Release of P-TEFb from the Super Elongation Complex promotes HIV-1 latency reversal

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INTRODUCTION

The persistence of HIV-1 in long-lived latent reservoirs during suppressive antiretroviral therapy (ART) remains one of the principal barriers to a functional cure [1]. Blocks to transcriptional elongation play a central role in maintaining the latent state and several latency reversal strategies focus on the release of positive transcription elongation factor (P-TEFb) from sequestration by negative regulatory complexes, such as the 7SK complex and BRD4 [2]. Another major cellular reservoir of P-TEFb is in Super Elongation Complexes (SECs), which play broad regulatory roles in host gene expression, but it is unknown if the release of P-TEFb from SECs is a viable latency reversal strategy.

Here, we demonstrate that the SEC is not required for HIV-1 replication in primary CD4+ T cells and that a small molecular inhibitor of the interaction between P-TEFb and the SEC (termed KL-2) increases viral transcription [3]. KL-2 acts synergistically with several latency reversing agents (LRAs) to reactivate viral transcription in several cell line models of latency in a manner that is, at least in part, dependent on the viral Tat protein. Finally, we demonstrate that KL-2 enhances viral reactivation in peripheral blood mononuclear cells (PBMCs) from people living with HIV on suppressive ART, most notably in combination with non-canonial NFkB agonists. Taken together, these results suggest that the release of P-TEFb from cellular SECs may be a novel route for HIV-1 latency reactivation [4].

Knock-out of Super Elongation Complex members does not decrease HIV-1 infection in primary CD4+ T cells

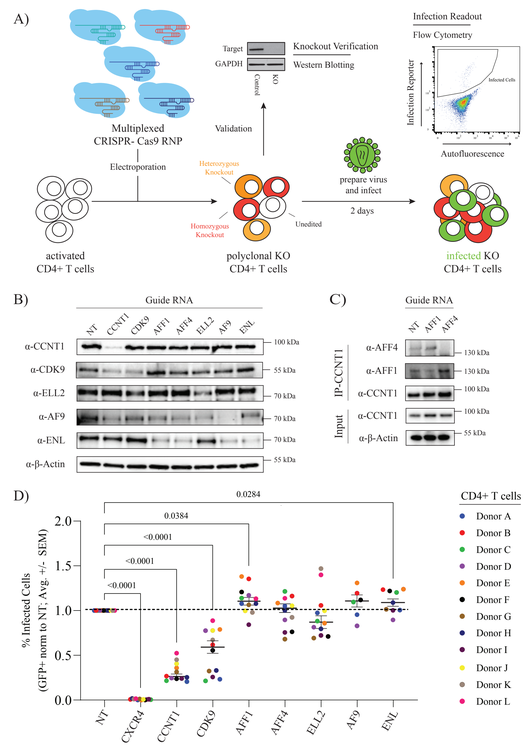


Figure 1 | Knock-out of Super Elongation Complex members does not inhibit HIV-1 infection of primary CD4+ T cells. A) Experimental schematic of multiplex CRISPR-Cas9 gene editing in primary CD4+ T cells. **B)** Immunoblots to assess CCNT1, CDK9, ELL2, AF9, and ENL knock-out efficiency in primary CD4+ T cells relative to a non-targeting (NT) control (1 donor). **C)** Immunoblots to assess AFF1 and AFF4 knock-out efficiency in primary CD4+ T cells relative to a NT control following immunoprecipitation of CCNT1 (1 donor). **D)** Percent infected (GFP+) primary CD4+ T cells (normalized to the donor-matched NT control) 48 hours after challenge with HIV-1 NL4-3 Nef-IRES-GFP. Each dot represents the average of technical triplicates; the black line represents the mean of means ± standard error of means. n = 12 donors for NT, CXCR4, CCNT1, CDK9, AFF1, AFF4, and ELL2; n=6 donors for AF9; and n=9 donors for ENL. Statistics were calculated by 2-way ANOVA with Dunnett's Multiple Comparison Test.

