Supporting Information

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SI Text

Identification of Lysines in Tat That Affect CycT1-Dependent Tat–TAR Interactions. Every Lys residue in the two contexts was mutated individually to Arg (maintaining the charge) or Gln (neutralizing the charge), which both eliminate possible acetylation (Fig. S1). After cotransfection of each HIV-1 Tat mutant with the corresponding HIV-1 TAR luciferase reporter, we found that any change to Lys-28 or Lys-41 in the AD severely reduced activation, whereas only Gln mutations at Lys-50 or Lys-51 in the HIV-1 RBD, which remove the charge, reduced activation (Fig. S1*A*). The results are consistent with previous experiments suggesting that acetylation of Lys-28 and Lys-41 may regulate Tat activation (1, 2), while the electrostatic potential of Lys-50 and Lys-51, but not their acetylation, is important (3). In the context of the BIV Tat–TAR interaction, Lys-41 in the AD also is essential (Fig. S1*B*), whereas removing the charge at Lys-59 in the BIV RBD has a 2-fold effect, consistent with its role in BIV TAR recognition (4, 5). In striking contrast to the HIV-1 RBD context, Lys-28 is not important for HBTat (Fig. S1*B*), suggesting that Lys-28 is needed only for CycT1-dependent TAR binding.

Both Lys-28 and Lys-41 are essential for HIV-1 Tat-mediated activation, but our studies focused on the role of Lys-28, and not Lys-41, as Lys-28 appears to have a role in ternary-complex formation. Lys-41, which is essential for both CycT1-dependent and -independent RNA binding modes, appears to be important for maintaining the proper Tat conformation (2, 6), consistent with its requirement for both HIV-1 Tat- and HBTat-mediated activation (Fig. S1).

Lentiviral Tat GenBank Accession Numbers. HIV-1 Tat was as described (7). BIV Tat (NC_001413) (8), JDV Tat (Q82854) (9), and SIVcpz Tat (ABD36895) were from the TAN2.69 isolate (10). SIVagm Tat (AAC57055) was from the TAN-1 isolate (11), SIVmac Tat (AAB54040) (12), and HIV-2 Tat (NP_056842) (13).

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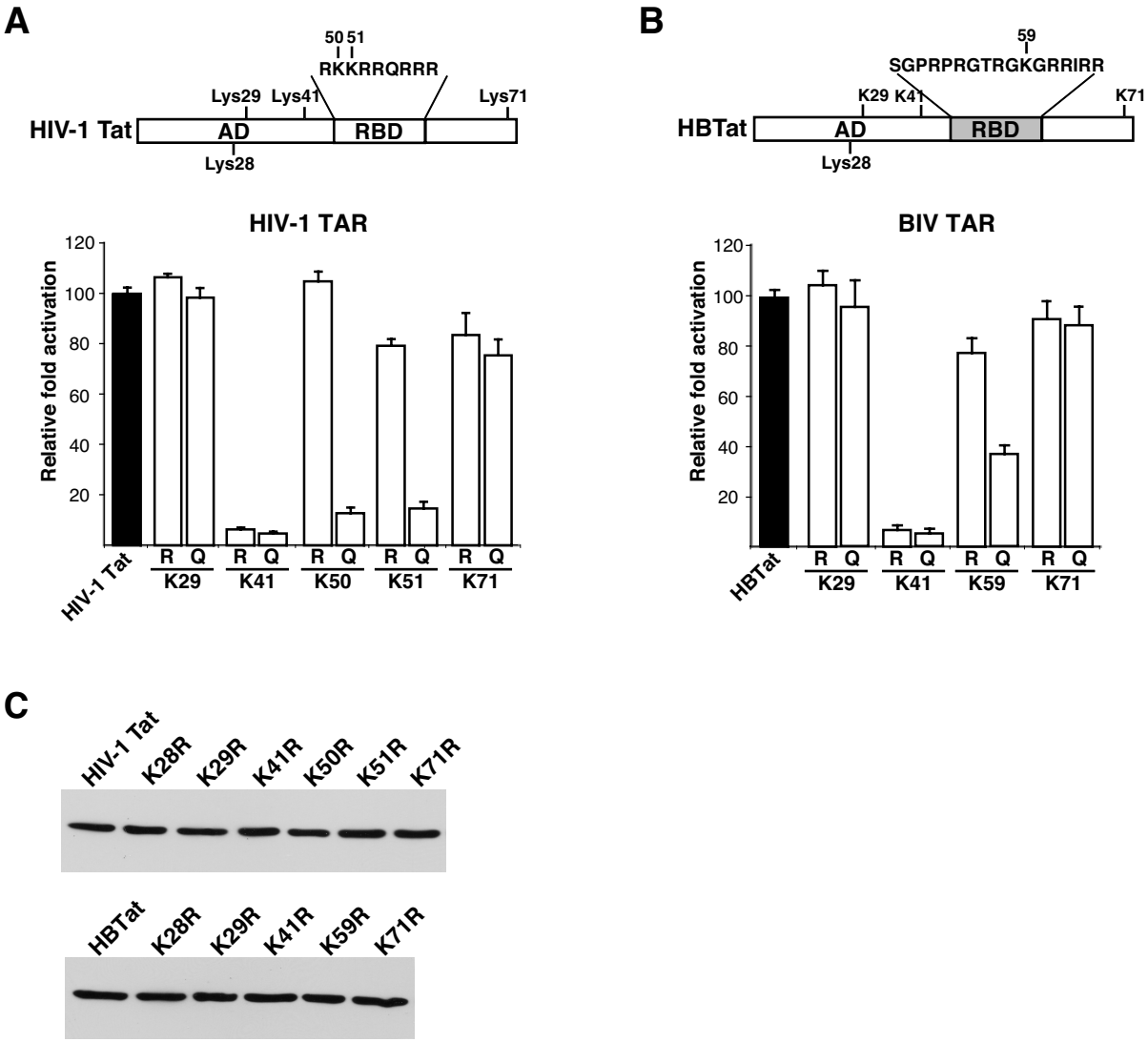

