Supplemental Materials

Supplemental Methods:

Primary cultures of brain ECs, pericytes and astrocytes

Primary cultures of mouse brain ECs and pericytes were prepared from 6-8-week old C57BL/6J mice purchased from Vital River Laboratory Animal Technology Company (Beijing, China), as previously described[1]. ECs were isolated and cultured in endothelial cell medium (ScienCell, CA, USA) at 37 ℃ with a humidified atmosphere of 5% CO_2/95% air. The medium was changed every 3 days. When the aim was to culture pericytes, cells were maintained in pericyte medium (ScienCell, CA, USA).

Mouse cerebral astrocytes were obtained from postnatal day 1 mice. Meninges were removed and cortical pieces were dissociated in astrocyte culture medium (DMEM supplemented with 10% FBS). Dissociated cells were seeded into cell culture plates. Plates with confluent cultures were shaken at 37℃ overnight. First or second passages were used in the experiments.

Construction of in vitro BBB model

The *in vitro* BBB model was constructed according to the protocol described before[2]. Briefly, pericytes were seeded on the bottom side of the collagen-coated polyester membrane of the inserts (Millipore, MA, USA). The cells were let to adhere to the membrane surface for overnight, then ECs were seeded on luminal side of the inserts placed in the well of the 24-well culture plates containing astrocytes.

Monitoring of CBF

Regional CBF was monitored by laser Doppler flowmetry (PeriFlux System 5000; Perimed, Sweden)[3]. After 120 minutes MCAO, the monofilament was gently withdrawn in order to restore blood flow. Changes in CBF were recorded and presented as percent change compared to before monofilament insertion.

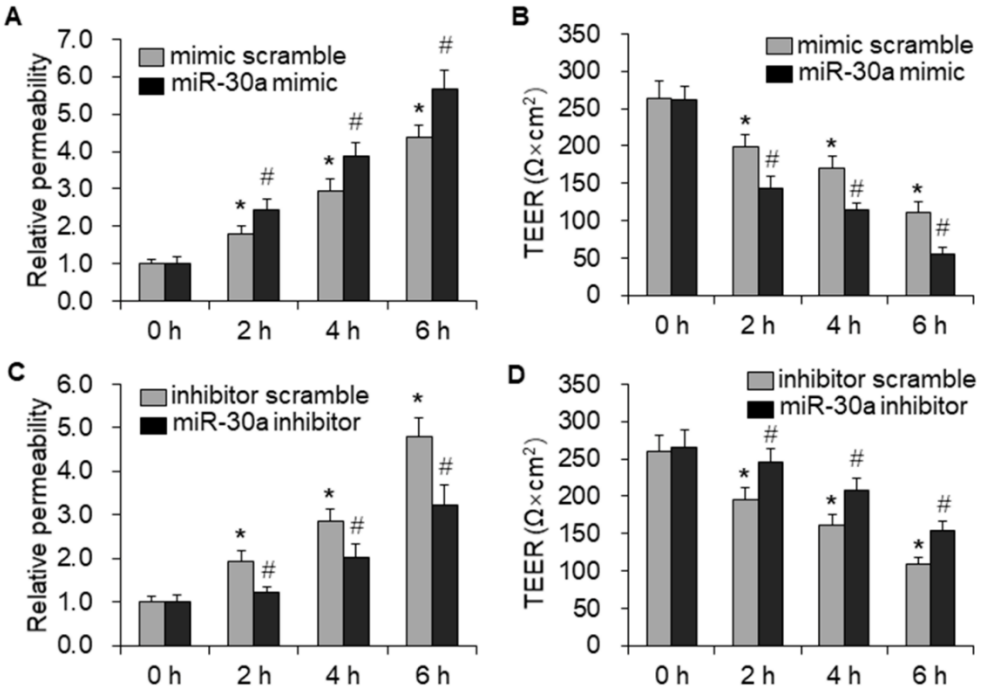
References

1. Tigges U, Welser-Alves JV, Boroujerdi A, et al. A novel and simple method for culturing pericytes from mouse brain. *Microvasc Res* 2012; 84(1): 74–80.
2. Nakagawa S, Deli MA, Kawaguchi H, et al. A new blood–brain barrier model using

