

Fig. S1: Normal brain architecture was found in Nissl-stained sections of wild type (WT) and small ubiquitin-like modifier knockdown (TG) mouse brains. Brains were perfusion-fixed with paraformaldehyde and embedded in paraffin. Brains were serially sectioned at 5 μ m. After rehydration, sections were stained with 0.1% cresyl violet solution for 15 minutes and then dehydrated. Images were taken using the Axio observer Z1 microscope. Multiple images were stitched together automatically by the tiling function of the software. Scale bar: 1 mm.

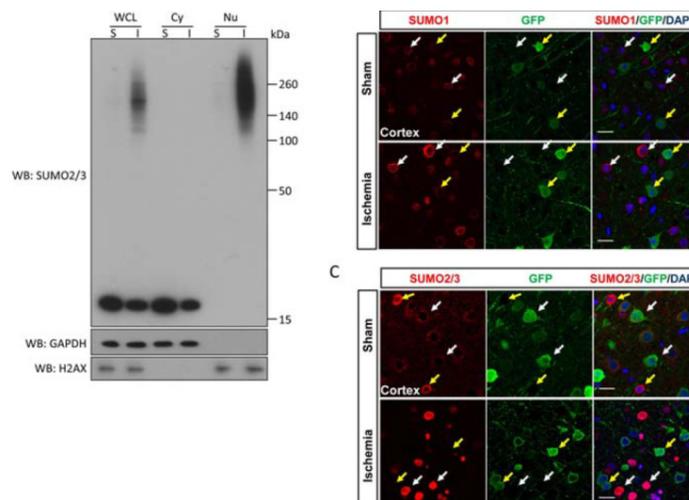


Fig. S2: Small ubiquitin-like modifier (Sumo)-1 and Sumo2/3 immunoreactivities were markedly reduced in green fluorescent protein (Gfp)-positive cortical neurons in postischemic brains. **(A)** Sumo2/3 conjugated proteins induced by transient global cerebral ischemia were accumulated in nuclei. C57BL/6 mice were subjected to sham surgery (S) or 10-minute forebrain ischemia and 1 hour of reperfusion (I), as described elsewhere.¹ Whole cell lysates (WCL), cytoplasmic (Cy) and nuclear (Nu) fractions were prepared from cortical tissues and analyzed by Western blotting. Free and conjugated Sumo2/3 were confined to cytoplasmic and nuclear fractions, respectively. Glyceraldehyde 3-phosphate dehydrogenase (Gapdh) and H2A histone family, member X, (H2ax) were used as cytoplasmic and nuclear marker proteins. **(B,C)** Effect of Sumo1–3 miRNA expression of **(B)** *Sumo1* and **(C)** *Sumo2/3* immunoreactivities in cortical neurons after global cerebral ischemia. The Sumo knockdown mice were subjected to sham surgery or 10-minute forebrain ischemia and 1 hour of reperfusion. Paraffin-embedded brain sections were used for immunohistochemistry. When brains were stressed by transient ischemia, the effects of Sumo1–3 miRNA expression on Sumo1–3 miRNAs (Gfp-negative cells, white arrows) exhibited strong Sumo1 and Sumo2/3 immunoreactivities after ischemia. In contrast, Sumo1 and Sumo2/3 immunoreactivities were markedly reduced in postischemia cells expressing Sumo1–3 miRNAs (Gfp-positive cells, yellow arrows). Furthermore, the strong Sumo2/3 immunoreactivity (Sumo2/3-conjugated proteins) in nuclei of postischemic cells not expressing *Gfp* was almost completely absent in Gfp-positive cells. Scale bar: 20 μ m. WB = Western blot.

Reference

1. Yang W, Sheng H, Warner DS, et al. Transient global cerebral ischemia induces a massive increase in protein sumoylation. *J Cereb Blood Flow Metab* 2008;28:269-79.