Scaffold attachment factor B suppresses HIV-1 infection of CD4+ T cells by preventing binding of RNA polymerase II to HIV-1’s long terminal repeat

The 5’ end of the HIV, type 1 (HIV-1) long terminal repeat (LTR) promoter plays an essential role in driving viral transcription and productive infection. Multiple host and viral factors regulate LTR activity and modulate HIV-1 latency. Manipulation of the HIV-1 LTR provides a potential therapeutic strategy for combating HIV-1 persistence. In this study, we identified an RNA/DNA-binding protein, scaffold attachment factor B (SAFB1), as a host cell factor that represses HIV-1 transcription. We found that SAFB1 bound to the HIV-1 5’ LTR and significantly repressed 5’-driven viral transcription and HIV-1 infection of CD4+ T cells. Mechanistically, SAFB1-mediated repression of HIV-1 transcription and infection was independent of its RNA- and DNA-binding capacities. Instead, by binding to phosphorylated RNA polymerase II, SAFB1 blocked its recruitment to the HIV-1 LTR. Of note, SAFB1-mediated repression of HIV-1 transcription from proviral DNA maintained HIV-1 latency in CD4+ T cells. In summary, our findings reveal that SAFB1 binds to the HIV-1 LTR and physically interacts with phosphorylated RNA polymerase II, repressing HIV-1 transcription initiation and elongation. Our findings improve our understanding of host modulation of HIV-1 transcription and latency and provide a new host cell target for improved anti-HIV-1 therapies.

HIV-1 latency because of reversible silencing of transcription from proviral DNA represents a major obstacle for virus eradication (1, 2). HIV-1 transcription driven by the viral 5’ LTR promoter and viral latency can be modulated by numerous host factors, including nucleus-located factors such as the bromodomain containing 4 (BRD4) and the Brahma-associated factor (BAF) complex (3–7). Therefore, agonists or antagonists for host factors involved in the epigenetic modification of HIV-1 LTR or the function of viral Tat protein have been employed in the activation of latently infected proviral DNA (8–10), which is the first step of “shock and kill.”

SAFB1 was first identified as a scaffold or matrix attachment region (S/MAR) DNA binding protein (11) that is mainly located in the nucleus and extensively expressed in multiple vertebrate tissues (11–14). Increased attention has recently been shifted to SAFB1-mediated RNA binding and protein–protein interactions and functions (12). SAFB1 contains several domains that mediate its crucial function in DNA repair, processing of mRNA, protein–protein interactions, as well as interaction with chromatin-modifying complexes (15, 16). Its N terminus contains the scaffold attachment factor box (SAF box), which mediates binding to adenine- and thymine-rich S/MARs of DNA elements, downstream of which are the high similarity region, the RNA recognition motif, and a nuclear localization signal. The C terminus contains the glutamate- and arginine-rich region and the Arg/Gly-rich region containing an RGG motif (15, 17, 18).

SAFB1 has been identified as a tumor suppressor that modulates tumorigenesis-related gene expression through association with other host proteins or by binding directly to different gene promoters. For instance, SAFB1 binds and represses estrogen receptor α–mediated trans-activation via indirect association with histone deacetylation and interaction with TATA-box binding protein associated factor 15 (TAF15), a member of the basal transcription machinery (19). Additionally, SAFB1 also binds to and represses expression of the androgen receptor (20) and androgen receptor–driven transcription, which is thought to be involved in the control of tumorigenesis of prostate cancer (20). Moreover, by forming a complex with other host proteins, X-ray repair cross-complementing 5 (XRCC5) and Brahma-related gene 1 (BRG1), SAFB1 binds to domain; DHS, DNase hypersensitive site; HA, hemagglutinin; TNFa, tumor necrosis factor α; hnRNP, heterogeneous nuclear ribonucleoprotein; IP, immunoprecipitation.
SAFB1 suppresses HIV-1 infection in CD4⁺ T cells

SAFB1 is a DNA- and RNA-binding protein that participates in regulating multiple cellular transcriptional events (15). To investigate whether SAFB1 regulates HIV-1 transcription and infection, lentivirus vectors containing SAFB1-specific short hairpin RNAs (shRNAs) were used to transduce Jurkat T or HEK293T cells to stably knock down SAFB1 expression (Fig. 1A). We found that SAFB1 knockdown enhanced HIV-1 replication in primary CD4⁺ T cells. Our findings facilitate a better understanding of host modulation of HIV-1 transcription and latency and might provide a new host target for HIV-1 cure strategies.

Results

SAFB1 suppresses HIV-1 infection in CD4⁺ T cells

SAFB1 suppresses HIV-1 infection in CD4⁺ T cells

Here we report that SAFB1 dramatically repressed HIV-1 infection and proviral DNA in T cells. SAFB1 knockdown significantly increased HIV-1 latency, as demonstrated by a 36- to 58-fold enhancement for viral infection in Jurkat T cells and 12- to 20-fold in HEK293T cells (Fig. 1B and C). Our findings facilitate a better understanding of host modulation of HIV-1 transcription and latency and might provide a new host target for HIV-1 cure strategies.
32-fold enhancement of viral infection (Fig. 1D). SAFB1 knockdown did not significantly alter the transcription of other host genes (Fig. S1).

