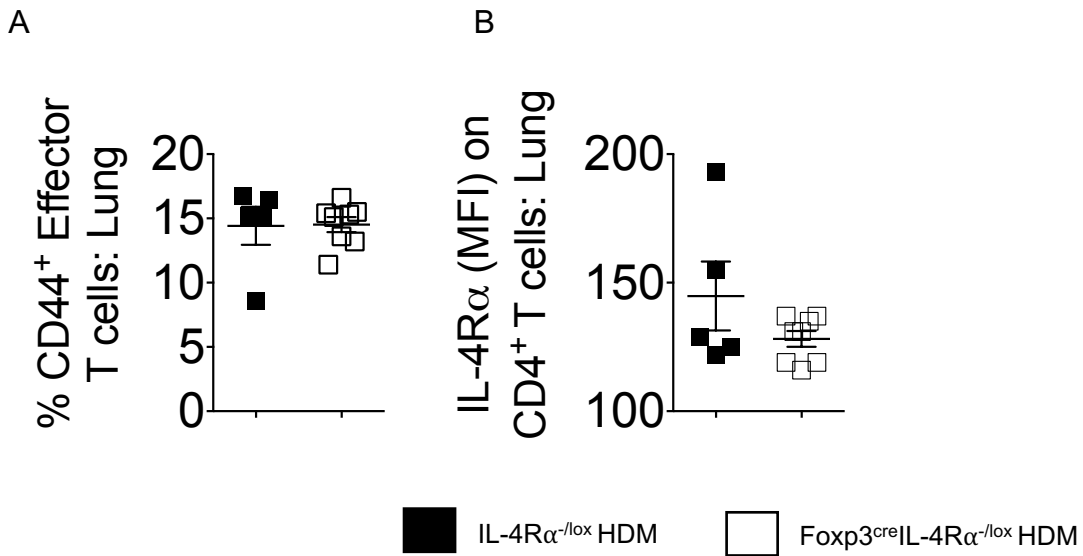


Supplemental Figure 1. Gating strategy for CD4⁺ CD25⁺ FoxP3⁺ T regulatory cells in both the mLN and lung tissue.

We counted live cells by trypan blue and excluded dead cells by 7AAD or Qdot605 live/dead stain marker. We excluded doubles and gated on lymphocytes. To get to T reg cells, we surface stained with CD19-CD3⁺, followed by CD4⁺CD8⁻CD25⁺. To detect Foxp3, we fixed the cells and permeabilised with eBioscience Foxp3 kit before staining for intracellular transcriptional factor Foxp3.

