## Supplemental figure legends

**Supplementary Figure 1: HIV outgrowth in CD4**<sup>+</sup> **T-cells of HIV-infected untreated PLWH.** The VOP was performed as described in <u>Figure 1A</u> using memory CD4<sup>+</sup> T-cells from ART-untreated PLWH (n=5; <u>Supplemental Table 1, HIV+#14-18</u>). Shown are levels of integrated HIV-DNA (mean±SD of PCR triplicates) measured *ex vivo* (**A**); as well as soluble HIV-p24 levels measured by ELISA at days 3 (1 replicate/donor), 6 (2 replicate/donor), 9 (4 replicate/donor), and 12 (8 replicate/donor) post-culture (**B**) and intracellular HIV-p24 levels measured by flow-cytometry at day 12 post-stimulation (**C**). Each symbol represents one experimental splitting replicate, with a total of 8 splitting replicates/study participant at day 12.

**Supplementary Figure 2: Reproducibility of the VOP.** The VOP, performed as in Figure 1A, was repeated in two independent experiments using memory CD4<sup>+</sup> T-cells from three HIV+ART individuals (ART#1, ART#5, and ART#7; <u>Table 1</u>). Shown are (A) the frequencies of HIV-p24<sup>+</sup> T-cells at day 12 post-culture in the 1<sup>st</sup> (open triangles) and 2<sup>nd</sup> (filled triangles) experiment and (B) the correlation between the results generated in these two independent experiments calculated using SC (p and r values) and LR (p and r2 values) models. Each symbol represents one splitting replicate well/study participant.

**Supplementary Figure 3: ATRA** accelerates/increases HIV outgrowth in a dose-dependent manner. The VOP was performed as described in <u>Figure 1A</u> with memory CD4<sup>+</sup> T-cells from one study participant (HIV+ART #5; <u>Supplemental Table 1</u>) in the presence of different doses of ATRA (10, 100, 1,000, and 10,000 nM) or the equivalent

volumes of DMSO as control. Shown are levels of HIV-p24 in cell-culture supernatants collected at days 3, 6, 9 and 12 post-culture. The red arrow indicates the optimal ATRA concentration (100 nM) subsequently used in <u>Figures 3-5</u>.

Supplemental Figure 4: ATRA boosts HIV outgrowth. The VOP was performed as described in Figure 1A with memory CD4<sup>+</sup> T-cells from HIV+ART individuals (n=9; Supplemental Table 1) and in the presence or in the absence of ATRA (100 nM). Shown are heatmap representations of HIV-p24 levels in cell-culture supernatants collected at days 3, 6, 9, and 12 post-stimulation in all n=9 donors. The heatmaps reflect the magnitude of soluble HIV-p24 levels *per* well and *per* donor at different time points (red for the highest and blue for the lowest HIV-p24 levels as indicated in the scale). The absolute HIV-p24 levels are indicated on the heat-map cells for positive wells.

**Supplemental Figure 5: Calculation of IUPMs in the ATRA-based QVOA.** The QVOA was performed in the presence or in the absence of ATRA (100 nM), as depicted in <u>Figure 4A</u>. Shown are **(A)** HIV-p24 quantification results in the splitting replicates from one representative donor (HIV+ART #12) and **(B)** a summary of HIV-p24 positivity in the original wells and the mode of IUPM calculation for all donors.