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CRISPR-mediated activation of latent HIV-1 expression

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ABSTRACT

Complete eradication of HIV-1 infection is impeded by the existence of cells that harbor chromosomally integrated but transcriptionally inactive provirus. These cells can persist for years without producing viral progeny, rendering them refractory to immune surveillance and antiretroviral therapy and providing a permanent reservoir for the stochastic reactivation and reseeding of HIV-1. Strategies for purging this latent reservoir are thus needed to eradicate infection. Here we show that engineered transcriptional activation systems based on CRISPR/Cas9 can be harnessed to activate viral gene expression in cell line models of HIV-1 latency. We further demonstrate that complementing Cas9 activators with latency-reversing compounds can enhance latent HIV-1 transcription, and that epigenome modulation using CRISPR-based acetyltransferases can also promote viral gene activation. Collectively, these results demonstrate that CRISPR systems are potentially effective tools for inducing latent HIV-1 expression and that their use, in combination with antiretroviral therapy, could lead to improved therapies for HIV-1 infection.

INTRODUCTION

Since its identification in 1983, HIV has remained a global pandemic. An estimated 35 million people worldwide are currently living with HIV type 1 (HIV-1), and an estimated 39 million people have died of AIDS-related illness since the onset of the epidemic (see UNAIDS report: http://unaids.org/globalreport/Global_report.htm). Strict adherence to combination antiretroviral therapy (cART) has significantly improved the quality of life and life expectancy of many infected individuals, transforming HIV-1 into a chronic, manageable illness in the industrialized world. However, despite its durable capacity to inhibit viral replication, cART does not cure HIV-1 infection since it cannot address the residual transcriptionally silent but replication-competent population of virus hidden within certain cells[1-3]. This so-called latent reservoir is primarily established when infected $CD4^+$ T cells revert to a resting memory state[4,5]. The resulting cells are largely non-permissive for viral gene expression and therefore do not generate new viral progeny[5,6]. Because latent HIV-1 is refractory to both immunological surveillance and cART intervention, its existence poses a major obstacle to complete viral eradication[7-9].

Though the mechanisms underlying latency are complex[10-13], several strategies have been developed to activate this HIV-1 reservoir[12], thereby rendering it susceptible to cART eradication. Because latent virus responds to T cell activation signals, proinflammatory cytokines – such as interleukin 2 (IL-2)[14] and IL-7[15] – can be used to induce its emergence from resting $CD4^+$ T cells. Protein kinase C (PKC) agonists that stimulate viral gene expression via NF-κB signaling[16] – including bryostatin[17], prostratin[18,19] and other synthetic analogs[20,21] – are also candidates for this strategy. Additionally, because histone deacetylation can induce repressive changes in chromatin structure at the HIV-1 promoter, histone deacetylase (HDAC) inhibitors have emerged as a promising approach for reversing latency[22,23].

Cytokine therapy, however, has been unable to completely purge latent virus[24,25] and has even led to toxicity[26] and/or long-term depletion of $CD4^+$ T cells[27]. Additionally, PKC agonists may be unable to activate latent HIV-1 in repressive chromatin, and to date several HDAC inhibitors have demonstrated

sub-optimal results in clinical trails[28,29]. The use of high levels of HDAC inhibitors may also affect global gene expression[30], potentially leading to unwanted side effects[31]. These limitations underscore the need for new strategies capable of stimulating latent HIV-1 expression in a robust and targeted manner.

The emergence of versatile genome modulation and editing technologies[32,33] – such as those based on zinc-finger domains[34], TAL effector proteins[35], and the RNA-guided CRISPR/Cas9 system[36] – can offer new means to combat HIV-1 infection. For example, the relative ease with which these tools can be configured, as well as their broad versatility, has endowed investigators with the ability to confer HIV-1 resistance upon primary cells[37] and stem cells[38-40] via modification of the *CCR5* gene. Indeed, this approach recently showed evidence of efficacy in a Phase I clinical trial[41]. Other strategies facilitated by this technology include the disruption[42] and excision[43-45] of proviral DNA from HIV-infected cells, the disruption of endogenous host factors critical for HIV-1 integration[46,47], and the attenuation of HIV-1 replication via transcriptional repression[48,49]. Among the three major DNA-targeting systems, CRISPR/Cas9 can readily be directed to nearly any genomic locus via RNA-DNA complementary base pairing using a chimeric single guide RNA (sgRNA)[50]. Though this system is typically used for inducing DNA cleavage[50-52], it can be co-opted for transcriptional modulation by fusing a catalytically inactivated variant of the Cas9 nuclease (referred to as dCas9)[53] with a transcriptional activator[54-56] or repressor[57] domain. Because the only major restriction for CRISPR/Cas9 target site selection is the protospacer adjacent motif (PAM)[50], which is recognized by the Cas9 protein and located immediately downstream of the sgRNA target site, CRISPR transcription activation systems[54,55,58-62] have the potential for driving the expression of nearly any gene, including stimulating viral gene expression within latent HIV-1 reservoirs for shock-and-kill treatments. Importantly, unlike approaches that rely on the Cas9 nuclease to disrupt HIV-1 provirus, CRISPR-based activators pose no significant risk of introducing mutations within the host genome[63] and have the added advantage of providing potentially reversible activity.

