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Supplemental Information

Exosome-Shuttled circSHOC2 from IPASs Regulates Neuronal Autophagy and Ameliorates Ischemic Brain Injury via the miR-7670-3p/SIRT1 Axis

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Supplemental figure legends

Supplement Fig. 1. IPAS-EXOs provide neuroprotection both *in vitro* and *in vivo*. (A) CCK-8 assays showing neuronal survival after pretreatments with different concentrations (5, 10, 20, 40 μg/ml) of IPAS-EXOs and subsequent OGD treatments. The cellular survival in the sham group was set to 100%. (B) TTC staining showing the volumetric changes of cerebral infarctions in mice. (C) Neurological scores showing the levels of neurobehavioral recovery of mice treated with PBS or IPAS-EXOs (*P < 0.05).

Supplement Fig. 2. The quantification of TUNEL double-labeled cells in the brain tissue of each group (*P < 0.05).

Supplement Fig. 3. LDH assays and TUNEL assays confirmed that circSHOC2 confers a neuroprotective effect *in vitro*. (A) LDH assays of neurons treated with IPAS-EXOs, si-circSHOC2-EXOs, or control siRNA-NC EXOs after OGD (*P < 0.05). (B) LDH assays in control neurons, neurons treated with IPAS-EXOs, neurons treated with si-circSHOC2-EXOs, and neurons treated with circRNA-NC EXOs. (C) Numbers of TUNEL+ neurons treated with IPAS-EXOs, si-circSHOC2-EXOs, and control siRNA-NC EXOs after OGD (*P < 0.05).

Supplement Fig. 4. circSHOC2 mimics regulated OGD-induced neuronal apoptosis via promoting autophagy. (A) Stable Gag-LC3-expressing cells were co-transfected with

a circSHOC2 mimics or circRNA-NC, and autophagy was tested under OGD or normal conditions for 6 h. Quantitative analysis of the amount of Gag-LC3 accumulation per cell was performed (*P < 0.05). (B) Western blot analysis of LC3 in the circSHOC2 mimics group and circRNA-NC group (*P < 0.05). (C) Western blot analysis of TIMM23 and SQSTM1 in the circSHOC2 mimics group and circRNA-NC group (*P < 0.05).

Supplement Fig. 5. circSHOC2 reduces OGD-induced neuronal death via the miR-7670a-3p/SIRT1 axis. (A) Neurons were co-transfected with a circSHOC2 mimic, si-circSHOC2, miR-7670-3p mimic, or siRNA-7670-3p. Cells were treated with OGD for 6 h (*P < 0.05). The expression of SIRT1 was analyzed by Western blotting. (B) Number of TUNEL+ neurons treated with circSHOC2, siRNA-SIRT1 and control siRNA-NC after OGD (*P < 0.05). All siRNA sequences were shown in Supplementary Table 1.

Supplement Table 1. The primers used for qRT-PCR in this study. **(Table S1)**

The primer sequences of gene-specific primers used for real-time RT-PCR:

circSHOC2 Forward: 5′-AAAACTTCACCTTCAACACCTGTGAAAGGGACTCC-3′,

circSHOC2 Reverse: 5′-AAACCAAACTGTCAGAATGGTAGATAAGAATAGTT-3′;

GAPDH Forward: 5′- TCGTGGAAGGACTCATGACC -3′;

GAPDH Reverse: 5′- AGGCAGGGATGATGTTCTGG -3′.

The primer Sequences of siRNA:

si-circSHOC2 sence : GGGCCGGAAGUGGUAGGGGCGUCGGAAGAAGGGTT;

si-circSHOC2 antisence: CCCUUCUUCCGACGCCCCUACCACUUCCGGCCCTT.

The primer Sequences of siRNA-7670-3p:

siRNA-7670-3p sense: 5′-UGGAUUUGUACCAUUCUUCUG-3′,

siRNA-7670-3p antisense: 3′-GAAGAAUGGUACAAAUCCAAG-5′.

