Supplementary figure 1

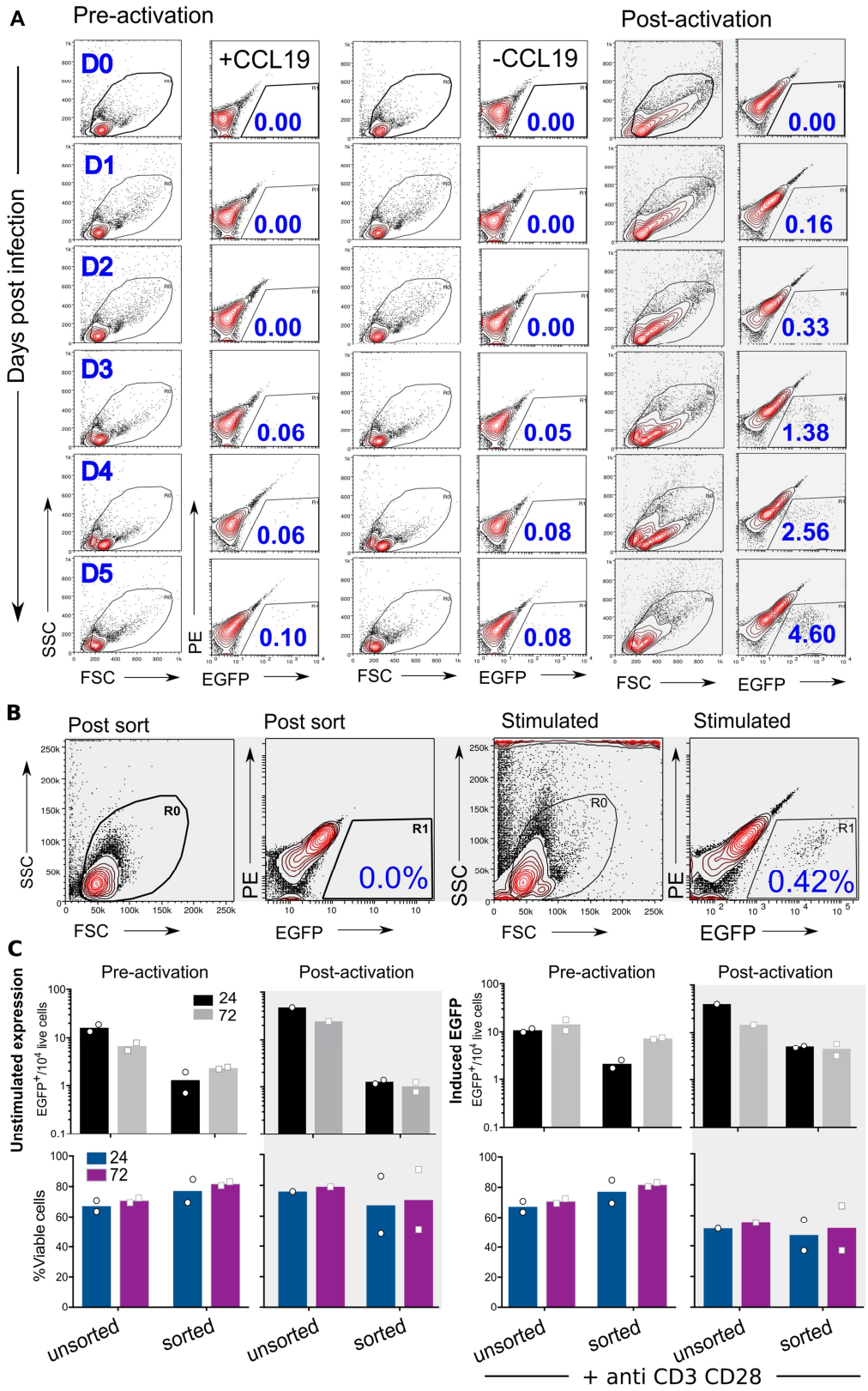

Figure S1: Kinetics of EGFP expression after infection of T cells in pre- and post-activation models of latency

