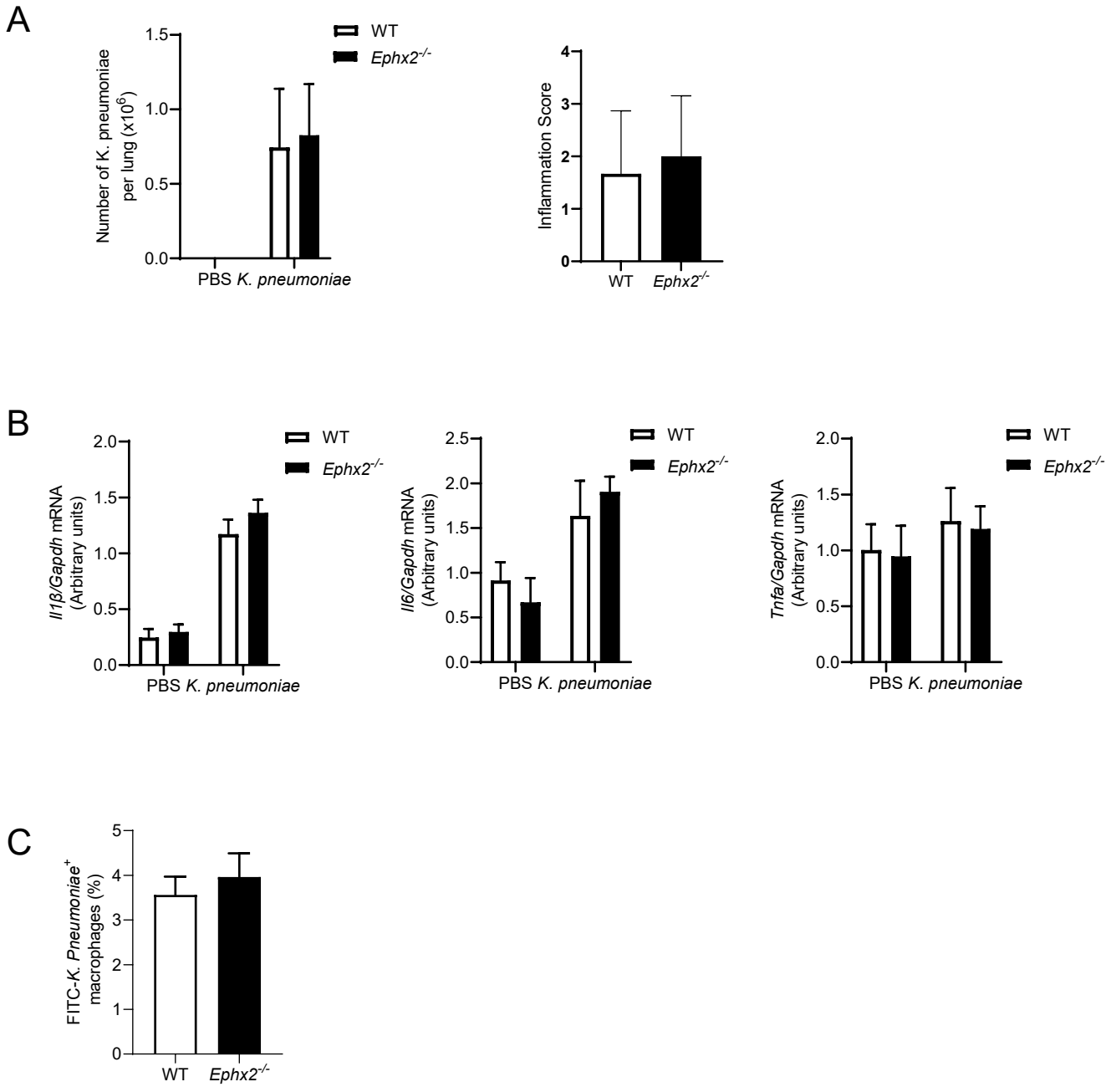
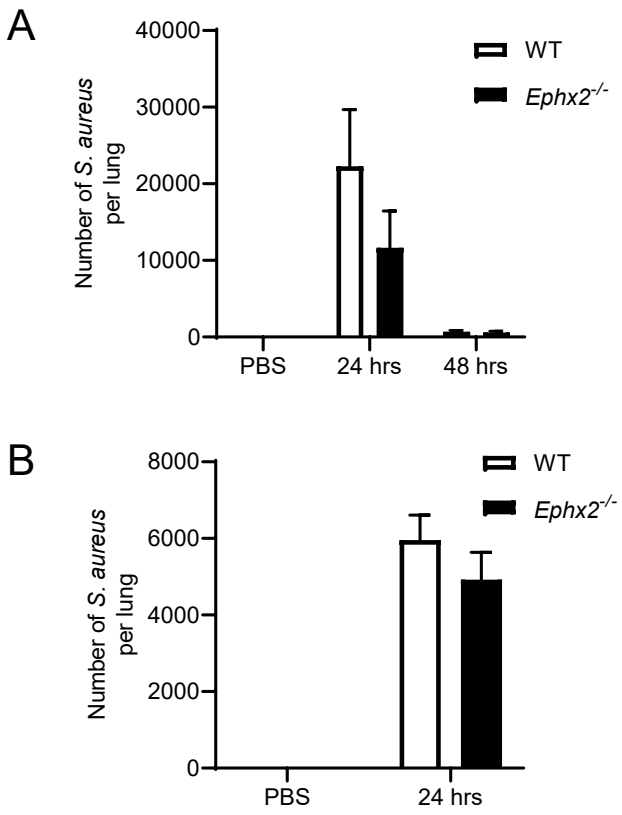
Supplemental Figure S1



