Cell Reports, Volume *27*

Supplemental Information

Transcriptional Circuit Fragility Influences HIV Proviral Fate

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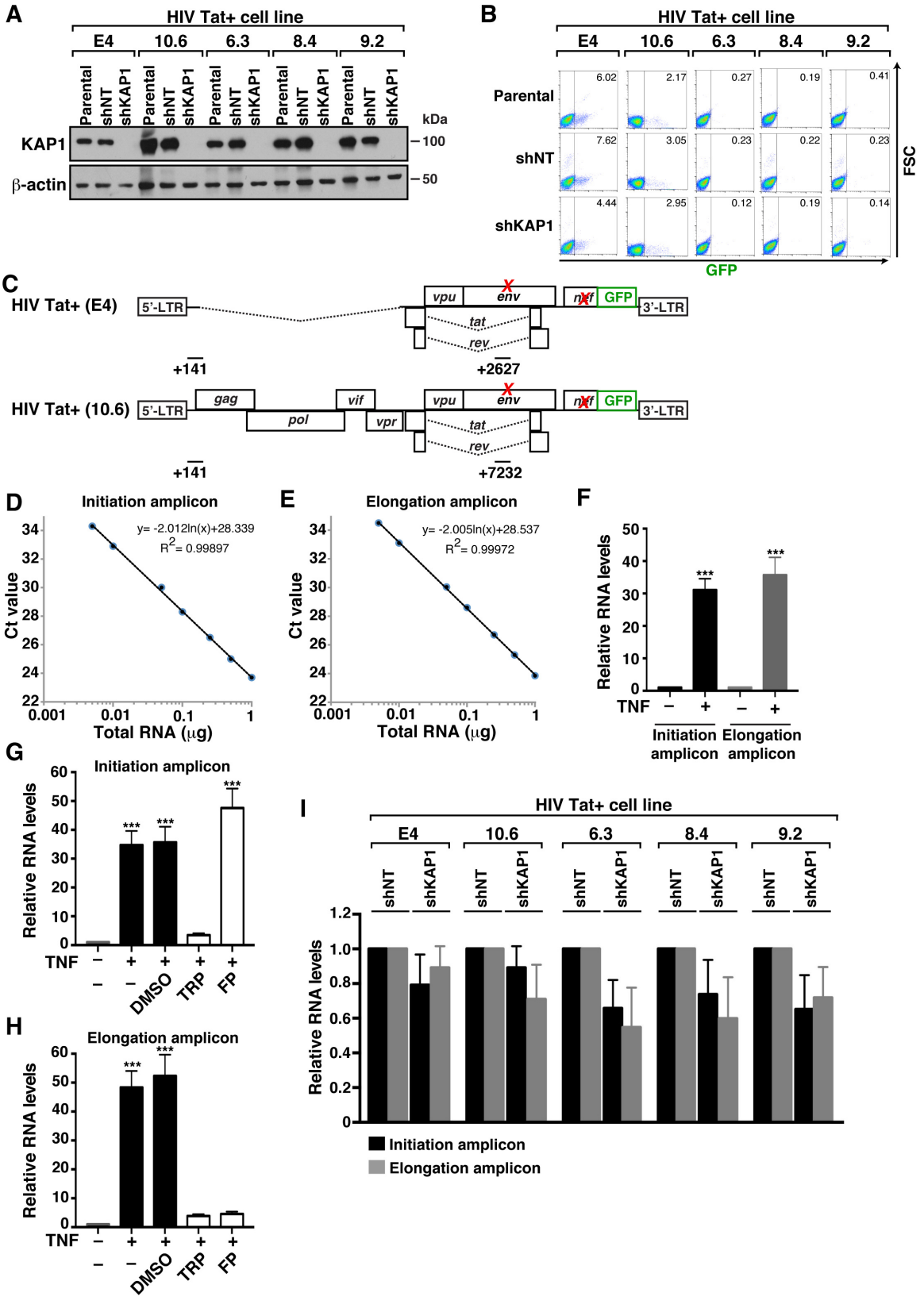

Figure S1. Loss of KAP1 Does not Appear to Affect Basal HIV Transcription. Related to Figure 2

