Cardiac arrest triggers hippocampal neuronal death through autophagic and apoptotic pathways

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Legends

Figure S1. Autophagosome formation following CA examined by transmission electron microscopy (TEM). (AA) normal hippocampal neurons; (AB) Mitochondrial swelling 1.5 h after ROSC; (AC) Growth and presence of double-membrane structure which contains mitochondria, or vacuoles (C-shaped), and chromatin condensation were observed at 3 h after ROSC. (AD) Increased number of vacuoles (C-shaped) or double membranes structure and lysosomes was observed at 6 h after ROSC. (AE) At 12 h after ROSC, cell exhibited apoptotic features including blebbing, cell shrinkage, and chromatin pyknosis. (AF) Cells exhibited both apoptosis and necrosis 24 h after ROSC. (B) Quantified autophagosomes lysosomes and intact mitochondria, in the CA and sham groups. n = 6. *$p < 0.05$ vs. sham group; N, nucleus; Broad arrows represent autophagosomes; Narrow arrows denote lysosomes; Black arrowheads indicate mitochondria; Scale bar = 1μm.

Figure S2. Representative fluorescent images of doubly-stained LC3-II (green) with NeuN (red) in the hippocampal CA1 subregion at 1.5, 3, 6, 12 and 24 h after ROSC and DAPI counterstaining of nuclei (blue). LC3-II staining was indistinct in the CA1 of the sham rats. 6-24 h after ROSC,

the staining was intense in the cytosol of CA1 showing a punctate appearance. After 6-24 h following ROSC, LC3 was predominantly seen in NeuN-expressing pyramidal neurons of the hippocampal CA1, confirmed by superimposed images from the same field. Scale bar, 50 μm.

Figure S3. Representative images of dual-labeled fluorescence of p53 (red) with Beclin-1 (green) in the hippocampal CA1 subregion at 3, 6, 12 and 24 h after ROSC and DAPI counterstaining of nuclei (blue). p53 and Beclin-1 staining was indistinguishable in the CA1 subregion of the non-ischemic brains (sham). 3-24 h after ROSC, the staining was stronger and appeared punctate in the cytosol of the CA1. 3-24 h after ROSC, p53 was predominantly localized to Beclin-1-expressing pyramidal neurons in CA1, confirmed by superimposed images from the same field. Scale bar, 50 μm.

Figure S4. Effects of 3-MA on hippocampal injury following CA at different time points after ROSC. Rats were pre-treated with 600 nmol 3-MA at 1.5, 3, and 6 h after ROSC and were euthanized for fluorescent morphological analyses at 12 h after ROSC. (1) Sham treatment. (2) CA + DMSO treatment. (3) CA + 3-MA treatment 1.5 h after ROSC. (4) CA + 3-MA treatment 3 h after ROSC. (5) CA + 3-MA treatment 6 h after ROSC. Representative images of dual-label fluorescence of LC3-II (green) with NeuN (red) in CA1 at 12h post-resuscitation and counterstaining of nuclei with DAPI (blue). LC3-II staining was indistinct in the CA1 of the sham animals. 12 h after ROSC, the intensity and punctate appearance increased in the cytosol of CA1. Rats treated with 3-MA at 3 h and 6 h after ROSC were compared with those treated with 3-MA 1.5 h after ROSC. NeuN immunoreactivity (red) marked the neurons. LC3-II were predominantly localized to NeuN-expressing pyramidal neurons in CA1, confirmed using superimposed images

from the same field. Scale bar, 50 μm.

