Anti-LPS antibodies protect against *K. pneumoniae* by empowering neutrophil mediated clearance without neutralizing TLR4

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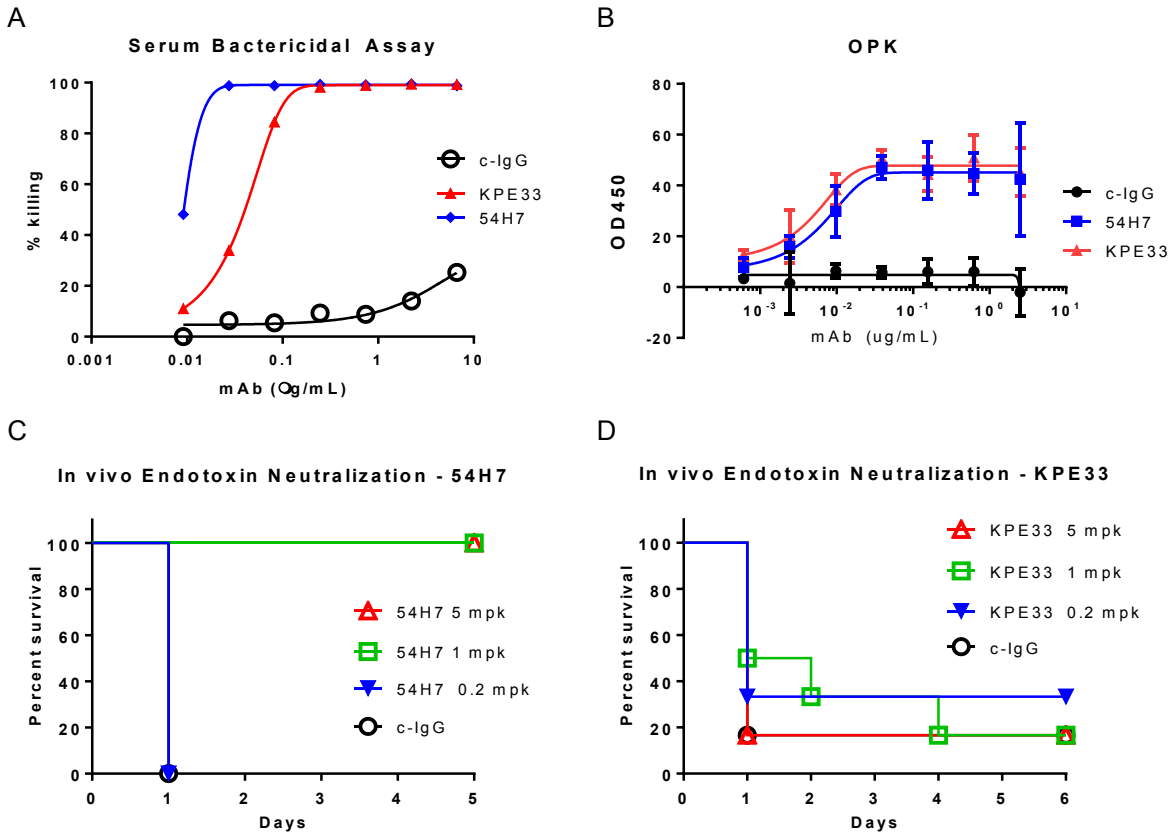
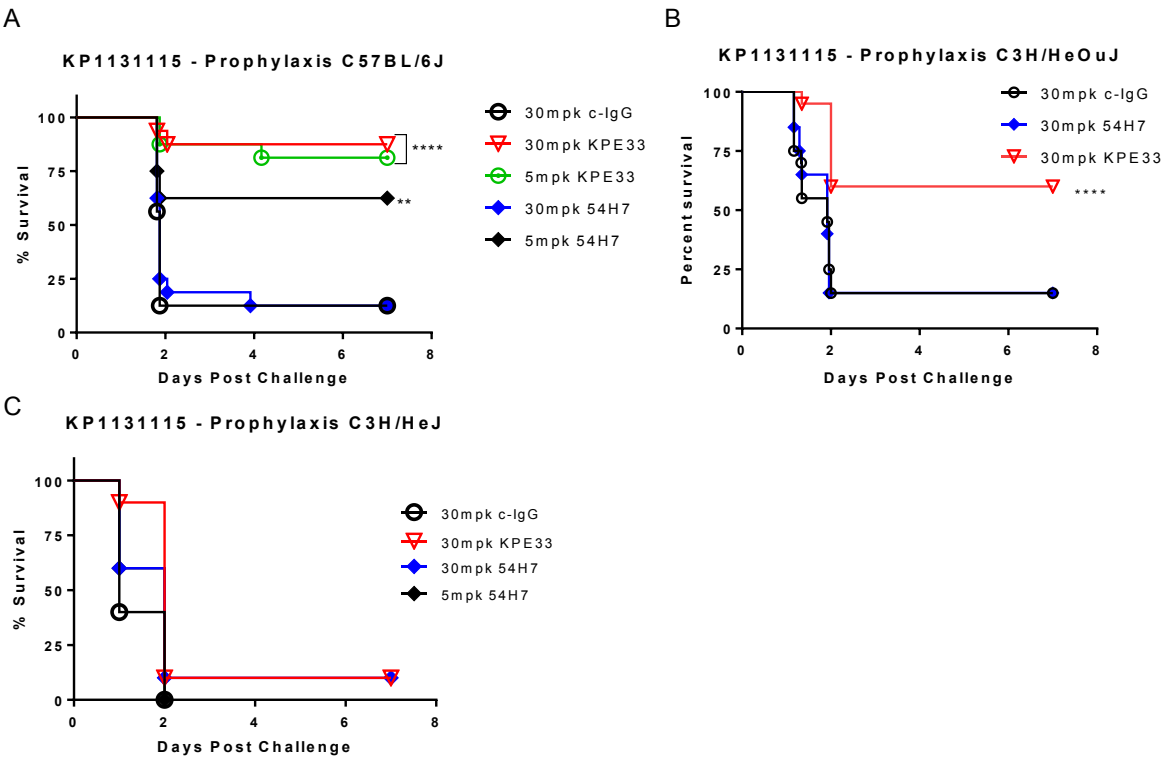
Conflict of Interest Statement:

