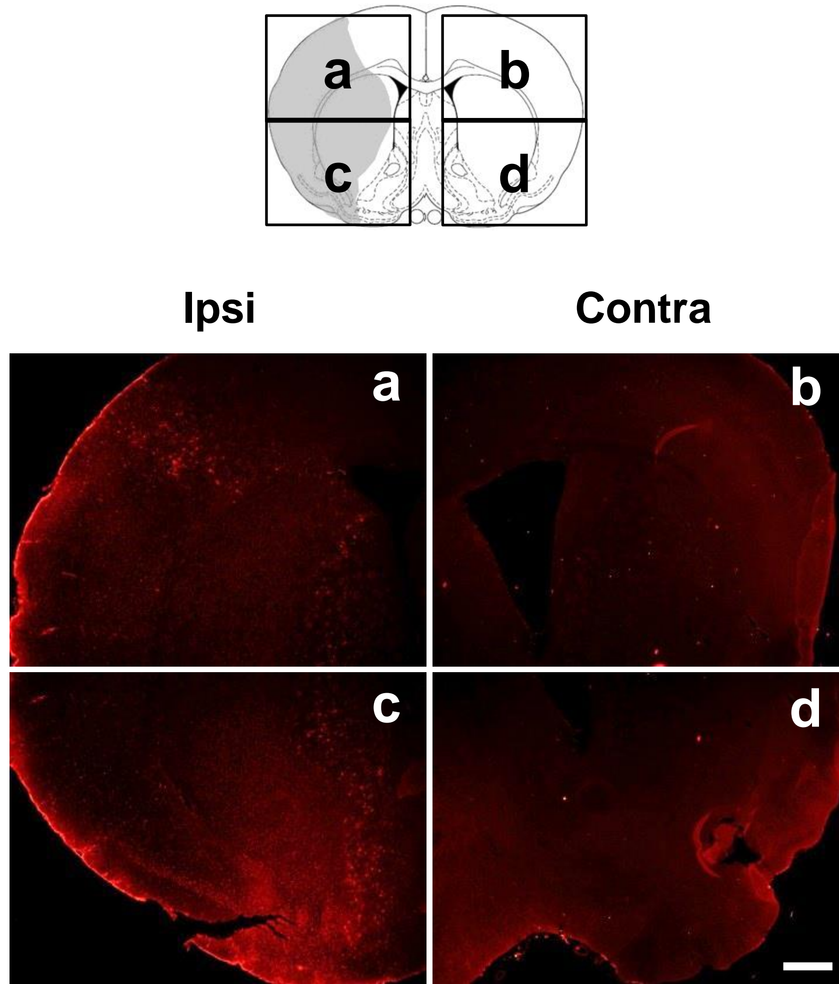
Supplementary Table I. DNA sequences of the primers used for RT-PCR.

