. ****$P$<0.0001 WT/AAV-miR-146a-5p vs. WT/AAV-Ctrl. (B) The expression level of miR-146a-5p in DG region

infected with AAV-146a-5pi. n=10 per group. $^{\&\&\&\&}P<0.0001$ CUMS/AAV-miR-146a-5pi vs. CUMS/AAV-Ctrl. (C) Representative confocal microscopic images of immunostainings for $Sox2^{+}$ cells in the DG regions. Scale bar, 50μm. n=6 per group. $^{*}P<0.05$ WT/AAV-miR-146a-5p vs WT/AAV-Ctrl, $^{\&}P<0.05$ CUMS/AAV-miR-146a-5pi vs CUMS/AAV-Ctrl. Data represent means ± SEMs. One-way ANOVA with Tukey's post-hoc test for multiple comparisons involving >2 groups (A -C). Wild type, WT. Chronic unpredicted mild stress, CUMS. Control, Ctrl.

**Figure. S13**

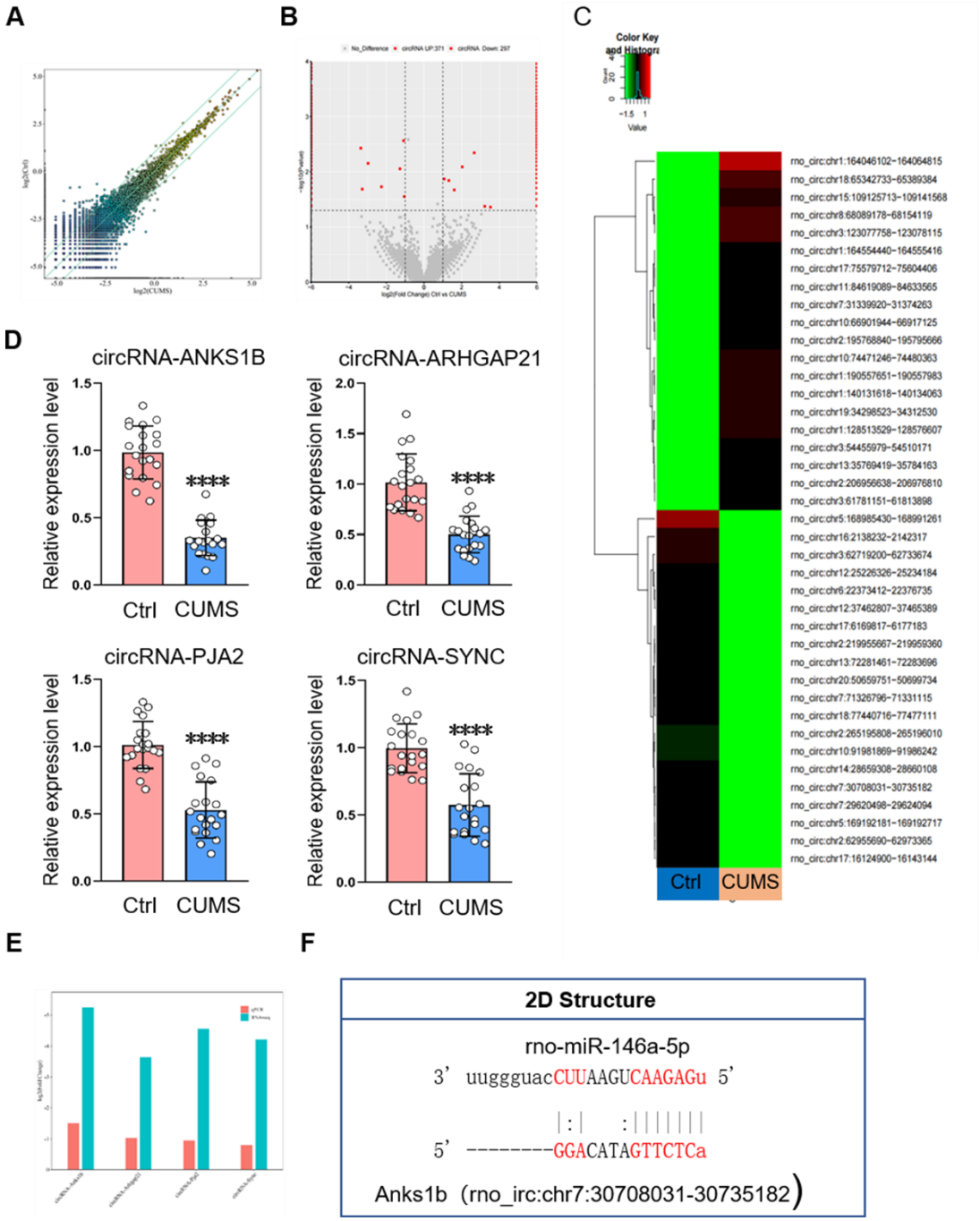


**Figure. S13**. **Differences and characterizations in circRNA expression profiles between control and CUMS rats.** (A) Differentially expressed circRNAs were displayed on a scatter plot. The middle green line refers to no difference between the two groups. And the flank green lines represent 2.0fold changes. The miRNAs above