A) Pre-activation latency was established by infection of resting CD4+ T cells cultured alone or with CCL19. Post-activation latency was established by activating naïve T cells with antiCD3/CD28 for 7 days before infection. Both T cells were infected with X4-EGFP virus and forward and side scatter (FSC and SSC) and EGFP expression monitored daily for 5 days. Higher levels of EGFP expression were found in post-activation compared to pre-activation latency. B) Cells from post-activation cultures were sorted at day 7 post infection and EGFP- cells were analysed by flow cytometry immediately or following stimulation with anti CD3/CD28 for 3 days (stimulated). FSC and SSC and EGFP expression are shown. C) EGFP- negative cells were collected after sorting and cultured for 24 (black) and 72 hours (grey) and compared to unsorted cultures stimulated with anti-CD3/anti-CD28. EGFP expression and viability of cells are shown. Data represents 2 matched donors from one experiment. Percentage of viable cells in unsorted and sorted cells cultured for 72 hours (squares, unstimulated expression, purple) or following anti-CD3/ anti-CD28 stimulation (+aCD3/CD28) at 24hrs (blue circles, bar). The post-activation latency is shown by grey shading.

Supplementary figure 2

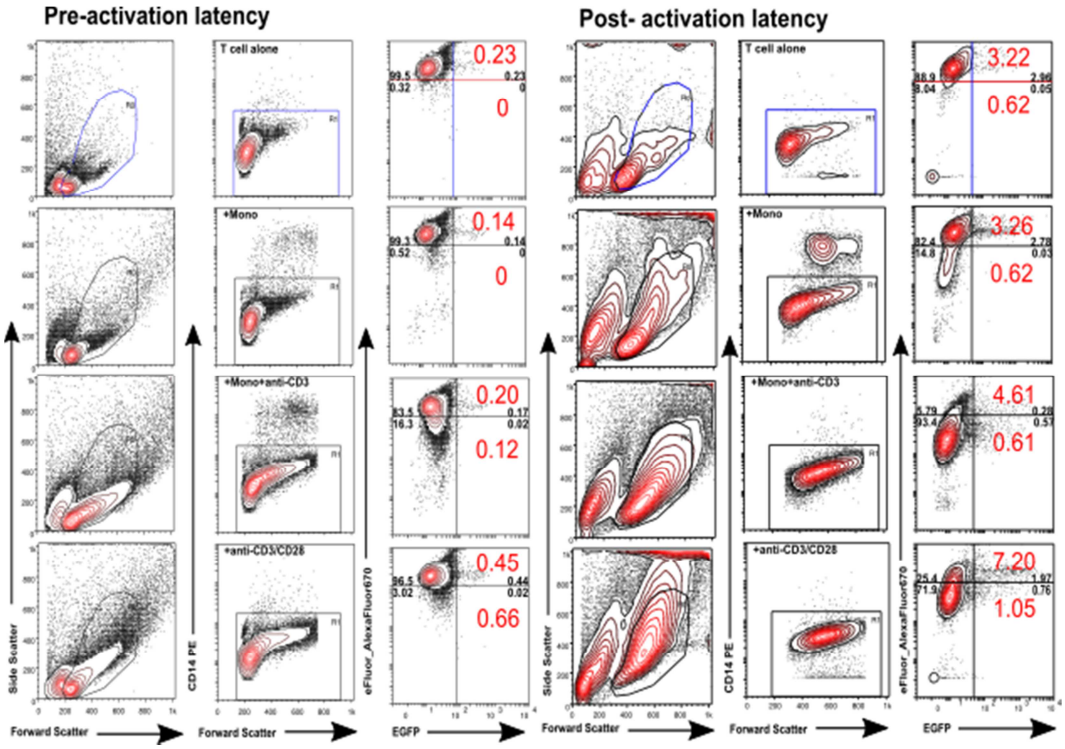

Figure S2: EGFP expression following activation in proliferating and non-proliferating latently infected cells.

The gating strategy to measure EGFP expression and cellular proliferation in latently infected cells post stimulation is shown. Live cells were defined using forward versus side scatter. Monocytes were excluded by gating for CD14 PE negative. The EGFP expression was measured against proliferation detected by reduced AlexaFluor 670. Data shown as the percentage of EGFP expression in top right as eFluor670^{hi}EGFP+, bottom right as eFluor670^{lo}EGFP+, top left eFluor670^{hi}EGFP- and bottom left eFluor670^{lo}EGFP-. Plots are representative of EGFP expression and proliferation on T cell alone (unstim), monocytes, monocytes/anti-CD3 and anti-CD3/anti-CD28 stimulation. The plots show the same donor tested in both pre- and post-activation latency models. The percentage of EGFP in proliferating

(eFlour670^{lo}) and non-proliferating (eFlour670^{hi}) cells is shown in red. Scatterplots represent analyses of 1 of 6 donors.