To further confirm the inhibitory role of SAFB1, we performed HIV-1 infection in PHA-P–activated primary CD4+ T cells (Fig. 1E). The endogenous SAFB1 in PHA-P–activated primary CD4+ T cells was successfully knocked down by transduction with lentiviruses containing SAFB1-specific shRNA for 3 days (Fig. 1F). The cells were further infected with replication-competent HIV

\[ \text{V}_{\text{NL4-3}} \text{ viruses for an additional 5 and 7 days.} \]

SAFB1 knockdown increased HIV-1 replication, as demonstrated by more viral particles synthesizing and releasing using anti-p24Gag Western blotting and p24Gag capture ELISA assays (Fig. 1, F and G). The harvested cell culture supernatants were further titrated in TZM-bl indicator cells, and a significantly increased viral infectivity from the supernatant of SAFB1 knockdown cells was observed (Fig. 1H). shR-SAFB1 treatment did not change the specific infectivity of the virus because viral infectivity (Fig. 1H) and viral production (Fig. 1G) increased in parallel.

We also performed a siRNA experiment to specifically interfere with SAFB1 expression in HEK293T cells and demonstrated the inhibitory role of SAFB1 on infection of HIV-Luc/VSV-G (Fig. S2A). In addition, we also overexpressed SAFB1 in HEK293T cells by transfection with the SAFB1-expressing plasmid pCDNA3.1-HA/SAFB1 for 24 h and then infected the cells with HIV-Luc/VSV-G for an additional 48 h. The results showed that SAFB1 overexpression significantly inhibited HIV-1 infection (Fig. S2B). Knockdown of SAFB1 by using lentiviruses containing SAFB1-specific shRNAs or SAFB1 overexpression in Jurkat cells did not alter cell proliferation (Fig. S3, A and B), which was assessed by using the MTT method as described previously (23). Taken together, these data demonstrate that SAFB1 is a negative host factor that inhibits HIV-1 infection.

**SAFB1 blocks HIV-1 transcription**

Having demonstrated the inhibitory role of SAFB1 on HIV-1 replication, we next investigated which step of the virus life cycle was blocked. Jurkat cells with SAFB1 stable knockdown were infected with HIV-Luc/VSV-G for 24 h, and then cellular DNA and mRNA were isolated. The integrated proviral gag DNA quantified with Alu-PCR showed similar levels in SAFB1 knockdown cells as in cells transfected with an off-target control (Fig. 2A, left panel). When quantified for the production of HIV-1 gag mRNA, SAFB1 knockdown increased the expression of gag mRNA by 10-fold (Fig. 2A, right panel). These data suggest that SAFB1 repressed the transcription of HIV-1 proviral DNA.

The initiation and elongation of HIV-1 LTR–driven transcription can be monitored by qPCR with specific primers (5). SAFB1 knockdown increased viral mRNAs of all lengths. In particular, elongated viral mRNAs, including proximal, intermediate, and distal transcripts, showed the most substantial elevation, a 6.9- to 10.3-fold increase (Fig. 2B). These data suggest that SAFB1 hinders HIV-1 LTR–driven transcription, particularly the transcriptional elongation step.

**Figure 2. SAFB1 represses HIV-1 transcription.** A, SAFB1 knockdown promotes HIV-1 transcription. SAFB1 stable knockdown HEK293T cells were infected with HIV-Luc/VSV-G (5 ng of p24Gag) for 24 h. Then the integrated HIV-1 gag DNA was quantified with Alu-PCR (left panel), and the transcribed gag mRNA levels were quantified with quantitative reverse transcription PCR. The Actin gene was used for normalization. B, SAFB1 knockdown significantly increased HIV-1 5’ LTR–driven RNA transcription initiation and elongation. The total amounts of mRNAs, as assessed by qPCR with specific primers, were used to quantify the initiation and elongation of HIV-1 transcription. Data are presented as mean ± S.D. Results are representative of at least three independent experiments. **, \( p < 0.01 \); ***, \( p < 0.001 \). Pro, proximal; Int, intermediate; Dis, distal.

**The C-terminal Arg/Gly-rich domain is required for SAFB1-mediated HIV-1 inhibition**

SAFB1 contains several functional domains that mediate its crucial cellular roles (15, 16). To investigate which domain is required for SAFB1-mediated HIV-1 inhibition, truncated SAFB1 mutants were constructed (Fig. 3A). HEK293T cells were transfected with truncated or WT SAFB1-expressing plasmids. These truncations did not alter its nucleoplasm location, as observed under a confocal microscope (Fig. 3B). The transfected HEK293T cells were further infected with HIV-Luc/VSV-G for an additional 48 h. Deletion of the C-terminal Arg/Gly-rich domain abolished SAFB1’s inhibitory role of HIV-1 infection, whereas the other truncated forms did not affect anti-HIV-1 activity (Fig. 3, C and D). These data demonstrate that the Arg/Gly-rich domain is required for SAFB1-mediated HIV-1 inhibition.

**SAFB1 associates with phosphorylated RNA pol II to impede its recruitment to the LTR**

The RGG motif mediates interactions of SAFB1 with multiple nuclear factors (14, 19). The finding that the deletion of the Arg/Gly-rich domain abolished SAFB1’s inhibitory role of HIV-1 infection led us to investigate whether SAFB1-repressed HIV-1 transcription occurred by affecting the functions of other host proteins that are essential for HIV-1 infection.

SAFB1 has been reported to be capable of interacting with the C-terminal domain (CTD) of RNA pol II and exist in the RNA pol II transcriptional complex (18, 24). It is possible that SAFB1 represses HIV-1 transcription by modulating RNA pol II function. To investigate this, we first determined the potential of SAFB1 binding with the HIV-1 LTR promoter. The HIV proviral 5’ LTR is organized into three strictly positioned nucleosomes (Nuc-0, Nuc-1, and Nuc-2) separated by two intervening enhancer regions, DNase hypersensitive site 1 (DHS-1) and DHS-2, which provide binding sites for multiple host transcription factors that either positively or negatively regulate transcription (25). We performed a ChIP analysis in HIV-1–infected Jurkat T cells with anti-SAFB1–specific anti-
SAFB1 suppresses HIV-1 infection in CD4+ T cells

bodies and analyzed the products using specific primers targeting the HIV-1 LTR Nuc0, DHS, Nuc1, or Nuc2 regions. We found associations between SAFB1 and the HIV-1 LTR Nuc1 and Nuc2 regions (Fig. 4A).

