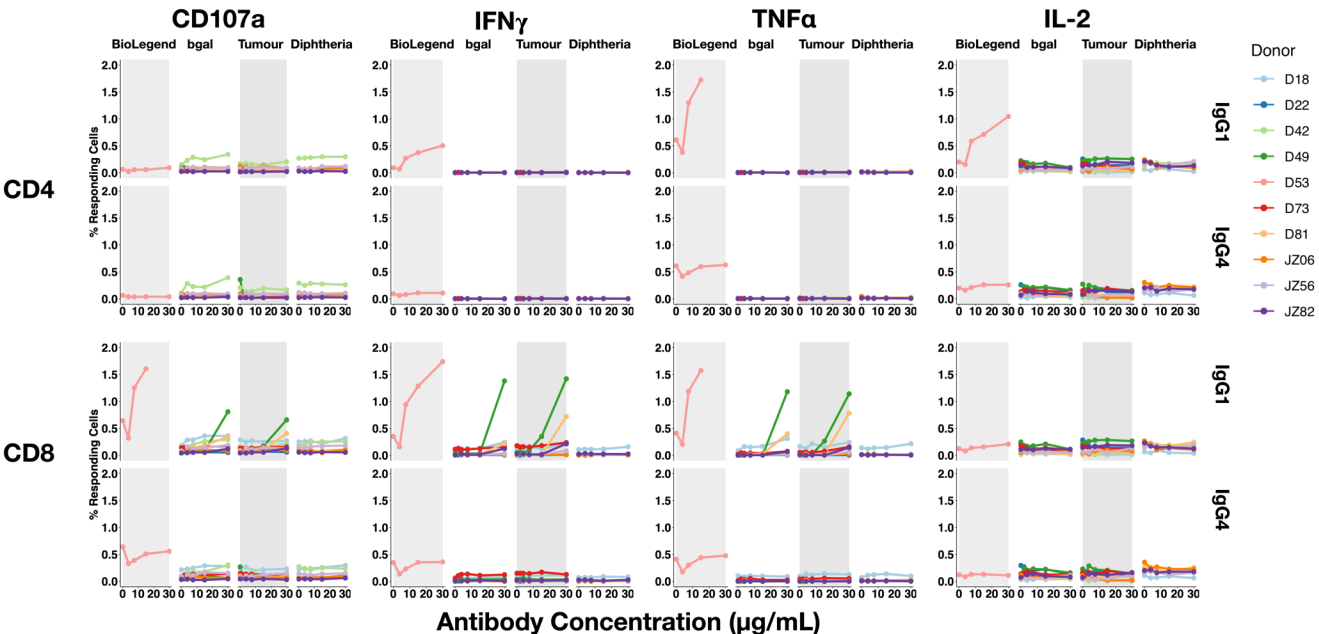
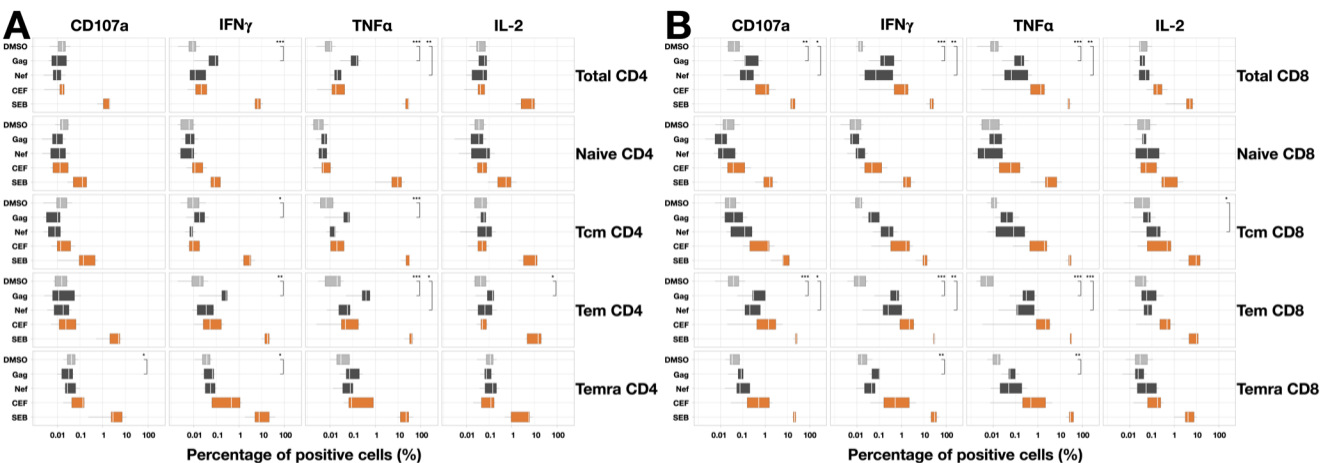