Here we demonstrate that CRISPR-based transcription activators can be configured to induce latent HIV-1 expression, and that combining these CRISPR tools with latency-reversing compounds can lead to robust activation of viral gene expression in cell-based models of HIV-1 latency.

RESULTS

CRISPR/Cas9 transcription activation systems induce gene activation from the HIV-1 LTR

We have investigated whether CRISPR-based transcription factors can reverse latency by stimulating gene expression from the HIV-1 long terminal repeat (LTR) promoter[64]. The HIV-1 subtype B LTR is 634 bp in length and can be subdivided into three regions **(Fig. 1a and Supplementary Fig. 1)**: a 454-nucleotide (nt) segment upstream of the transcriptional start site (TSS) termed U3, which includes binding sites for host transcription factors that drive HIV-1 gene expression as well other *cis*-acting DNA elements; a 96-nt repeat region downstream of the TSS referred to as R, which encodes the trans-activation response (TAR) element; and an 84-nt segment downstream of the R domain designated U5.

We designed seven sgRNA to overlap with key features of the LTR **(Fig. 1a,b)**. The U3 region was targeted by sgRNAs 1-5. sgRNAs 4 and 5, in particular, were designed to overlap with the binding sites for NF-κB and Sp-1, respectively, since these transcription factors contribute directly to viral gene expression and latency[65-69]. The R region, which encodes a repressive mRNA hairpin structure (TAR) that inhibits RNA pol II processivity[70,71] in the absence of the viral Tat protein, was targeted by sgRNA 6. Finally, the U5 region was targeted by sgRNA 7.

We initially evaluated the ability of two distinct CRISPR complexes to induce gene activation: (i) dCas9-VP64[54,55] and (ii) the synergistic activation mediator (SAM) complex[61]. dCas9-VP64 comprises dCas9 fused with a tetrameric repeat of the herpes simplex virus VP16 transactivation domain (VP64)[72] **(Fig. 1c)**. The SAM system similarly contains dCas9-VP64, but also: (a) a modified sgRNA harboring an aptamer that binds to the MS2 bacteriophage coat protein and (b) a tripartite fusion protein

consisting of the MS2 protein, the NF-κB trans-activating subunit p65, and the activation domain from the human heat-shock factor 1 protein (HSF1) (MS2–p65–HSF1) **(Fig. 1c)**. Both complexes function by recruiting cellular transcription factors[73] and chromatin-remodeling proteins[73,74] to targeted genomic loci, leading to transcriptional activation.

To assess the ability of each Cas9-based complex to stimulate gene expression, we co-transfected human embryonic kidney (HEK) 293T cells with either dCas9-VP64 or SAM, as well as a reporter plasmid containing the full-length HIV-1 subtype B promoter upstream of an EGFP reporter gene **(Fig. 1a)**. Both dCas9 complexes were tested with each of the seven individual sgRNAs, and the level of Cas9-mediated gene activation was directly correlated with EGFP fluorescence. We observed a minimal increase in EGFP expression in cells transfected with dCas9-VP64, with only sgRNA 3 yielding a significant increase ($p < 0.05$) in the number of EGFP positive cells **(Fig. 1d)**. Surprisingly, "tiling" the LTR promoter with multiple sgRNAs, which has been shown to lead to increased activation of endogenous genes in HEK293T cells[54,55], had minimal effect on EGFP expression in this transient reporter system **(Supplementary Fig. 2)**.

In contrast to the dCas9-VP64 complex, HEK293T cells co-transfected with reporter plasmid and the SAM system, showed a 12- to 24-fold increase in the number or percentage of EGFP positive cells, with the most robust levels of activation observed using sgRNAs 1, 2, and 3 **(Fig. 1d)**. As previously reported[61], no substantial increase in SAM-mediated activation was observed by tiling the promoter with multiple sgRNAs **(Supplementary Fig. 3)**. As a control for target specificity, we evaluated EGFP expression in cells co-transfected with reporter plasmid and SAM encoding a random sgRNA library. No significant increase in EGFP was observed in transfected cells **(Supplementary Fig. 4)**, indicating that SAM induced specific activation from the HIV-1 LTR promoter.

Reactivation of latent HIV expression by CRISPR activator complexes

We next evaluated whether SAM could reactivate viral gene expression in established cell line models of HIV-1 latency. Specifically, we sequentially infected the Jurkat-derived lymphocytic cell lines J-Lat 9.2 and J-Lat 10.6[75] with lentiviruses encoding each SAM element **(Fig. 2a)**. J-Lat cells harbor an integrated but transcriptionally silent HIV-1 provirus that expresses GFP in lieu of the *nef* and *env* genes but still recapitulates the natural transcriptional complexity of the HIV promoter, thereby mimicking HIV latency. We specifically chose the J-Lat 9.2 and 10.6 cell lines since they display distinct gene activation thresholds arising from differences in chromatin accessibility at the HIV-1 LTR[76]. Specifically, J-Lat 9.2 is a strongly repressed clone that requires high transcriptional induction to overcome its chromatin environment, whereas J-Lat 10.6 cells possess a lower activation threshold.

