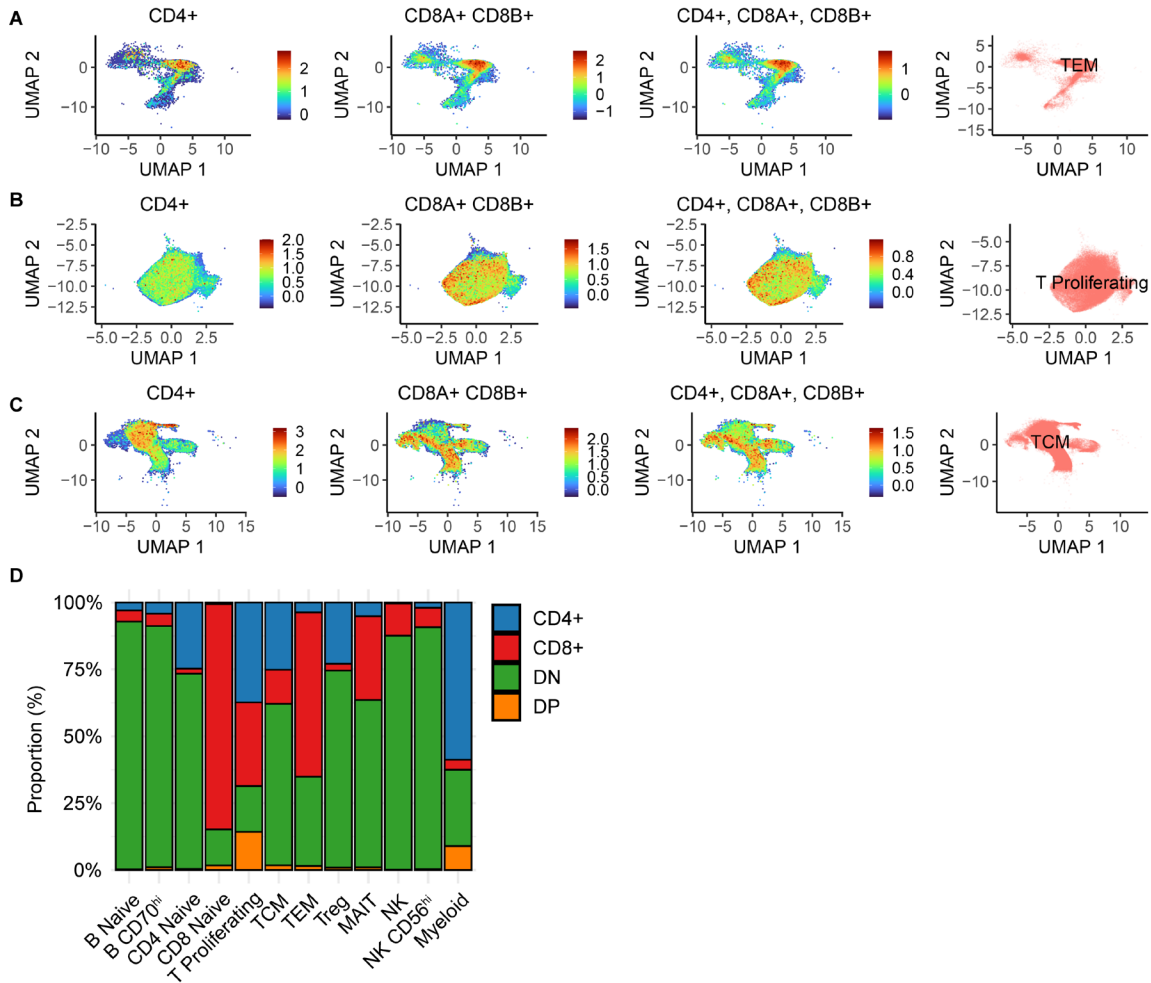
