A:

Adult male SD rats (n = 183 rats)

- Sham (n = 30 rats)
- ICH+Vehicle-1 (n = 30 rats)
- ICH+ILG 10 mg/kg (n = 30 rats)
- ICH+ILG 20 mg/kg (n = 30 rats)
- ICH+ILG 40 mg/kg (n = 30 rats)

- mNSS score (n = 24 rats)
- Event blue (n = 12 rats)

- Brain water content (n = 12 rats)
- H&E staining (n = 12 rats)
- FJC staining (n = 12 rats)

- 24 h after ICH (n = 6 rats)
- 72 h after ICH (n = 6 rats)

B:

Adult male SD rats (n = 122 rats)

- Sham (n = 30 rats)
- ICH (n = 30 rats)
- ICH+Vehicle-1 (n = 30 rats)
- ICH+ILG 20 mg/kg (n = 30 rats)

- RT-qPCR (n = 6 rats)
- WB (n = 6 rats)
- H&E / IF (n = 6 rats)
- ELISA (n = 6 rats)
- Other objects (n = 6 rats)

- NLRP3, ASC, Caspase-1, IL-1β, IL-18, NQO1, HO-1
- Nrf2 pathway
- NF-κB pathway
- NLRP3 pathway
- IL-1β, IL-18

C:

Adult male SD rats (n = 137 rats)

- Sham (n = 24 rats)
- ICH+Vehicle-2 (n = 24 rats)
- ICH+Control scramble siRNA (n = 24 rats)
- ICH+Nrf2 siRNA (n = 24 rats)
- ICH+Nrf2 siRNA+Vehicle-1 (n = 18 rats)
- ICH+Nrf2 siRNA+ILG 70 mg/kg (n = 18 rats)

- Effectiveness of Nrf2 siRNA (n = 6 rats)

- RT-qPCR, WB

- mNSS score (n = 18 rats)

- WB (n = 6 rats)
- Brain water content (n = 6 rats)
- FJC staining (n = 6 rats)

- Total NF-κB p65, NLRP3, ASC, Caspase-1, IL-1β
MRI and Hematoma Volume Evaluation

Experiment design and groups:

Thirty rats were used in this study. Rats were randomly divided into five groups, namely, sham group, ICH + vehicle-1 24 h, ICH + ILG 20 mg/kg 24 h, ICH + vehicle-1 72 h, ICH + ILG 20 mg/kg 72 h. At the corresponding time points, rats were performed magnetic resonance imaging (MRI) and then hematoma volume evaluations were carried out (n = 6).

Methods:

MRI was conducted at 24 h and 72 h after ICH induction using a 7.0-T small-animal PharmaScan 70 / 16 MRI scanner (Bruker, USA) as previously performed[1, 2]. The rats were anesthetized with isoflurane and T2-weighted images (T2WI) were obtained using following parameters: Resolution matrix = 256 × 256, Field of view (FOV) = 35 mm × 35 mm, Slice number = 10, Slice thickness = 0.8 mm, slice gap = 0, TR / TE = 2500 ms / 33 ms. A skillful technician performed the image acquisition in a blinded manner[1, 2].

After MRI being performed, rats were deeply anesthetized and sacrificed to obtain whole brain, then serially sliced (2 mm thickness) anterior and posterior to the needle.
entry site (identifiable on the brain surface). Coronal brain slices were photographed with a digital camera to get digital images. Then, the images were analyzed and hematoma volume was computed by using an Image J software package (National Institutes of Health, Baltimore, MD)[3, 4].

References:


E:

Additional file 2

Effects of ILG on the hematoma volume and expansion at 24 h and 72 h after ICH (A, B) and effects of ILG on the number of CD68+, Iba-1+ cells in the perihematomal brain tissue at 24 h after ICH (C-E). Representative MRI T2WI images (A) and quantitative analyses of hematoma volume (B) (n = 6 rats / group). Representative microscopic images (C) and quantitative analyses of CD68+, Iba-1+ cells (D, E) (n = 6 rats / group). Scale bar = 20 μm. Values are reported as means ± SD. ** p < 0.01, * p
Additional file 3

Mechanism diagram. Underlying molecular mechanisms of ILG’s neuroprotective effects on the early brain injury after ICH induction. ILG alleviated the early brain injury following ICH may be involved in the regulation of ROS and/or NF-κB on the activation of NLRP3 inflammasome pathway by the triggering of Nrf2 activity and the induction of Nrf2-mediated antioxidant system.