We observed GFP expression in ~30% and ~5% of J-Lat 9.2 cells stably expressing SAM with sgRNAs 4 and 6, respectively **(Fig. 2b)**. Additionally, we observed reactivation in up to 85% of J-Lat 10.6 cells expressing SAM with sgRNA 6 **(Fig. 2c)**. As an efficient positive induction control, we used tumor necrosis factor alpha (TNF-α), a proinflammatory cytokine that stimulates HIV expression through activation of NF-κB, but whose *in vivo* toxicity[77] precludes its use as a therapeutic. TNF-α treatment yielded ~18% and ~82% GFP positive J-Lat 9.2 and 10.6 cells, respectively **(Supplementary Fig. 5)**. Compared to an empty sgRNA cassette, each sgRNA we tested induced an increase ($p < 0.05$) in the number of GFP positive J-Lat 10.6 cells **(Fig. 2c)**. However, only two sgRNA (4 and 6) induced a significant increase ($p < 0.05$ for both) in the number of GFP positive cells in the more repressed J-Lat 9.2 cell line **(Fig. 2b)**. Taken together, these data indicate that CRISPR/Cas9-based transcription activation systems delivered via lentivirus can stimulate latent HIV gene expression, but that differences in chromatin accessibility at the HIV-1 LTR can affect the ability of Cas9 to stimulate transcription.

Combining CRISPR activators with an HDAC inhibitor and prostratin synergistically increases latent HIV activation

HDAC inhibitors, such as valproic acid[78] or suberoylanilide hydroxamic acid (i.e., SAHA or Vorinostat),[29] are capable of partially reversing the effects of chromatin silencing and alleviating HIV latency. We hypothesized that such compounds could also enhance the ability of Cas9 to induce HIV-1 expression, particularly in repressive chromatin contexts. To this end, we treated J-Lat 9.2 and 10.6 cells stably expressing SAM with increasing doses of SAHA **(Fig. 3a)**, a compound that inhibits the Class I HDAC isotypes 1, 2, 3, and 8. SAHA was recently shown to be safe and well tolerated in a Phase I clinical trial, though it was unable to provide a long-term increase in HIV-1 expression in patients[28].

Incubation of SAM-expressing J-Lat cells with SAHA led to a robust and dose-dependent increase in the number of GFP positive cells **(Fig. 3b)**. Specifically, reactivation was evident in ~70% of J-Lat 9.2 cells with sgRNA 4, and ~80% of J-Lat 10.6 cells with sgRNAs 3, 5, 6, and 7 after treatment with 4 μM SAHA, substantially higher than control cells expressing an empty sgRNA expression cassette **(Fig. 3b)**. Depending on the sgRNA used, high-doses of SAHA led to a 2- to 5-fold increase in activation in J-Lat 9.2 cells **(Fig. 3b)**, indicating that CRISPR-based transcriptional modulators and HDAC inhibitors can act synergistically to reactivate HIV-1.

We next investigated whether the use of multiple latency reversing compounds in combination with Cas9 could further increase HIV-1 expression. We treated J-Lat 9.2 and 10.6 cells expressing SAM with escalating doses of SAHA and 2 μM prostratin **(Fig. 3a)**. The latter small molecule stimulates IKK-dependent phosphorylation and degradation of Iκ-Bα[79], leading to the rapid nuclear translocation of NF-κB and activation of latent provirus[18,19]. Depending on the sgRNA used, SAM-expressing J-Lat 9.2 cells co-treated with SAHA plus prostratin showed a 2- to 3-fold increase in the number of GFP positive cells compared to those treated with SAHA only **(Fig. 3c)**. Notably, sgRNAs 4 and 6 stimulated GFP expression in up to 80% of J-Lat 9.2 cells treated with SAHA and prostratin **(Fig. 3c)**. J-Lat cells

expressing SAM and treated with prostratin in the absence of SAHA, however, showed less activation than those treated with both compounds **(Supplementary Fig. 6)**.

Interestingly, J-Lat 10.6 cells expressing SAM with sgRNA 4 – whose target site overlaps with the binding sites for NF-κB – showed a marked decrease in GFP positive cells after treatment with prostratin **(Fig. 3c)**, indicating the possibility that SAM and NF-κB may be competing for LTR binding sites, and that this interplay led to suboptimal viral gene expression. Collectively, these results indicate that HIV latency reversing compounds can enhance the efficacy of Cas9-mediated activation of HIV-1, particularly in repressive chromatin contexts.

