HIV-1 Nef-associated Factor 1 Enhances Viral Production by Interacting with CRM1 to Promote Nuclear Export of Unspliced HIV-1 gag mRNA

HIV-1 depends on host-cell-encoded factors to complete its life cycle. A comprehensive understanding of how HIV-1 manipulates host machineries during viral infection can facilitate the identification of host targets for antiviral drugs or gene therapy. The cellular protein Naf1 (HIV-1 Nef-associated factor 1) is a CRM1-dependent nucleo-cytoplasmic shuttling protein, and has been identified to regulate multiple receptor-mediated signal pathways in inflammation. The cytoplasm-located Naf1 can inhibit NF-κB activation through binding to A20, and the loss of Naf1 controlled NF-κB activation is associated with multiple autoimmune diseases. However, the effect of Naf1 on HIV-1 mRNA expression has not been characterized. In this study we found that the nucleus-located Naf1 could promote nuclear export of unspliced HIV-1 gag mRNA. We demonstrated that the association between Naf1 and CRM1 was required for this function as the inhibition or knockdown of CRM1 expression significantly impaired Naf1-promoted HIV-1 production. The mutation of Naf1 nuclear export signals (NESs) that account for CRM1 recruitment for nuclear export decreased Naf1 function. Additionally, the mutation of the nuclear localization signal (NLS) of Naf1 diminished its ability to promote HIV-1 production, demonstrating that the shuttling property of Naf1 is required for this function. Our results reveal a novel role of Naf1 in enhancing HIV-1 production, and provide a potential therapeutic target for controlling HIV-1 infection.

This work was supported by grants (to J.-H. W.) from the Interdisciplinary and Collaboration Team of the Chinese Academy of Sciences, the Natural Science Foundation of China (No. 81572001), the National Basic Research Program of China (973 Program) (2011CB510004), the National Grant Program on Key Infectious Disease (2014ZX10001003), the NSFC-NIH Joint Grant (81561128009), and Grants AI120209 and AI104483 (to L. W.) from the NIH/NIAID. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication. The authors declare that they have no conflicts of interest with the contents of this article. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

† This article contains supplemental Movies A and B.

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1 To whom correspondence should be addressed: Inst. Pasteur of Shanghai, Chinese Academy of Sciences, 320 Yueyang Rd., Shanghai 200031, China. Tel.: (86)-21-54923120; E-mail: jh_wang@siibs.ac.cn.
In this study, we revealed a novel role of the host protein Naf1 (HIV-1 Nef-associated factor 1) in promoting Rev/CRM1 function during HIV-1 production. Naf1 is also known as virion-associated nuclear shuttling protein and has been identified as a HIV-1 Nef-binding protein that increases the expression of cell surface CD4 (34). Naf1 is also known as A20-binding inhibitors of NF-κB activation (ABIN-1) (35–36), and three ABIN proteins have been identified to bind with the deubiquitinating protein A20 for inhibiting NF-κB activation (37–40).

Here we found that Naf1 is associated with CRM1 for nucleo-cytoplasmic shuttling and promotes the nuclear export of unspliced mRNA of HIV-1 gag. The novel role of cellular factor Naf1 in regulating HIV-1 production could provide a potential therapeutic target for controlling HIV-1 infection.

**Experimental Procedures**

**Cells and Virus Stock**—HEK293T cells were cultured in DMEM medium (Hyclone) containing 10% fetal bovine serum (FBS) (Hyclone) and 100 units/ml penicillin and 100 μg/ml streptomycin. Calcium-phosphate-mediated transfection of HEK293T cells was used to generate virus stock as described previously (41). Pseudotyped single-cycle infectious HIV-H131/VSV-G was cotransfected with the plasmid pHIV/H131 and the expression plasmid vesicular stomatitis virus G (VSV-G) protein. Harvested supernatants of transfected cells that contained viral particles were filtered and titrated with VSV-G protein for 4 h (2 ng p24gag), and after washing, cells were further cultured for 24 h. Total RNA from the cells was extracted using TRIzol reagent (Life Technologies). Nuclear and cytoplasmic RNA fractions were purified by using a PARIS Protein and RNA isolation kit (Ambion, Life Technologies) following the manufacturer’s protocol.