the top green line and below the bottom green line indicate more than 2.0fold changes between two groups. (B) Differentially expressed circRNAs were displayed on a volcano plot. The red represents the differentially expressed circRNAs with statistical significance, respectively ($P<0.05$). Gray indicates no significant difference. (C) Heat map of the top 20 differentially up- and down-expressed circRNAs between control and CUMS rats. (D) Relative expression levels of circRNAs in DG tissue. n=20 per group. ****$P<0.0001$ CUMS vs. Ctrl. (E) Comparison between circRNAs sequencing data and qPCR results were consistent with that obtained in the sequencing results. (F) Predicted putative seed-matching sites between circANKS1B and miR-146a-5p. Data represent means ± SEMs. Student t-tests for comparisons between the two groups (D). Chronic unpredicted mild stress, CUMS. Control, Ctrl.

**Figure. S14**

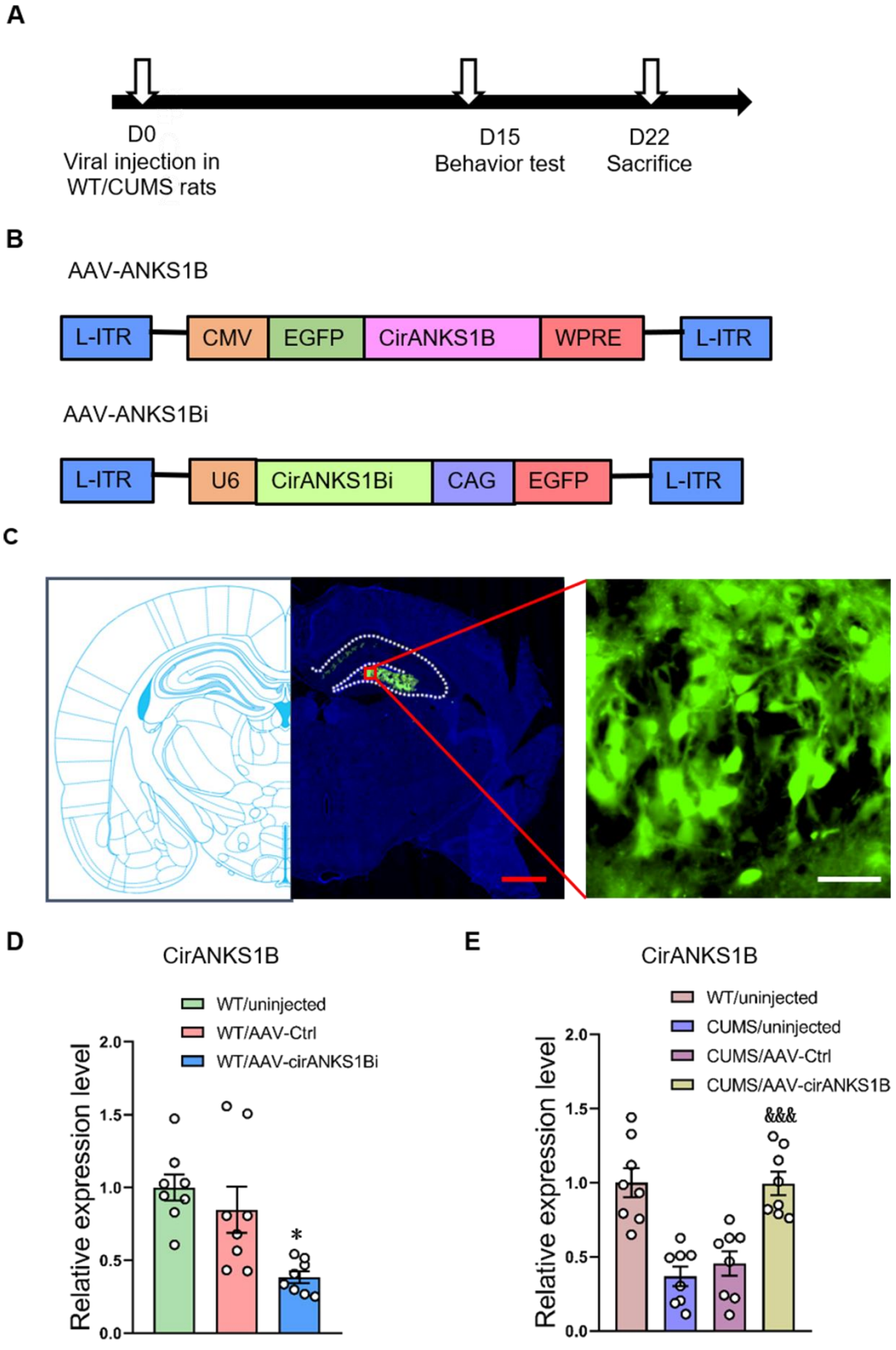


**Figure. S14. Estimation of expression level of viral constructs through**

**Stereotactic injection.** (A) Schematics of AAV vectors engineered to overexpress circANKS1B, circANKS1B shRNA, and their corresponding controls. (B) Experimental paradigm for determining behavioral responses of rats infected with the virus. (C) Representative