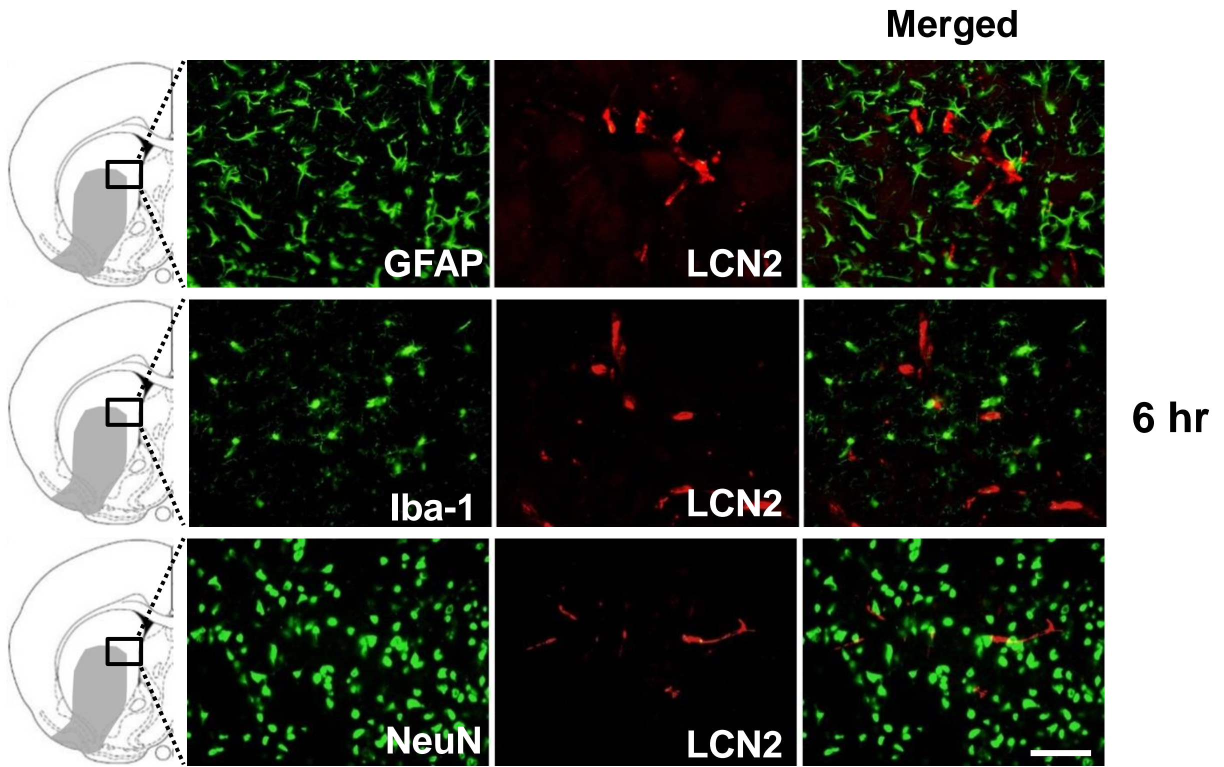
Mouse cDNAs	RT-PCR methods	Primer sequences	GenBank accession No.
β-actin	Real-time & Traditional	Forward, 5´-ATCCGTAAAGACCTCTATGC-3´ Reverse, 5´-AACGCAGCTCAGTAACAGTC-3´	NM_007393
Ccl2	Real-time	Forward, 5´-TCAGCCAGATGCAGTTAACG-3´ Reverse, 5´-GATCCTCTTGTAGCTCTCCAGC-3´	NM_011333
Cxcl1	Real-time	Forward, 5´-CACACTCAAGAATGGTCGCGA-3´ Reverse, 5´-TTGTCAGAAGCCAGCGTTCAC-3´	NM_008176
Cxcl10	Real-time	Forward, 5´-AAGTGCTGCCGTCATTTTCT-3´ Reverse, 5´-GTGGCAATGATCTCAACACG-3´	NM_021274
Icam-1	Real-time	Forward, 5´-AGATCACATTCACGGTGCTG-3´ Reverse, 5´-CTTCAGAGGCAGGAAACAGG-3´	NM_010493
Il-1β	Real-time	Forward, 5´-AGTTGCCTTCTTGGGACTGA-3´ Reverse, 5´-TCCACGATTTCCCAGAGAAC-3´	NM_008361
Lcn2	Real-time	Forward, 5´-CCCCATCTCTGCTCACTGTC-3´ Reverse, 5´-TTTTTCTGGACCGCATTG-3´	NM_008491
Tnf-α	Real-time	Forward, 5´-ATGGCCTCCCTCTCAGTTC-3´ Reverse, 5´-TTGGTGGTTTGCTACGACGTG-3´	NM_013693
24p3r	Traditional	Forward, 5´-AATGACTCTCACGGGGATTG-3´ Reverse, 5´-AGTGGTGGGGATGACTTCAG-3´	NM_021551
Lcn2	Traditional	Forward, 5´-ATGTCACCTCCATCCTGGTC-3´ Reverse, 5´-CACACTCACCACCCATTCAG-3´	NM_008491