Fig. S1. Activities of Tat lysine mutants. (*A*) (*Upper*) Schematic of HIV-1 Tat and the Tat–TAR–CycT1 complex. Lys residues (K) mutated are shown. The position of Lys-28 is indicated in the scheme, but the data with these mutants are presented in *Results* and Fig. 1 in the main text. (*Lower*) Transcription activities of wild type Tat, K-to-R (R), and K-to-Q (Q) mutants after cotransfection with the HIV-1 TAR reporter in HeLa cells. Activities were normalized to a Renilla luciferase control and expressed as relative fold activation compared with reporter alone. Values also were normalized to wild-type Tat to facilitate comparison between HIV-1 Tat and HBTat. (*B*) (*Upper*) Schematic of HBTat (a fusion between HIV-1 Tat AD and BIV RBD) and the BIV Tat–TAR complex. Lys residues (K) mutated are shown. (*Lower*) Experiments were performed with a BIV TAR reporter and analyzed as in *A*. (*C*) The expression level of all Tat proteins was similar as judged by Western blot analysis with an anti-Tat serum.

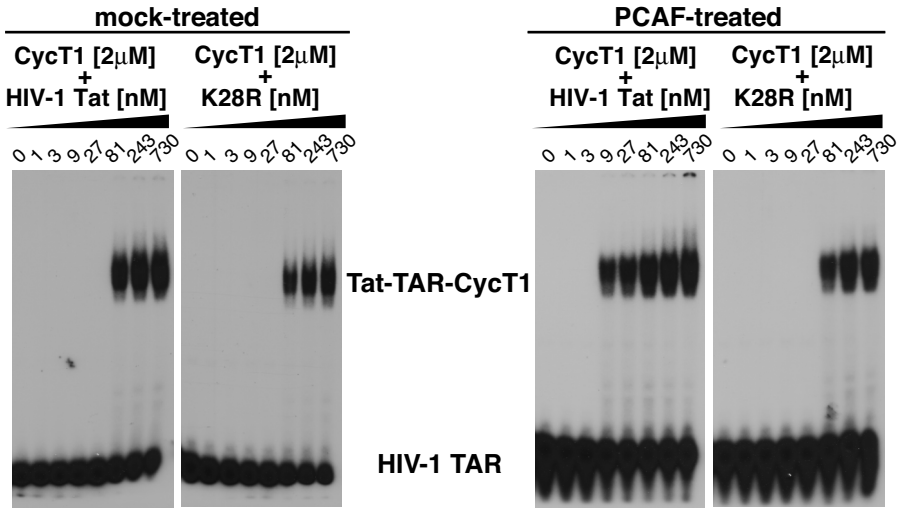

Fig. S2. Acetylation of HIV-1 Tat Lys-28 by PCAF enhances assembly of ternary complexes. RNA-binding gel-shift assays with labeled HIV-1 TAR, 2μM His-tagged CycT1, and increasing amounts of HIV-1 Tat or K28R mutant. Ternary complex formation was assessed with nonacetylated HIV-1 Tat or K28R mutant (mock-treated) or PCAF-treated proteins.