We next performed a co-immunoprecipitation assay in Jurkat T cells to detect the association of SAFB1 with RNA pol II. Specific antibodies against SAFB1, unphosphorylated RNA pol II (8WG16), and phosphorylated RNA pol II at serine 2 of the CTD (pSer-2) were used for immunoprecipitation. SAFB1 showed an association with the phosphorylated (Fig. 4B, bottom panel) but not the unphosphorylated RNA pol II (Fig. 4B, top panel). SAFB1 is known to be a DNA/RNA-binding protein (26). To prove the direct interaction between SAFB1 and phosphorylated RNA pol II, an extensive nuclease, benzonase, was used to treat the cell lysates to rapidly hydrolyze nucleic acids. The co-immunoprecipitation assay showed a stable association of SAFB1 with the pSer-2 form of RNA pol II despite benzonase treatment (Fig. 4C and Fig. S4).

We have demonstrated the binding between SAFB1 and the HIV-1 LTR Nuc1 and Nuc2 regions (Fig. 4A), which are also the sites on LTR regions responsible for binding with phosphorylated RNA pol II. Thus, the association of SAFB1 with phosphorylated RNA pol II might hinder the latter being recruited to HIV-1 LTR regions. Indeed, knockdown of endogenous SAFB1 significantly increased recruitment of pSer-2 RNA pol II to the HIV-1 LTR Nuc-1 and Nuc-2 regions, as shown in the pSer-2 RNA pol II ChIP assay by using HIV-1–infected Jurkat cells (Fig. 4D).

Having demonstrated that deletion of the C-terminal Arg/Gly-rich domain abolished SAFB1–mediated HIV-1 inhibition, we then investigated whether deletion of the Arg/Gly-rich domain could affect its association with phosphorylated RNA pol II. The HA-tagged plasmid expressing full-length or Arg/Gly-rich domain-deleted (∆Arg/Gly) SAFB1 was transfected into Jurkat T cells, and then co-immunoprecipitation of SAFB1 or its mutant with the endogenous phosphorylated RNA pol II (Fig. 4E). As expected, SAFB1-∆Arg/Gly was unable to inhibit HIV-1 infection of Jurkat T cells (Fig. 4F). Taken together, these results demonstrate that SAFB1 associates with phosphorylated RNA pol II to impede its recruitment to the LTR.

SAFB1 represses HIV-1 reactivation from an integrated HIV-1 proviral DNA

HIV-1 latency is characterized mainly by reversible silencing of LTR-driven transcription of an integrated provirus (1, 27, 28). The inhibitory effect of SAFB1 on HIV-1 transcription suggests a potential role for SAFB1 in HIV-1 latency. Thus, we sought to investigate the effect of endogenous SAFB1 on the expression of an integrated HIV-1 proviral DNA in CD4+ T cells. A latently HIV-1–infected Jurkat T cell clone (C11) harboring an HIV-1 proviral DNA encoding GFP was used (29). These cells can be reactivated upon stimulation with TNFα, vorinostat, or trichostatin A to express GFP as an indication of HIV-1 reactivation from latency (29, 30). The endogenous SAFB1 in C11 cells was successfully knocked down by using specific shRNA (Fig. 5A). SAFB1 knockdown increased HIV-1 reactivation despite the presence or absence of stimulation with TNFα (Fig. 5B).

To confirm that the observed effect of SAFB1 on HIV-1 gene expression is not an artifact in a single cell line, we performed
SAFB1 suppresses HIV-1 infection in CD4+ T cells

Figure 4. SAFB1 associates with phosphorylated RNA pol II to impede its recruitment to LTR. A, association of SAFB1 with the HIV-1 5′ LTR. HEK293T cells were infected with HIV-luc/VSV-G for 24 h, cross-linked, and sonicated and then subjected to an SAFB1 ChIP assay. Fragments of the LTR covering nucleotides 40–902, which contain Nuc0, DHS, Nuc-1 and Nuc-2, were amplified by qPCR. B and C, SAFB1 interacts with pSer-2 RNA pol II. Jurkat T cells were lysed, treated with benzonase, and then incubated with the indicated antibodies and protein A or G magnetic beads. The immunoprecipitates were detected by Western blotting with specific antibodies, and the nucleotides from cell lysates were electrophoresed on 1% agarose gel and stained with ethidium bromide. 15 kbp and 7.5 kbp indicate the DNA marker used in the gel (C). D, SAFB1 knockdown promotes the enrichment of pSer-2 RNA pol II on the HIV-1 LTR. SAFB1 stable knockdown Jurkat T cells were infected with HIV-luc/VSV-G (5 ng of p24gag) for 48 h. After being cross-linked and sonicated, the Jurkat T cells were subjected to a ChIP assay with pSer-2 RNA pol II antibody. Fragments of the LTR covering nucleotides 40–902, which contain Nuc0, Nuc-1, and Nuc-2, were amplified by qPCR. E, deletion of the Arg/Gly-rich domain abolishes SAFB1 association with pSer-2 RNA pol II. Jurkat cells were transfected with a pCDNA3.1-HA/SAFB1-Arg/Gly–expressing plasmid and then lysed. The cell lysates were used for IP with anti-HA antibodies, and protein expression was detected by immunoblotting (IB) with the indicated antibodies. F, deletion of the Arg/Gly-rich domain abolishes SAFB1-mediated HIV-1 inhibition. Jurkat cells were transfected with pCDNA3.1-HA/SAFB1-Arg/Gly–expressing plasmid and then infected with HIV-luc/NL4-3 (20 ng of p24gag) for an additional 48 h. Viral infection was measured by detecting the luciferase value. Results are representative of three independent repeats. Data are presented as mean ± S.D. *, p < 0.05; **, p < 0.01; ***, p < 0.001 as determined by an unpaired Student’s t test.

