SUPPLEMENTAL MATERIAL

Genetic inhibition of RIPK1 reduces cell death and improves functional outcome after intracerebral hemorrhage in mice

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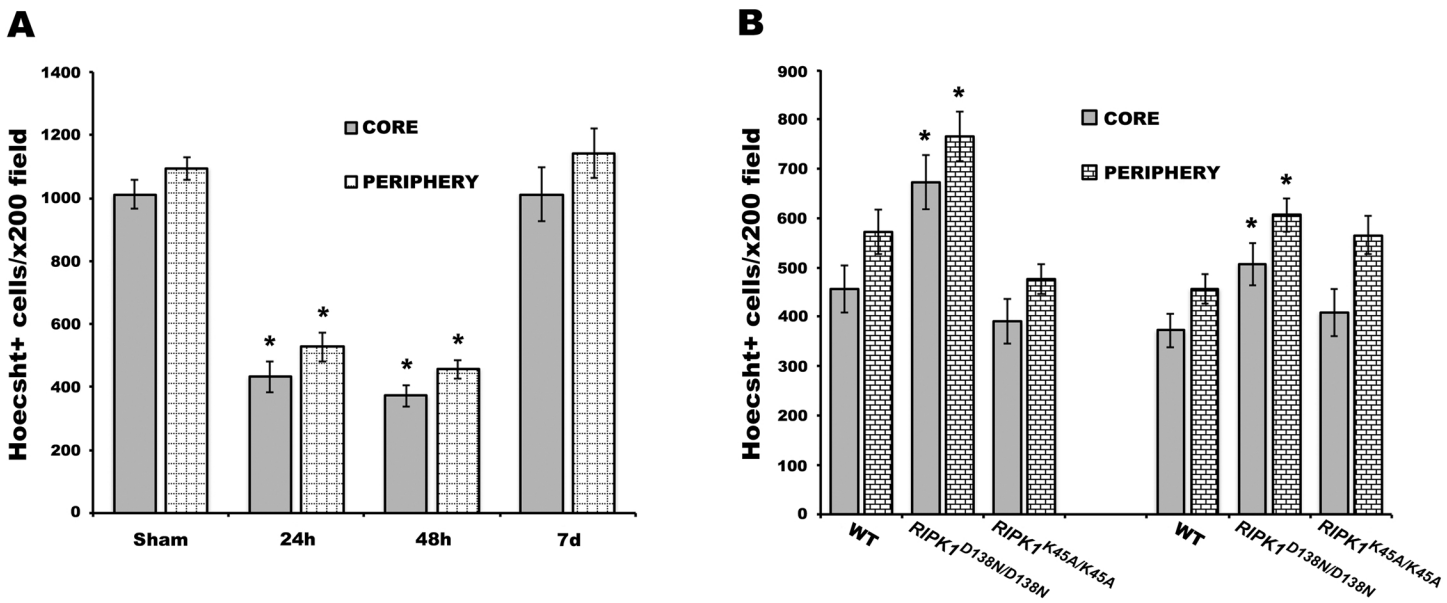
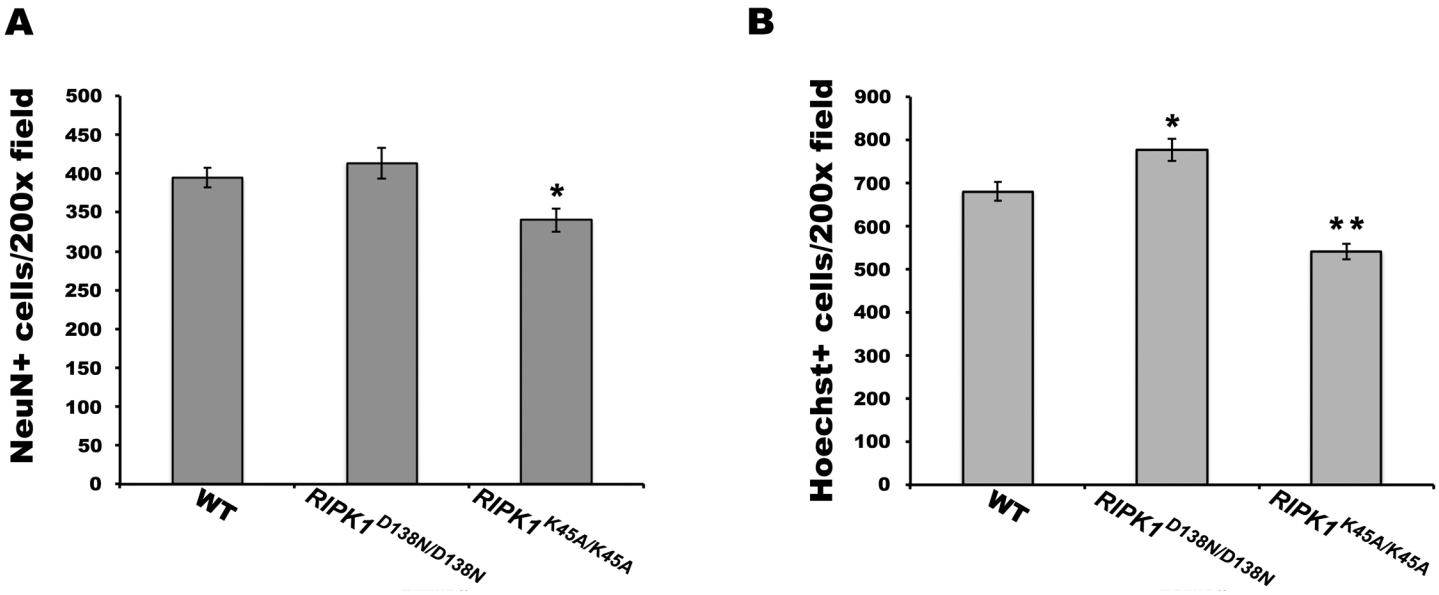
Supplemental Figures



