Identification of the cellular transcription factor KLF16 as a new HIV-1 silencing factor

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RESULTS 1

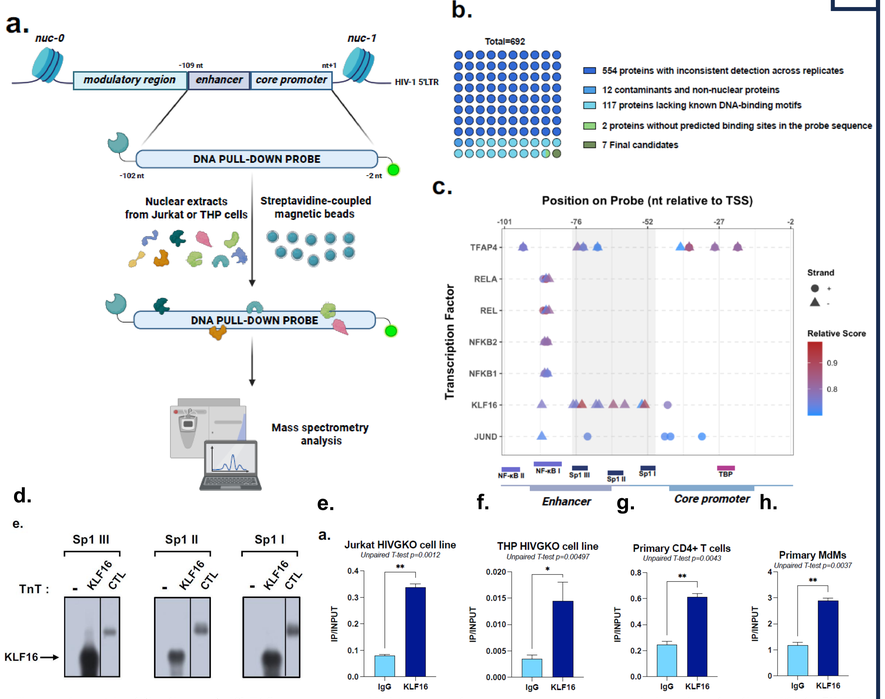


Figure 1 – Identification of KLF16 as a new HIV-1 regulator binding *in vitro* and *in vivo* to the HIV-1 5'LTR
(a) DNA pull-down assays using a biotinylated probe spanning the HIV-1 5'LTR enhancer and U3 core promoter were performed with Jurkat or THP-1 nuclear extracts, followed by mass spectrometry analysis. **(b)** KLF16 sequence coverage identified by mass spectrometry. **(c)** Predicted binding sites of the seven candidate transcription factors within the HIV-1 5'LTR enhancer–core promoter region relative to the TSS+1, with strand orientation and JASPAR-predicted binding scores indicated. **(d)** EMSA using radiolabeled probes corresponding to the three Sp1 sites incubated with purified KLF16 protein. **(e-h)** ChIP assays were performed in Jurkat HIVGKO, THP HIVGKO, primary CD4$^+$ T cells, and monocyte-derived macrophages infected with HIV-1 using anti-KLF16 or control IgG; results are shown as IP/INPUT ratios. One representative of three independent experiments is shown.

BACKGROUND

Despite effective antiretroviral therapy, HIV-1 persistence in latent reservoirs of T-lymphoid and myeloid origins remains a major obstacle to a cure. The molecular mechanisms of HIV-1 latency are complex and multifactorial involving numerous blocks to viral gene expression, including transcriptional and epigenetic blocks. Deciphering the underlying mechanisms that drive HIV-1 into latency is critical for targeted HIV cure efforts.

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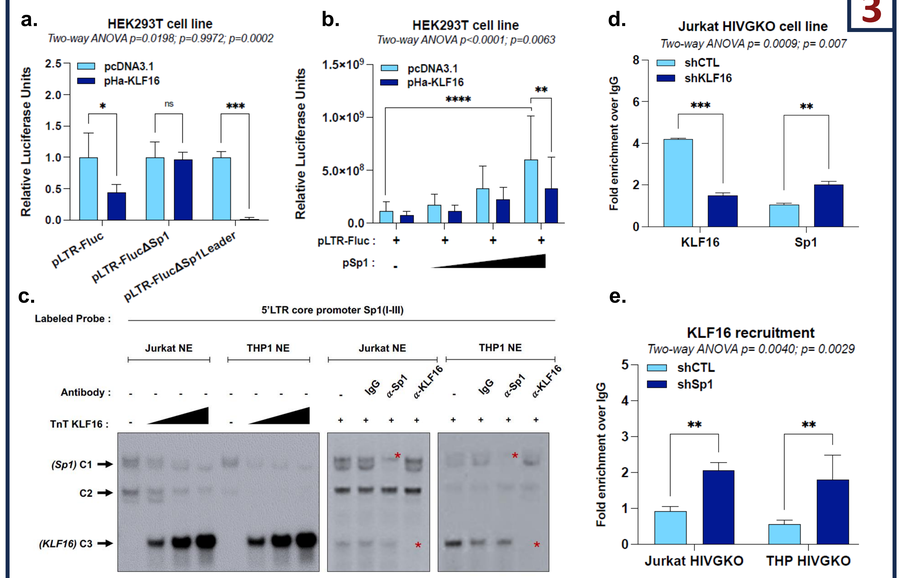