coronal section showing the expression of AAV-eGFP in DG regions of rats. (D) The expression level of circANKS1B in DG region infected with AAV-ANKS1Bi. n=8 per group. *$P$<0.05 WT/AAV-cirANKS1Bi vs. WT/AAV-Ctrl. (E) The expression level of circANKS1B in DG region infected with AAV- ANKS1B. n=8 per group. $^{\&\&\&}P$<0.001 CUMS/AAV-cirANKS1B vs. CUMS/AAV-Ctrl. Data represent means ± SEMs. One-way ANOVA with Tukey's post-hoc test for multiple comparisons involving >2 groups (D-E). Chronic unpredicted mild stress, CUMS. Control, Ctrl. Wild type, WT.

**Figure. S15**

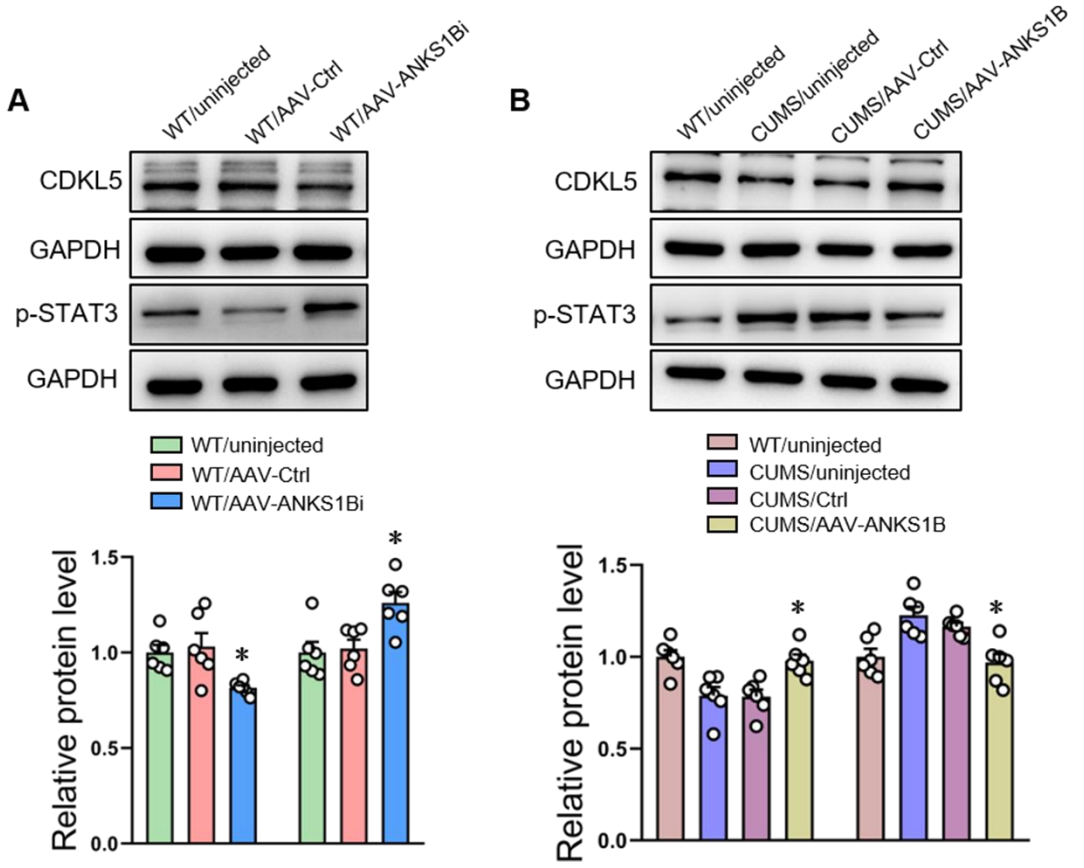


**Figure. S15. Estimation of expression level of CDKL5 and p-STAT3 through Stereotactic injection.** (A) Representative western blot images showing relative protein levels of CDKL5 and Phosphorylation of STAT3 in AAV-ANKS1Bi infected DGs of WT rats. n=6 per group. **P*<0.05 WT/AAV-ANKS1Bi vs WT/AAV-Ctrl. (B) Representative western blot images showing relative protein levels of Cdkl5 and Phosphorylation of STAT3 in AAV-ANKS1B infected DGs of CUMS rats. n=6 per group. **P*<0.05 CUMS/AAV-ANKS1B vs CUMS/AAV-Ctrl rat. Data represent means ± SEMs. One-way ANOVA with Tukey's post-hoc test for multiple comparisons involving >2 groups (A, B). Control, Ctrl.

