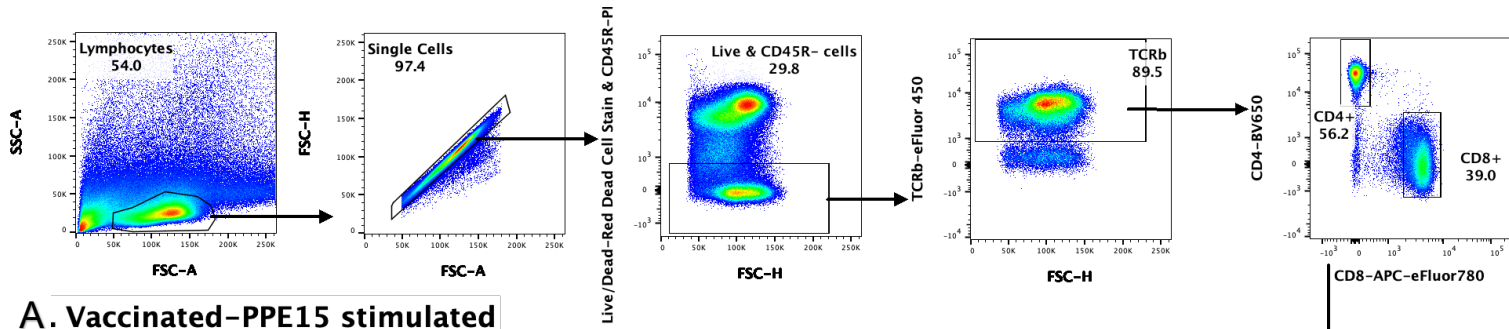
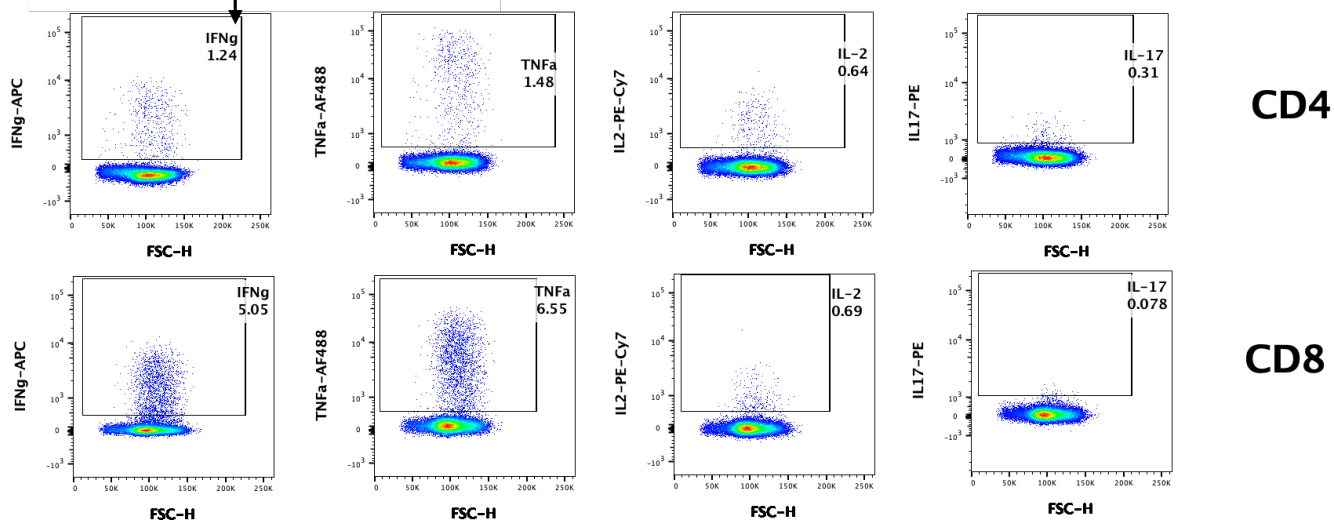


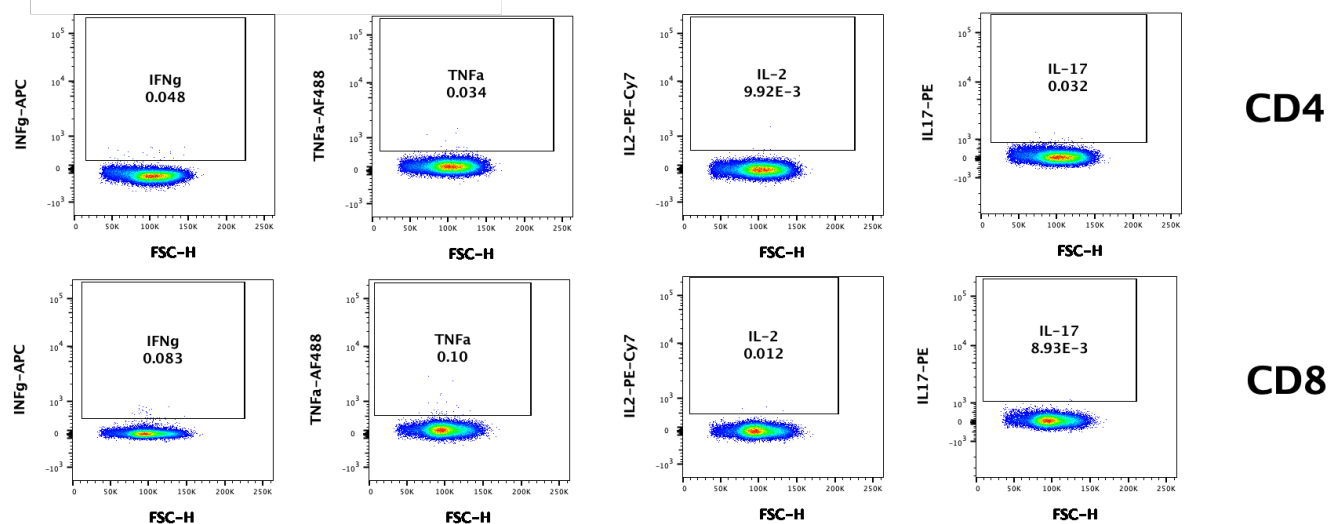
Supplementary Information



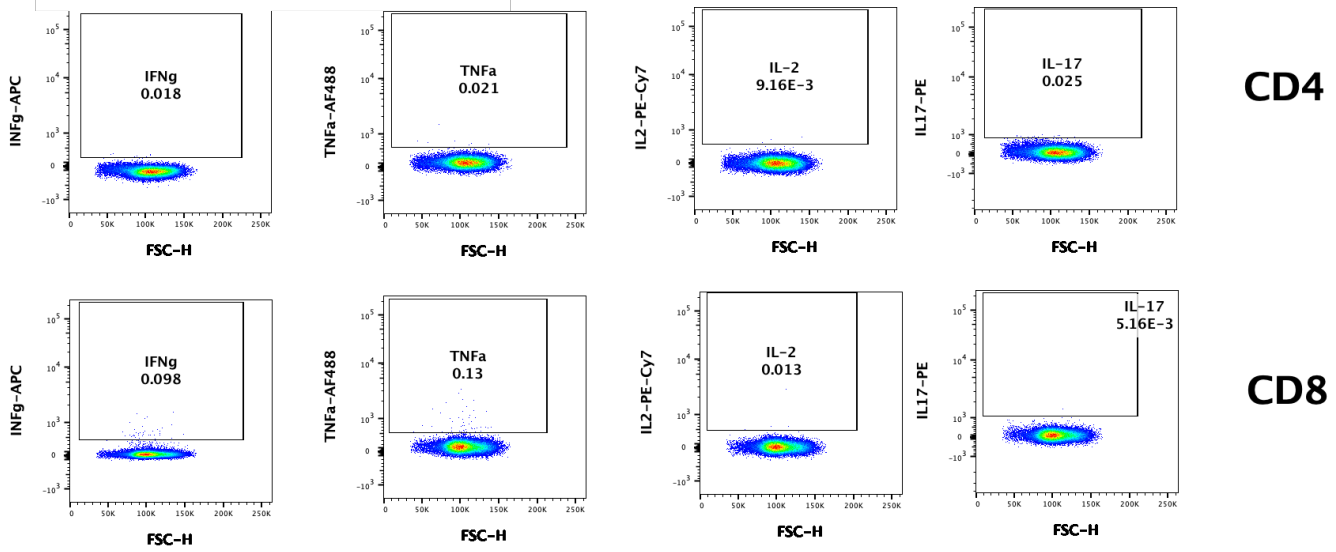
A. Vaccinated-PPE15 stimulated



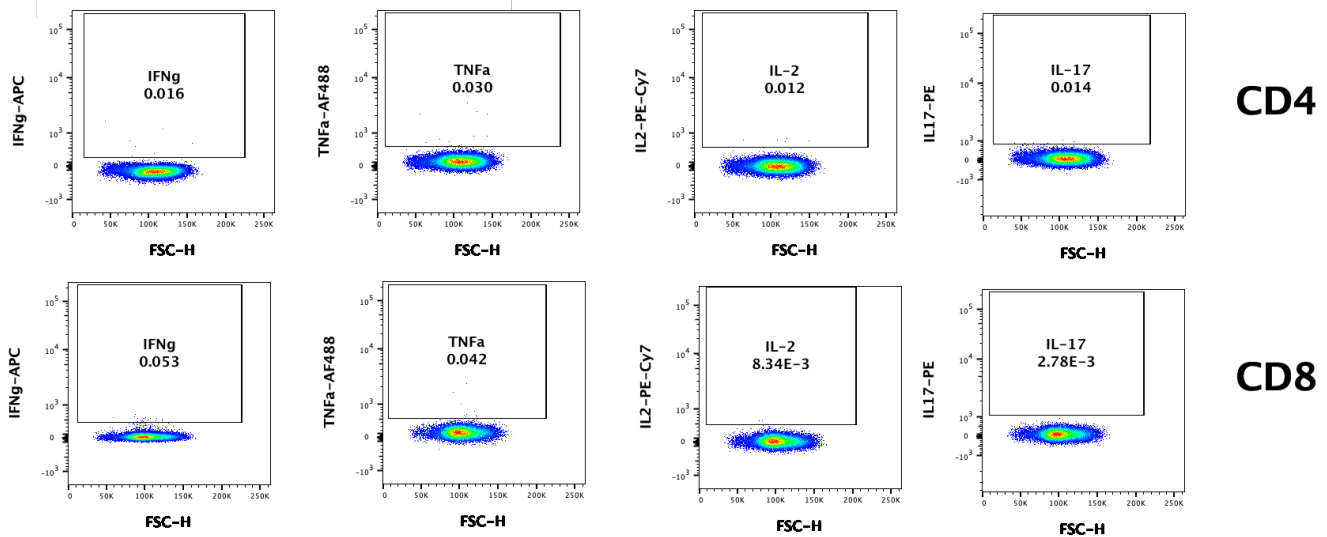
B. Naive-PPE15 stimulated



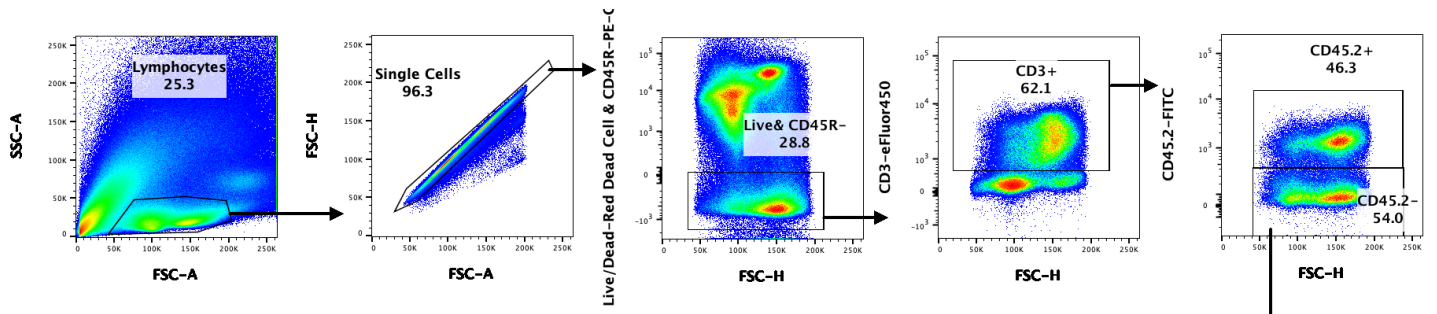
C. Vaccinated-Media



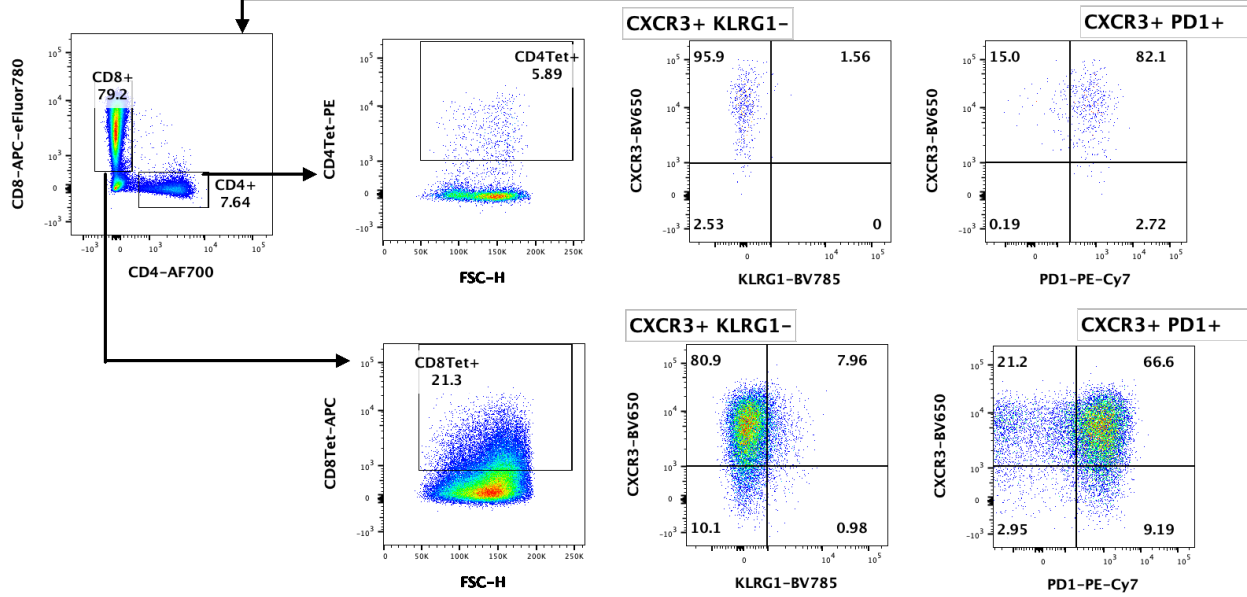
D. Naive-Media