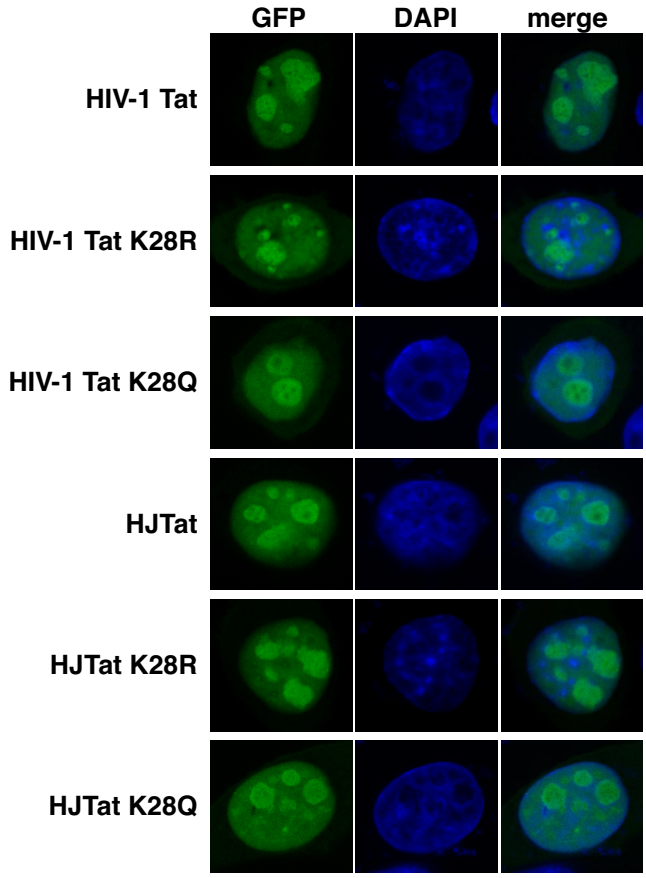

Fig. S3. Mutation of Lys-28 does not affect Tat subcellular localization. HeLa cells were transiently transfected with the indicated GFP-tagged Tat or mutants. Confocal images of each GFP-tagged fusion, DAPI staining, and combined images (merge) are shown. Microscopy was performed as reported (10).

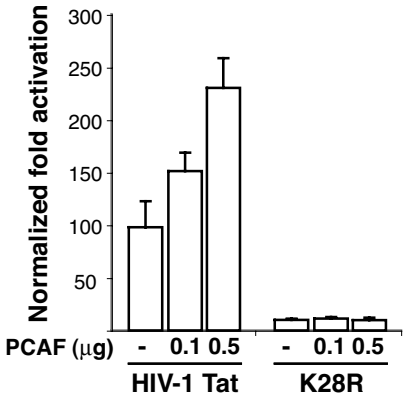

Fig. S4. PCAF-mediated stimulation of HIV-1 Tat activity is Lys-28-dependent. Transcription activation of HIV-1 Tat or K28R mutant on the HIV-1 TAR reporter and stimulation by increasing amounts of PCAF expression plasmid transfected into HeLa cells.

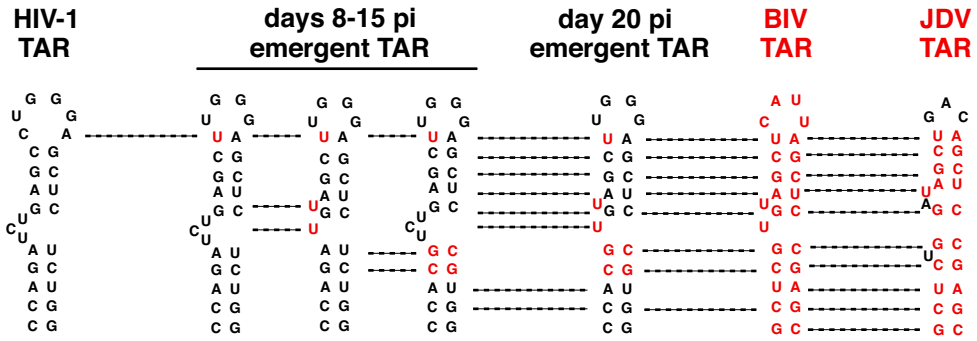

Fig. S5. Mutations in HIV-1 TAR acquired during virus replication with an HJTat K28R mutant. From left to right, sequences of wild-type HIV-1 TAR, emergent HIV-1 TARs, and wild-type bovine (BIV and JDV) TAR elements. The mutated positions in HIV-1 TAR that correspond to BIV or JDV sequences are shown in red.

