Airway delivery of both a BCG prime and adenoviral boost drives CD4 and CD8 T cells into the lung tissue parenchyma

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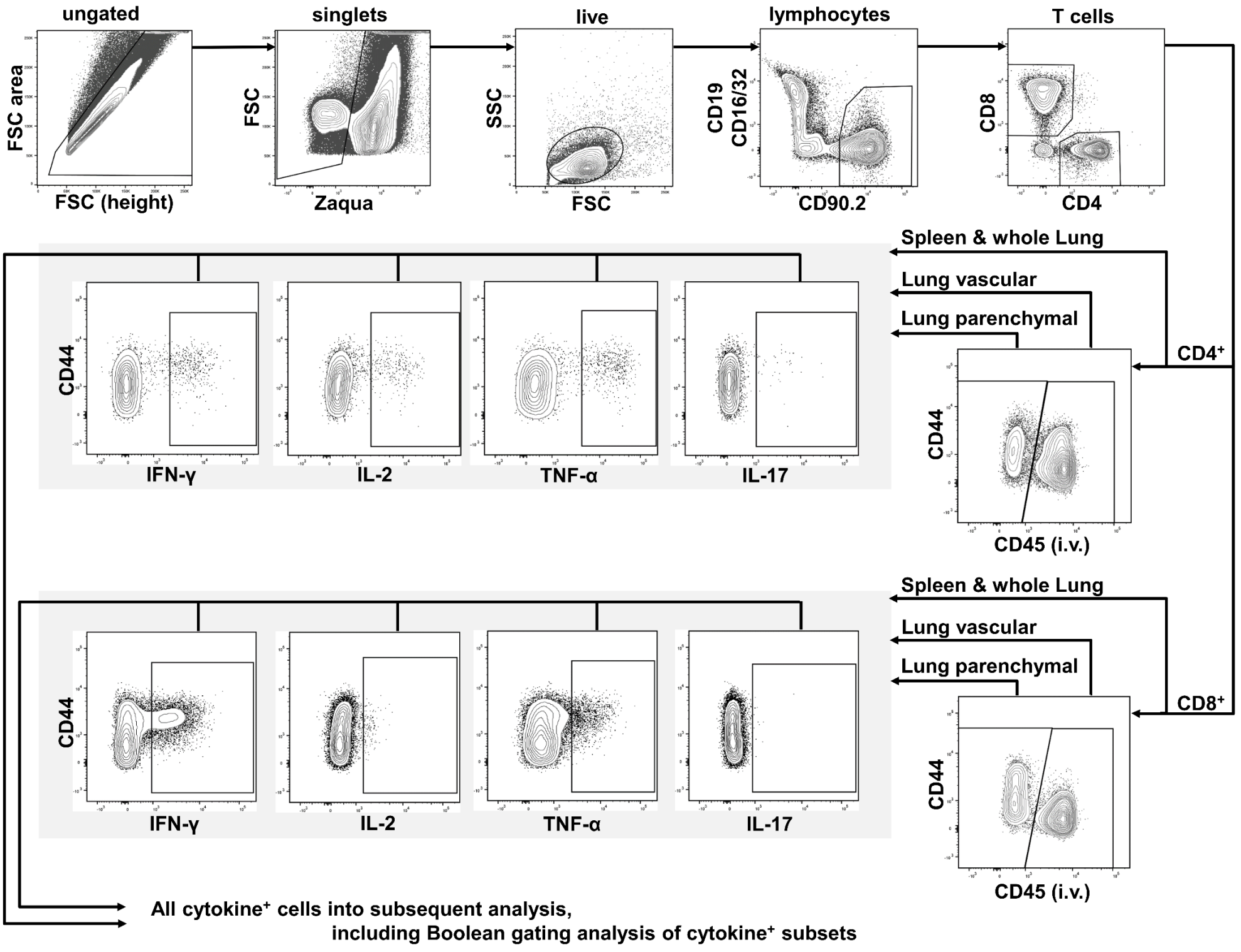
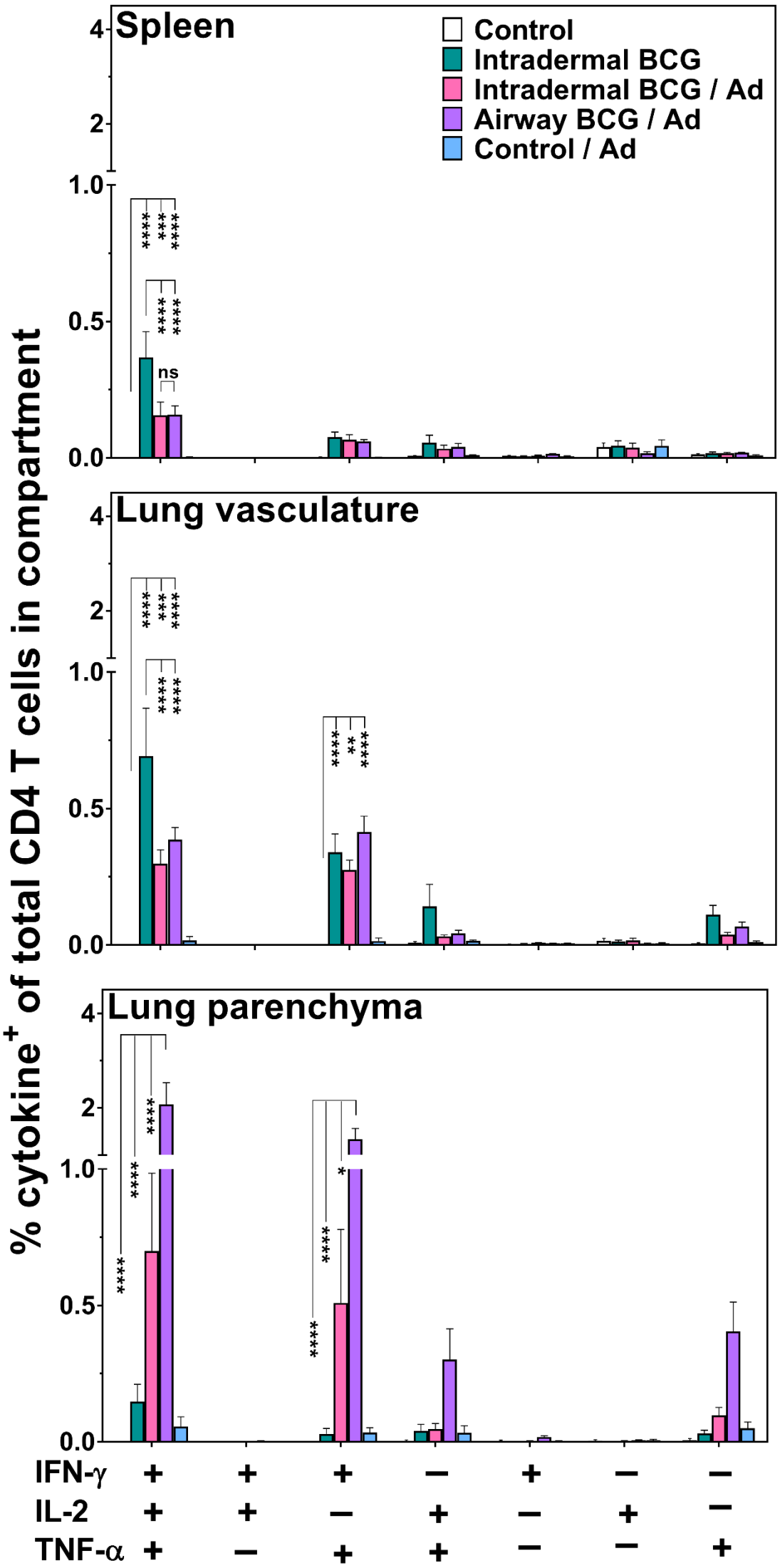
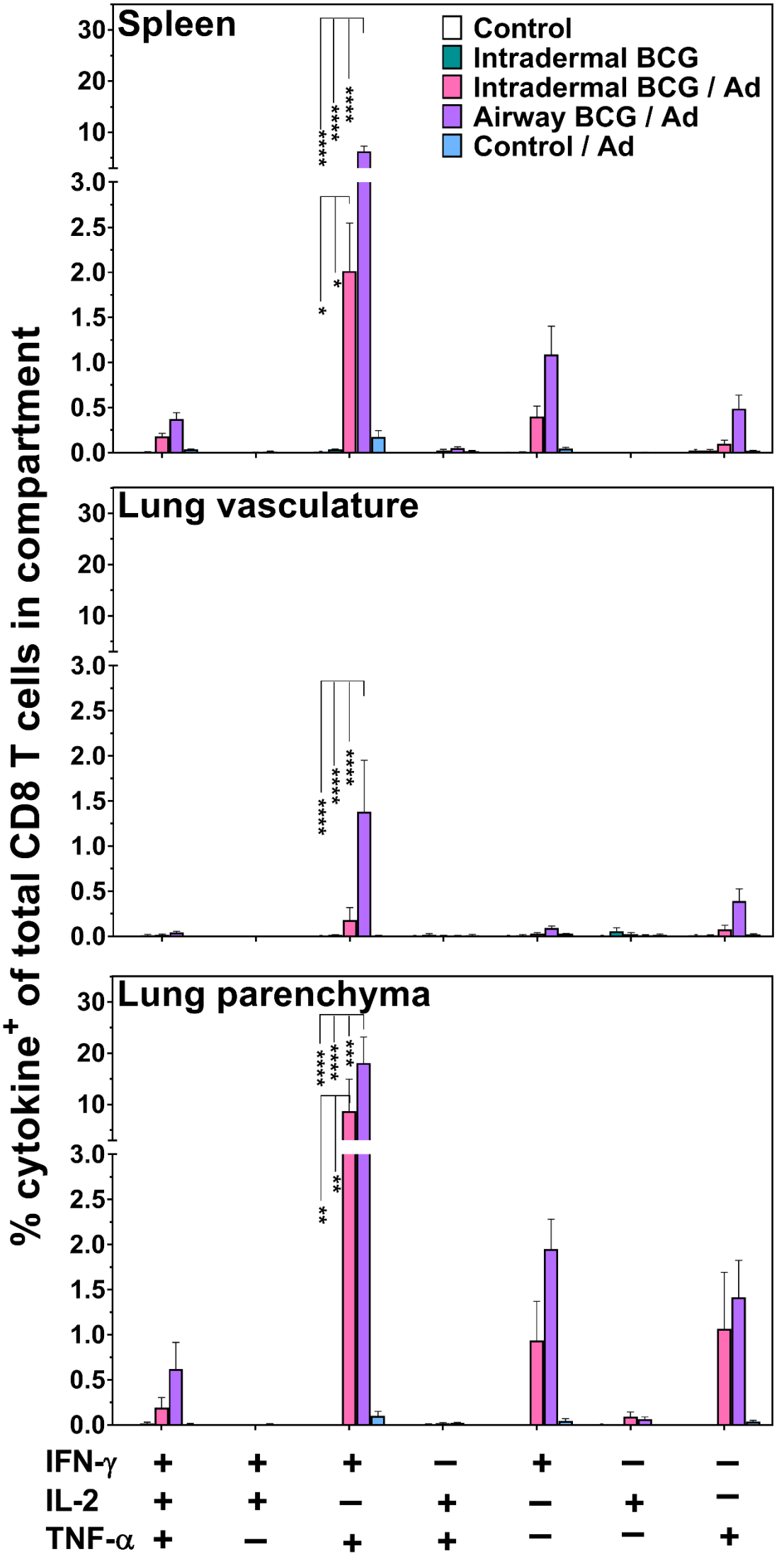


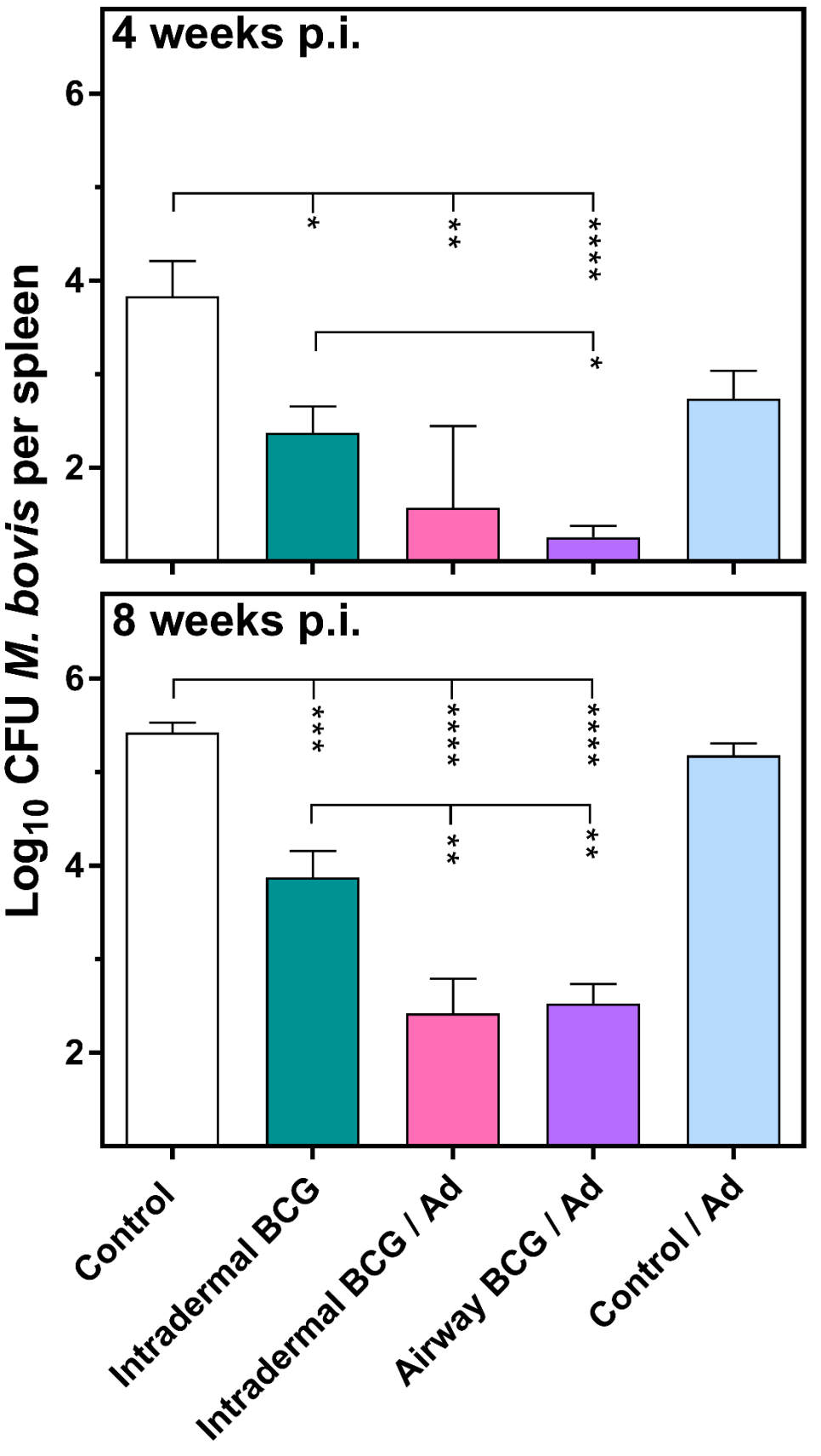
Supplementary Figure S1. Flow cytometry gating strategy. Gating strategy used for identification of CD4 and CD8 T cells producing IFN-γ, TNF-α, IL-2, and IL-17 alone or in any combination. Cells were gated on singlets followed by live cells and then lymphocytes. T cells were identified as CD90.2^{+} CD19^{-} CD16/32^{-} before gating on CD4^{+} or CD8^{+} cells. Where lung-derived CD4 or CD8 T cells were additionally subdivided into