(A) Western blots of the different HIV Tat+ cell-based models created as described in **Figure 2**.
(B) Flow cytometry analysis of the indicated cell-based models from panel A. FSC, forward scatter. The number in the quadrant denotes the average percentage of GFP+ cells from three independent experiments.
(C) Scheme of the HIV proviruses with the position of the amplicons used in RT-qPCR assays. The top scheme corresponds to the HIV Tat+ (E4) provirus, while the bottom scheme corresponds to HIV Tat+ (10.6, 6.3, 8.4, and 9.2) proviruses. The position of the initiation amplicon (+141) is indicated. The position of the elongation amplicon in the E4 proviral genome is +2627 respective to the TSS, and +7232 in the other proviruses because they contain full-length genomes.
(D-E) Standard curves for RT-qPCR assay. Total RNA from the HIV Tat+ (E4) clone treated with 25 ng/mL TNF for 16 hr was serially diluted and seven aliquots between 0.005 ng and 1 μg were converted to cDNA using individual RT reactions before performing qPCR assays with the initiation (+141) and elongation (+2627) amplicons. While the initiation amplicon only measures short, promoter-proximal transcripts, the elongation amplicon measures promoter-distal transcripts. PCR amplifications were performed in 20 μL reaction mixtures containing 10 μL of SYBR green master mix, primers and 2 μL of cDNA. The plot demonstrates linear reverse transcription for the concentrations of RNA tested without any effect of RNA input beyond RT capacity. In this situation, both HIV short and long target transcripts (as well as the internal control *ACTB* (data not shown)) have linear RT efficiencies across all starting concentrations of RNA tested. The qPCR plots show threshold Ct values (y-axis) as a function of increasing RNA concentrations (x-axis).
(F) Relative HIV RNA levels of HIV Tat+ (E4) clone treated (+) or not (-) with 25 ng/mL TNF for 2 hr by RT-qPCR using the initiation and elongation amplicons, and normalized to *ACTB* (mean ± SEM; n = 3).
(G) Quantification of short transcripts with the initiation amplicon (+141) from total RNA from the HIV Tat+ (E4) clone isolated after treatment with 25 ng/mL TNF alone for 2 hr or pre-treated with Triptolide (TRP), Flavopiridol (FP) or vehicle (DMSO) for 30 min before the addition of TNF. Relative HIV RNA levels were normalized to *ACTB* (mean ± SEM; n = 3).
(H) Quantification of long transcripts with the elongation amplicon (+2627) from total RNA from the HIV Tat+ (E4) clone isolated after treatment with 25 ng/mL TNF alone for 2 hr or pre-treated with TRP, FP or DMSO for 30 min before the addition of TNF. Relative HIV RNA levels were normalized to *ACTB* (mean ± SEM; n = 3).
(I) Relative HIV RNA levels: initiation (black bars) and elongation (grey bars) transcripts quantified by RT-qPCR and normalized to *ACTB* (mean ± SEM; n = 3).
Statistical significance in all panels was determined using unpaired Student's *t*-test. **P* < 0.05, ***P* < 0.005, ****P* < 0.0005.

