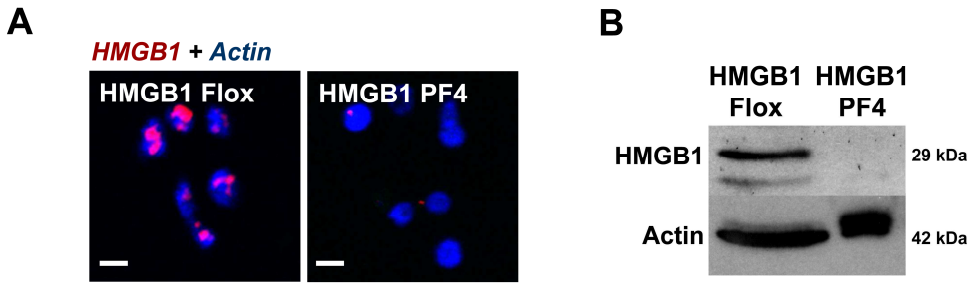
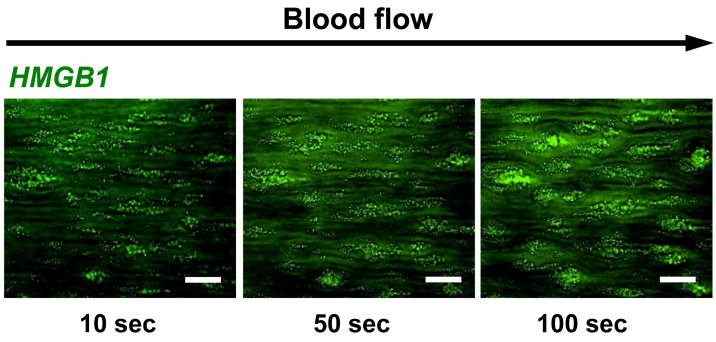
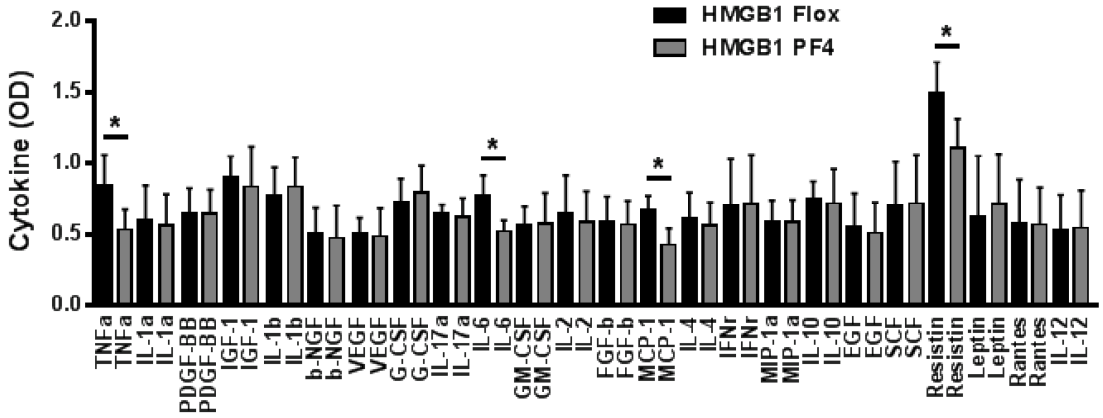
Supplementary Data



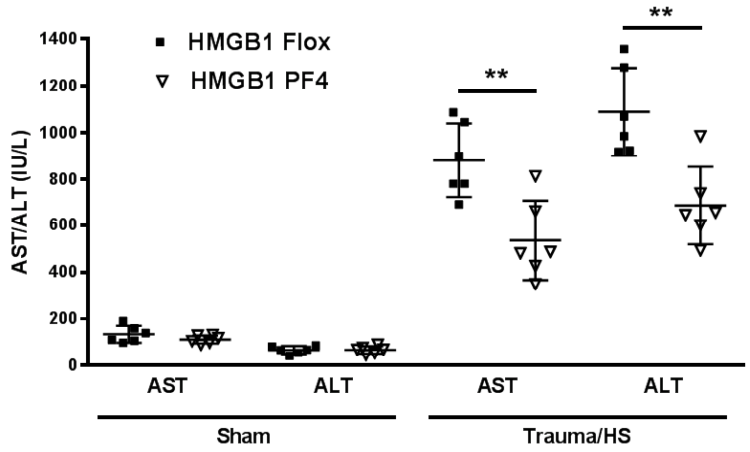
Supplemental Figure 1. Platelet-specific ablation of HMGB1. Platelet-specific ablation of HMGB1 is confirmed by (A) immunofluorescence staining of isolated platelets from *HMGB1 Flox* and *HMGB1 PF4* mice and (B) Western blot analysis of PRP derived from each mouse strain. N ≥ 3 mice per group. Representative images of three separate experiments. *Scale bar*, 3 μm.



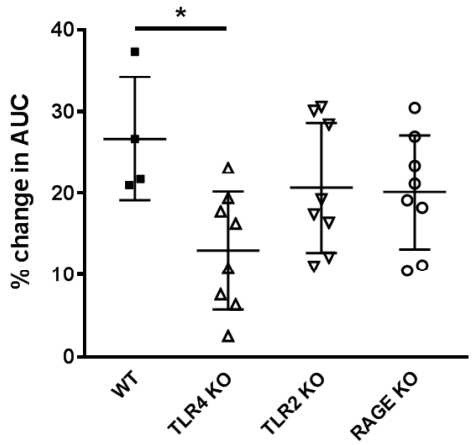
Supplemental Figure 2. HMGB1 expression during thrombus formation. Expression of HMGB1 during thrombus formation is demonstrated by perfusion of human blood, which has been preincubated with a labeled anti-HMGB1 specific antibody (green), through a flow chamber over a collagen-coated surface with high shear rates (1700 s^{-1}) (100 second investigation period). *Scale bar*, 70 μm. Representative images of three separate experiments.