Super Elongation Complex disruptor KL-2 increases HIV-1 infection in primary CD4+ T cells

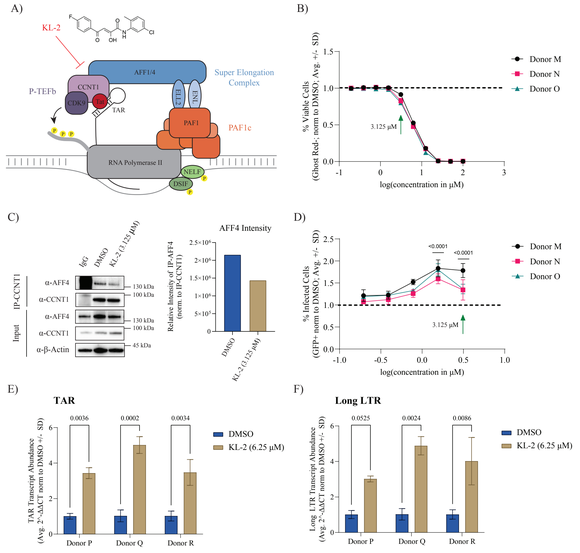


Figure 2 | Super Elongation Complex disruptor KL-2 increases HIV-1 infection in primary CD4+ T cells. A) Model of Tat-mediated recruitment of P-TEFb to paused RNA Pol II at sites of nascent HIV-1 transcription. **B)** Percent viable primary human CD4+ T cells (normalized to the donor-matched DMSO control) after 72 hours of treatment with increasing concentrations of KL-2 as measured by amine dye staining and flow cytometry. Data represent the average ± SD of technical triplicates; the green arrow indicates the concentration chosen for downstream experiments. **C)** Immunoblots for AFF4 and CCNT1 following CCNT1 immunoprecipitation from primary CD4+ T cells treated for 24 hours with either DMSO or 3.125 μM KL-2 (1 donor). Quantification is shown on the right. **D)** Percent infected (GFP+) primary CD4+ T cells (normalized to the donor-matched DMSO control) 48 hours after challenge with HIV-1 NL4-3 Nef-IRES-GFP in the presence of increasing concentrations of KL-2 (24 hours pre-treatment prior to challenge). Data represent the average ± SD of technical triplicates (n = 3 donors); statistics were calculated relative to the DMSO control by two-way ANOVA and Sidak's Multiple Comparison test. **E)** Relative transcript levels of HIV-1 TAR and **F)** long LTR to human b-Actin in activated primary CD4+ T cells after 48 hours challenge with HIV-1 in the presence or absence of a 24 hour pretreatment with KL-2. Data represent the mean of means of 3 biological replicates in technical duplicate ± standard error; statistics calculated by two-way ANOVA with Sidak's Multiple Comparison test.

KL-2 enhances latency reversing agent activity in J-Lat cells

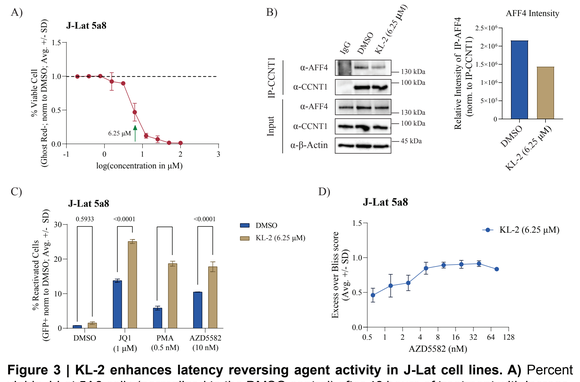


Figure 3 | KL-2 enhances latency reversing agent activity in J-Lat cell lines. A) Percent viable J-Lat 5A8 cells (normalized to the DMSO control) after 48 hours of treatment with increasing concentrations of KL-2 as measured by amine dye staining and flow cytometry. Data represent the average ± SD of technical triplicates; the green arrow indicates the concentration chosen for downstream experiments. **B)** Immunoblots for AFF4 and CCNT1 following CCNT1 immunoprecipitation from J-Lat 5A8 cells treated for 24 hours with either DMSO or 6.25 μM KL-2. Quantification is shown on the right. **C)** Percent reactivated (GFP+) J-Lat 5A8 cells (normalized to the DMSO control) after 48 hours of treatment with the indicated compounds in the presence or absence of KL-2. Data represent the average ± SD of technical triplicates; statistics were calculated by two-way ANOVA with Sidak's Multiple Comparison test. D) Excess over Bliss scores for latency reactivation in J-Lat 5a8 cells treated with combinations of KL-2 and AZD5582 in biological duplicate with the mean excess over Bliss score +/- SD between replicates shown.