All authors are employees of, or have received funding from, MedImmune, a member of the AstraZeneca group.

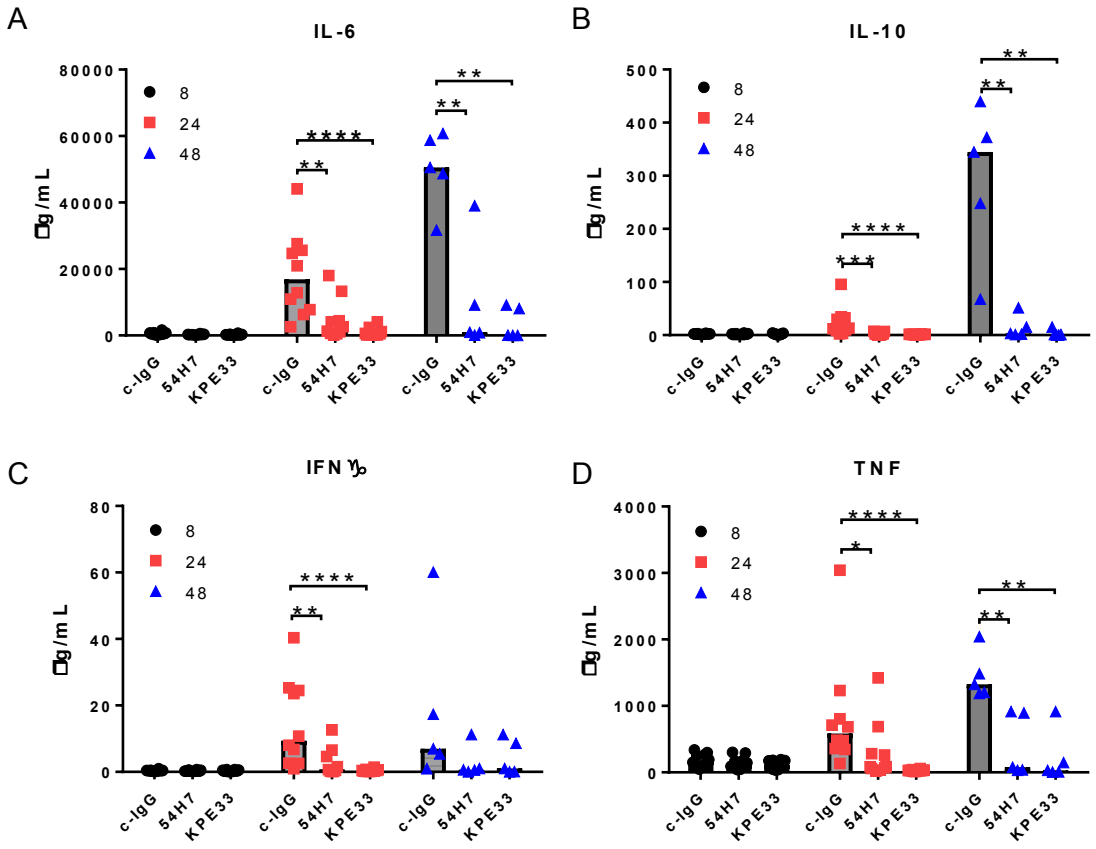
Supplemental Figures



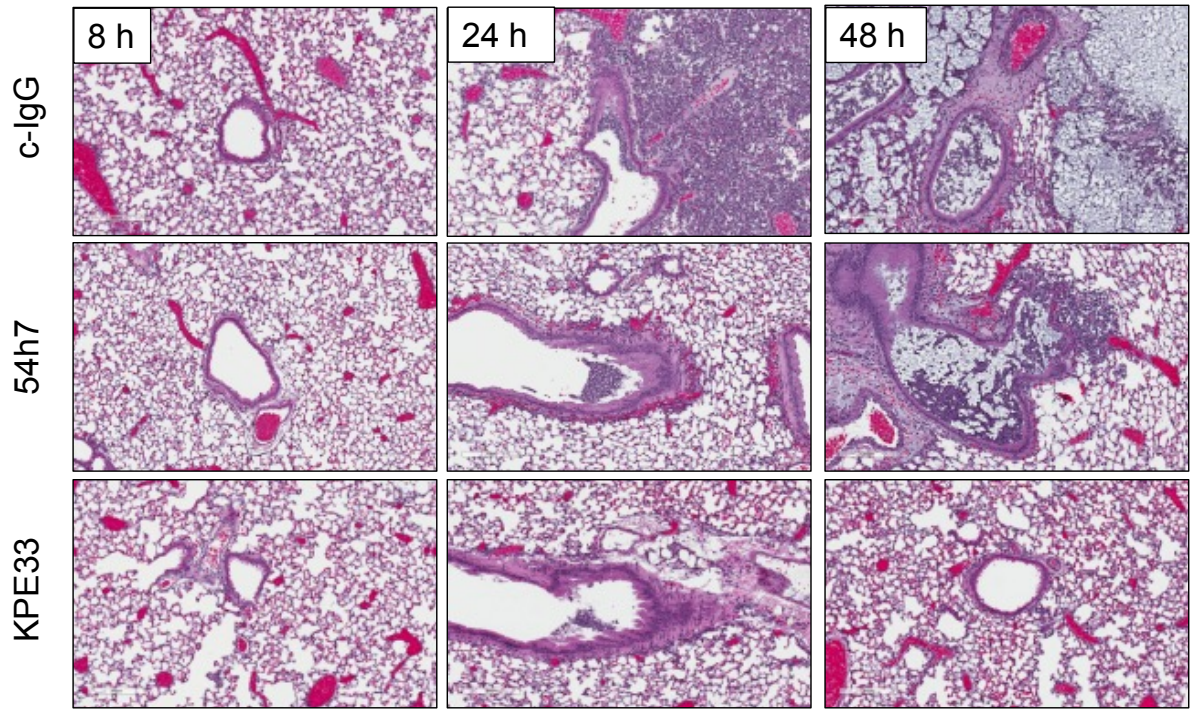
Supplemental Figure 1: Characterization of anti-LPS antibodies. (A) Killing of *K. pneumoniae* in baby rabbit serum containing increasing concentrations of mAb. (B) Opsonophagocytic killing (OPK) of baby rabbit serum opsonized *K. pneumoniae* by neutrophil like cells (HL-60) in the presence of increasing concentrations of mAb. (C) Survival of C57BL/6 mice prophylactically immunized with various doses of 54H7 24h prior to lethal endotoxin IV challenge (N = 6 per group). (D) Survival of C57BL/6 mice prophylactically immunized with various doses of KPE33 24h prior to lethal endotoxin IV challenge (N = 6 per group). Data representative of at least 2 independent experiments.