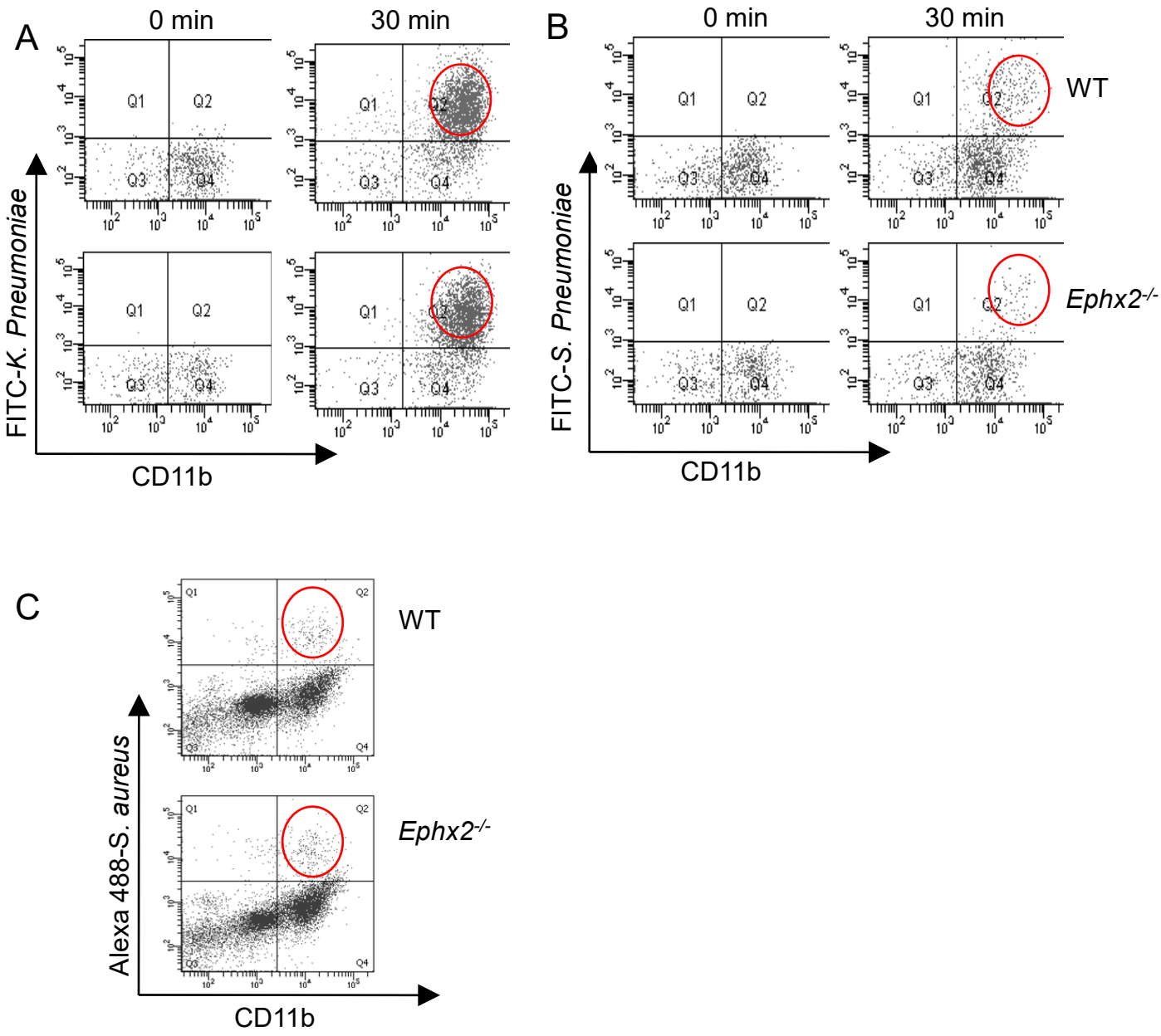
Supplemental Figure S1. *Ephx2*$^{-/-}$ mice exhibit normal clearance of *K. pneumoniae*. WT and *Ephx2*$^{-/-}$ mice were inoculated with a low dose of *K. pneumoniae* (1 x 10^3 CFU/mouse, half that used in the experiment shown in Figure 1). (A) After 48 hours, there were no differences in the number 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 *Tnfα* 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