Reactivation of latent HIV by epigenome editing using CRISPR/Cas9-based acetyltransferases

Multiple mechanisms contribute to HIV latency, including repressive local chromatin effects stemming from histone deacetylation of the nucleosomes (nuc-0 and nuc-1) that form within the LTR[80]. While HDAC inhibitors such as SAHA can stimulate latent HIV expression by promoting an open chromatin environment around the provirus promoter, their activity can also influence the expression of host genes. In contrast, emerging epigenome-modifying technologies[81] have the potential to directly alter the chromatin structure of the LTR and thereby affect target gene expression in a site-specific manner. For instance, fusion of dCas9 with the catalytic histone acetyltransferase core domain of the human E1A-associated protein p300 (dCas9-p300) was recently shown to facilitate transcriptional activation via targeted acetylation of histone H3 in the promoter region of targeted genes **(Fig. 4a)**[82]. We thus hypothesized that dCas9-p300 could also reactivate latent HIV-1 expression by acetylation of nuc-0 or nuc-1. To examine this possibility, we evaluated GFP expression in J-Lat 10.6 cells nucleofected with expression vectors encoding dCas9-p300 and the aforementioned LTR-targeted sgRNAs. In particular, sgRNAs 1, 2, and 3 mediate binding near nuc-0, whereas the target sites for sgRNAs 6 and 7 overlap with nuc-1 **(Fig. 1a)**. dCas9-p300 introduced into cells by nucleofection induced significant gene expression when targeted by sgRNAs 2, 4, 5, and 6 ($p < 0.05$ for each) **(Fig. 4b)**, achieving levels of

activation comparable to each corresponding SAM complex (which were ~2-fold lower than by lentiviral-mediated expression, see **Fig. 2**). In the case of sgRNA 2, whose target site overlaps with nuc-0, dCas9-p300 induced a significant increase ($p < 0.05$) in HIV-1 expression over SAM **(Fig. 4b)**. Likely due to their more repressive transcriptional environment, no significant increase in activation was observed when J-Lat 9.2 cells were nucleofected with dCas9-p300 (data not shown). However, these findings collectively indicate that dCas9-p300 offers strong potential to stimulate transcription from the HIV-1 LTR.

DISCUSSION

The presence of residual latent but replication-competent HIV-1 reservoirs is a major hurdle impeding viral eradication. Recent work has indicated that HIV expression can be induced using zinc-finger[83] and TAL effector-based[84] transcription factors engineered to bind the LTR promoter. Here we show that CRISPR/Cas9-based transcriptional effectors – which can be retargeted to nearly any DNA sequence without protein engineering – can reactivate viral gene expression in cell line models of HIV-1 latency. Using LTR-targeted sgRNAs, we tested the ability of two distinct Cas9 complexes to stimulate transcription: dCas9-VP64[54,55] and the synergistic activation mediator (SAM) complex[61], which consists of dCas9-VP64 and an accessory transactivation domain designed to recruit a complementary suite of transcription and chromatin remodeling factors. We found that SAM activated gene expression from the LTR in transient reporter assays at levels that exceeded dCas9-VP64. This result is consistent with recent data indicating that, for some genes, first-generation dCas9-based transcription factors induce only modest levels of transcription[53-57].

While all seven LTR-targeted sgRNAs induced robust expression from the full-length LTR promoter in the context of transient reporter assays, they demonstrated variable activity when challenged with proviral DNA. Only sgRNAs 4 and 6, whose target sites overlap with the binding sites for NF-κB and the TAR element, induced significant changes in expression in J-Lat 9.2, with the latter possibly

through a mechanism that enhances transcriptional elongation. Despite the use of identical sgRNAs, different levels of Cas9-mediated activation were also observed between these cell lines (especially with sgRNA 4), suggesting that epigenetic and/or genetic[85,86] factors can influence the ability of Cas9 to stimulate transcription. However, when combined with latency reversing compounds, such as SAHA and prostratin, SAM-induced HIV-1 transcription at levels that exceeded those observed using TNF-α simulation, particularly in J-Lat 9.2 cells. Interestingly, we observed reduced transcription in J-Lat 10.6 cells expressing SAM with sgRNA 4 and treated with prostratin. Possible explanations for this include competition for LTR binding sites between Cas9 and prostratin-activated NF-κB, or an unforeseen inhibitory effect between endogenous NF-κB protein and the transactivation domains present in the SAM complex.

The SAM system used here[61] contains both the activation domain of the HSF1 protein and the NF-κB trans-activating subunit p65, which can stimulate transcriptional elongation during proviral activation[87]. This notwithstanding, we observed no increase in HIV activation in J-Lat cells expressing dCas9-VP64, MS2-p65-HSF1, and an empty sgRNA cassette, indicating that HIV-1 expression was due to sgRNA targeting to the LTR. The modularity of the tripartite MS2–p65–HSF1 fusion protein could be exploited in the future to create a Cas9 activator complex specifically tailored for $CD4^+$ T cells or other cell types relevant to HIV latency.

We also show that epigenome editing using a Cas9 acetyltransferase (dCas9-p300)[82] can lead to a significant increase in GFP expression in J-Lat 10.6 cells. dCas9-p300-induced changes in transcription similar to SAM for multiple sgRNAs including sgRNA 2, which overlaps with a nucleosome (nuc-0) that contributes to proviral silencing. Because SAM and dCas9-p300 function through complementary mechanisms, emerging Cas9 orthologs[88] could be co-opted and used in tandem for multiplexed induction of viral gene expression via epigenome editing and promoter-directed transcriptional activation. Future studies are necessary to determine the effectiveness of these technologies in primary cell models of HIV-1 latency, as well as the DNA-binding specificity of each

