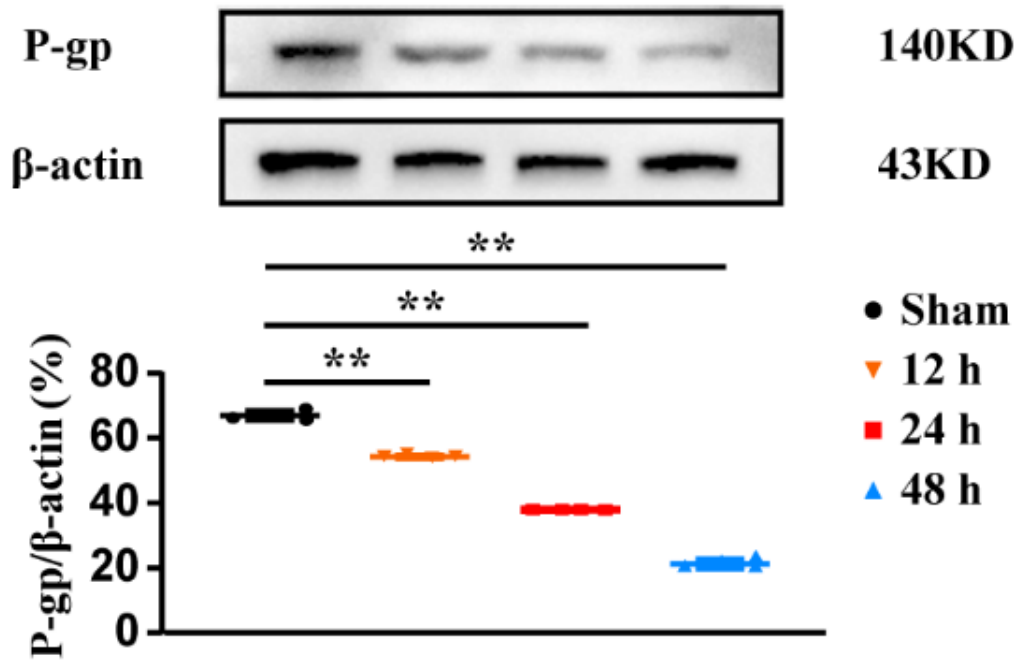


SUPPLEMENTARY DATA

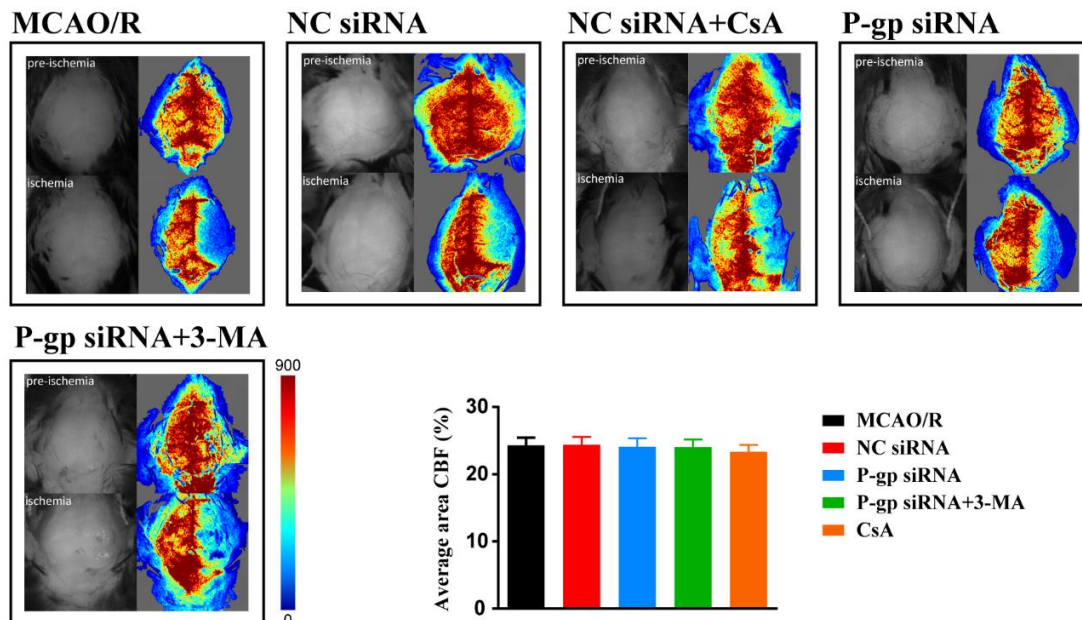
**P-Glycoprotein Aggravates Blood Brain Barrier  
Dysfunction in Experimental Ischemic Stroke by  
Inhibiting Endothelial Autophagy**