Supplementary figure 3

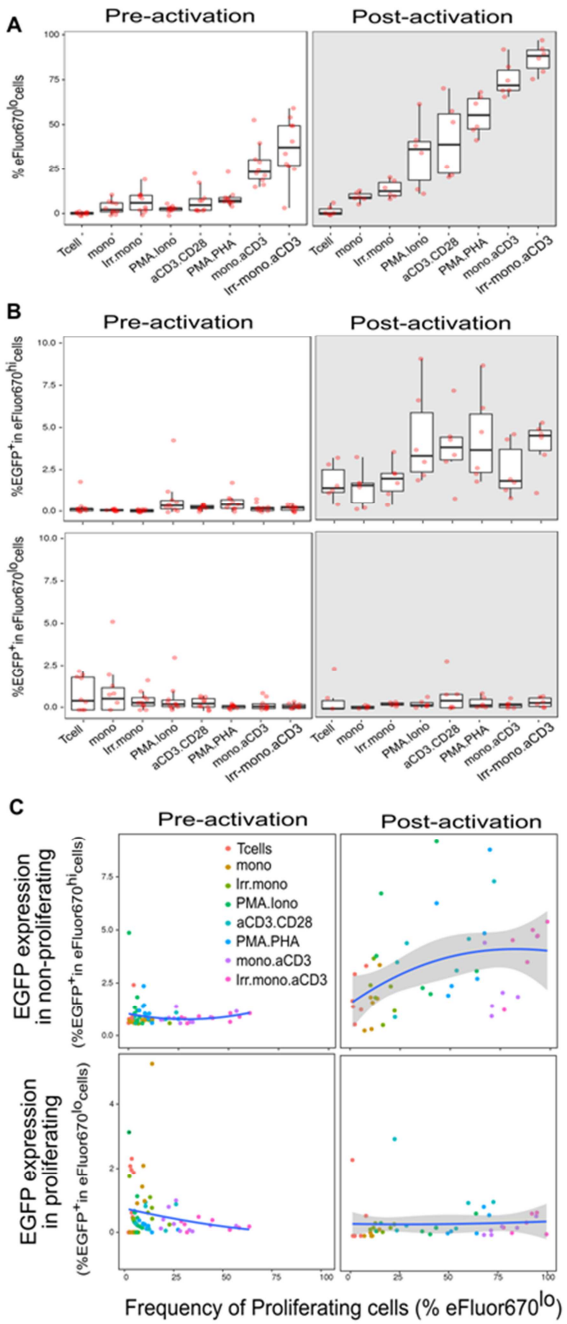

Figure S3: EGFP expression in proliferating and non-proliferating latently infected cells following activation.

A) Percentage of proliferating (eFluor670^{lo}) cells in response to different activating stimuli in pre- and post- activation latency, **B)** Distribution of EGFP expression in non-proliferating (eFluor670^{hi}) and proliferated (eFluor670 ^{lo}) cells. Each point represents a single donor. The boxplots show 25 and 75 percentiles, median and range. Grey shades represent latently infected cells in the post-activation latency model. **C)** The frequency of EGFP expressed cells following stimulation in proliferating (eFluor670^{lo}) and non-proliferating (eFluor670^{hi}) cells plotted against the frequency of proliferated (eFluor670^{lo}) cells in post- and pre-activation model. Each point represents a single donor.

Supplementary figure 4

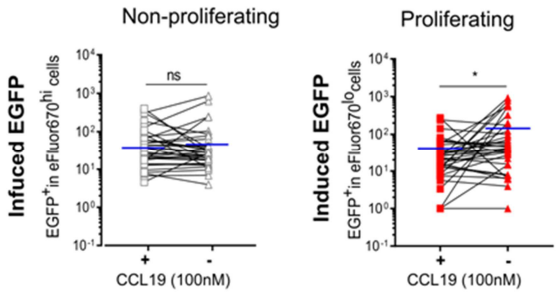

Figure S4: Effect of CCL19 on induced HIV expression in non-proliferating and proliferating latently infected T cells.

