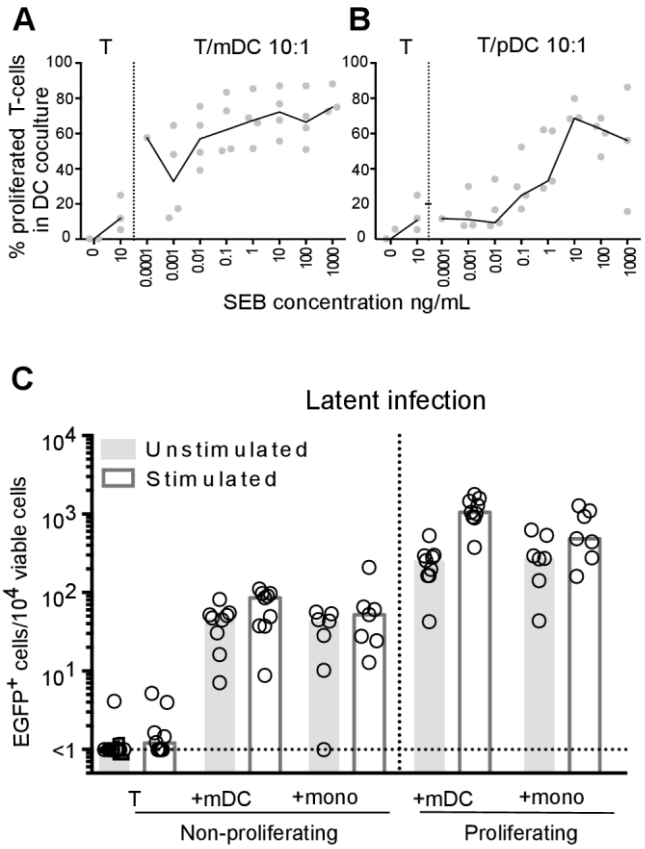
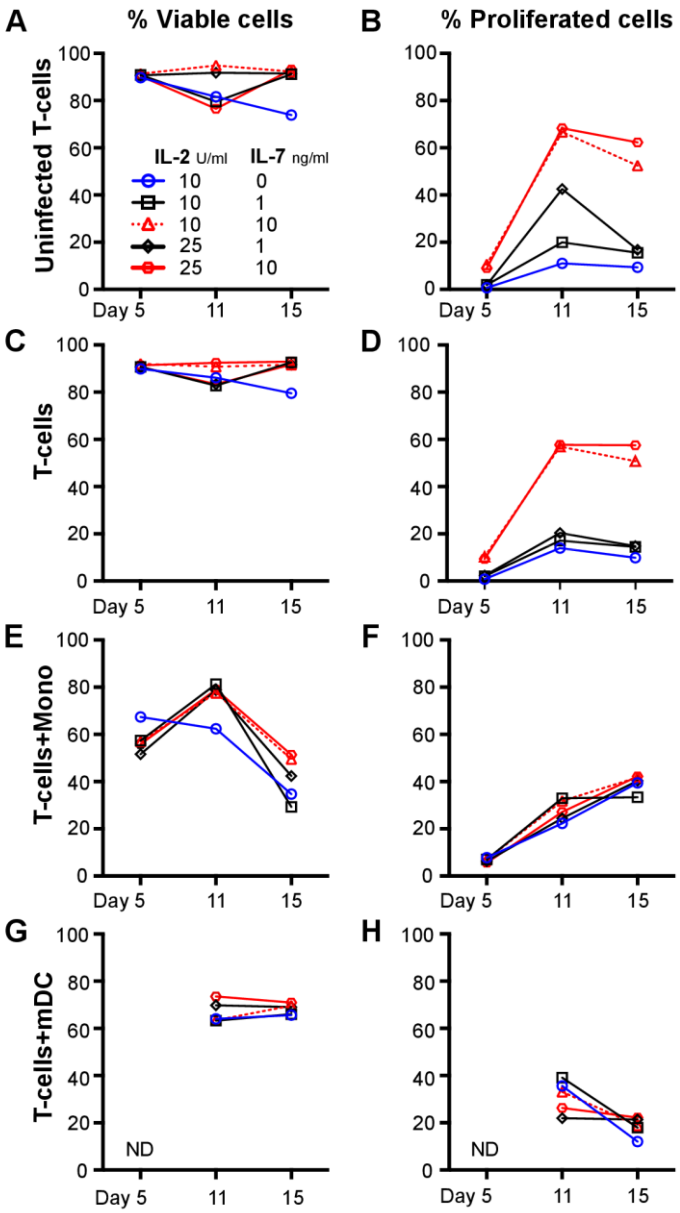
Supplemental Data