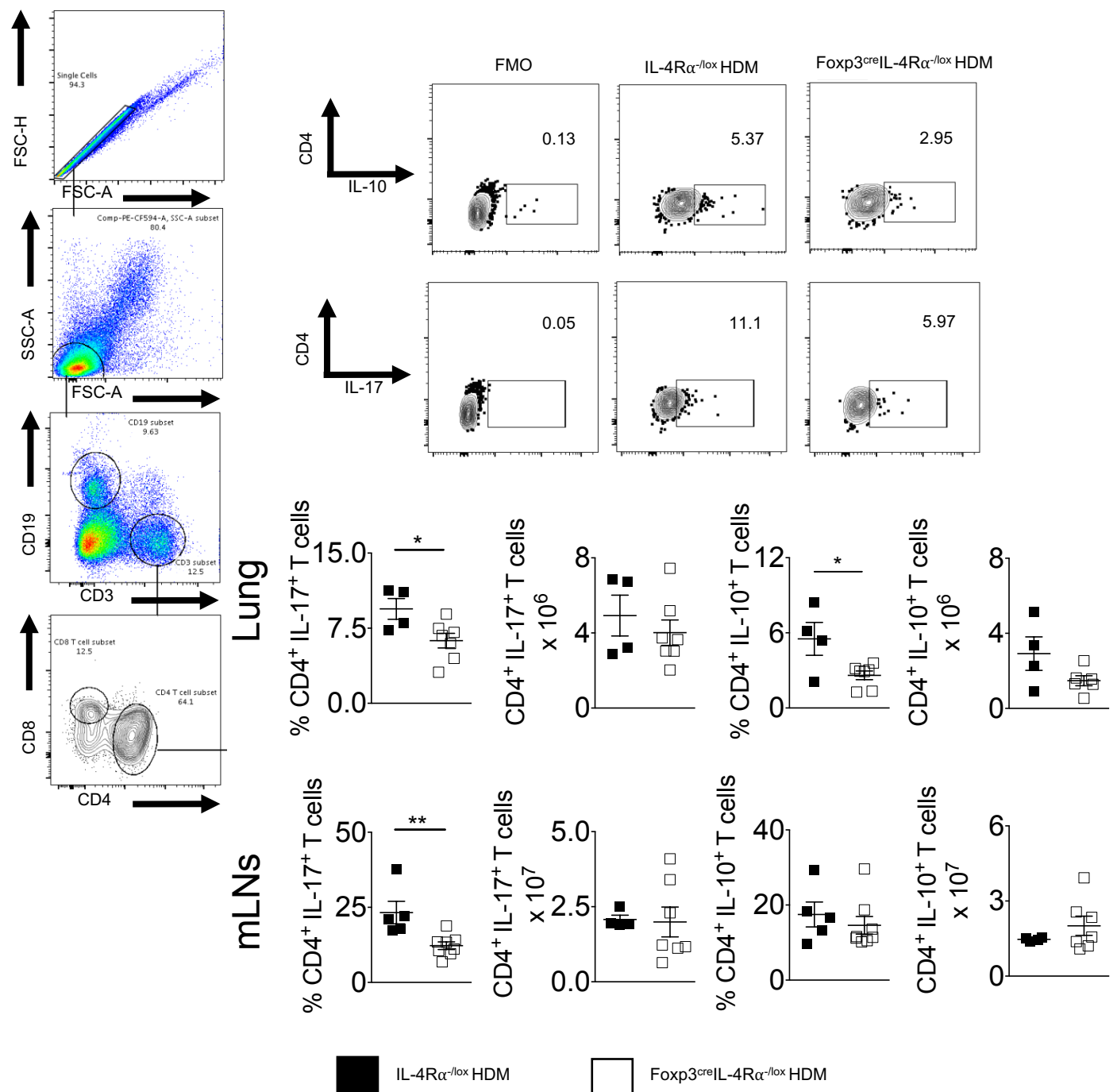


Supplemental Figure 2. Lung CD4 T cells are not impacted by lack of IL-4R α signalling on T regs.

(A) Effector phenotype of CD4 T cells (CD3⁺CD4⁺CD44⁺CD62L⁻)

(B) Expression of IL-4R α in lung CD4 T cells (CD3⁺CD4⁺CD44⁺CD62L⁻)