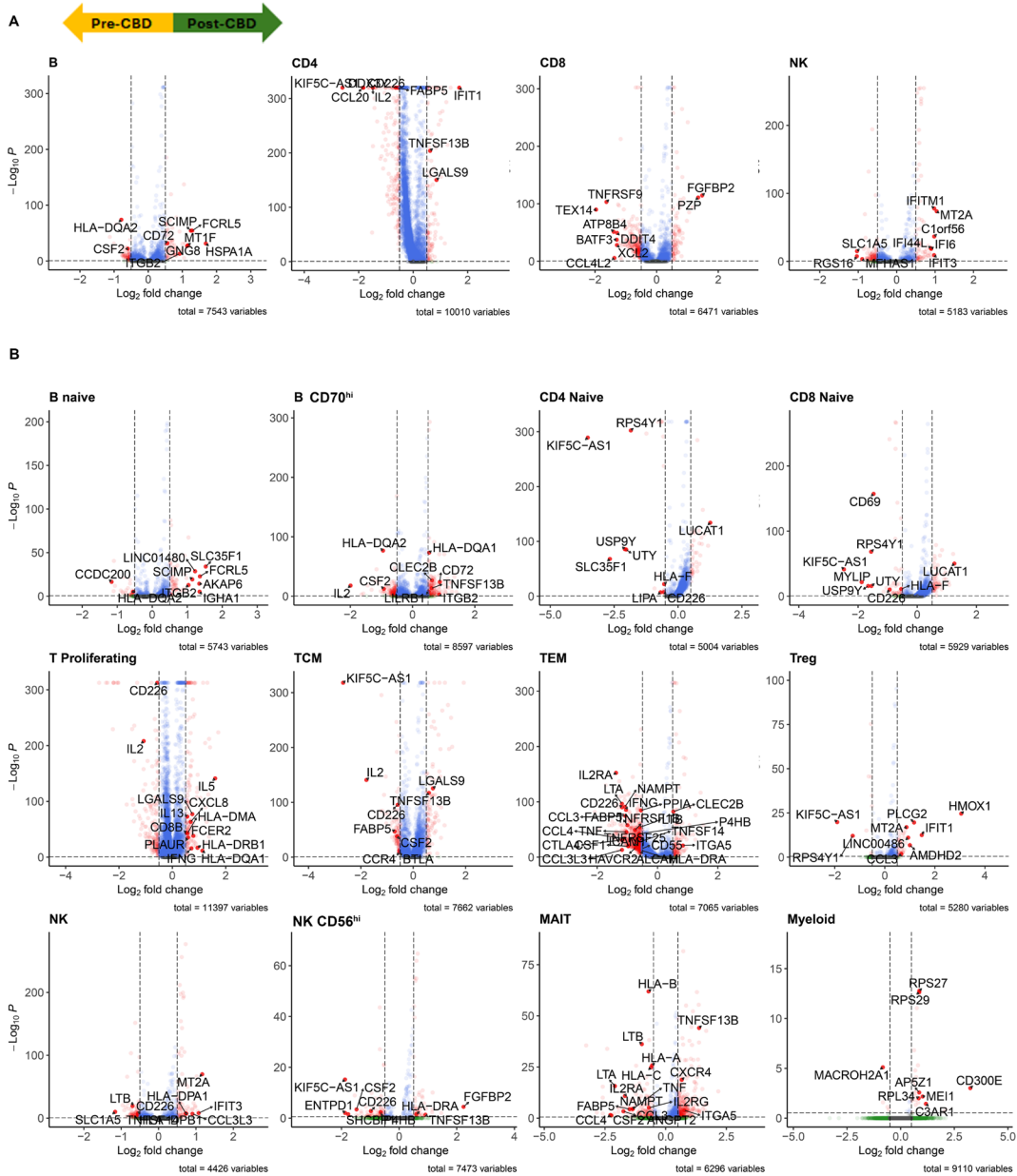


