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**Figure S1**. Confirmation of the pMCAO model. (**A to B**) The infarct volume was identified by TTC staining. The red part is the intact brain tissue and the white part is the infarction. (**C to D**) The establishment of pMCAO was further confirmed by the assessment of brain water content and neurological scores. Data are presented as mean±SEM from 4 rats in each group. \*\*\**p*<0.001, \*\**p*<0.01, \**p*<0.05 vs. sham group. (**E**) The time-dependent change of MAP2 levels from 3 h to 48 h following pMCAO. (**F**) Quantification of the MAP2 protein in the cortex extracts. Statistical comparisons were carried out with ANOVA followed by Tukey’s test. Data are presented as mean±SEM from 4 rats in each group. \*\*\**p*<0.001 vs. sham group. (**G**) Quantification of RBFOX3-positive cells shown in Fig. 3A. Data are presented as mean±SEM from 4 rats in each group. \*\*\**p*<0.001, \*\**p*<0.01 vs. sham group. (**H**) The time-dependent change of cell viability detected by MTT assay in primary neurons with OGD treatment. Data are presented as mean±SEM from 3 independent experiments. \*\*\**p*<0.001, \*\**p*<0.01 vs. CON group.

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**Figure S2**. Identification of the cell types in which LC3 and TFEB are expressed. (**A to C**) Immunofluorescence images showing the distribution of the autophagic marker LC3 in neurons and other brain cells in the ipsilateral cortex of sham-operated rats and pMCAO-treated rats after 24 h, as detected by laser confocal microscopy using antibodies against LC3 (red), RBFOX3 (green), GFAP (green) and AIF1 (green). DAPI is used to label the nuclei (blue). Scale bar: 20 μm. High-magnification images of the boxed areas are shown in the inserts; scale bar: 10 μm. (**D and E**) Immunofluorescence images showing the distribution of TFEB in microglia (AIF1 positive) or astrocytes (GFAP positive) in the ipsilateral cortex in sham-operated rats and pMCAO-treated rats, as detected by laser confocal microscopy using antibodies against TFEB (red) and AIF1 (green) or GFAP (green). DAPI is used to label the nuclei (blue). Scale bar: 20 μm. (**F and G**) The average optical density of red fluorescence in the confocal image, which represents TFEB expression. Data are presented as mean±SEM from 4 independent experiments. \*\*\**p*<0.001 vs. sham or CON group. (**H and I**) The mRNA level of *Tfeb* normalized to CON was detected from 3 h to 48 h after pMCAO and from 1 h to 24 h after OGD. Data are presented as mean±SEM from 4 independent experiments. \*\*\**p*<0.001 vs. CON group; \*\**p*<0.01 vs. sham group.

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**Figure S3**. Permanent ischemic insult results in the translocation of MiT/TFE3 family members to the nucleus. (**A**) The time-dependent changes of MITF, TFEC and TFE3 expression from 3 h to 48 h after pMCAO in nuclear and cytoplasmic cortex protein extracts. TUBB1/β-tubulin was used as a cytoplasm loading control. LMNB was used as a nuclear loading control. (**B to G**) Quantification of the immunoblotted proteins in the cortex extracts. Statistical comparisons were carried out with ANOVA followed by Tukey’s test. Data are presented as mean±SEM from 4 rats in each group. \*\**p*<0.01, \**p*<0.05 vs. sham group. (**H**) Knockdown of *Tfeb* has no effect on the other MiT/TFE3 family members as monitored by immunoblotting. (**I to K**) Quantification of the immunoblotted proteins in the cortex extracts. Statistical comparisons were carried out with ANOVA followed by Tukey’s test. Data are presented as mean±SEM from 4 rats in each group.

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**Figure S4**. The construction of AAV vectors for overexpressing and knocking down TFEB in the cortex of rats. (**A**) Top: the AAV-TFEB cassette with a neuron-specific promoter, *Syn1*. Bottom: delivery of the AAV-TFEB particles into cortex. Scale bar: 500 μm. (**B**) Top: the AAV-GFP-sh*Tfeb* cassette with a neuron-specific promotor, *Camk2a*. Bottom: delivery of the GFP-sh*Tfeb* particles into cortex. Scale bar: 500 μm. (**C**) Representative confocal images showing expression of TFEB (red) in Flag-positive neurons in the cortex of sham-operated or pMCAO-treated rats injected with GFP- or TFEB-expressing vectors. Scale bar: 20 μm. (**D**) Representative confocal images showing expression of TFEB (red) in GFP-positive neurons in the cortex of sham-operated or pMCAO-treated rats injected with GFP-Scramble or sh*Tfeb* vectors; scale bar: 20 μm. The boxed inserts are magnified images of TFEB-expressing neurons; scale bar: 10 μm. (**E and G**) Immunoblots of TFEB in nuclear extracts from cortex. LMNB was used as a loading control. (**F and H**) Quantification of the immunoblotted proteins in the cortex extracts. Statistical comparisons were carried out with ANOVA followed by Tukey’s test. Data are presented as mean±SEM from 4 rats in each group. \*\*\**p*<0.001, \**p*<0.05 vs. sham group. ###*p*<0.001 vs. pMCAO group.