

SUPPLEMENTARY MATERIAL

Circulating *Mycobacterium tuberculosis* DosR latency antigen-specific, polyfunctional, regulatory IL10⁺ Th17 CD4 T-cells differentiate latent from active tuberculosis.

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Supplementary Figure Legends

Supplementary Figure 1: Gating strategy to identify cytokine secreting CD4 and CD8 T cells.

Representative data of PBMCs from Rv1737c-stimulated IGRA⁺ individual is shown. Sequential gating included: (A) Singlets were gated using FSC-A and FSC-H; (B) live Avid^{low} cells (C) gating on FSC-A and SSC-A to discriminate Lymphocytes. (D) CD3⁺ cells; (E) CD4⁺ and CD8⁺ T cells. (F and G) Quadrant gating on CD4⁺ and CD8⁺ T cells based on CD45RA and CD27 expression: naïve, T_{CM}, T_{EM} and TEMRA; region gates identify single and double positive populations for: IFN γ vs IL2 (H & I), IFN γ vs TNF α (J & K), IFN γ vs IL17A (L & M) and IFN γ vs MIP1 β (N & O) respectively.

Supplementary Figure 2: CD8 T cell frequencies to Mtb-specific and common recall antigens.

PBMCs from subjects with LTBI, PTB and EPTB (N=6) were cultured overnight in a standard ICS assay in the presence or absence of (a) secretory (Ag85A/B, TB10.4, ESAT6/CFP10 & PPD), (b) DosR (Rv1733c, Rv1737c, Rv2029 & Rv2628) and (c) common recall (CMVpp65 & CandidaMP65) antigens. CD3⁺CD8⁺ T cells were analysed for intracellular expression of IFN γ , TNF α , IL2, IL17A and MIP1 β . Box-and-whisker plots show the range in frequencies of total cytokine-positive CD8⁺ T cells with the horizontal bar within the box showing the median. One-Way ANOVA was used to determine between group statistical significance: *p<0.05; **p<0.01; ***p<0.001.

Supplementary Figure 3: Comparison of regulatory IL10⁺ and proinflammatory IFN γ ⁺ Th17 responses in IGRA⁺ and EPTB subjects to individual DosR latency antigens analyzed by COMPASS and SPICE.

PBMCs from subjects with LTBI and EPTB (N=10) were cultured overnight in a standard ICS assay in the presence or absence of DosR (Rv1733c, Rv1737c, Rv2029 & Rv2628) antigens. CD3⁺CD4⁺ T cells were analysed for intracellular expression of IFN γ , IL17A, IL17F, IL22, IL10, TNF α , IL2 and MIP1 β . Boolean gates were created from the 8 individual cytokine (listed above) gates in FlowJo to divide responding cells into 256 distinct subsets corresponding to all possible combinations of these functions. These were analysed using COMPASS or SPICE software. (a) Boxplots of data is shown for the EPTB and IGRA⁺ groups of probability scores of antigen-specific response for each subject for each DosR antigen, averaged across regulatory or proinflammatory Th17 functional profiles, either IL10⁺ or IFN γ ⁺ cells together with all combinations of IL17A, IL17F and IL22 respectively. Probabilities are derived from a linear model fit to ICS data using the Th17 panel. A p-value is shown testing for a difference between groups (two-sided t-test). (b) Box-and-whisker plots show the range in frequencies with horizontal bar