the same experiment in the latently HIV-1-infected CEM cell clone ACH2, which contains a single copy of proviral DNA per cell (31). A point mutation in Tat response element within the LTR region in ACH2 cells makes them unresponsive to Tat stimulation for viral reactivation, whereas viruses can be reactivated from these cells by other reagents, such as TNFα (32). Additionally, continuous viral reactivation at a low level was observed in ACH2 cells (33). The endogenous SAFB1 in ACH2 cells was knocked down by using lentiviruses containing SAFB1-specific shRNAs (Fig. 5C). The cells were then treated with or without TNFα or the latency-reversing agent vorinostat (SAHA). SAFB1 knockdown significantly increased HIV-1 reactivation with or without stimulation (Fig. 5D). These results demonstrate a role of SAFB1 in maintaining viral latency by its repression of HIV-1 reactivation from the integrated proviral DNA.

Discussion

Accumulating data have identified a crucial role of heterogeneous nuclear ribonucleoprotein (hnRNP) family members in modulating HIV-1 replication (34–36). HnRNP family proteins have a highly conserved RNA binding motif, and the majority of family members mediate HIV-1 repression by affecting viral RNA-related processes, such as decreasing HIV-1 RNA stability or impairing viral RNA splicing (37–39). SAFB1 has a similar highly conserved RNA binding motif and is considered a member of the hnRNP family. In our study, we found that SAFB1-mediated HIV-1 inhibition was not due to its binding to RNA, as deletion of the RNA recognition motif did not affect SAFB1-mediated HIV-1 repression; instead, it is by SAFB1 binding to the HIV-1 5′ LTR region and direct association with phosphorylated RNA pol II that SAFB1 impedes RNA pol II’s recruitment to the LTR and consequently represses HIV-1 transcription initiation and elongation (Fig. 5E). Our findings reveal a new mechanism of hnRNP-mediated HIV-1 inhibition and highlight the importance of analyzing protein–protein interactions to fully elucidate SAFB1 functions.

SAFB1 can bind to a promoter to regulate gene expression (26, 40). Although we have revealed binding of SAFB1 with the HIV-1 LTR Nuc-1 and Nuc-2 regions, SAFB1-mediated HIV-1 inhibition was not attributed to its capacity for binding with DNA, as deletion of the SAF box that mediates binding to the S/MAR of DNA elements did not affect SAFB1’s inhibitory role in HIV-1 infection. Binding of SAFB1 with the HIV-1 LTR Nuc-1 and Nuc-2 region might facilitate the association with RNA pol II. The scaffold attachment factor A (SAAF) protein is another member of these SAFB family proteins and can specifically target the 3′ LTR of HIV-1 mRNA and blocks the cytoplasmic accumulation of HIV-1 mRNAs (41).

Phosphorylation of RNA pol II at Ser-2 sites restarts HIV-1 transcription. Several nuclear factors, such as the positive transcription elongation factor (P-TEFb) complex, affect SAFB1 transcription elongation by interaction with RNA pol II. SAFB1 has been reported to be associated with the CTD of RNA pol II.
SAFB1 suppresses HIV-1 infection in CD4+ T cells

SAFB1 also shows an interaction with the enhancer of zeste homolog 2 (EZH2), a component of polycomb repressive complex 2 (PRC2), and this interplay may facilitate the transition of chromatin from a repressive to an active state and contribute to the activation of skeletal muscle gene expression during myogenic differentiation (42). In our study, we also investigated the potential modulation of SAFB1 on EZH2 functions. EZH2 catalyzes the addition of methyl groups to histone H3 at lysine 27 (H3K27me3); we found that SAFB1 depletion in Jurkat cells did not affect the H3K27me3 level (data not shown), implying that SAFB1 does not repress HIV-1 replication through modulation of EZH2 functions.

Reversible silencing of transcription driven by the viral 5’ LTR promoter is the major determinant for the establishment and maintenance of HIV-1 post-integrational latency (1, 2). HIV-1 transcription can be modulated by numerous host factors. By using latently HIV-1–infected C11 cells and ACH2 cells, we demonstrated that SAFB1 repressed HIV-1 reactivation from an integrated proviral DNA and maintains HIV-1 latency. Resting CD4+ T cells isolated from patients under combination antiretroviral therapy are another cellular model for studying HIV-1 latency (43) that may provide an alternative way to further validate our results. Taken together, in this study, we have identified a novel role of SAFB1 for repressing HIV-1 transcription and infection. Our findings facilitate a better understanding of host modulation of HIV-1 transcription and latency, and they might provide a new host cell target for HIV-1 cure strategies.

