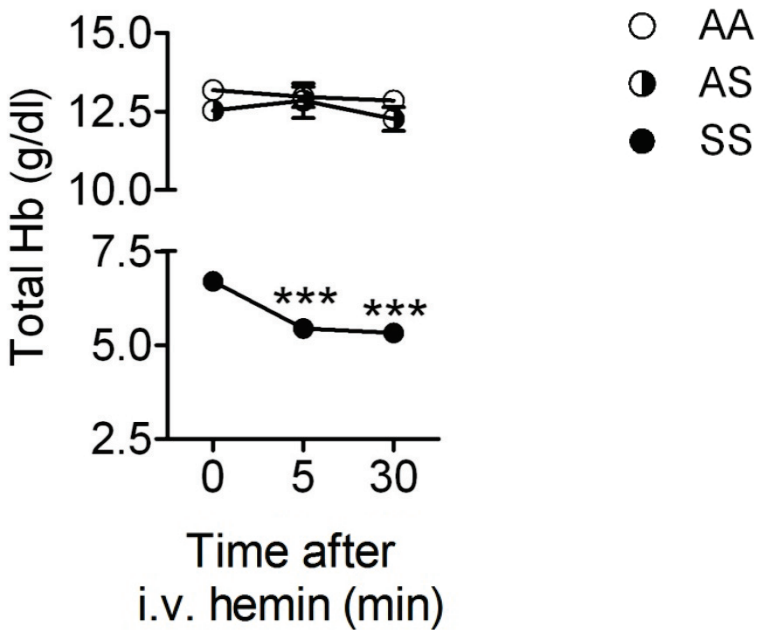
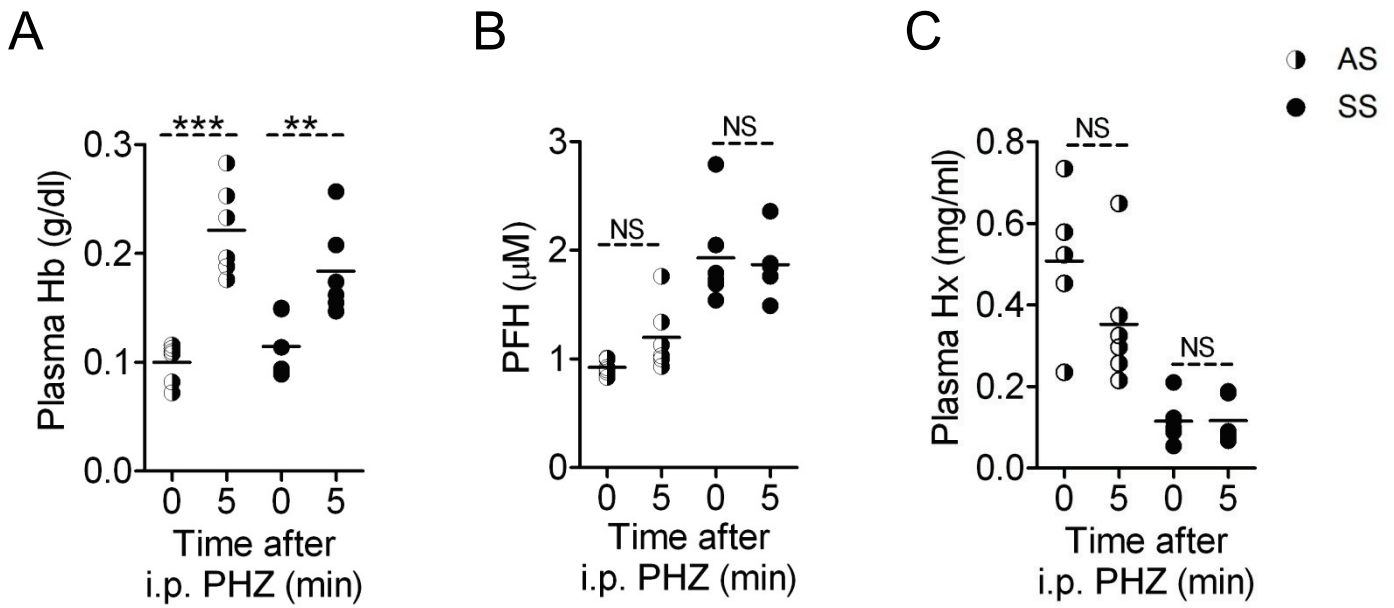
Table S1. Hematological details of wild-type and chimeric sickle mice