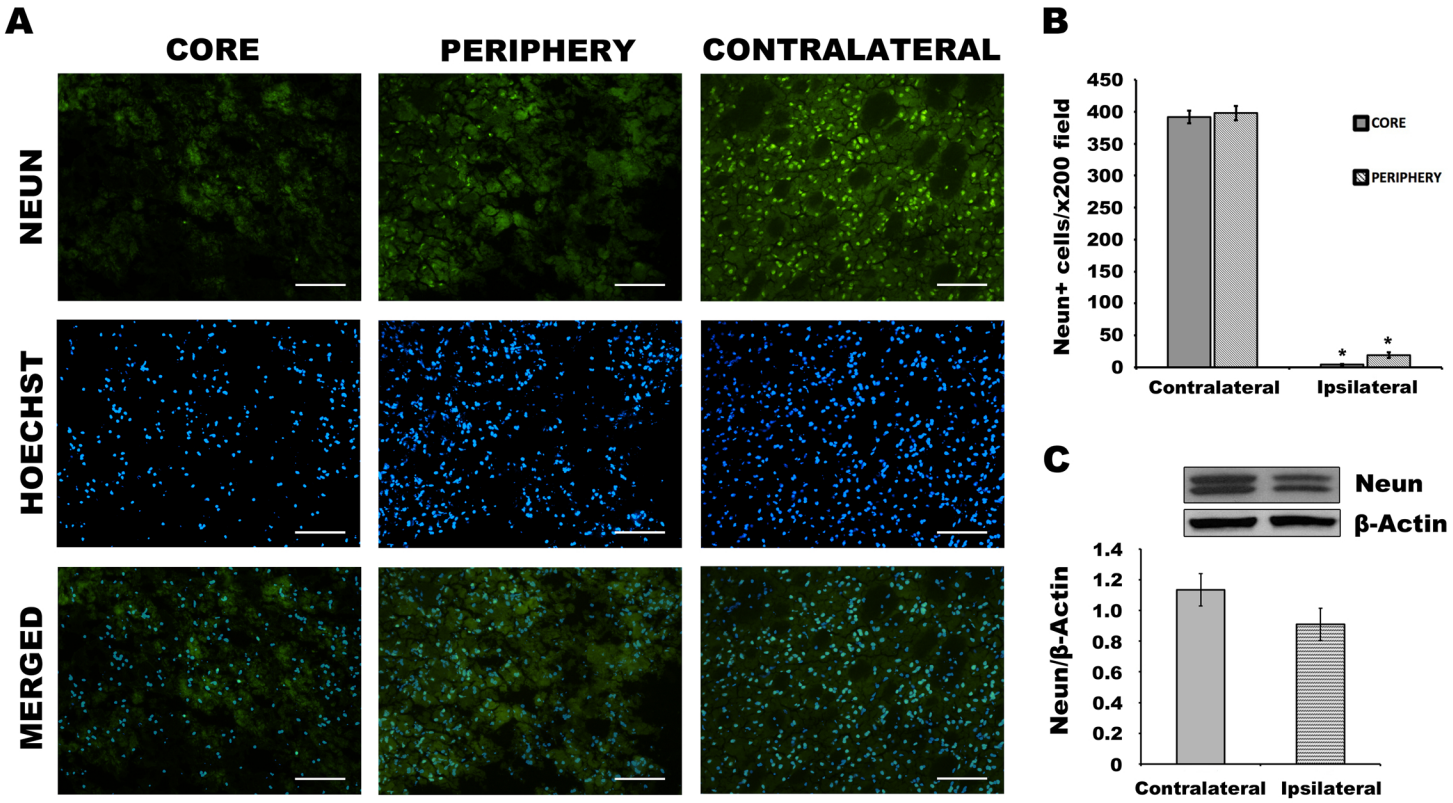
Supplemental Figure I.

