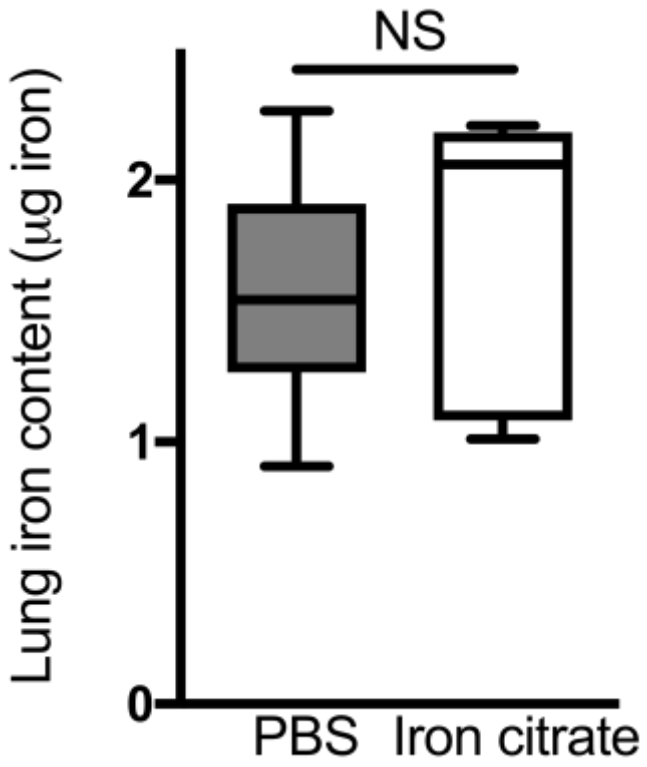

Figure S1. Lung iron content in wildtype mice treated with PBS or iron citrate. Mice were treated with either PBS or ferric ammonium citrate (iron) 30 minutes prior to intrapulmonary *K. pneumoniae* challenge and again 24 hours thereafter; lungs were harvested on day 2 of infection. Lung iron content was determined using the ferrozine assay. . Box and whisker plots show median (line within box), upper and lower quartiles (upper and lower box boundaries), and total range (bars); *n* = 6-5 per group; NS, not significant (*t*-test).

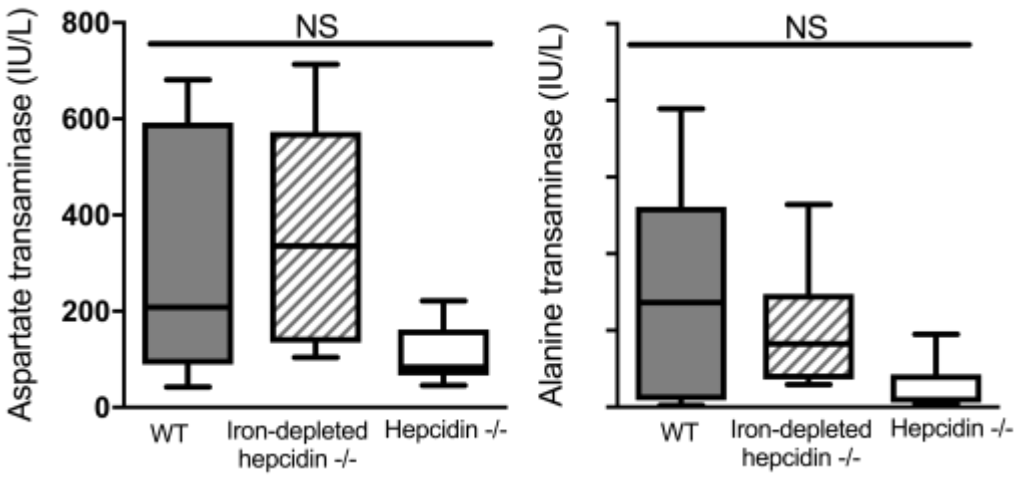

Figure S2. Aspartate transaminase (AST) and alanine transaminase (ALT) in wildtype and hepcidin -/- mice. Wildtype (WT) mice were maintained on standard diet, and hepcidin -/- mice were maintained on wither a prolonged iron-deficient diet (Iron depleted hepcidin -/-) or standard diet prior to intratracheal inoculation with *K. pneumoniae.* Samples were harvested on day 2 of infection. Box and whisker plots show median (line within box), upper and lower quartiles (upper and lower box boundaries), and total range (bars); *n*=6-9 per group; NS=not significant, one way ANOVA.