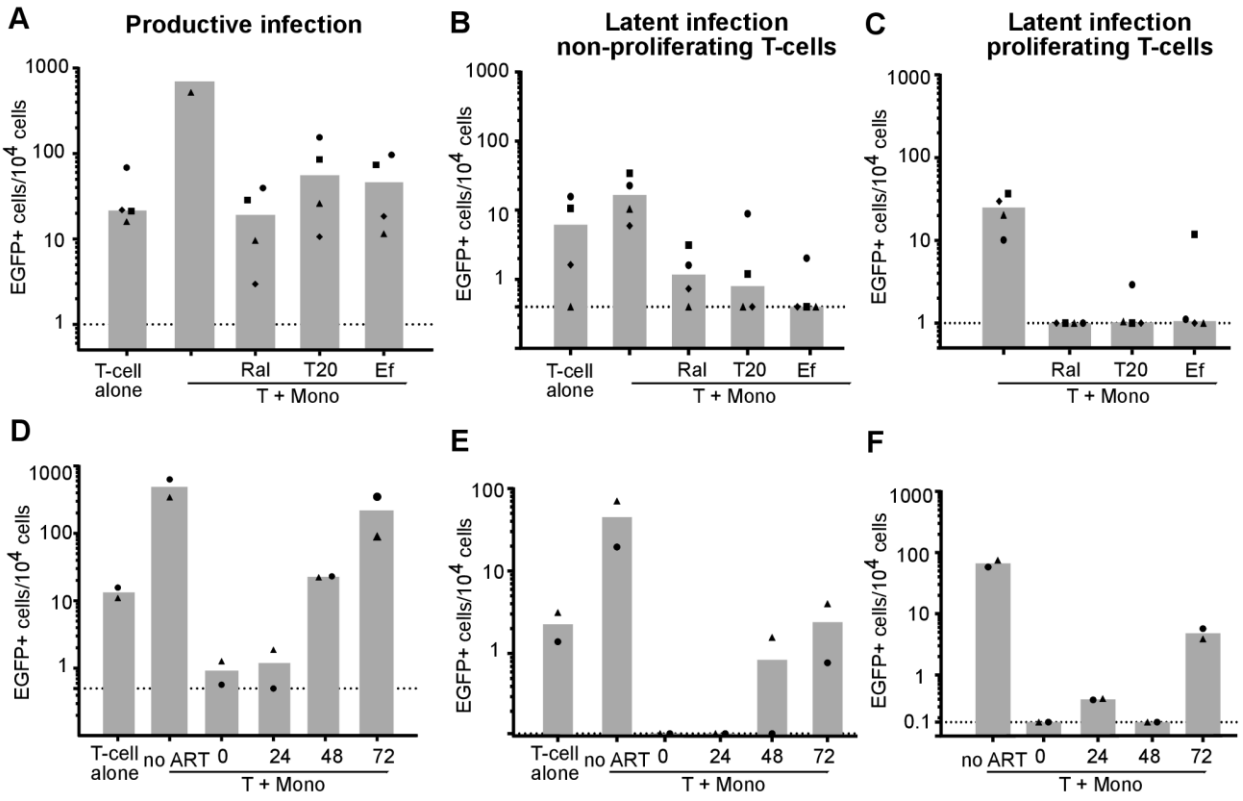


Supplemental Figure S1. Measurement of latency in proliferating T-cells in cocultures with DC and increasing concentrations of staphylococcal enterotoxin B (SEB).
The optimal concentration of SEB to induce CD4+ T-cell proliferation was determined in T-cell-DC cocultures. eFluor670 labelled CD4+ T-cells were cocultured with A. mDC (T/mDC); or B. pDC (T/pDC) at a ratio of 10:1 with increasing concentrations of SEB (0-1000ng/ml; n=4). Resting CD4+ T-cells cultured with SEB (0 and 10 ng/ml) were used as a control (T). The solid line represents the median of 4 individual experiments; symbols represent results from individual donors. C Measurement of HIV latency by EGFP expression after stimulation with anti-CD3/anti-CD28+IL-7. eFluor670 labelled CD4+ T-cells were cultured alone, with mDC or monocytes (mono) at a ratio of 10:1 and then infected with CCR5-EGFP reporter virus. At day 5, sorted non-productively infected, non-proliferating (eFluor670hiEGFP-) and proliferating (eFluor670lo EGFP-) T-cells were either left unstimulated (grey columns) or stimulated with anti-CD3/CD28+IL-7 to reactivate latency (open columns). Both unstimulated and stimulated cells were cultured in the presence of IL-2 and an integrase inhibitor (L8 or raltegravir). Total post-integrated latency was calculated by subtraction of unstimulated EGFP expression from stimulated EGFP expression (n=6-8). Columns represent the median, symbols represent results from individual donors, and the dotted line represents the limit of detection.