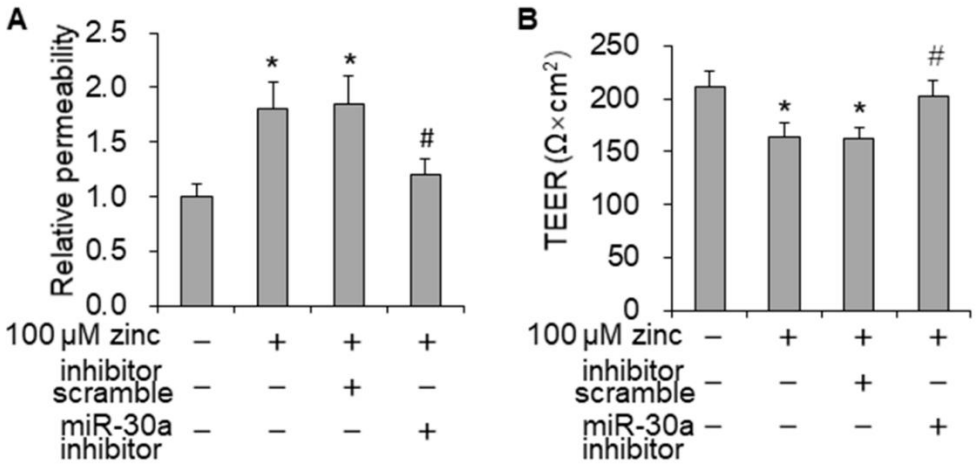
primary rat brain endothelial cells, pericytes and astrocytes. *Neurochem Int* 2009; 54: 253–263.

3. Esposito E, Mandeville ET, Hayakawa K, et al. Effects of normobaric oxygen on the progression of focal cerebral ischemia in rats. *Exp Neurol* 2013; 249: 33–38.