**Plasmids and Short Hairpin (shRNA) Vectors**—The human Naf1 gene TNIP1 was cloned into pEGFP-N1 (pEGFP-Naf1), or pCMV-tag3B vector with a Myc tag at the amino terminus (pCMV-Myc-Naf1), or pCDH-CMV-MCS-EFl1-Puro vector (Clontech) with a FLAG tag at the carboxyl terminus (pCDH-CMV-Maf1-Flag). The point mutations in the Naf1 NES and NLS were generated by using PCR-based mutagenesis on pCMV-Myc-Naf1, respectively. The human CRM1 gene was cloned into pcDNA3.1 with a HA tag at the carboxyl terminus (pcDNA3.1-CRM1-HA), or pCDH-CMV-MCS-EFl1-Puro vector (pCDH-CMV-CRM1-Flag). HIV-1 Rev gene was also cloned into pcDNA3.1 vector (pcDNA3.1-Rev-HA). Naf1 and CRM1 shRNA, as well as scramble shRNA were cloned into pLKO.1-Puro vector (Addgene), and the sequences were as follows: scramble shRNA (off-target): 5′-CCGGTTTCTCCGAACGTGTCACGTATCTCGAGATACGTGACACGTTCGGGGAATTTGT-3′ (sense), 5′-AATCTCAAAAATCTCCTCAGCTGCTTCT--3′ (antisense); TNIP1 (TNFα-induced protein 3-interactive protein 1, Naf1) shRNA: 5′-CCGGATTACGAGTCTCGAGATCGTAGTCTCGAGATCTGGAGCTGCTGAAATTTGG-3′ (sense), 5′-AATCTCAAAAATCTCCTCAGCTGCTTCT--3′ (antisense); CRM1 shRNA: 5′-CCGGGCTTCACACCATCAAGTGAATTGCTCGAGCATCTCAGCTGAGGATTGAGTTTT-3′ (sense), 5′-AATCTCAAAAATCTCCTCAGCTGCTTCT--3′ (antisense). Naf1-stably-knocking-down HEK293T cells were developed by administrating Naf1-specific shRNA via puromycin screened/lentiviral vector. HIV-1-derived expression vector pH131 was a kind gift from Dr. Derya Unutmaz (New York University), and this vector was derived from HIV-1-eGFP construct with the deletions of nef, vif, vpr, vpu, and env (43–44).

**Transfection and HIV-1 Infection Assays**—Transfection was performed by using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. At 6 h post-transfection, the medium containing the mixture of plasmids and transfection reagents was replaced with fresh DMEM supplemented with 10% FBS, and then cells were further cultured for 24 h. For infection, Naf1-stable-knocking-down or off-target HEK293T cells were inoculated with HIV-H131/VSV-G for 4 h (2 ng p24gag), and after washing, cells were further cultured for 24 h, then culture supernatants and cell lysates were harvested to quantify viral production by p24gag capture ELISA.

**Cytotoxicity Assay by MTT Method**—LMB cytotoxicity was assessed by MTT method as described previously (45). Briefly, HEK293T cells (2 × 10⁴) seeded on a 96-well plate were transfected with pCMV-Myc-Naf1 or empty vector and pHIV/H131 plasmid for 18 h. Various concentrations of LMB were added for an additional 6 h of cell culture. 20 μl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (the stock concentration is 5 mg/ml) was added and incubated at 37 °C for 4 h. Then 100 μl of 10% SDS (sodium dodecyl sulfate)-50% DMF (N,N′-dimethyl formamide) was added and incubated until the formazan was dissolved completely. The plates were read on a Bio-Tek ELx 800 enzyme-linked immunosorbent assay (ELISA) reader at 595/630 nm. The cell viability (% of untreated control) was calculated by measuring absorbance values.