Cas9 activator[89] and the exact epigenetic modifications induced by dCas9-p300. In addition, successful clinical translation of this approach necessitates the safe and effective delivery of CRISPR transcriptional activators into cells that harbor latent HIV-1. Neither SAM nor dCas9-p300 (~7.5 kb and ~6.1 kb in length, respectively) can be packaged into a single adeno-associated virus vector. As an alternative, non-integrated lentiviruses could be used to facilitate their delivery *ex vivo*, as these vectors have enabled zinc-finger nuclease-mediated modification of the *CCR5* gene in patient-derived resting $CD4^+$ T cells.[90] *In vivo* delivery of Cas9-sgRNA could prove challenging, however. The use of dCas9-VP64 ribonucleoprotein[91,92] conjugates engineered to specifically recognize[93], and subsequently be internalized by $CD4^+$ T cells, or emerging lentiviral vectors capable of infecting human lymphocytes upon systemic delivery[94] could help to overcome this limitation.

Finally, as with RNA interference-based gene therapies for HIV-1[95,96], CRISPR-mediated transcriptional activation of HIV-1 could be vulnerable to viral escape, as recent work has indicated that even the latent reservoir can become populated with escape mutants[97]. However, due to the ease with which sgRNA can be designed, CRISPR-resistant strains of HIV-1 could be addressed through the use of new sgRNA tailored for specific escape mutants. These sgRNA could conceivably be introduced into cells already expressing a dCas9 variant via an aptamer-based bridge design similar to those previously described for combating HIV-1 resistance using RNAi[98,99]. The use of a CRISPR-based cocktail consisting of multiple sgRNA from the onset of treatment could also be used to help block HIV-1 escape.

In summary, we show that CRISPR activation systems have the potential to induce HIV-1 expression in cell-based models of latency. CRISPR systems, in combination with cART, may lead to new treatments for HIV-1 infection.

METHODS

Plasmid construction

pLV-dCas9-VP64-Blast (Addgene plasmid #61425), pLV-MS2-p65-HSF1-Hygro (Addgene plasmid #61426), and pLV-sgRNA(MS2)-Zeo (Addgene plasmid #61427)[61] were gifts from Feng Zhang. pcDNA-dCas9-VP64 (Addgene plasmid #47107), pSP-sgRNA (Addgene plasmid #47108)[54] and pcDNA-dCas9-p300 (Addgene plasmid #61357)[82] were gifts from Charles A. Gersbach.

Reporter plasmid encoding the HIV-1 subtype B LTR upstream of the EGFP gene (LTR-EGFP) was previously described[13]. Oligonucleotides encoding sgRNA target sites were custom ordered (Elim Biopharm), phosphorylated by T4 polynucleotide kinase (New England Biolabs), hybridized and ligated into the *Bbs*I and *Bsm*BI restriction sites of pSP-gRNA and pLV-sgRNA(MS2)-Zeo, respectively. Correct construction of each sgRNA was verified by sequence analysis. Oligonucleotides used in this study are shown in **Supplementary Fig. 7**.

Cell culture

HEK293T and Jurkat cells were obtained from American Type Culture Collection (ATCC). J-Lat 9.2 and J-Lat 10.6 cells were obtained from the National Institutes of Health (NIH) AIDS Reagent Program. HEK293T cells were maintained in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% (vol/vol) fetal bovine serum (FBS; Life Technologies) and 1% (vol/vol) antibiotic-antimycotic (Anti-Anti; Life Technologies) in a humidified 5% CO_2 atmosphere at 37°C. Jurkat and J-Lat cells were maintained in RPMI 1640 medium (Life Technologies) supplemented with 10% FBS and 1% Anti-Anti in a humidified 5% CO_2 atmosphere at 37°C.

Lentivirus production and infections

HEK293T cells were seeded onto 10-cm plates at a density of 2 x 10^6 cells per plate in serum-containing medium. At 24 h after seeding, cells were transfected with 10 µg of pLV-dCas9-VP64-Blast,

pLV-MS2-p65-HSF1-Hygro, or pLV-sgRNA(MS2)-Zeo, as well as 5 µg of pMDL g/p RRE, 3.5 µg of pMD2.G, and 1.5 µg of pRSV-Rev using polyethylenimine (PEI), as described[100]. At 48 h and 72 h after transfection, cell culture medium was harvested, concentrated by ultracentrifugation (L8-55M Ultracentrifuge; Beckman Coulter), and resuspended in 200 µl of PBS with 20% sucrose. Lentivirus was stored in single-use aliquots at -80° C.

For infections, J-Lat 9.2 and 10.6 cells were seeded onto 24-well plates at a density of 2 x 10^5 cells per well in serum-containing medium. Lentiviral infections were performed sequentially. At 24 h after seeding, cells were infected with either LV-dCas9-VP64-Blast, LV-MS2-p65-Hygro, or LV-sgRNA(MS2)-Zeo using a multiplicity of infection (MOI) of 0.1. After 1 week, infected cells were selected with either 10 µg/ml of blasticidin (Sigma) for LV-dCas9-VP64-Blast, 1 mg/ml of hygromycin (Sigma) for LV-MS2-p65-Hygro, or 100 µg/ml of zeocin (Sigma) for LV-sgRNA(MS2)-Zeo. Each selection was performed for 1 week. Seven days after the final selection, cells were washed once with PBS, and GFP expression was evaluated by flow cytometry (BD LSR Fortessa X-20; BD Biosciences). For each sample, 10,000 live events were collected, and data were analyzed using FlowJo (Tree Star, Inc.).

