SUPPLEMENTAL FIGURES

Supplemental Fig. 1. T-lymphocytes increase in the alveolar compartment after IT LPS. BAL cells harvested at intervals after IT LPS were evaluated by FACS. $CD3^+$ cells were analyzed for CD4 and CD8 expression with gating into quadrants as shown. $CD4^+$, $CD8^+$, and $CD4^-CD8^-$ cells increased in the alveolar compartment after LPS.

Supplemental Fig. 2. T- lymphocytes do not increase in the lung parenchyma after LPS. BAL was performed at designated intervals after IT LPS to eliminate alveolar compartment cells. Lung tissue mononuclear cells were then isolated and evaluated by FACS. CD3+ cells were examined for expression of CD4 and CD8. The abundance of $CD4^+$, $CD8^+$, and $CD4^-CD8^-$ cells did not change after IT LPS.

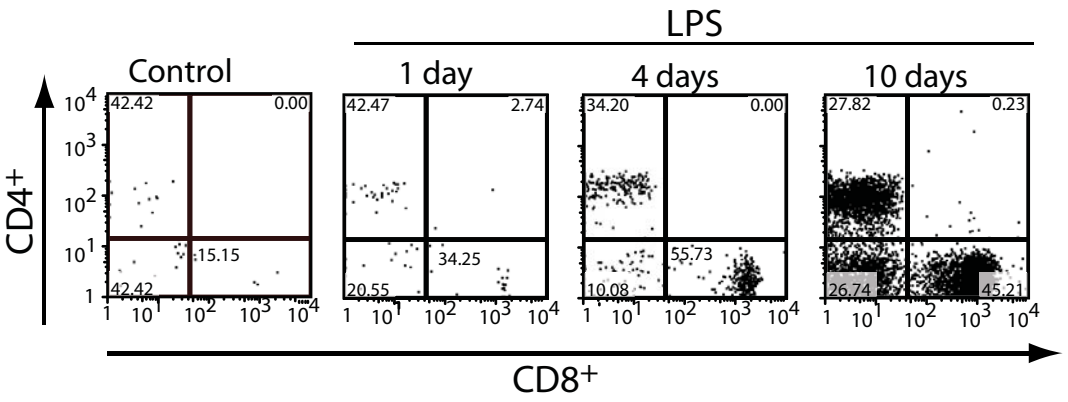
Supplemental Fig. 3 Flow cytometry reveals coexpression of Foxp3 and FR4 in CD4+CD25+ cells from the alveolar compartment during the course of lung injury after IT LPS compared to naïve splenocytes.

Supplemental Fig. 4. Neutrophil chemokines were not different between WT and Rag-$1^{-/-}$ mice. Mice received IT LPS or sterile water (control), and designated groups of Rag-$1^{-/-}$ mice received $CD4^+CD25^-$ or $CD4^+CD25^+$ cells one hour after LPS. BAL was harvested at times as noted, and BAL fluid was analyzed for Keratinocyte Chemokine (KC), Granulocyte-colony stimulating factor (G-CSF), or Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) using mouse cytokine multiplex assay. n=4/group; * $p < 0.05$ vs. Rag-$1^{-/-}$ at each time point.

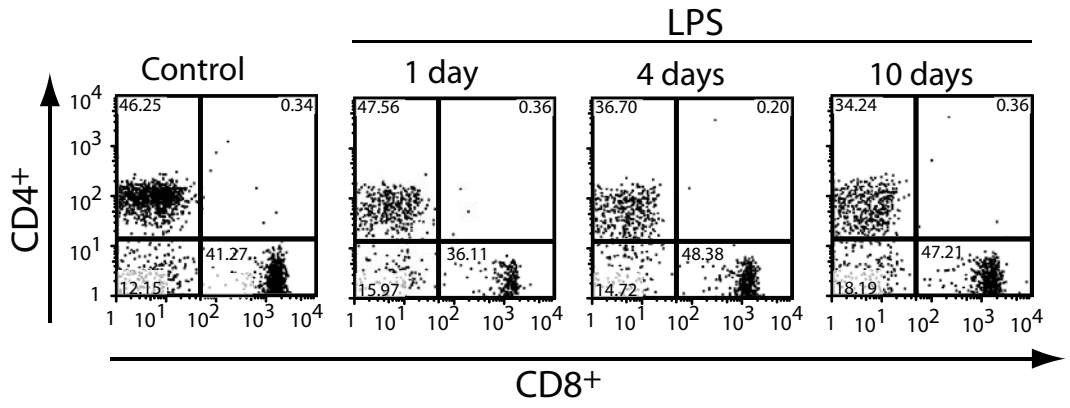
Supplemental Fig. 5. Alveolar neutrophils persist in the absence of Tregs. WT and Rag-$1^{-/-}$ mice were exposed to IT LPS, and BAL was performed on day 4 to harvest a mixed population of neutrophils and macrophages; WT BAL also had lymphocytes. The % neutrophils in BAL fluid was determined (pre-culture), and BAL fluid was plated. As designated, media alone was added to BAL from WT and Rag-$1^{-/-}$ mice, or either $CD4^+CD25^-$ or $CD4^+CD25^+$ cells were added to the mixed cell culture. At 24 and 48 hours after harvest and co-culture, the % remaining neutrophils was determined. Co-cultures containing Tregs (WT and Rag-$1^{-/-}$ with CD4+CD25+ cells added) had

