

## **Supplemental Digital Content 2**

### **Materials and Methods**

#### **Western blot analysis**

At 6 h, 24 h, 3 d and 7 d after reperfusion, the mice (n=5 per time point per group) were deeply anesthetized with 10% chloral hydrate (350 mg/kg) and the ischemic penumbras were microdissected according to established protocols in rodent models of unilateral proximal MCAO. The tissue was homogenized on ice in RIPA lysis buffer (Beyotime, China) with 1 mM phenylmethylsulfonyl fluoride, and Western blotting was performed in accordance with the standard protocol. The following primary antibodies were used: an anti-TREM2 rabbit antibody (1:200 dilution, Santa Cruz Biotechnology, USA), an anti-iNOS rabbit antibody (1:500 dilution, Abcam, USA), an anti-liver arginase rabbit antibody (1:1000 dilution, Abcam, USA), an anti-IL-6R rabbit antibody (1:200 dilution, Santa Cruz Biotechnology, USA), an anti-BDNF rabbit antibody (1:500 dilution, Santa Cruz Biotechnology, USA), and an anti-tubulin rabbit antibody (1:2000 dilution, Beyotime, China). Appropriate horseradish peroxidase-conjugated goat anti-rabbit and rabbit anti-goat secondary antibodies (1:20,000 dilution, Beyotime, China) were also used. Semi-quantitative analysis of the blots was performed via densitometry followed by quantification using NIH Image software (NIH Image version 1.61). Each sample was subjected to immunoblotting three times, and the final optical density value (relative to that for the internal standard) represents the average of these three separate analyses.

## **Immunohistochemistry**

At 6 h, 3 d, and 7 d after reperfusion, the mice (n=3 for each group) were deeply anesthetized and transcardially perfused with normal saline and 4% paraformaldehyde. To ensure that homologous areas of injury were sampled between animals, parallel sets of sections from -3.0 to -5.0 mm from Bregma (covering the infarct area) were collected. After three washes in phosphate-buffered saline (PBS), the 13- $\mu$ m-thick coronal sections were permeabilized and blocked for 60 min with 0.1% Triton X-100, 5% goat serum, and 1% bovine serum albumin (BSA) in PBS. After the coronal sections were washed with PBS, they were incubated for 24 h at 4°C with the following primary antibodies: an anti-Arg-1 rabbit polyclonal antibody (1:50 dilution, Abcam, USA), an anti-TREM2 rabbit antibody (1:50 dilution, Santa Cruz Biotechnology, USA) and an anti-OX42 mouse monoclonal antibody (1:50 dilution, Santa Cruz Biotechnology, USA). After the sections were washed three times with PBS, they were incubated with FITC-labeled goat anti-mouse IgG (1:100 dilution, Molecular Probes Inc., Life Technologies) for 1 h and then incubated with Alexa Fluor 594-labeled goat anti-rabbit IgG (1:500 dilution, Invitrogen) for 2 h at room temperature in the dark. Sections incubated without primary or secondary antibodies served as negative controls. After the sections were washed with PBS, fluorescence images of the cells were obtained via confocal microscopy.

## **Transfection of siRNA into the mouse brain**

Briefly, under chloral hydrate anesthesia (350 mg/kg), a stainless steel cannula was stereotaxically implanted unilaterally in the cerebral ventricle. The stereotaxic coordinates were 0.4 mm posterior and 1.0 mm lateral to bregma and 2.5 mm below the surface of the

skull. According to the manufacturer's instructions, 5  $\mu$ l of TREM2-siRNA (QIAGEN, Valencia, CA) or control-siRNA (QIAGEN, Valencia, CA) duplex was diluted into 100  $\mu$ l siRNA Transfection Medium, and the siRNA duplex solution was added directly into the diluted HiPerFect Transfection Reagent using a pipette. Then, the solutions were mixed gently by pipetting up and down, and the mixture was incubated for 15-45 minutes at room temperature. Then, 2  $\mu$ l of the diluted mixture was stereotaxically delivered into the lateral ventricle ipsilateral to the MCAO. After the mice had recovered from the anesthesia, they were returned to their cages and given ad libitum access to food and water.

### **Neurobehavioral evaluation and infarct measurement**

At 3 d after reperfusion, a 5-point scoring system modified from Longa was used by a blinded observer to assess neurological deficiency. The rating scale was as follows: 0=no deficit; 1=failure to extend left forepaw; 2=decreased grip strength of left forepaw; 3=circling to left by pulling the tail; and 4=spontaneous circling. Then, the mice were deeply anesthetized, the brains were removed, and the infarct volume was measured according to the protocol described previously (n=8). The brain sections were stained with a standard 2% 2,3,5-triphenyltetrazolium chloride solution (Sigma-Aldrich, St. Louis, MO) for 10 min at 37°C, followed by overnight immersion in 4% paraformaldehyde. The stained sections were photographed with a digital camera (Canon G11, Canon Inc., Japan). Unstained areas were defined as the infarct and were measured using image analysis software (Adobe Photoshop 8.0 CS for Windows) by an investigator blinded to the experimental grouping. Infarct volume was quantified as follows: relative infarct volume=(contralateral area-ipsilateral non-infarct

area)/contralateral area.

