Figures and figure supplements

Distinct chromatin functional states correlate with HIV latency reactivation in infected primary CD4+ T cells

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Figure 1. Second generation of dual-fluorescence HIV-1 reporter, HIV\textsubscript{GKO}, to quantify stable latency. (A) Schematic representation of first (top: HIV\textsubscript{DuoFluoI}) and second generation (bottom: HIV\textsubscript{GKO}) of dual-labeled HIV-1 reporters. (B) Representative experiment of HIV\textsubscript{GKO} virus titration in Uninfected Double Negative Latent Productive

Donor 1

Donor 2

Gag-p55

Gag-p24

Vif

Actin

Fold Change (\textDelta\text{ACT})

US (Gag)

SS (Env)

MS (Tat, Rev)

Uninfected Double Negative Latent Productive

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activated primary CD4+ T cells (4 days post-infection). Primary CD4+ T cells were activated with αCD3/CD28 beads + 20 U/mL IL-2 for 3 days before infection with different amounts of HIV_GKO (input, ng/p24) and analyzed by flow cytometry 4 days post-infection. (C) Comparison of HIV_DuoFluo and HIV_GKO infection profiles by flow cytometry in activated primary CD4+ T-cells (4 days post-infection). Cells were treated as in (B). (D) Comparison of GKO-WT-LTR and GKO-ΔU3 3’LTR infection profiles by flow cytometry in cells treated as in (B). (E, F) Primary CD4+ T cells were treated as in (B). At 4 days post-infection, double-negative, productively infected, and latently infected cells were sorted out, and (E) the total RNA isolated from each population was subjected to Taqman RT-qPCR analysis (Source Data - Figure 1). Unspliced (US), singly spliced (SS), and multiply spliced (MS) HIV-1 mRNAs were quantified relative to cellular GAPDH. (F) Western blot analysis of each population.

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Comparison of HIV\textsubscript{GKO} and HIV\textsubscript{DuoFluoI}. Titration of HIV\textsubscript{GKO} input revealed that latently and productively infected cells increased proportionately as viral input increased (Figure 1—figure supplement 1A and B), as reflected by steady ratios of latent/productive (Figure 1—figure supplement 1C). On the contrary, increasing HIV\textsubscript{DuoFluoI} input does not lead to a proportional increase of latent and productive populations (Figure 1—figure supplement 1A and B), as reflected on the ratios latent/productive (Figure 1—figure supplement 1C). Briefly, CD4\textsuperscript{+} T-cells were purified from blood of one representative healthy donor and activated for 72 hr with \textgreek{a}CD3/CD28 beads and 100 U/ml IL-2 before infection with serial dilutions of either HIV\textsubscript{GKO} or HIV\textsubscript{DuoFluoI}. Four days post-infection, the percentage of latent cells (csGFP- mKO2+, Figure 1—figure supplement 1A) and productive cells (csGFP+, Figure 1—figure supplement 1AB) were quantified by FACS for both dual-fluorescence viruses, and ratios of latent versus productive populations were calculated (Figure 1—figure supplement 1C).

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Figure 2. LRAs efficacy in patient samples is predicted by activity in HIV<sub>GKO</sub> latently infected cells. (A) Intracellular HIV-1 mRNA levels in rCD4s, obtained from infected individuals and treated ex vivo with a single LRA or a combination of two LRAs for 24 hr in presence of raltegravir, presented as fold induction relative to DMSO control. (n = 4, mean ±SEM) (Figure 2—source data 1). (B) Intracellular HIV-1 mRNA levels in HIV<sub>GKO</sub> latently infected CD4<sup>+</sup> T-cells, and treated with a single LRA or a combination of two LRAs for 6 hr in presence of raltegravir, presented as fold induction relative to DMSO control. (n = 3 (different donors), mean ±SEM, paired t-test) (Figure 2—source data 1). (C) Correlation between intracellular HIV-1 mRNA levels quantified in either 6 hr stimulated HIV<sub>GKO</sub> latently infected CD4<sup>+</sup> T-cells from different donors, or 24 hr stimulated rCD4s from HIV infected patients, with a single LRA or a combination of two LRAs in presence of raltegravir.