Pre-activation latency was established in resting CD4+ T cells with and without CCL19 (100nM). Antiretrovirals were added 48hrs post infection. The EGFP- cells were sorted and cultured with activation stimulus for 3 days. The induced EGFP expression was determined in the 2 populations by FCM. The number of EGFP expressing cells in non-proliferating (eFluor670^{hi}) and proliferating (eFluor670^{lo}) cells in the pre-activation latency model with CCL19 (square) or without (triangle) are shown. p*≤0.05, as determined by Wilcoxon matched pairs signed rank test**.** Each point represents a single donor in a matched condition. The median is shown by blue line.

Supplementary figure 5

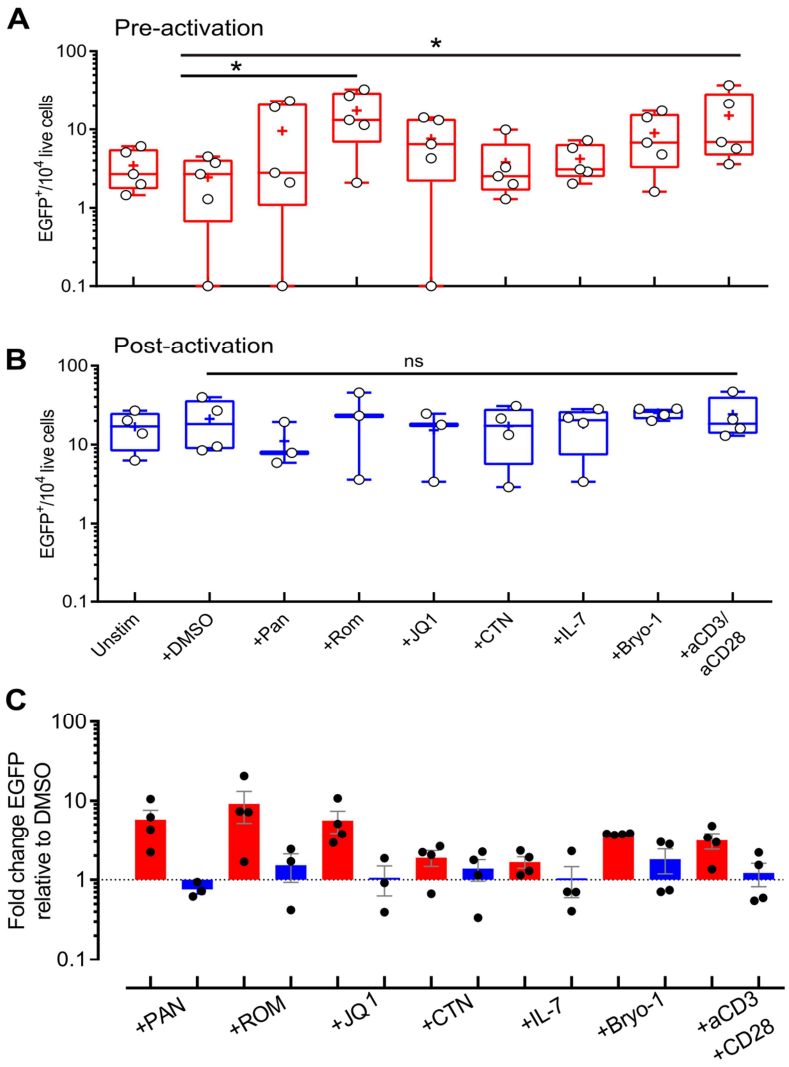

Figure S5: Response to LRA in pre- and post-activation latency.

Non-EGFP expressing cells sorted from the pre- activation **A)** and post-activation models **B)** were co-cultured with a panel of LRAs including the HDACs inhibitors panobinostat (30nM) and romidepsin (40nM), BET inhibitor JQ1 (10nM), PKC agonist Bryostatin-1 (10nM), histone methyltransferase inhibitor; Chaetocin, CTN (10nM), and γc-cytokine IL-7 (50ng/ml). AntiCD3/antiCD28 beads (at 1:1 ratio) were used as a positive control. To measure the background expression cells were also cultured in DMSO or media containing antivirals only

(unstim). EGFP expression was measured 72hrs post activation. Each point represents mean values of two technical replicates. The boxplots show 25 and 75 percentiles, median and range. **C)** Fold change in EGFP expression in sorted non-EGFP expressing cells from pre-activation (red) and post-activation (blue) following stimulation with LRA. Fold change was determined against DMSO control. Each dot indicates an individual donor, error bars represents mean ±SEM. p*≤0.05, as determined by Student's t-test on log transformed data.