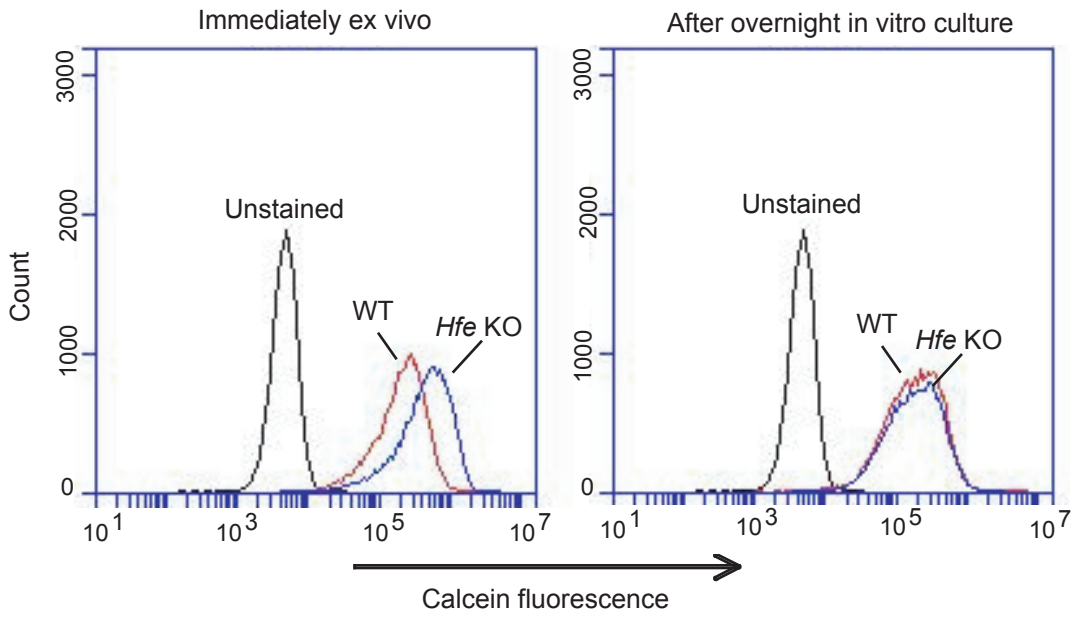
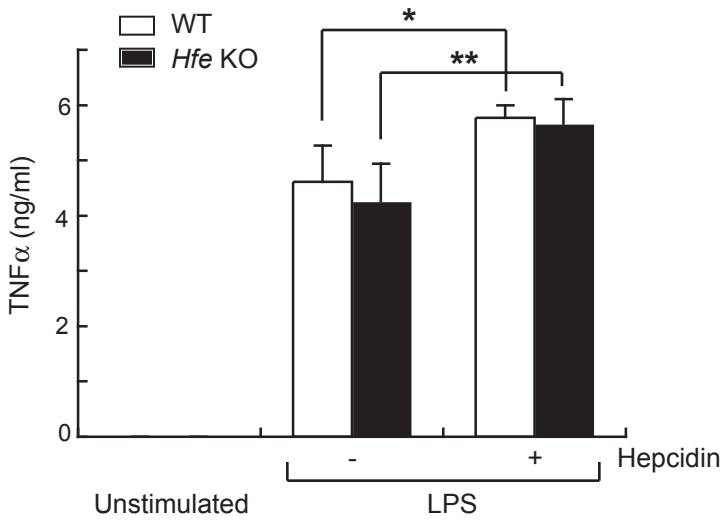
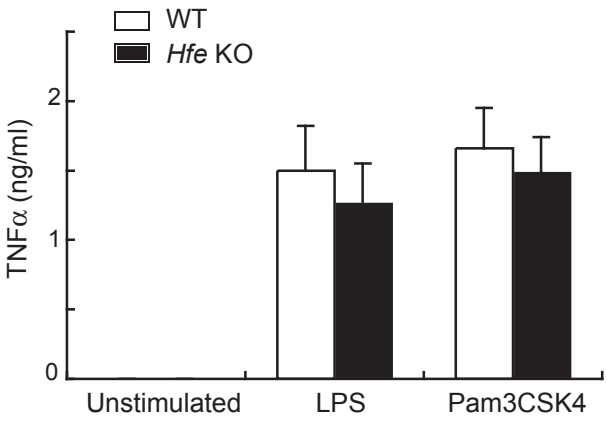


