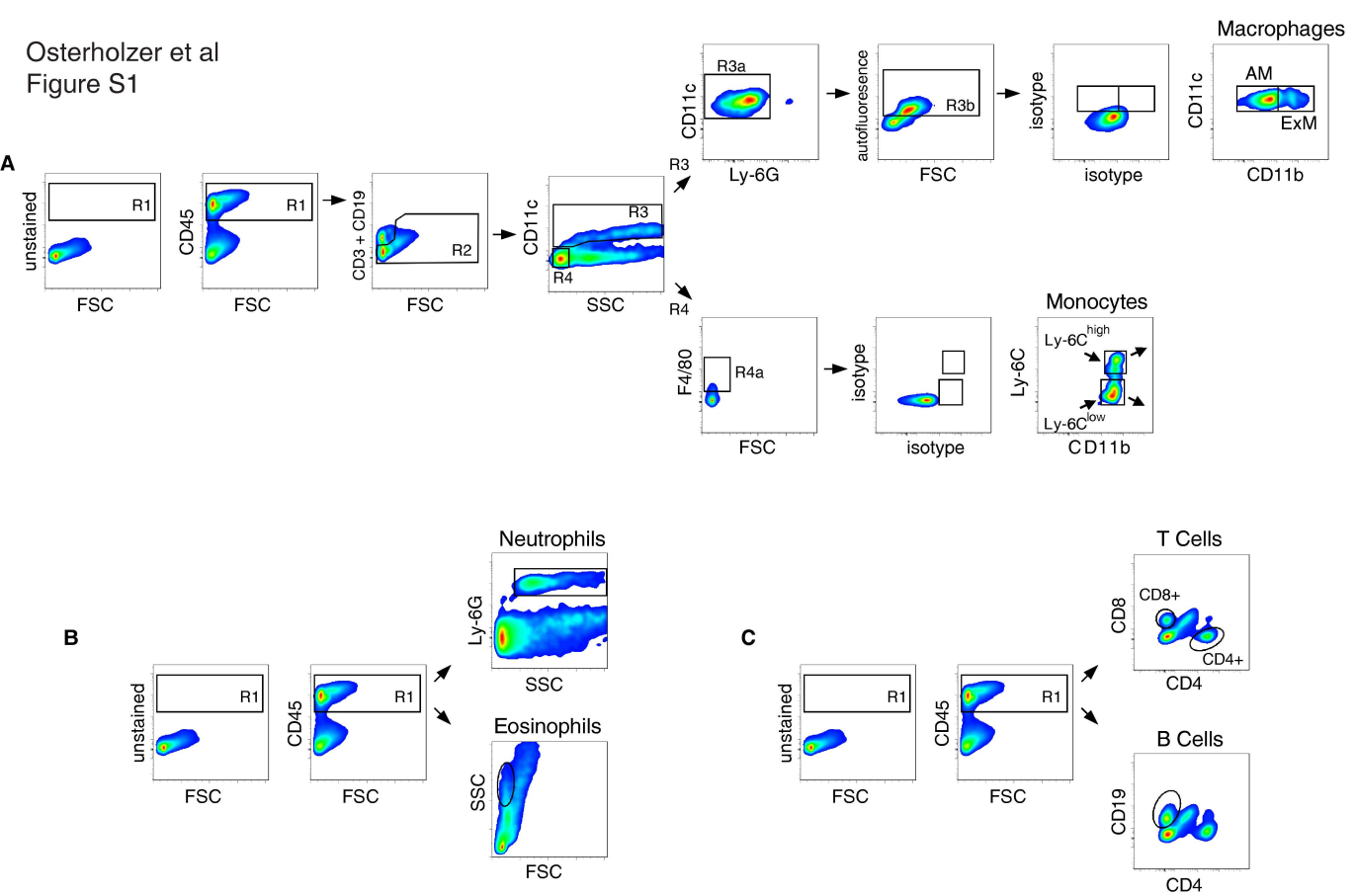

Figure S1. Gating strategy used to identify lung leukocyte subsets in mice with targeted injury to type II AEC. Diphtheria toxin was administered per protocol for 14 days to DTR+ mice. Lung cells were isolated, stained with specific antibodies, and analyzed by flow cytometric analysis as described in Materials and Methods. Preliminary plots of FSC vs SSC eliminate red blood cells, debris, and cell clumps (not shown). (A) Gating strategy used to identify macrophage and monocyte subsets. Initial dot plots of isotype controls and FSC vs CD45 and gate R1 identifies CD45+ cells. An R1 plot of FSC vs CD3 and CD19 (combined) and gate R2 excludes lymphocytes (defined as FSC^{low} CD3+ or CD19+ cells). An R2 plot of SSC vs CD11c and gate R3 next identifies CD11c+ cells (includes macrophages and dendritic cells) whereas gate R4 identifies SSC^{low} CD11c- cells (includes monocytes). Thereafter, an R3 plot of Ly-6G vs CD11c and gate R3a excludes residual Ly-6G+ granulocytes. Next, an R3a plot of FSC vs FL-3 (488-nm blue laser) and gate R3b distinguishes large autofluorescent ($FSC^{mod/high}$ AF+) macrophages from $FSC^{low/mod}$ AF- dendritic cells. Within the R3b gate, plots of isotype controls and CD11b vs. CD11c distinguish AM (as AF+ CD11b+ CD11c-, gate AM) and ExM (as AF+ CD11b+ CD11c+, gate ExM). Within the R4 gate (SSC^{low} CD11c- cells), a plot of F4/80 vs FSC and gate R4a identifies F4/80+ monocytes. Within the R4a gate, plots of isotype controls and Ly-6C vs CD11b identifies Ly-$6C^{high}$ CD11b+ monocytes (gate Ly-$6C^{high}$) and Ly-$6C^{low}$ CD11b+ monocytes (gate Ly-$6C^{low}$). (B) Gating strategy to identify granulocytes. Initial