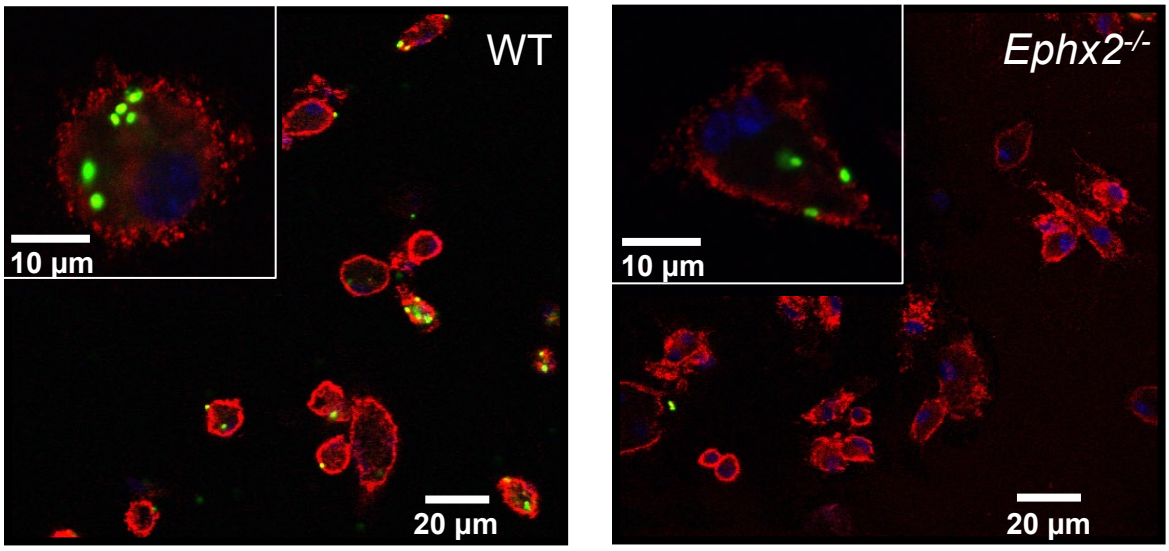
Supplemental Figure S2. *Ephx2* disruption does not alter lung clearance of *S. aureas*. (A) WT and *Ephx2*$^{-/-}$ mice were inoculated with 6.6 x 10^7 CFU/lung of *S. aureus* and number of bacteria was determined at 24 and 48 hours, n=3-4 per group. (B) WT and *Ephx2*$^{-/-}$ mice were inoculated with 3.3 x 10^7 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



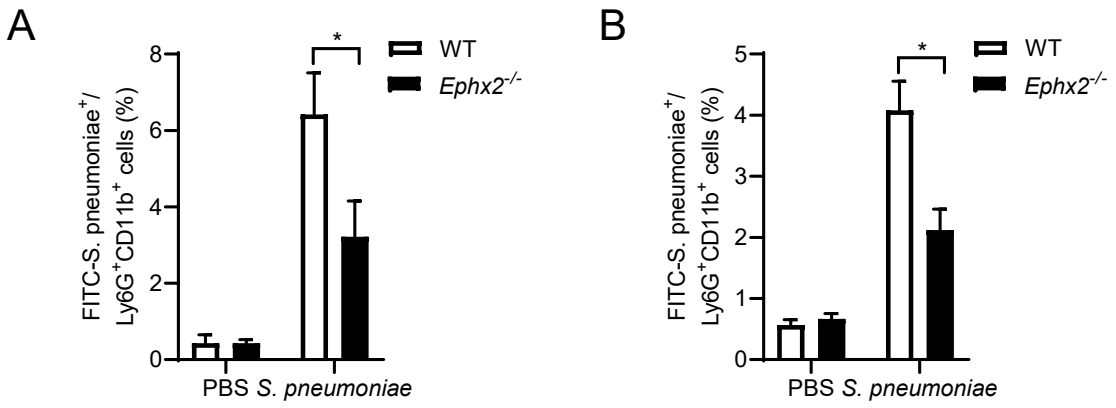
Supplemental Figure S3. Representative FACS analysis of WT and *Ephx2*$^{-/-}$ macrophage phagocytosis of *K. pneumoniae, S. pneumoniae* and *S. aureus in vitro*. Representative FACS results for the data in manuscript Figure 4a, 4b and 4c. WT and *Ephx2*$^{-/-}$ peritoneal macrophage were incubated with 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