**Liangliang Huang<sup>1,#</sup>, Yan Chen<sup>1,#</sup>, Rui Liu<sup>1</sup>, Binbin Li<sup>1</sup>, Xuan Fei<sup>1</sup>, Xiang Li<sup>1</sup>, Ge Liu<sup>1</sup>, Yunman Li<sup>1\*</sup>, Baohui Xu<sup>2</sup>, Weirong Fang<sup>1\*</sup>**

## SUPPLEMENTARY DATA



**Supplementary Figure 1. Expressions of P-glycoprotein at 12, 24 or 48 h after intracerebroventricular injection.** Mice were intracerebroventricularly injected with P-glycoprotein (P-gp) or negative control (NC) siRNA (1.5  $\mu$ L/10 g body weight) prior to MCAO/R surgery. Twenty-four hours after the surgery, brains were harvested for Western-blotting assay. Western-blotting images and quantification of P-gp levels in brain cortex (n = 4). One-way ANOVA followed by the post hoc Tukey test. All data are mean  $\pm$  SD, \**P*<0.05, \*\**P*<0.01 between two groups.

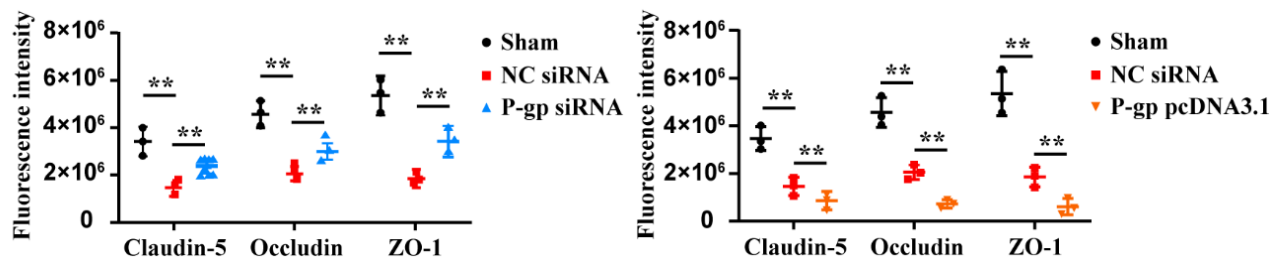


**Supplementary Figure 2. Middle cerebral artery occlusion surgery decreased the cerebral blood flow of mice.** Representative speckle contrast images and quantification for cerebral blood flow (CBF) before and after middle cerebral artery occlusion surgery in differentially treated mice (n = 6). Mann-Whitney test. All data are shown as mean  $\pm$  SD, *P*>0.05 between two groups.

# SUPPLEMENTARY DATA

**Supplementary Table 1.** The primers of the genes tested.

Target gene	Primer type	Sequence
Mouse TNF- $\alpha$	Forward	GCACCACCATCAAGGACTCA
	Reverse	TCGAGGCTCCAGTGAATTTCG
Mouse IL-1 $\beta$	Forward	GCCTCGTGCTGTCCGGACC
	Reverse	TGTCGTTGCTTGGTTCTCCTTG
Mouse MMP-2	Forward	TCTGGTGCTCCACCACATACT
	Reverse	CTGCATTGCCACCCATGGTAAACA
Mouse MMP-9	Forward	GCCCTGGAACCTCACACGACA
	Reverse	TTGGAAACTCACACGCCAGAAG
Mouse $\beta$ -actin	Forward	CATCCGTAAAGACCTTTGCCAAC
	Reverse	ATGGTGCCACCGATCCACA



**Supplementary Figure 3. P-glycoprotein affects tight junction proteins degradation following endothelial oxygen glucose deprivation/reperfusion.** Endothelial cells (bEnd.3) were transfected with P-gp or negative (NC) siRNA, P-gp or NC pcDNA3.1, or un-transfected, and then subjected to either oxygen glucose deprivation/reperfusion (OGD/R) treatment or normal culture conditions. Twenty-four hours thereafter, cells were harvested for immunofluorescence staining. Quantification of tight junction proteins in endothelial cells as representative images showing in figure 5E and 6E (n = 3). Mann–Whitney test. All data are shown as mean  $\pm$  SD, \* $P < 0.05$ , \*\* $P < 0.01$  between two groups.