

Supplementary

Supplementary Methods:

Primary antibodies for single-labelled immunohistochemistry

Primary antibodies include mouse monoclonal antibody against MLKL (diluted 1:2000; MilliporeSigma, Burlington, MA, USA, Cat# MABC604, RRID: AB_2820284), rabbit monoclonal antibody against phosphorylated MLKL (p-MLKL) (Ser345; diluted 1:2000; Abcam, Cambridge, MA, USA, Cat# ab196436, RRID: AB_2687465), and mouse monoclonal antibody against neuronal nuclei (NeuN; diluted 1:6000; MilliporeSigma, Cat# MAB377, RRID: AB_2298772).

Primary antibodies for double-fluorescent immunohistochemistry

Primary antibodies include rabbit monoclonal antibody against p-MLKL (Ser345; diluted 1:200; Abcam, Cat# ab196436, RRID: AB_2687465), mouse monoclonal antibody against NeuN (diluted 1:3000; MilliporeSigma, Cat# MAB377, RRID: AB_2298772), mouse monoclonal antibody against Iba-1 (diluted 1:100; Wako, Chuo-ku, Osaka, Japan, Cat# 016-26721, RRID: AB_2811160), mouse anti-GFAP (diluted 1:3000; MilliporeSigma, Cat# MAB360, RRID: AB_11212597), Cy3-conjugated goat anti-rabbit IgG antibody (diluted 1:100; MilliporeSigma, Cat# AP132C, RRID: AB_92489), and FITC-conjugated goat anti-mouse IgG antibody (diluted 1:100; MilliporeSigma, Cat# AP308F, RRID: AB_92634).

Primary antibodies for western blotting

The primary antibodies include mouse polyclonal antibody against MLKL (diluted 1:1000; MilliporeSigma, Cat# MABC604, RRID: AB_2820284), rabbit monoclonal antibody against p-MLKL (Ser345; diluted 1:1000; Abcam, Cat# ab196436, RRID: AB_2687465), rat monoclonal antibody against IL-1R1 (diluted 1:1000; Abcam, Cat# ab8154, RRID: AB_306312), mouse monoclonal antibody against glyceraldehyde 3-phosphate dehydrogenase (GADPH; diluted 1:10,000; Proteintech Group, Chicago, IL, USA, Cat# 60004-I-Ig, RRID: AB_2107436), and rabbit monoclonal antibody against Na⁺/K⁺-ATPase (diluted 1:1000; Cell Signaling Technology, Danvers, MA, USA, Cat# 3010, RRID: AB_2060983).

Primary antibodies for immunoprecipitation

The primary antibodies include rabbit monoclonal antibody against RIP3 (diluted 1:1000; Prosci, Fort Collins, CO, USA, Cat# 2283, RRID: AB_203256), rat monoclonal antibody against IL-1R1 (diluted 1:1000; Abcam, Cat# ab8154, RRID:

AB_306312), and rabbit monoclonal antibody against p-MLKL (diluted 1:1000; Abcam, Cat# ab196436, RRID: AB_2687465).

