

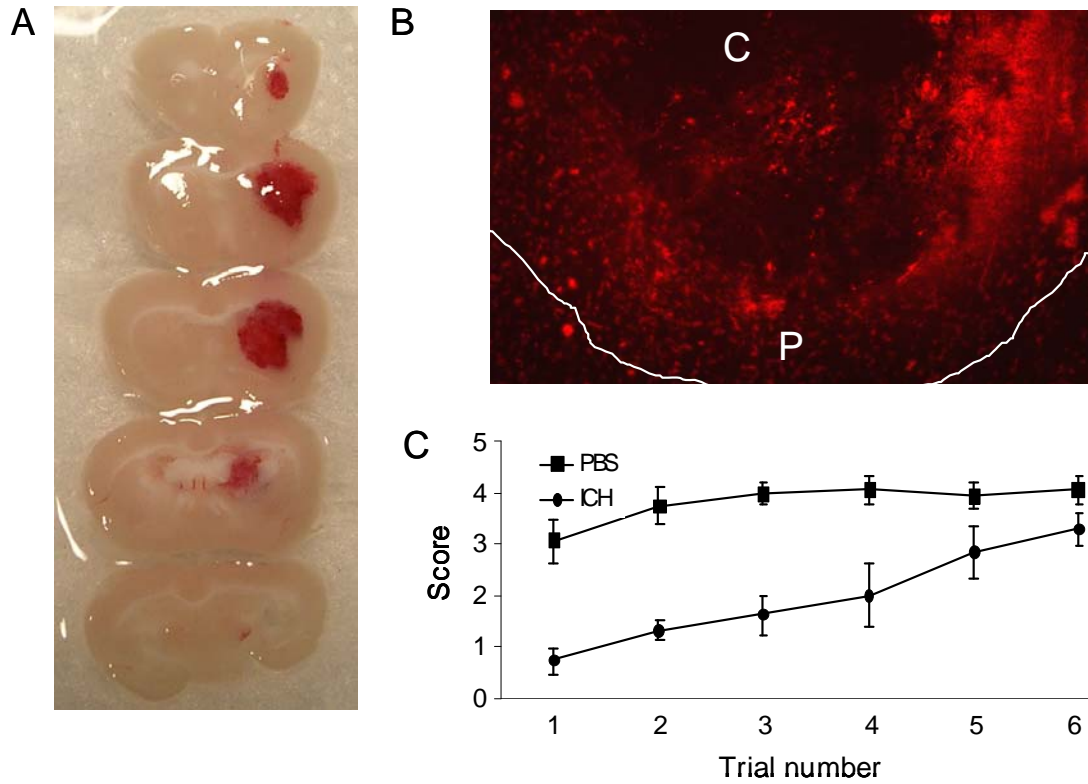
SUPPLEMENTARY MATERIALS

Supplemental Table 1: Summary of Experiments

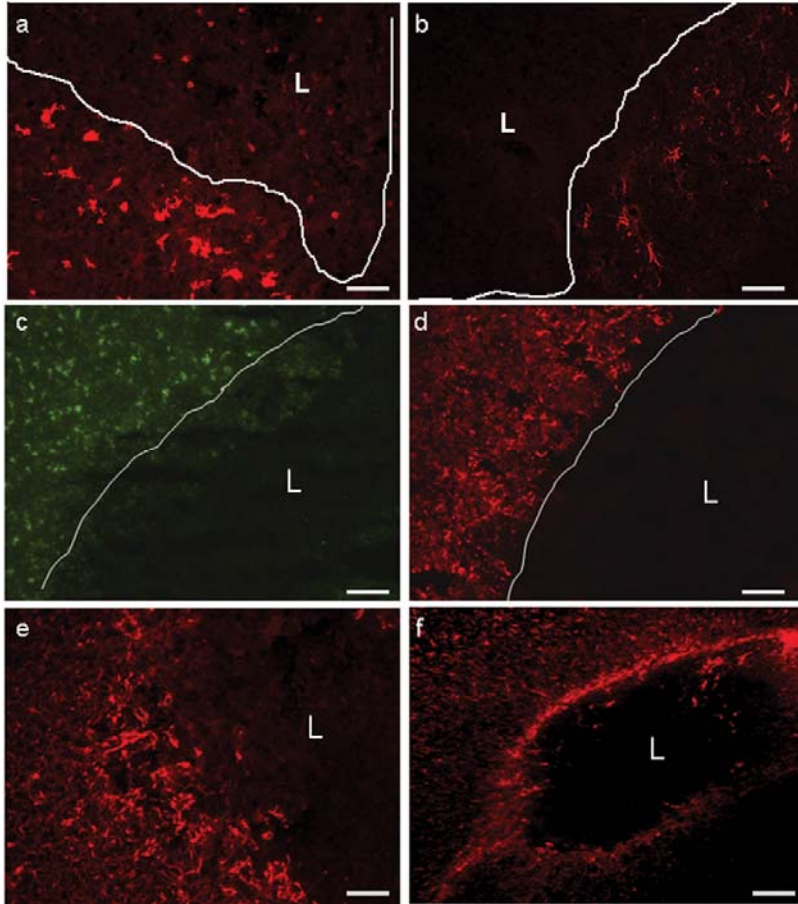
Experiment	Time points examined	Number of mice
PI+ cell time course PI+ cells: RIPK3 KO vs WT	3, 6, 24, 48, 72 h and 7 d 24 h	4-5/group, total 29 5/group
Immunohistochemistry: GFAP, IBA-1, NeuN, HMGB1	6, 24, 48, 72 h	3-5/group
TUNEL labeling Caspase substrate labeling	6, 24, 48 h	4-8/group ^a
Wire grip testing	1- 7 days	8/group
Electron microscopy	24 h	3
PI+ cell survival, resealing	24-48 and 48-72 h	4-5/group