**Table S1. The top 12 up-regulated and down-regulated expressed miRNAs ranked by P-value in serum-derived exosomes produced from control and CUMS rats**

| miRNA ID | log2 (foldchange) | P-value | miRNA ID | log2 (foldchange) | P-value |
|---|---|---|---|---|---|
| miRNAs upregulated | | | miRNAs downregulated | | |
| **rno-miR-146a-5p** | 1.6312 | 0.002193 | rno-miR-1-3p | -2.9663 | 1.04E-06 |
| rno-miR-187-3p | 2.2387 | 0.0041260 | rno-miR-1b | -3.0321 | 2.57E-06 |
| rno-miR-122-5p | 2.283 | 0.004604 | rno-miR-206-3p | -2.6015 | 1.28E-05 |
| rno-miR-429 | 1.8147 | 0.0146116 | rno-miR-133a-3p | -1.7835 | 0.001038 |
| rno-miR-192-5p | 1.7775 | 0.0165811 | rno-miR-542-3p | -12.9304 | 0.001958 |
| rno-miR-486 | 1.6663 | 0.0176033 | rno-miR-133b-3p | -1.9624 | 0.00475 |
| rno-miR-484 | 2.0131 | 0.0214115 | rno-miR-149-5p | -1.717 | 0.034851 |
| rno-miR-27a-5p | 1.491 | 0.0214272 | rno-miR-24-1-5p | -11.9792 | 0.038419 |
| rno-miR-129-5p | 1.8089 | 0.0256463 | rno-miR-6329 | -11.9629 | 0.03898 |
| rno-miR-185-5p | 1.6643 | 0.0335633 | rno-miR-341 | -11.902 | 0.043148 |
| rno-miR-200b-3p | 1.5095 | 0.0378972 | rno-let-7b-3p | -12.1555 | 0.043672 |
| rno-miR-191a-5p | 1.1623 | 0.0484238 | rno-miR-322-3p | -1.4519 | 0.047895 |

**Table S2. Primer sequences of target genes used for quantitative real-time PCR in this study**

| Gene/CircRNA ID | Species | Suppliers | Product ID or Primer sequences |
|---|---|---|---|
| miR-146a-5p | Rat | GeneCopoeia | RmiRQP0196 |
| miR-122-5p | Rat | GeneCopoeia | RmiRQP0056 |
| miR-133a-3p | Rat | GeneCopoeia | RmiRQP0166 |
| miR-206-3p | Rat | GeneCopoeia | RmiRQP0308 |
| miR-1b | Rat | GeneCopoeia | RmiRQP0044 |
| miR-187-3p | Rat | GeneCopoeia | RmiRQP0251 |
| miR-16 | Rat | GeneCopoeia | RmiRQP0227 |
| miR-451 | Rat | GeneCopoeia | RmiRQP0509 |
| RsnRNA U6 | Rat | GeneCopoeia | RmiRQP9003 |
| Circ-ANKS1B rno_circ:chr7:30708031-30735182 | Rat | Ribobio | Forward: AAAATGGAAGCCAGAGTGTAGGA |
| | | | Reverse: GGGAAGTAACCTTTGTTGTCTGG |
| Circ-ARHGAP21 rno_circ:chr17:87795320-87797427 | Rat | Ribobio | Forward: AAGAGAATGGAAACAGAGGAGGTG |
| | | | Reverse: ACGATTCGTCTTCGGACGGT |
| Circ-PJA2 rno_circ:chr9:112023471-112027242 | Rat | Ribobio | Forward: TTGACAAAGACGAAGACAGGAATC |
| | | | Reverse: GCAGCGTCTATGCTTCCAGTT |
| Circ-SYNC rno_circ:chr5:147482283-147483432 | Rat | Ribobio | Forward: CGCTCGTGAGGCAAAAACA |
| | | | Reverse: TGAGTCCTGAAGAGGAAAACTGG |

## Supplementary Methods and Materials

### Animals

Male Wistar rats (120-140g) were purchased from the animal center of Shandong University (Shandong, China) and randomly settled into groups. Rats were raised in single cages and maintained on a 12 h light/dark cycle at 22-25℃ with free access to food and water. All experimental procedures were reviewed and approved by the Ethics Committee of Shandong College, and strictly abide by the International Guiding Principles of Animal Research formulated by the Council of the International Medical Science Organization.

### CUMS model

Rats were exposed to a variable sequence of stressors consisting of 24-h food deprivation followed by 24-h water deprivation, overnight illumination, swimming (4℃, 5 min), wet bedding (24h), tail clip (1 min), titled cage (24h), physical restraint (2h), noise (2h) and shaking (1h). Stressors were administered daily in a random sequence for five weeks. The sucrose preference and forced swimming tests were then used to assess depression-like behaviors in these rats.