Cell count data using Hoechst dye for nuclear staining in wild type and RIPK1 mutant mice. (A) Time course of cell loss after intracerebral hemorrhage (ICH) in wild type (WT) mice assessed by Hoechst stain *p < 0.0001 vs. the corresponding sham-injured brain region. (B) Cell count data at 24 and 48 h after ICH in WT and RIPK1 mutant mice. Compared to WT, *RIPK1*$^{D138N/D138N}$ mice had more Hoechst+ cells remaining in hemorrhagic brain regions at both time points. *p < 0.05 vs. WT.



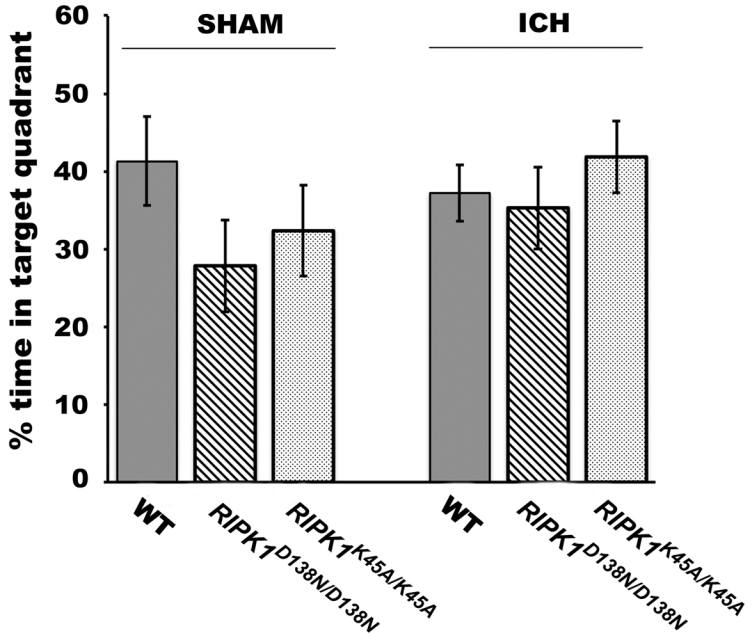
Supplemental Figure II.

Assessment of neurons (A) and total nucleated cells (B) in contralateral striatum of wild type (WT) and RIPK1 mutant mice assessed by NeuN and Hoechst staining, respectively, at 24 h after ICH. *RIPK1*$^{K45A/K45A}$ mice had approximately 10% less NeuN+ cells vs. WT (*$p < 0.05$ vs. WT) and 20% Hoechst+ cells (*$p < 0.05$, **$p < 0.005$ vs. WT).