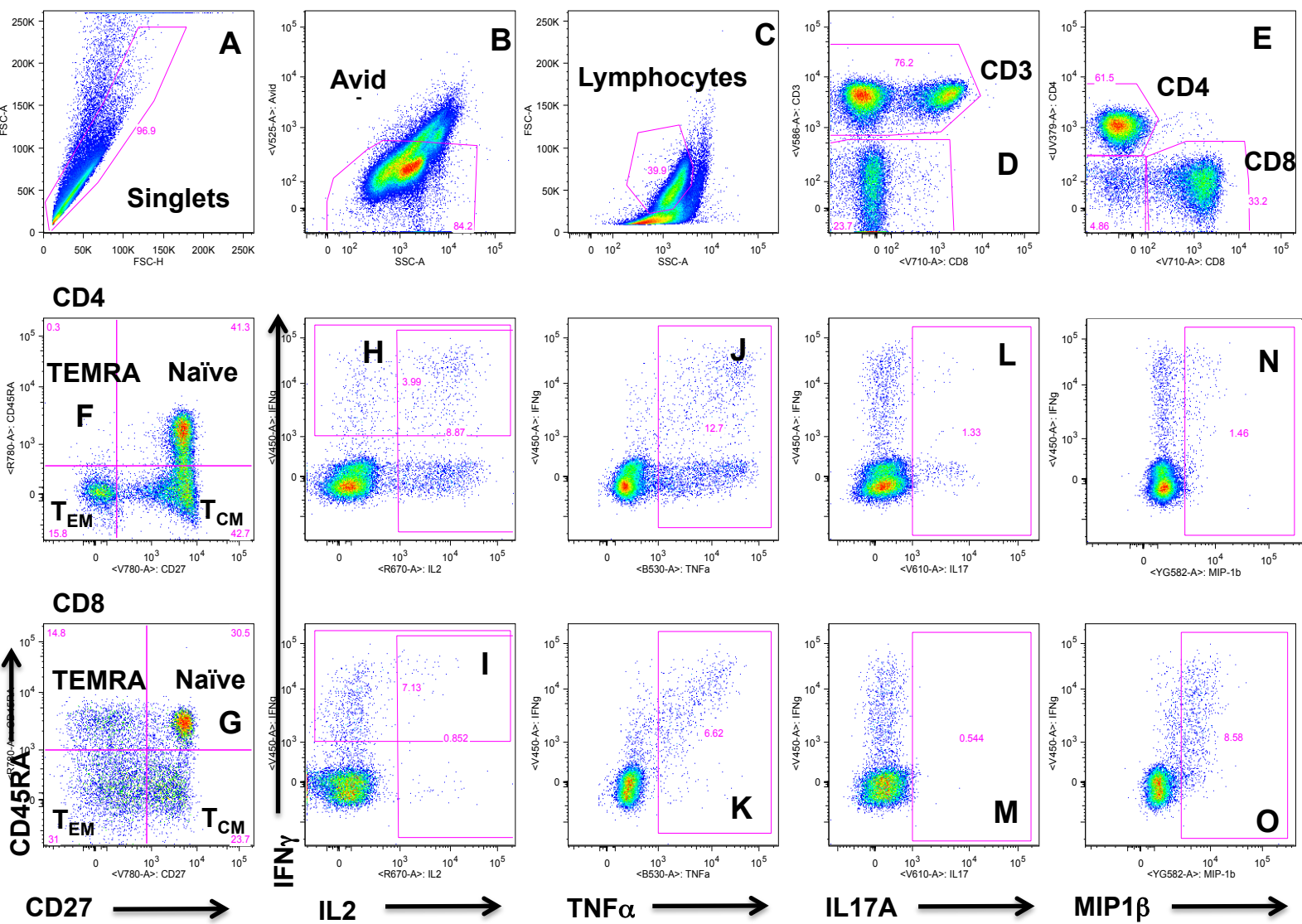
representing the median of regulatory Th17 subsets (IL10⁺IL17A⁺, IL10⁺IL17F⁺ and IL10⁺IL22⁺) on the left panel and proinflammatory Th17 subsets (IFN γ ⁺IL17A⁺, IFN γ ⁺IL17F⁺ and IFN γ ⁺IL22⁺) on the right panel, induced by individual DosR antigen stimulation analysed by SPICE. Statistical differences between groups was calculated by Mann-Whitney U test: *p<0.05; **p<0.01; ***p<0.001.

Supplementary Figure 4: Gating strategy to identify cytokine secreting immune cells in BAL.