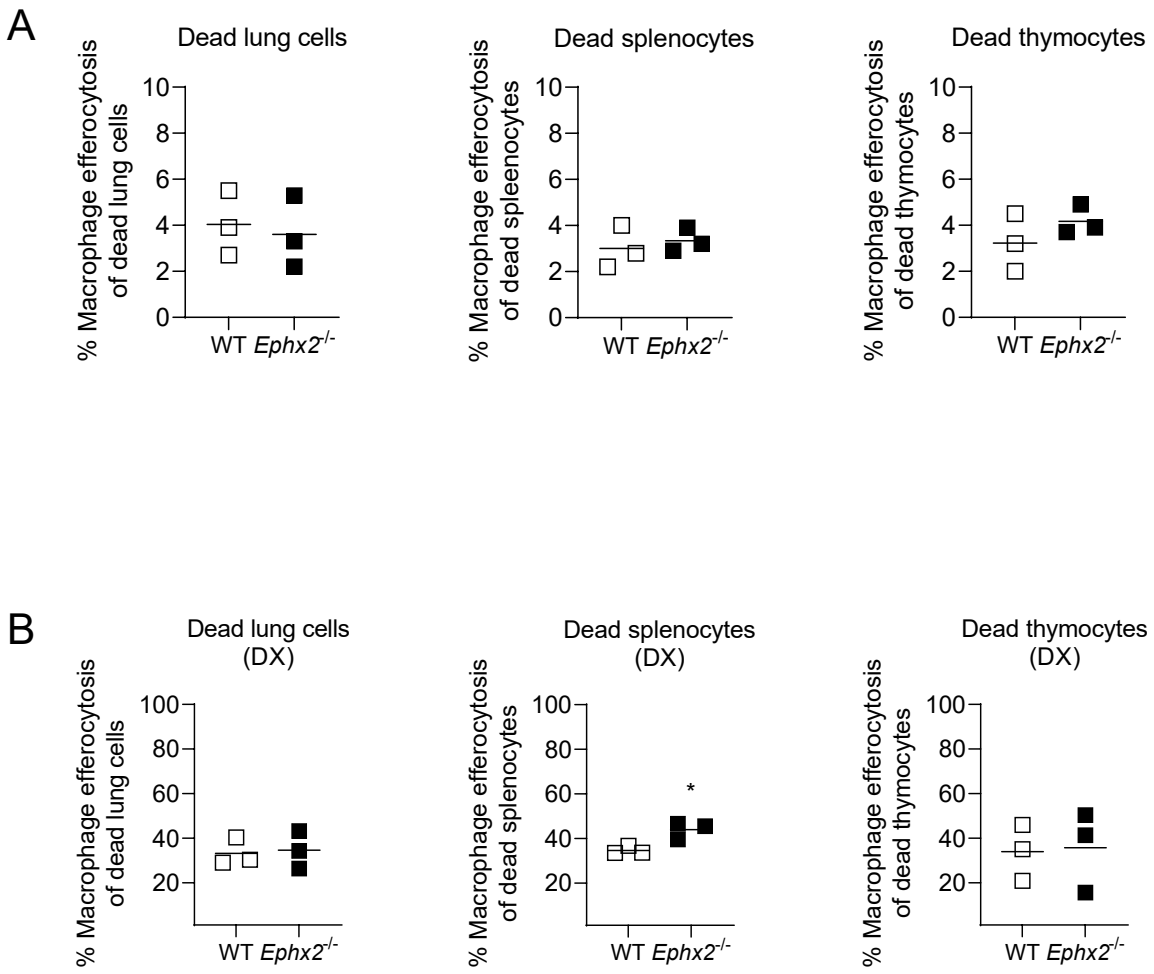
Supplemental Figure S4. Immunofluorescence analysis of WT and *Ephx2*$^{-/-}$ macrophage phagocytosis of *S. pneumoniae in vitro*. Confocal images of macrophages treated as in Figure 4B and Supplemental Figure 3B show internalization of *S. pneumoniae.* Immunofluorescent staining of FITC-*S. pneumoniae* (green), DAPI (nuclei, blue), and CD11b (red) are shown.

Supplemental Figure S5



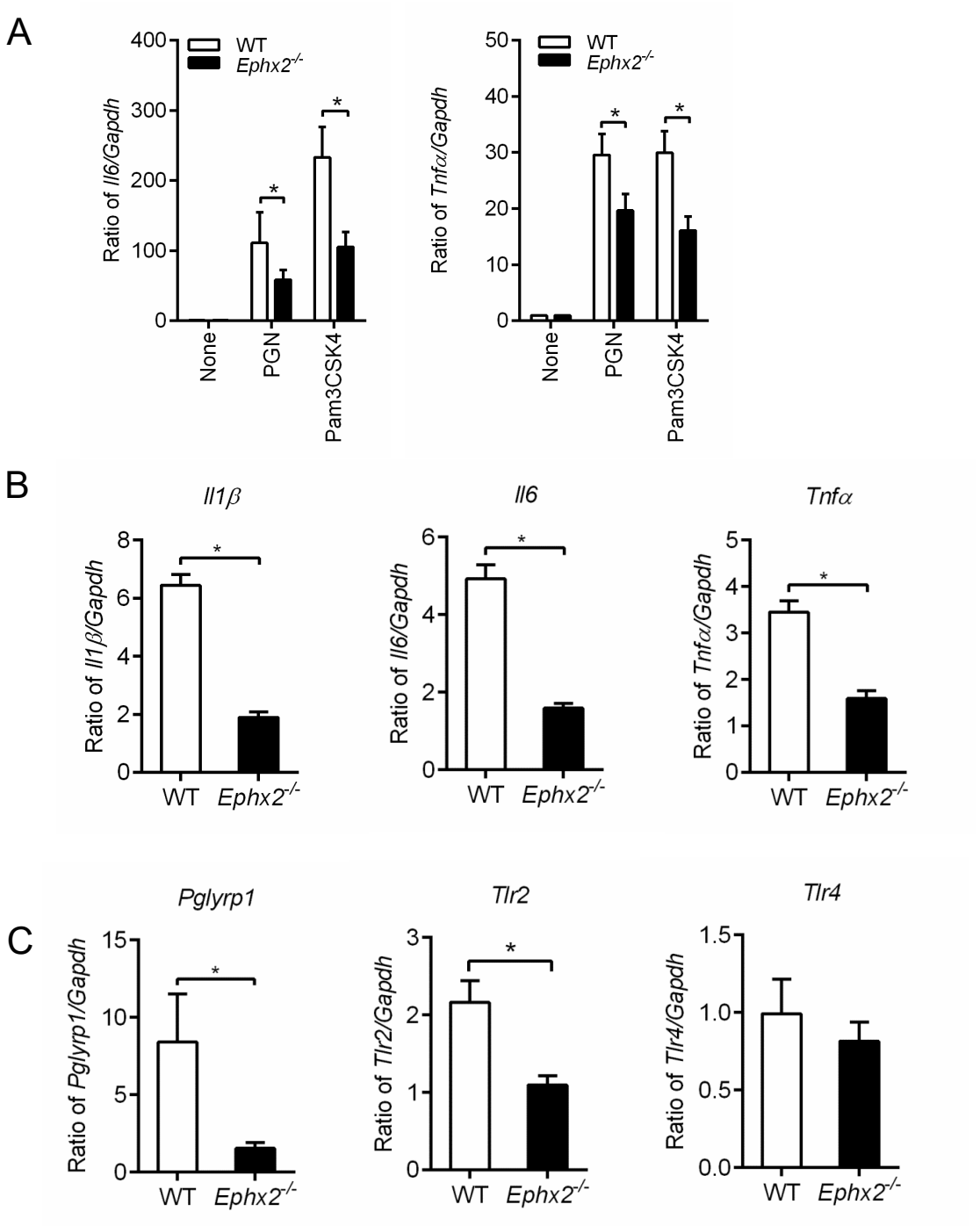
Supplemental Figure S5. *Ephx2*$^{-/-}$ neutrophils have reduced phagocytosis of *S. pneumoniae in vivo*. WT and *Ephx2*$^{-/-}$ mice were inoculated with *S. pneumoniae.* Twelve hours after inoculation, neutrophils (Ly6G+CD11b+ cells) from WT and *Ephx2*$^{-/-}$ lungs (A) and BALF (B) were stained with 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



