

Online Data Supplement:

IL-33-mediated Eosinophilia Protects Against Acute Lung Injury

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The authors have declared that no conflicts of interest exist.

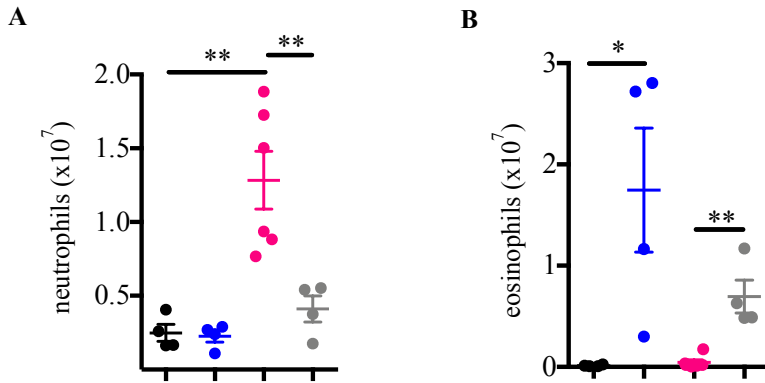
Running Title: Eosinophilia protects against lung injury

Supplemental Methods:

Lung digestion: Lungs were processed as previously described (1, 13). Mice from all groups were euthanized on the same day and lungs were perfused with sterile, cold PBS. Lungs were digested with 150 U/ml of Collagenase D (Sigma-Aldrich) for 1 hour at 37°C in DMEM media (Invitrogen) supplemented with 5% FCS. Red blood cells were briefly lysed with ACK lysis buffer. For flow cytometry staining, 500,000 cells in single suspension were resuspended in 100 µl of PBS containing 0.1% sodium azide and 0.2% bovine serum albumin. Cells were stained in the same manner as used for the BAL.

Supplementary Figure E1

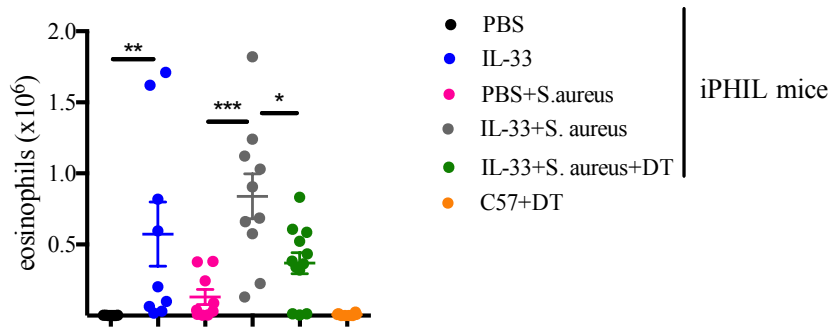
Lung digest (18 hours p.i.)



Supp Fig E1 IL-33 pretreatment induces lung eosinophilia while inhibiting *S. aureus*-induced neutrophilia during pneumonia. C57BL/6 mice were treated intratracheally with PBS, IL-33, PBS+*S. aureus* or IL-33+*S. aureus*. All groups were euthanized and flow cytometry was performed on lung digests at 18 hours p.i. Shown are graphs depicting **A**, neutrophil or **B**, eosinophil counts. Significance was determined using a two-way ANOVA, with a tukey post-hoc test. Graphs are representative of 2 independent experiments, with sample sizes of 3-6 per group.

Supplemental Figure E2

BAL total cell numbers at 18 hours post-infection demonstrating eosinophil reduction in IL-33 treated iPHIL mice



Supp Fig E2. Airway eosinophil depletion in IL-33 treated iPHIL mice. On days -3 to -1, iPHIL mice were treated i.t. with PBS or IL-33. *S. aureus*+Diphtheria toxin (DT) mice received DT intraperitoneally on day -1. Mice were infected intratracheally with *S. aureus* on day 0. Shown are BAL eosinophil total cell numbers quantified using flow cytometry. All groups were euthanized at 18 hours p.i. As a negative control, C57BL/6 mice were treated with DT alone. Eosinophils were defined as singlet, live, CD45⁺Ly6G^{lo}CD11c⁻SiglecF⁺SSC^{hi}. Statistical analysis was performed with a one-way ANOVA. Graph is pooled from 3 independent experiments, with n=7-12 per group. *p<0.05; **p<0.01; ***p<0.001.