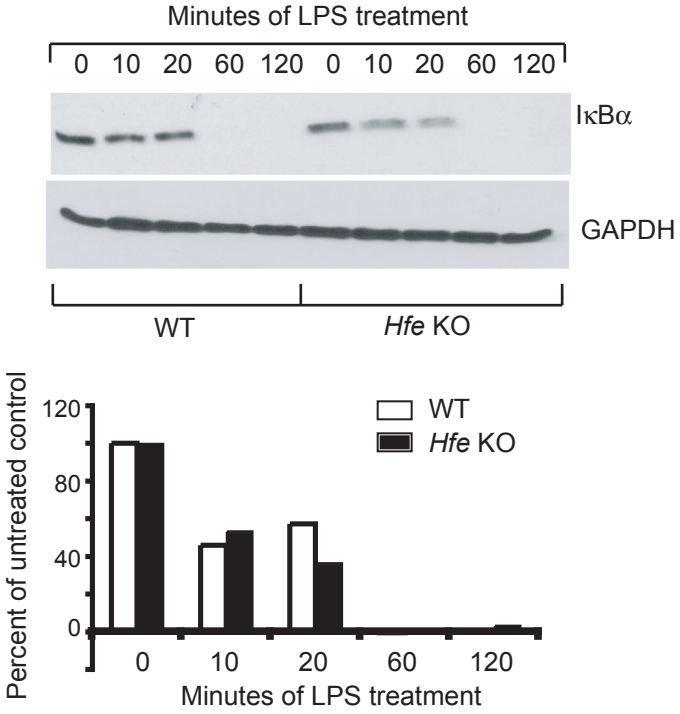
Supplemental Figure 1. Change in calcein fluorescence of *Hfe* KO macrophages after in vitro culture. WT and *Hfe* KO peritoneal macrophages were harvested and calcein fluorescence evaluated by flow cytometry either immediately after collection (left panel) or after overnight culture in vitro (right panel).



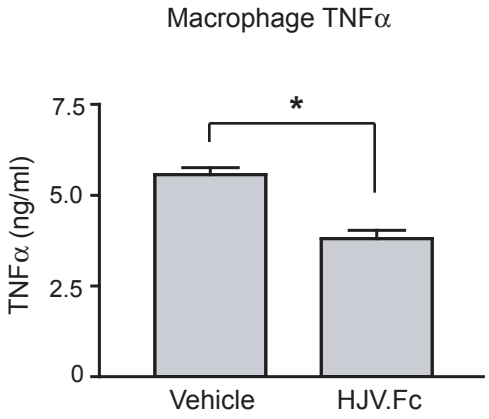
Supplemental Figure 2. Effect of hepcidin on LPS-induced macrophage TNFα production. WT and *Hfe* KO peritoneal macrophages were cultured overnight in the presence or absence of 700 nM hepcidin and then stimulated with 100 ng/ml of LPS for 3 hours. Supernatants were collected and analyzed by ELISA for TNFα. *p = 0.006, **p = 0..002 (n = 6 stimulations in each group from 2 separate experiments). Data represent mean +/- SD.



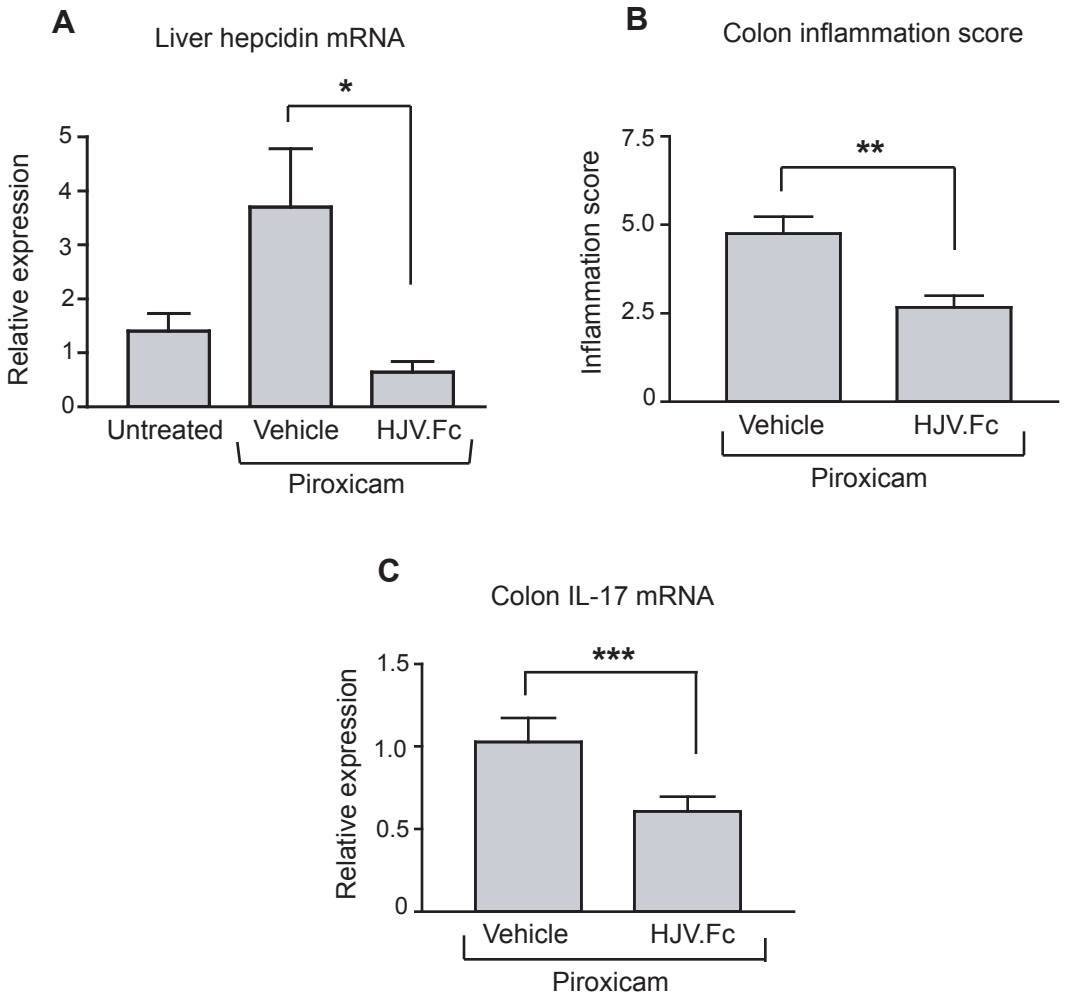
Supplemental Figure 3. Responses of bone marrow-derived macrophages to TLR4 and TLR2 stimulation. Bone marrow-derived macrophages from WT and *Hfe* KO mice were stimulated with 100 ng/ml of LPS or 1 μg/ml of Pam3CSK4 for 6 hours. Supernatants were collected and analyzed by ELISA for TNFα. (n = 7 for LPS stimulations and 6 for Pam3CSK4 from 2 separate experiments). Data represent mean +/- SD.