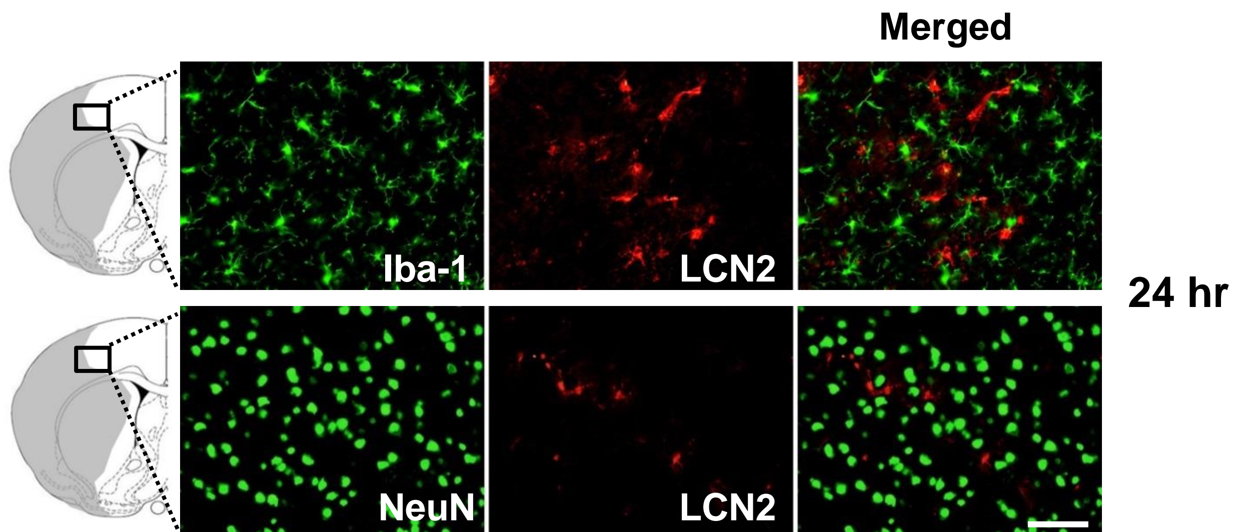
Supplementary Fig. I



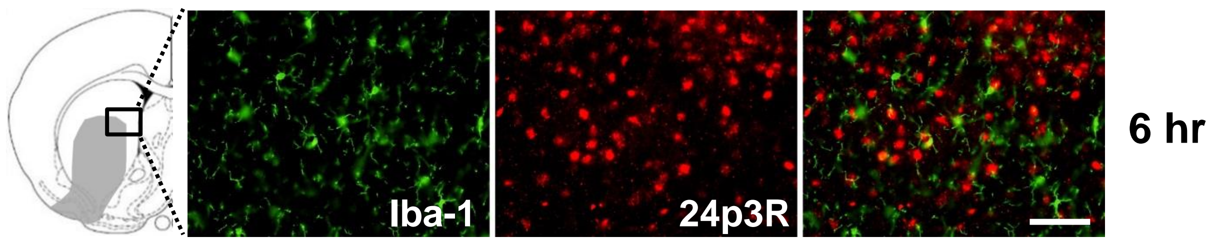
Supplementary Figure I. Induction of LCN2 protein in the whole brain after ischemia/reperfusion. Immunofluorescence staining and the subsequent examination with low magnification revealed an overview of the LCN2 expression in the whole brain at 24 hr after reperfusion. The shaded area indicates the infarcted region. Scale bar = 500 μm. Contra = contralateral hemisphere; Ipsi = ipsilateral hemisphere.