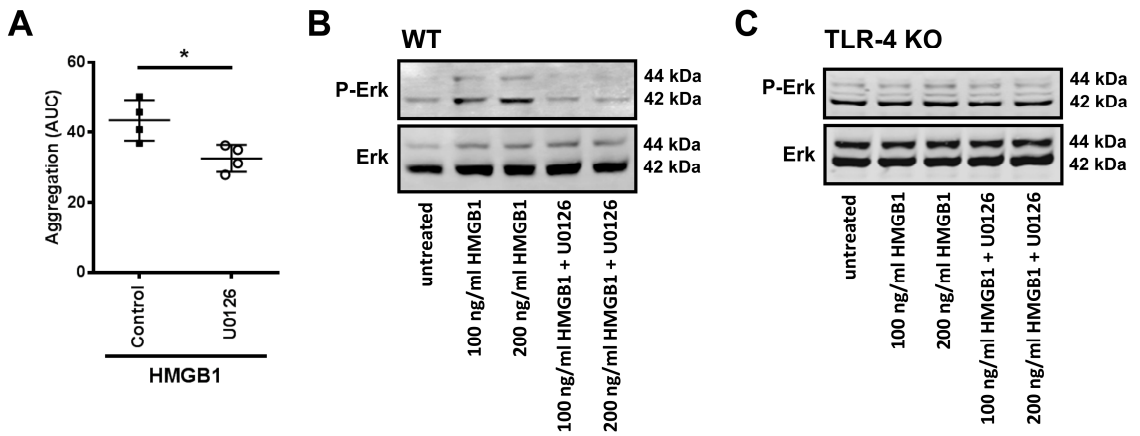
Supplemental Figure 3. Serum levels of circulating cytokines in *HMGB1 PF4* and *Flox* control mice following experimental trauma/hemorrhagic shock. Circulating cytokines in *HMGB1 PF4* (grey bars) and *Flox* control mice (black bars) subjected to experimental trauma/hemorrhagic shock are determined. Data show mean ± SD of the results from at least two separate experiments and N = 4 mice per group. * $p<0.05$ (Student's t test).



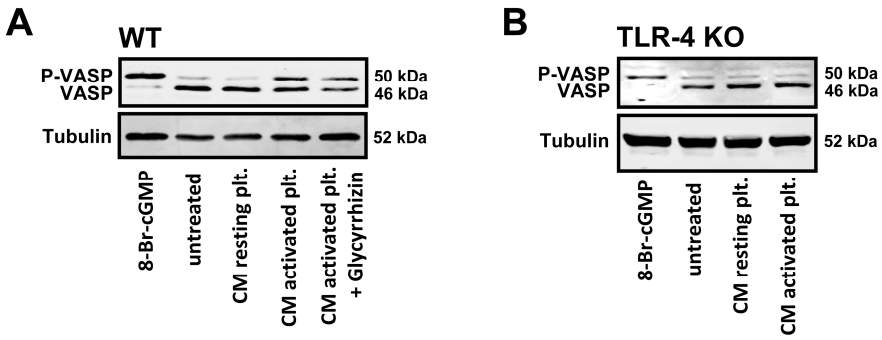
Supplemental Figure 4. Serum transaminase levels in *HMGB1 PF4* and *Flox* control mice following experimental trauma/hemorrhagic shock. Measurement of hepatic injury in control (sham) mice and mice subjected to experimental trauma/hemorrhagic shock by quantification of serum transaminases (AST and ALT). *HMGB1 PF4* and *Flox* control mice as indicated. Data show mean ± SD of the results from at least three separate experiments and N = 6 mice per group. ** $p<0.01$ (Student's t test).



Supplemental Figure 5. HMGB1 promotes platelet aggregation via TLR4. Collagen-induced platelet aggregation in the presence of rHMGB1 (200 ng/ml) in WT, TLR4$^{-/-}$, TLR2$^{-/-}$, and RAGE$^{-/-}$ mice (quantified as AUC). Data show mean ± SD of the results from at least three separate experiments and N≥4 mice per group. * $p<0.05$ (1-way ANOVA with Tukey's post-hoc test).



Supplemental Figure 6. HMGB1 induces activation of MAPK/ERK in platelets via TLR4, which promotes platelet aggregation. (A) CRP-induced platelet aggregation in the presence of rHMGB1 (200 ng/ml) is inhibited by the MEK/ERK inhibitor U0126 (10 μM). (B) rHMGB1 (100-200 ng/ml) induces phosphorylation of MAPK/ERK in WT, but not in TLR4$^{-/-}$ platelets. Data show mean ± SD of the results from at least two separate experiments and N≥3 mice per group. * $p<0.05$ (Student's t test).



Supplemental Figure 7. Conditioned media from activated, but not resting platelets activate platelet cGKI via HMGB1/TLR4. Conditioned media (CM) from CRP-activated, but not resting WT platelets induce VASP phosphorylation in WT platelets, which is reversed following glycyrrhizin pretreatment or in TLR4$^{-/-}$ platelets. Representative images of two separate experiments and N = 4 mice per group.