**Nucleo-cytoplasmic HIV-1 RNA Fractionation and Quantification**—HEK293T cells were co-transfected with pCMV-Myc-Naf1 (0.4 μg) or pCMV-tag3B vector with pHIV/H131 (0.4 μg) for 24 h. HEK293T cells with stable Naf1 knockdown or the off-target control were inoculated with HIV-H131/VSV-G for 4 h (4 ng p24gag), and after washing, cells were further cultured for 24 h. Total RNA from the cells was extracted using TRIzol reagent (Life Technologies). Nuclear and cytoplasmic RNA fractions were purified by using a PARIS Protein and RNA isolation kit (Ambion, Life Technologies) following the manufacturer’s protocol. U6 snRNA and 18S rRNA were used as nuclear or cytoplasmic RNA control, respectively.

RNA was reverse transcribed into cDNA using the PrimeScript RT Reagent Kit with gDNA Eraser (Takara). Real-time PCR was performed using the Thunderbird SYBR qPCR Mix (Toyobo) with pre-denaturation at 95 °C for 1 min, amplification with 40 cycles of denaturation (95 °C, 15 s), and annealing (60 °C, 45 s), on the ABI 7900HT Real-Time PCR system. The data were analyzed by green-based SYBR, semi-quantified and normalized with β-actin. HIV-1 transcriptional products of unspliced RNA (gag), and multiply spliced RNA (tat-rev) were detected, respectively, and relative expression was calculated. The primers were listed as below: β-actin-forward, 5′-GGGGAATCTGTGCTGTGACAT-3′, β-actin-reverse, 5′-CTCGAGGAGTCTCTGTGATCT-3′; Gag-forward, 5′-GGTGGAGAAAATCTTCTAGCGAG-3′, Gag-reverse, 5′-CTCGAGGAGTCTCTGTGATCT-3′; Tat-rev-forward, 5′-CTCGAGGAGTCTCTGTGATCT-3′; Tat-rev-reverse, 5′-CTCGAGGAGTCTCTGTGATCT-3′; 18S rRNA-forward, 5′-CAGCGACCTCCACTCTCT Commission of the European Communities-3′; 18S rRNA-reverse, 5′-TGCAGAGAGATACTTTCGACG-3′; U6-5′-AATGTGACAATTTTG-3′; 18S rRNA-forward, 5′-CAGCGACCTCCACTCTCT 18S rRNA-reverse, 5′-TGCAGAGAGATACTTTCGACG-3′; U6-5′-AATGTGACAATTTTG-3′;
Naf1 Regulates HIV-1 Production

FIGURE 1. Naf1 expression is required for efficient HIV-1 production. A, endogenous Naf1 expression in HEK293T cells was knocked down with specific shRNA and detected by immunoblotting. B and C, knock-down of Naf1 significantly suppresses HIV-1 production in comparison to off-target control in HEK293T cells transfected with HIV-1-based expression vector pH131 (B), or infected with HIV-H131/VSV-G vector (C), and HIV-1 production was quantified by measuring p24gag amount 24 h later in cell culture supernatants and cell lysates. D and E, Naf1 overexpression enhances HIV-1 production. HEK293T cells were transfected with pCMV-Myc-Naf1, and Naf1 expression was detected by immunoblotting (D). In Naf1-overexpressed cells, viral p24 production was increased (E, F and G, Naf1 complementation in stable Naf1-knocking-down HEK293T cells restores HIV-1 production. Naf1 expression was complemented in stable Naf1-knocking-down HEK293T cells by transfected with pCMV-Myc-Naf1 for 24 h (F), and cells were transfected with the pHIV/H131 plasmid for additional 24 h and viral production was quantified by p24gag assay (G). GAPDH was used as a loading control (A, D, F). One representative of at least four independent repeats for each result is shown. The results were analyzed by the paired Student's t test; **, p < 0.01 and ***, p < 0.001 were considered statistically significant.

snRNA-forward, 5'-CTCGCCCTCGCCGCAGCA-3', U6 snRNA-reverse, 5'-AACGCCCTCAAGATTTGCGT-3'.