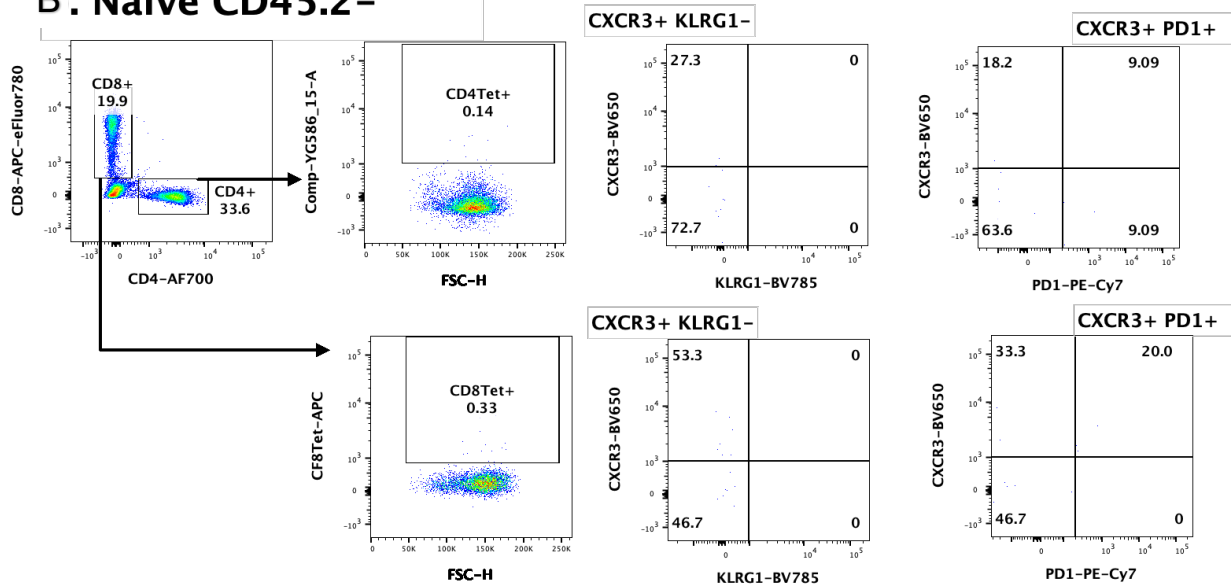
Supplementary Figure 1: Flow cytometry gating strategy for T cell surface and intracellular cytokine staining. Splenocytes from (A) vaccinated and (B) control animals were stimulated with PPE15 peptide pool to assess cytokine production. Background subtraction was performed using splenocytes from the same animals incubated with media (C, D). Lymphocytes were gated based on forward scatter (FSC) and side scatter (SSC). Following the exclusion of doublets, live CD45R⁻ cells were selected. TCR β ⁺ cells were further gated into CD4⁺ or CD8⁺ subsets. Cytokine production, including IFN- γ , TNF- α , IL-2, and IL-17, was assessed for each of the CD4⁺ and CD8⁺ T cell populations.



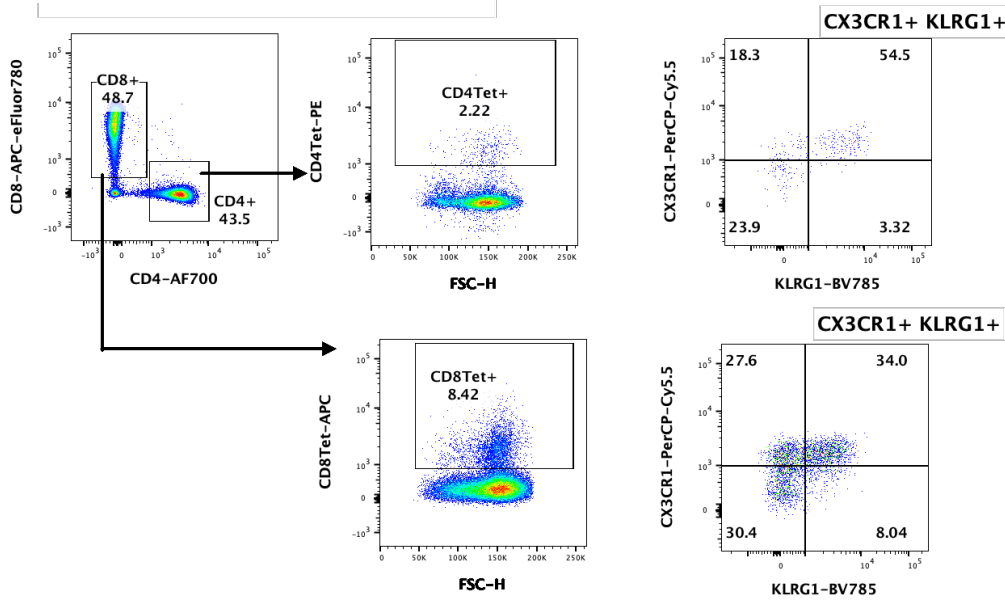
A. Vaccinated CD45.2-



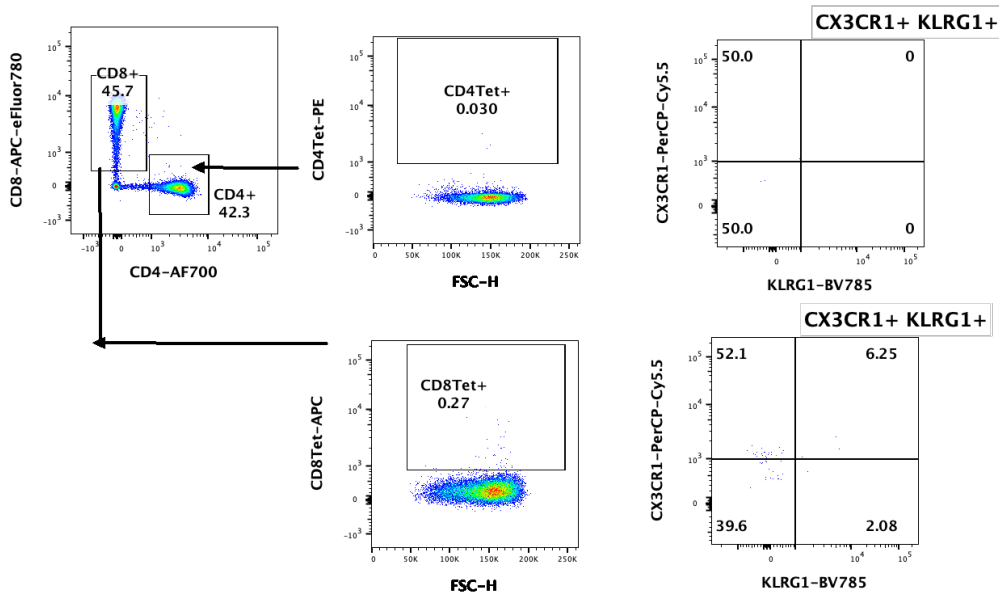
B. Naive CD45.2-