### Behavioral Tests

After the five-week period of stress administration, behavioral tests for assessing depression were conducted as described previously[1]. For the sucrose preference test, rats were placed individually in cages and provided with two bottles of sucrose

solution (1%, w/v) located at opposite sides of the cage at identical heights for the first 24-h period. For the second 24 h period one bottle of sucrose solution was replaced with a bottle containing tap water. Immediately after, rats were deprived of water and food for 24h. On the fourth day, sugar water consumption was measured over a 3 h period when rats were given two bottles (100 ml each) with one containing 1% sucrose in water and the other only tap water. Bottle positions were reversed after 1.5 hours. Sucrose preference was defined as sucrose consumption/ [water consumption + sucrose consumption] ×100% during the 3-h test.

The forced swimming test has been established as an effective method to assess depression-like behaviors in rats. The procedure for this test consists of placing the rats individually in a glass cylinder (height: 80 cm, diameter: 30 cm) filled with 40 cm of water (25℃) for 15 min on day 1. After 24 h, rats were again individually placed in the cylinder for a 5 min period and immobility and swimming times were recorded for each rat. Immobility time was defined as only exposing the head above the surface of the water while floating.

**Cerebrospinal fluid (CSF) samples**

CSF samples were collected as described previously with slight modifications[2]. Rat were anesthetized with 2.5% isoflurane and placed in the stereotaxic instrument. A sagittal incision of the skin was made inferior to the occiput to expose the foramen magnum. A sterile cotton swab was then used to absorb any blood on the dura mater. A penetrating capillary tube was inserted into the cisterna magna through the dura

mater to enable CSF to flow into the capillary tube. The CSF sample was frozen on dry ice and then transferred to a -80 ℃ freezer.

**Serum samples**

A blood sample was obtained from the abdominal aorta of anesthetized rats using a 10ml syringe. The blood sample was stored at 4℃ for 3-4 h, then centrifuged at 5000 rpm at 4℃ for 10 min followed by centrifugation at 13000 rpm for 10 min at 4 ° C. The upper phase of serum was collected and shipped on dry ice to the RiboBio Biotechnology Company (Guangzhou, China) for exosome isolation and miRNA sequencing.

**Microglia depletion**

To deplete microglia in vivo, rats were treated with the CSF1R inhibitor PLX3397 (Selleck Chemicals, USA) which mixed into the standard chow. The dose and administration route were as described previously with minor modifications[3]. The PLX3397 was administered at 600 ppm as well as the control group received standard chow on daily for 14 days.

**BV-2 cell culture**

BV-2 is a clonal mouse microglia cell line with the functional and morphological characteristics of microglia[4]. The cells were purchased from Iennio (Guangzhou, China), and cultured in DMEM supplemented with 10% FBS and 1%

penicillin/streptomycin (Thermo Fisher Scientific) at 37℃ in 5% CO2. The cells were transferred to serum-free medium and then exposed to LPS (100 ng/ml) for 24 h to isolate exosomes from cell culture supernatants.

**Primary neural cell culture**

Primary NSCs were isolated from P0 to P1 neonatal rats as described previously with minor modifications[5]. Briefly, the cortex and hippocampus were removed from the brain and placed in cold Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/L glucose. The meninges were carefully cleared and discarded. Tissues were triturated in proliferation medium [DMEM/F12 supplemented with 1%penicillin/streptomycin (Gibco), 2%B27 supplement (Gibco), 20 ng/ml recombinant human EGF (Gibco) and 20 ng/ml recombinant human FGF2 (Gibco)] and obtain a single cell suspension. The cell suspension was filtered through a 70-μm cell strainer (Millipore, Billerica, MA) and cells were counted using a hemocytometer, followed by plating on 6-well tissue culture plates at a density of 1000,000 cells/well and incubated at 37℃ in 5% CO2. Cells were cultured as neurospheres in complete proliferation media and half of the medium was refreshed every 3 days.

Primary culture of mixed glial cell was prepared from newborn P1 rat pup as described previously[6,7]. In brief, the brain of animals (P0-P1 rat) was extracted and placed into the ice-cold DMEM medium with 4.5 g/L glucose. The removal of the meninges with forceps should be carefully. Then, the brain tissue was trypsinized with 0.25% trypsin and DNase for 5-10 min at room temperature to obtain the cell

suspension through a 70-μm cell strainer. The cells were seeded into poly-L-lysine-coated flasks with DMEM/F12 medium supplemented with 10% FBS. After in vitro culture for 10–14 days, microglia cells were isolated from mixed glial cultures by gently shaking the flasks for 30 min on an orbital shaker at 37 ℃, while astrocyte was layer on the flask surface. The purity of the microglia or astroglia preparation were identified by staining with rabbit anti-Iba1 and rabbit anti-GFAP, respectively.

