

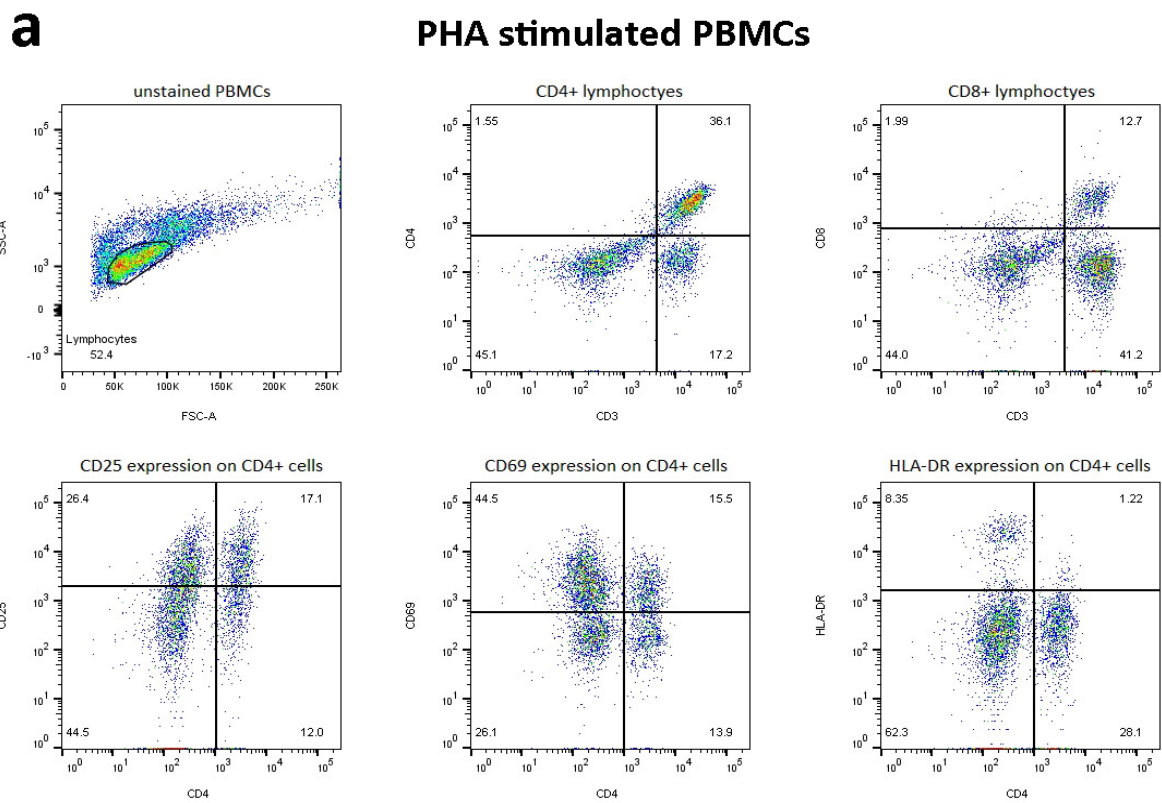
Supplementary Information

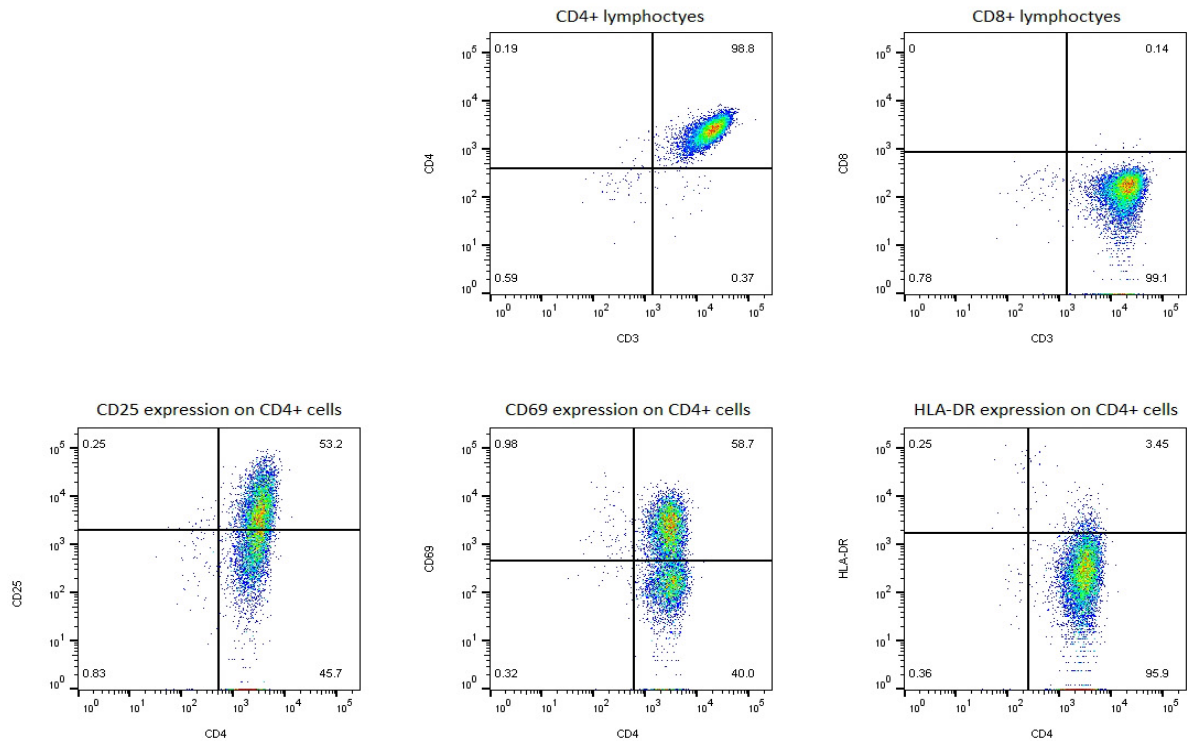
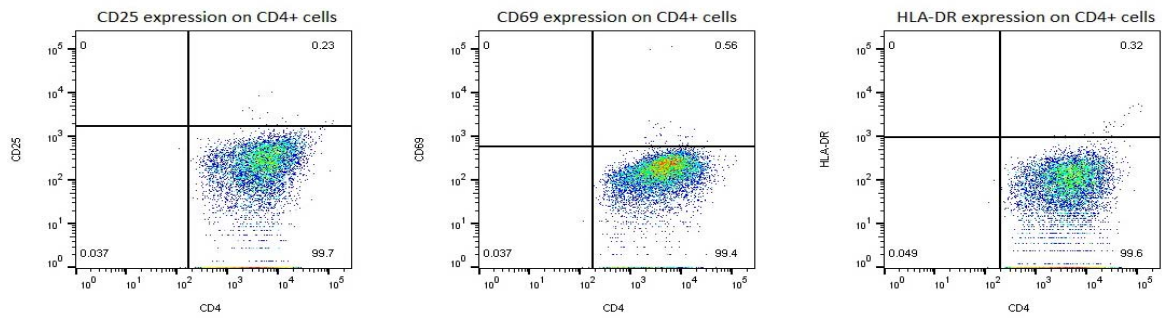
A highly reproducible quantitative viral outgrowth assay for the measurement of the replication-competent latent HIV-1 reservoir