Mouse Type	Total Hb (g/dl)	Retics (%)	RBC (x 10^6 / µl)	WBC (x 10^3 / µl)	Hct (%)
B6TLR4$^{+/+}$	14.88±0.24	3.55±.14	9.65±0.53	6.26±0.77	41.21±2.06
SS$^{TLR4+/+}$	7.73±0.16	48.35±2.54	6.79±0.17	16.29±1.9	29.77±0.6
B6TLR4$^{-/-}$	14.23±0.11	3.2±.18	9.56±0.26	6.2±0.8	40.02±1.09
SS$^{NH/TLR4-/-}$	7.88±0.24	42.84±2.05	7.02±0.26	16.21±1.0	30.73±0.91
SS^{WT}	7.18±0.32	64.26±.48	6.83±0.33	22.01±2.5	30.05±1.35

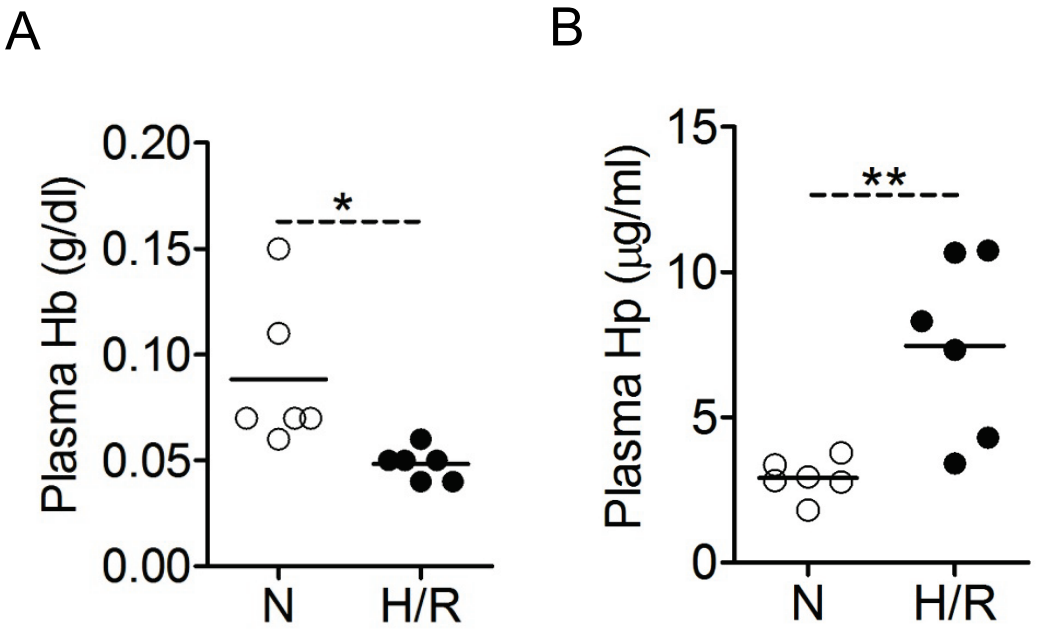
Values are expressed as mean ± SEM (n=3).



