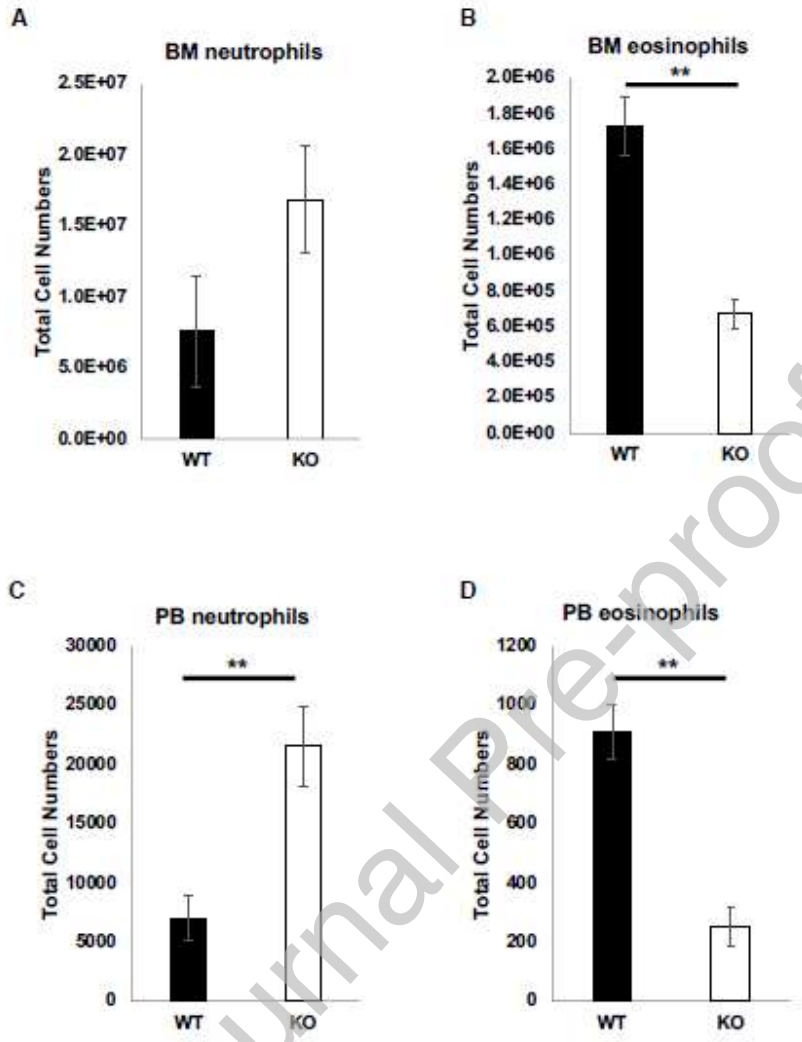
Supplemental Materials

Supplemental Figure 1



Supplemental Figure 1

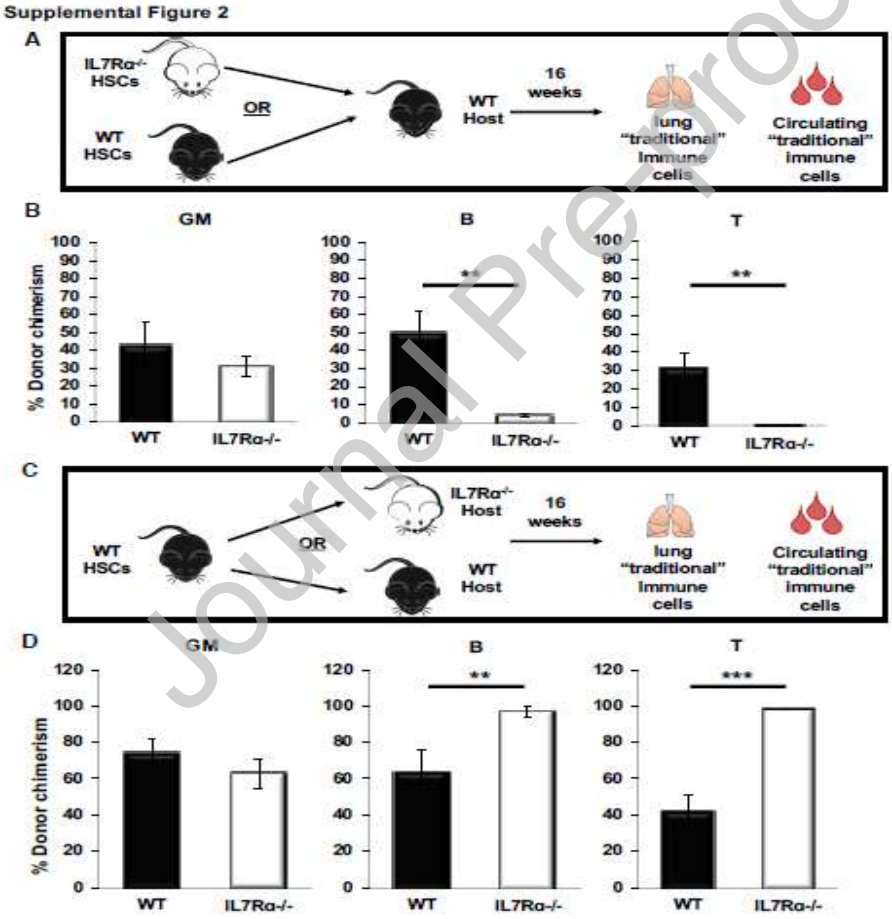
Neutrophil and eosinophil homeostasis are altered in the bone marrow (BM) and peripheral blood (PB) of IL7Rα$^{-/-}$ mice. Quantification of total cells per 25ul of PB or 1 leg (for BM) of WT (black) or IL7Rα$^{-/-}$ (white) adult mice. Error bars are SEM, WT n=3 and IL7Rα$^{-/-}$ n=4 representing two independent experiments. *P<0.05, **P<0.005, ***P<0.001.

A, Neutrophil numbers were not significantly different in the BM of Il7Rα$^{-/-}$ mice. Quantification of neutrophils (Live, CD3-CD4-CD5-CD8-B220-Ter119-CD45+Ly6g+CD11b+) per 1 leg of wild type (WT) (black) or IL7Rα$^{-/-}$ (white) adult mice.

B, Eosinophil numbers were significantly reduced in the BM IL7Rα$^{-/-}$ mice. Quantification of eosinophils (Live, CD3-CD4-CD5-CD8-B220-Ter119-CD45+Ly6g-CD11b+SiglecF^{mid}CD11c-) per 1 leg of wild type (WT) (black) or IL7Rα$^{-/-}$ (white) adult mice.

C, Neutrophil numbers were significantly increased in the PB of IL7Rα$^{-/-}$ mice. Quantification of neutrophils (Live, CD3-CD4-CD5-CD8-B220-Ter119-CD45+Ly6g+CD11b+) per 25ul of wild type (WT) (black) or IL7Rα$^{-/-}$ (white) adult blood.