Primary neurons cells were isolated from P1 rat fetuses as described previously[8-10]. Briefly, the brains were rapidly removed and placed in the 10-cm dish with ice-cold DMEM medium with 4.5 g/L glucose. The meninges were removed and then transferred to the centrifugation tubes containing 0.25% trypsin for 5-10 min at room temperature. The dissociated cells were resuspended by DMEM/F12 (containing 10% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin), and then seeded into 6-well plates pre-coated with poly-D-lysine (Millipore-Sigma) at a density of 1X $10^6$/well. After 4 h, the neurobasal medium (containing 2% B27 supplement (Gibco), 1% Gluta-Max (Gibco) glutamine, 100 U·mL-1penicillin and 100 mg·mL-1 streptomycin) was applied as the culture medium. Cells were grown at 37 ℃ in 5% CO2 and half of the medium was refreshed every 3 days. 5μM of cytosine arabinoside (Ara-c, Sigma) was added at 3th day of culture to control the proliferation of non-neuronal cells. The purity of the neurons was identified by staining with rabbit anti-MAP2, a neuron biomarker.

**BrdU staining of NSCs**

NSCs were treated with BrdU (10 μM) for 24 hours at 37℃ in 5% CO2 and then fixed with 70% ethanol for 5 minutes at room time[4]. Then, the cells were pretreated with 1.5M HCl for 30 min at 37℃, the Primary antibody was anti-BrdU (1:1000, CST Technology)

**Co-culture of NSCs with BV-2 -derived exosomes**

To determine the impact that microglia-derived exosomes have on neurogenesis in vitro, we use a co-culture assay of 24-well plates as described previously with minor modifications[10,11]. In brief, BV-2 cells were cultured in serum-free medium and pretreated with GW4869 (10 μM) for 30min prior to the application of LPS (100ng/ml) for 24 hours. Before exosomal treatment, NSCs were incubated for 4 hours in poly L-lysine-coated plates and the medium were changed to fresh proliferation media (for the proliferation study), or differentiation media (Neurobasal-A media, 2%B27, and 1x penicillin/streptomycin, Invitrogen, for the differentiation study). The collected exosomes derived from BV-2 cell supernatant were added into the medium of NSCs.

**Lentivirus transfection**

The recombinant lentivirus (LV) used to knock-out miR-146a-5p was purchased from GENEchem (Shanghai, China), and transfected into BV-2 cells according to the manufacturer's instructions. The expression levels of miR-146a-5p were detected by qPCR analysis after 72 h.

**Flow cytometry**

Multiple Cell Types retrieved from rat hippocampal tissue was performed as described previously with minor modifications[12-14]. Briefly, rats were anesthetized and perfused with PBS solution to eliminate blood contamination from brains. Hippocampal tissue from rat brain were dissected. Enzymatic digestion was performed with Neural Tissue Dissociation Kit (P) (Miltenyi Biotec) according to manufacturer protocol. Dissociated cells were re-suspended in PBS buffer with 0.5% BSA, following by through a 70 μm cell strainer to remove any undigested tissue chunk.

The cell pellet consisting of a mixture of all brain cells was further subjected to magnetic cells sorting for microglia enrichment using CD11b/c microbeads (Miltenyi Biotec) according to manufacturer protocol. Microglia, astroglial and neurons were positively selected from cell suspensions using anti-CD11b, anti-GFAP, and anti-NeuN antibody. Samples were analyzed with the FACSAria III flow cytometer (BD Biosciences, San Jose, CA, USA). Subsequent data analysis was performed with FlowJo software v.7.6.1 (Ashland, OR, USA).

**Exosome isolation and identification**

Rat serum and cell culture supernatants were collected and exosomes purified following a sequence of several centrifugation and filtration procedures as described previously[15]. Briefly, for serum, 2 ml of dilute serum with an equal volume of PBS were centrifuged for 30 min at 2000g. The supernatant was then centrifuged at

12000g for 45 min and 110000g for 2 h. Pellets were re-suspended in 1 ml PBS, followed by filtration through a 0.22-um filter to eliminate cellular debris. For purification of exosomes, the supernatant was ultra-centrifuged at 110000g for 70 min, followed by a wash of the exosomes pellet with 1 ml PBS and another centrifugation at 110000g for 70 min. Cell culture supernatants were centrifuged at 300g for 10 min, 2,000g for 10 min and 10,000g for 30 min to remove extraneous cells, dead cells and cellular debris. Next, the supernatant was filtered through a 0.22μm filter (Millipore-Sigma) to remove dead cells and particles larger than 200nm. Exosomes were subsequently isolated from the supernatant by centrifugation at 100,000g for 70 min. The pellet was re-suspended with PBS and centrifuged at 100000g for 70 min (Ultracentrifuge, Beckman Coulter, XPN-100). The exosome pellet was then re-suspended in 100ul PBS and stored at –80 ℃.