## Supplemental Figures and Legends



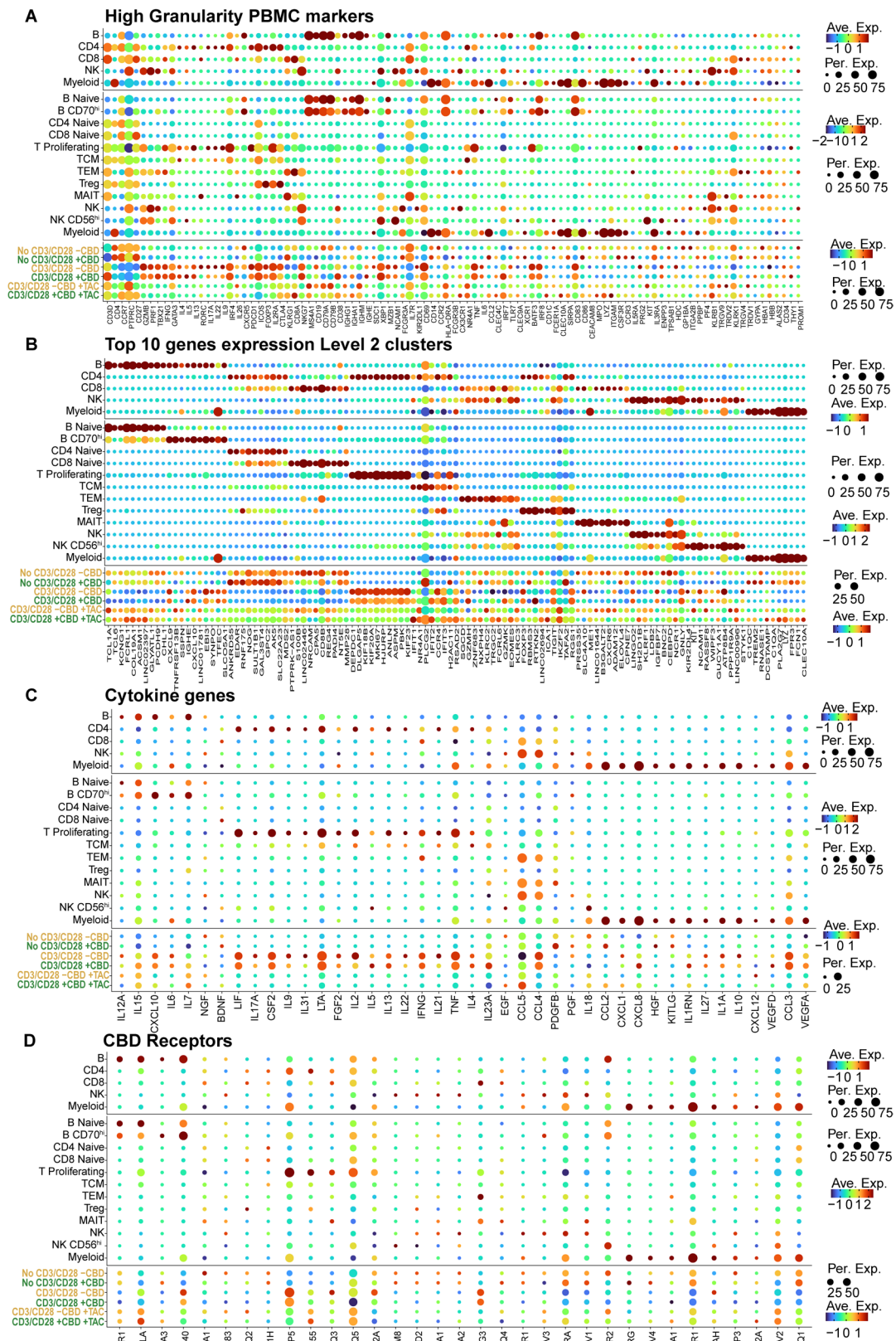
### Supplemental Figure 1. Phenotype-Driven Annotation of T-Cell Clusters.