Supplementary Figures

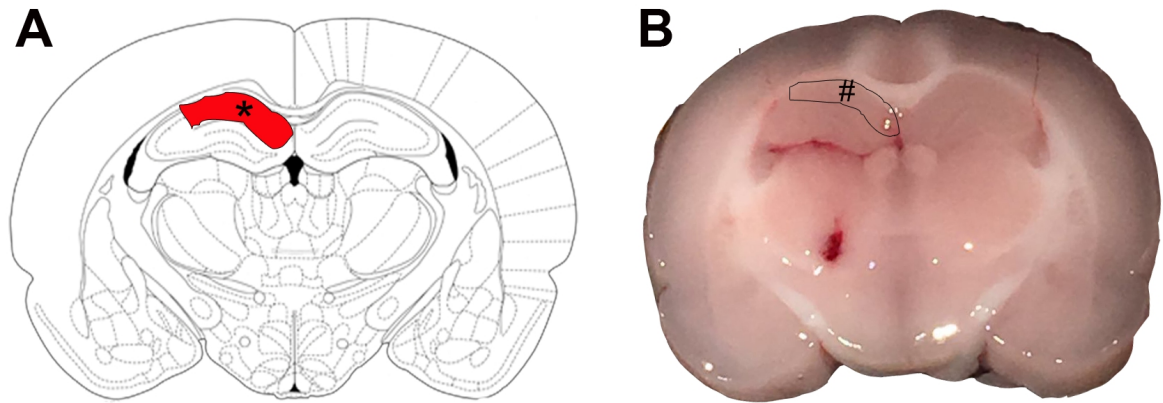


Fig. S1 Schematic illustration of hippocampal CA1 region. A (*red area) and B (#light brown area) indicates hippocampal CA1 region, which is located within the area from bregma -2.30 mm to -4.52 mm based on the rat brain atlas by Paxinos and Watson (1997).

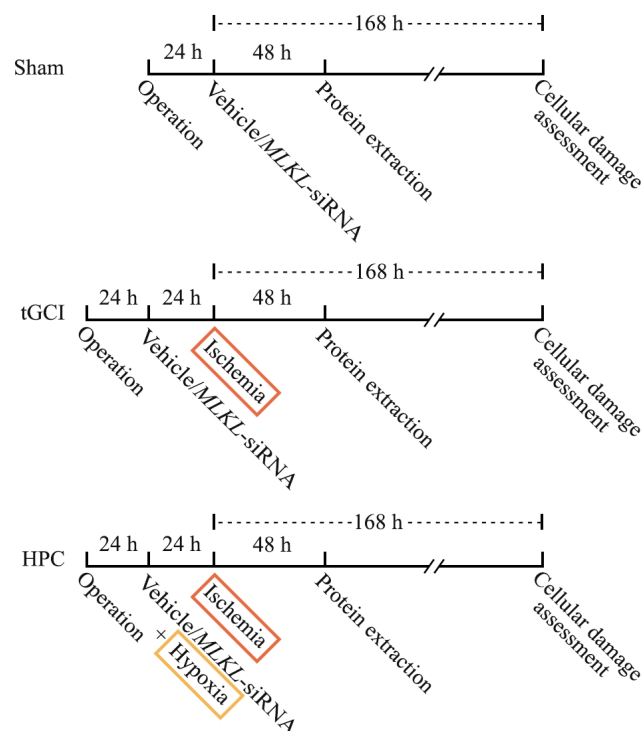


Fig. S2 Design of experiments in which rats were stereotactically injected bilaterally with MLKL-siRNA in the dorsal CA1 pyramidal layer and subjected to either Sham or tGCI with or without hypoxia.