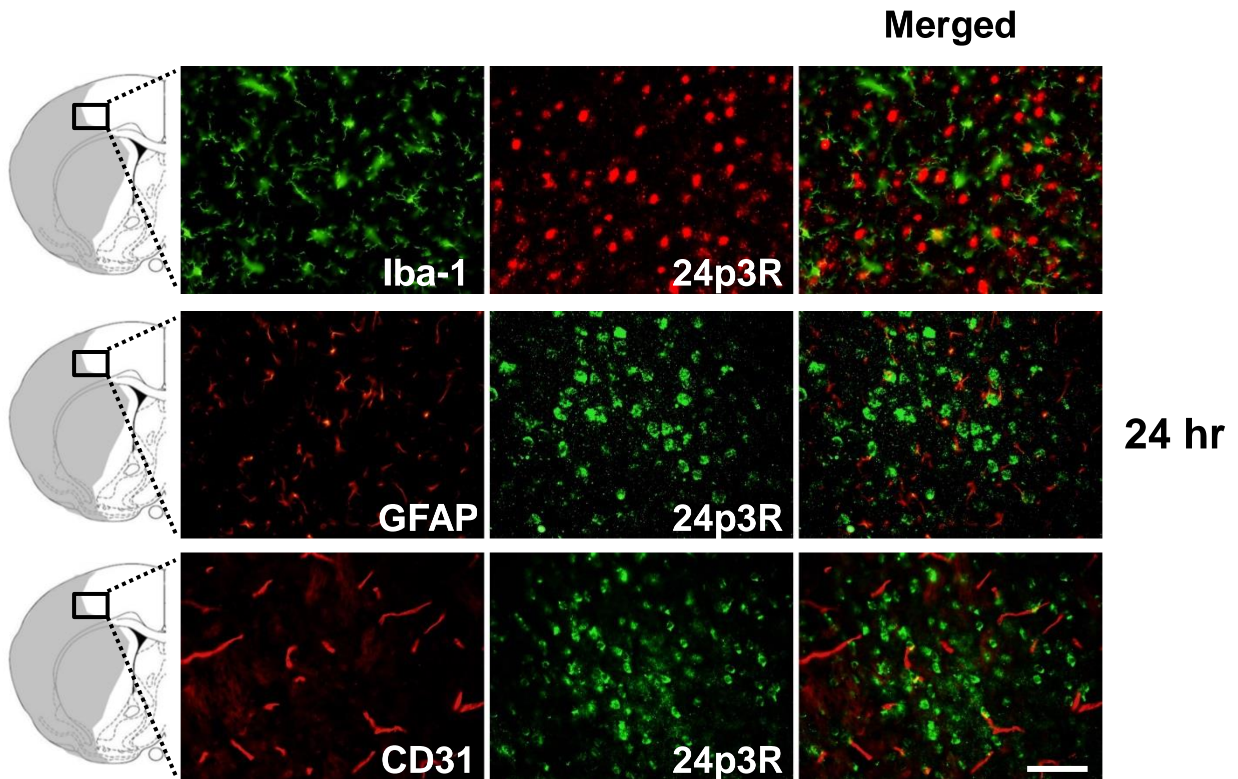
Supplementary Fig. IIAB





Supplementary Fig. IICD

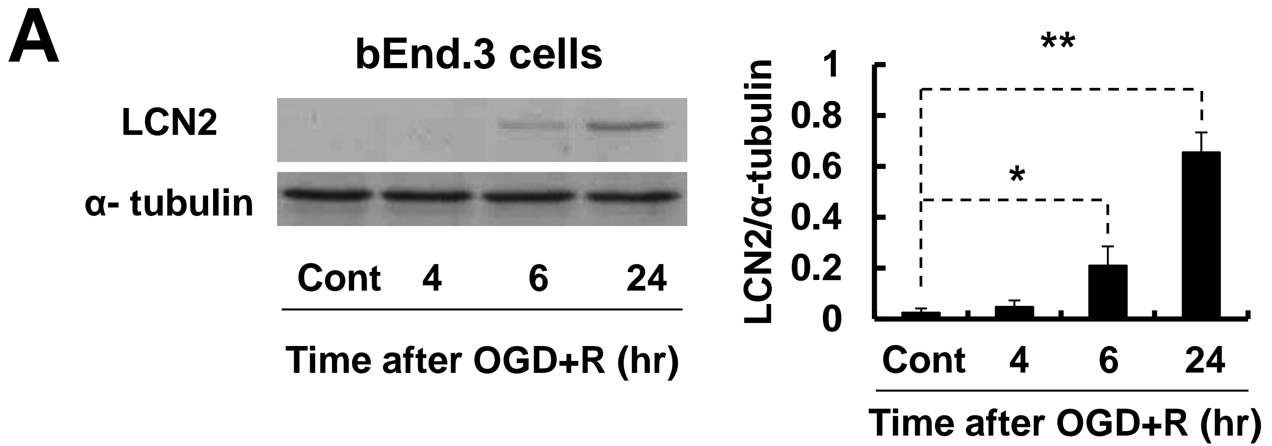




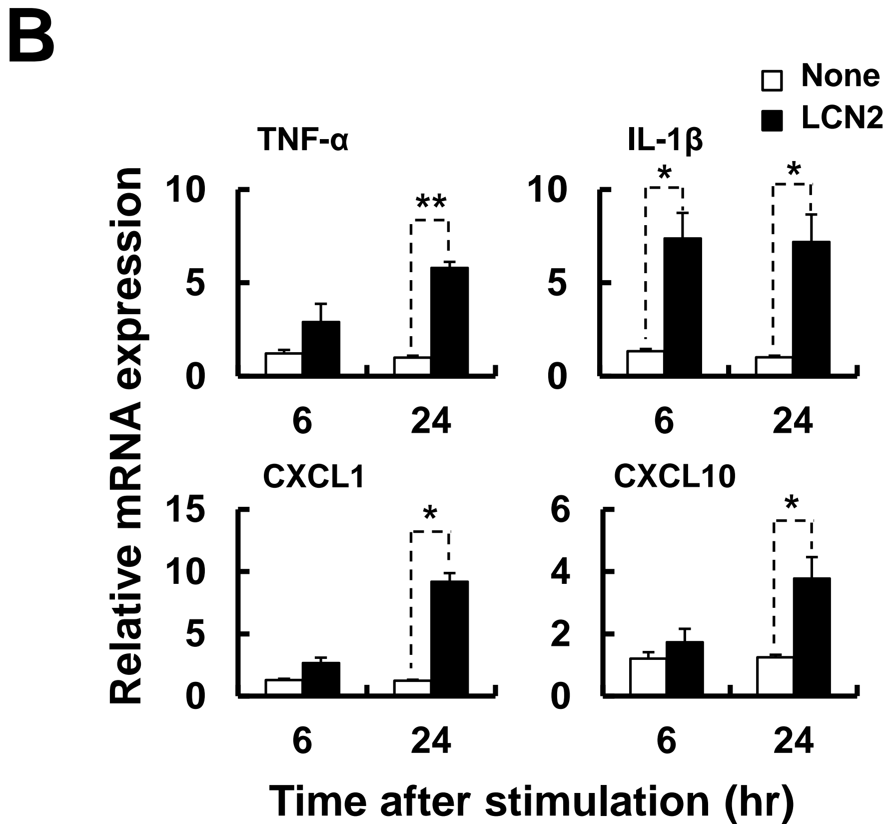
Supplementary Figure II. Localization of LCN2 and 24p3R within ipsilateral hemispheres after ischemia/reperfusion. Double-immunofluorescence staining for LCN2 or 24p3R and GFAP (astrocyte marker), Iba-1 (microglia marker), NeuN (neuron marker) and CD31 (endothelial cell marker). LCN2 expression was not localized in astrocytes, microglia, or neurons at 6 hr after reperfusion (**A**), or in microglia and neurons at 24 hr after reperfusion (**B**). The localization of 24p3R in microglia was not detected at 6 hr after reperfusion (**C**) or in microglia, astrocytes, or endothelial cells at 24 hr after reperfusion (**D**). Scale bar = 100 µm. The shaded area indicates the infarcted region. Results are representative of more than three independent experiments. Co-localization is indicated by arrowheads.