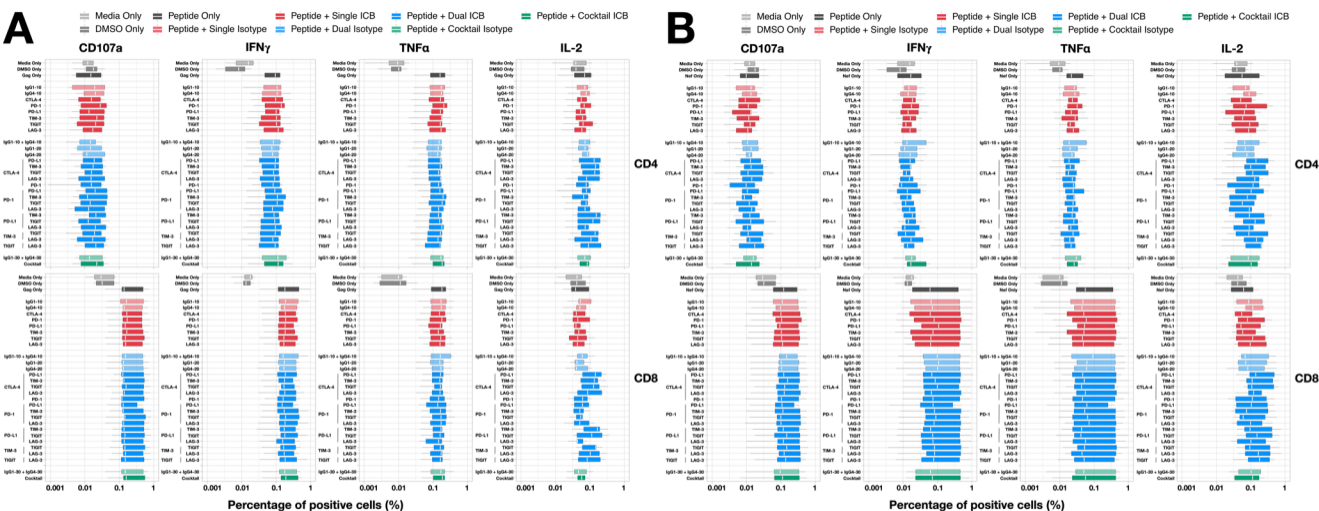
SUPPLEMENTAL FIGURE 1. Sequential gating strategy for total T-cells and subsets. **(A)** Lymphocytes were identified by low forward scatter (FSC) and low side scatter (SSC) gates, followed by FSC-A and FSC-H to identify singlets. Live CD3 were identified using a dump channel that consisted of cells positive for CD14, CD19 and live/dead stain in aqua. Gating was then performed on total CD4⁺ and CD8⁺ T-cells following by CD45RA and CCR7. This approach defined naive (CD45RA⁺ CCR7⁺), central memory (CD45RA⁻ CCR7⁺), effector memory (CD45RA⁻ CCR7⁻) and effector memory with CD45RA (CD45RA⁺ CCR7⁻) CD4⁺ or CD8⁺ T cells. **(B)** Representative gating for CD4⁺ and CD8⁺ T-cells expressing CD107a, IFN γ , TNF α and IL-2 following incubation with either DMSO, HIV Gag/Nef peptides, CEF peptide and SEB.



SUPPLEMENTAL FIGURE 2. Frequency of cytokine+ T-cells in the presence of IgG1 and IgG4 isotype controls. The frequency of T cells that produce CD107a, IFN γ , TNF α or IL-2 were quantified following incubation of PBMC from healthy donors with four isotype controls including BioLegend isotype or antibodies that recognise B-galactosidase, tumor or diphtheria antigens shown for IgG1 (top panel) or IgG4 (bottom panel) and CD4+ (upper row) and CD8+ (lower row) T cells. Each donor is represented by a different colour. Any dose-dependent increase in the frequency of cytokine+ cells indicates non-specific stimulation.



SUPPLEMENTAL FIGURE 3. Intracellular cytokine staining in response to HIV peptides in CD4⁺ and CD8⁺ T-cell subsets. The frequency of cells that produced CD107a, IFN γ , TNF α and IL-2 in blood from PWH on ART stimulated with negative controls (grey; DMSO); HIV peptide pools (black; Gag and Nef) and positive controls (orange; CEF, Cytomegalovirus, Epstein Barr Virus and Influenza peptides and SEB, staphylococcal enterotoxin B) in **(A)** CD4⁺ and **(B)** CD8⁺ T cells. Data are summarised with box plots indicating the median and inter-quartile range for the 9 participants. Statistical significance between HIV peptides and DMSO was determined by Wilcoxon Signed-Rank tests. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$



SUPPLEMENTAL FIGURE 4. Frequency of cytokine+ T cells in response to HIV peptides. Total CD4+ and CD8+ T-cells collected from PWH on ART were incubated with media alone (light grey), DMSO (dark grey) or peptide alone (black) antibodies to ICs either alone (red), as dual combinations (blue) or a cocktail of six antibodies (green) following incubation with overlapping peptides to either **(A)** Gag or **(B)** Nef. Data are summarised with box plots indicating the median and inter-quartile range for the 9 participants.