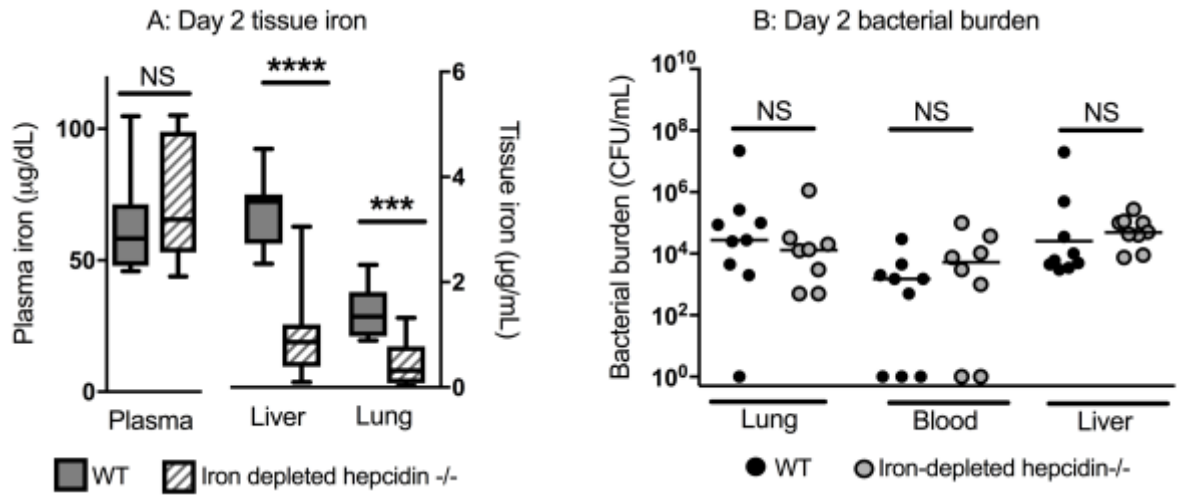

Figure S3. Iron-depleted hepcidin-/- mice are not susceptible to infection compared to wildtype mice. Wildtype (WT) mice maintained on standard diet and hepcidin -/- maintained on a prolonged iron-deficient diet were infected intratracheally with *K. pneumoniae* and samples were harvested on day 2 of infection. (A) Liver, lung, and plasma iron content in wildtype and iron-depleted hepcidin -/- mice. Box and whisker plots show median (line within box), upper and lower quartiles (upper and lower box boundaries), and total range (bars); *** and **** denote p <0.001 and p <0.0001 respectively; NS, not significant (*t*-test). (B): Lung, blood, and liver bacterial burden in wildtype and iron-depleted hepcidin -/- mice. Horizontal lines represent median and each circle represents one animal; animals with no detectable bacteria are reported to have a bacterial burden of 1 colony forming units on the logarithmic scale; NS, not significant (Mann-Whitney test). *N=8-9* per group in both panels.