Supplementary Fig. III

A

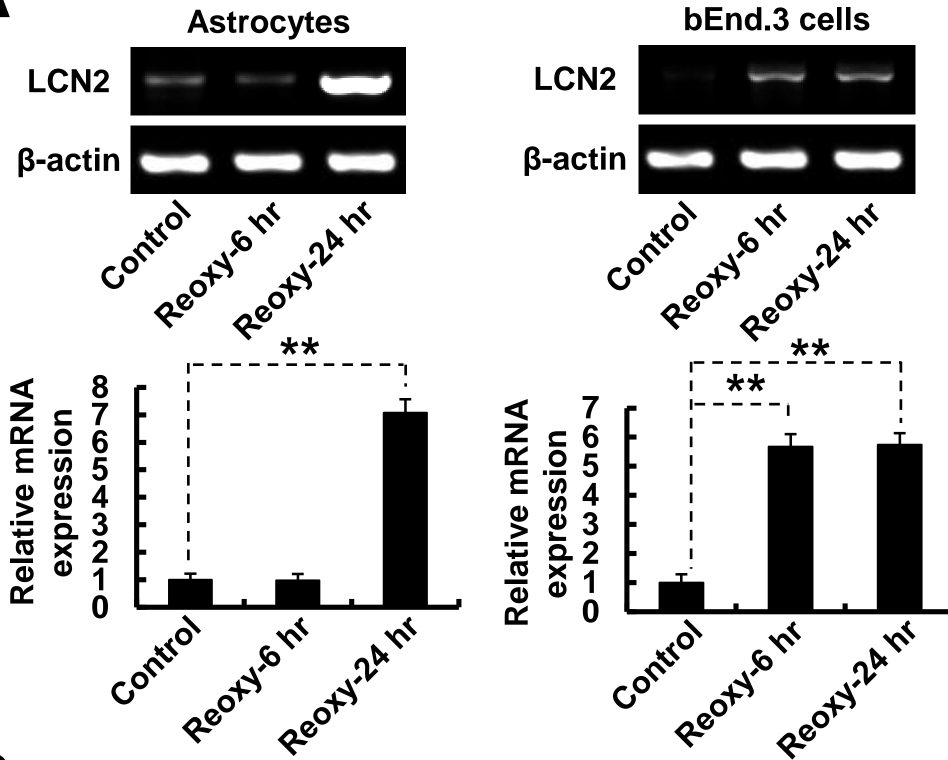


B



Supplementary Figure III. Effects of LCN2 on endothelial cells. A, Western blot analyses revealed the induction of LCN2 protein in bEnd.3 cells after OGD+R (*left*). Control bEnd.3 cells did not undergo OGD. Results were normalized to α-tubulin, and are presented as means ± SDs (n = 3) (*right*), *P < 0.05, **P < 0.01. **B**, Relative mRNA expression of proinflammatory cytokines (TNF-α and IL-1β) and chemokines (CXCL1 and CXCL10) in bEnd.3 cells at 6 and 24 hr after LCN2 stimulation (1.0 µg/ml) was evaluated by Real-time RT-PCR (*n* = 3 per group). GAPDH was used as an internal control. The nucleotide sequences of the primers used were as follows: GAPDH, forward, 5´-TGGGCTACACTGAGCACCAG-3´, reverse, 5´-GGGTGTCGCTGTTGAAGTCA-3´. Results are means ± SDs, *P < 0.05, **P < 0.01.