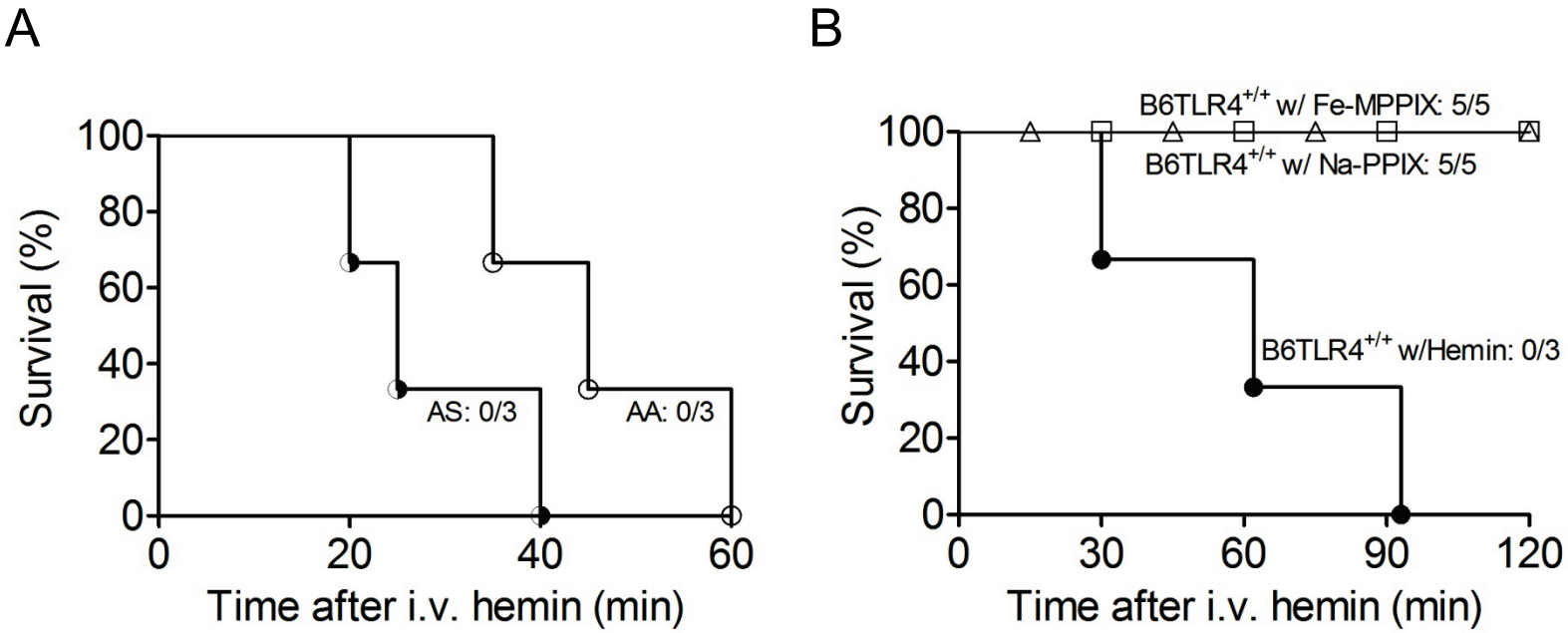
Supplementary Figure 1. Extracellular hemin causes acute hemolysis in sickle mice. Total hemoglobin (Hb) level declined sharply in SS mice challenged with hemin but not in control mice (n=6-9) ; ***p<0.001.



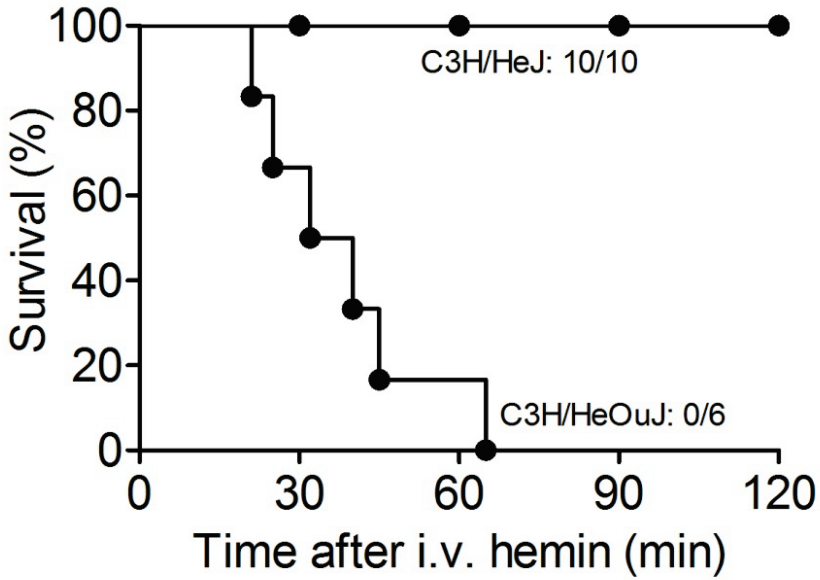
Supplementary Figure 2. Phenylhydrazine (PHZ) hemolysis model. Mice were injected (i.p.) with PHZ (100 mg/kg). **(A)** Increased level of plasma Hb confirms acute intravascular hemolysis in both control (AS) and SS mice. **(B, C)** PHZ did not change plasma free heme (PFH) and plasmahemopexin (Hx) significantly in either group of mice (n=6) .