Immunoprecipitation and Immunoblotting Assays—HEK293T cells (8 × 10⁵) were co-transfected with pCDH-CMV-Naf1-Flag (2.5 μg) and pcDNA3.1-Rev-HA (2.5 μg), or pCDH-CMV-CRM1-Flag (2.5 μg) and cCMV-Myc-Naf1 (2.5 μg) or pcDNA3.1-Rev-HA for 24 h. Then cells were collected and lysed with IP lysis buffer with protease inhibitor mixture (Sigma-Aldrich). Immunoprecipitations were performed with anti-Flag M2 magnetic beads (Sigma-Aldrich) following the manufacturer’s protocol. Nuclear and cytoplasmic protein fractions were purified by NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific) as the manufacturer’s description. For immunoblotting, samples were first lysed with 1% Triton X-100 at 37 °C for 1 h, then were denatured in loading buffer at 100 °C for 5 min. Samples were electrophoresed on a 10% SDS-PAGE gel, and the gels were transferred to nitrocellulose membranes and blocked with 5% nonfat dry milk (NFDM) for 1 h and probed with the appropriate antibodies. The antibodies of anti-Myc, anti-Flag, anti-HA, anti-tubulin, anti-Histone3.1, and anti-GAPDH were purchased from Abmart.

Confocal Microscopy—HEK293T cells were seeded on the Cell Imaging Dish (35 × 10 mm) (Eppendorf), and transfected with pEGFP-Naf1 (0.2 μg) for 24 h as above. Three-dimensional reconstruction was performed using Nikon A1+ laser scanning confocal microscope, and live-cell imaging was performed with Leica TCS SP8 laser scanning confocal microscope. Nuclei were indicated with Hoechst 33258 (Sigma).

Statistical Analysis—Statistical analysis was performed using a paired t test with SigmaStat 2.0 software (Systat Software, San Jose, CA).

Results

Naf1 Expression Is Essential for HIV-1 Production—To better understand the effect of Naf1 on HIV-1 replication, we first investigated the role of Naf1 in HIV-1 production. The expression of endogenous Naf1 in HEK293T cells was successfully knock-down by using specific shRNA (Fig. 1A), then cells were transfected with the HIV-1-derived expression vector H131, and viral production was monitored by measuring the p24gag from both cell culture supernatants and cell lysates. The vector pH131 was derived from the HIV-1-eGFP construct with the deletions of nef, vif, vpr, vpu, and env, but this vector possesses HIV-1 rev and tat gene (43–44). The interference with Naf1 expression significantly reduced p24gag production (Fig. 1B).

To verify the necessity of Naf1 for HIV-1 production during viral infection, the HIV-derived H131 vector was used to generate VSV-G pseudotyped, single-cycle HIV-H131/VSV-G. HEK293T cells with or without knock-down of Naf1 were infected with HIV-H131/VSV-G and HIV-1 production was examined by measuring p24gag. Similar results indicate that knockdown of Naf1 significantly impaired HIV-1 production (Fig. 1C).

In contrast, when Naf1 was overexpressed (Fig. 1D), enhanced HIV-1 p24gag production from both supernatants and cell lysates was observed (Fig. 1E). Furthermore, when Naf1 expression was complemented in HEK293T cells with stable Naf1 knockdown (Fig. 1F), HIV-1 p24gag production from both supernatants, and cell lysates was recovered (Fig. 1G). Together, these data demonstrate that Naf1 expression is required for efficient HIV-1 production in virus-producing cells.