Clusterization of CD8+ and CD4+ Overlap Clustering of T cell populations based on transcriptional profiles. UMAP visualization shows clustering of T cells, with annotations highlighting major phenotypic subsets. In some cases, CD8+ and CD4+ T cells are resolved into distinct clusters, while in others, they are grouped. This indicates that clustering is primarily driven by phenotypic features rather than strict lineage (CD8/CD4) identity, reflecting shared functional or transcriptional programs across subsets. The feature plots are showing this effect occurring in (A) T effector memory (TEM), (B) T Proliferating and (C) T central memory (TCM). (D) Subtype composition of T cell clusters. Expression of CD4, CD8A, and CD8B was used to classify cells into four categories: CD4+, CD8+, double-negative (DN), lacking both CD4 and CD8 expression, or double-positive (DP), co-expressing CD4 and CD8. Stacked bar plots display the proportion of these subtypes within each cluster.



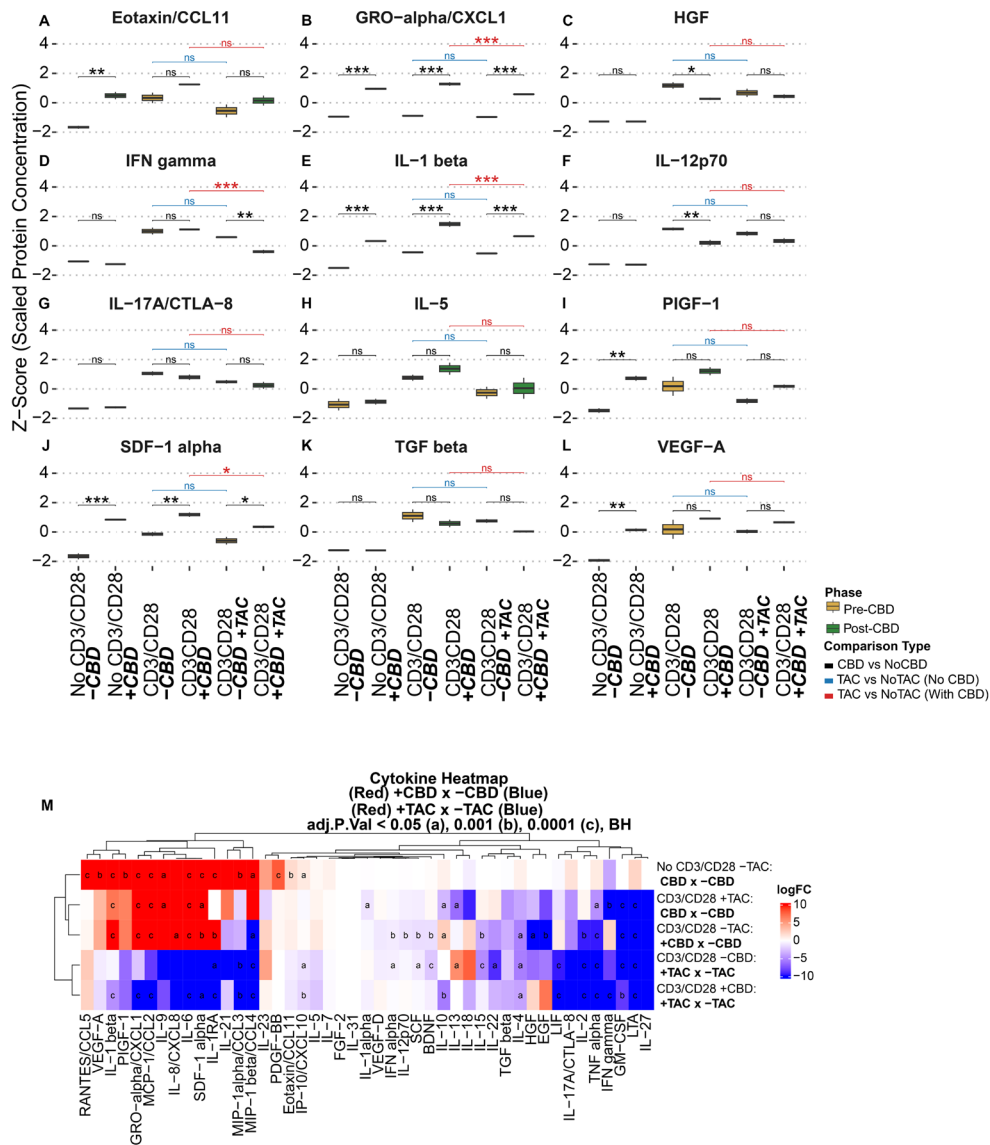
**Supplemental Figure 2. Differential expression genes (DEGs) in CD3/CD28 -CBD versus CD3/CD28 +CBD.**