D, Eosinophil numbers were significantly reduced in the PB of IL7Rα$^{-/-}$ mice. Quantification of eosinophils (Live,CD3-CD4-CD5-CD8-B220-Ter119-CD45+Ly6g-CD11b+SiglecF^{mid}CD11c-) per 25ul of wild type (WT) (black) or IL7Rα$^{-/-}$ (white) adult blood.



Supplemental Figure 2

A, Schematic depicting the transplantation experimental setup used to determine cell extrinsic mechanisms regulating "traditional" mature myeloid and lymphoid cell development. 500 WT or

IL7Rα$^{-/-}$ HSCs were transplanted into a ¾ sublethally irradiated wild type (WT) GFP recipient. After 16 weeks post transplant, the peripheral blood was harvested and mature immune cells were analyzed via flow cytometry for donor chimerism.

B, IL7R deletion did not alter the ability of HSCs to reconstitute "traditional" circulating myeloid cells, but did display reduced capacity to generate B and T lymphocytes. Percent donor chimerism of granulocyte/macrophage (GM) (Gr1+CD11b+), B lymphocytes (B220+), and T lymphocytes (CD3+) in the peripheral blood of the same transplanted mice as in Figure 3B. Error bars are SEM, WT n = 5 IL7Rα$^{-/-}$ n=6 from 2 independent experiments. ** = p<0.05 , **P<0.005, ***P<0.001.

C, A schematic depicting the transplantation experimental setup used to determine cell extrinsic mechanisms regulating "traditional" circulating myeloid and lymphoid cells.