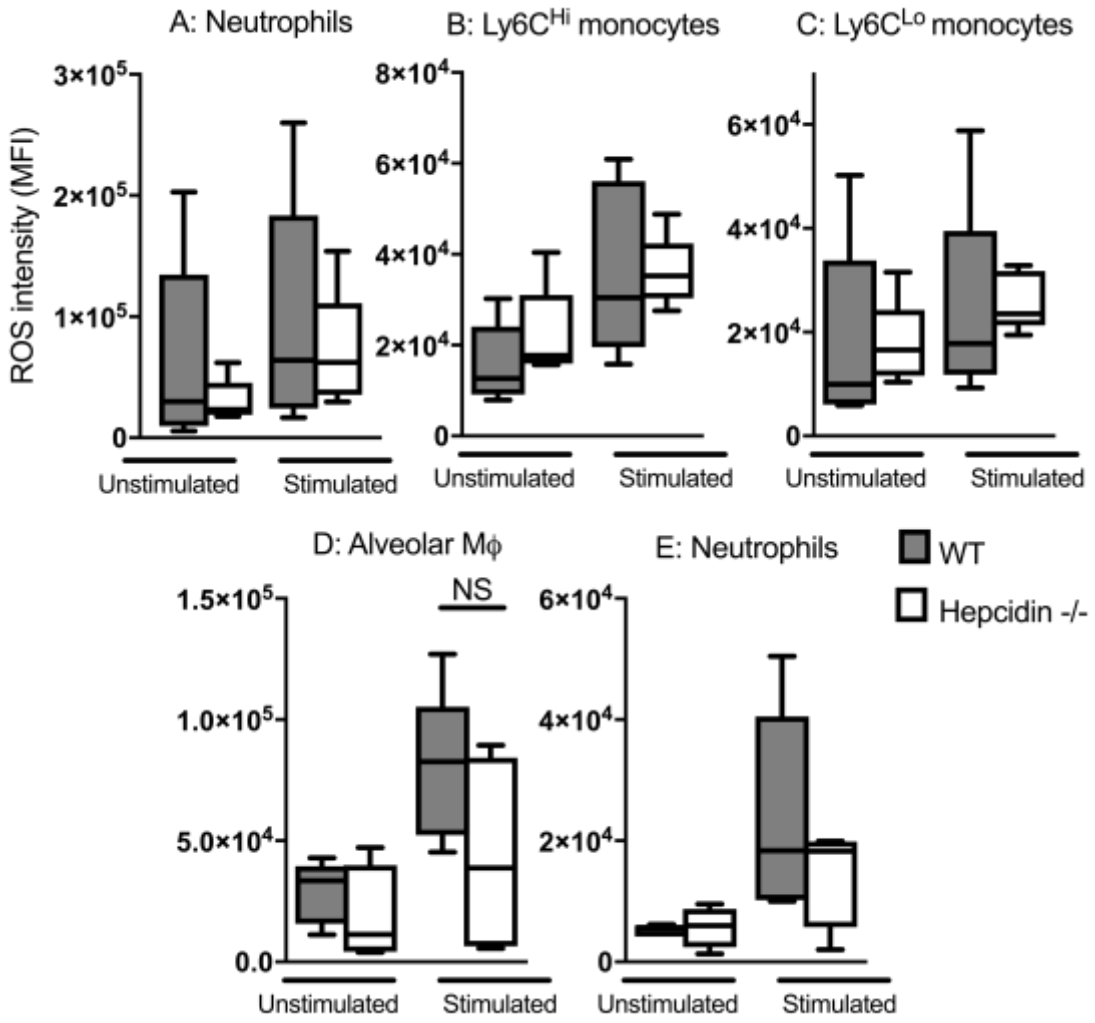

Figure S4. Reactive oxygen species (ROS) production in infected mice. ROS production in indicated cell types from in the blood and bronchoalveloar lavage fluid (BAL) of wildtype and hepcidin -/- mice 2 days following infection. Box and whisker plots show median (line within box), upper and lower quartiles (upper and lower box boundaries), and total range (bars); *n* = 4-5 per group; NS, not significant (*t*-test) for comparing ROS production between WT and hepcidin-/- mice.

Supplementary methods:

Iron depletion protocol: Hepcidin-deficient mice were weaned to iron deficient chow at 22 days of age for 3-4 weeks before use in experiments.

Determination of AST and ALT: Plasma AST and ALT were measured using commercial kits according to the manufacturer instructions (Liquid AST Reagent set and Liquid ALT Reagent Set; Pointe Scientific, Canton, MI).

Flow cytometry: Cell populations were isolated and stained as described in the main methods. After extracellular staining, cells were washed in FA buffer once and prepared for reactive oxygen species staining using DCFDA (2',7'–dichlorofluorescein diacetate) with a commercial kit (Abcam, Cambridge, UK) according to the manufacturer's instructions. Cells were stained using 2 µM DCDFA for 15 minutes at 37°C. TBHP (Tert-butyl-hydrogen peroxide) was added to a final concentration of 50 µM for stimulated cells. Cells were incubated for an additional 45 minutes at 37°C. Data were acquired immediately after incubation on a FACS Canto II instrument using BD FACS Diva software (version 8.0; BD Biosciences) and analyzed with FlowJo software (version 8.8.6; Tree Star, Ashland, Oregon).