Supplementary Information

Prophylactic treatment of Curcumin in a rat model of depression by attenuating hippocampal synaptic loss

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Supplementary Methods

Electrophysiology

Rats were deeply anesthetized with 4% pentobarbital, the brains were quickly removed and cut into 300-μm-thick coronal slices with a vibrating microtome (Leica) while being maintained in an oxygenated chilled cutting solution consisting of 119 mM choline chloride, 30 mM Glucose, 26 mM NaHCO3, 7 mM MgSO4, 2.5 mM KCl, 1 mM NaH2PO4, 1 mM CaCl2, 3 mM sodiumpyruvate, 1.3 mM sodium L-ascorbate and 1 mM kynurenicacid at pH 7.40. Hippocampus slices were transferred as quickly as possible to a recovery solution consisting of 85 mM NaCl, 24 mM NaHCO3, 4 mM MgCl2, 2.5 mM KCl, 1.25 mM NaH2PO4, 0.5 mM CaCl2, 25 mM glucose and 50 mM sucrose at 32°C for a minimum of one hour before recording. Whole-cell patch-clamp recordings were used to record spontaneous excitatory postsynaptic currents (sEPSCs), Miniature excitatory post-synaptic currents (mEPSCs) and spontaneous burst activity in hippocampal CA1 neurons. The glass micropipette (3-6 m ω) was filled with an inner solution consisting of 130 mM CsMeSO4, 10 mM CsCl, 4 mM NaCl, 1 mM MgCl2, 5 mM MgATP, 5 mM EGTA, 10 mM HEPES, 0.5 mM Na3GTP, 10 mM phosphocreatine and 4 mM QX-314. During recordings, the slices were continuously perfused with an artificial cerebrospinal fluid (ACSF) consisting of 120 mM NaCl, 3.5 mM KCl, 2.5 mM CaCl2, 1.3 mM MgSO4, 1.25 mM NaH2PO4, 26 mM NaHCO3 and 10 mM glucose at a temperature of 31-33°C and a flow rate of ~ 2ml/min. For sEPSC recordings, neurons were clamped at -70 mV in the presence of AP-5 (50 mM) and picrotoxin (50 mM) in the ACSF. During recordings of mEPSCs, tetrodotoxin (1 μM)

was added to the standard extracellular solution to block spontaneous action potentials. Results were analyzed using a Mini Analysis Program and all the chemicals used in these electrophysiological recordings were purchased from Sigma.

Golgi Staining

Brains were collected and processed for Golgi staining according to the protocol provided with the FD Rapid GolgiStain™ Kit (PK401, FD Neuro-Technologies, USA). The brains were cut into coronal slices (100μm) using a vibratome (VT 1000S, Leica), and transferred to gelatin-coated microscope slides, dehydrated in absolute alcohol, cleared in xylene and cover slipped for light microscopic observation. Stained pyramidal neurons from the hippocampal CA1 region were examined and captured using Nikon microscopy (Japan). Images at ×100 magnification were used for dendritic spine analysis. The density of dendritic spines was calculated as the number of dendritic spines per 10μm of dendrite length. All image processing was performed using Fiji (Image J, NIH)

Reverse transcription PCR and real-time quantitative PCR

Total RNA was extracted from hippocampal CA1 tissue with use of the RNA rapid extraction kit (Axygen, USA) according to the manufacturer's protocol. The primeScript RT reagent kit (TaKaRa, Japan) was then used to reverse-transcribe this RNA into cDNA, which was subsequently amplified by PCR using specific primers (Table S1). PCR products were assessed using electrophoresis on a 3% agarose gel and

images were obtained using the Gel Image Analysis System (Bio-rad, USA). All bands were analyzed with use of ImageJ software and normalized to GAPDH.

Total RNA from exosomes and CA1 tissue were isolated using the miRNeasy Micro Kit (QIAGEN 217084) and q-PCR for detection of mature miR-146a-5p was performed with two replicates using the All-in-OneTM miRNA qRT-PCR Detection Kit (GeneCopoeia, QP018, USA) according to the manufacturers' instructions. Real-time PCR was performed with use of the Bio-Rad IQ5 Real Time PCR System (Bio-Rad, USA). The PCR conditions consisted of: 95 ℃ × 10 min, followed by 40 cycles of 95 ℃ × 10 s, 55 ℃ × 20 s and 72 ℃ × 15 s. The relative fold change in expression of miR-146a-5p was determined using the 2− (ΔΔCt) method of the Bio-Rad IQ5 Software (Bio-Rad, USA).