For exosomes identification, protein levels of serum-derived exosomes were measured with use of the BCA protein assay (Thermo Scientific, MA). Equal volumes of Exosomes (10ul) were run on 10% SDS-PAGE gels and transferred to PVDF membranes. The antibodies included anti-CD63 (1:200, Santa Cruz Biotechnology), anti-CD13 (1:200, Santa Cruz Biotechnology), anti-CD14 (1:200, Santa Cruz Biotechnology), anti-Alix(1:200, Santa Cruz Biotechnology), anti-CD81 (1:500, Abcam), anti-GM130 (1:200, Santa Cruz Biotechnology), anti-Albumin (1:5000, proteintech), anti-TMEM119 (1:500, ABclonal), anti-MCT1 (1:500, Bioss), anti-IL-1β (1:1000, Abcam), anti-CD-11b (1:1000, Abcam) and anti-β-tubulin (1:1000, Cell Signaling Technology). Membranes were incubated with anti-HRP secondary

antibodies (1:5000, Bioworld) and detected with use of an ECL kit (Thermo Scientific, MA). Protein band intensities were quantified with use of Image-J software and normalized to CD63.

Exosomes were analyzed using electron microscopy as described previously with slight modifications[10,15]. In short, a drop of freshly purified exosomes were deposited onto a paraffin membrane and covered with a 200-mesh copper mesh for 20 min. After washing with PBS, the copper mesh was fixed in 1% glutaraldehyde for 5 minutes and positively stained with uranyl acetate for 5 min, followed by methyl cellulose-uranyl acetate for 10 min. Remove excess liquid gently by using an absorbing paper and let it air dry for 5 min. Images of exosomes were obtained using a JEM-1011 electron microscope (JEOL, Japan) at an accelerating voltage of 80 kV.

Exosomes were re-suspended within 1 ml of filtered PBS and stored on ice. Exosome sizes were determined using a ZETASIZER Nano series-Nano-ZS (Malvern, England). The exosomal markers, CD63 and CD81, were analyzed using antibodies (CD63-Antibody-FITC BD 557288, CD81-Antibody-PE BD 555676) with a BD Accuri C6 flow cytometer.

**Exosome labeling**

Freshly isolated exosomes were labeled using the PKH67 Green Fluorescent Cell Linker Kit (Sigma-Aldrich, USA) with minor modifications of the manufacturers' instructions. Exosomes were re-suspended in 100ul Sterile PBS and added to 300ul Diluent C. This mixture was then added to 4ul of PKH67 and incubated for 4 min in

the dark. Finally, 300ul of 1% BSA solution was added to stop the reaction, followed by filtration through a 0.22-μm filter to remove excess dye. The labeled exosomes were purified using the SBI, EXOQ5A-1 kit, and the exosome pellet was diluted in 20ul PBS for use in exosome uptake experiments. Rats were injected bilaterally with 2.0 - 2.5μl of purified and concentrated exosomes and brain sections were collected 4 h after exosome injection.

**Exosome miRNA sequencing**

Exosome miRNA sequencing determinations were performed by the RiboBio Biotechnology Company (Guangzhou, China). Exosomal RNA was isolated by using the HiPure Liquid miRNA Kit/HiPure Serum/Plasma miRNA Kit (Megan, China). The Qubit®2.0 (Life Technologies, USA) and Agilent 2200 TapeStation (Agilent Technologies, USA) were individually used to assess the quantity and integrity of Exosomal RNA yield. Exosomal RNA of each sample (50ng) was used to prepare small RNA libraries by NEBNext® Multiplex Small RNA Library Prep Set for Illumina (NEB, USA) according to the manufacturers' instructions. The libraries were sequenced using HiSeq 2500 (Illumina, USA) with a single-end 50 bp.

**Tissue transcriptome sequencing**

Total RNA was isolated from tissues using the Trizol kit (Invitrogen) according to the manufacturers' instructions. RNA integrity (RIN) was accessed with use of Agilent 2200 TapeStation (Agilent Technologies, USA). Purified library products were

evaluated using the Agilent 2200 TapeStation and Qubit®2.0 (Life Technologies, USA) and then sequenced using the HiSeq 2500 and HiSeq 3000 (Illumina, USA) platform at the RiboBio Company (Guangzhou, China).

**Dual luciferase assay**

We designed a dual luciferase assay to assess the binding of miR-146a-5p to its target gene, KLF4. The experimental plasmid was constructed by OBIO Technology (Shanghai). Briefly, after 48 hours of transfection, the 293Tcells were collected and assayed for luciferase activity using an Enzyme standard instrument (Spark 10M, TECAN). The results were normalized to the ratio between firefly activity and renilla luciferase activity. Results were analyzed from three independent experiments and each experiment was performed in sextuplicate.