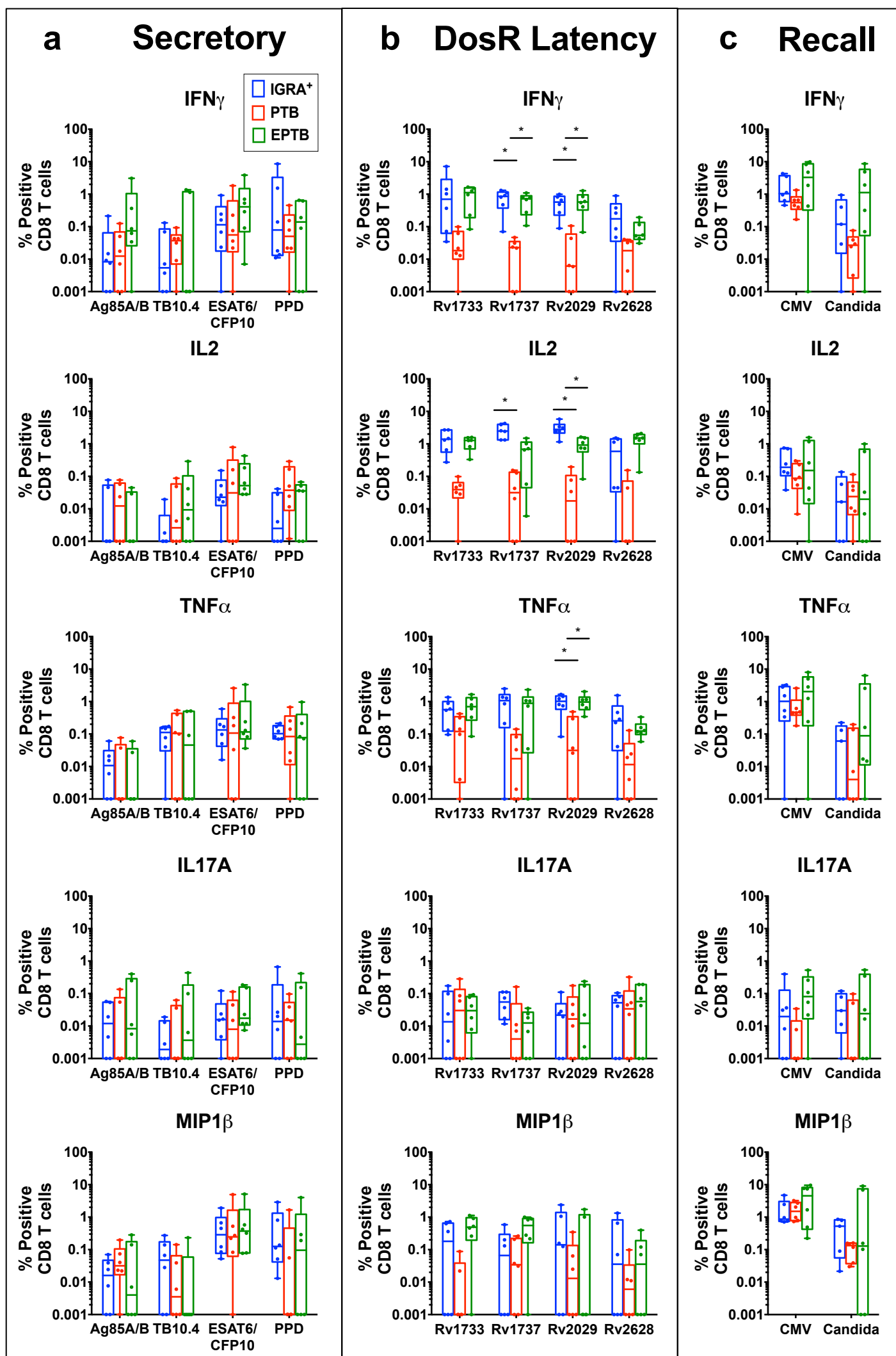
Representative data of freshly isolated unstimulated BAL from a PTB is shown. Sequential gating included: (A) Lymphocytes based on FSC-A and SSC-A; (B) singlets (based on FSC-A and FSC-H; (C) Live (Avid^{low}) cells; (D) CD3⁺ cells; (E & F) CD4⁺, CD8⁺ and CD4⁻CD8⁻ T cells. Quadrant gating on CD4⁺, CD8⁺ and CD4⁻CD8⁻ cells represent single and double positive populations for; IFN γ vs IL17A (G, H, I, J); IFN γ vs TNF α (K, L, M & N); IFN γ vs MIP1 β (O, P, Q & R); IFN γ vs IL10 (S, T, U & V); and IFN γ vs IL2 (W, X, Y & Z) respectively.