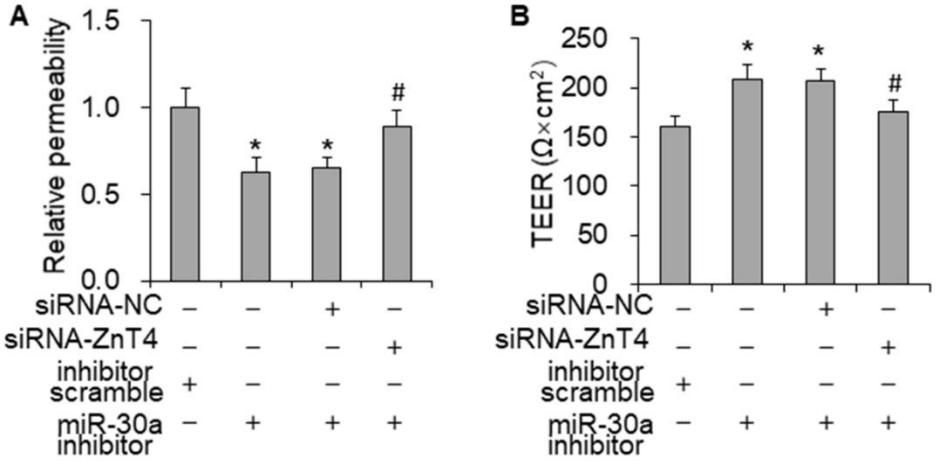
Supplemental Figures



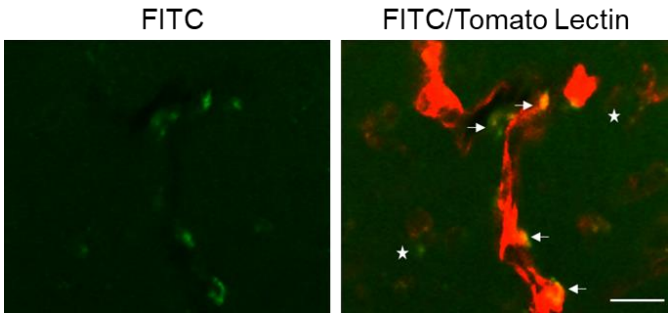
Supplementary Figure 1. Effects of miR-30a on the barrier function in BBB model *in vitro*. The primary cultures of ECs, pericytes and astrocytes were used to construct *in vitro* BBB model. (**A** and **C**) After the triple cell coculture model was treated with miR-30a mimic, miR-30a inhibitor or their controls, the integrity of the endothelial barrier was evaluated by quantifying the FITC-BSA permeability. (**B** and **D**) TEER was evaluated after miR-30a mimic or miR-30a inhibitor treatment. $^{*}P<0.05$ vs. 0 h mimic scramble or inhibitor scramble group, $^{\#}P<0.05$ vs. mimic scramble or inhibitor scramble at the same time after OGD, n=4/group.



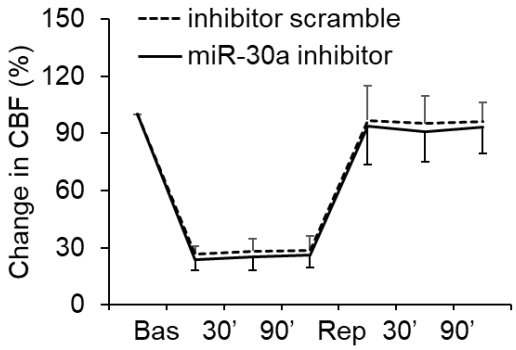
Supplementary Figure 2. Effect of miR-30a on zinc-mediated BBB disruption. The integrity of the endothelial barrier was evaluated by quantifying the FITC-BSA permeability (**A**) and TEER (**B**) after 2 h OGD. $^{*}P<0.05$ vs. OGD; $^{\#}P<0.05$ vs. OGD + zinc. n=4/group.



Supplementary Figure 3. ZnT4 plays an important role in miR-30a-mediated endothelial barrier integrity. (**A** and **B**) The barrier integrity of the triple cell coculture model increased after miR-30a inhibition, and this effect of miR-30a inhibitor was reversed by ZnT4 siRNA. $^{*}P<0.05$ vs. inhibitor scramble. $^{\#}P<0.05$ vs. miR-30a inhibitor. n=4/group.



Supplementary Figure 4. The neurovascular location of FITC-conjugated miR-30a inhibitor after intravenous administration. Distribution of injected FITC-conjugated miR-30a inhibitor was assessed at 72h after reperfusion. Fluorescence microscopy imaging detected FITC-positive oligonucleotides (green, arrows) associated with cerebral microvessels. Some FITC-positive oligonucleotides also crossed the BBB and were seen in the brain parenchyma (stars). Scale bar=20μm.



Supplementary Figure 5. CBF during ischemia/reperfusion. MiR-30a inhibitor had no significant effect. n=5/group.

Supplementary Table 1. Physiological parameters (Mean±SD)

Variables	inhibitor scramble	miR-30a inhibitor
Body weight (g)	302.6 ± 13.5	305.4 ± 12.1
BP (mm Hg)	107.4 ± 11.1	109.2 ± 12.4
pH	7.37 ± 0.09	7.39 ± 0.06
pCO2 (mm Hg)	38.6 ± 3.5	40.2 ± 5.2
pO2 (mm Hg)	107.2 ± 14.9	110.8 ± 17.8

Blood pressure (BP) was monitored through a femoral artery, blood gas was analyzed at 72 h after reperfusion. n=5/group. No significant differences were noted between the two groups.