### **TUNEL staining**

Three days after reperfusion, neuronal apoptosis in the ischemic penumbra was assessed in situ by terminal deoxynucleotide transferase-mediated dUTP nick-end labeling (TUNEL) staining as described in our previous studies (n=7 for each group). Briefly, regions of interest were observed via light microscopy at 100x magnification, and the total numbers of positively stained cells in these regions were counted and are expressed as cells per mm<sup>2</sup>.

### **Primary culture of microglia**

Using previously reported methods, mixed glia were prepared from the whole brains of postnatal day 1 C57BL/6 mice. Briefly, the meninges were removed, and the cells were dissociated by 8 min of enzymatic digestion with 0.25% trypsin. The mixed glia were then plated on poly-L-lysine-coated 24-well plates at a density of  $1 \times 10^5$  cells per well in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS) and incubated at 37°C. The cells were fed every 3-4 d with fresh DMEM/F-12 with 10% FBS. After 15–18 d, microglia were isolated from the mixed glial cultures by using a rocking device and were allowed to rest for 24 h prior to treatment. As determined via immunocytochemistry, the purity of the microglia in culture was 95%.

### **Co-culturing HT22 cells and N9 cells using the transwell system**

Three experimental systems were used: (1) a transwell system (Corning, China), which

enables contact-independent communication through diffusible factors; (2) a conditioned medium transfer system; and (3) a co-culture system that allows cell-cell communication via direct microglial factors. N9 cells were seeded on transwell inserts, and HT22 cells were cultured in 6-well culture plates in the lower compartment. To evaluate the effects of the HT22 cells on the survival of the N9 cells, we divided the cells into three groups: 1) control-vector group: N9 cells transformed with the control recombinant lentivirus were seeded on transwell inserts for 3 d. 2) TREM2-vector group: N9 cells transformed with recombinant lentivirus containing the gene encoding full-length mouse TREM2 were seeded on transwell inserts for 3 d. 3) TREM2-siRNA group: N9 cells transformed with TREM2-siRNA were seeded on transwell inserts for 3 d. Then, microglia and the HT22 cells were co-cultured for 24 h.

#### **Determination of apoptotic rate by flow cytometry**

N9 cells were seeded on transwell inserts, and HT22 cells were cultured in 24-well culture plates in the lower compartment. The N9 cells were transfected with lentivirus for 72 h, the HT22 cells were treated with OGD for 4 h, and then the cells were co-cultured for 24 h in an incubator. The HT22 cells were centrifuged at 1000 rpm for 5 min. After the cells were washed twice with ice-cold PBS, they were resuspended in binding buffer at a density of  $1 \times 10^6$  cells/ml. Then, 5  $\mu$ l of a fluorescein 5-isothiocyanate [2-(3,6-dihydroxy-9H-xanthen-9-yl)-5-isothiocyanatobenzoic acid; FITC]-conjugated anti-annexin-V antibody and 2  $\mu$ l of propidium iodide (PI) solution were added to 100  $\mu$ l of binding buffer. After thorough mixing and a 15-min incubation at room temperature in the

dark, the apoptotic rate (n=5) was assessed via flow cytometry (BD, USA).

### **Cell proliferation and cytotoxicity assay**

N9 cells were plated at a density of  $3 \times 10^4$  cells/well in 96-well plates and pre-incubated for 24 h in a humidified incubator (37°C, 5% CO<sub>2</sub>). At 72 h after the cells were transfected with lentivirus, we subjected the microglia to OGD for 4 h. Cell injury was then evaluated via the CCK-8 assay after 24 h (n=8). CCK-8 solution (10 µl) was added to each well of the plate, which was then incubated in the incubator for 4 h. Finally, the absorbance was measured at 450 nm using a microplate reader.

### **Lactate dehydrogenase (LDH) release**

N9 microglia were plated at a density of  $2 \times 10^4$  cells/well in 24-well plates. After the treatments, the supernatant of each well (n=6) was removed to assess LDH release by using the LDH assay kit according to the manufacturer's instructions. In brief, 100 µl of cell-free supernatant, 250 µl of buffer, and 50 µl of coenzyme were mixed to form a homogeneous solution, and the supernatant was incubated with this reaction mixture for 15 min at 37°C. Next, 250 µl of 2,4-dinitrophenylhydrazine was added to the mixture, which was then incubated for an additional 15 min at 37°C in the dark. Finally, 2.5 ml of 400 mM NaOH was added to the mixture to stop the reaction. After 3 min, the absorbance of the mixture was determined at 440 nm by spectrophotometry. The absorbances of a sample blank, a standard, and a standard blank were measured at the same time.

### **Statistical analysis**

Brain sections were examined by two independent and blinded investigators. SPSS 13.0 for Windows (SPSS Inc., Chicago, IL) was used to conduct statistical analyses. All values, except for neurological scores, are presented as the mean±SD and were analyzed by one-way analysis of variance. Between-group differences were detected using Tukey's multiple comparisons tests. The neurological deficit scores are expressed as the median (interquartile range) and were analyzed using the Kruskal-Wallis test followed by the Mann-Whitney U test with Bonferroni correction. Sample size was estimated based on our previous experience.<sup>22,23</sup>  $P<0.05$  was considered statistically significant.