Immunofluorescent staining

For immunohistochemistry, rats were deeply anesthetized using 4% pentobarbital. The brains were collected after transcardial perfusion with 0.9% NaCl followed by 4% paraformaldehyde (PFA), and then post-fixed overnight in 4% PFA at 4 °C. PFA-fixed tissues were transferred to 30% sucrose for 3 days and frozen serial coronal sections (40 μm) were cut for immunofluorescent staining. Sections were washed in PBS and embedded in a blocking solution (5% BSA and 0.2% Triton X-100 in PBS) for 1 h. Primary antibodies were incubated overnight at 4 ℃ and secondary antibodies for 1h at room temperature. All slices were counterstained with 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI) (1:1000, Thermo Fisher Scientific, USA) during

the final incubation step. The antibodies used were rabbit anti-8-OHdG (1: 400, Abcam, Cambridge, UK), rabbit anti-active caspase-3 (1: 300, Abcam, USA), mouse anti-NeuN (1: 400, Cell Signaling Technology, USA), rabbit anti-ionized calcium binding adaptor molecule-1 (Iba-1) (1: 500, WAKO, Japan), Alexa Fluor594 donkey anti-rabbit IgG (1:1000, Invitrogen) and Alexa Fluor488-conjugated goat anti-mouse (1: 200, ProteinTech, USA). MitoSOX Red fluorescent dye (10 μM, Abcam, USA) was used to examine mitochondrial ROS levels as performed at room temperature in the dark for 15min. Hoechst 33258 (C0031) was purchased from Solarbio (Beijing, China) and stained for 5 min. All images were acquired using a Zeiss confocal microscope (LSM 880, Germany) or Andor microscopy (Dragonfly 200, UK) and analyzed using ImageJ software.

Western blotting

For western blot, hippocampal CA1 tissues were homogenized in RIPA buffer with a protease and phosphatase inhibitor. Protein (20 μg) within each lane was separated using a 10% SDS–PAGE gel and transferred for western blot analysis. Rabbit anti-phospho-ERK (1: 1000, Cell Signaling Technology, USA), rabbit anti-PSD-95 (1: 1000, Cell Signaling Technology, USA), rabbit anti-Syn (1: 1000, Cell Signaling Technology, USA), rabbit anti-Syt1 (1: 1000, Cell Signaling Technology, USA), mouse anti-Nlg1 (1:1000, Abcam), anti-CD63 (1:200, Santa Cruz Biotechnology), anti-CD81 (1:500, Abcam), rabbit anti-CD11b (1:1000, Abcam), rabbit anti-CD45 (1:1000, Abcam), rabbit anti-Nrf2 (1: 1000, ProteinTech, USA), rabbit anti- HO-1(1: 1000, Cell