Supplementary Figure 5: Comparison of spontaneous cytokine expression in blood from diverse clinical groups versus BAL from PTB.

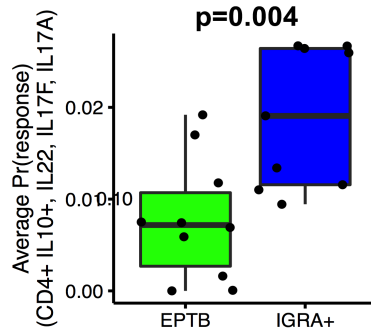
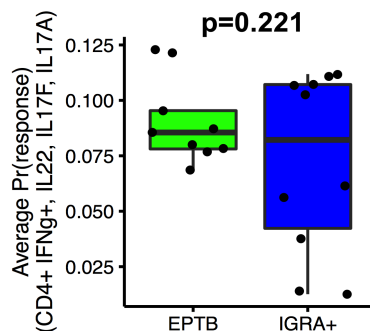
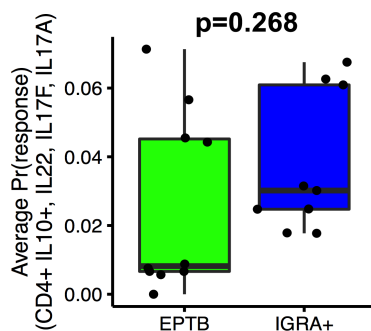
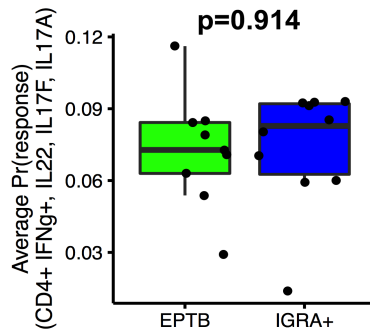
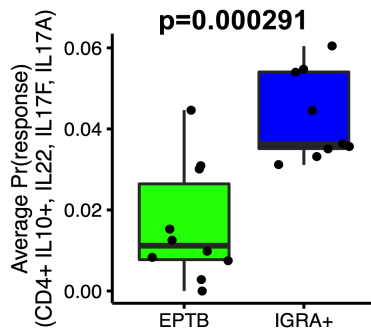
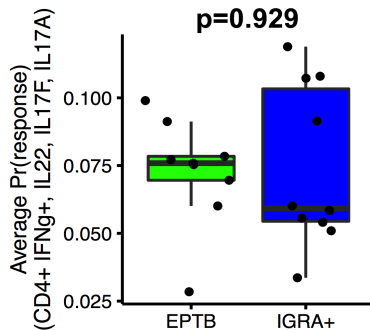
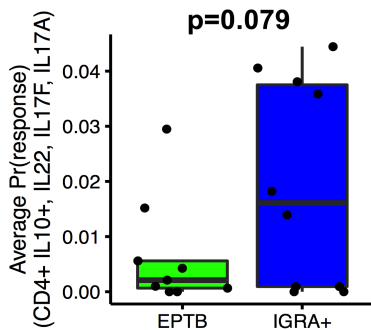
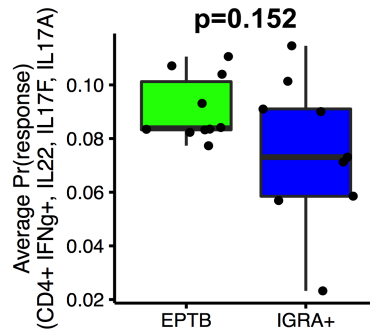
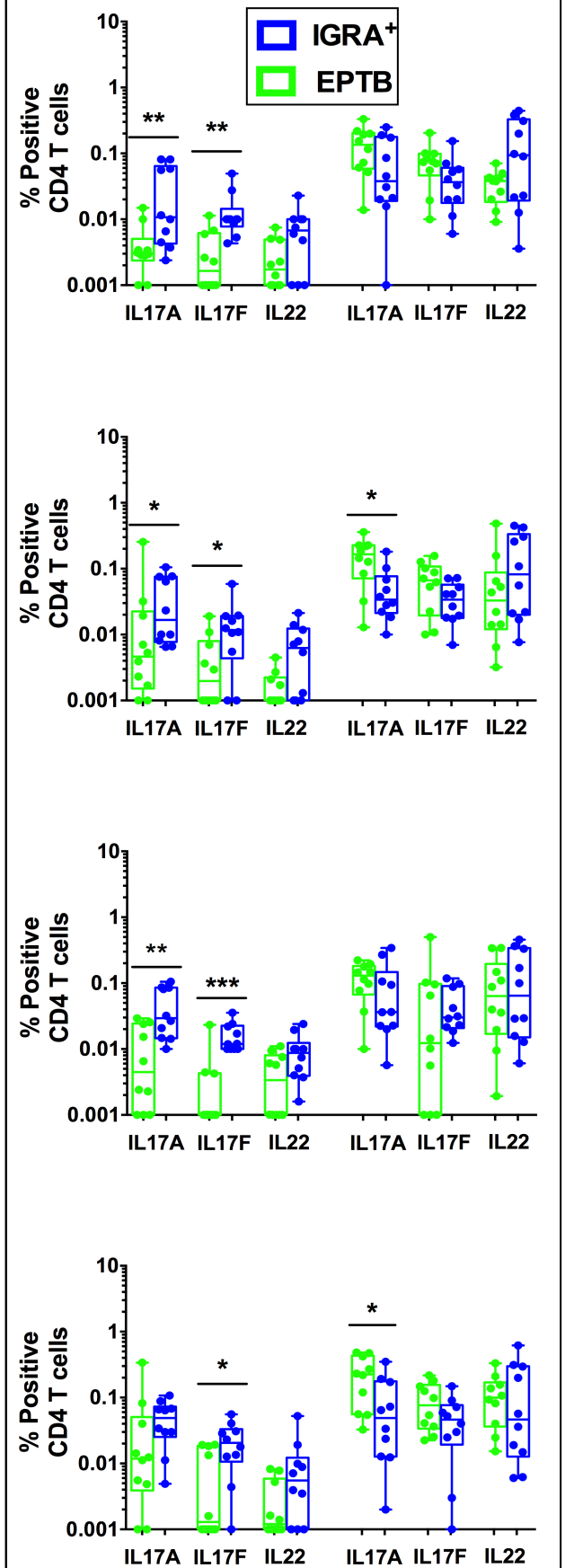
(a) Box-and-whisker plots show the range in frequencies (horizontal bar within the box showing the median) of spontaneous cytokine expressing cells in BAL (pink) and matched PBMC (red) of PTB patients along with blood responses in EPTB (green) and IGRA⁺ (blue) subjects. N=7 for BAL and N=12 for IGRA⁺, PTB and EPTB. One-Way ANOVA was used to determine statistical significance between groups and corrected for multiple comparisons using Bonferroni's test. ***p<0.001; ****p<0.001. (b) Box-and-whisker plots show the range in frequencies with horizontal bar representing the median of antigen-induced IL17A expressing cells (after subtracting spontaneous release) in BAL (blue) and matched PBMC (red).