Supplemental Figure 2: Increasing concentrations of LPS neutralizing mAb fail to protect against *K. pneumoniae* pneumonia. (A) Survival of C57BL/6 mice prophylactically immunized 24h prior to infection (5e7 CFU KP113115) with c-IgG or anti-LPS mAbs KPE33 and 54H7. (B) Survival of C3H/HeOuJ mice prophylactically immunized 24h prior to infection(5e7 CFU KP1131115). (C) Survival of C3H/HeJ mice prophylactically immunized 24h prior to infection (5e7 CFU KP113115) with c-IgG or anti-LPS mAbs KPE33 and 54H7. Statistical significance was determined by log rank test (Survival, ** $p = 0.0093$, **** $p < 0.0001$ vs. c-IgG). (A-C) Data combined from 2 independent experiments ($N \geq 16$).

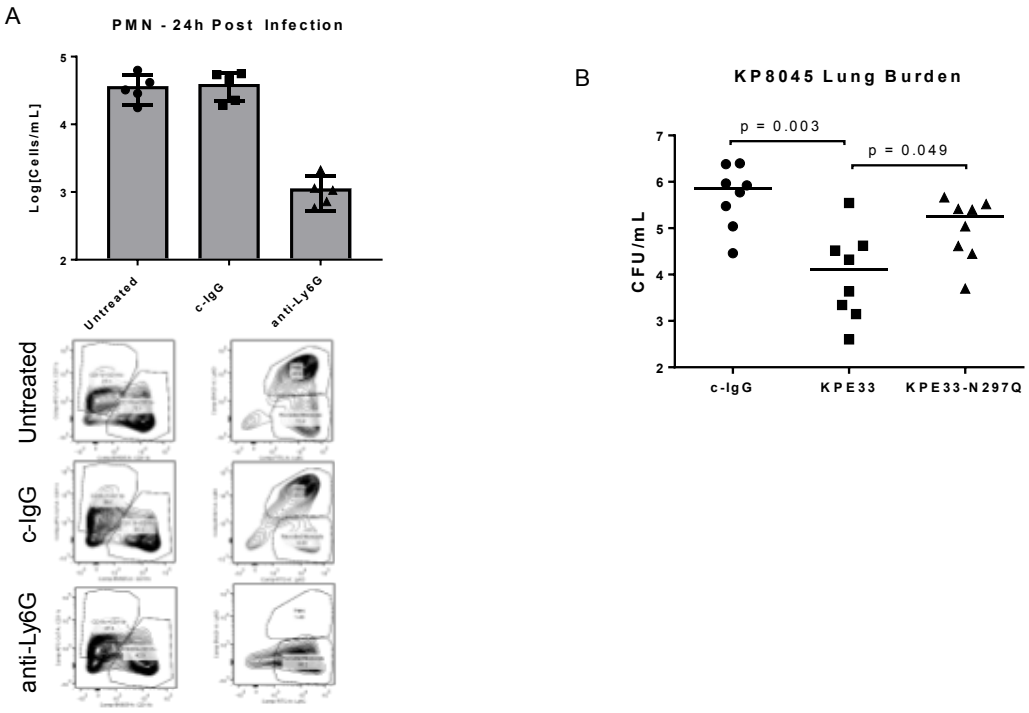


Supplemental Figure 3: Anti-LPS mAbs reduce levels of cytokines in the lung. (A-D) Multiplex ELISA measurement of cytokine expression 8, 24, and 48h following infection of mice with *K. pneumoniae* (1e4 CFU KP8045). mAbs were delivered 24h prior to infection. Statistical significance ($p < 0.05$) was determined by ANOVA followed by Dunn's test (* $p < 0.05$, ** $p < 0.01$. *** $p < 0.001$, **** $p < 0.0001$ mAb vs c-IgG). Data representative of at least 2 independent experiments.