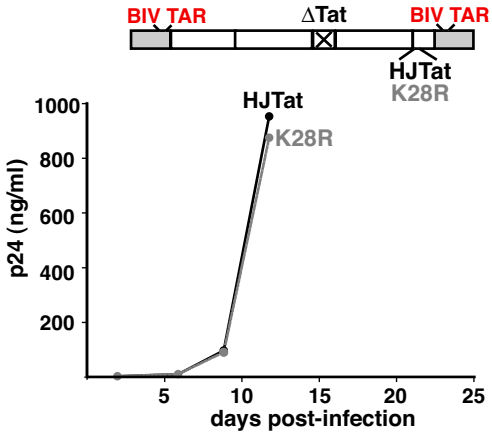

Fig. S6. SupT1 cells were infected with two hybrid viruses that use the BIV TAR interaction, and p24 levels at the postinfection times indicated were monitored by ELISA.

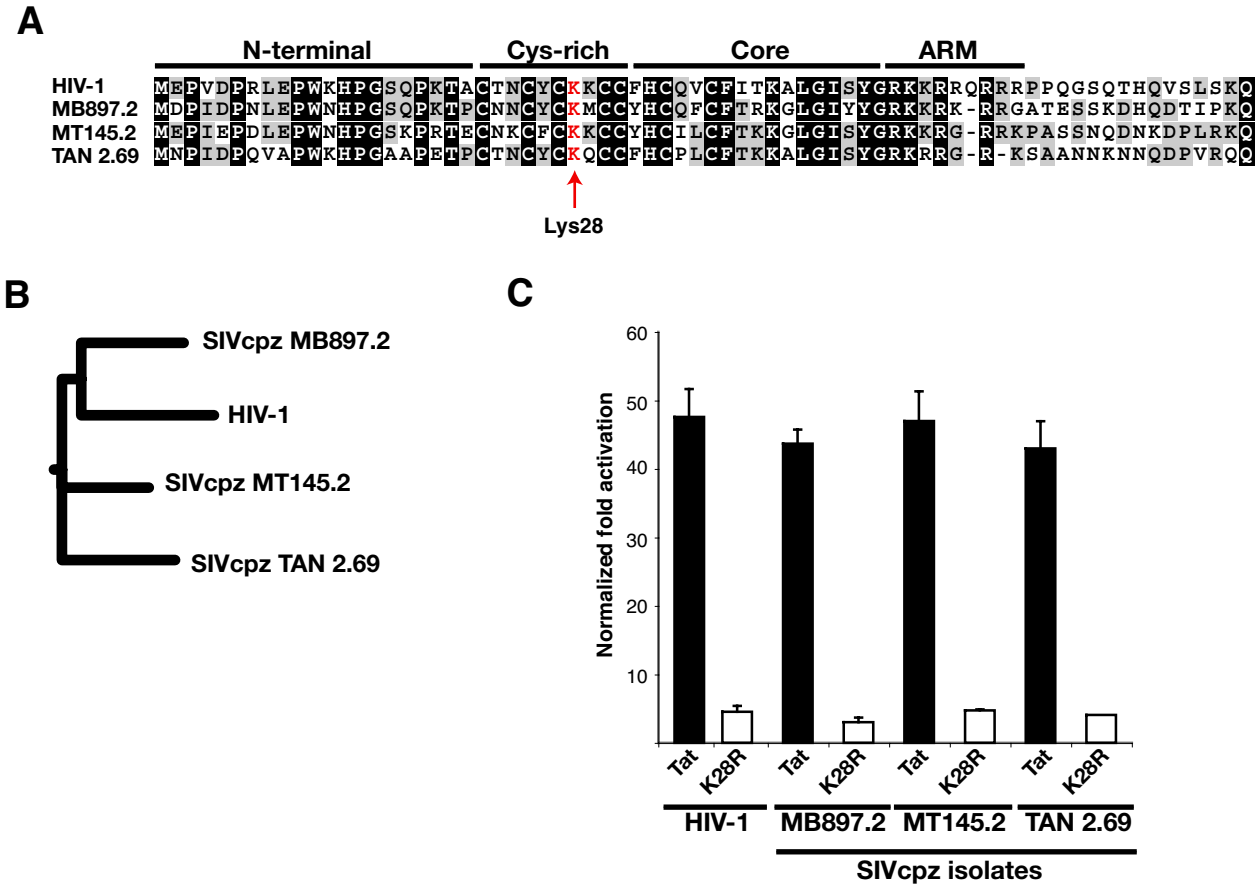

Fig. S7. Lys-28 is required in different SIVcpz isolates. ClustalW alignment of HIV-1 Tat and three SIVcpz Tat AD sequences (14) with their domain structure indicated on the top (N-terminal, Cys-rich, core, and ARM). The arrow indicates the position of the acetylated Lys-28 in HIV-1 Tat. (*B*) Rooted phylogeny dendogram showing their genetic relationships. (*C*) HeLa cells were cotransfected with HIV-1 TAR reporter and the indicated Tat (HIV-1 or SIVcpz) or K28R mutants, and transcription activation was determined after 48 h.