D, WT HSCs robustly reconstituted "traditional" circulating myeloid and lymphoid cells in an IL7Rα$^{-/-}$ recipient. Percent donor chimerism of granulocyte/macrophage (GM) (Gr1+CD11b+), B lymphocytes (B220+), and T lymphocytes (CD3+) in the peripheral blood of the same transplanted mice as in Figure 3D. Error bars are SEM, WT n = 5, IL7Rα$^{-/-}$ n=13 from 4 independent experiments. * = p<0.05, **P<0.005, ***P<0.001.

Methods

Mice

All animals were housed and bred in the AALAC accredited vivarium at UC Santa Cruz and group housed in ventilated cages on a standard 12:12 light cycle. All procedures were approved by the UCSC Institutional Animal Care and Use (IACUC) committees. IL7Rα-Cre[6,16] and Flk2-Cre (Benz et al 2008) mice, obtained under fully executed Material Transfer Agreements, were crossed to homozygous Rosa26^{mTmG} females[17] to generate "switch" lines, all on the C57Bl/6 background. WT C56Bl/6 mice were used for controls and for all expression experiments. Adult male and female mice were used randomly and indiscriminately, with the exception of FlkSwitch mice, in which only males were used because of more uniformly high floxing in male than in female mice.

Tissue and cell isolation

Mice were sacrificed by CO_2 inhalation. Adult peripheral blood was collected by femoral artery knick. Lungs were dissected, manually dissociated, and incubated in 1x PBS(+/+) with 2% serum, 1-2mg/ml collagenase IV (Gibco) with 100U/ml Dnase1 for 1- 2 hours. Following incubation, all tissues were passed through a 16g needle ~10X followed by a 19g needle ~10X to make a single cell suspension, and then filtered through a 70 μM filter to obtain a single cell suspension. Cells were pelleted by centrifugation (1200g/ 4 degrees C /5 minutes). Numbers neutrophils, eosinophils, and B lymphocytes were analyzed and compared from the same tissue preparations from the same mice.

Flow Cytometry

Cell labeling was performed on ice in 1X PBS with 5 mM EDTA and 2% serum[6]. Analysis was performed on a customized BD FACS Aria II and analyzed using FlowJo. Antibodies used: cd45.2-PB (BioLegend-109820), Ter119-PGP (BioLegend-116202), CD3-PGP (BioLegend-100202), CD4-PGP (BioLegend-100402), CD5-PGP (BioLegend-100602), CD8-PGP (BioLegend-100702), B220-PGP (BioLegend-103202), CD11b-PGP (BioLegend-101202), Gr1-PGP (BioLegend-108402), Ly6g-APC (BiolLegend-127614), CD11b-PeCy7 (BiolLegend-101216), SiglecF-BV786 (BioLegend-740956), CD11c-APC-Cy7 (BioLegend-117323), B220-APC Cy7 (BioLegend-103224), CD45.2-A700 (Biolegend-109822), CD19-BV786 (Biolegend-115543), GAR-PE Cy5 (Life Technologies- A-10691).

Transplantation Assays

Transplantation assays were performed as previously described[11,18,30–34]. Briefly, sorted cells were isolated from wild type (WT) or IL7Rα knockout mice (IL7Rα$^{-/-}$) donor bone marrow. WT recipient mice aged 8-12 weeks were sublethally irradiated (750 rad, single dose) using a Faxitron CP-160 (Faxitron). Under isofluorane-induced general anesthesia, sorted cells were transplanted retro-orbitally.

Cytokine Analysis

Serum was harvested from wild type or IL7R$^{-/-}$ mice via cardiac puncture. Several hundred microliters of blood was collected and transferred to a 1.5ml anti-coagulant free tube and allowed to clot at room temp for 30 min. Blood was centrifuged at 3,000 rpm for 10 min at 4C.

Serum was carefully transferred to a fresh tube and stored at -80C until shipment to Eve Technologies (Calgary, Canada) for analysis..

Quantification and Statistical Analysis

Number of experiments, n, and what n represents can be found in the legend for each figure. Statistical significance was determined by two-tailed unpaired student's T-test. All data are shown as mean ± standard error of the mean (SEM) representing at least two to three independent experiments. Power calculations for cytokine analysis indicated that 3 mice was sufficient to achieve a statistical power of 80% at 0.9-0.95 confidence level (https://epitools.ausvet.com.au/twomeanstwo).