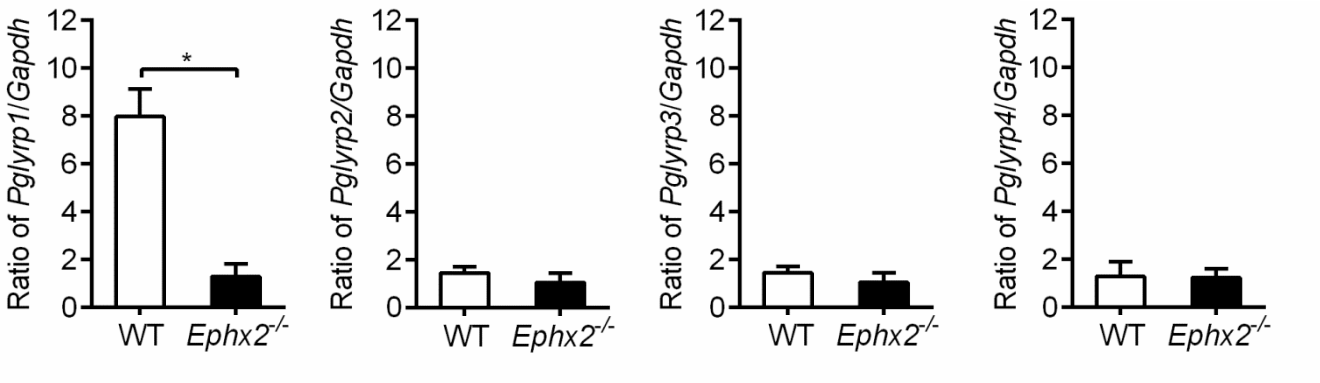
Supplemental Figure S6. Efferocytosis of dead cells by macrophages is not altered by *Ephx2* disruption. Efferocytosis of lung cells, splenocytes and thymocytes killed by heat (5 minutes boiling) (A) or 6-hour treatment with 5 μM dexamethasone (DX) (B) by WT and *Ephx2*$^{-/-}$ macrophages, n=3 per group; *p<0.05.