dot plots of isotype controls or CD45 vs FSC and gate R1 identifies CD45+ cells. An R1 plot of Ly-6G vs SSC identifies neutrophils (Ly-$6G^{high}$ $SSC^{mod/high}$). A separate R1 plot of FSC vs SSC identifies eosinophils ($FSC^{low/mod}$ SSC^{high}). Eosinophils are also Ly-$6G^{mod}$ (not shown). (C) Gating strategy to identify lymphocytes. Initial dot plots of isotype controls and CD45 vs FSC and gate R1 identifies CD45+ cells. An R1 plot of CD4 vs CD8 identifies CD4+ T cells and CD8+ T cells. A separate R1 plot of CD4 vs CD19 identifies CD19+ B cells.

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Figure S2

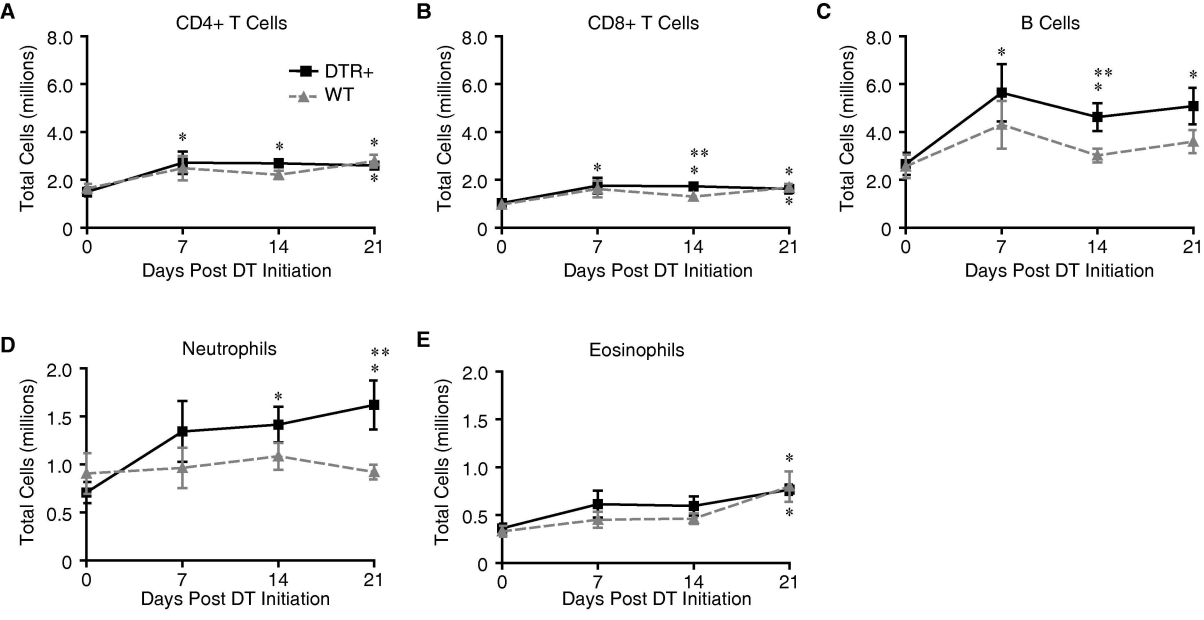

Figure S2. Accumulation of lymphocytes and granulocytes in mice with targeted injury to type II AEC. (A-E) Diphtheria toxin was administered per protocol for 14 days to either WT (DTR-) or DTR+ mice. Lung cells isolated from untreated mice (D0) and at 7, 14, and 21 days after the onset of DT treatment were stained with specific antibodies and analyzed by flow cytometric analysis as described in Materials and Methods and Supplemental Figure S1. Total numbers of CD4+ T cells (A), CD8+ T cells (B), B cells (C), neutrophils (D), and eosinophils (F), present in the lungs of WT or DTR+ mice at each timepoint. Total leukocyte subset numbers were calculated by multiplying the frequency of each population by the total number of CD45+ lung leukocytes at each time point. Data represent mean ± SEM of 8-12 mice assayed individually per time point. * $p<0.05$ ANOVA vs. Day 0 (untreated) of mice of the same DTR expression profile; ** $p<0.05$ when values from WT mice or DTR+ mice were compared against each other at the designated time point.