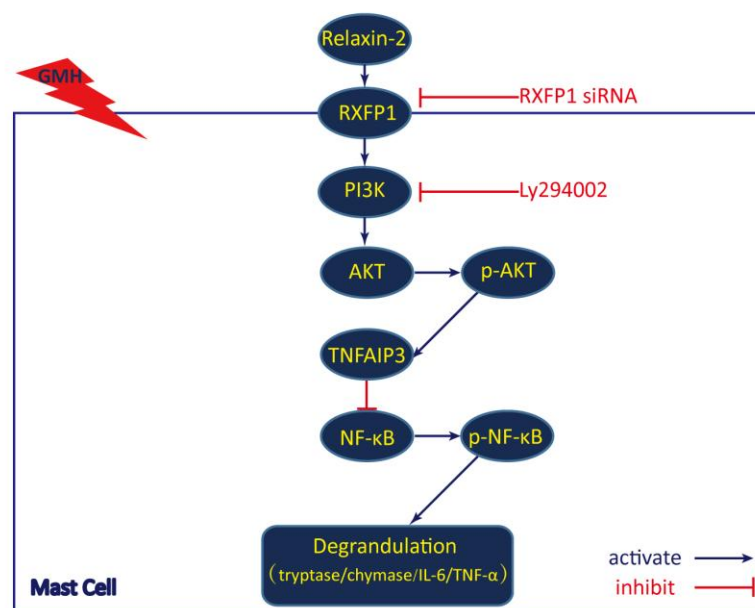


Rh-relaxin-2 Attenuates Degranulation of Mast Cells by Inhibiting NF- κ B through PI3K-

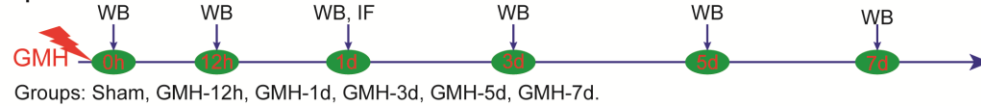
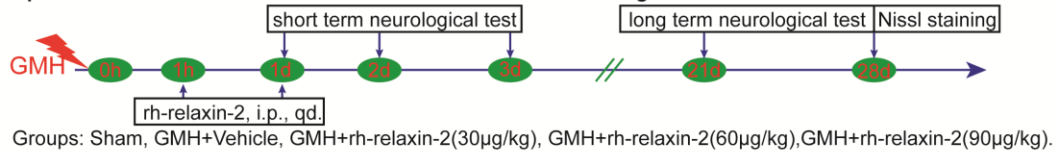
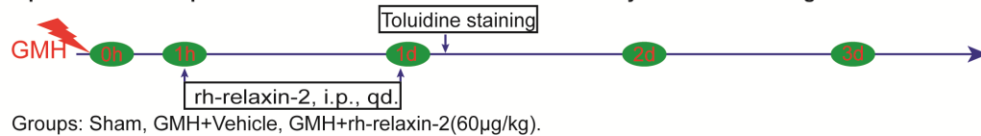
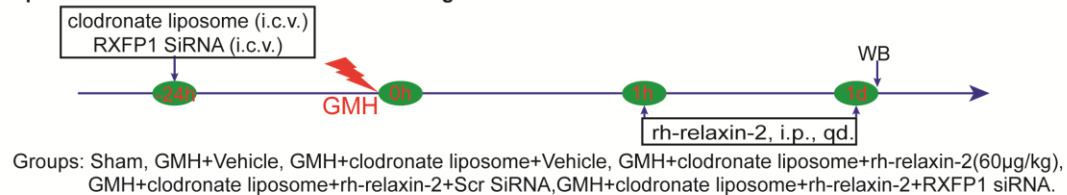
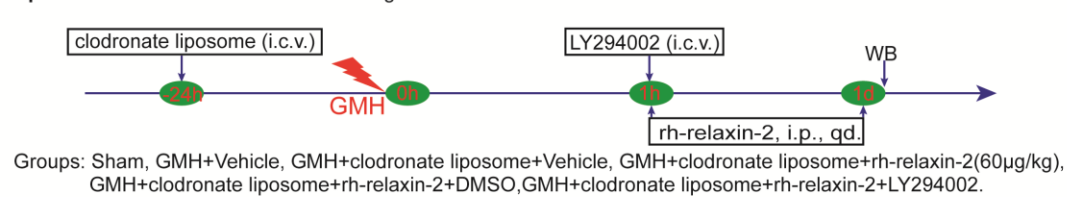
AKT/TNFAIP3 Pathway in An Experimental Germinal Matrix Hemorrhage Rat Model

Peng Li^{a, †}, Gang Zhao^{a, b, c, †}, Fanfan Chen^{d, †}, Yan Ding^a, Tianyi Wang^a, Shengpeng Liu^a, Weitian Lu^a,