For small molecule treatments, ten days after the final selection, J-Lat cells expressing the SAM complex were seeded into a 96-well plate at a density of 4 x 10^4 cells per well with 0.5, 1.0, 2.0, or 4.0 µM SAHA (Sigma) in the presence of absence of 2.0 µM prostratin (Sigma). At 24 h after treatment, cells were harvested, and EGFP expression was measured by flow cytometry. For each sample, 10,000 live events were collected, and data were analyzed using FlowJo.

Transient transfections

HEK293T cells were seeded into 96-well plates at a density of 4 x 10^4 cells per well in serum-containing medium. For SAM, at 16-24 h after seeding, cells were transfected with 20 ng of LTR-EGFP, 60 ng of pLV-dCas9-VP64-Blast, 60 ng of pLV-MS2-p65-HSF1-Hygro, and 60 ng of pLV-

sgRNA(MS2)-Zeo using PEI. For dCas9-VP64 transfections, at 16-24 h after seeding, cells were transfected with 20 ng of LTR-EGFP, 90 ng of pcDNA-dCas9-VP64, and 90 ng of pSP-sgRNA using PEI. Transfection efficiency was measured to be >90%.

For nucleofections, J-Lat cells were seeded into a T-25 cell culture flask at a density of 1 x 10^5 cells per ml. For SAM, at 24 h after seeding, 2 x 10^5 cells per nucleofection were centrifuged at 90 x *g* for 10 min at room temperature and resuspended in Nucleofector Solution SE (Lonza) with 650 ng of pLV-dCas9-VP64-Blast, 650 ng of pLV-MS2-p65-HSF1-Hygro, 650 ng of pLV-sgRNA(MS2)-Zeo, and 40 ng of pEntry-CMV-puro-mTagBFP, which was used as a transfection control. For dCas9-p300, 2 x 10^5 cells per nucleofection were resuspended in Nucleofector Solution SE with 1 μg of pcDNA-dCas9-p300 Core, 1 μg of pSP-sgRNA, and 40 ng of pEntry-CMV-puro-mTagBF. Cells were transferred to a 16-well Nucleocuvette Strip (Lonza) and electroporated using the 96-well Shuttle Device (Lonza) with the program CL-120, according to the manufacturer's instructions. Transfection efficiency was measured to be ~20%.

At 48 h after nucleofection, cells were washed once with PBS, and GFP expression was evaluated by flow cytometry. For each sample, 10,000 live events were collected, and data were analyzed using FlowJo.

Statistical analysis

Data represents the mean (data points) and standard deviation (error bars) of three independent replicates. Statistical significance was calculated using a one-tailed independent two-sample Student's *t*-test (Microsoft Excel 2013).

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AUTHOR CONTRIBUTIONS

P.L., T.G., and D.V.S. conceived the study; P.L. and T.G. designed and performed the experiments; P.L., T.G., and D.V.S. wrote the manuscript.

ADDITIONAL INFORMATION

Supplementary Information accompanies this paper.

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FIGURE LEGENDS

Fig. 1. **CRISPR transcriptional activators induce gene expression from the HIV-1 LTR (a)** Schematic of the EGFP reporter system used to evaluate Cas9-mediated gene activation and the architecture of the HIV-1 subtype B long terminal repeat (LTR) promoter. Grey circles indicate approximate locations of nucleosome-0 (nuc-0) and nuc-1 in the LTR. Red triangles indicate location of the sgRNA target site. Black arrow denotes the transcriptional start site (TSS). Yellow squares and orange circles indicate NF-κB and Sp-1 binding sites, respectively **(b)** sgRNA target sites from the LTR. PAM highlighted blue. **(c)** Cartoon illustrating the potential modes of transcriptional activation by dCas9-VP64 and SAM. Black arrows indicate VP64- and MS2-p65-HSF1-mediated recruitment of cellular transcription factors and the chromatin-remodeling proteins. **(d)** Fold-increase in the percentage of EGFP positive HEK293T cells after transfection with EGFP reporter plasmid and expression vectors-encoding dCas9-VP64 with sgRNA (blue) or SAM with sgRNA (red). EGFP expression was normalized to cells transfected with reporter plasmid, dCas9-VP64 and an empty sgRNA expression cassette. EGFP expression was measured 48 h after transfection, and was observed in 0.5 ± 0.6% of negative control cells. Error bars indicate s.d. (n = 3; *p-value < 0.005; **p-value < 0.001; ***p-value < 0.0001; Student's t-test).