**BrdU Injection and Immunofluorescence Staining**

BrdU (5-bromo-2'-deoxyuridine, Sigma) was administered via an intraperitoneal injection (50kmg/kg) for five days at both the 2th week after virus injection, twice every day at 8 h intervals. BrdU was dissolved in normal saline (0.9%, pH7.4) and maintained in the dark to protect it from light. Coronal sections of the hippocampus (30 μm) were collected from each brain. For BrdU staining, tissue sections were pretreated with 1M HCl for 30 min at 45℃, then washed 3 times with PBS before primary antibody incubation. Primary antibodies were anti-BrdU (1:100, Bioworld Technology), anti-doublecortin (anti-DCX) (1:200, Cell Signaling Technology) and

anti-NeuN (1:500, Cell Signaling Technology). Secondary antibodies were Alexa Fluor 488 or 546 conjugated (1:500, Abcam). Fluorescence images were acquired with use of confocal microscopy (LSM880; Zeiss).

**Immunofluorescence**

Rats were anesthetized with sodium phenobarbital (30 mg/kg) using an intraperitoneal injection and perfused with 4% PFA through the heart. Brains were removed and fixed in 4% PFA overnight, then transferred to 30% sucrose solution for two days. Frozen coronal sections of the hippocampus were cut with a thickness of 30um and stored at −20 °C. The sections were incubated overnight at 4 °C with primary antibodies. Primary antibodies used were anti-Sox2 (1:100; Bioworld), anti-doublecortin (anti-DCX) (1:200, Cell Signaling Technology), anti-Nestin (1:20, Thermo Fisher) and anti-NeuN (1:1000, Abcam). Secondary antibodies were alexa-405 (1:1000, Abcam) and alexa-568 (1:1000, Invitrogen). Fluorescence images were obtained with use of confocal microscopy (LSM880; Zeiss) and processed by ZEN software.

For immunofluorescence staining of cells, the cells were fixed in 4%PFA for 15min, followed by treatment with 3%BSA for 1h at room temperature. They were then incubated overnight at 4℃ with primary antibodies, and then incubated with secondary antibodies for 1 h at room temperature after 24h. Fluorescence images were acquired with use of confocal microscopy (Dragonfly 200; Andor).

**Western blotting**

Protein was isolated from rat tissue by homogenization with lysis buffer containing protease and phosphatase inhibitors. Protein samples (20ug) were fractionated on SDS-PAGE gels by electrophoresis and then transferred onto PVDF membranes. Membranes were incubated with 5% non-fat milk for 1 h at room temperature and overnight with primary antibodies at 4℃. Primary antibodies were anti-CDKL5 (1:500, Abcam), anti-KLF4 (1:1000, Abcam) and anti-GAPDH (1:4000, Proteintech). Secondary antibodies were anti-HRP antibodies (1:5000, Bioworld). Bands on the membranes were detected with use of the Enhanced Chemiluminescence kit (ECL, Thermo Fisher) and quantified using Image-J software. The primary antibody GAPDH was used as a loading control.

**RNA preparation and Quantitative real-time PCR**

Total RNA of exosomes, CSF, cells and tissues were isolated using the miRNeasy Micro Kit (QIAGEN 217084) according to the manufacturer's protocol. RNA purity and concentration were determined with use of a Nano Drop ND-1000 spectrophotometer (Nano Drop Thermo, Wilmington, DE). Expression levels of miRNA were determined with use of quantitative real-time RT-PCR. The All-in-One$^{TM}$ miRNA qRT-PCR Detection Kit (GeneCopoeia, QP018, USA), the HiSoript II 1$^{st}$ Strand cDNA Synthesis Kit (Vazyme, Nanjing) and ChamQ SYBR Qpcr Master Mix (Vazyme, Nanjing) were used to quantify expression levels of circRNA by the Bio-Rad IQ5 Real Time PCR System. Bio-rad's IQ5 Software was used to analyze the PCR reaction data and the sequences of primers are listed in Supplementary Table 3.

**Hippocampus Slices Preparation and Electrophysiological Recordings**

Slices were cutting in cutting solution (pH 7.40) containing (in mM) 119 choline chloride, 30 Glucose, 26 NaHCO3, 7 MgSO4, 2.5 KCl, 1 NaH2PO4, 1 CaCl2, 3 sodiumpyruvate, 1.3 sodium L-ascorbate and 1 kynurenicacid. Then, slices were transferred as quickly as possible to a recovery solution containing. (in mM) 85 NaCl, 24 NaHCO3, 4 MgCl2, 2.5 KCl, 1.25 NaH2PO4, 0.5 CaCl2, 25 glucoses and 50 sucrose to recover for 30 min at 30 ℃. The glass micropipettes (4–6 MΩ) were filled with an internal solution containing (in mM) 130 CsMeSO4, 10 CsCl, 4 NaCl, 1 MgCl2, 5 MgATP, 5 EGTA, 10 HEPES, 0.5 Na3GTP, 10 phosphocreatine and 4 QX-314, with a pH of 7.35. During recordings, slices were continuously perfused with an artificial cerebral spinal fluid (ACSF) contained (in mM) 120 NaCl, 3.5 KCl, 2.5 CaCl2, 1.3 MgSO4, 1.25 NaH2PO4, 26 NaHCO3 and 10 glucoses.

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