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Figure 3. Few HIV_{GKO} latently infected primary CD4^{+} T cells are reactivated. (A) Schematic of experimental procedure with primary CD4^{+} T cells. Briefly, CD4^{+} T cells were purified from blood of healthy donors and activated for 72 hr with αCD3/αCD28 beads and 100 U/ml IL-2 before infection with HIV_{GKO}. Five days post-infection, latently infected cells (csGFP- mKO2+) cells were sorted, put back in culture overnight and stimulated with different LRAs in presence of raltegravir for 24 hr before performing FACS analysis. (B) Percentage of GFP^{+} cells is shown after stimulation of latently infected CD4^{+} T-cells with LRAs (n = 4 (different donors), mean +SEM, paired t-test) (Figure 3—source data 1). (C) Histogram plot of percent live cells for each drug treatment (n = 3 (different donors), mean + SEM, paired t-test) (Figure 3—source data 1). p-value: *p<0.05, **p<0.01 relative to DMSO.

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Figure 4. Low-level latency reactivation is not explained by low cellular responses to activation signals. T-cell activation patterns between double negative, reactivated (RLIC) and non-reactivated (NRLIC) latently infected cells. Briefly, CD4+ T-cells were purified from blood of four healthy donors and activated for 72 hr with αCD3/CD28 beads and 20 U/ml IL-2 before infection with HIVGKO. At 4 days post-infection, csGFP- were sorted, cultured overnight and stimulated with αCD3/CD28 in presence of raltegravir. At 24 hr post-treatment, cells were stained for CD25, CD69, and HLA-DR activation markers before performing FACS analysis. (n = 4, mean ±SEM, paired t-test; *p<0.05; **p<0.01) (Figure 4—source data 1).

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Figure 4—figure supplement 1. 24 hr treatment effectively activate primary CD4+ T cells. Quantification of T-cell activation-associated surface markers after 24 hr stimulation. Briefly, CD4+ T-cells were purified from blood of two healthy donors and activated for 72 hr with αCD3/CD28 beads and 100 U/ml IL-2 before infection with HIV_GKO. Five-days post-infection, latently infected cells (csGFP- mKO2+) were sorted, cultured overnight and stimulated with either αCD3/CD28 or bryostatin + panobinostat in presence of raltegravir. 24 hr post-treatment, cells were stained for CD25 and CD69 activation markers before performing FACS analysis.

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Figure 5. Relative expression of HIV-1 integration targeted genes for each population, before or after TCR activation. (A) Scatter chart showing primary CD4+ T-cell gene expression changes after 48 hr of stimulation with αCD3/CD28 beads. Integration sites displayed outside of the two solid gray lines were targeted genes whose expression is at least ± twofold differentially expressed after 48 hr stimulation. Plot points size can be different, the bigger the plot point is, the more integration events happened within the same gene. (B) Fraction of integration sites from the different populations PIC, RLIC or NRLIC, integrated within genes whose expression is at least ± twofold differentially expressed after 48 hr of αCD3/CD28 stimulation (**p<0.01; ***p<0.001; two-proportion z test) (Figure 5—source data 1). (C) Relative expression of genes targeted by HIV-1 integration in PIC, RLIC or NRLIC before TCR stimulation and after αCD3/CD28 stimulation (n = 3, mean ±SEM, paired t-test). ***p<0.001; ****p<0.0001. (Figure 5—source data 2).

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Figure 6. Insertion landscapes of HIV-1. (A) Proportion of mapped insertions that are in genic or intergenic regions. (Figure 6—source data 1). (B) Proportion of integration sites in transcribed regions with high (top 1/8), medium (top 1/4–1/8), low expression (top 1/2–1/4), trace (bottom 1/2) or silent (0) expression. (Figure 6—source data 1). (C) Proportion of unique genic integration sites located in introns, exons, UTR or promoters. (Figure 6—source data 1). (D) Transcriptional orientation of integrated HIV-1 relative to host gene. (Figure 6—source data 1). p-value: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 using two-proportion z test.
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Figure 7. Epigenetics marks and nuclear localization of HIV-1 integration sites. (A) 500 bp centered on HIV-1 integration sites for each population were analyzed for the presence of H3K4me1 (active enhancers), H3K36me3 (active transcribed regions), H3K9me3 and H3K27me3 (repressive marks of transcription), and DNA accessibility (DNAseI). (Figure 7—source data 1). (B) Nuclear localization of HIV-1 integration sites. Quantification was based on inside a LAD (=1) or outside (=0), which means the Y axis represents the fraction of integrations within a LAD. (Figure 7—source data 1). (n = 3–4 ENCODE donors, mean ±SEM, paired t-test). *p<0.05; **p<0.01; ***p<0.001).

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