Figure 5. SAFB1 represses HIV-1 reactivation from an integrated HIV-1 proviral DNA. A and B, SAFB1 knockdown promoted HIV-1 reactivation in C11 cells. A, C11 cells were infected with lentiviruses containing SAFB1 shRNA or an off-target control for 72 h. SAFB1 knockdown was determined by Western blotting. B, cells were stimulated with or without TNFα for an additional 24 h, and HIV-1 reactivation was measured by detecting GFP expression. The percentage of GFP+ cells and the mean fluorescence intensity (MFI) were calculated. C, SAFB1 knockdown in ACH2 cells. ACH2 cells were infected with lentiviruses containing SAFB1-specific or off-target shRNA for 72 h, during which puromycin was added for selection 24 h post-infection. SAFB1 knockdown from four independent repeats was detected by Western blotting. D, cells were stimulated with TNFα or SAHA for an additional 24 h. Viral reactivation was detected by quantifying the produced viral particles in the supernatant with p24capture ELISA. Data from four independent repeats are shown. Data are presented as mean ± S.D. *, p < 0.05; **, p < 0.01; ***, p < 0.001. E, schematic illustration of SAFB1 inhibiting HIV transcription. SAFB1 bound to the HIV-1 5’ LTR region and directly associates with the phosphorylated RNA pol II to impede RNA pol II recruitment to the LTR and consequently repress HIV-1 transcription.
SAFB1 suppresses HIV-1 infection in CD4\(^+\) T cells

**Experimental procedures**

**Cells**

Human peripheral blood mononuclear cells from healthy donors were purchased from the Blood Center of Shanghai (Shanghai, China). Resting CD4\(^+\) T cells were isolated from peripheral blood mononuclear cells by using anti-CD4 antibody–coated magnetic beads (Miltenyi Biotec) as described previously (44). CD4\(^+\) T cells were cultured in the presence of 20 international units/ml recombinant IL-2 (R&D Systems) and activated with 5 \(\mu\)g/ml phytohemagglutinin-P (PHA-P) (Sigma-Aldrich) for 3 days. The latently HIV-1–infected CD4\(^+\) CEM cell clone ACH2 contains a single copy of proviral DNA per cell (31, 45) and was provided by Dr. Shi-Bo Jiang (Fudan University, Shanghai, China). Latently HIV-1–infected Jurkat T cells (C11 clone) were provided by Dr. Huan-Zhang Zhu (Fudan University, Shanghai, China). Cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 \(\mu\)g/ml streptomycin.

**Virus stock**

Single-cycle infectious HIV-Luc/VSV-G and HIV-luc/NL4-3 were produced by calcium phosphate–mediated co-transfection of HEK293T cells with pLAI-Env-Luc and an expression plasmid of vesicular stomatitis virus G (VSV-G) protein or envelope protein (Env) of HIV-1 NL4-3 as described previously (46–48). Replication-competent HIV\(_{NL4-3}\) virus was obtained by transfection with HIV-1 vectors of pNL4-3. Plasmids were provided by Dr. Li Wu (Ohio State University, Columbus, OH). Harvested supernatants that contained viral particles were filtered and quantified with p24\(^{\text{Gag}}\) capture ELISA (49). Calcium phosphate–mediated transfection of HEK293T cells was used to generate shRNA lentiviruses as described previously (50).

**Viral titration in TZMB1 indicator cells**

HIV production was titrated in TZMB1 indicator cells, which contain an LTR-driven luciferase reporter, as described previously (47). Briefly, equal amounts of cell culture supernatant containing replication-competent HIV were used to infect TZMB1 cells for 48 h, and HIV infection was detected by measuring the luciferase activity.

**Transfection with plasmids, shRNAs, and siRNAs**

The HA-tagged SAFB1 expression plasmid pCDNA3.1-HA/SAFB1 was constructed and used to generate deletion mutations. SAFB1-specific shRNA and off-target shRNA were cloned into the PLKO.1-puro shRNA expression vector. The sequences of shRNAs were as follows: off-target, 5'-TTC TCC GAA CGT GTC ACG TAT-3'; sh-SAFB1, 5'-AAC AAG GCT ATG TCT TGT TAG-3'. siRNAs used for SAFB1 knockdown were synthesized (GenePharma, Shanghai, China). The sequences of siRNAs were as follows: off-target, 5’-UUC UUC GAA CGU GUC ACG UTT-3’; si-SAFB1-1, 5’-GUU AUC CUG ACG AAA UUG A-3’; si-SAFB1-2, 5’-AAG GCU AUG UUC UGU UAG GAG-3’. Plasmids and siRNAs were transfected into HEK293T cells by Lipofectamine 2000 (Invitrogen) and Jurkat T cells by an electroporation system (NEPA 21, NEPA GENE).

**Real-time PCR**

Total cellular RNA from different treated HEK293T cells was isolated by TRIzol reagent (Invitrogen) and reverse-transcribed into complementary DNA using ReverTra Ace qPCR RT Master Mix (Toyobo). Total DNA was abstracted with the QIAamp DNA Blood Mini Kit (Qiagen). Real-time PCR was performed using the Thunderbird SYBR qPCR Mix (Toyobo) on the ABI 7900HT real-time PCR system. Primers were as follows: Gag forward, 5’-GTT TGG AAA ATC TCT AGC AGT GG-3’; Gag reverse, 5’-CGC TCT CGC ACC CAT CTC-3’; Actin forward, 5’-GGG AAA TCG TGC GTG ACA T-3’; Actin reverse, 5’-GTC AGG CAG CTC GTA GCT CTT-3’. The integrated HIV-1 proviral DNA was quantified using a two-step Alu-PCR as described previously (46, 51). The following primers and probe were used for Alu-PCR: Alu forward, 5’-AGC CTC CCG AGT AGC TGG GA-3’; Alu reverse, 5’-TGC TGG GAT TAC AGG CTT GAG-3’; First gag reverse, 5’-CAA TAT CAT ACG CCG AGA GTG CGC G CTT CAG CAA G-3; second LTR forward, 5’-TTG TTA CAC CCT ATG AGC CAG C-3’; second tag reverse, 5’-CAA TAT CAT ACG CCG AGA GTG C-3; probe, 5’-(FAM)-AAG TAG TGT GTG CCC TTC TGT GTG ACT C-(TAMRA)-3’. HIV-1 transcription initiation and elongation were assessed by qPCR with specific primers as described previously (5).