Fig. S1

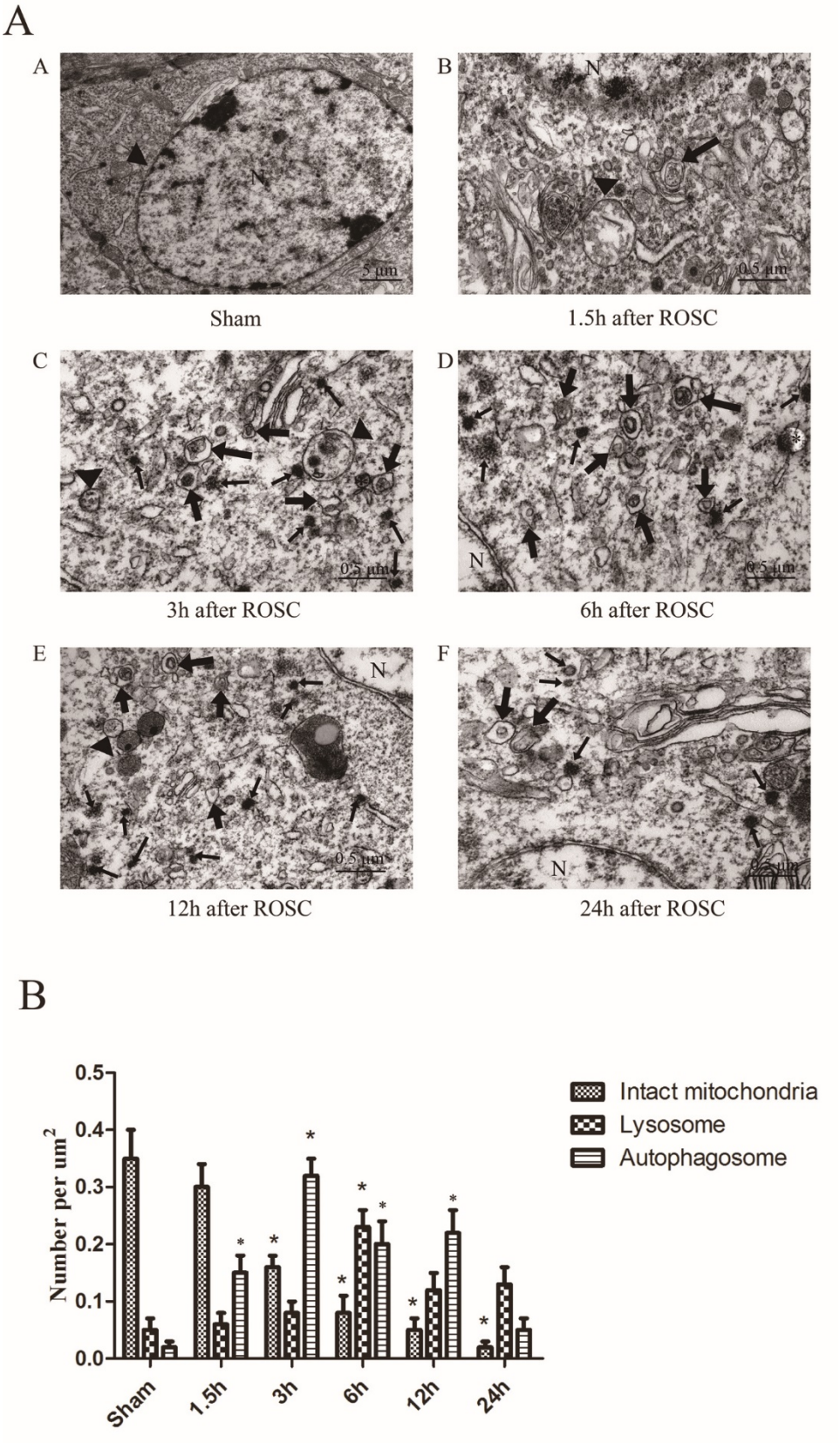

A
A
N
5 μm
Sham
B
N
0.5 μm
1.5h after ROSC
C
0.5 μm
3h after ROSC
D
N
0.5 μm
6h after ROSC
E
N
0.5 μm
12h after ROSC
F
N
0.5 μm
24h after ROSC
B
Number per um2
0.5
0.4
0.3
0.2
0.1
0.0
Sham
1.5h
3h
6h
12h
24h
Intact mitochondria
Lysosome
Autophagosome