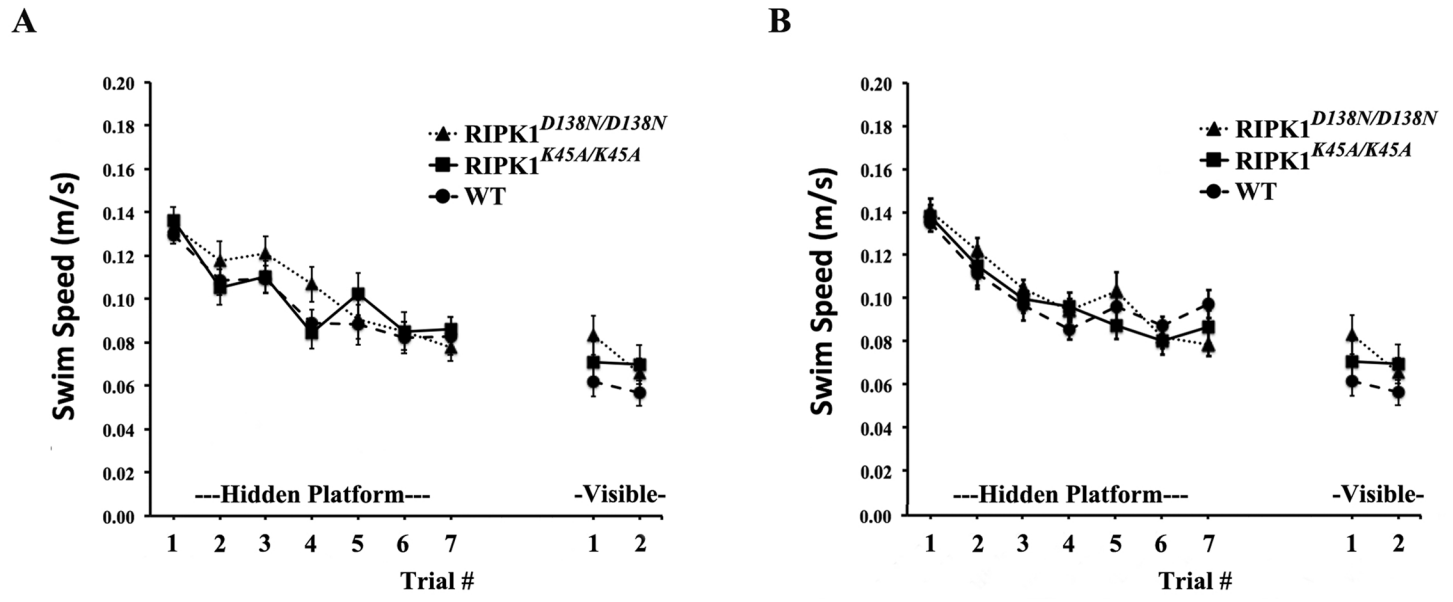
Supplemental Figure III

Loss of NeuN immunoreactivity in hemorrhagic and contralateral brain regions at 24 h after intracerebral hemorrhage. (A) Immunohistochemical detection of NeuN in hemorrhagic brain regions shows loss of NeuN staining compared to Hoechst+ cells and NeuN+ cells in the contralateral striatum. (B) Quantitation of NeuN+ cells in peripheral and core regions of the ICH as well as contralateral striatum. *$p < 0.001$ vs. contralateral striatum. (C) Western blot analysis of NeuN in contralateral and ipsilateral striatum showing no difference in expression after ICH.