Signaling Technology, USA), rabbit anti- COX-2(1: 1000, Cell Signaling Technology, USA), rabbit anti-GAPDH (1: 3000, ProteinTech, USA), rabbit anti- Bcl-2(1: 1000, Cell Signaling Technology, USA), rabbit anti- Bax(1: 1000, Cell Signaling Technology, USA), rabbit anti- PARP(1: 1000, Cell Signaling Technology, USA), mouse anti-β-tubulin (1: 3000, Santa Cruz Biotechnology, USA), mouse anti-β-actin (1: 3000, Santa Cruz Biotechnology, USA) and highly sensitive ECL reagent (GE Healthcare, UK) were all used as antibodies. All bands were quantified with use of Image J.
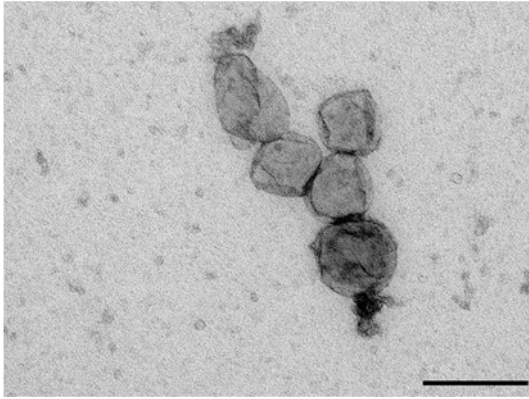
Figure S1
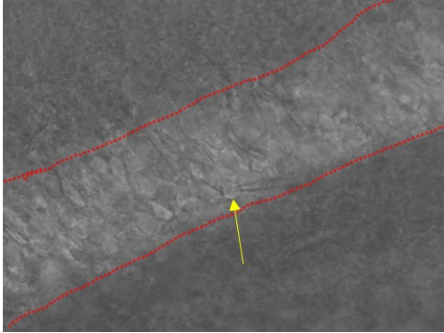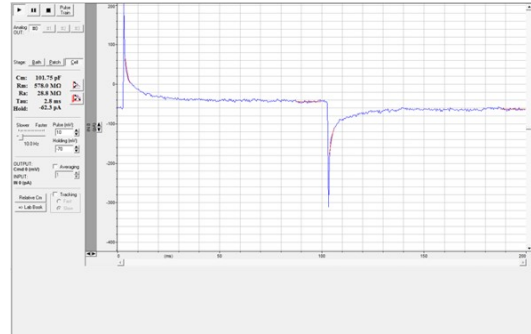
Figure S1 Representative images of electrophysiological recordings. A. Representative microscopic images showing the CA1 neurons of electrophysiological recordings in rats. B. Representative images showing the activities of CA1 neurons in electrophysiological recordings of rats.
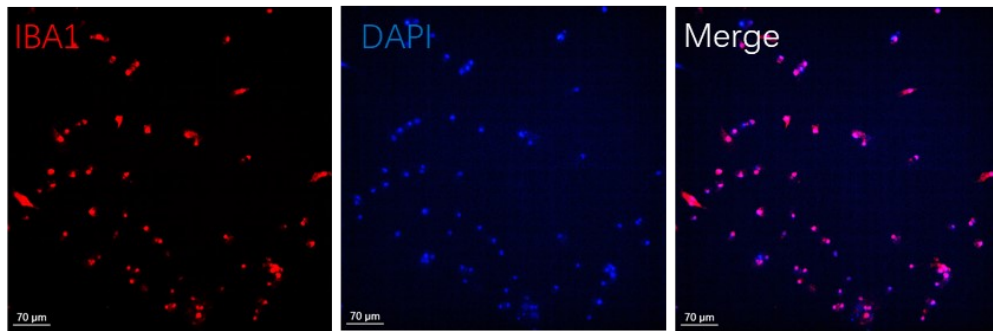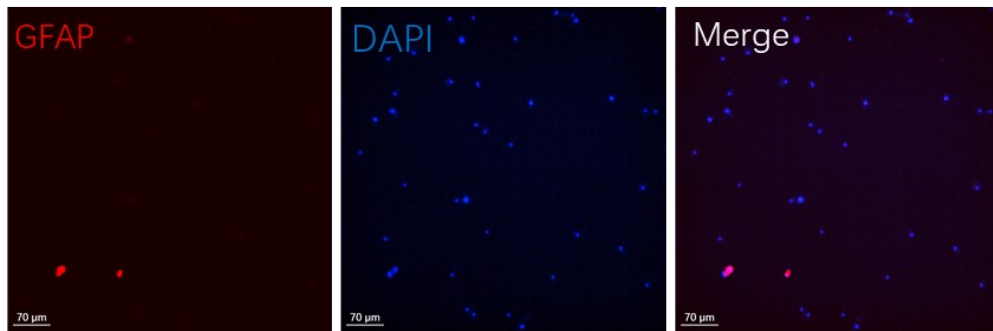
Figure S2