KL-2 enhances enhances transcriptional elongation across multiple J-Lat lines

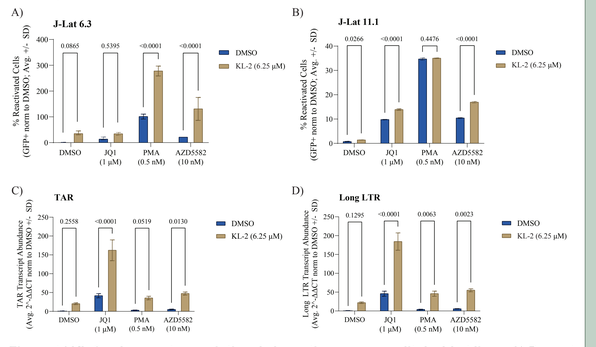


Figure 4 | KL-2 enhances transcriptional elongation across mulitple J-Lat lines. A) Percent reactivated (GFP+) J-Lat 6.3 cells or **B)** J-Lat 11.1 cells (normalized to the DMSO control) after 48 hours of treatment with the indicated compounds in the presence or absence of KL-2. Data represent the average ± SD of technical triplicates; statistics were calculated by two-way ANOVA with Sidak's Multiple Comparison test. **C)** Relative transcript levels of HIV-1 TAR and **D)** long LTR to human b-Actin in J-Lat 5A8 cells after 48 hours of treatment with the indicated compounds in the presence or absence of KL-2 (normalized to the DMSO control). Data represent the mean of means of 3 biological replicates in technical duplicate ± standard error; statistics were calculated by two-way ANOVA with Sidak's Multiple Comparison test.

KL-2 latency reversing activity is Tat- and cell line-dependent

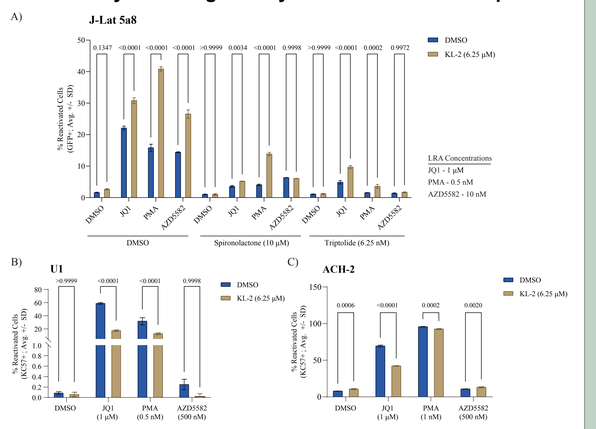


Figure 5 | KL-2 latency reversing activity is Tat- and cell line-dependent. A) Percent reactivated (GFP+) J-Lat 5a8 cells after 48 hours of treatment with indicated compounds in the presence or absence of KL-2 and the Tat inhibitors Spironolactone and Triptolide. **B)** Percent reactivated (KC57-FITC+) Tat-deficient U1 cells after 48 hours of treatment with indicated compounds in the presence or absence of KL-2. **C)** Percent reactivated (KC57-FITC+) TAR-deficient ACH-2 cells after 48 hours of treatment with indicated compounds in the presence or absence of KL-2. For all panels, the data represent the average ± SD of technical triplicates; statistics were calculated by two-way ANOVA with Sidak's Multiple Comparison test.