Fig. S2

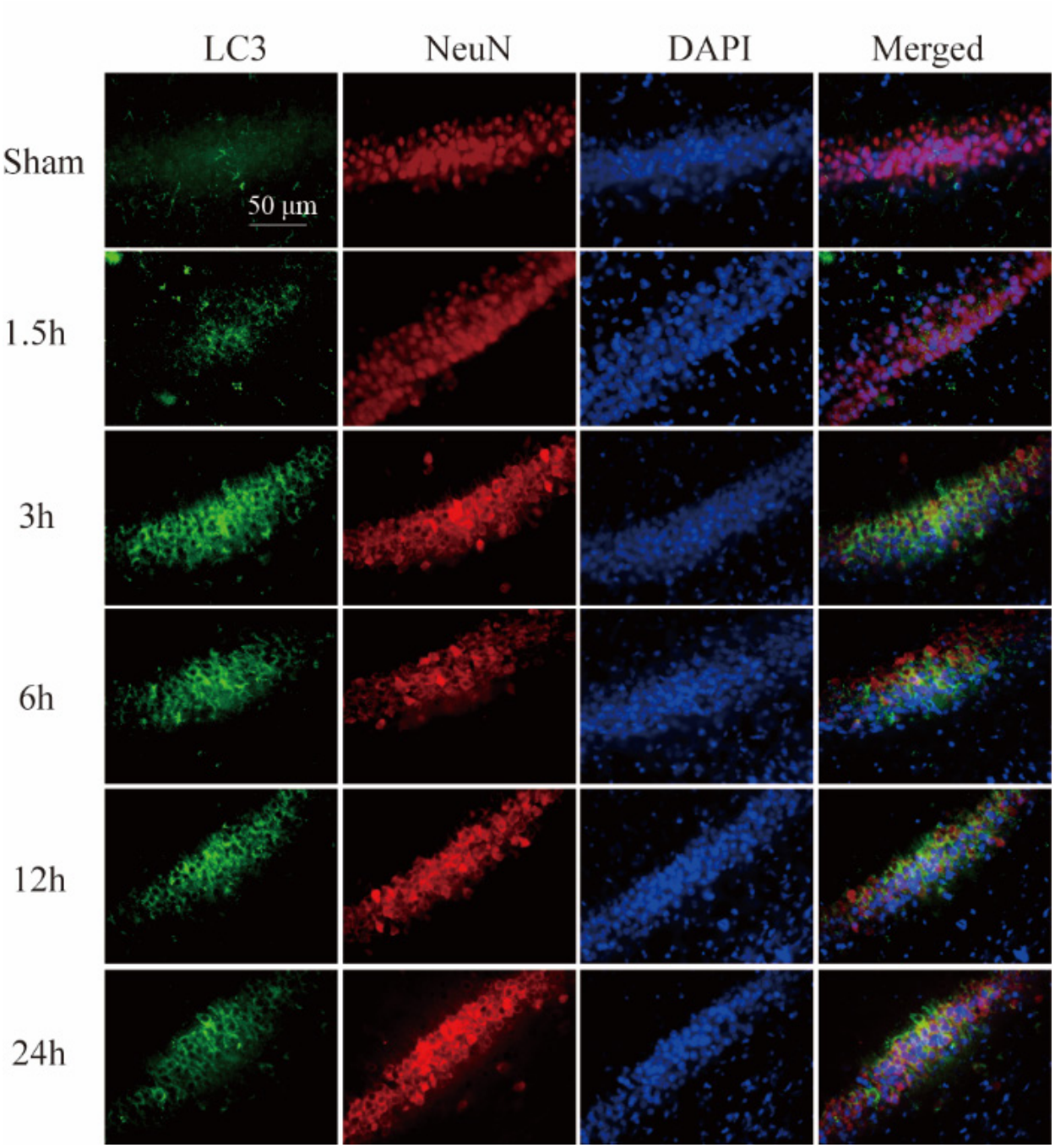


Fig. S3

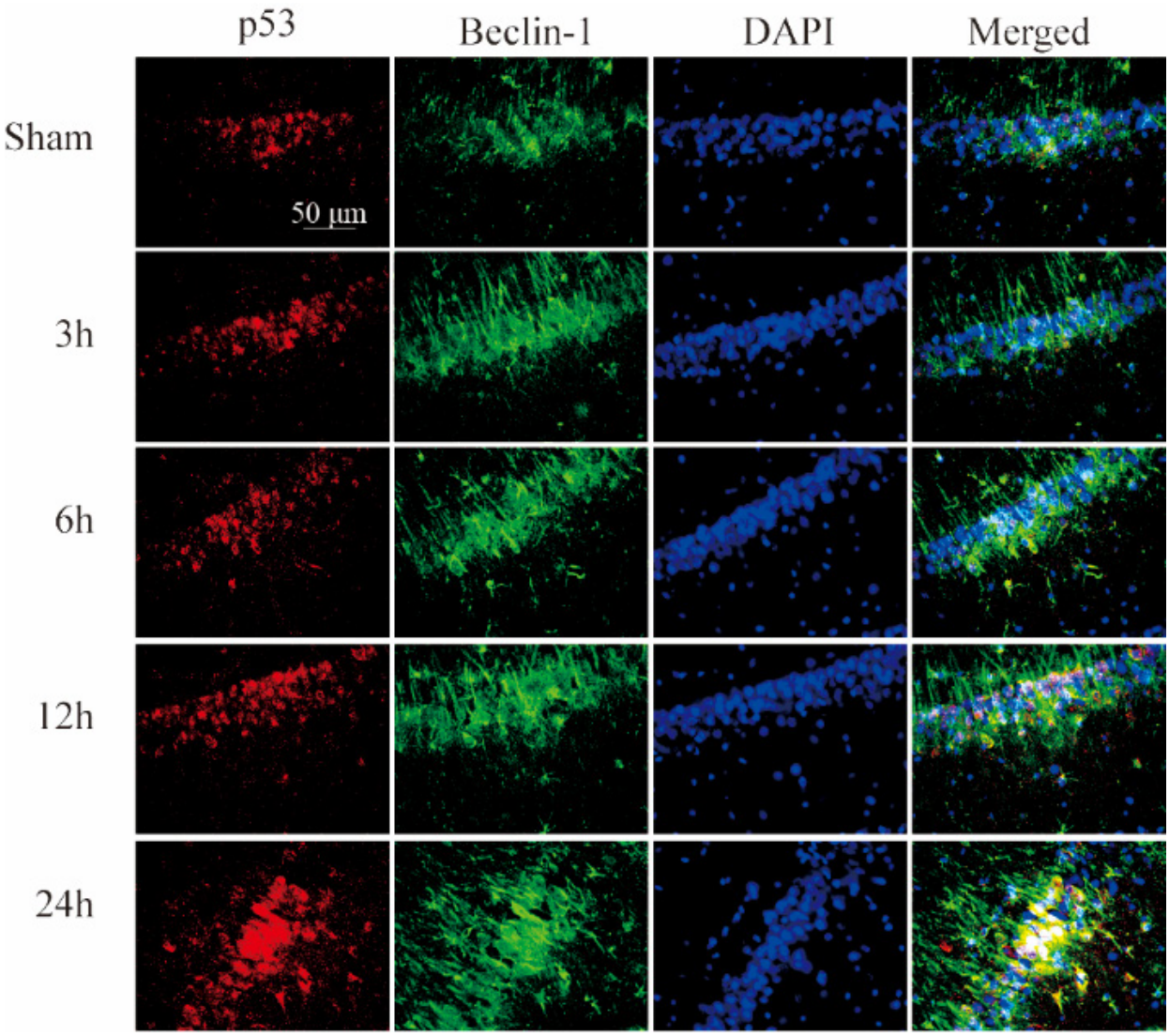