Figure 3 – KLF16 competes with Sp1 for HIV-1 5'LTR binding
(a) HEK293T cells were transfected with p-LTR-Fluc, pLTR-FlucΔSp1, or pLTR-FlucΔSp1Leader together with pHA-KLF16, and luciferase activity was normalized to protein content. **(b)** HEK293T cells were transfected with p-LTR-Fluc and increasing amounts of pSp1 in the presence of pHA-KLF16, and luciferase activity was normalized to protein content. **(c)** EMSA using radiolabeled probes spanning the three Sp1 sites of the HIV-1 5'LTR was performed with Jurkat or THP-1 nuclear extracts in the presence or absence of increasing recombinant KLF16; supershifted complexes are indicated. **(d-e)** ChIP assays were carried out in shCTL-, **(d)** shKLF16-transduced Jurkat HIVGKO cells or **(e)** shSp1-transduced Jurkat and THP HIVGKO using anti-KLF16, anti-Sp1, or control IgG. One representative of three independent experiments is shown.

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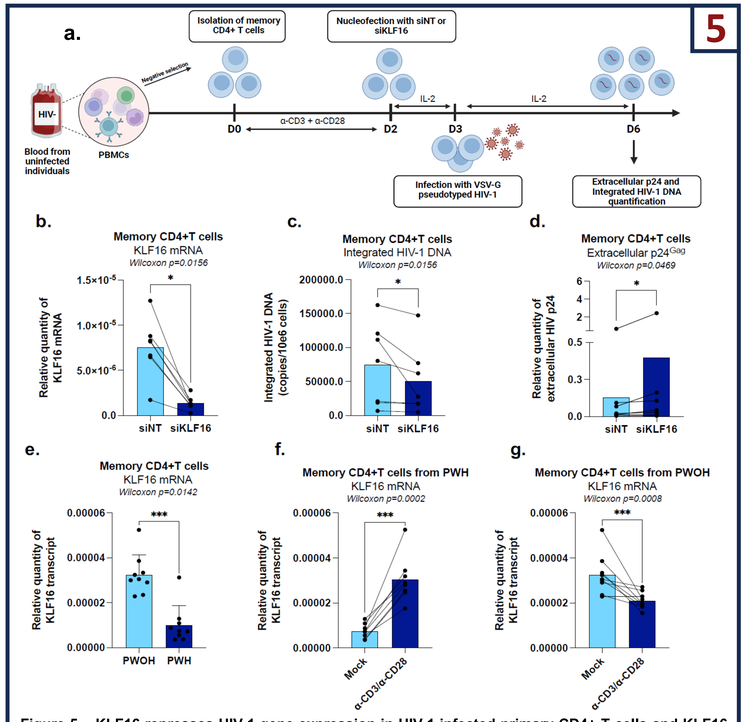
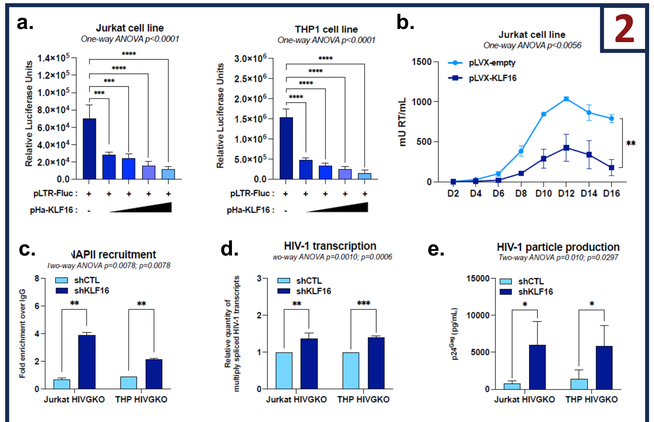


Figure 5 – KLF16 represses HIV-1 gene expression in HIV-1 infected primary CD4+ T cells and KLF16 expression is upregulated following TCR-triggering in memory CD4+ T cells from PWH.
(a) Memory CD4$^+$ T cells from PBMCs of HIV-uninfected donors were activated with α-CD3/α-CD28 for 3 days, nucleofected with control (siNT) or KLF16-targeting siRNA (siKLF16), cultured with rhIL-2, and infected with VSV-G–HIV-1. **(b)** KLF16 mRNA were measured by RT-qPCR 24h post-nucleofection. **(c)** Integrated HIV DNA was quantified by nested real-time PCR 3 days post-infection. **(d)** p24 levels were measured by ELISA 3 days post-infection (n=7 donors). **(e-g)** Memory CD4$^+$ T cells from people without HIV (PWOH, n=9) or ART-treated people with HIV (PWH, n=9) were stimulated or not with α-CD3/α-CD28 for 18 h. KLF16 mRNA were quantified by RT-qPCR *ex vivo* in **(e)** PWOH and PWH, **(f)** PWH only, and **(g)** PWOH only.