**Co-IP assay**

HEK293T cells were collected and lysed with radioimmune precipitation assay buffer (20 mM Tris (pH 7.5), 150 mM NaCl, 1% Triton X-100, and protease inhibitor mixture) for 1 h on ice. 2 \(\mu\)g of antibodies was added to the lysis buffer and incubated overnight at 4 °C, followed by incubation with 20 \(\mu\)l of protein A or G magnetic beads for 3 h. Immunoprecipitates were obtained after washing four times and identified by immunoblotting.

**ChIP assay**

Cells were cross-linked with 1% formaldehyde for 10 min at room temperature and quenched with 0.125 mol/liter glycine for 5 min. After lysis, chromatin was sheared by use of a sonicator for a total of 12 min (2 s on and 6 s off) on ice to obtain DNA fragments of 200 to 500 bp. Five percent of the total sheared chromatin DNA was used as input DNA. Other sheared chromatin was incubated overnight at 4 °C with an antibody against pSer-2 RNA pol II (Abcam), SAFB1 (Abcam), or IgG (Cell Signaling Technology), followed by incubation with 40 \(\mu\)l of protein A or G magnetic beads for 3 h. After washing and reversing the cross-link, the input and immunoprecipitated DNA were purified and analyzed by real-time PCR using primers specifically targeting the HIV-1 LTR Nuc0, DHS, and Nuc2 regions, which have been described previously (6): Nuc0 forward, 5’-TGG ATC TAC CAC ACA ACA GAG G-3’; Nuc0 reverse, 5’-GTA CTA ACT TGA AGC ACC ACC T-3’; DHS forward, 5’-AAG TTT TCT GAG GAC CTC CTA GC-3’; DHS reverse, 5’-CAC ACC TTC CTG GAA AGT C-3’; Nuc1 forward, 5’-TCT CGT GCT AAC TAG GGA ACC-3’; Nuc1 reverse, 5’-CTA AAA GGG TCT GAG GGA TCT C-3’; Nuc2 forward, 5’-AGA GAT GGG TCG GAG AGC-3’; Nuc2 reverse, 5’-ATT AAC TGC GAA TCG TTC TAG C-3’.
SAFB1 suppresses HIV-1 infection in CD4+ T cells

Confocal microscopy

HEK293T cells were cultured on poly-lysine–coated glass coverslips and transfected with HA-tagged SAFB1 or truncated SAFB1 for 24 h. Cells were then fixed with 4% paraformaldehyde (Sigma–Aldrich), permeated with 0.3% Triton X-100, and blocked with 10% goat serum. Specific antibodies for the HA tag (Abcam) were incubated with cells overnight at 4 °C, followed by secondary antibodies of Alexa 555–labeled IgG (Invitrogen). Nuclei were stained with 4′,6-diamidino-2-phenylindole (Invitrogen). Slides were mounted with fluorescent mounting medium (Dako) and observed using a laser-scanning confocal microscope (Olympus FV-1200).

Statistical analysis

Statistical analysis was performed using paired or unpaired Student’s t test with SigmaStat 2.0 (Systat Software, San Jose, CA).

Author contributions—L. M. and L. S. data curation; L. M., X. J., and J.-H. W. formal analysis; L. M. and L. S. methodology; L. M. and J.-H. W. writing–original draft; X. J. validation; X. J. and J.-H. W. writing–review and editing; S.-D. X. and J.-H. W. supervision; S.-D. X. and J.-H. W. project administration; J.-H. W. conceptualization; J.-H. W. funding acquisition.

Acknowledgments—We thank Dr. Li Wu, Dr. Huan-Zhang Zhu, and Dr. Shi-Bo Jiang for kind gifts of cells and reagents.