A

Figure S2 Identification of exosomes. Representative electron micrograph images of exosomes. Scale bar: 500nm. N=6/group.
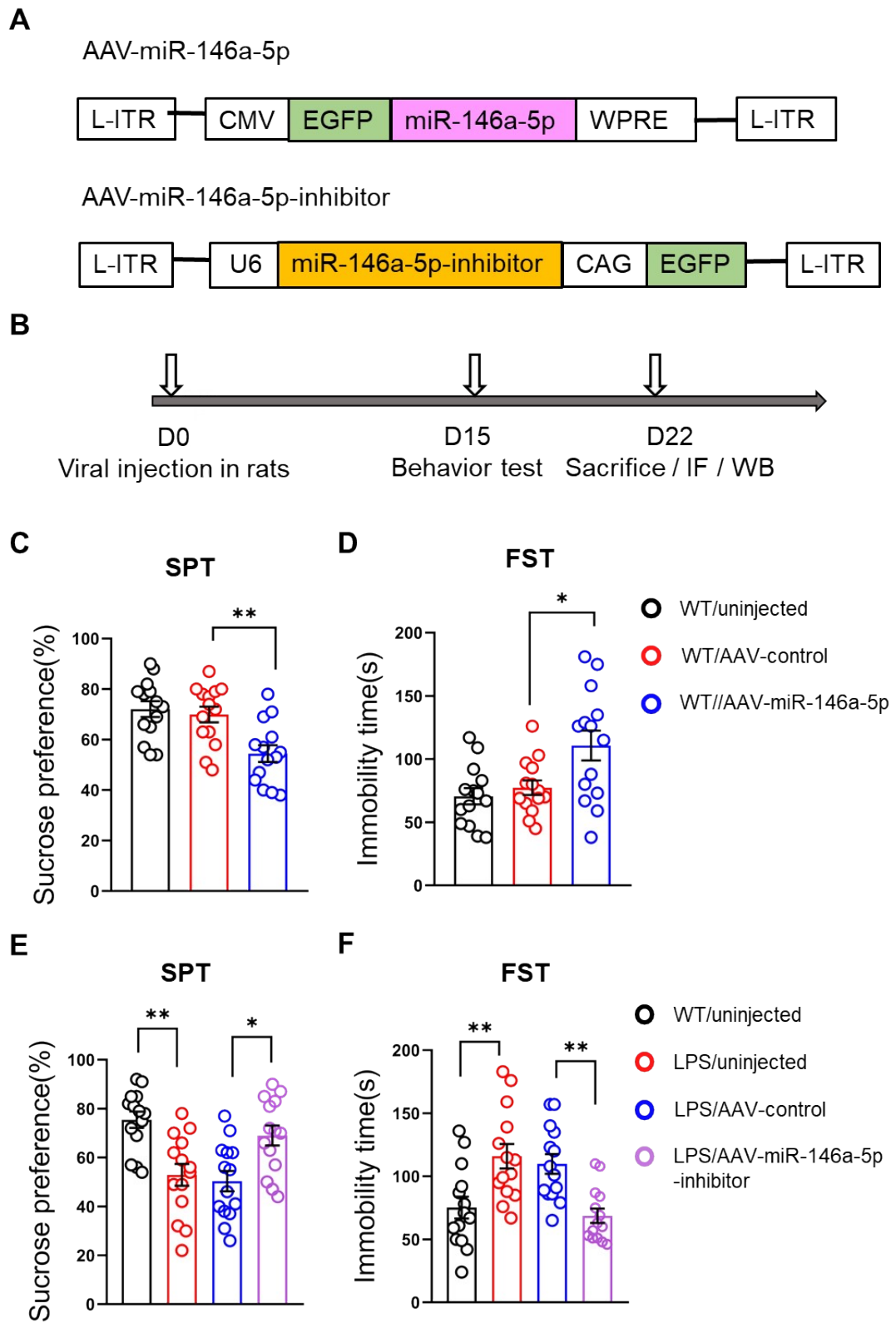
A

B

Figure S3

Figure S3. Identification of primary cultured microglia. A. Representative confocal microscopic images showing the expression of microglia in rats. Scale bar: 70um.B. Representative confocal microscopic images showing the expression of astroglia in rats. Scale bar: 70um.

Figure S4

A

AAV-miR-146a-5p

| L-ITR |—| CMV | EGFP | miR-146a-5p | WPRE |—| L-ITR |

AAV-miR-146a-5p-inhibitor

| L-ITR |—| U6 | miR-146a-5p-inhibitor | CAG | EGFP |—| L-ITR |

B

D0
Viral injection in rats

D15
Behavior test

D22
Sacrifice / IF / WB

C SPT

D FST

○ WT/uninjected
○ WT/AAV-control
○ WT//AAV-miR-146a-5p

E SPT

F FST

○ WT/uninjected
○ LPS/uninjected
○ LPS/AAV-control
○ LPS/AAV-miR-146a-5p
-inhibitor

Figure S4. Estimation of viral constructs and Stereotactic injection in hippocampal CA1. A. Diagram of the AAV constructs for the miR-146a-5p-inhibitor or miR-135a-5p. B. Estimation of expression level of viral constructs through Stereotactic injection. C-D. Overexpression of miR-146a-5p in WT rats prevented the decreases in sucrose consumption in the SPT(C) and reversed the increases in immobility times in the FST(D). E-F. Knockdown of miR-146a-5p in LPS rats prevented the decreases in sucrose consumption in the SPT(E) and reversed the increases in immobility times in the FST(F). N=14/group (C-F). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. one-way ANOVA with Bonferroni post-hoc analysis.

Table S1. PCR primers used in this study

Gene	Forword (5'→3')	Reverse (5'→3')
IL-1β	AAG ATG AAG GGC TGC TTC CAA ACC	ATA CTG CCT GCC TGA AGC TCT TG
IFN-γ	ATT CAT GAG CAT CGC CAA GTT C	TGA CAG CTG GTG AAT CAC TCT GA
TNF-α	TGA TCG GTC CCA ACA AGG A	TGC TTG GTG GTT TGC TAC GA
Caspase3	GGA GCT TGG AAC GCG AAG AA	ACA CAA GCC CAT TTC AGG GT
Bax	TCT TCA AAC TGC TGG GCC ATT	CTT GTC ACC TGC CTG ACT GC
GAPDH	AGT GCC AGC CTC GTC TCA TA	GGT AAC CAG GCG TCC GAT AC