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GENOME-WIDE CRISPR INHIBITION SCREEN IDENTIFIES A NEW HIV-1 SILENCING FACTOR SLTM

Rachel N. Kim, Savannah F. Pedersen, Jack A. Collora, Ya-Chi Ho
Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, Connecticut

BACKGROUND

HIV-1 persistence in latent reservoirs remains a major obstacle to cure. We postulate that HIV-1 silencing factors suppress HIV-1 reactivation, and inhibition of HIV-1 silencing factors will increase HIV-1 reactivation and guide drug designs.

METHODS/RESULTS

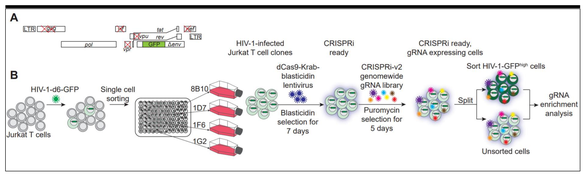

Figure 1. Experimental scheme of genome-wide CRISPRi screen. (A) The HIV-1-d6-GFP reporter virus used. **(B)** Scheme of the CRISPRi screen preformed. The sgRNA library targets 5 sgRNAs/gene for 18,905 genes.

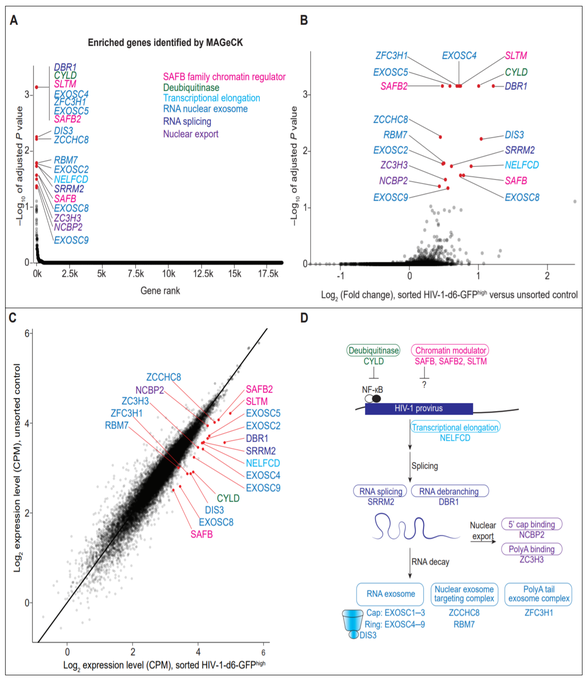

Figure 2. CRISPRi screen identifies 18 HIV-1 silencing factors. (A) Rank of sgRNAs enriched in the GFP^{high} population identified by MAGeCK. Red dots indicate 18 sgRNAs with adjusted P values <0.05. **(B)** Significance (adjusted P values) versus level of enrichment (log2 fold change) of sgRNAs identified by MAGeCK. **(C)** Expression levels for sgRNA enrichment in sorted GFP^{high} cells versus unsorted control. **(D)** Functional roles of the 18 HIV-1 silencing factors.