References

1. Margolis, D. M. (2010) Mechanisms of HIV latency: an emerging picture of complexity. Curr. HIV/AIDS Rep. 7, 37–43 CrossRef Medline
2. Mbonye, U., and Karin, J. (2014) Transcriptional control of HIV latency: cellular signaling pathways, epigenetics, happenstance and the hope for a cure. Virology 454, 328–339 Medline
3. Lusic, M., and Giacca, M. (2015) Regulation of HIV latency by chromatin structure and nuclear architecture. J. Mol. Biol. 427, 688–694 CrossRef Medline
4. Francis, A. C., Di Primio, C., Allouch, A., and Cereseto, A. (2011) Role of phosphorylation in the nuclear biology of HIV-1. Curr. Med. Chem. 18, 2904–2912 CrossRef Medline
5. Zhu, J., Gaia, G. D., John, S. P., Pertel, T., Chin, C. R., Gao, G., Qu, H., Walker, B. D., Elledge, S. J., and Brass, A. L. (2012) Reactivation of latent HIV-1 by inhibition of BRD4. Cell Rep. 2, 807–816 CrossRef Medline
6. Rafati, H., Parra, M., Hakre, S., Moshkin, Y., Verdin, E., and Mahmoudi, T. (2011) Repressive LTR nucleosome positioning by the SAF-B complex is required for HIV latency. PLoS Biol. 9, e1001206 CrossRef Medline
7. Mahmoudi, T. (2012) The SAF-B complex and HIV latency. Transcription 3, 171–176 CrossRef Medline
8. Manson McManany, M. E., Hakre, S., Verdin, E. M., and Margolis, D. (2014) Therapy for latent HIV-1 infection: the role of histone deacetylase inhibitors. Antivir. Chem. Chemother. 23, 145–149 Medline
9. Schwartz, C., Bouchat, S., Marban, C., Gautier, V., Van Lint, C., Rohr, O., and Le Douce, V. (2017) On the way to find a cure: purging latent HIV-1 reservoirs. Biochem. Pharmacol. 146, 10–22 CrossRef Medline
10. Li, Z., Guo, J., Wu, Y., and Zhou, Q. (2013) The BET bromodomain inhibitor JQ1 activates HIV latency through antagonizing Brd4 inhibition of Tat-transactivation. Nucleic Acids Res. 41, 277–287 CrossRef Medline
11. Renz, A., and Fackelmayer, F. O. (1996) Purification and molecular cloning of the scaffold attachment factor B (SAF-B), a novel human nuclear protein that specifically binds to S/MAR-DNA. Nucleic Acids Res. 24, 843–849 CrossRef Medline
12. Norman, M., Rivers, C., Lee, Y. B., Idris, J., and Uney, J. (2016) The increasing diversity of functions attributed to the SAFB family of RNA–DNA-binding proteins. Biochem. J. 473, 4271–4288 CrossRef Medline
13. Alfonso-Parra, C., and Maggert, K. A. (2010) Drosophila SAF-B links the nuclear matrix, chromosomes, and transcriptional activity. PLoS ONE 5, e10248 CrossRef Medline
14. Debril, M. B., Dubuquoy, L., Feige, J. N., Wahl, W., Desvergne, B., Auwerx, J., and Gelman, L. (2005) Scaffold attachment factor B1 directly interacts with nuclear receptors in living cells and represses transcriptional activity. J. Mol. Endocrinol. 35, 503–517 CrossRef Medline
15. Garee, J. P., and Oesterreich, S. (2010) SAFB1’s multiple functions in biological control: lots still to be done! J. Cell. Biochem. 109, 312–319 CrossRef Medline
16. Drakoulis, S., Lyberopoulou, A., Papathanassiou, M., Mylonis, I., and Georgatsou, E. (2017) Enhancement of rudimentary homologue interacts with scaffold attachment factor B at the nuclear matrix to regulate SR protein phosphorylation. FEBS J. 284, 2482–2500 CrossRef Medline
17. Altmeyer, M., Toledo, L., Gudjonsson, T., Grofte, M., Rask, M. B., Lukas, C., Akimov, V., Blagoev, B., Bartek, J., and Lukas, J. (2013) The chromatin scaffold protein SAFB renders chromatin permissive for DNA damage signaling. Mol. Cell 52, 206–220 CrossRef Medline
18. Nayler, O., Strätling, W., Bourquin, J. P., Stagglar, J., Lindemann, L., Jasper, H., Hartmann, A. M., Fackelmayer, F. O., Ullrich, A., and Stamms, S. (1998) SAF-B protein couples transcription and pre-mRNA splicing to SAF/MAR elements. Nucleic Acids Res. 26, 3542–3549 CrossRef Medline
19. Townsend, S. M., Kang, K., Lee, A. V., and Oesterreich, S. (2004) Structure–function analysis of the estrogen receptor α corepressor scaffold attachment factor-B1: identification of a potent transcriptional repression domain. J. Biol. Chem. 279, 26074–26081 CrossRef Medline
20. Mukhopadhyay, N. K., Kim, J., You, S., Morello, M., Hager, M. H., Huang, W. C., Ramachandran, A., Yang, J., Cinar, B., Rubin, M. A., Adam, R. M., Oesterreich, S., Di Vizio, D., and Freeman, M. R. (2014) Scaffold attachment factor B1 regulates the androgen receptor in concert with the growth inhibitory kinase MST1 and the methyltransferase EZH2. Oncogene 33, 3235–3245 CrossRef Medline
21. Lin, J., Xu, P., LaVallee, P., and Hoidal, J. R. (2008) Identification of proteins binding to E-Box/Ku86 sites and function of the tumor suppressor SAFB1 in transcriptional regulation of the human xanthine oxidoreductase gene. J. Biol. Chem. 283, 29681–29689 CrossRef Medline
22. Liu, H. W., Banerjee, T., Guan, X., Freitas, M. A., and Parvin, J. D. (2015) The chromatin scaffold protein SAFB1 localizes SUMO-1 to the promoters of ribosomal protein genes to facilitate transcription initiation and splicing. Nucleic Acids Res. 43, 3605–3613 CrossRef Medline
23. Ren, X. X., Wang, H. B., Li, C., Jiang, J. F., Xiong, S. D., Jin, X., Wu, L., and Wang, J. H. (2016) HIV-1 Nef-associated factor 1 enhances viral production by interacting with CRM1 to promote nuclear export of unspliced HIV-1 gag mRNA. J. Biol. Chem. 291, 4580–4588 CrossRef Medline
24. Melnik, S., Deng, B., Papantonis, A., Baboo, S., Carr, J. I., and Cook, P. R. (2011) The proteomes of transcription factories containing RNA polymerase I or II or III. Nat. Methods 8, 963–968 CrossRef Medline
25. Verdin, E., Paras, P. J., and Van Lint, C. (1993) Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcription activation. EMBO J. 12, 3249–3259 Medline
26. Omura, Y., Nishio, Y., Takemoto, T., Ikeuchi, C., Sekine, O., Morino, K., Maeno, Y., Obata, T., Ugi, S., Maegawa, H., Kimura, H., and Kashigawa, A. (2009) SAFB1, an RBMX-binding protein, is a newly identified regulator of hepatic SREBP-1c gene. BMB Rep. 42, 232–237 CrossRef Medline
27. Cary, D. C., Fujinaga, K., and Peterlin, B. M. (2016) Molecular mechanisms of HIV latency. J. Clin. Invest. 126, 448–454 CrossRef Medline
28. Hakre, S., Chavez, L., Shirakawa, K., and Verdin, E. (2011) Epigenetic regulation of HIV latency. Curr. Opin. HIV AIDS 6, 19–24 CrossRef Medline
29. Qu, X., Wang, P., Ding, D., Li, L., Wang, H., Ma, L., Zhou, X., Liu, S., Lin, S., Wang, X., Zhang, G., Liu, S., Liu, L., Wang, J., Zhang, F., et al. (2013) Zinc-finger–nucleases mediate specific and efficient excision of HIV-1 proviral DNA from infected and latently infected human T cells. Nucleic Acids Res. 41, 7771–7782 CrossRef Medline
SAFB1 suppresses HIV-1 infection in CD4+ T cells