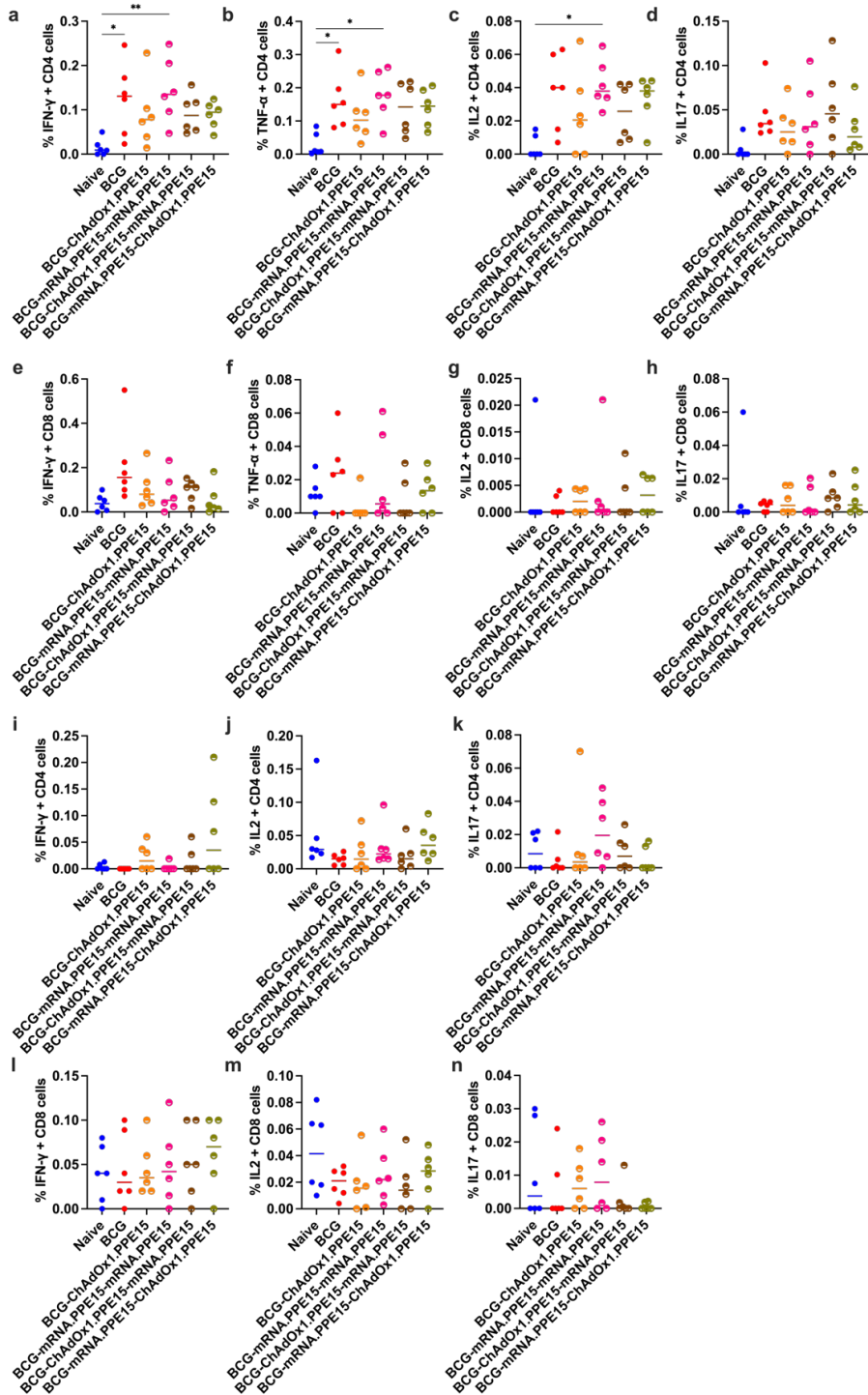
C. Vaccinated CD45.2+



D. Naive CD45.2+



Supplementary Figure 2: Flow cytometry gating strategy for lung resident memory T cells. PPE15 I-A(b) and H-2D(b) tetramers were used in combination with intravascular staining with α CD45. Lymphocytes were gated based on forward scatter (FSC) and side scatter (SSC). After the exclusion of doublets, live CD45R- cells were selected. CD3⁺ cells were