The volcano plots show red dots for genes that have average log<sub>2</sub>FC > 0.5 and an adjusted P-value < 0.05 (Wilcoxon and Benjamini-Hochberg). Blue dots correspond to adjusted average log<sub>2</sub>FC < -0.5 and adjusted P-value < 0.05. Green genes correspond to an average log<sub>2</sub>FC > 0.5 and adjusted P-value > 0.05. Grey dots correspond to an average log<sub>2</sub>FC < 0.5 and adjusted P-value > 0.05. Negative x-axis genes are downregulated, CD3/CD28 post-CBD, and positive x-axis genes are upregulated CD3/CD28 post-CBD. (A) DEGs at annotation Level 1: B, CD4, CD8, NK and Myeloid (B) DEGs at annotation Level 2: B naive, B CD70<sup>hi</sup>, CD4 Naive, CD8 Naive, T Proliferating, T Central Memory (TCM), T effector memory (TEM), T regulatory (Treg), Mucosal-associated invariant T (MAIT), Natural Killer (NK), and Natural Killer CD56<sup>+</sup> (NK CD56<sup>hi</sup>).



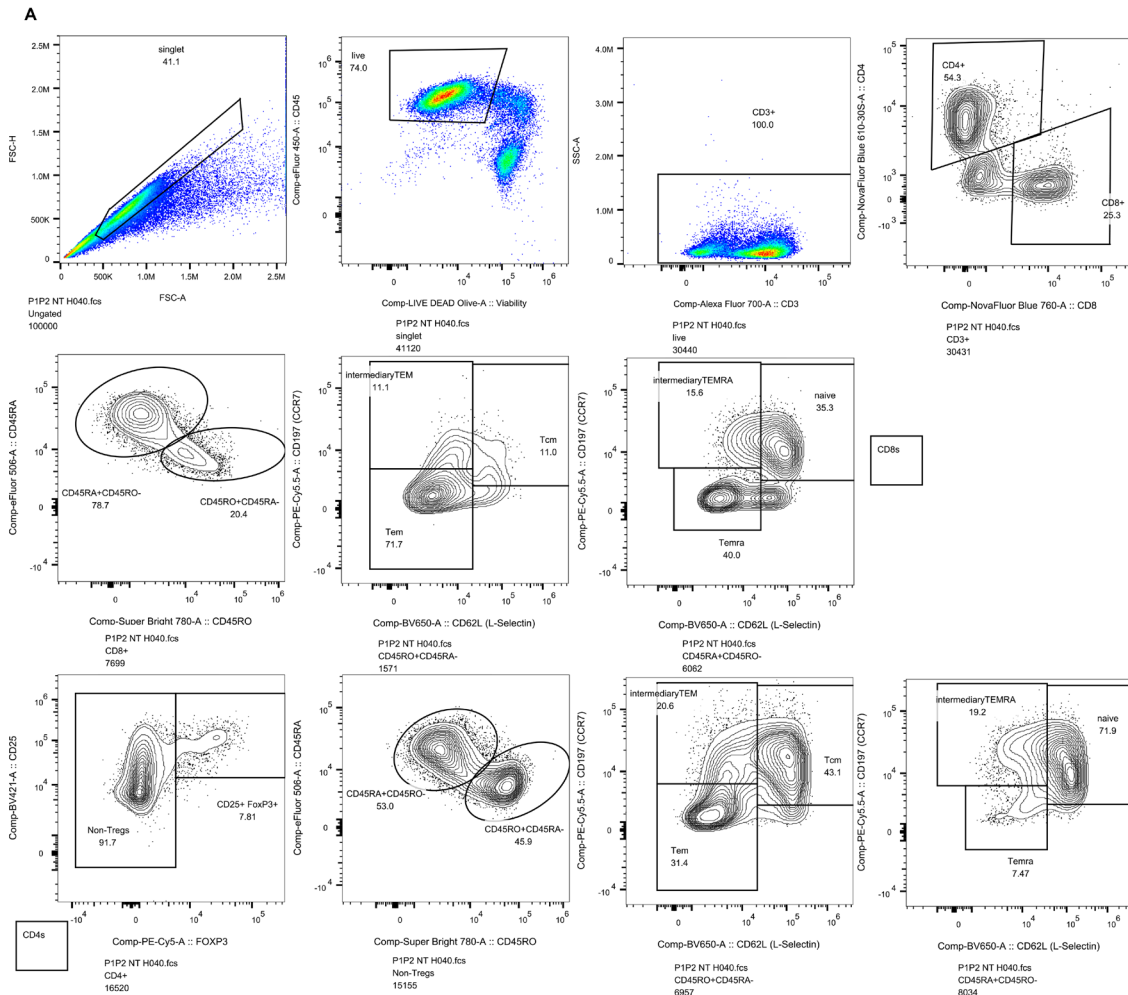
### Supplemental Figure 3. Comparative Gene-Set Dotplots Across Three Cell Annotations.