30. Ding, D., Qu, X., Li, L., Zhou, X., Liu, S., Lin, S., Wang, P., Liu, S., Kong, C., Wang, X., Liu, L., and Zhu, H. (2013) Involvement of histone methyltransferase GLP in HIV-1 latency through catalysis of H3K9 dimethylation. *Virology* **440**, 182–189 CrossRef Medline

31. Schaub, M. C., Lopez, S. R., and Caputi, M. (2007) Members of the heterogeneous nuclear ribonucleoprotein H family activate splicing of an HIV-1 splicing substrate by promoting formation of ATP-dependent spliceosomal complexes. *J. Biol. Chem.* **282**, 13617–13626 CrossRef Medline

32. O'Hagan, A., Bandarenko, N., Schmitz, J. L., Bosch, R. J., Landay, A. L., Coffin, J. M., and Margolis, D. M. (2008) Valproic acid without intensified antiviral therapy has limited impact on persistent HIV infection of resting CD4+ T cells. *AIDS* **22**, 1131–1135 CrossRef Medline

33. Wolf, D., Witte, V., Clark, P., Lichtenheld, M. G., and Baur, A. S. (2008) HIV Nef enhances Tat-mediated viral transduction through a hnRNP-K-nucleated signaling complex. *Cell Host Microbe* **4**, 398–408 CrossRef Medline

34. Jean-Philippe, J., Paz, S., Lu, M. L., and Caputi, M. (2014) A truncated hnRNP A1 isoform, lacking the RGG-box RNA binding domain, can efficiently regulate HIV-1 splicing and replication. *Biochim. Biophys. Acta* **1839**, 251–258 CrossRef Medline

35. Woolaway, K., Atasi, K., Emili, A., and Cochrane, A. (2007) HnRNP E1 and E2 have distinct roles in modulating HIV-1 gene expression. *Retrovirology* **4**, 28 CrossRef Medline

36. Archin, N. M., Eron, J. J., Justement, J., Rabson, A., Duh, E., Kehrl, J. H., and Fauci, A. S. (1989) Tumor necrosis factor a induces expression of human immunodeficiency virus in a chronically infected T-cell clone. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 2365–2368 CrossRef Medline

37. Folks, T. M., Clouse, K. A., Justement, J., Rabson, A., Duh, E., Kehrl, J. H., and Fauci, A. S. (1989) Tumor necrosis factor a induces expression of human immunodeficiency virus in a chronically infected T-cell clone. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 2365–2368 CrossRef Medline

38. Mallal, S., Lewin, S. R., and Cameron, P. U. (2017) HIV integration sites in human CD4+ T cells. *AIDS* **31**, 182–189 CrossRef Medline

39. Emili, S., Van Lint, C., Fischle, W., Paras, P., Jr., Ott, M., Brady, J., and Fischle, W. (2009) Transcriptional restriction of human immunodeficiency virus type 1 gene expression in undifferentiated human primary monocytes. *J. Virol.* **83**, 2928–2937 CrossRef Medline

40. Jiang, A. P., Jiang, J. F., Wei, J. F., Guo, M. G., Qin, Y., Guo, Q. Q., Ma, L., Liu, B. C., Wang, X., Veazey, R. S., Ding, Y. B., and Wang, J. H. (2015) Human mucosal mast cells capture HIV-1 and mediate viral trans-infection of CD4+ T cells. *J. Virol.* **89**, 8050–8062 CrossRef Medline

41. Jiang, A. P., Jiang, J. F., Wei, J. F., Guo, M. G., Qin, Y., Guo, Q. Q., Ma, L., Liu, B. C., Wang, X., Veazey, R. S., Ding, Y. B., and Wang, J. H. (2015) Human mucosal mast cells capture HIV-1 and mediate viral trans-infection of CD4+ T cells. *J. Virol.* **89**, 2928–2937 CrossRef Medline

42. Caputi, M., Mayeda, A., Krainer, A. R., and Zahler, A. M. (1999) hnRNP A/B proteins are required for inhibition of HIV-1 pre-mRNA splicing. *EMBO J.* **18**, 4060–4067 CrossRef Medline

43. O'Brien, M. C., Ueno, T., Jahan, N., Zajac-Kaye, M., and Mitsuya, H. (1995) IFN-γ represses HIV-1 gene expression induced by anti-cancer agents in latently HIV-1-infected ACH2 cells. *Biochem. Biophys. Res. Commun.* **207**, 903–909 CrossRef Medline

44. Wolf, D., Witte, V., Clark, P., Lichtenheld, M. G., and Baur, A. S. (2008) HIV Nef enhances Tat-mediated viral transduction through a hnRNP-K-nucleated signaling complex. *Cell Host Microbe* **4**, 398–408 CrossRef Medline

45. Schaub, M. C., Lopez, S. R., and Caputi, M. (2007) Members of the heterogeneous nuclear ribonucleoprotein H family activate splicing of an HIV-1 splicing substrate by promoting formation of ATP-dependent spliceosomal complexes. *J. Biol. Chem.* **282**, 13617–13626 CrossRef Medline

46. Caputi, M., Mayeda, A., Krainer, A. R., and Zahler, A. M. (1999) hnRNP A/B proteins are required for inhibition of HIV-1 pre-mRNA splicing. *EMBO J.* **18**, 4060–4067 CrossRef Medline