subsequently gated into (A, B) CD45.2- (lung parenchymal) and (C, D) CD45.2+ (intravascular) populations. These populations were then gated for CD4⁺ and CD8⁺ T cells, followed by further gating for CD4⁺ PPE15 I-A(b) and CD8⁺ H-2D(b) tetramer-positive cells. CD45.2-, tetramer-positive cells were analysed for resident memory markers by gating on CXCR3⁺KLRG1⁻ or CXCR3⁺PD1⁺ populations. CD45.2+, tetramer positive CD4⁺ and CD8⁺ T cells were gated on CX3CR1⁺KLRG1⁺. Representative plots shown from lung cells from vaccinated (A, C) and from (B, D) naive animals.



Supplementary Figure 3: Comparable spleen and lung cell responses across vaccinated groups following stimulation with PPD-T. Immune responses were quantified in the lung and spleen four weeks after the last vaccination following cells stimulation with PPD-T. Flow cytometric analysis of spleen (a-d) CD4+, (e-h) CD8+ T cell producing IFN- γ , TNF- α , IL-2, and IL-17. Responses from lung (i-k) CD4+, (l-n) CD8+ T cells producing IFN- γ , IL-2, and IL-17. Each symbol represents response from 1 animal, n=6 per group. The bar indicates the median value for each group. Statistical analyses were performed using Kruskal-Wallis, followed by Dunn's multi-comparison test to evaluate differences between groups. *p<0.05, **p<0.01