The sequences of SIRT1 mRNA primers were:

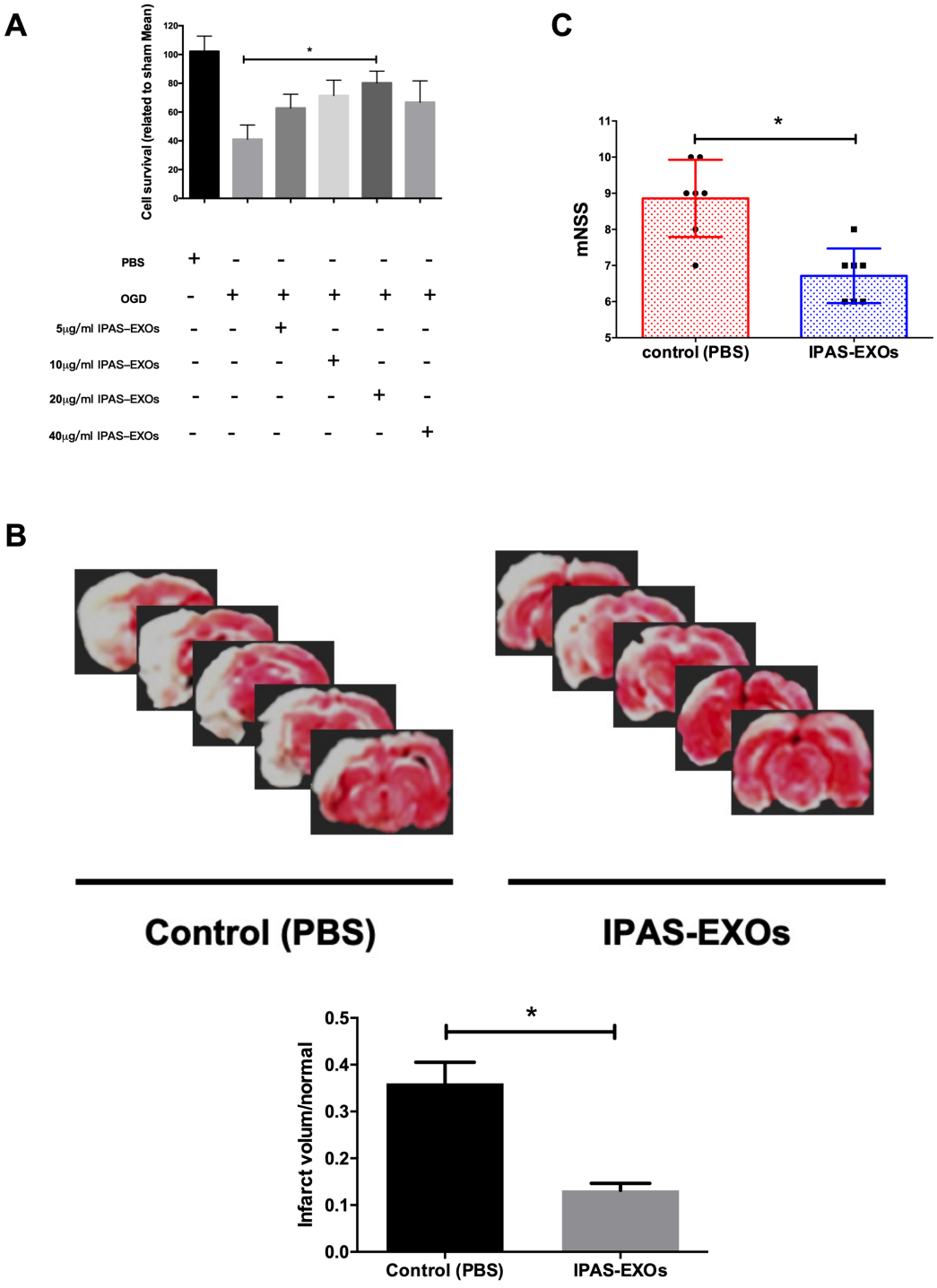
Forward, 5′- TAGACACGCTGGAACAGGTTGC-3′;

Reverse, 5′- CTCCTCGTACAGCTTCACAGTC-3′.