Supplemental Figure S7



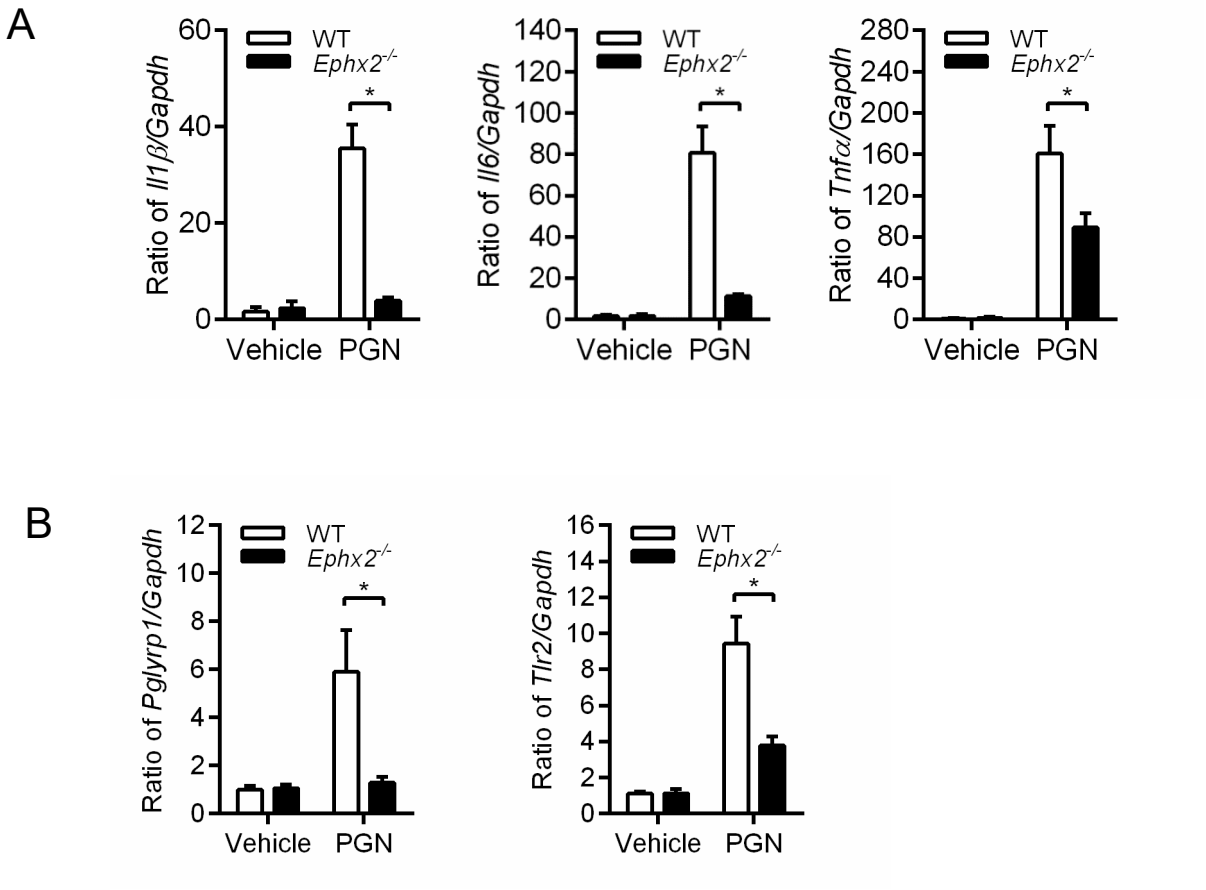
Supplemental Figure S7. Effects of PGN on expression of cytokines and pattern recognition receptors in WT and *Ephx2*$^{-/-}$ peritoneal macrophages. Peritoneal macrophages were isolated from WT and *Ephx2*$^{-/-}$ mice and treated with 10 µg/ml PGN or 10 µg/ml Pam3CSK4 for 4 hours. Both PGN- and Pam3CSK4 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



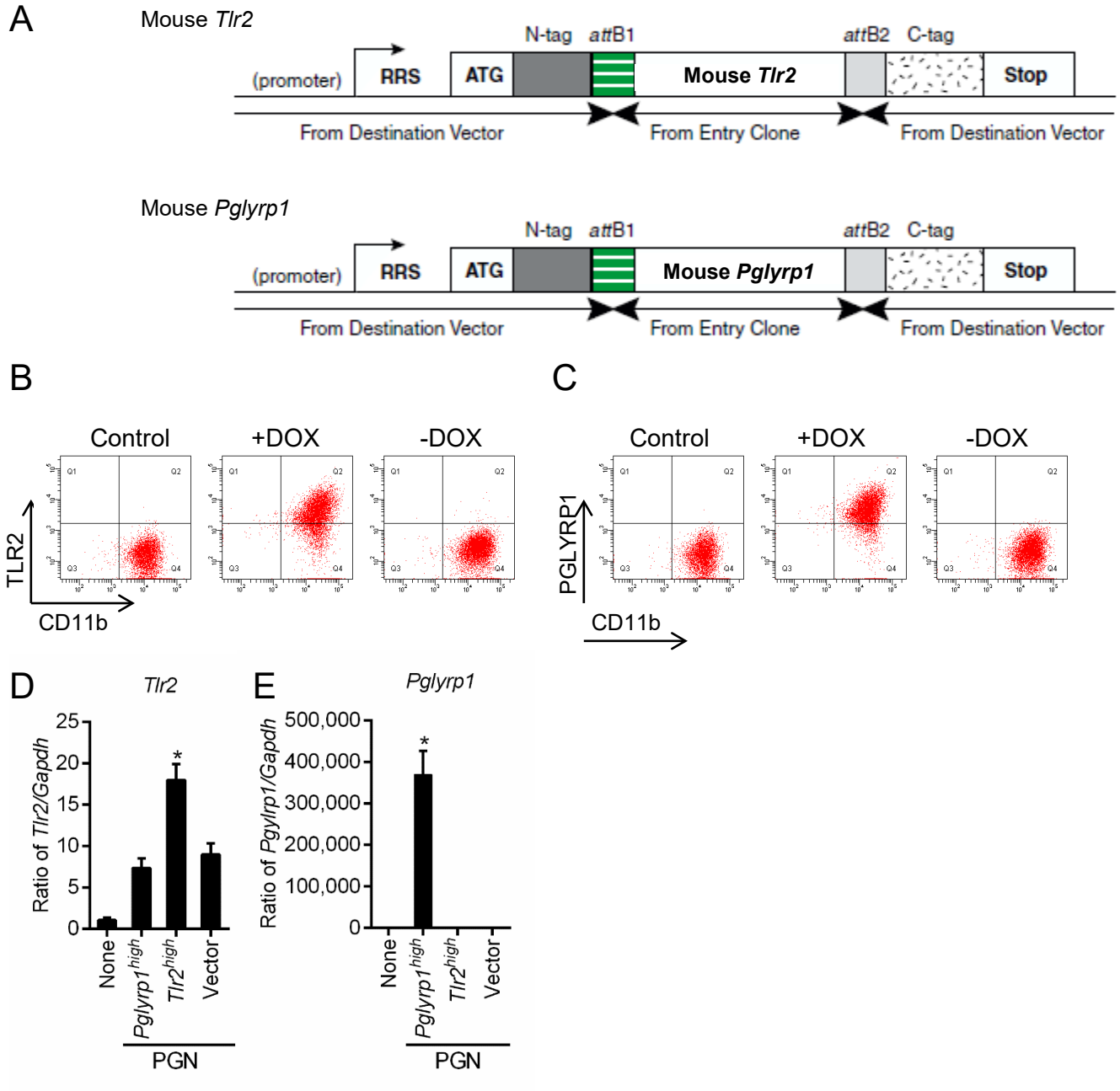
Supplemental Figure S8. Peptidoglycan recognition protein (*Pglyrp*) expression in lung macrophages. Lung macrophages ($CD11b^+F4/80^+$) from WT and *Ephx2*$^{-/-}$ mice were stimulated with PGN (10 μg/ml) for 4 hours. *Pglyrp1*, *Pglyrp2*, *Pglyrp3* and *Pglyrp4* transcript levels were quantified by real time quantitative RT-PCR and normalized to *Gapdh*. Data represent mean ± SEM, n=3 per group; *p<0.05.

