# Supporting Information

## Yang et al. 10.1073/pnas.0809536106

### SI Materials and Methods

**Plasmids and Site-Directed Mutagenesis.** To construct the HIV-1 LTR reporter, a 789-bp fragment containing the 5′ LTR, including the untranscribed region of Gag from the reference strain NL4-3, was amplified by PCR using primers containing KpnI and XhoI restriction sites. The PCR product was then subcloned upstream of the luciferase gene in the pGL4.11 plasmid (Promega) to generate the wild-type HIV-1 LTR reporter vector (wt-LTR-Luc). Site-directed mutagenesis was performed by using the QuickchangeII XL Kit (Stratagene) to generate the NF-κB-site-mutated LTR reporter (mκB-LTR-Luc). Two 3-bp substitutions of the NF-κB-binding sites in the LTR enhancer region replaced the wild-type sequence 5′-GGGACTTTCCGCTGGGGACTTTCC-3′ (−106 to −83) with 5′-CTCACTTTCCGCTGCTCACTTTCC-3′ as previously reported (1). Numbering positions in the parenthesis represent the positions of nucleotides relative to the transcription start site (+1), based on the HIV-$1_{HXB2}$ sequence from the Los Alamos National Laboratory HIV Sequence Database (http://hiv.lanl.gov). The NF-κB-responsive reporter Igκ$_2$-IFN-GL4 was constructed by cloning the promoter region of Igκ$_2$-IFN-LUC (2) into the pGL4.11 vector. Igκ$_2$-IFN-GL4 contains 2 copies of the NF-κB site (5′-GGGGACTTTCC-3′) upstream of the IFN-β minimal promoter.

A series of mutant LTR reporters with point mutations or deletions were also derived from the mκB-LTR-Luc by site-directed mutagenesis. The mutation of the Ets-binding site (mEBS)-LTR-Luc contains the mutant NF-κB-binding sites in the core enhancer region (−106 to −83), and a 4-bp substitution of the EBS in the distal region of LTR, which replaces the wild-type sequence 5′-CGGATG-3′ (−145 to −150) with 5′-CTCTAG-3′ as previously reported (3). The delEBS-LTR-Luc contains the mutant NF-κB-binding sites and a deletion of nucleotides −455 to −134 that eliminates the EBS. In delEnh-LTR-Luc, the core enhancer region (−106 to −83) is deleted. The mEBS-delEnh-LTR-Luc reporter contains the core enhancer deletion (−106 to −83) and the 4-bp EBS substitution (−149 to −146). The Sp1TA-LTR-Luc lacks the region between −455 and −84, whereas the TA-LTR-Luc lacks the region between −455 and −44. The reporters are illustrated in Fig. 4*A*.

**Generation of Wild-Type and Mutant ΔVII-Ets-1 Expression Vectors.** A 1062-bp ΔVII-Ets-1 cDNA was cloned into the vector pmax-GFP (Amaxa) by replacing GFP with ΔVII-Ets-1 with KpnI and BglII restriction sites to generate the pmax-ΔVII-Ets-1 expression vector; pmax-Empty was generated by replacing the GFP with a pair of linker oligonucleotides: 5′-CTAACATGCCTGTTCGAAGGCA-3′ and 5′-GATCTGCCTTCGAACAGGCATGTTAGGTAC-3′. Expression vectors containing the transactivation domain (pmax-TAD-ΔVII-Ets-1, residues 1–243) and DNA-binding domain (pmax-DBD-ΔVII-Ets-1, residues 244–354) of ΔVII-Ets-1 were amplified by PCR, and subcloned into pmax-GFP. Point mutations were introduced into pmax-ΔVII-Ets-1 to create R304A and R307A of ΔVII-Ets-1 (pmax-mDBD-ΔVII-Ets-1). A pair of linker oligonucleotides, 5′-CATGGAGGACTACAAGGACGACGATGACAAGGGTAC-3′ and 5′-CCTTGTCATCGTCGTCCTTGTAGTCCTCCATGGTAC-3′, were inserted into the KpnI site of these vectors to encode expression of N-terminal FLAG-tagged ΔVII-Ets-1 variants.


1. Nabel G, Baltimore D (1987) An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature* 326:711–713.
2. Pomerantz JL, Denny EM, Baltimore D (2002) CARD11 mediates factor-specific activation of NF-kappaB by the T cell receptor complex. *EMBO J* 21:5184–5194.
3. Sheridan PL, et al. (1995) Activation of the HIV-1 enhancer by the LEF-1 HMG protein on nucleosome-assembled DNA in vitro. *Genes Dev* 9:2090–2104.