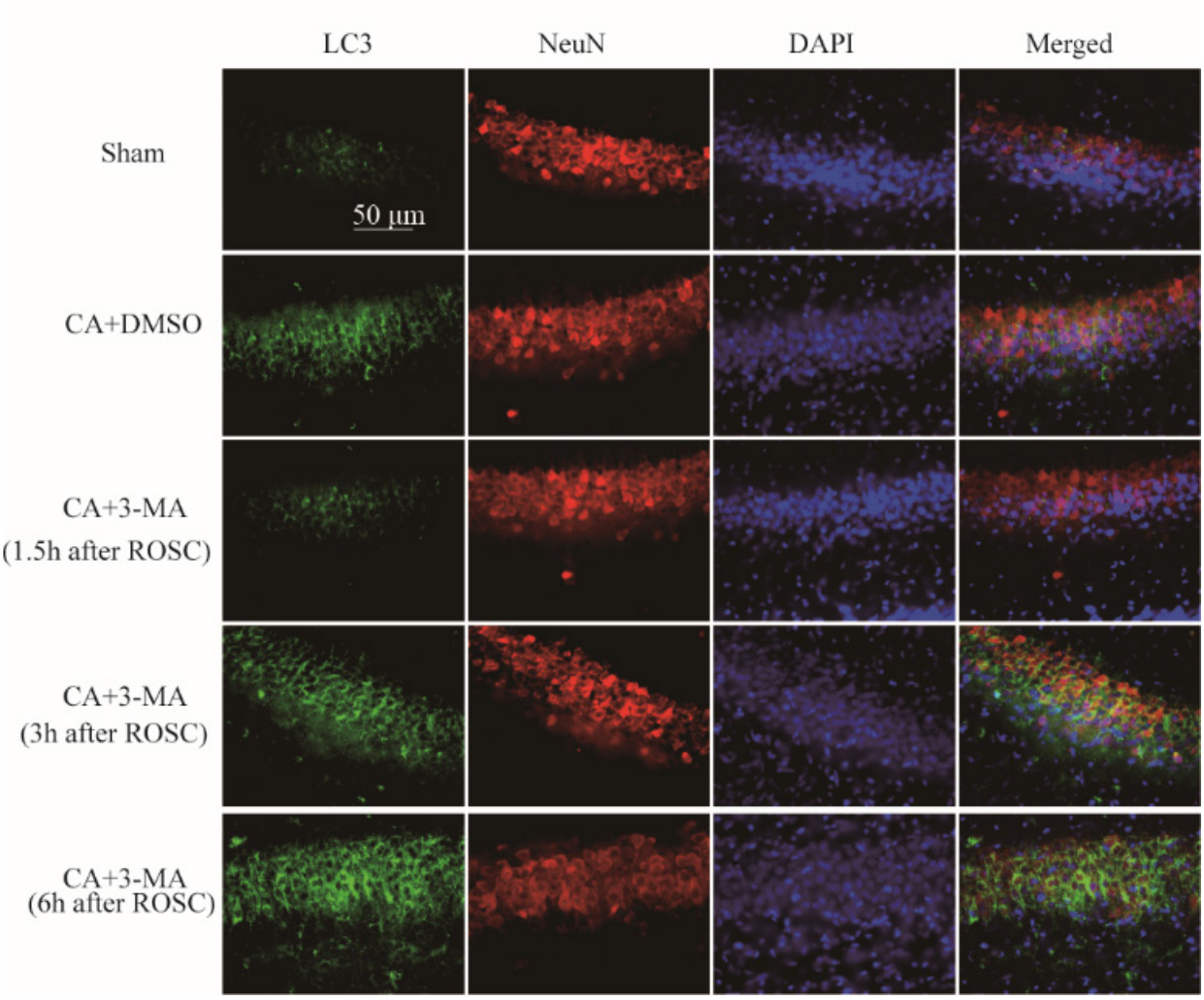


Fig. S4


LC3
NeuN
DAPI
Merged
Sham
50 μm
CA+DMSO
CA+3-MA
(1.5h after ROSC)
CA+3-MA
(3h after ROSC)
CA+3-MA
(6h after ROSC)