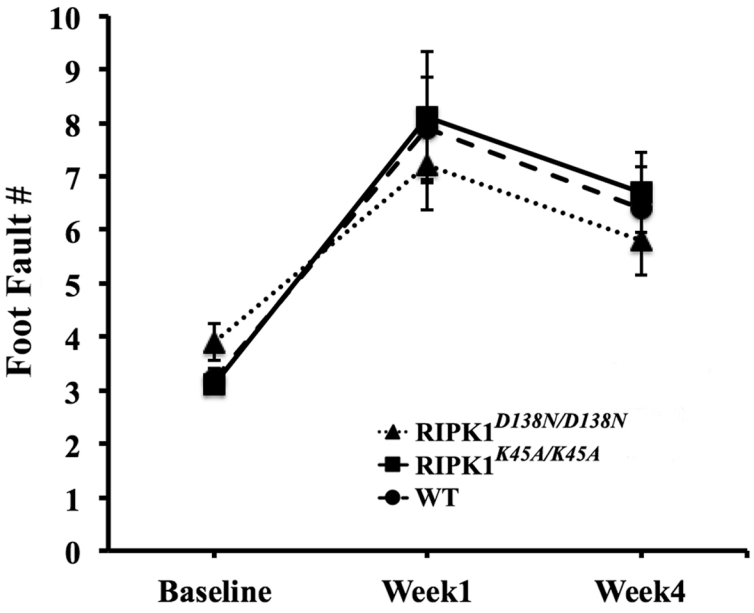
Supplemental Figure IV.

Morris water maze probe trial data. No differences were observed among sham injured groups and among intracerebral hemorrhage (ICH) groups in percent time in the target quadrant, and no differences were observed between corresponding sham and injured groups. n = 9-10/group for sham, n = 15-17/group for ICH.



Supplemental Figure V.

Swim speed data for Morris water maze analyses did not differ among groups in (A) sham (p = 0.74 for group, RM ANOVA) and (B) ICH (p = 0.82 for group, RM ANOVA) groups. n = 9-10/group for sham, n = 15-17/group for ICH.



Supplemental Figure VI.

Sensorimotor deficits before and after autologous blood intracerebral hemorrhage (ICH) in wild type and RIPK1 mutant mice. Mice were assessed at baseline for stepping errors (foot faults) on a grid walk test and then again at 1 and 4 weeks after ICH. ICH induced robust deficits in all groups compared to baseline ($p < 0.001$ for time, RM ANOVA) but there were no group differences ($p = 0.22$ for group, RM ANOVA).

Week 1: WT: $p < 0.0005$ vs. baseline; *RIPK1*$^{D138N/D138N}$: $p < 0.005$ vs. baseline; *RIPK1*$^{K45A/K45A}$: $p < 0.001$ vs. baseline.

Week 4: WT: $p < 0.001$ vs baseline, *RIPK1*$^{D138N/D138N}$: $p < 0.02$ vs. baseline, *RIPK1*$^{K45A/K45A}$: $p < 0.0005$ vs. baseline.n = 10/group.

Supplemental Tables

Experiment	Time points	Number of mice	Randomization	Blinding
PI and Hoechst positivity time course (Figure 1A, B and Supplemental Figure IA)	6h 24h, 48h, 72h and 7d after ICH	(n=6) x 5groups =30 mice	Simple (Random digit)	Group allocation
PI disappering over the time (Figure 1C)	24h after ICH: 6h, 12h and 24h after PI injection 48h after ICH: 6h, 12h and 24h after PI injection	(n=6) x 3groups =18 mice (n=6) x 3groups =18 mice	Simple (Random digit)	Group allocation
HMGB1 and NeuN Immunohistochemistry (Figure 2A and Supplemental Figure IIIA, B)	24h after ICH	n=3 mice	Simple (Random digit)	Group allocation
HMGB1 and NeuN Tissue-Western blotting (Figure 2B and Supplemental Figure III-C)	24h after sham and 24h after ICH	(n=4 sham) + (n=7 ICH) = 11 mice	Simple (Random digit)	Group allocation
HMGB1 CSF-Western blotting (Figure 2C)	24h after sham and 24h after ICH	(n=3 sham) + (n=3 ICH) = 6 mice	Simple (Random digit)	Group allocation
PI and FJB positivity (Figure 3A, B and Figure 4A, B), Hoechst positivity (Supplemental Figure IB and IA) and NeuN positivity (Supplemental Figure IA) WT vs *RIPK1*$^{D138N/D138N}$ and *RIPK1*$^{K45A/K45A}$	24h after ICH	(n=9 WT) + (n=6 *RIPK1*$^{D138N/D138N}$) + (n=6 *RIPK1*$^{K45A/K45A}$) = 21 mice	Simple (Random digit)	Group allocation
PI and FJB positivity (Figure 3C, D and Figure 4C, D): WT vs *RIPK1*$^{D138N/D138N}$ and *RIPK1*$^{K45A/K45A}$	48h after ICH	(n=6 WT) + (n=6 *RIPK1*$^{D138N/D138N}$) + (n=6 *RIPK1*$^{K45A/K45A}$) = 18 mice	Simple (Random digit)	Group allocation
GSK' 963 inhibitor (Figure 5)	24h after ICH	(n=6 vehicle) + (n=6 GSK' 963) = 12 mice	Simple (Random digit)	Group allocation
Sham body weight loss, Morris water maze (MWM) (Figure 6A, C and Supplemental Figure IVA)	Day 0-17 after sham for weight loss, 3-4 weeks after sham for MWM	(n=10 WT) + (n=9 *RIPK1*$^{D138N/D138N}$) + (n=10 *RIPK1*$^{K45A/K45A}$) = 29 mice	Simple (Random digit)	Group allocation, concealment procedure
ICH body weight loss, MWM and foot fault (Figure 6B, D and Supplemental Figure IVB, V and VI)	Day 0-17 after ICH for weight loss, 4 weeks after sham for MWM, Day 0, 7 and 28 after ICH for foot fault	(n=16WT) + (n=17 *RIPK1*$^{D138N/D138N}$) + (n=15 *RIPK1*$^{K45A/K45A}$) = 48 mice	Simple (Random digit)	Group allocation
		TOTAL = 214 mice		