Naf1 Enhances the Nuclear Export of Unspliced HIV-1 gag mRNA—To examine the mechanism of Naf1-promoted HIV-1 production, the potential effect of Naf1 on viral transcription was investigated. HIV-1-based expression vector H131 was transfected into Naf1-overexpressing HEK293T cells. Time-course analysis showed that Naf1 overexpression did not affect the expression of total HIV-1 gag mRNA (Fig. 2A), suggesting...
that Naf1 mediates post-transcriptional enhancement of HIV-1 production.

Naf1 has been shown to be a nucleo-cytoplasmic shuttling protein (46), which prompted us to investigate the potential effect of Naf1 on nuclear export of transcribed HIV-1 mRNA (Fig. 2B). The pHIV/H131 plasmid was transfected into Naf1-overexpressing or vector control HEK293T cells, and the cytoplasmic and nuclear fractions were separated and the levels of HIV-1 mRNA were quantified (Fig. 2D). U6 snRNA and 18S rRNA was used as nuclear or cytoplasmic RNA control, respectively. C: cytoplasm, N: nucleus. D, Naf1 overexpression enhances the nuclear export of HIV-1 unspliced gag mRNA. Naf1-overexpressing or parental (pCMV-Myc vector) HEK293T cells were transfected with HIV/H131 for 24 h, then cells were collected and total mRNA were isolated, and HIV-1 gag mRNA were quantified as above, the relative cytoplasmic and nuclear distribution of HIV-1 mRNA were calculated, and 4 repeats were summarized. The horizontal lines in the dot plot (low panels) indicate the mean with S.D. E, Naf1 knock-down decreases the nuclear export of HIV-1 unspliced gag mRNA. The Naf1-stable-knock-down HEK293T or off-target cells were infected with the HIV-H131/VSV-G vector for 24 h, and the cytoplasmic/nuclear distribution of HIV-1 mRNA was monitored and the relative values were calculated. ***, p < 0.001 was considered significant as determined by the paired Student’s t test.

To confirm this finding, a single-cycle infectious HIV-H131/VSV-G vector was used to infect HEK293T cells with stable knock-down, of Naf1, and the nucleo-cytoplasmic distribution of HIV-1 mRNA was measured. Naf1 knock-down decreased the nuclear export of unspliced mRNA of HIV-1 gag from 33% to 18%, but only slightly altered the nuclear export of multiple-spliced HIV-1 tat-rev mRNA from 74% to 77% (Fig. 2E). We have observed that these viral unspliced RNAs at 24 h post-transfection or -infection were predominantly located in the nucleus (Fig. 2, D and E), which might suggest the different export dynamic for unspliced viral mRNA compared with multiple-spliced viral mRNA. Together, these data suggest that Naf1 expression facilitates efficient nuclear export of unspliced HIV-1 gag mRNA.