those residing in the parenchyma or vasculature, CD45^{-} and CD45^{+} (intravascular stained) cells were gated, respectively. The use of a bivariate plot vs. CD44 provided increased definition of this and subsequent gating. Antigen-specific CD4 or CD8 T cells were then identified by their production of IFN-γ, TNF-α, IL-2 or IL-17. Boolean gating was then used to identify all cells producing any combination of one or more of these cytokines (termed cytokine^{+}) as well as the individual cytokine^{+} CD4 and CD8 T cell subsets defined by the different simultaneous combinations.



Supplementary Figure S2. Frequency of individual functional subsets of cytokine producing CD4 T cells. Cytokine$^+$ CD4 T cells derived from the spleen, lung vasculature and lung parenchyma were identified as per Fig. 2 and then subdivided into the seven possible individual subsets defined by the different simultaneous combinations of IFN-γ, IL-2 and TNF-α production. Data represent the frequency of each cytokine producing subset as a percentage of the all CD4 T cells in the organ/compartment. Bars represent the mean ±SEM (n = 5-11). * p <0.05, ** p <0.01, *** p <0.001 **** p <0.0001, 2-way ANOVA with Tukey's post-hoc test.



Supplementary Figure S3. Frequency of individual functional subsets of cytokine producing CD8 T cells. Cytokine$^+$ CD8 T cells derived from the spleen, lung vasculature and lung parenchyma were identified as per Fig. 4 and then subdivided into the seven possible individual subsets defined by the different simultaneous combinations of IFN-γ, IL-2 and TNF-α production. Data represent the frequency of each cytokine producing subset as a percentage of the all CD4 T cells in the organ/compartment. Bars represent the mean ±SEM (n = 5-11). * p <0.05, ** p <0.01, *** p <0.001 **** p <0.0001, 2-way ANOVA with Tukey's post-hoc test.



Supplementary Figure S4. Bacterial burden in the spleen following challenge. Groups of mice were immunised and then challenged with ~200 CFU *M. bovis* as per schedule in Fig. 1. Four and eight weeks later the spleens of individual mice in equivalent groups were removed, homogenised and bacteria enumerated. Data represent the mean Log_{10} CFU ±SEM (n = 7-14). * p <0.05, ** p <0.01, *** p <0.001 **** p <0.0001, 1-way ANOVA with Tukey's post-hoc test.