Supplemental Figure S9



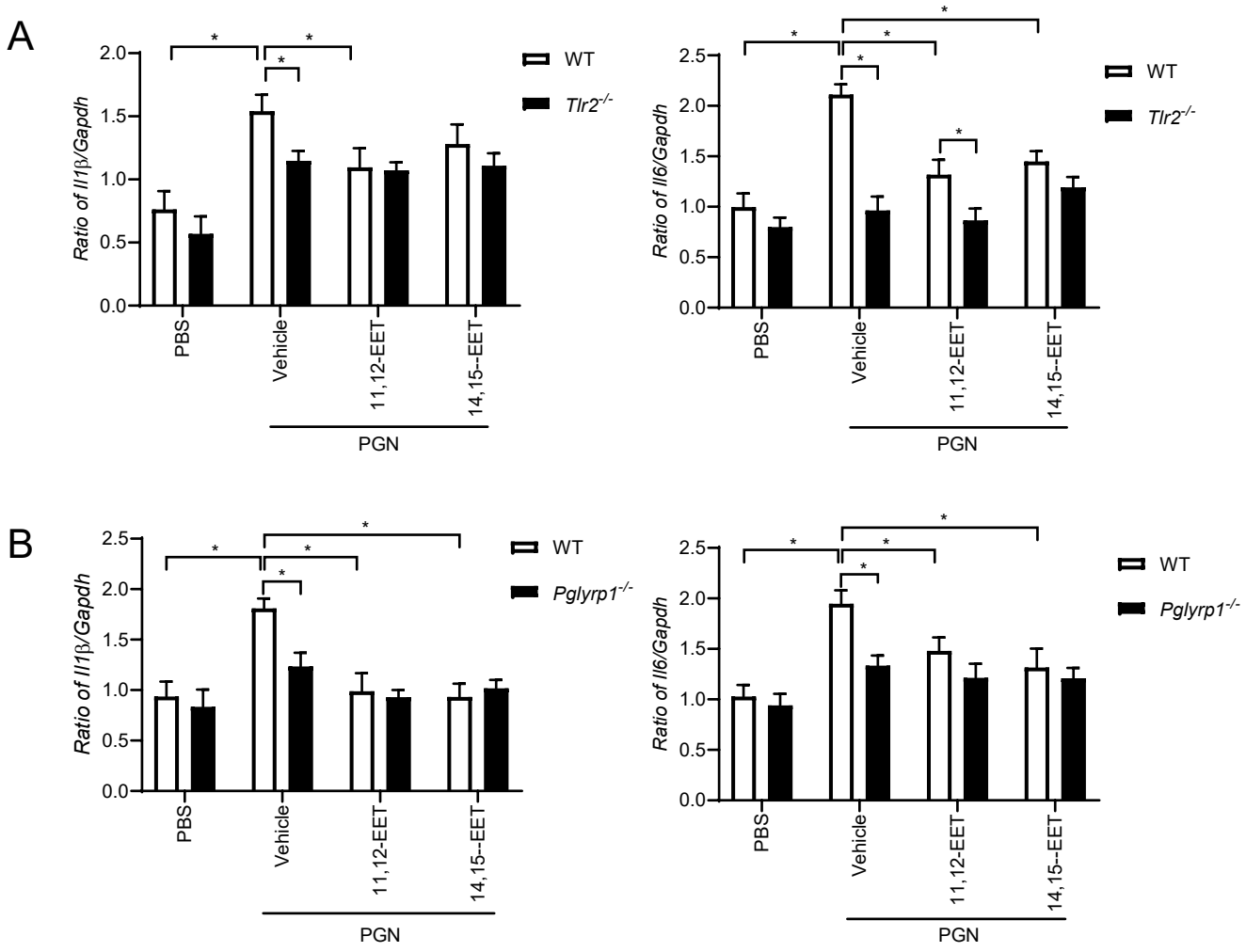
Supplemental Figure S9. *In vitro* treatment of WT and *Ephx2*$^{-/-}$ lung macrophages with peptidoglycan (PGN). Isolated lung macrophages from WT and *Ephx2*$^{-/-}$ mice were treated with vehicle or PGN for 4 hours. mRNA expression of cytokines *Il1β*, *Il6* and *Tnfα* (A) and pattern recognition receptors *Pglyrp1 and Tlr2* (B) normalized to *Gapdh* are shown. Data represent mean ± SEM, n=6 per group; *p<0.05.