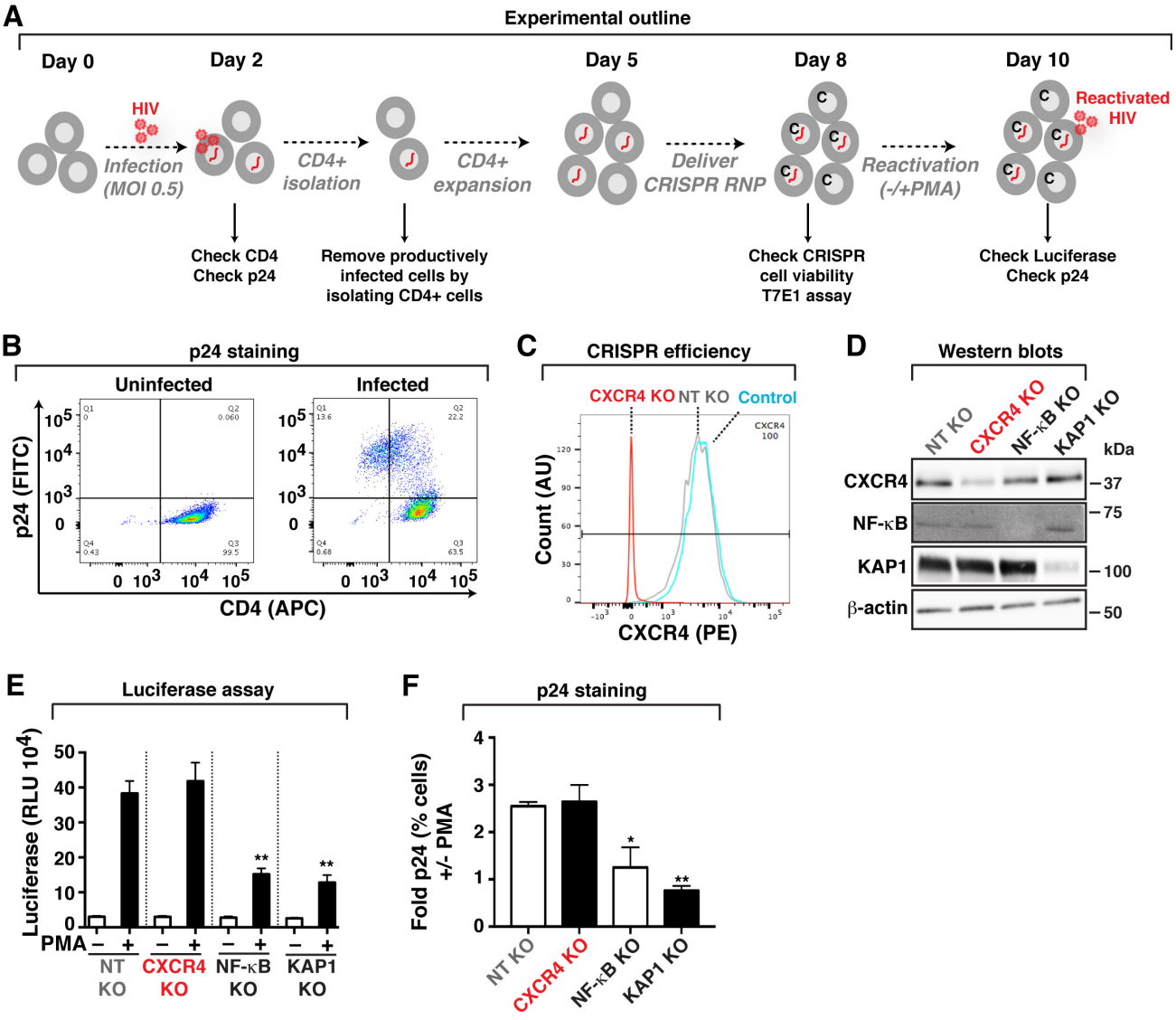

Figure S2. KAP1 is Required for Latent HIV Reactivation in Response to Immune Signaling in Cell-based Models. Related to Figure 3

(A) Experimental outline through which CD4+ SUPT1 cells were used for infections with replication defective, pseudotyped HIV (pNL4-3-delta*Env*-nLuc-2A*Nef*-VSVG), and then used for CRISPR-Cas9–mediated knockout (KO) of CXCR4, NF-κB (p65 subunit) and KAP1, followed by reactivation assays. RNP, Cas9-gRNA RiboNucleoProtein (RNP) complex. C, indicates cells containing the Cas9-gRNA RNP complex.

(B) FACS plots (CD4, HIV p24) of mock infected (uninfected) and HIV-infected cells as in panel (A).

(C) FACS plots (CXCR4) in control SUPT1 cells (not nucleofected) and SUPT1 nucleofected with Cas9-gRNA complexes for targeting CXCR4 and a non-target (NT) negative control. AU, arbitrary units.

(D) Western blots of SUPT1 cells containing KO of specific host cell factors generated as in panel (A) with the indicated antibodies.

(E) Luciferase assay of SUPT1 cells containing KO of specific host cell factors generated as in panel (A) and treated with PMA or vehicle (DMSO). Luciferase is expressed as relative luciferase units (RLU).
(F) p24 staining of SUPT1 cells containing KO of specific host cell factors generated as in panel (A) and treated with PMA or vehicle (DMSO). The fold change in p24 staining (+/- PMA) is indicated.
Statistical significance in panels (E) and (F) was determined using unpaired Student's *t*-test. $*P < 0.05$, $**P < 0.005$, $***P < 0.0005$.

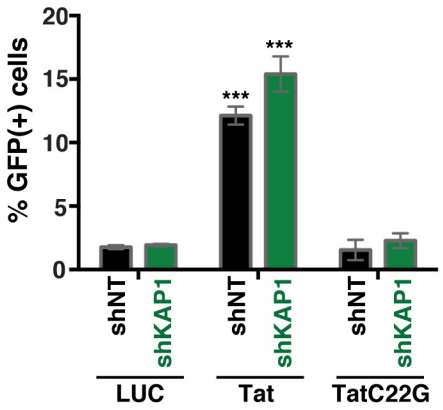

Figure S3. Tat, but not a non-functional mutant, reactivates a latent HIV Tat- provirus. Related to Figure 5

Quantification of GFP+ cells (percentage) in the Jurkat HIV Tat- (2B2D) shNT and shKAP1 cell lines transduced with pTRIP lentiviruses (5 ng p24) expressing firefly luciferase (LUC) as negative control, wild-type Tat or the C22G non-functional mutant. Statistical significance between Tat or TatC22G and LUC samples was determined using unpaired Student's *t*-test. **P* < 0.05, ***P* < 0.005, ****P* < 0.0005.