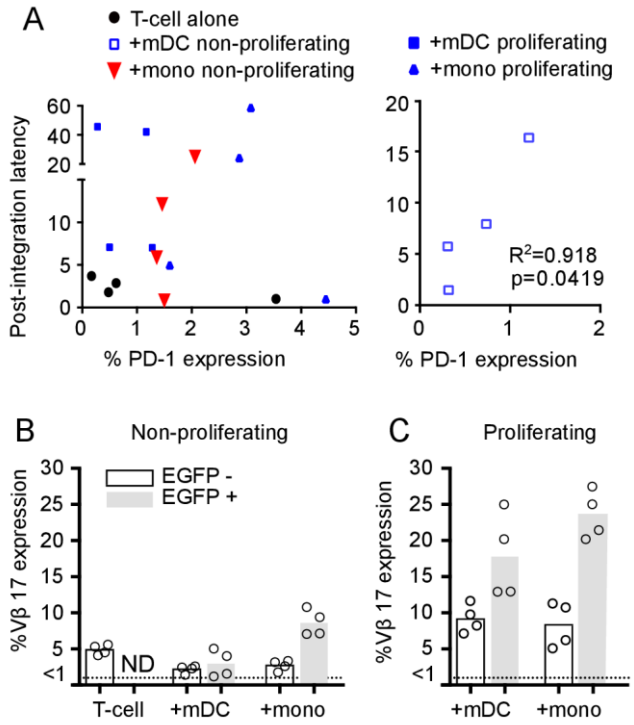


Supplemental Figure S2. Titration of IL-2 and IL-7 in T cells cultured alone or with mDC or monocytes.

We measured cell viability (A, C, E, G) and cell proliferation (B, D, F, H) in A, B. uninfected resting memory T-cells C, D. EGFP reporter virus infected T-cells, with E, F. monocytes and G, H. mDC. Along with uninfected T-cells, all cultures were treated with different combinations or IL-2 and IL-7; ○ 10U/ml IL-2 + 0ng/ml IL-7, □ 10U/ml IL-2 + 1ng/ml IL-7, △ 10U/ml IL-2 + 10ng/ml IL-7, ◇ 25U/ml IL-2 + 1ng/ml IL-7, 25U/ml IL-2 + 10ng/ml IL-7. Each line represents a different condition, n=1.



Supplemental Figure S3. T-cells were cultured alone or cocultured with monocytes at a ratio of 10:1. Cells were infected with EGFP reporter virus. A-C.
Cocultures were either untreated or treated with the integrase inhibitor raltegravir (Ral) at the time of infection or fusion inhibitor (T20) or non-nucleoside reverse transcription inhibitor (NNRTI; Efavarinz (Ef)) 24 hours after infection. A. Productive infection was measured at day 5 post-infection. At day 5 cell cultures were sorted into uninfected, non-proliferating (eFluor670hiEGFP-) or proliferating (eFluor670loEGFP-) CD4+ T-cells. D-F. In another set of experiments combination of Ral, T20, Ef (ARV) was added to cultures at 24, 48 and 72 hours post-infection and productive and latent infection quantitated as in figure S1C. Columns represent the median; symbols represent results from individual donors.



Supplemental Figure S4. Correlation of latency with expression of immune checkpoints and specific TCR Vβ expression.

A Correlation of latent infection with PD-1 expression in non-proliferating and proliferating CD4+ T-cells from mDC and mono co-cultures. A correlation was seen with latency and PD-1 expression in non-proliferating CD4+ T cells in mDC cocultures but not in proliferating CD4+ T cells.

B, C CD4+ T-cells labelled with eFluor670 were cultured alone, or with either mDC or monocytes (mono) at a ratio of 10:1 and then infected with a CCR5-EGFP reporter virus. At day 5 post-infection expression of TCR Vβ 17 was quantified on **B** non-productively infected EGFP+ (grey) and **C** non-productively infected, EGFP- (clear) CD4+ T-cells (n=4). ND = not done. Increased expression of TCR Vβ 17 was seen in proliferating cells and in EGFP+ T cells in monocyte cocultures.