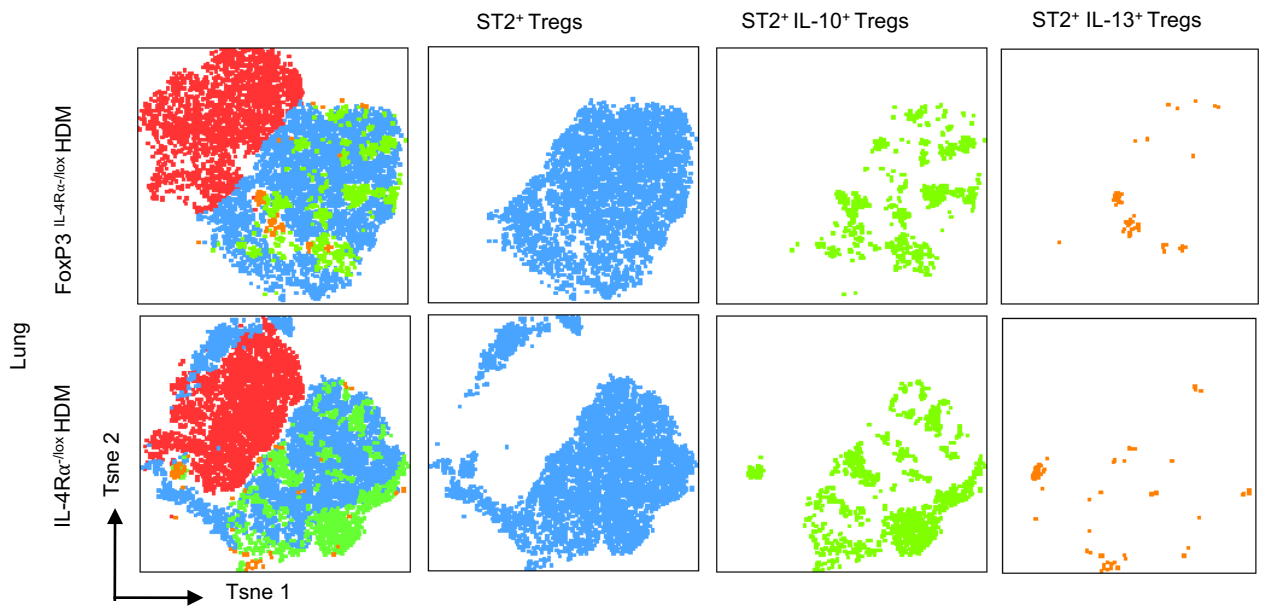
Related to Figure 2



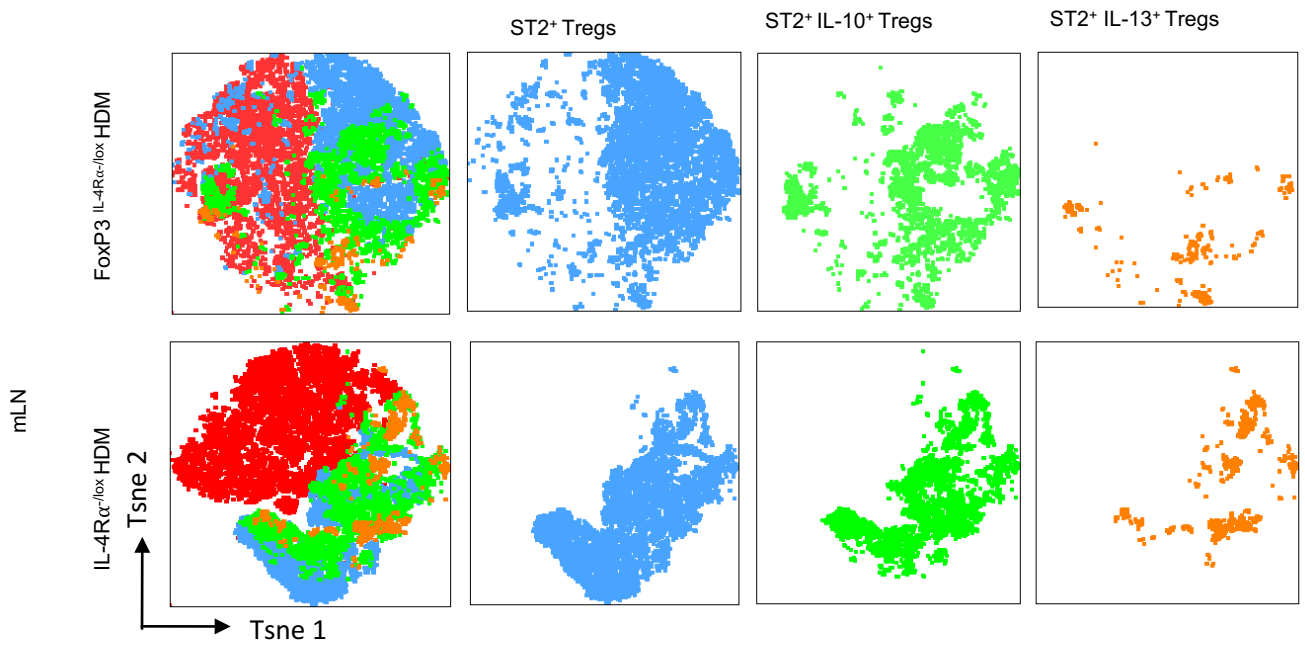
Supplemental Figure 3. Flow cytometry plots and gating strategy for CD4⁺ T cells producing intracellular IL-10 and IL-17 after 5 hr stimulation with PMA/ionomycin and monensin. Cellular numbers of cytokine producing CD4⁺ T cells calculated based on percentages.

Data shown is mean \pm SDs 1 representative experiment of 3 independent experiments (n=4-7). Significant differences between groups are represented as *p < .05. Part of Figure 4.