The Dot plot shows the expression of the genes across 3 annotation levels: Level 1 (B, CD4, CD8, NK and Myeloid), Level 2 with 12 cell types (B Naive, B CD70<sup>hi</sup>, CD4 Naive, CD8 Naive, T proliferating, TCM, TEM, Treg, MAIT, NK, NK CD56<sup>hi</sup> and Myeloid), and Level 3 for the 6 conditions: No CD3/CD28 -CBD, No CD3/CD28 +CBD, CD3/CD28 -CBD, CD3/CD28 +CBD, CD3/CD28 -CBD +TAC and CD3/CD28 +CBD +TAC. **(A)** PBMC markers with high granularity to identify the main immune cell population. **(B)** Top 10 differentially expressed genes (DEGs, unbiased) for each cluster based on Level 2 annotation, but plotted for all three annotation levels. **(C)** cytokine proteins using the respective gene symbol. **(D)** CBD receptors were used in our analyses to filter the pathway analyses.



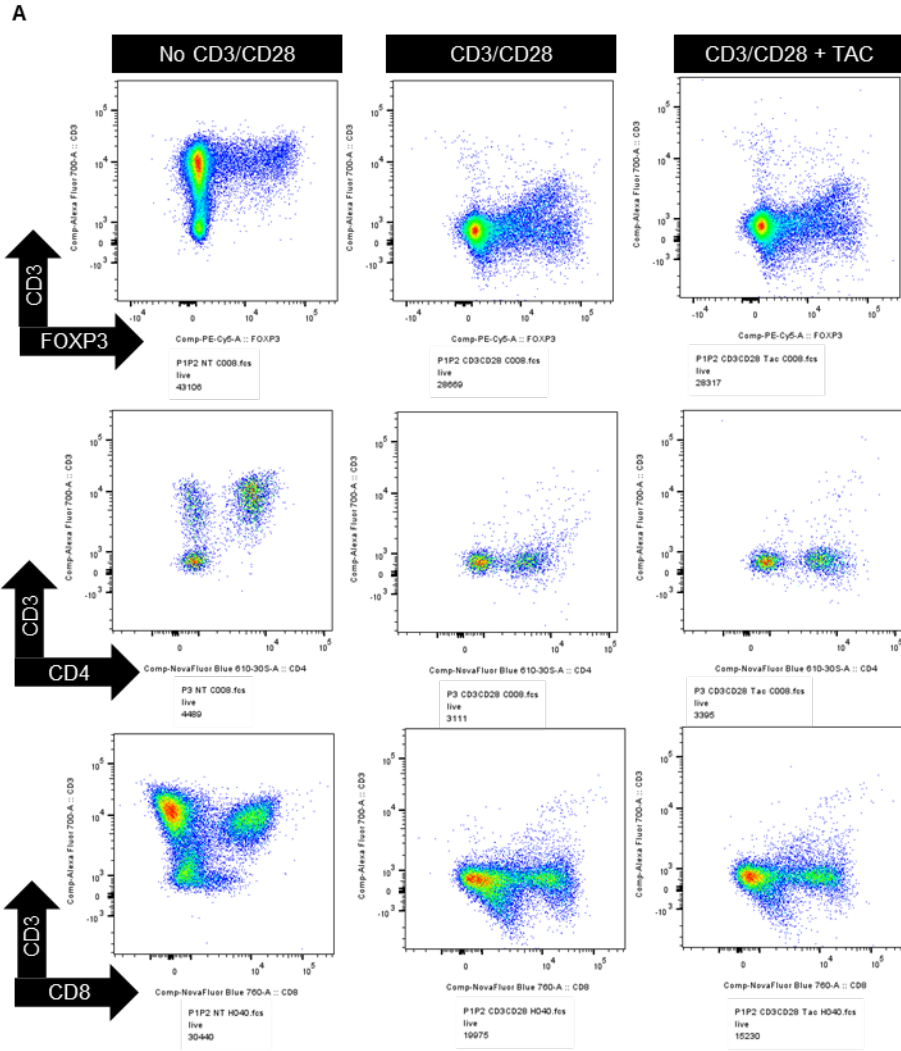
**Supplemental Figure 4. Additional cytokine and chemokine levels in the (±CD3/CD28), (±CBD), and (±TAC) conditions.**

Each panel presents box plots comparing cytokine levels in the pre-CBD (yellow) and post-CBD (green) conditions across three experimental groups: No Stimulation (No CD3/CD28), Stimulation alone (CD3/CD28), and Stimulation with Tacrolimus (CD3/CD28 + TAC). Protein concentrations are shown as z-score normalized values (pg/ml). Symbols indicate