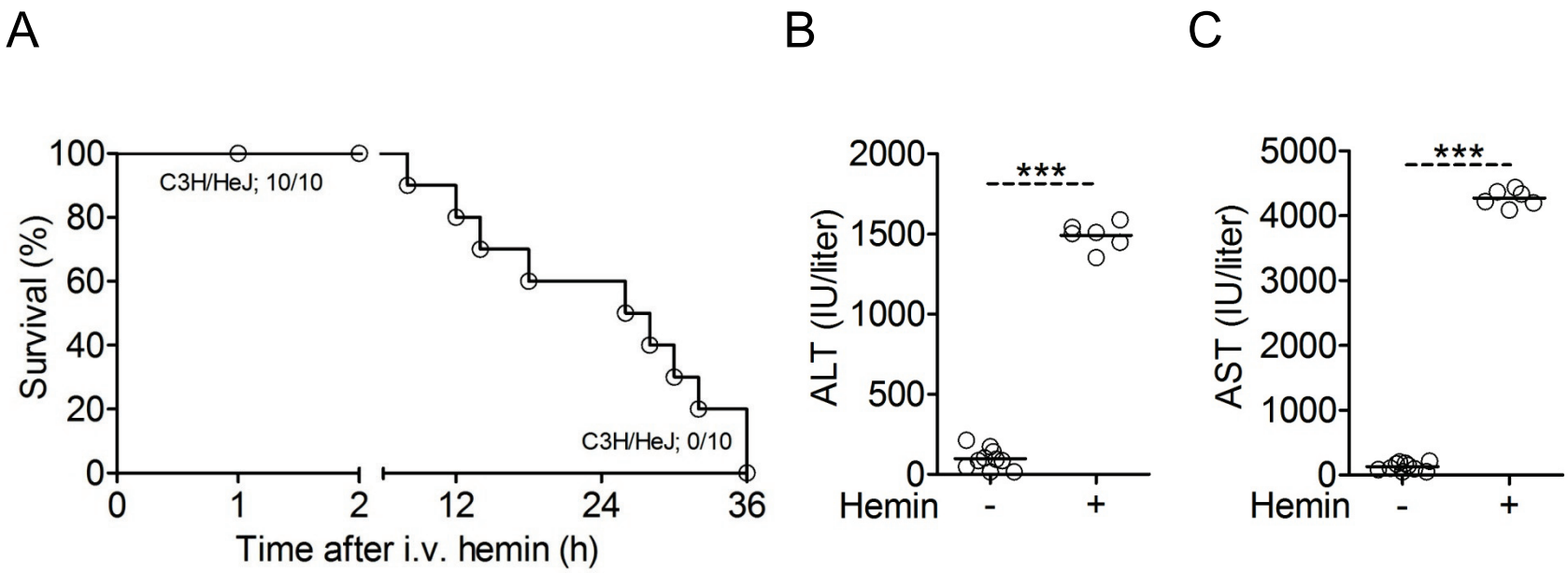
Supplementary Figure 3. Concentration of plasma hemoglobin (Hb) and haptoglobin (Hp) following hypoxia/reoxygenation (H/R). Level of **(A)** Plasma Hb was reduced significantly, concomitant with significant elevation of **(B)** Plasma Hp. *p<0.05 and **p<0.01.



