



Supplementary Figure 1: Gating strategy for multiparameter intracellular cytokine analysis (ICS).

Representative dot plots from one of three independent experiments performed on buffy coats from three healthy donors are shown, illustrating the sequential gating strategy used to analyze intracellular cytokines within CD3+, CD4+, and CD8+ T cell subpopulations.

(A) Sequential gating workflow: (1) the TIME gate selects the stable instrumental acquisition window; (2) doublets are excluded to retain singlets; (3) live cells are identified by excluding dead cells (LIVE/DEAD Fixable Aqua negative); (4) lymphocytes are defined using forward scatter (FS-A) versus side scatter (SS); (5) CD3+ T cells are gated; (6) CD4+ and CD8+ T cell subsets are further defined. (B) Intracellular cytokine analysis within the CD3+ gate showing IFN- γ , IL-4, IL-17, and IL-10 in non-treated (NT) and SEB-stimulated samples. The same gating and analysis strategy was applied in parallel to CD4+ and CD8+ T cell subsets and across all experimental conditions.