FIGURE 2. Naf1 expression promotes nuclear export of unspliced HIV-1 gag mRNA. A, effect of Naf1 on HIV-1 transcription. HEK293T cells were transfected with pCMV-Myc-Naf1 or empty vector and pHIV/H131 for 24 h. Cells were harvested at the indicated times post-transfection, and total mRNAs were isolated, and the levels of HIV-1 gag mRNA were quantified with qRT-PCR and normalized to β-actin mRNA. B, scheme for detection of cytoplasmic/nuclear mRNA. Cells were collected for isolation of cytoplasmic and nuclear mRNA, and the relative ratios of cytoplasmic and nuclear mRNA (unspliced gag mRNA or multi-spliced tat-rev mRNA) were analyzed by qRT-PCR. C, U6 snRNA and 18S rRNA was used as nuclear or cytoplasmic RNA control, respectively. C: cytoplasm, N: nucleus. D, Naf1 overexpression enhances the nuclear export of HIV-1 unspliced gag mRNA. Naf1-overexpressing or parental (pCMV-Myc vector) HEK293T cells were transfected with HIV/H131 for 24 h, then cells were collected and total mRNA were isolated, and HIV-1 gag mRNA were quantified as above, the relative cytoplasmic and nuclear distribution of HIV-1 mRNA were calculated, and 4 repeats were summarized. The horizontal lines in the dot plot (low panels) indicate the mean with S.D. E, Naf1 knock-down decreases the nuclear export of HIV-1 unspliced gag mRNA. The Naf1-stable-knock-down HEK293T or off-target cells were infected with the HIV-H131/VSV-G vector for 24 h, and the cytoplasmic/nuclear distribution of HIV-1 mRNA was monitored and the relative values were calculated. ***, p < 0.001 was considered significant as determined by the paired Student’s t test.
Naf1 Association with CRM1 Is Required for Augmenting HIV-1 Production—It has been reported that Naf1 nucleo-cytoplasmic shuttling is CRM1-dependent (46). To confirm the association between Naf1 and CRM1, co-immunoprecipitation assays were performed. The expression plasmids encoding FLAG-tagged Naf1 and HA-tagged Rev were co-transfected into HEK293T cells, then cells were harvested and lysed for immunoprecipitation with anti-Flag beads, and then immunoblotting was performed with specific antibodies against FLAG (Naf1), CRM1, or HA (Rev), respectively (Fig. 3A). Similarly, we
also co-transfected HEK293T cells with FLAG-tagged CRM1 and Myc-tagged Naf1 or HA-tagged Rev for co-immunoprecipitation (Fig. 3B). Our results confirmed the association between Naf1 and CRM1, and showed that Naf1 did not interact directly with HIV-1 Rev (Fig. 3, A and B).

To investigate whether association with CRM1 is required for Naf1 augmentation of HIV-1 production, the specific inhibitor LMB was used to block CRM1-dependent nuclear export pathway. LMB is known to bind to CRM1 to prevent the formation of nuclear export complex (47–49). We found that LMB treatment significantly impaired Naf1-promoted HIV-1 production in both supernatants and cell lysates (Fig. 3, C and D). LMB treatment did not cause apparent cytotoxicity (Fig. 3E). Similarly, when CRM1 expression in HEK293T cells was knocked-down with specific shRNA, Naf1-augmented HIV-1 production was significantly attenuated (Fig. 3, F and G). The CRM1 knock-down was confirmed by Western blotting (Fig. 3H). Conversely, CRM1 overexpression significantly increased

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Naf1-mediated augmentation of HIV-1 production (Fig. 3, I, J, and K). Together, these data demonstrate the requirement of CRM1 for Naf1-promoted HIV-1 production.

Disruption of NESs or NLS of Naf1 Attenuates the Enhancement for HIV-1 Production—Naf1 is a nucleo-cytoplasmic shuttling protein with characteristic intracellular trafficking (46). To confirm the intracellular trafficking of Naf1, the Naf1-GFP-expressing plasmid pEGFP-Naf1 was transfected into HEK293T cells, and Naf1 cellular localization was monitored. Naf1-GFP was distributed both in the cytoplasm and the nucleus as observed by confocal microscopy (Fig. 4A). When the transfected HEK293T cells were fractionated into the cytoplasmic and nuclear components, the distribution of Naf1 in both cytoplasm and nucleus was confirmed by Western blotting (Fig. 4B). The nucleo-cytoplasmic shuttling of Naf1 was also observed by time-lapse imaging (supplemental Videos A and B).

The leucine-rich NES in bearing proteins, like HIV-1 Rev, mediates CRM1 recruitment for nuclear export (14, 47, 50). Based on the analysis of amino acid sequence, Naf1 was predicted to contain four putative NESs that interact with CRM1 (51). When point mutations were introduced into NESs (Fig. 4, C and D), the mutation at NES1, 2, or 3, but not NES4, significantly attenuated Naf1-mediated enhancement of HIV-1 p24\(\text{ag}^{\text{ag}}\) production (Fig. 4F), suggesting that NES4 might be inadequately required for modulating Naf1 nuclear export. These data confirm that the interaction between Naf1 and CRM1 is required for Naf1-promoted HIV-1 production.