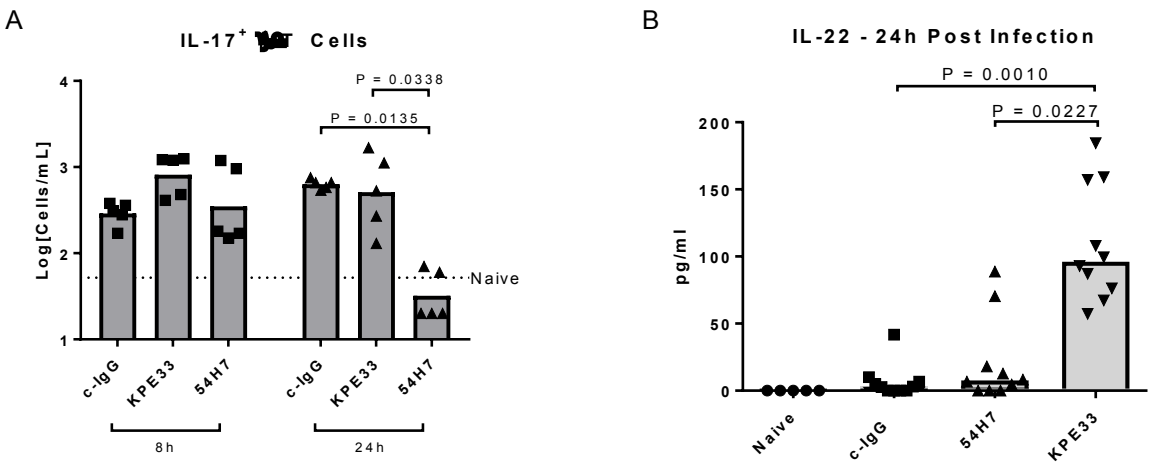


Supplemental Figure 4: Hematoxylin and eosin stained lung sections from prophylactically immunized mice.

Hematoxylin and eosin stained lung sections from prophylactically immunized C57BL/6 mice (54H7, KPE33 or c-IgG 24h prior to infection). Lungs were harvested 8, 24, or 48h post infection with *K. pneumoniae* (1e4 CFU KP8045). Magnification 200x.

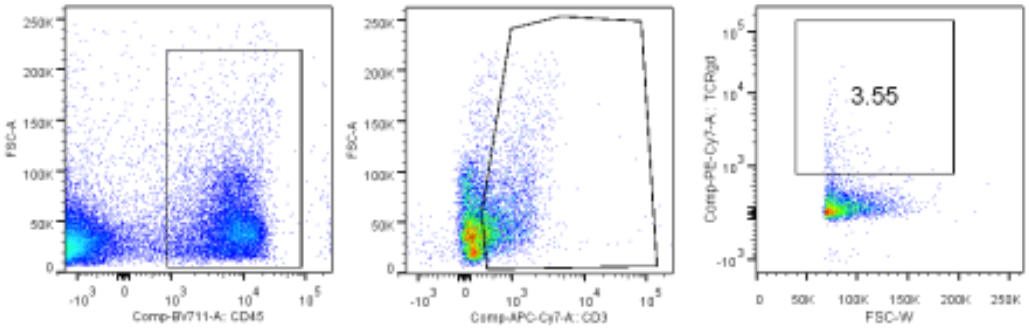


Supplemental Figure 5: Inhibiting Fc interaction with KPE33 prevents bacterial clearance. (A) Numbers of neutrophils (PMNs) recovered from the lungs of mice treated 24h prior to infection with anti-Ly6G or c-IgG then infected with KP8045 (24h). Representative FACS plots shown below. (B) Numbers of *K. pneumoniae* recovered from the lungs of prophylactically immunized C57BL/6 mice 24h prior to infection (24h, 1e4 CFU KP8045). Statistical significance determined by ANOVA with post hoc Dunn's test. Data representative of at least 2 independent experiments.



Supplemental Figure 6: LPS neutralization impairs IL-17 signaling in the lung.

(A) Numbers of IL-17$^+$ γδTCR$^+$ Tcells in the lungs of mice prophylactically treated with c-IgG, KPE33 or 54H7 8 and 24h following infection. (B) Levels of IL-22 in the BALF 24h following infection of mice prophylactically treated with c-IgG, 54H7 or KPE33. Statistical significance determined by ANOVA with post hoc Dunn's test. Data representative of at least 2 independent experiments.



Supplemental Figure 7: FACS gating strategy.