Supplementary Fig. IV



B

Neurons

Astrocytes

bEnd.3 cells

24p3R

β-actin

Control

Reoxy-6 hr

Reoxy-24 hr

Relative mRNA expression

0

0.2

0.4

0.6

0.8

1

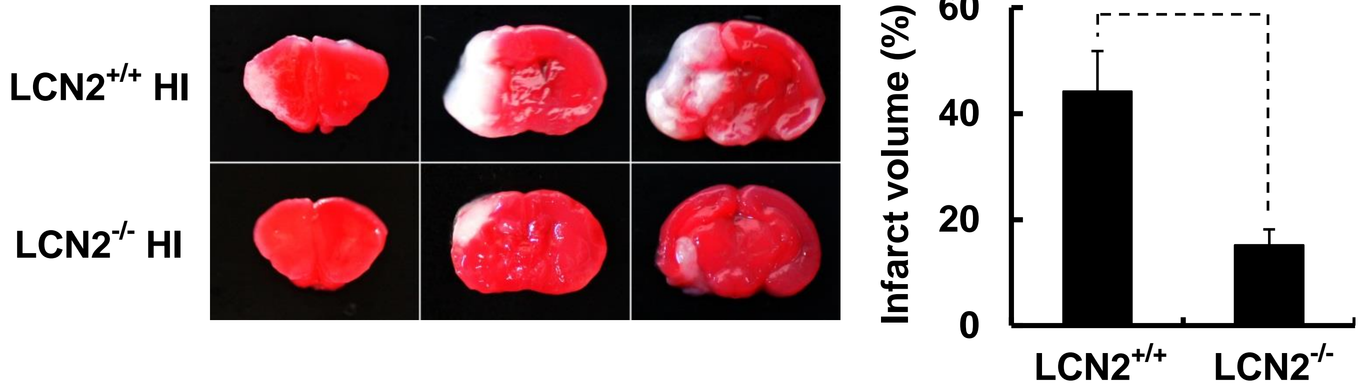
1.2

**

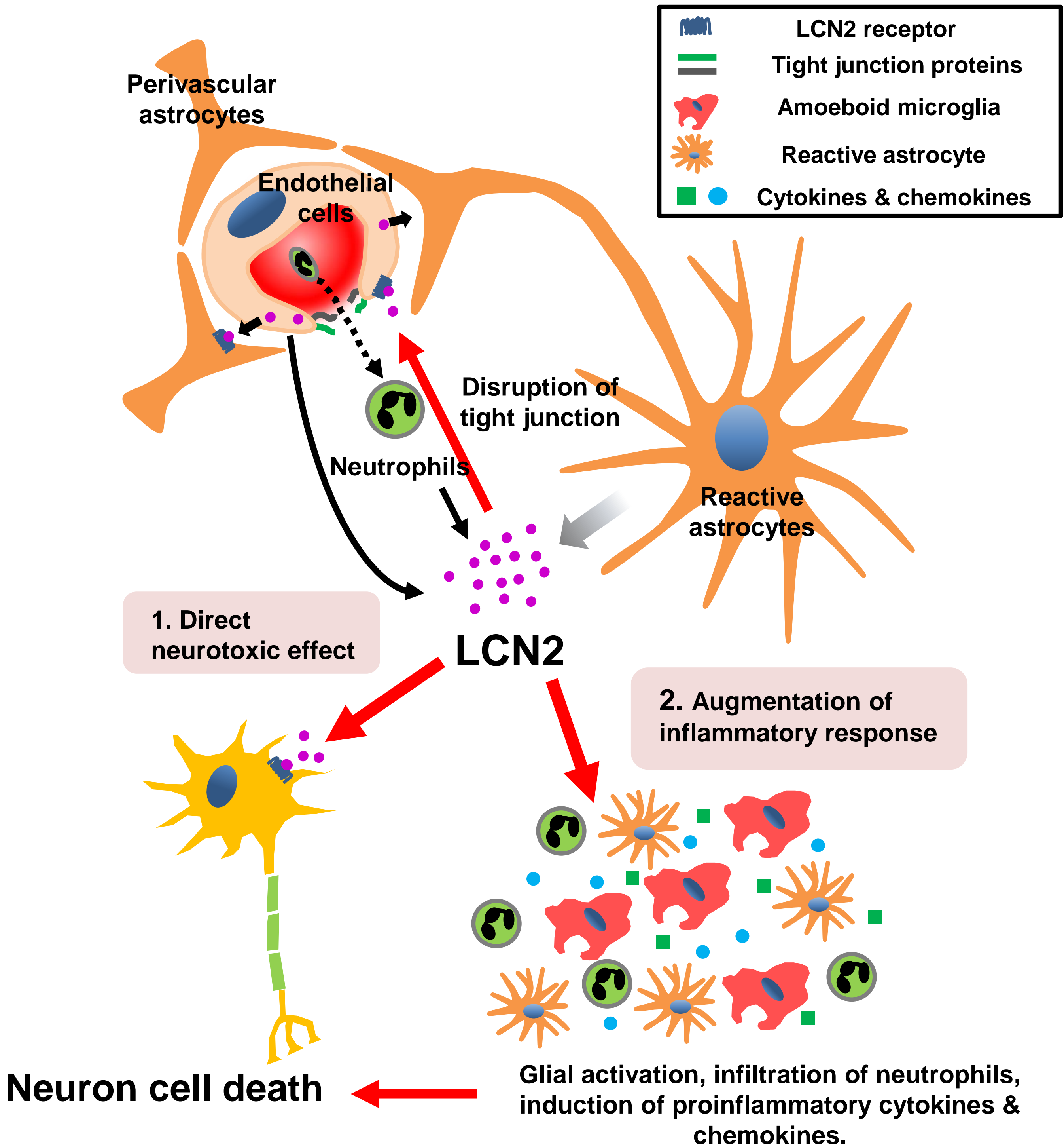
*

Supplementary Figure IV. The expression of LCN2 and 24p3R after oxygen-glucose deprivation. **A.** Levels of LCN2 mRNA expression in the primary astrocyte cultures or bEnd.3 cells were assessed by traditional RT-PCR after OGD+R. β-actin was used as an internal control. The results are representative of three independent experiments (*upper*) or means ± SDs (n = 3 per group) (*lower*), $^{**}P < 0.01$. **B**. Levels of 24p3R mRNA expression in the primary neurons, astrocyte cultures, and bEnd.3 cells were determined by traditional RT-PCR after OGD+R. β-actin was used as an internal control. The results are representative of three independent experiments (*upper*) or means ± SDs (n = 3 per group) (*lower*), $^{**}P < 0.01$.

Supplementary Fig. V



Supplementary Figure V. LCN2 deficiency reduces infarct volume after hypoxic-ischemia in neonatal mice. In hypoxic-ischemic (HI) brain injury model, at 48 hr after reoxygenation, infarct volume was compared between $LCN2^{+/+}$ and $LCN2^{-/-}$ mice by TTC staining of coronal sections (n = 5 per group). Results are means ± SDs, $^{**}P < 0.01$.



Supplementary Figure VI. Proposed role of LCN2 in brain injury after ischemia/reperfusion. Two putative mechanisms underlying LCN2 contribution to ischemic brain damage are illustrated: 1) LCN2 secreted by reactive astrocytes, endothelial cells, and neutrophils directly contributes to neuronal cell death by interacting with its receptor (24p3R); 2) LCN2 amplifies diverse events associated with neuroinflammation, such as glial activation, neutrophil infiltration, and the induction of proinflammatory cytokines and chemokines. LCN2 may also contribute to neuroinflammation by increasing BBB permeability via the disruption of tight junctions.