



Supplemental Figure 4. CD4⁺ and CD8⁺ T-cell responses against EBV and CMV (A) antigens and gating strategies to determine (A) JCV responsive CD4 cell subsets and (B) HIV reservoirs by HIV Flow. (A) Irrelevant peptide NY-ESO1 (New York Esophageal-Squamous-cell-carcinoma-1): negative control; CMV [pp65, Immediate-early-1 (IE1)] and EBV [Epstein-Barr nuclear antigen (EBNA), BamHI-Z-fragment leftward open reading frame-1 (BZLF1)]. (B) Lymphocytes were identified based on light scatter properties, singlets were selected based on forward scatter-area vs. height, and viability based on dye exclusion. CD4 and CD8 subsets were identified among total CD3⁺ T cells PBMC stimulated with JCV peptide pools as described in the Methods section. Non-Naïve CD4⁺T-cells were identified by extracellular staining and sorted into CFSE^{high}/CFSE^{low} subsets. (C) Sorted CFSE^{high/low} non-naïve CD4⁺T-cells, were resuspended in culture media, stimulated with the Dynabeads for 42 hours and then stained for CD4, CD3, p24 KC67-PE, and p24 28B7.