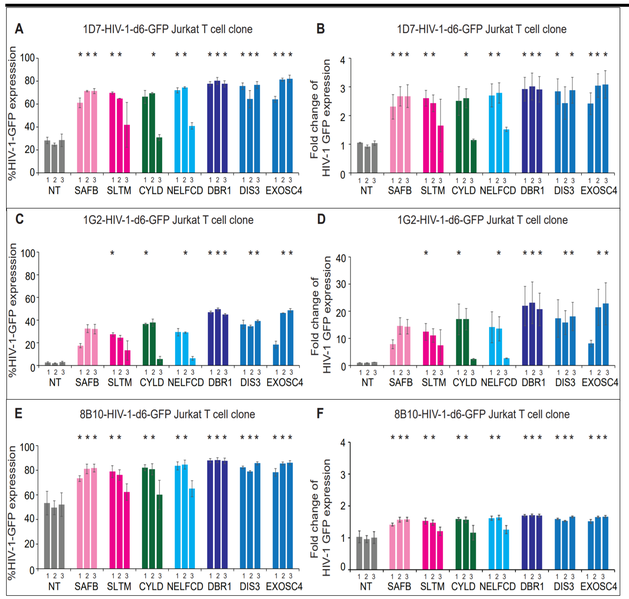

Figure 3. Knocking down HIV-1 silencing factors increases HIV-1 expression. HIV-1-driven GFP expression **(A, C, E)** and fold change compared with NT sgRNA transduced controls **(B, D, F)** in three different HIV-1-d6-GFP-Jurkat T cell clones. Bar graphs show mean ± SEM. P values calculated by two-tailed Student's t-test using data from two independent experiments. *, P < 0.05.

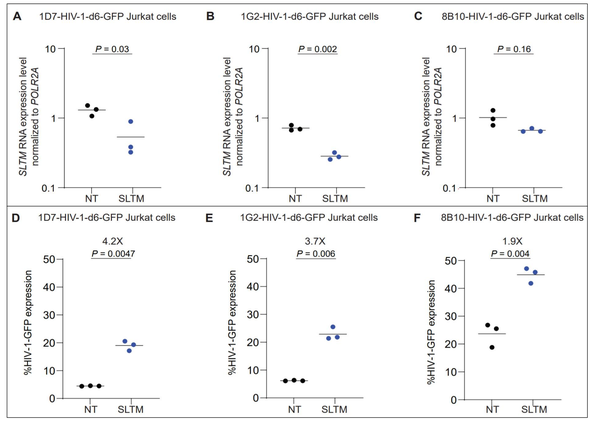

Figure 4. SLTM suppression increases HIV-1 expression in HIV-1-d6-GFP Jurkat T cell clones enriched using antibiotic selection. (A-C) *SLTM* RNA expression levels normalized to the housekeeping gene *POLR2A*, as measured by qRT-PCR. **(D-F)** HIV-1-driven GFP expression levels in HIV-1-d6-GFP Jurkat T cell clones. P values calculated by two-tailed Student's t-test.

METHODS/RESULTS

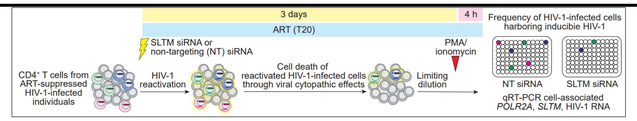

Figure 5. Experimental scheme for siRNA knockdown of HIV-1 silencing factors ex vivo.

Table 1. Clinical demographics of study participants

Participant	Age	Sex	Race	ART	Duration of viral suppression (months)	CD4 count (/µl)	Viral load (copies/µl)
5004	46	M	White	ABC/DTG/3TC	100	737	<20
5005	41	M	AA	TDF, ABC/DTG/3TC	47	498	<20
5006	40	M	AA	EVG/c/FTC/TAF	187	784	<20
5007	63	M	AA	BIC/FTC/TAF	40	552	<20
5008	35	M	AA	BIC/FTC/TAF	41	797	<20
5009	62	M	AA	BIC/FTC/TAF	68	583	<20
5010	53	M	AA	BIC/FTC/TAF	41	569	<20
5011	43	M	AA	DTG/3TC	48	667	<20
5012	33	M	AA	EVG/c/FTC/TAF	73	1,322	<20
5013	55	M	AA	BIC/FTC/TAF	48	828	<20

*AA African American; 3TC, lamivudine; ABC, abacavir; BIC, bictegravir; /c, cobicistat; DTG, dolutegravir; EVG, elvitegravir; FTC, emtricitabine; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil

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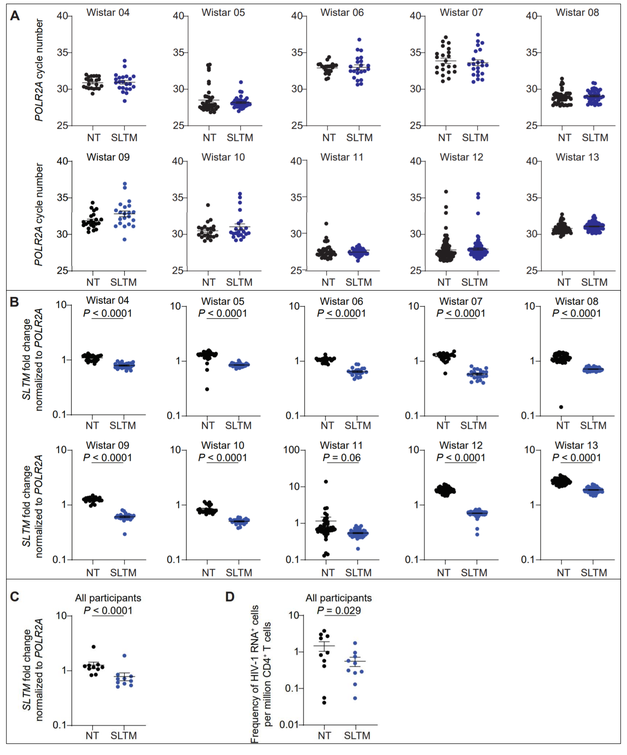

Figure 6. SLTM knockdown reduces frequency of inducible HIV-1-infected cells ex vivo. (A) Cell viability estimated by the expression level of housekeeping gene *POLR2A* by qRT-PCR. **(B-C)** The SLTM knockdown measured by *SLTM* RNA expression level normalized to *POLR2A*. **(D)** Cell-associated HIV-1 RNA expression measured by polyadenylated HIV-1 qRT-PCR. Frequency of inducible HIV-1-infected cells calculated by IUPM calculator. P values calculated by two-tailed Student's t-test.

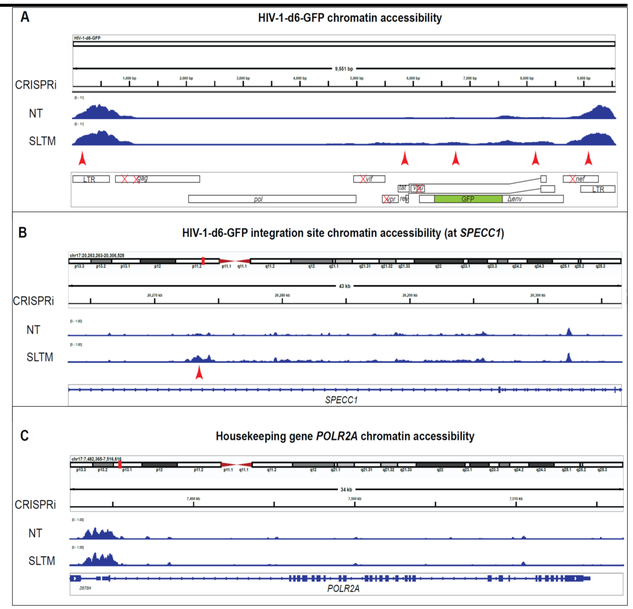

Figure 7. Knocking down SLTM increases chromatin accessibility at HIV-1 provirus. The 1D7-HIV-1-d6-GFP cell line clone was first transduced with CRISPRi lentiviruses, then NT or SLTM sgRNA, and enriched by antibiotic selection. HIV-1-d6-GFP integrates into host gene *SPECC1*. Chromatin accessibility measured by ATAC-seq. **(A)** Compared with the NT control, CRISPRi-mediated SLTM knockdown increases chromatin accessibility in HIV-1-d6-GFP, mainly at the 3' genome and the U3 region of HIV-1 LTR (red arrowheads). **(B)** CRISPRi-mediated SLTM knockdown increases chromatin accessibility of SPECC1 at HIV-1 integration site. **(C)** CRISPRi-mediated SLTM knockdown does not affect chromatin accessibility in genes outside of HIV-1 integration site, like the housekeeping gene *POLR2A*.

CONCLUSIONS

The genome-wide CRISPRi screen identified 18 HIV-1 silencing factors. Combined with validation in HIV-1-infected T cell clones containing HIV-1 in different integration sites, we identified the epigenetic and transcriptional regulator SLTM as a novel HIV-1 silencing factor. Overall, our study indicates that SLTM inhibition reactivates HIV-1 in vitro and induces cell death of HIV-1-infected cells ex vivo.

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