statistical comparisons: black denotes differences between pre- and post-CBD conditions; blue indicates comparisons between CD3/CD28–TAC and CD3/CD28 +TAC in the absence of CBD; and red indicates the same comparison in the presence of CBD. The statistical significance is represented as follows: P-value < 0.05 a, P-value < 0.001 b, P-value < 0.0001 c. All p-values were calculated using the limma package with Benjamini–Hochberg false discovery rate (FDR) correction. Analytes shown include: (A) EOTAXIN/CCL11, (B) GRO-alpha/CXCL1, (C) HGF, (D) IFN gamma, (E) IL-1 beta, (F) IL-12p70, (G) IL-17A/CTLA-8, (H) IL-5, (I) PGF1, (J) SDF-1, (K) TGF beta, and (L) VEGF-A. The heat map (M) provides an integrated overview of cytokine and chemokine responses across the same experimental comparisons depicted in the box plots. Each row corresponds to a specific group-wise comparison—namely, pre-CBD versus post-CBD, CD3/CD28 –TAC versus CD3/CD28 +TAC in the absence of CBD, and CD3/CD28 –TAC versus CD3/CD28 +TAC in the presence of CBD—aggregated across all measured cytokines. This visualization enables the identification of coordinated immunological patterns and potential treatment-related effects, offering a comprehensive, systems-level perspective on the modulatory impact of CBD and Tacrolimus.