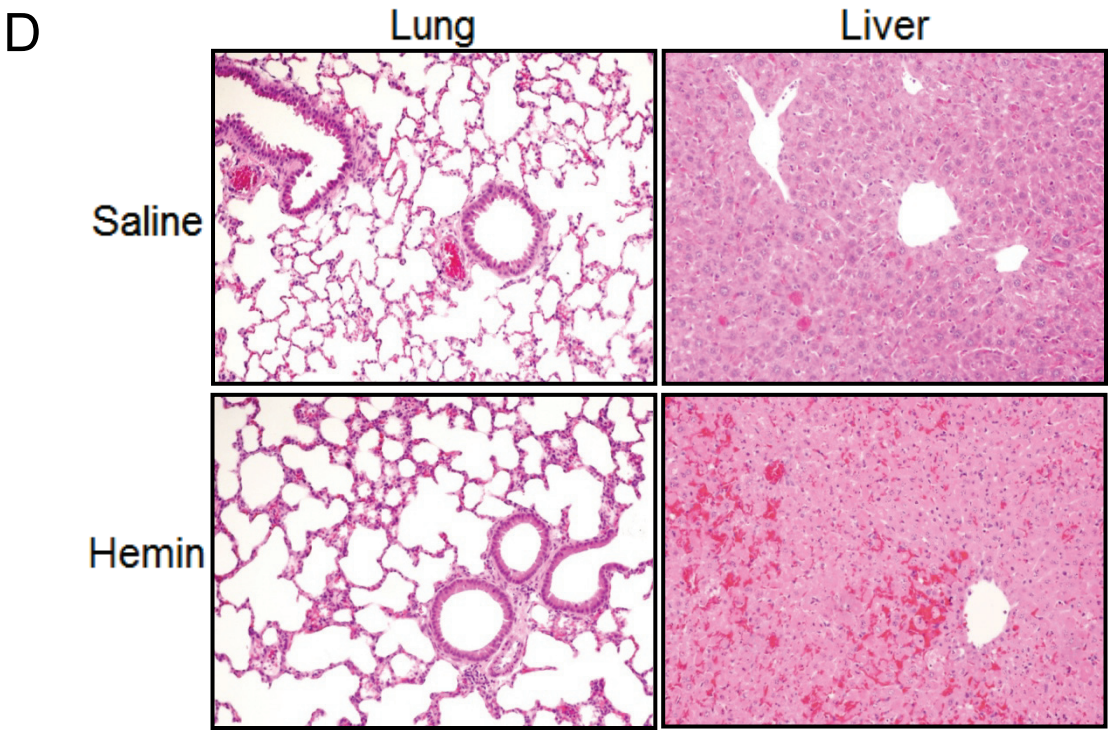
Supplementary Figure 4. Hemin toxicity is independent of SCD and is not replicated by hemin analogues. **(A)** Survival rate of AA and AS mice (n=3) challenged with 210 μmoles/kg hemin. **(B)** Survival rate of B6TLR4$^{+/+}$ mice challenged with 210 μmoles/kg of analogues of hemin (NaPPIX and Fe-MesoPPIX) or hemin (n=3-5).



Supplementary Figure 5. Lack of acute hemin toxicity in TLR4 null mice. Survival rate of C3H/HeJ (n=10) and wild type control, C3H/HeOuJ (n=6) challenged with 210 μmoles/kg hemin.



Supplementary Figure 6



Supplementary Figure 6. Hepatic hemin toxicity in TLR4 mutant mice. (A) Survival rate of C3H/HeJ mice challenged with hemin. Note that none of the mice died in the 2 hours window associated with acute hemin toxicity in the murine model of ALI reported in this study. Level of (B) Plasma ALT and (C) Plasma AST in C3H/HeJ mice at baseline (-) and at 24 hours following hemin challenge (+) indicating severe hepatic injury. (D) Representative photomicrograph of H&E stained postmortem lung and liver sections of C3H/HeJ mice that succumbed 30 hours after being challenged. Note the lack of injury in the lung and the severe damage in the liver. Original magnification: 100X; ***p<0.001.