Fig. 2. SAM-mediated activation of latent HIV-1 expression in cell line models of latency (a) Diagram of the HIV-1 proviral genome in J-Lat cells. Grey boxes indicate genes whose expression has been disabled. **(b and c)** Percentage of GFP positive **(b)** J-Lat 9.2 and **(c)** J-Lat 10.6 cells stably expressing SAM with sgRNA. "Empty" indicates J-Lat cells expressing SAM with an empty sgRNA expression cassette. Dotted line indicates the percentage of naïve GFP positive **(b)** J-Lat 9.2 and **(c)** J-Lat 10.6 cells after treatment with 20 ng/μL tumor necrosis factor α (TNF-α). GFP fluorescence was

measured 14 days after final infection. Error bars indicate s.d. (n = 3; **p-value < 0.001; ***p-value < 0.0001; Student's t-test).

Fig. 3. SAHA and prostratin enhance SAM-mediated activation of latent HIV-1 expression (a) Schematic illustrating experimental setup. Naïve or SAM-expressing J-Lat cells were treated with SAHA alone or in the presence of prostratin. **(b and c)** Percentage of GFP positive J-Lat 9.2 (upper) and J-Lat 10.6 (lower) cells stably expressing SAM and treated with **(b)** increasing amounts of SAHA only or **(c)** increasing amounts of SAHA with 2 μM prostratin. "Empty" indicates J-Lat cells expressing SAM with an empty sgRNA expression cassette. Dotted line indicates the percentage of naïve GFP positive J-Lat cells after treatment with 20 ng/μL of TNF-α. Cells were treated 17 days after final infection, and GFP expression was measured 24 h after treatment. Error bars indicate standard deviation (n = 3).

Fig. 4. Reactivation of latent HIV-1 expression using CRISPR-based acetyltransferases (a) Cartoon illustrating the potential mode of action by a CRISPR-based acetyltransferase **(**dCas9-p300) on the HIV LTR. Grey circles indicate approximate locations of nuc-0 and nuc-1 in the LTR **(b)** Fold-increase in the percentage of GFP positive J-Lat 10.6 cells after nucleofection with SAM and sgRNA (dark blue) or dCas9-p300 with sgRNA (teal). Data were normalized to J-Lat 10.6 cells nucleofected with SAM or dCas9-p300 and an empty sgRNA expression cassette. GFP fluorescence was measured 48 h after nucleofection. Error bars indicate standard deviation (n = 3; *** p-value < 0.0001; Student's t-test).