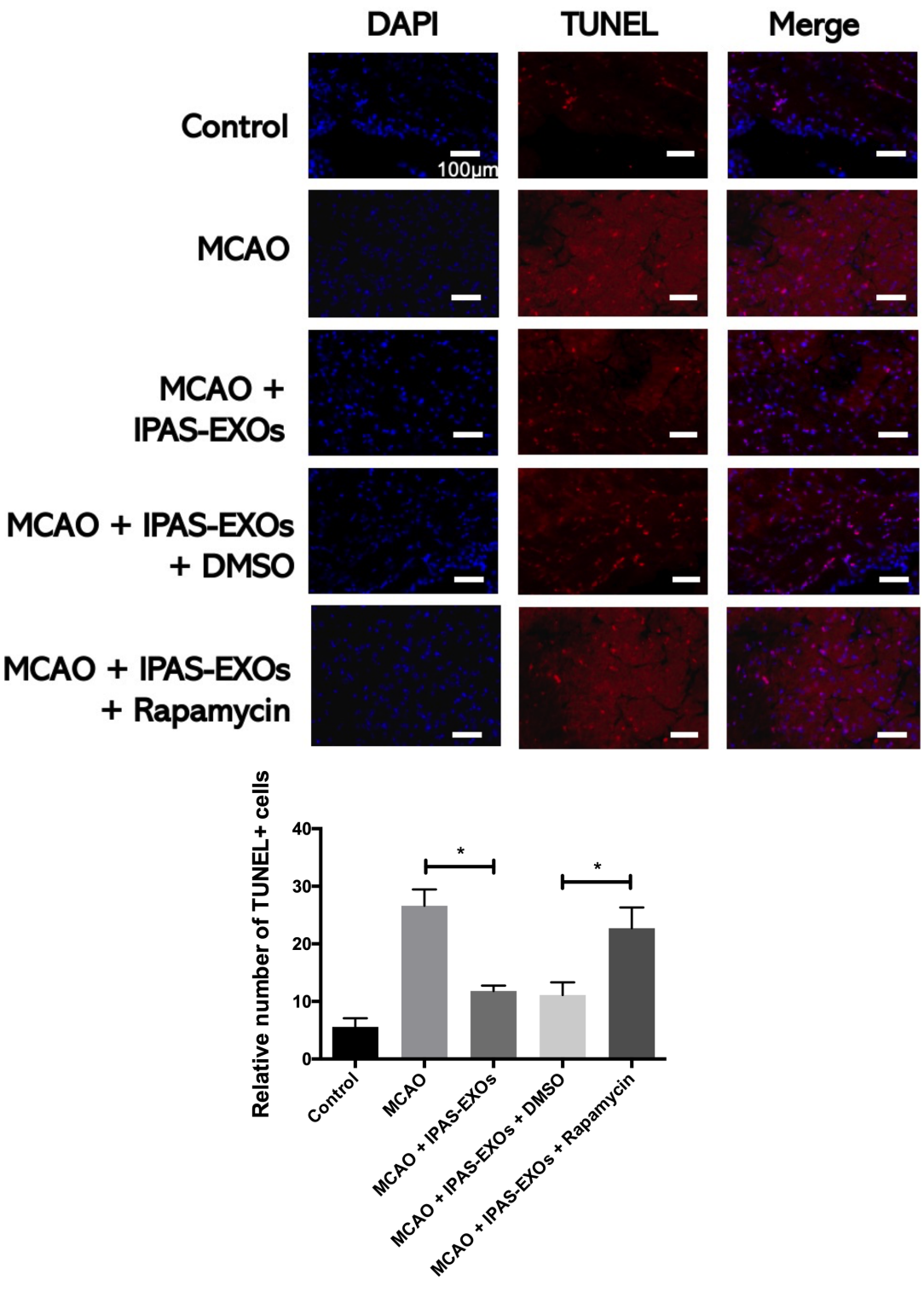
The siRNA sequences were used to silence SIRT1 expression:

SIRT1-siRNA sense: 5′-AGAGUUGCCACCCACACCU-3′,

SIRT1-siRNA antisense: 5′-AGGUGUGGGUGGCAACUCU-3′.