significantly fewer neutrophils remaining at 48 hours than the co-cultures in the absence of Tregs.

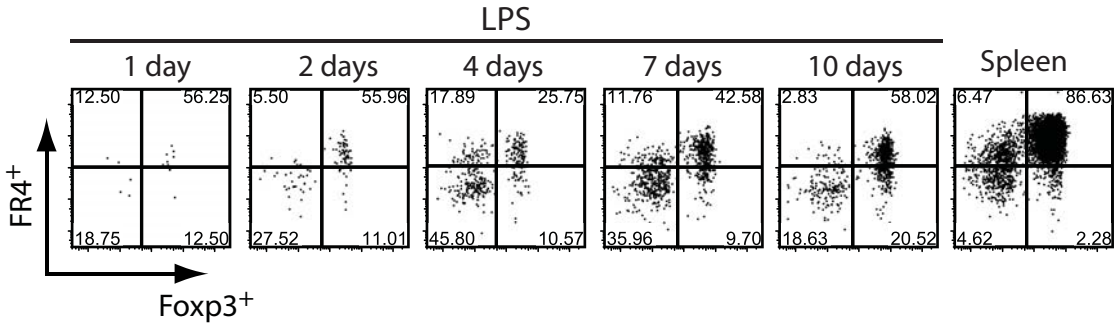
† $p < 0.05$ vs. Rag-$1^{-/-}$ / media only at each time point.



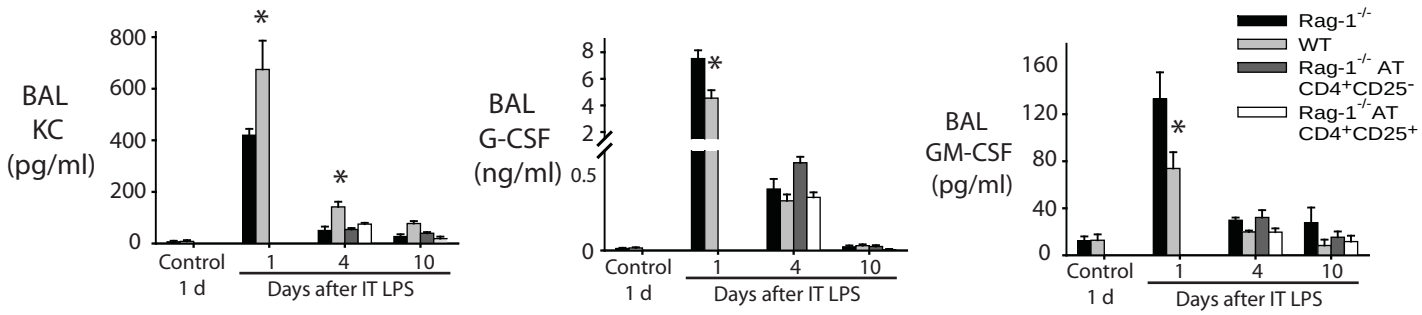
Supplemental Fig. 1



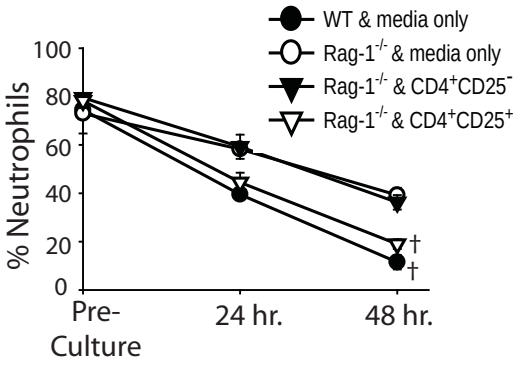
Supplemental Fig. 2



Supplemental. Fig. 3



Supplemental Fig. 4



Supplemental Fig. 5