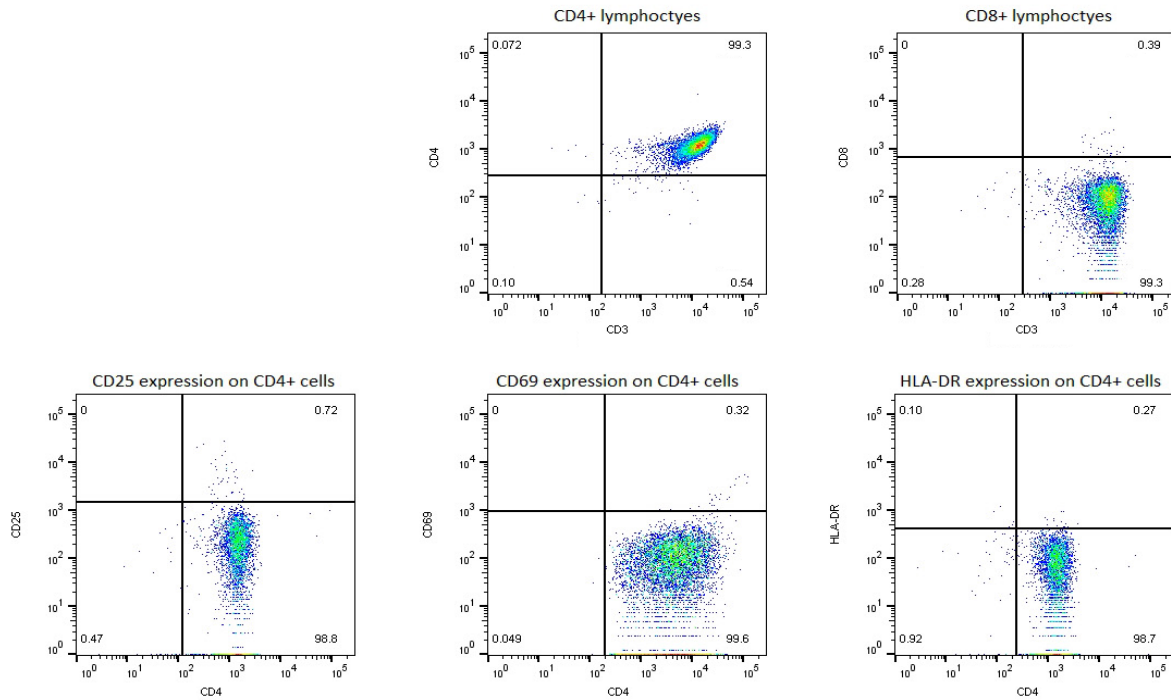
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Supplementary Figure 1



b**Purified total CD4+ T cells****C****Purified resting CD4+ T cells after activated cell depletion**

d**Resting CD4+ T cells purified in a single step**

Supplementary Figure 1. Purification of resting CD4⁺ T cells from highly activated PBMCs with the one-step custom antibody kit and the standard two-step procedure. PBMCs were stimulated for 3 days with 10 U/ml IL-2 and 1 µg/ml PHA-L. Cells were stained with either anti-CD3-PerCP/Cy5.5, anti-CD4-FITC and anti-CD8-Pacific Blue or anti-CD4-FITC, anti-CD25-PE/Cy7, anti-CD69-Pacific Blue and anti-HLA-DR-APC to analyse their purity by flow cytometry. **(a)** Mitogen stimulation resulted in high expression levels of CD25 and CD69 and moderate expression of HLA-DR on lymphocytes. **(b)** Total CD4⁺ T cells were isolated with a commercial isolation kit yielding highly purified CD4⁺ T cells and FACS analysis revealed an expression of CD25 and CD69 on >50% of CD4⁺ T cells and HLA-DR on >3% of CD4⁺ T cells. **(c)** Activated cells were depleted with FITC conjugated antibodies against activation markers CD25, CD69 and HLA-DR using anti-FITC magnetic beads. This resulted in highly purified resting CD4⁺ T cells. **(d)** Resting CD4⁺ T cells were obtained in a single step using a custom antibody cocktail with a yield and purity identical to the standard two-step procedure.