Supplement Figure 1 (Figure S1). IPAS-EXOs provide neuroprotection both *in vitro* and *in vivo*.



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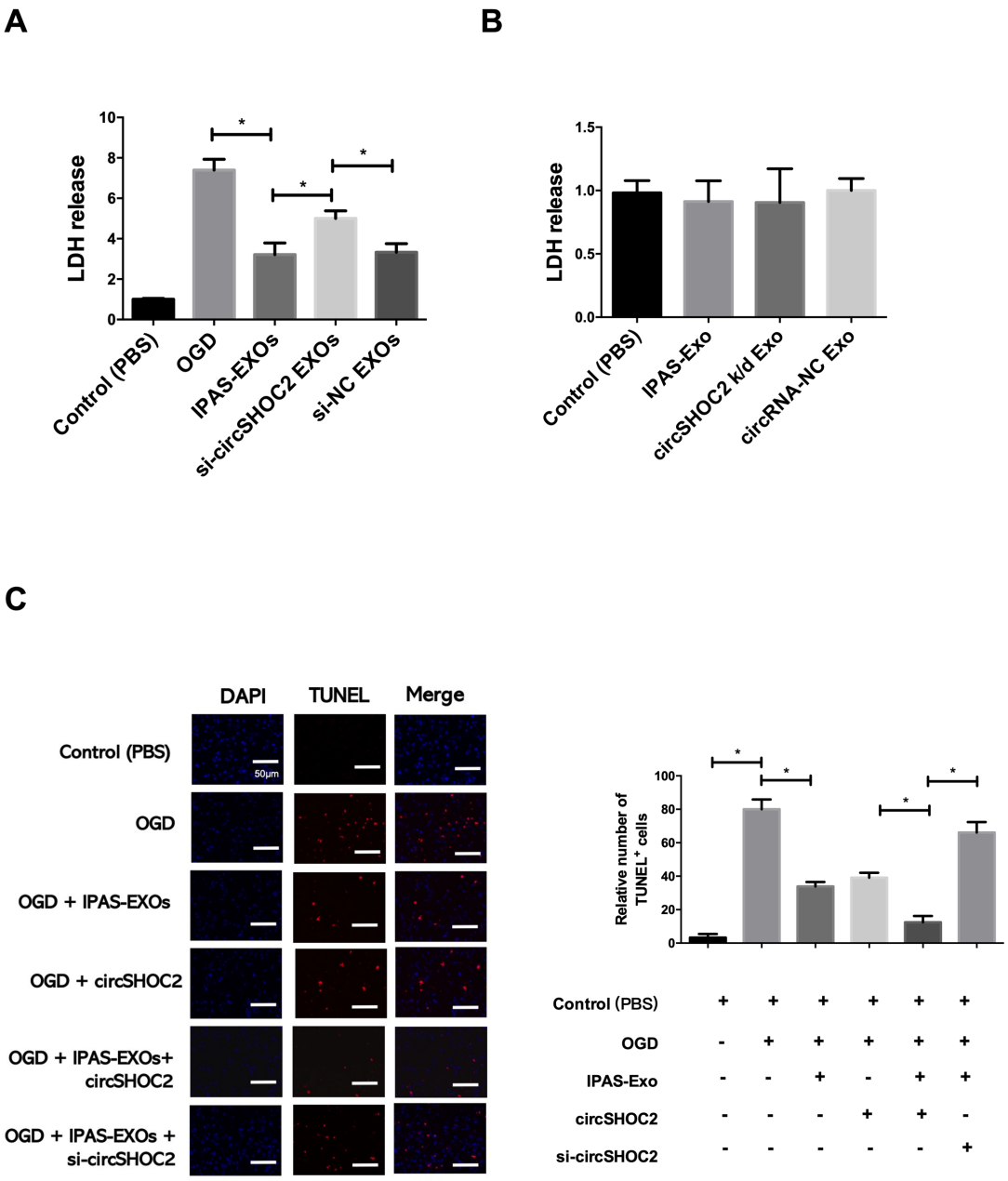