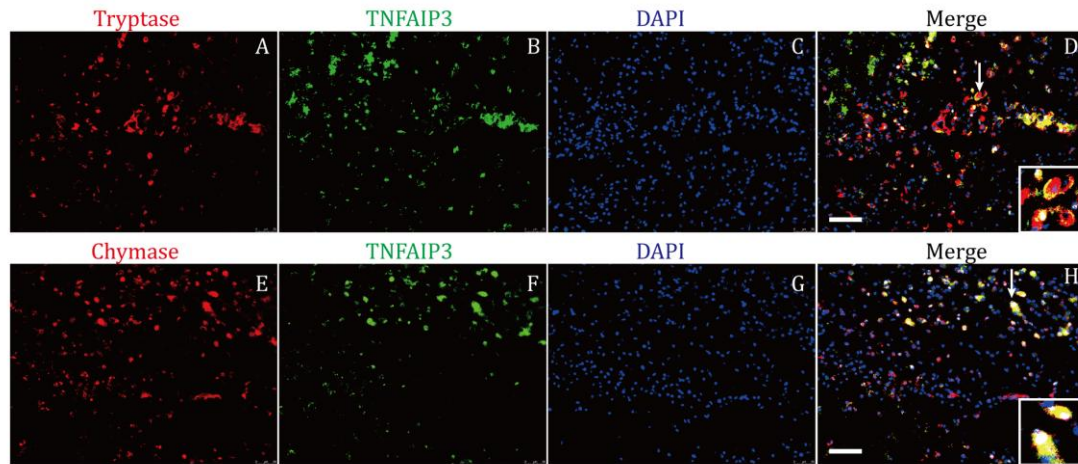
Weilin Xu^a, Jerry Flores^a, Umut Ocak^a, Tongyu Zhang^a, John H. Zhang^{a, e}, Jiping Tang^{a, *}



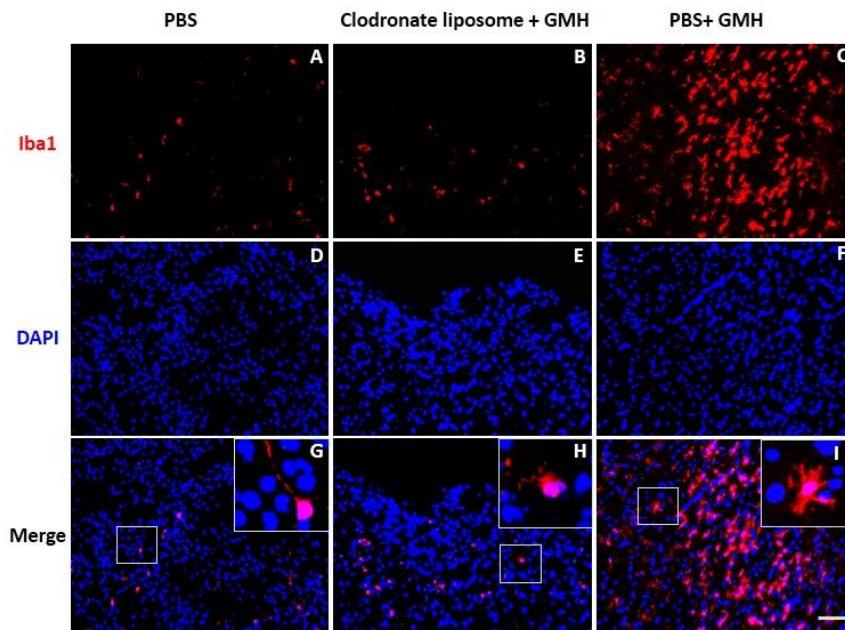
Supplementary Figure 1. Proposed mechanism.

Experiment 1. The time course and cellular localization of RXFP1 and TNFAIP3**Experiment 2. The effect of rh-relaxin-2 after GMH in short and long term outcome****Experiment 3. The quantification of active mast cells after GMH by Toluidine staining****Experiment 4. RXFP1 siRNA abolished anti-degranulation/neuroinflammation effect of rh-relaxin-2 after GMH****Experiment 5. LY294002 reversed anti-degranulation/neuroinflammation effect of rh-relaxin-2 after GMH**

Supplementary Figure 2. Experimental design.



Supplementary Figure 3. The cellular localization of TNFAIP3 in the perihematoma area of brains. Representative of double immunofluorescence staining showed that TNFAIP3 (B and F) was expressed on mast cells marked with Tryptase (A) and Chymase (E) on the first day after GMH. n=6. Scale bar=50 μ m.



Supplementary Figure 4. Clodronate liposome inhibits the response of microglia in GMH rat model. Representative of immunofluorescence staining showed the expression of Iba1 in clodronate liposome + GMH group (B, H) was inhibited compare to PBS + GMH group (C, I) on the first day after GMH. n=3, scale bar=50 μ m.

Groups	numbers
Experiment 1 (time course)	
naive	6
GMH(12h,1d,3d,5d,7d)	30
Experiment 1 (IF)	
sham	6
GMH+vehicle	6
GMH+rh-relaxin-2(60μg/kg)	6
Experiment 2 (outcome study)	
sham	7+3
GMH+vehicle	7+3
GMH+rh-relaxin-2(30μg/kg)	7
GMH+rh-relaxin-2(60μg/kg)	7+3
GMH+rh-relaxin-2(90μg/kg)	7
Experiment 3 (Toluidine Staining)	
sham	6
GMH+vehicle	6
GMH+rh-relaxin-2(60μg/kg)	6
Experiment 4 (mechanism)	
Sham	6
GMH+Vehicle	6
GMH+clodronate liposome+Vehicle	6
GMH+clodronate liposome+rh-relaxin-2(60μg/kg)	6
GMH+clodronate liposome+rh-relaxin-2+Scr siRNA	6
GMH+clodronate liposome+rh-relaxin-2+RXFP1 siRNA	6
GMH+clodronate liposome+rh-relaxin-2+DMSO	6
GMH+clodronate liposome+rh-relaxin-2+LY294002	6
Experiment 5 (clodronate liposome function)	
PBS	3
clodronate liposome + GMH	3
PBS + GMH	3
Total	173

Supplementary Table 1. Animal use in each experimental group.