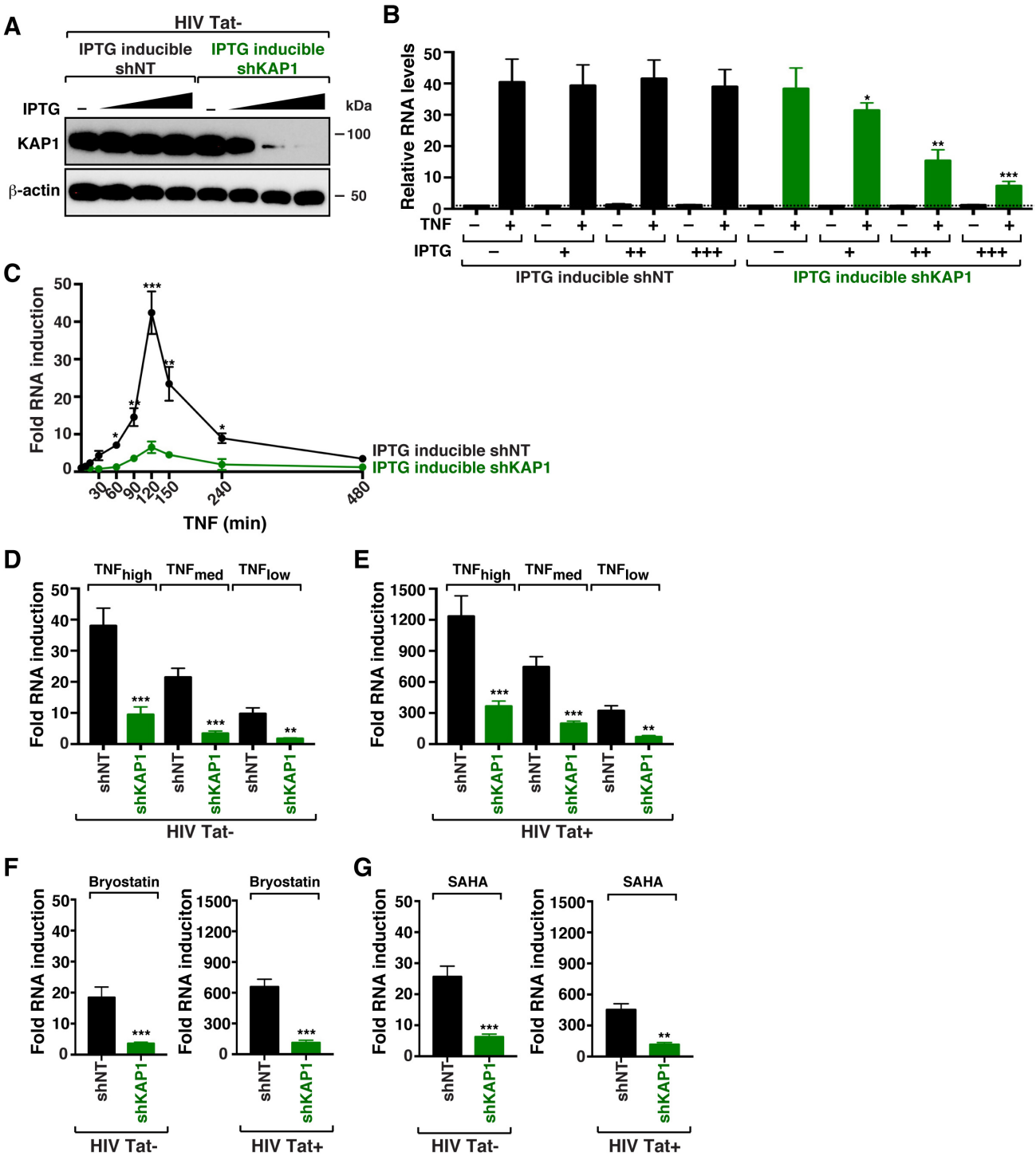

Figure S4. Effects of KAP1 protein levels and graded, diverse immune stimuli on activation of the host and viral phases. Related to Figure 7

(A) Western blots of the HIV Tat- (2B2D) IPTG-inducible shNT and shKAP1 cell lines untreated (-) or treated with increasing IPTG concentrations (1, 10, and 100 μM) for 2 days.