Naf1 has also been predicted to contain a C terminus-located, lysine-enriched, classical NLS (51) (Fig. 4C), and the mutation of NLS retained Naf1 in the cytoplasm (Fig. 4E). Additionally, the NLS mutant lost its capacity for promoting HIV-1 production (Fig. 4F), suggesting that the intact NLS is required for Naf1-mediated enhancement for HIV-1 production. These data indicate that the nucleus and cytoplasm shuttling property of Naf1 is required for promoting HIV-1 production.

Discussion

Our results reveal a novel role of cellular factor Naf1 in enhancing HIV-1 production. Naf1 associates with cellular CRM1 for promoting nuclear export of unspliced HIV-1 gag mRNA. The association of Naf1 and CRM1 is required for Naf1-promoted HIV-1 production, and the inhibition of CRM1 activity or knockdown of its expression hence attenuated Naf1’s function in enhancing HIV-1 production. Naf1 was predicted to contain four putative NESs that mediate CRM1 recruitment for nuclear export (51). Naf1 also contains a C terminus-located, lysine-enriched, classical NLS (51). Our mutagenesis of NES1–3 and NLS indicates that the shuttling property of Naf1 is essential for promoting viral production, as the disruption of NESs or NLS of Naf1 attenuates the enhancement of HIV-1 production. The mutation at NES4 did not significantly diminish viral production, suggesting a dispensable role of the predicted NES4 in regulating Naf1 trafficking.

It has been reported that Naf1 expression inhibited NF-κB activation and HIV-1 infection (37, 46, 51, 53). Naf1 associates with A20, a zinc finger protein with deubiquitinase activity, and facilitates A20-mediated de-ubiquitination of NEMO/IKK\(\gamma\) and subsequent NF-κB inhibition in response of TNF-α (40, 54, 56). HIV-1 LTR contains the NF-κB binding sites, and the suppression of Naf1 on NF-κB activation may account for the inhibition of HIV-1 promoter LTR-driven gene expression and viral infection. Naf1 can be incorporated into HIV-1 virions and interacts with the matrix protein of HIV-1 (46). HIV-1 matrix is a 17-kDa myristoylated protein derived from Gag precursor polyprotein and identified as a key component of HIV-1 pre-integration complex to contribute to its nuclear localization (57–59). Notably, HIV-1 matrix is also involved in the viral assembly by directing Gag and Gag-Pol polyproteins to the plasma membrane during viral production (55). These data may suggest that Naf1 possesses multifaceted roles in regulating HIV-1 infection.

Naf1 is ubiquitously expressed in human tissues and cell types, particularly in peripheral blood lymphocytes (46). Naf1 can regulate multiple receptor-mediated signal pathways for modulating inflammation (52), which may interact with HIV-1 replication. Further study the potential relationship of Naf1-modulating cellular signaling with enhanced HIV-1 production would be informative to better understand Naf1 functions and mechanisms in viral infection. Overall, our data demonstrate the novel role of cellular factor Naf1 in regulating HIV-1 production, and this finding might facilitate the identification of host targets for antiviral drugs or gene therapy.

Author Contributions—J. W., X. R., and H. W. conceived the project. J. W., X. R., and L. W. designed the study and wrote the manuscript. X. R., H. W., C. L., and J. J. conducted the experiments. J. W., X. R., and L. W. analyzed the results. S. X. and J. X. advised the study. All authors reviewed the results and approved the final version of the manuscript.

Acknowledgments—We thank Derya Unutmaz for the pH131 vector and Nikon and Leica for tech support.

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