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Results 1 - KLF16 represses viral gene expression
(a) Jurkat or THP-1 cells were transfected with p-LTR-Fluc and increasing amounts of KLF16 (pHA-KLF16); luciferase activity was normalized to protein content. **(b)** Jurkat cells stably expressing KLF16 (pLVX-KLF16) or pLVX-empty were infected with HIV-1, and viral production was measured by SG-PERT. Jurkat HIVGKO and THP HIVGKO cells transduced with shCTL or shKLF16 were used for **(c)** RNA Pol II recruitment by ChIP-qPCR, **(d)** RT-qPCR analysis of HIV-1 multiply spliced (MS) mRNAs, and **(e)** viral production by p24 ELISA of culture supernatants. One representative experiment out of three independent replicates is shown.

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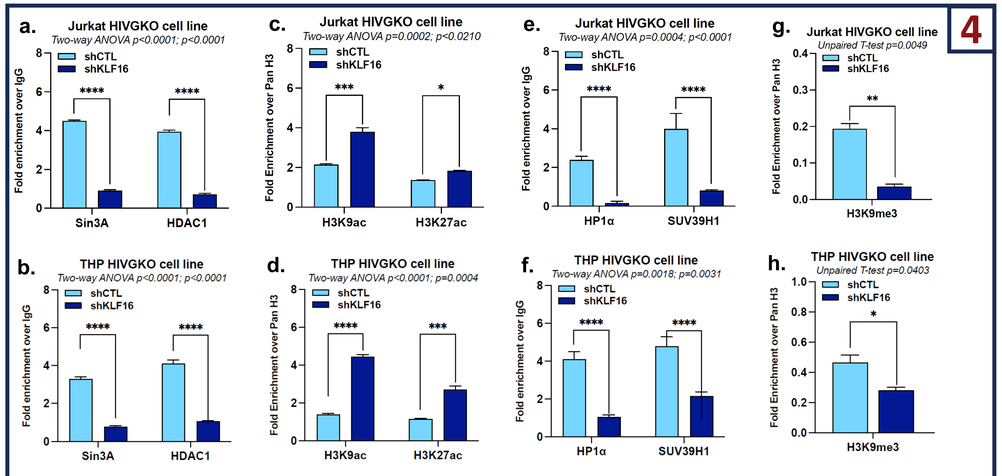
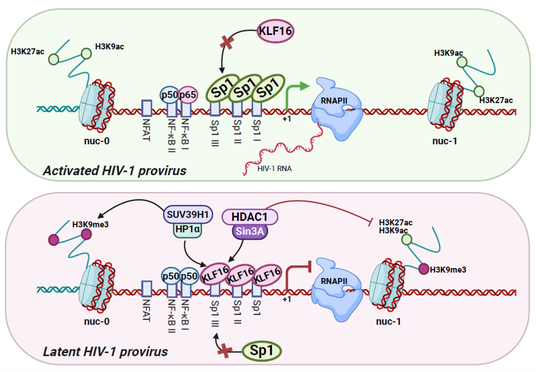


Figure 4 – KLF16 modulates the epigenetic profile of the HIV-1 5'LTR to repress viral gene expression
Jurkat or THP-1 HIVGKO cells transduced with either shCTL or shKLF16 were used for chromatin immunoprecipitations with a non-specific IgG serving as background control or with **(a-b)** specific antibodies targeting Sin3A or HDAC1, or **(c-d)** specific antibodies targeting HP1α or SUV39H1 (enrichments over IgG). The same chromatin samples were used to assess the histone modification profile at the HIV-1 5'LTR using specific antibodies targeting histone H3 (Pan H3) or **(e-f)** H3K9ac and H3K27ac, or **(g-h)** H3K9me3 (enrichments over Pan H3). One representative experiment out of three independent replicates is shown.

CONCLUSION



Together, our results support a model in which KLF16 act as a novel and selective repressor of HIV-1 gene expression in both T-lymphocytes and myeloid cells. Under productive infection, the three tandem 5'LTR U3 promoter region Sp1 binding sites are occupied by Sp1, which activates HIV-1 transcription and inhibits KLF16 binding to the viral promoter. Under latent conditions, KLF16 binds to the Sp1 binding sites in the U3 region of the HIV-1 5'LTR and promotes recruitment of the Sin3A/HDAC1 and HP1α/SUV39H1 chromatin-modifying complexes, leading to removal of activating acetylation marks from histones H3 lysine residues H3K9 and H3K27 and to deposition of the repressive histone mark H3K9me3 on nucleosome-1 (nuc-1), respectively. These epigenetic modifications results in heterochromatin formation and HIV-1 transcriptional silencing. Overall, these findings provide evidence that targeting KLF16 or its downstream effectors represents a promising new avenue to enhance HIV-1 reactivation for shock-and-kill cure strategies.

PLAIN LANGUAGE SUMMARY

We identify the cellular transcription factor KLF16 as a new repressor of HIV-1 transcription and replication. Targeting this protein could help wake HIV-1 from its dormant state, which may support future strategies aimed at achieving a complete cure.