Supplemental Table I.

Descriptive table of experimental groups, the timeline of experimentation and total number of mice.

Stroke Online Supplement

Table I. Checklist of Methodological and Reporting Aspects for Articles Submitted to *Stroke* Involving Preclinical Experimentation

Methodological and Reporting Aspects	Description of Procedures
Experimental groups and study timeline	☑ The experimental group(s) have been clearly defined in the article, including number of animals in each experimental arm of the study. ☑ An account of the control group is provided, and number of animals in the control group has been reported. If no controls were used, the rationale has been stated. ☑ An overall study timeline is provided.
Inclusion and exclusion criteria	☑ A priori inclusion and exclusion criteria for tested animals were defined and have been reported in the article.
Randomization	☑ Animals were randomly assigned to the experimental groups. If the work being submitted does not contain multiple experimental groups, or if random assignment was not used, adequate explanations have been provided. ☑ Type and methods of randomization have been described. ☑ Methods used for allocation concealment have been reported.
Blinding	☑ Blinding procedures have been described with regard to masking of group/treatment assignment from the experimenter. The rationale for nonblinding of the experimenter has been provided, if such was not feasible. ☑ Blinding procedures have been described with regard to masking of group assignment during outcome assessment.
Sample size and power calculations	☑ Formal sample size and power calculations were conducted based on a priori determined outcome(s) and treatment effect, and the data have been reported. A formal size assessment was not conducted and a rationale has been provided.
Data reporting and statistical methods	☑ Number of animals in each group: randomized, tested, lost to follow-up, or died have been reported. If the experimentation involves repeated measurements, the number of animals assessed at each time point is provided, for all experimental groups. ☑ Baseline data on assessed outcome(s) for all experimental groups have been reported. ☑ Details on important adverse events and death of animals during the course of experimentation have been provided, for all experimental arms. ☑ Statistical methods used have been reported. ☑ Numeric data on outcomes have been provided in text, or in a tabular format with the main article or as supplementary tables, in addition to the figures.
Experimental details, ethics, and funding statements	☑ Details on experimentation including stroke model, formulation and dosage of therapeutic agent, site and route of administration, use of anesthesia and analgesia, temperature control during experimentation, and postprocedural monitoring have been described. ☑ Different sex animals have been used. If not, the reason/justification is provided. ☑ Statements on approval by ethics boards and ethical conduct of studies have been provided. ☑ Statements on funding and conflicts of interests have been provided.

Supplemental Table II

Checklist of methodological and reporting aspects for articles submitted to Stroke involving preclinical experimentation.