### Supplemental Figure 5. Representative flow-cytometry gating strategy used to delineate T-cell subsets.

(A) Live singlet lymphocytes were first selected by FSC-A × FSC-H and exclusion of the viability dye. Total T cells were identified as CD3<sup>+</sup> events and subsequently split into CD4<sup>+</sup> and CD8<sup>+</sup> lineages. Within the CD8 compartment, we resolved memory/effector phenotypes on the basis of CD45 isoforms: CD45RO<sup>+</sup> CD45RA<sup>-</sup> effector-memory (Tem), CD45RA<sup>+</sup> CD45RO<sup>-</sup> (Temra), and an intermediate CD45RA<sup>+</sup> CD45RO<sup>-</sup> pool that was further subdivided into intermediary Tem, Tcm (central memory) and naive/Temra subsets according to granzyme-A, TGF-β, CD127 and CD25 expression. CD4 T cells were first partitioned into regulatory (CD25<sup>high</sup> FoxP3<sup>+</sup>) and non-T-regulatory (CD25<sup>low</sup> FoxP3<sup>-</sup>) fractions; each fraction was then stratified in an identical CD45RO/CD45RA hierarchy yielding Tem, Tcm, intermediary Tem, naive and Temra populations. Median fluorescence of CD127 (IL-7Rα) and CD25, as well as granzyme-A<sup>+</sup> and TGF-β<sup>+</sup> functional overlays, are displayed for the indicated gates. Percentages shown correspond to the frequency of each subset within its parent population. Abbreviations: Tem, effector memory; Temra, terminally differentiated effector memory re-expressing CD45RA; Tcm, central memory; cTreg, conventional regulatory T cell. The participant in the condition No CD3/CD28 -CBD -TAC (P1P2 NT H040) starts with 30440 CD3<sup>+</sup> cells where 54.3% is CD4<sup>+</sup> and 25.3% is CD8<sup>+</sup>.



**Supplemental Figure 6. CD3 internalization following T-cell activation.**

Representative flow cytometry plots showing surface CD3 expression with FOXP3, CD4, and CD8 in paired samples with FOXP3, CD4, and CD8 under three conditions: No CD3/CD28 (left), stimulated with CD3/CD28 (middle), and stimulated with CD3/CD28 + tacrolimus (right). In unstimulated samples, FOXP3+, CD4+, and CD8+ cells coexpress CD3, confirming T-cell identity. After stimulation, CD3 surface intensity decreases across all subsets, consistent with receptor internalization following T-cell activation. This effect persists with tacrolimus treatment, although activation intensity differs.

## **Supplemental Tables**

**Supplemental Table 1. Participants Stratified Across Experimental Conditions and Technologies.**

**Supplemental Table 2. CellTiter-Glo® Data.**

**Supplemental Table 3. Differentially Expressed Genes (DEGs) Across the Three Annotation Levels.**

**Supplemental Table 4. Cell Proportion Forest Plot Pre-CBD versus Post-CBD.**

**Supplemental Table 5. Volcano Plots CD3/CD28 +CBD -TAC versus CD3/CD28 -CBD -TAC.**

**Supplemental Table 6. Dual-Contrast CBD Modulation Analysis in Effector-Memory T (TEM).**

**Supplemental Table 7. Pathway Analysis with KEGG for Effector-Memory T (TEM) cells.**

**Supplemental Table 8. Cell-Cell Communication Result Across Level 2 Annotation.**

**Supplemental Table 9. Network of CBD-Regulated Pathways in Effector-Memory T (TEM) Cells.**

**Supplemental Table 10. Dual-Contrast CBD Modulation Analysis in B CD70hi Cells.**

**Supplemental Table 11. Pathway Analysis with KEGG for B CD70hi Cells.**

**Supplemental Table 12 | Dual-Contrast CBD Modulation Analysis in Proliferating T cells.**

**Supplemental Table 13 | Pathway Analysis with KEGG for Proliferating T Cells.**

**Supplemental Table 14 | Differentially Expressed Genes (DEGs) Across the Three Annotation Levels.**

**Supplemental Table 15 | Cell Proportion Forest Plot with Tacrolimus.**

**Supplemental Table 16 | Cytokine Data.**

**Supplemental Table 17 | Flow Cytometry Cell Counts and Proportions.**