KL-2 enhances latency reversing agent activity in PBMCs from virally suppressed people living with HIV

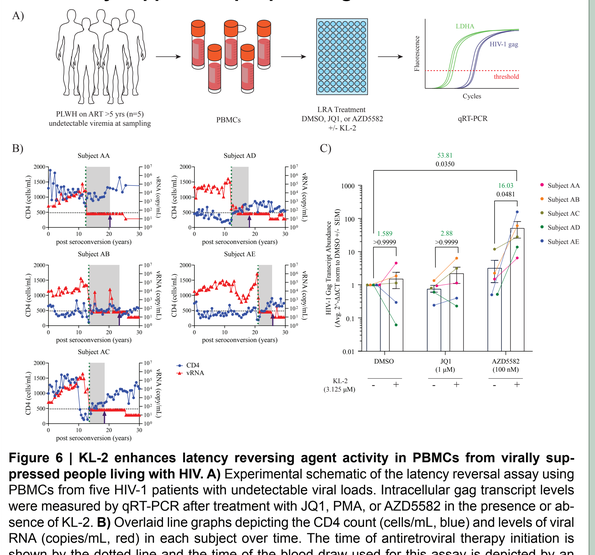
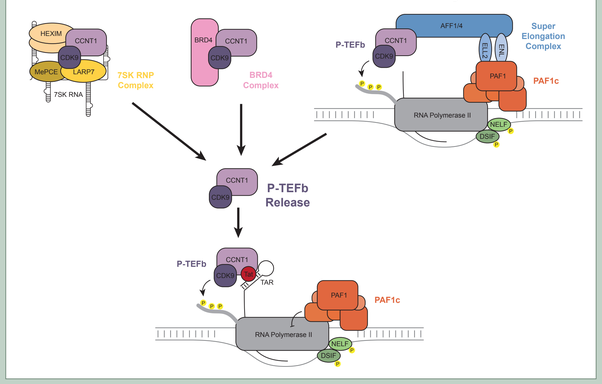


Figure 6 | KL-2 enhances latency reversing agent activity in PBMCs from virally suppressed people living with HIV. A) Experimental schematic of the latency reversal assay using PBMCs from five HIV-1 patients with undetectable viral loads. Intracellular gag transcript levels were measured by qRT-PCR after treatment with JQ1, PMA, or AZD5582 in the presence or absence of KL-2. **B)** Overlaid line graphs depicting the CD4 count (cells/mL, blue) and levels of viral RNA (copies/mL, red) in each subject over time. The time of antiretroviral therapy initiation is shown by the dotted line and the time of the blood draw used for this assay is depicted by an arrow. The gray boxes represent the cumulative time on antiretroviral therapy prior to sampling. **C)** Relative transcript levels of HIV-1 gag relative to human LDHA in patient PBMCs (n = 5 donors) after 48 hours of treatment with the indicated compounds in the presence or absence of KL-2. Data represent the average $2^{-\Delta\Delta Ct}$ [(Ct_{gag}-Ct_{LDHA})LRAs - (Ct_{gag}-Ct_{LDHA})DMSO] ± SEM of technical triplicates. Statistics were calculated by two-way ANOVA with Sidak's Multiple Comparison Test. The fold change in mean $2^{-\Delta\Delta Ct}$ is shown above for relevant comparisons in green text with corresponding p-values shown below in black text. The Institutional Review Board of Northwestern University approved the study (STU00022906-CR0008).

Mechanisms of P-TEFb release for HIV-1 latency reversal



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CONCLUSIONS

In this study, we demonstrate a potential new strategy for the enhancement of HIV-1 latency reversal through the release of P-TEFb from the cellular pool of Super Elongation Complexes.

1) HIV-1 does not rely on the SEC for transcription in activated, primary CD4+ T cells.

2) KL-2, a small molecule inhibitor of the interaction between the SEC and P-TEFb, is sufficient to enhance viral transcription and synergistically enhances the activity of other LRAs in cell line models of latency.

3) KL-2 increases HIV-1 *gag* expression in PBMCs from PLWH on suppressive ART, most notably in combination with the non-canonical NFkB agonist, AZD5582.