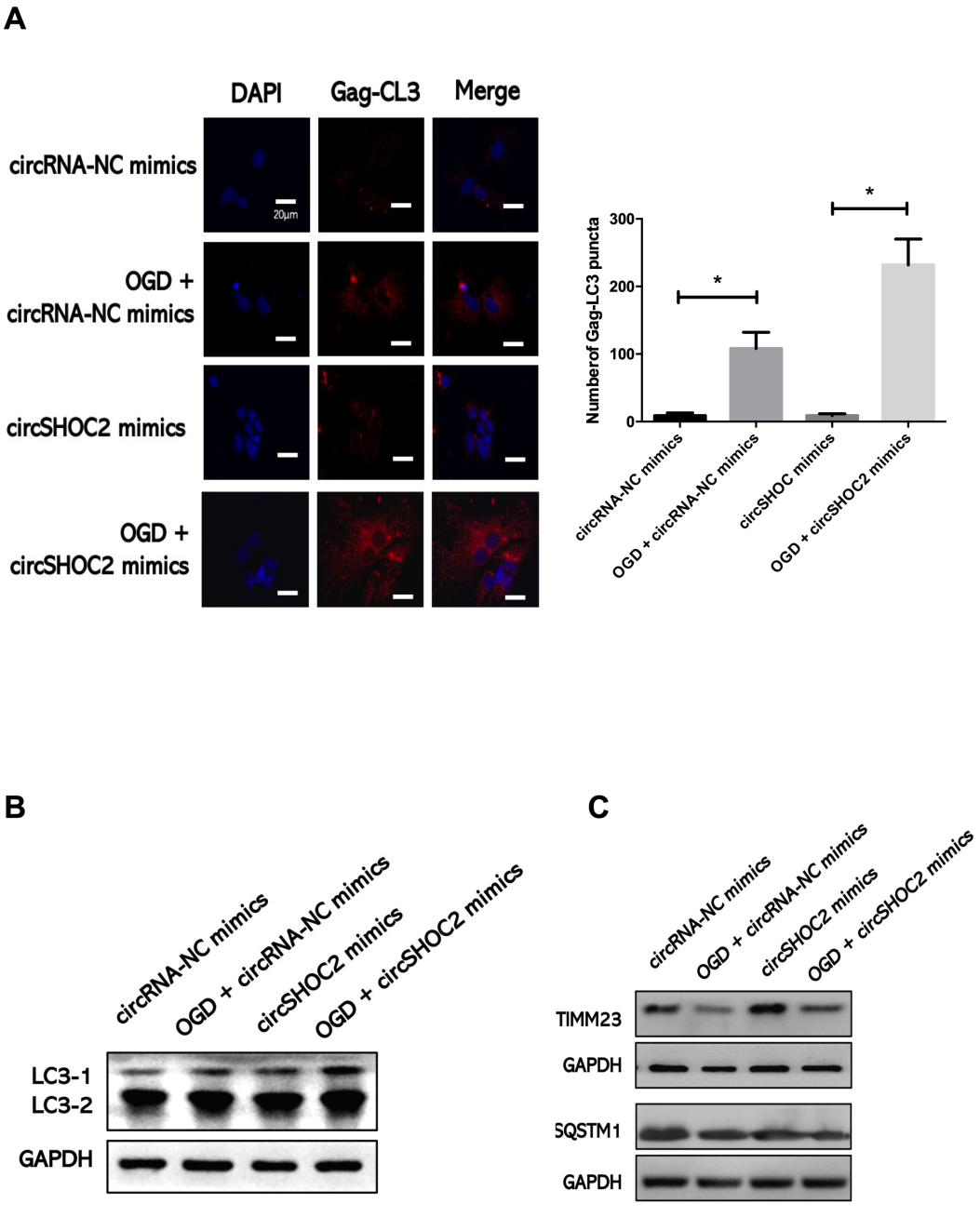
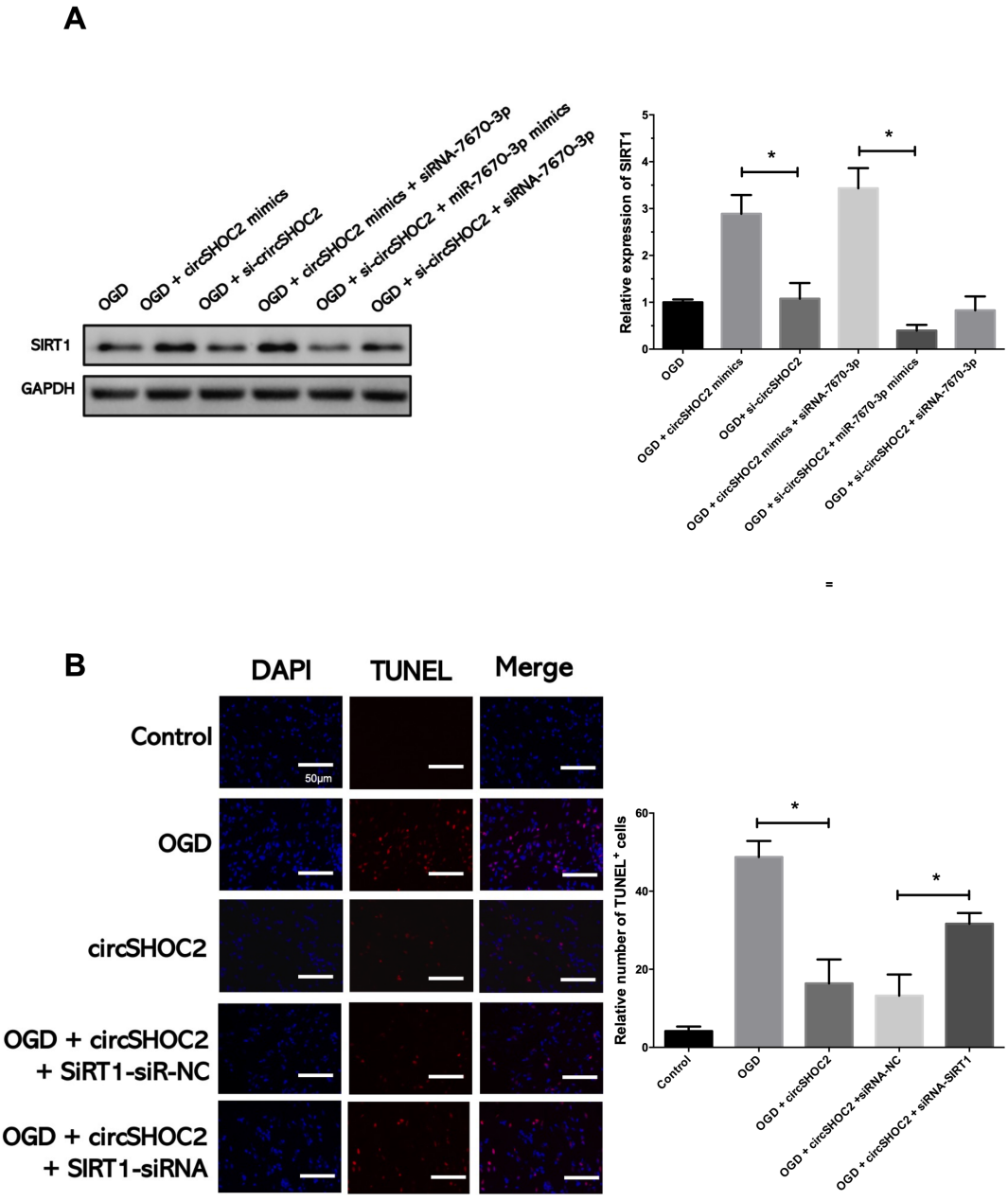


Supplement Figure 3 (Figure S3). LDH assays and TUNEL assays confirmed that circSHOC2 confers a neuroprotective effect *in vitro*.



Supplement Figure 4 (Figure S4). circSHOC2 mimics regulated OGD-induced neuronal apoptosis via promoting autophagy.



Supplement Figure 5 (Figure S5). circSHOC2 reduces OGD-induced neuronal death via the miR-7670a-3p/SIRT1 axis.