A



B

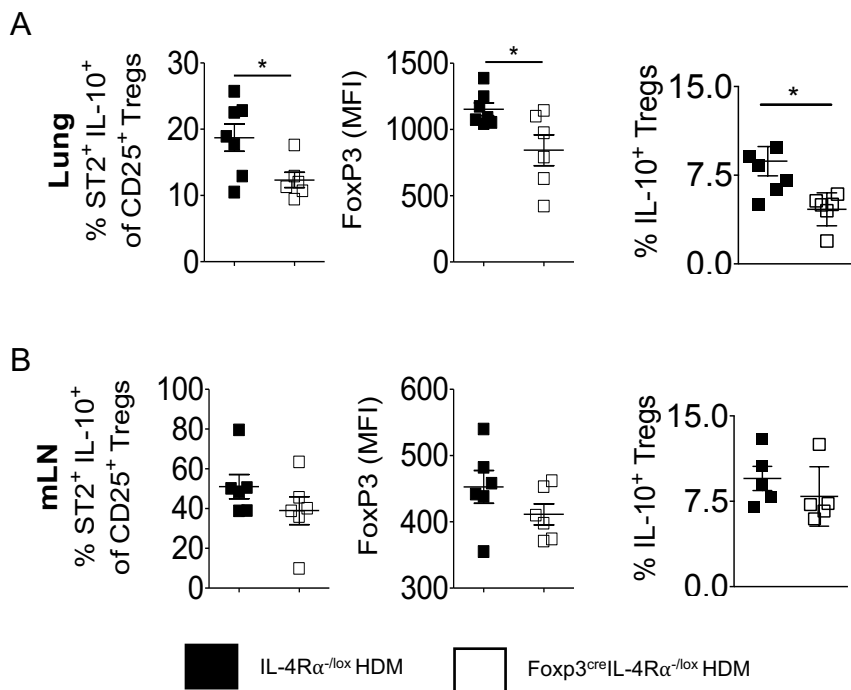


Supplemental Figure 4. tSNE dimensional analysis for the CD25⁺ FoxP3⁺ T regulatory cell compartment

(A) Lung and

(B) mLN.

Related to Figure 6



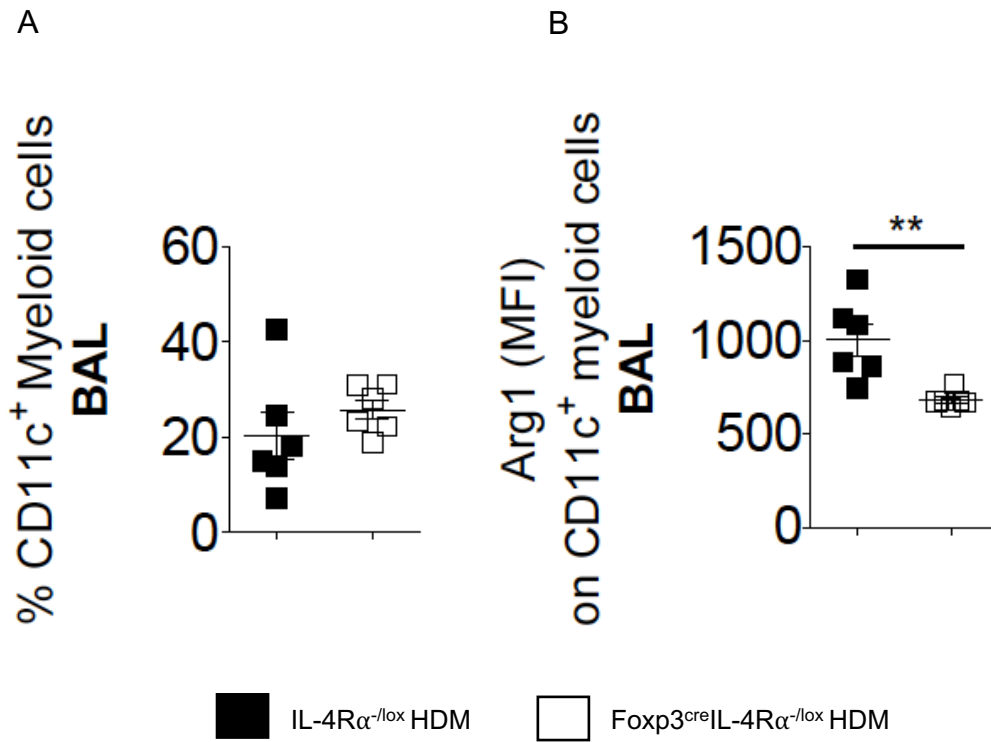
Supplemental Figure 5: Modulation of suppressive Treg compartment in the absence of IL-4R α signaling on T reg cells .

(A) % of ST2⁺IL-10⁺ CD25⁺ Treg cells (left panel), Foxp3 expression within the ST2⁺IL-10⁺ CD25⁺ Treg cells (middle panel), % IL-10 expression within CD25⁺ST2⁺ Treg cells (right panel) in the lung. (B) % of ST2⁺IL-10⁺ CD25⁺ Treg cells (left panel), Foxp3 expression within the ST2⁺IL-10⁺ CD25⁺ Treg cells (middle panel), % IL-10 expression within CD25⁺ST2⁺ Treg cells (right panel) in the mediastinal lymph nodes.

Data shows mean \pm SDs from 1 representative experiment of 3 independent experiments carried out (n= 5-8 per group). Significant differences are described as: *p < .05

MFI, Median fluorescence intensity; mLNs, mediastinal lymph nodes

Figure related to Figure 6.



Supplemental Figure 6: CD11c⁺ Myeloid cells are altered in the absence of IL-4R α signalling in T reg cells during HDM induced allergic asthma.

(A) Proportions of CD11c⁺ myeloid cells in BAL.

(B) Expression of arginase 1 on CD11c⁺ myeloid cells in BAL fluid