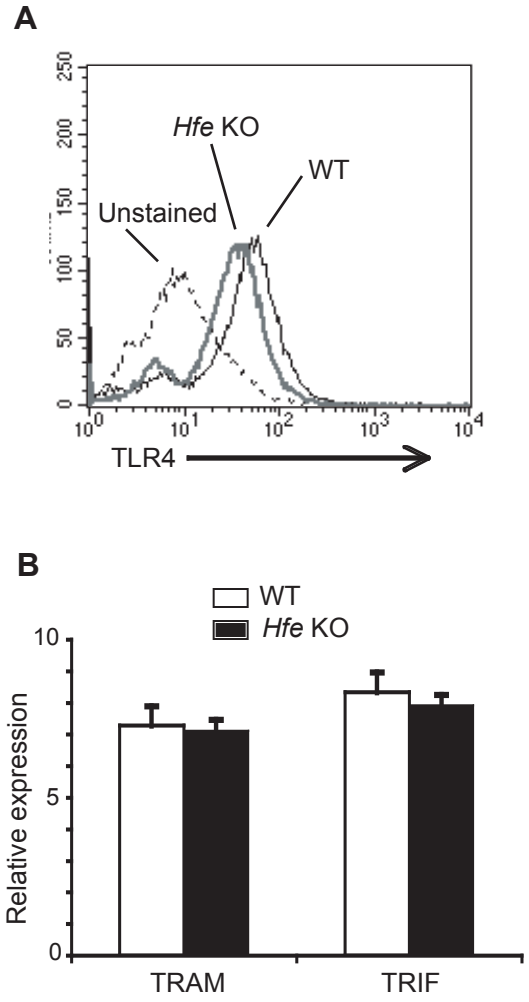
Supplemental Figure 4. LPS-induced activation of the NF-κB pathway is intact in *Hfe* KO macrophages. WT and KO macrophages were stimulated for the indicated times with 100 ng/ml LPS after treatment with 10 μg/ml of cycloheximide to inhibit resynthesis of IκBα. Cell lysates were prepared and analyzed by western blotting for IκBα and GAPDH (upper panel). The results were scanned and subjected to densitometry to obtain a measure of IκBα band intensity normalized to GAPH (lower panel). Similar results obtained in 3 separate experiments.



Supplemental Figure 5. Effect of HJV.Fc on LPS-induced macrophage TNFα production. Peritoneal macrophages from wild-type mice that were treated with either vehicle (PBS) or HJV.Fc were stimulated with 100 ng/ml of LPS for 3 hours. Supernatants were collected and analyzed by ELISA for TNFα. *p = 0.0002 (n = 6 stimulations in each group from 2 separate experiments). Data represent mean +/- SEM.



Supplemental Figure 6. HJV.Fc suppresses hepcidin up-regulation and intestinal inflammation in piroxicam-induced colitis. IL-10 KO mice were treated with piroxicam for 2 weeks and then injected with vehicle or HJV.Fc for an additional week. The animals were sacrificed and liver hepcidin mRNA measured by quantitative RT-PCR (A). Colon histopathology was evaluated by a blinded observer and an inflammation score assigned (B). Colon IL-17 mRNA was measured by quantitative RT-PCR (C). *p = 0.009 (n = 3 mice in untreated group, 4 in vehicle group, 6 in HJV.Fc.group). **p = 0.01 (n = 4 in vehicle group, 6 in HJV.Fc group). ***p = 0.05 (n = 4 in vehicle group, 5 in HJV.Fc group). Data represent mean +/- SEM.



Supplemental Figure 7. Expression of TLR4, TRAM and TRIF in WT and *Hfe* KO macrophages. WT and KO macrophages were analyzed by flow cytometry (A) or quantitative RT-PCR (B) to determine levels of TLR4, TRAM and TRIF. Results in (A) representative of 2 separate experiments and in (B), n = 3 mice in each group. Data in (B) represent mean +/- SD.