Figure 1

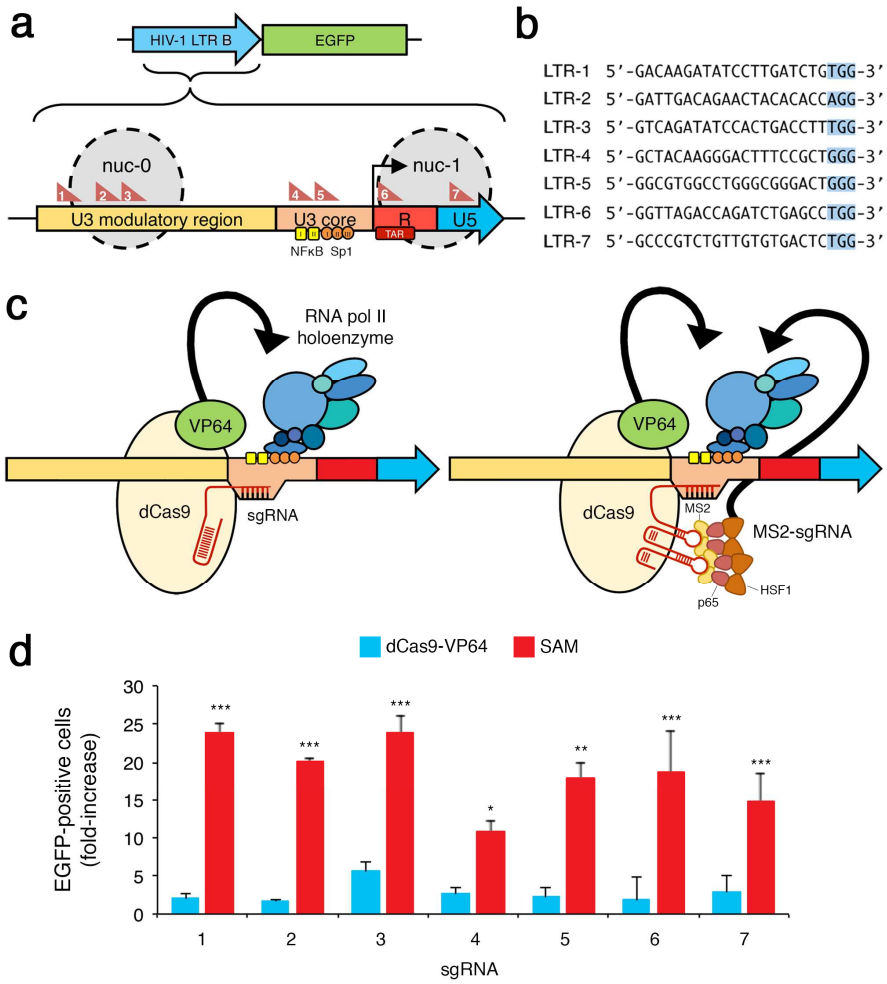

Figure 2

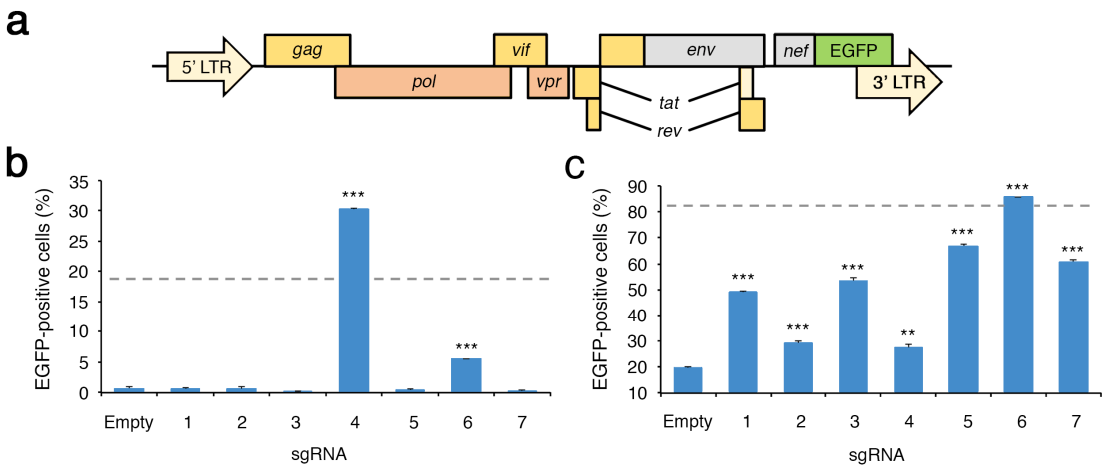

Figure 3

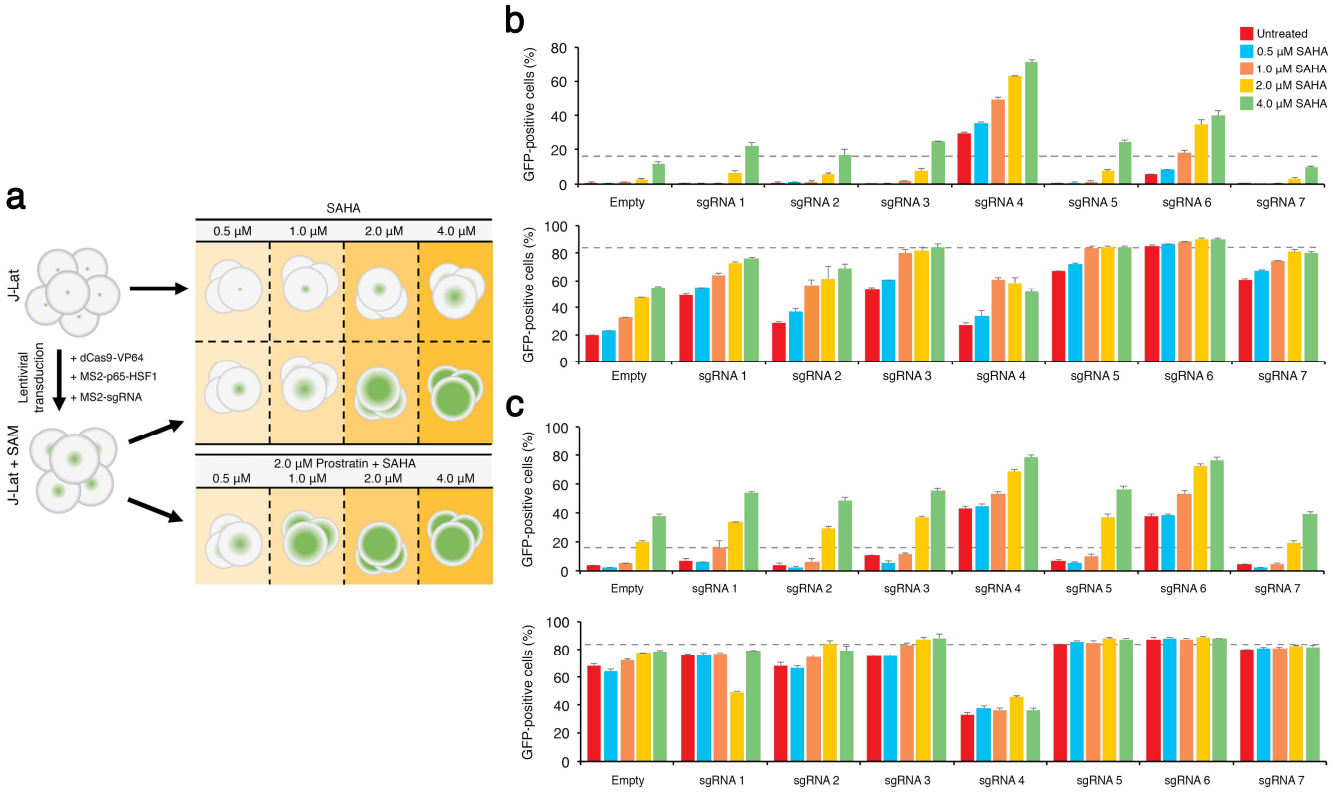

Figure 4