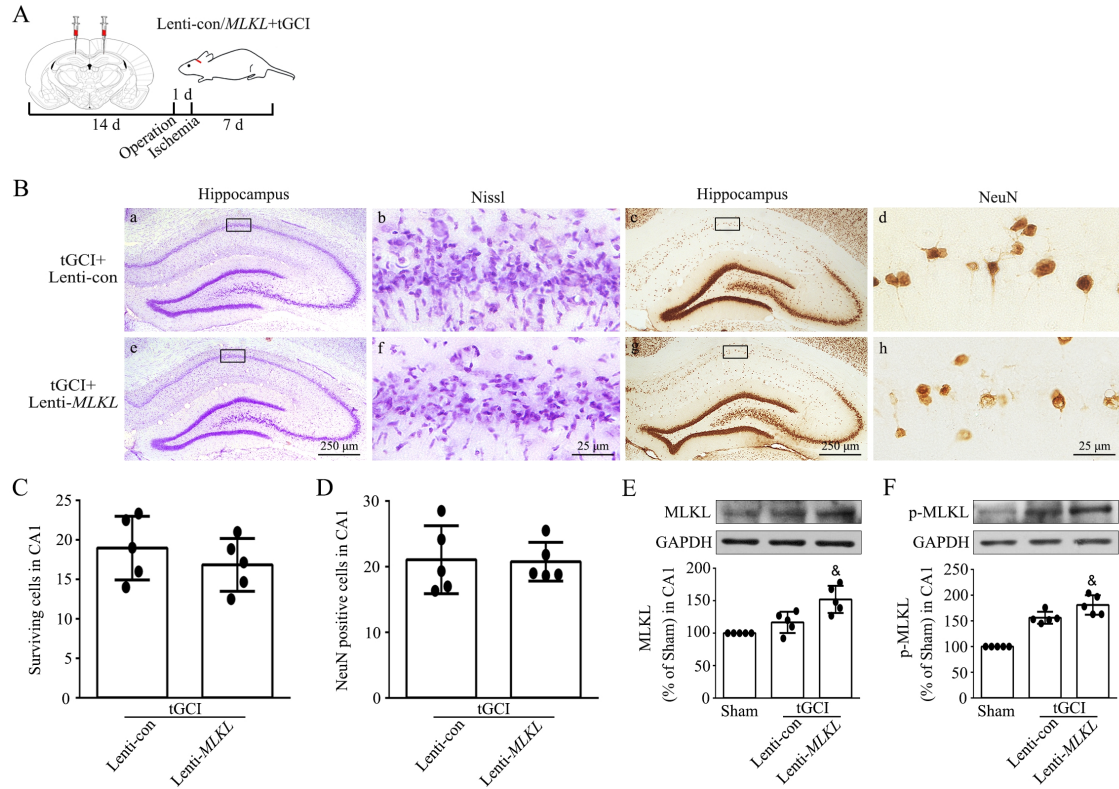


Fig. S3 Overexpression of MLKL increases phosphorylated MLKL, but not aggravates the neuronal damage in CA1 after tGCI. (A) Design of experiments in which rats were stereotactically injected bilaterally with MLKL lentiviral vectors in the dorsal CA1 pyramidal layer and subjected to tGCI. (B) Cresyl violet stained and NeuN immunostained hippocampal sections from rats administered bilaterally with either Lenti-control or Lenti-*MLKL* at 7 d after reperfusion of tGCI. Boxes indicate that the magnified regions displayed in the right panel. (C & D) Quantitative analyses of surviving cells and NeuN-positive cells in CA1. Each bar represents the mean $\pm$ S.D. (E & F) Representative immunoblots of MLKL and p-MLKL (Ser345) expression in CA1 after tGCI with either Lenti-control or Lenti-*MLKL* administration. The histogram presents the quantitative analyses of MLKL or p-MLKL protein. Data are expressed as percentage of value of Sham animals. Each bar represents the mean $\pm$ S.D. & p <0.05 vs. tGCI group with Lenti-con. Lenti-con, Lenti-Control, scrambled lentivirus vector; Lenti-*MLKL*, *MLKL*-carried lentivirus.

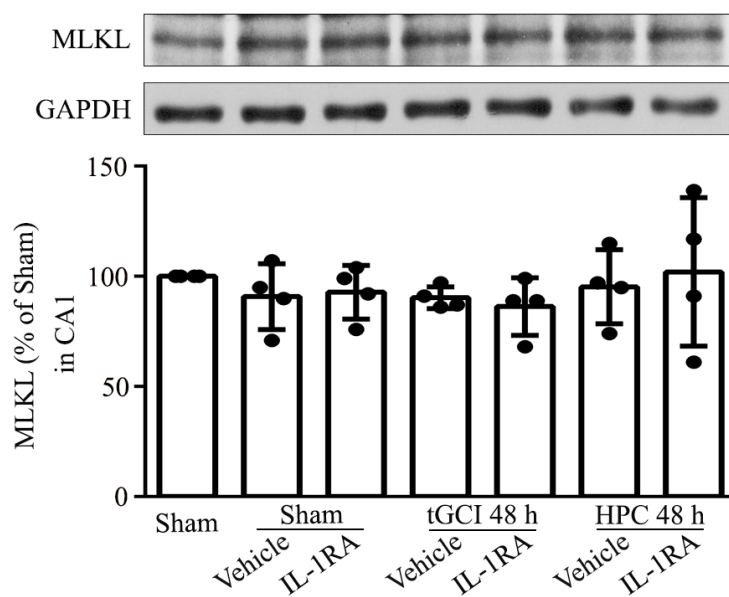


Fig. S4 Effects of pretreatment with IL-1RA on the MLKL expression in CA1 of tGCI rats with or without hypoxia using immunoblot analysis. Each bar represents the mean $\pm$ S.D. HPC, hypoxic preconditioning.