a: For quantitation of labeled cells at 24 h (n = 8) and 48 h (n = 4)

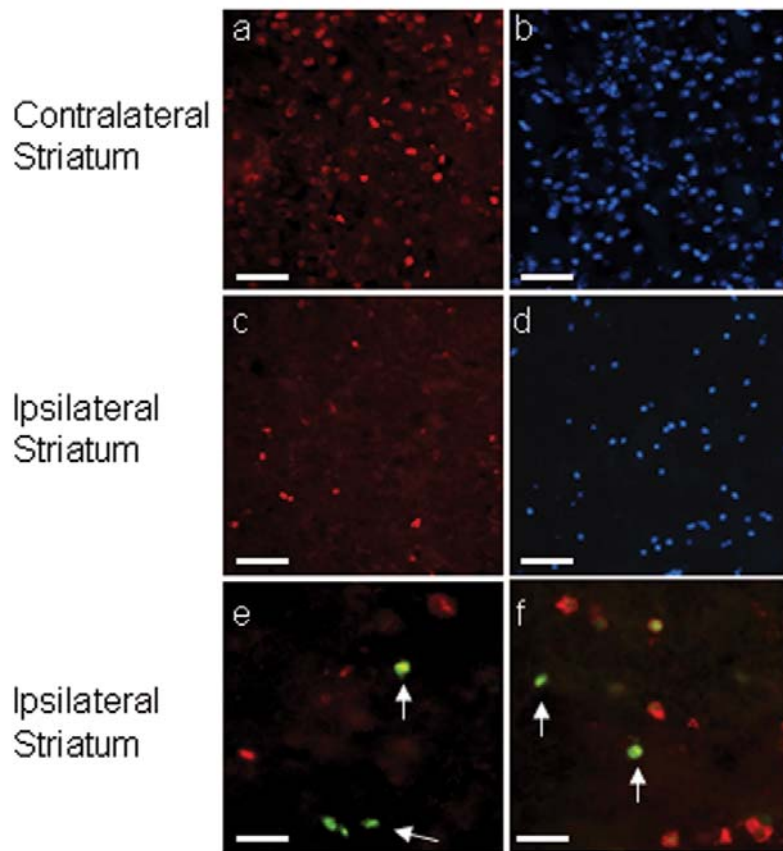
PI+, Propidium iodide-positive; IHC, Immunohistochemistry; GFAP, glial fibrillary acidic protein; IBA-1, ionized calcium binding adaptor molecule 1; NeuN, neuronal nuclear marker; TUNEL, terminal deoxynucleotidyl transferase mediated nick end labeling.



Supplemental Figure 1. (A) Intracerebral hemorrhage produced by collagenase injection. (B) Blood brain barrier damage assessed by Evans blue fluorescence in hemorrhagic brain. C, core; P, periphery; NI, normal brain. (C) Motor deficits assessed by the wire grip score after collagenase intracerebral hemorrhage. $p < 0.01$ for group.



Supplemental Figure 2: Localization of IBA-1+ cells (microglia/monocytes) and GFAP+ cells (astrocytes) after intracerebral hemorrhage. (a) IBA-1+ microglia and (b) GFAP+ astrocytes surround the periphery of the hemorrhagic lesion (L) as early as 6 h. At 72 h IBA-1+ cells (c) and GFAP+ cells (d) reside exclusively around the outer limits of the hemorrhagic lesion. GFAP+ Astrocytes surround the lesion at 7 (e), and some are observed entering the lesion core at 14 d (f). Scale bars: a, b 10 μ m; c, d 40 μ m; e, 5 μ m; f, 100 μ m



Supplemental Figure 3: HMGB1 is released after intracerebral hemorrhage. Mice were subjected to collagenase ICH and cells with plasmalemma damage were labeled with YOYO-1 (green) at 5-6 h. Mice were killed at 6 h and assessed for HMGB1 (red) release by immunostaining. (a-d) Lack of HMGB1 immunostaining in ipsilateral compared to contralateral striatum (a, c) is not fully explained by loss of cell density as evidenced by Hoechst staining (blue) (b, d). YOYO-1+ cells rarely colocalized with HMGB1, indicating release from necrotic cells (arrows in e, f). Scale bars, a-d, 100 μ m; e-f, 40 μ m.