Supplemental Figure S1. Ephx2⁻/⁻ mice exhibit normal clearance of K. pneumoniae. WT and Ephx2⁻/⁻ mice were inoculated with a low dose of K. pneumoniae (1 x 10³ CFU/mouse, half that used in the experiment shown in Figure 1). (A) After 48 hours, there were no differences in the number of K. pneumoniae bacteria or in lung inflammation score, n=6-19 per group. (B) 48 hours after K. pneumoniae inoculation, lungs from WT and Ephx2⁻/⁻ mice had similar Il1β, Il6 and Tnfa mRNA levels (normalized to Gapdh), n=3-7 per group. (C) In vitro phagocytosis of FITC-labeled K. pneumoniae by WT and Ephx2⁻/⁻ macrophages was similar using a reduced ratio (1:1) of K. pneumoniae:macrophage, n=10 per group. Data represent mean ± SEM.
Supplemental Figure S2. Ephx2 disruption does not alter lung clearance of S. aureas. (A) WT and Ephx2⁻/⁻ mice were inoculated with 6.6 × 10⁷ CFU/lung of S. aureus and number of bacteria was determined at 24 and 48 hours, n=3-4 per group. (B) WT and Ephx2⁻/⁻ mice were inoculated with 3.3 × 10⁷ CFU/lung of S. aureus and number of bacteria was determined at 24 hours, n=3-7 per group. Data represent mean ± SEM.
Supplemental Figure S3. Representative FACS analysis of WT and Ephx2−/− macrophage phagocytosis of *K. pneumoniae*, *S. pneumoniae* and *S. aureus* in *vitro*. Representative FACS results for the data in manuscript Figure 4a, 4b and 4c. WT and Ephx2−/− peritoneal macrophage were incubated with FITC-labeled *K. pneumoniae* (A), FITC-labeled *S. pneumoniae* (B) or Alexa 488-labeled *S. aureus* (C) at a 10:1 ratio of bacteria:macrophages and % phagocytosis was determined.
Supplemental Figure S4. Immunofluorescence analysis of WT and Ephx2-/- macrophage phagocytosis of S. pneumoniae in vitro. Confocal images of macrophages treated as in Figure 4B and Supplemental Figure 3B show internalization of S. pneumoniae. Immunofluorescent staining of FITC-S. pneumoniae (green), DAPI (nuclei, blue), and CD11b (red) are shown.
Supplemental Figure S5. *Ephx2^-/-* neutrophils have reduced phagocytosis of *S. pneumoniae* in vivo. WT and *Ephx2^-/-* mice were inoculated with *S. pneumoniae*. Twelve hours after inoculation, neutrophils (Ly6G+CD11b+ cells) from WT and *Ephx2^-/-* lungs (A) and BALF (B) were stained with FITC-labeled anti-*S. pneumoniae* antibodies and analyzed by FACS. A lower percentage of *Ephx2^-/-* neutrophils took up *S. pneumoniae* relative to WT neutrophils in both lung and BALF. Data represent mean ± SEM, n=3-5 per group; *p<0.05.
Supplemental Figure S6. Efferocytosis of dead cells by macrophages is not altered by Ephx2 disruption. Efferocytosis of lung cells, splenocytes and thymocytes killed by heat (5 minutes boiling) (A) or 6-hour treatment with 5 μM dexamethasone (DX) (B) by WT and Ephx2−/− macrophages, n=3 per group; *p<0.05.
Supplemental Figure S7. Effects of PGN on expression of cytokines and pattern recognition receptors in WT and Ephx2−/− peritoneal macrophages. Peritoneal macrophages were isolated from WT and Ephx2−/− mice and treated with 10 μg/ml PGN or 10 μg/ml Pam3CSK4 for 4 hours. Both PGN- and Pam3CSK4 increased mRNA levels of Il6 and Tnfα in WT macrophages, an effect that was attenuated in Ephx2−/− macrophages (A). In a similar but separate experiment, PGN-induced expression of Il1β, Il6 and Tnfα was attenuated in Ephx2−/− macrophages compared to WT (B). PGN-induced expression of the pattern recognition receptors Pglyrp1 and Tlr2, but not Tlr4, was attenuated in Ephx2−/− macrophages compared to WT (C). All data are normalized to Gapdh. Data represent mean ± SEM, n=5 per group; *p<0.05.
Supplemental Figure S8. Peptidoglycan recognition protein (Pglyrp) expression in lung macrophages. Lung macrophages (CD11b^+F4/80^+) from WT and Ephx2^−/−^ mice were stimulated with PGN (10 μg/ml) for 4 hours. Pglyrp1, Pglyrp2, Pglyrp3 and Pglyrp4 transcript levels were quantified by real time quantitative RT-PCR and normalized to Gapdh. Data represent mean ± SEM, n=3 per group; *p<0.05.
Supplemental Figure S9. *In vitro* treatment of WT and Ephx2\(^{-/-}\) lung macrophages with peptidoglycan (PGN). Isolated lung macrophages from WT and Ephx2\(^{-/-}\) mice were treated with vehicle or PGN for 4 hours. mRNA expression of cytokines *Il1β, Il6* and *Tnfα* (A) and pattern recognition receptors *Pglyrp1* and *Tlr2* (B) normalized to *Gapdh* are shown. Data represent mean ± SEM, n=6 per group; *p<0.05.
Supplemental Figure S10. Overexpression of Tlr2 and Pglyrp1 in primary macrophages using the pINDUCER20 Lentivirus system. (A) Tlr2–pINDUCER20 and Pglyrp1-pINDUCER20 plasmid constructs. Mouse peritoneal macrophages were infected with Tlr2 (B) or Pglyrp1 (C) lentivirus for 48 hours under G418 selection. Infected macrophages were then induced with doxycycline for 48 hours and analyzed by FACS. Lentivirus infected macrophages were stimulated with 10 µg/ml PGN for 4 hours and Tlr2 (D) or Pglyrp1 (E) transcripts were quantified. Data represent mean ± SEM, n=3 per group; *p<0.05 vs. vector alone.
Supplemental Figure S11. Suppression of *Il1β* and *Tnfa* mRNA levels after PGN treatment is reduced by *Tlr2* or *Pglyrp1* disruption and EET treatment. Peritoneal macrophages were isolated from *Tlr2*−/− (A), *Pglyrp1*−/− (B) or WT littermate control mice and treated for 4 hours with PBS or 10 μg/ml PGN, in the presence of vehicle, 1 μM 11,12-EET or 1 μM 14,15-EET. Expression of *Il1β* and *Il6* normalized to *Gapdh* was determined. Data represent mean ± SEM, n=9 per group; *p<0.05.
Supplemental Figure S12. Phagocytosis of *K. pneumoniae*, and induction of proinflammatory cytokines, *Pglyrp1* and *Tlr2* in human alveolar macrophages stimulated with LPS. (A) Human alveolar macrophages obtained from BALF were incubated with 2x10^6 *K. pneumoniae* and either vehicle, 1μM 11,12-EET or 1μM 14,15-EET for 30 min. Phagocytosis was then examined by flow cytometry. (B, C) Alveolar macrophages were incubated with LPS (1 μg/ml) and either vehicle, 1μM 11,12-EET or 1μM 14,15-EET for 4 hours. Expression of *Il1β*, *Il6* and *Tnfa* (B) or *Pglyrp1* and *Tlr2* (C) normalized to *Gapdh* was determined. n=4 per group. Data represent mean ± SEM.