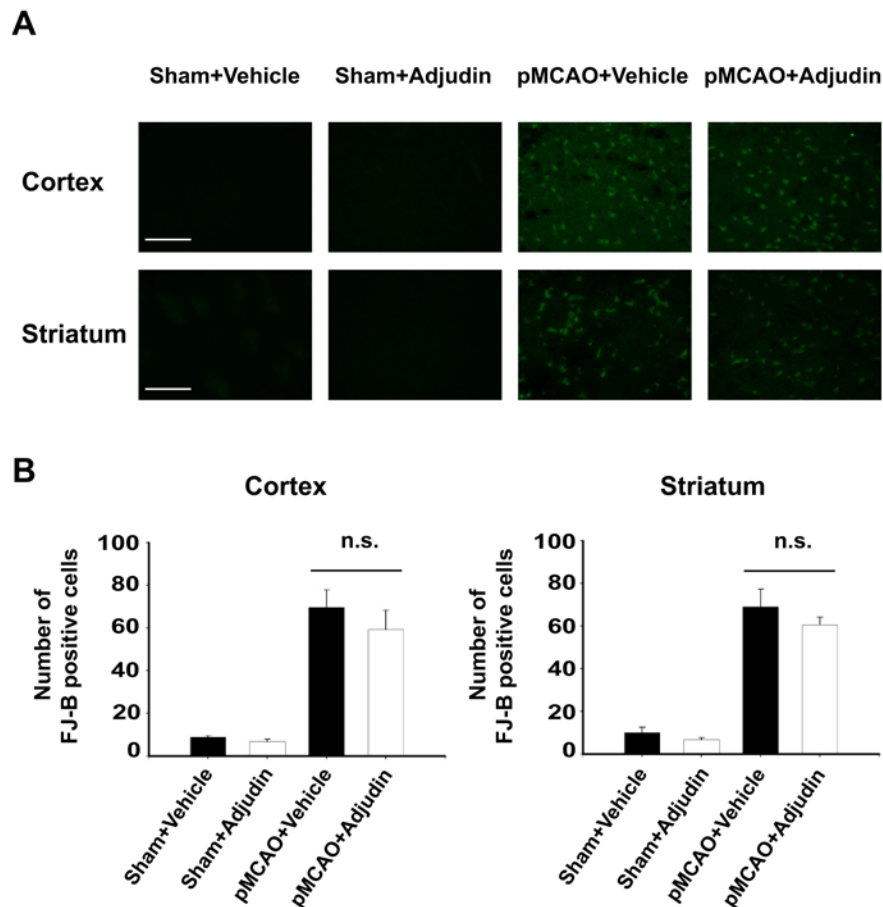
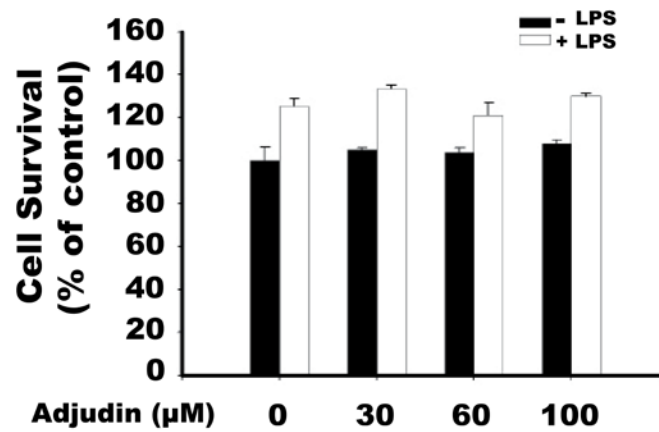


## Supplementary Figures



**Supplementary Figure.1.** Adjudin does not reduce the number of degenerating neurons after pMCAO. (A) Representative photographs of Fluoro-Jade B (*F-JB*) positive cells in cerebral cortex and striatum at 24 h after ischemia. Scale bar = 100  $\mu$ m. (B) The number of degenerating neurons is quantified (n=3-4 per group).



**Supplementary Figure.2.** Effect of Adjudin on the cell viability of primary mixed glial cultures. Cells were pretreated with indicated concentrations of Adjudin for 1 h and then stimulated with 200 ng/ml LPS for a 24-h incubation period. Cell viability was assessed by LDH assay. Bars represent the mean  $\pm$  SEM from three independent experiments.

## **Supplementary Materials and Methods**

### ***Primary mixed glial cultures***

Primary mixed glial cultures were isolated from cortex of post-natal day 1 Sprague Dawley rats. Briefly, cerebral cortex was dissected and then incubated in trypsin/EDTA at 37°C for 15 min. Tissue was dissociated by pipetting and centrifuged at 225 g for 5 min. The pellet was resuspended in DMEM and filtered through a nylon mesh. The resulting cell suspension was plated in 24-well plates with medium being changed every 2-3 days. Experiments were performed on these cells on days 12–16 of *in vitro* culture.

### ***Fluoro-Jade B (F-J B) staining***

Brain cryosections (20 µm) were fixed in 4% paraformaldehyde. The slides were immersed in a solution containing 1% sodium hydroxide in 80% alcohol for 5 min, followed by 2 min in 70% alcohol and 2 min in distilled water. Then the slides were transferred to a solution of 0.06% potassium permanganate for 10 min, rinsed in distilled water for 2 min and incubated in the 0.0004% staining solution for Fluoro-Jade® B (Millipore, Temecula, CA) for 20 min. Microscopic images were acquired using a laser-scanning confocal microscope (Leica TCS SP5 II, Germany).

## Supplementary Tables

### Detailed modified neurologic severity scores (mNSS):

	24 h after pMCAO				72 h after pMCAO												
	Vehicle (n=4)				Adjudin (n=4)				Vehicle (n=5)				Adjudin (n=5)				
<b>Raising the mouse by the tail</b>																	
1 Flexion of forelimb	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1 Flexion of hindlimb									1	1	1	1	1				
1 Head moved more than 10° to the vertical axis within 30s																	
<b>Walking on the floor</b>																	
0 Normal walk																	
1 Inability to walk straight																	
2 Circling toward the paretic side	2	2	2	2	2	2	2	2	2		2	2	2	2	2	2	2
3 Falling down to the paretic side										3							
<b>Beam balance tests</b>																	
0 Balance with steady posture																	0
1 Grasps side of beam						1								1	1		1
2 Hugs the beam and one limb falls down from the beam	2	2	2	2	2		2	2	2	2	2	2	2			2	
3 Hugs the beam and two limbs fall down from the beam, or spins on beam (>30s)																	
4 Attempts to balance on the beam but falls off (>20s)																	
5 Attempts to balance on the beam but falls off (>10s)																	
6 Falls off. No attempt to balance or hang on to the beam (<10s)																	
<b>Reflexes absence</b>																	
1 Pinna reflex (a head shake when touching the auditory meatus)																	
1 Corneal reflex (an eye blink when touching the cornea with cotton)																	
<b>Total Score</b>	5	5	5	5	5	4	5	5	6	7	6	6	6	4	4	5	4
<b>Average of total score</b>	5				4.75				6.2				4				