Supplemental Figure S10



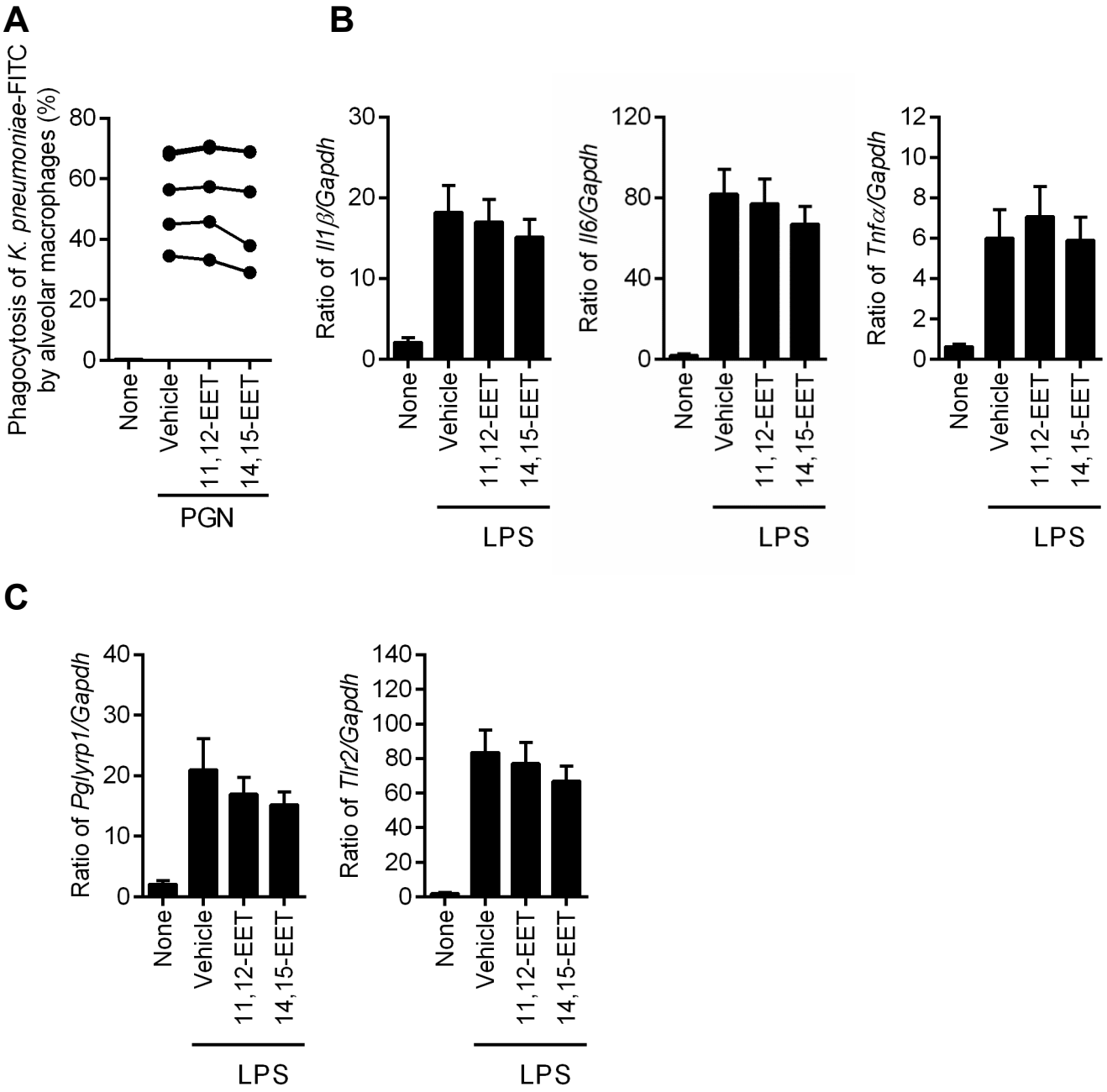
Supplemental Figure S10. Overexpression of *Tlr2* and *Pglyrp1* in primary macrophages using the pINDUCER20 Lentivirus system. (A) *Tlr2*–pINDUCER20 and *Pglyrp1*-pINDUCER20 plasmid constructs. Mouse peritoneal macrophages were infected with *Tlr2* (B) or *Pglyrp1* (C) lentivirus for 48 hours under G418 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



Supplemental Figure S11. Suppression of *Il1β* and *Tnfα* mRNA levels after PGN treatment is reduced by *Tlr2* or *Pglyrp1* disruption and EET treatment. Peritoneal macrophages were isolated from *Tlr2*$^{-/-}$ (A), *Plglyrp1*$^{-/-}$ (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



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 *Tnfα* (B) or *Pglyrp1* and *Tlr2* (C) normalized to *Gapdh* was determined. n=4 per group. Data represent mean ± SEM.