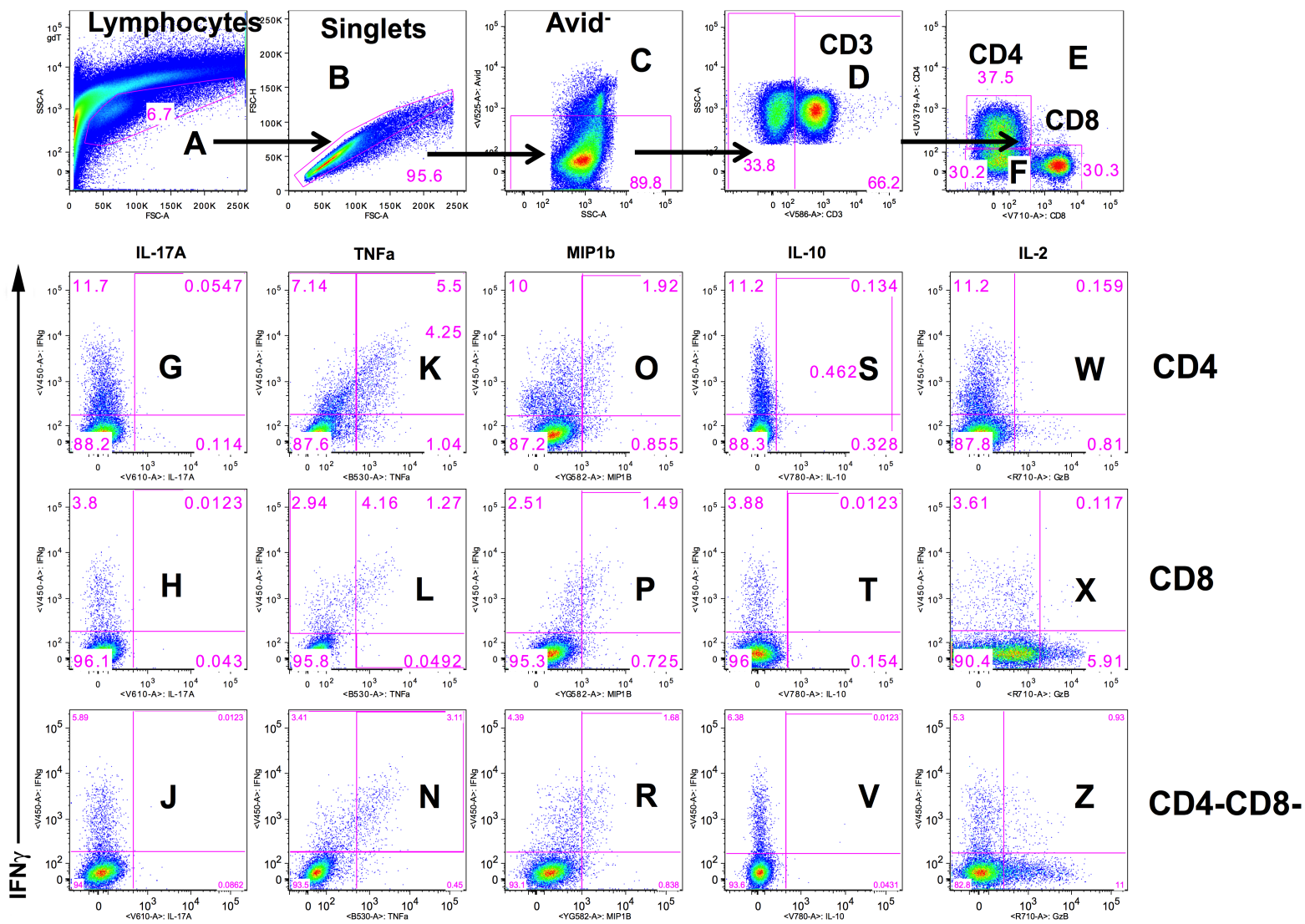


Supplementary Figure 1

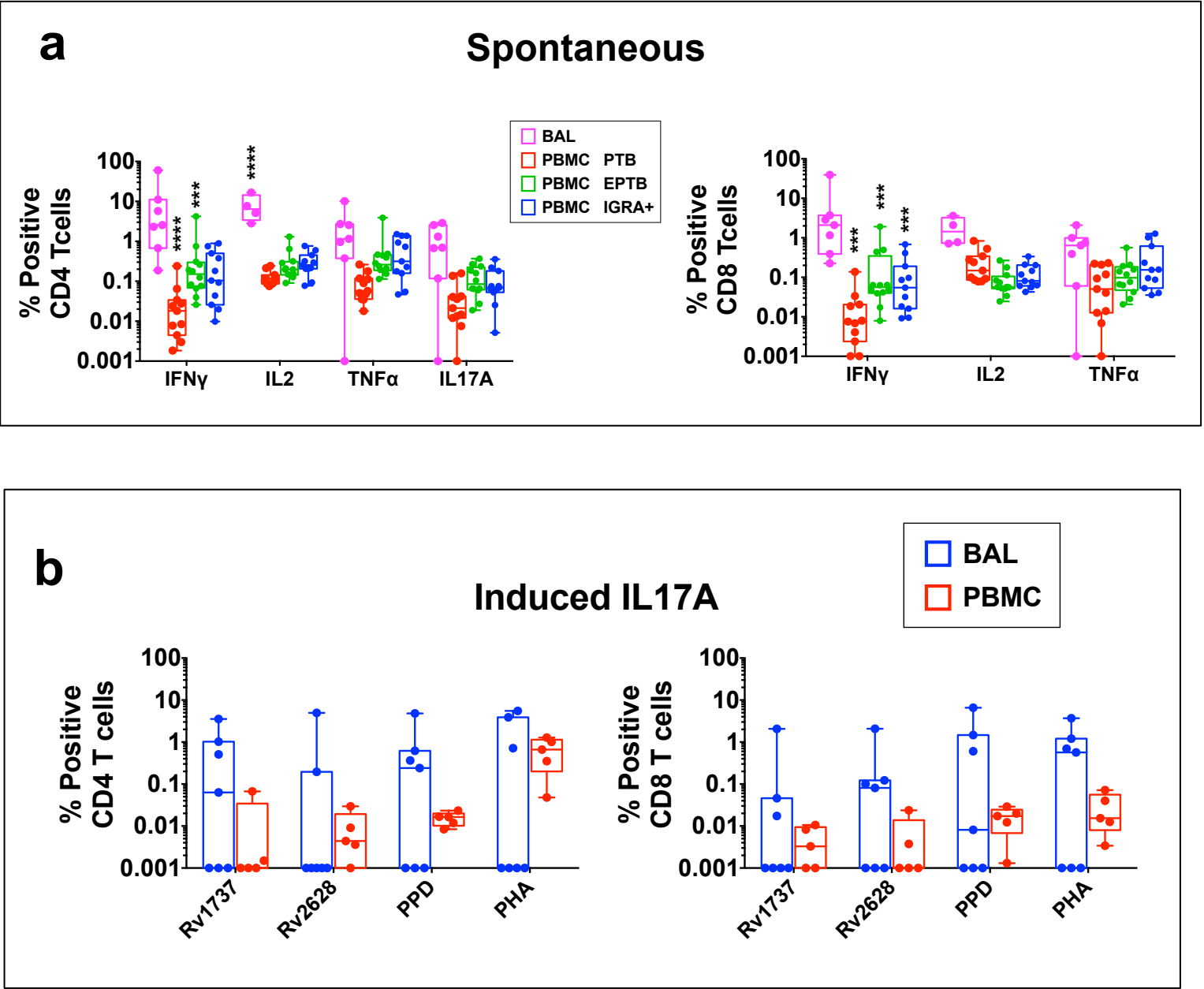


Supplementary Figure 2

a**COMPASS****IL10⁺/Th17****IFN γ ⁺/Th17****b****SPICE****IL10⁺/Th17****IFN γ ⁺/Th17**



Supplementary Figure 4



Supplementary Figure 5