

SUPPLEMENTAL MATERIAL

Table S1. Neurological impairment evaluation scale.

Neurological deficits	Scores
No observable neurological damage	0
Failure to fully extend the right forepaw or flex the left forelimb when suspended by the tail	1
Left shoulder adduction when suspended by the tail	2
Reduced resistance to a lateral push toward the left	3
Moving spontaneously but turning to the left when dragged by the tail	4
Rotating spontaneously or only to the left	5
Moving only in response to stimulus	6
No movement in response to stimulus	7
Death resulting from stroke	8

Table S2. Antibody information for Western blotting.

Anti-body Name	Source	Catalog Number	RRID
CARD3	Cell Signaling Technology	#4142	AB_2716277
Bcl-2	Cell Signaling Technology	#3498	AB_1903907
Bax	Cell Signaling Technology	#2772	AB_10695870
Bid	Cell Signaling Technology	#2003	AB_10694562
cleaved-caspase3	Cell Signaling Technology	#9661	AB_2341188
p-ERK	Cell Signaling Technology	#4370	AB_2315112
ERK	Cell Signaling Technology	#4695	AB_390779
p-JNK	Cell Signaling Technology	#4668	AB_823588
JNK	Cell Signaling Technology	#9258	AB_2141027
p-p38	Cell Signaling Technology	#4511	AB_2139682
p38	Cell Signaling Technology	#9212	AB_330713
IKBa	Cell Signaling Technology	#4814	AB_390781
p-IKK β	Cell Signaling Technology	#2694	AB_2122296
IKK β	Cell Signaling Technology	#8943	AB_11024092
p-p65	Cell Signaling Technology	#3033	AB_331284
p65	Cell Signaling Technology	#4764	AB_823578
TAK1	Cell Signaling Technology	#5206	AB_10694079
p-TAK1	Cell Signaling Technology	#9339	AB_2140096
GAPDH	Cell Signaling Technology	#2118	AB_561053

Anti-Mouse IgG	Jackson ImmunoResearch	#115-035-00	AB_10015289
H+L	Labs	3	
Anti-Rabbit IgG	Jackson ImmunoResearch	#111-035-00	AB_2313567
H+L	Labs	3	

Table S3. Primer sequences for PCR.

Gene	Forward	Reverse
<i>TNF-α</i>	TGACAAGCCTGTAGCCCAC	TAGCAAATCGGCTGACGGTG
<i>COX-2</i>	TCTCCCTGAAGCCGTACACA	AATGGTGCTCCAAGCTCTACC
<i>IL-1β</i>	TAATGAAAGACGGCACACCCA	GTTTCCCAGGAAGACAGGCT
<i>IL-6</i>	ATGAACTTGGACCTCTGCGG	GTCCACCACAGTTGCTGACT
<i>GAPDH</i>	GGTTGTCTCCTGCGACTTCA	CCCTGTTGCTGTAGCCGTAT

Figure S1. The rCBF (relative cerebral blood flow) of the different tMCAO (transient middle cerebral artery occlusion model) mice model was measured by a Doppler flowmeter (A). The expression of CARD3 in different tissues from CARD3-TG mice was measured by western blotting (B), GAPDH (glyceraldehyde-3-phosphate dehydrogenase) served as loading control. (C), in vivo, the mRNA levels of apoptotic factors in WT and CARD3-KO mice or NTG and neuron-specific CARD3-TG mice after reperfusion for 24 h were analyzed with qPCR. All data were normalized to GAPDH and shown as mean \pm SD. Comparison between groups were performed with Student's t-test, * p <0.05 vs the control group, $n=4$ mice per group. (D), in vitro, primary neurons separated from CARD3-KO or -TG mice were subjected to OGD (oxygen and glucose deprivation) for 1 hour and reperfusion for 24h. The mRNA levels of the inflammatory-related factors were analyzed with qPCR. All data were normalized to GAPDH and shown as mean \pm SD. Comparison between groups were performed with Student's t-test, * p <0.05 vs the control group. Three independent experiments were repeated. Bcl2 indicates B-cell lymphoma-2; Bax, Bcl2-Associated X; TNF- α , tumour necrosis factor alpha; COX-2, cyclooxygenase-2; IL-1 β , interleukin-1 β ; IL-6, interleukin-6.