(B) HIV RNA levels of the HIV Tat- (2B2D) IPTG-inducible shNT and shKAP1 cell lines from panel (A) untreated (-) or treated with three IPTG concentrations (1, 10, and 100 μM; +, ++, +++, respectively) for 2 days in the absence (-) and presence (+) of TNF stimulation for 2 hr and measured by RT-qPCR using the elongation amplicon (+2627) and normalized to *ACTB* (mean ± SEM; n = 3).
(C) Fold HIV RNA induction of the HIV Tat- (2B2D) IPTG-inducible shNT and shKAP1 cell lines from panel (A) treated with IPTG (100 μM) for 2 days in response to a time course TNF treatment and measured by RT-qPCR using the elongation amplicon (+2627) and normalized to *ACTB* (mean ± SEM; n = 3).
(D) Fold HIV RNA induction of HIV Tat- (2B2D) shNT and shKAP1 cell lines in the absence (-) and presence (+) of different amounts of TNF stimulation [TNF_{high} (25 ng/mL), TNF_{medium} (5 ng/mL), TNF_{low} (1 ng/mL)] for 2 hr and measured by RT-qPCR using the elongation amplicon (+2627) and normalized to *ACTB* (mean ± SEM; n = 3).
(E) Fold HIV RNA induction of HIV Tat+ (E4) shNT and shKAP1 cell lines in the absence (-) and presence (+) of different amounts of TNF stimulation [TNF_{high} (25 ng/mL), TNF_{medium} (5 ng/mL), TNF_{low} (1 ng/mL)] for 16 hr and measured by RT-qPCR using the elongation amplicon (+2627) and normalized to *ACTB* (mean ± SEM; n = 3).
(F) Fold HIV RNA induction of HIV Tat- (2B2D) and HIV Tat+ (E4) shNT and shKAP1 cell lines in the absence (-) and presence (+) of Bryostatin (10 nM) stimulation for 2 hr (HIV Tat-) or 16 hr (HIV Tat+) and measured by RT-qPCR using the elongation amplicon (+2627) and normalized to *ACTB* (mean ± SEM; n = 3).
(G) Fold HIV RNA induction of HIV Tat- (2B2D) and HIV Tat+ (E4) shNT and shKAP1 cell lines in the absence (-) and presence (+) of SAHA (500 nM) stimulation for 2 hr (HIV Tat-) or 16 hr (HIV Tat+) and measured by RT-qPCR using the elongation amplicon (+2627) and normalized to *ACTB* (mean ± SEM; n = 3).
Statistical significance in all panels was determined using unpaired Student's *t*-test. $*P < 0.05$, $**P < 0.005$, $***P < 0.0005$.

Cell line	Laboratory	Reference
Jurkat E4	Jonathan Karn	(Pearson et al., 2008)
Jurkat E4 NT shRNA (shNT)	Iván D'Orso	Created in this study
Jurkat E4 KAP1 shRNA (shKAP1)	Iván D'Orso	Created in this study
Jurkat E4 NELF-E shRNA (shNELF)	Iván D'Orso	Created in this study
Jurkat 10.6	Eric Verdin	(Jordan et al., 2003)
Jurkat 10.6 NT shRNA (shNT)	Iván D'Orso	Created in this study
Jurkat 10.6 KAP1 shRNA (shKAP1)	Iván D'Orso	Created in this study
Jurkat 10.6 NELF-E shRNA (shNELF)	Iván D'Orso	Created in this study
Jurkat 6.3	Eric Verdin	(Jordan et al., 2003)
Jurkat 6.3 NT shRNA (shNT)	Iván D'Orso	Created in this study
Jurkat 6.3 KAP1 shRNA (shKAP1)	Iván D'Orso	Created in this study
Jurkat 8.4	Eric Verdin	(Jordan et al., 2003)
Jurkat 8.4 NT shRNA (shNT)	Iván D'Orso	Created in this study
Jurkat 8.4 KAP1 shRNA (shKAP1)	Iván D'Orso	Created in this study
Jurkat 9.2	Eric Verdin	(Jordan et al., 2003)
Jurkat 9.2 NT shRNA (shNT)	Iván D'Orso	Created in this study
Jurkat 9.2 KAP1 shRNA (shKAP1)	Iván D'Orso	Created in this study
Jurkat 2B2D	Jonathan Karn	(Pearson et al., 2008)
Jurkat 2B2D NT shRNA (shNT)	Iván D'Orso	Created in this study
Jurkat 2B2D KAP1 shRNA (shKAP1)	Iván D'Orso	Created in this study
Jurkat 2B2D (IPTG) NT shRNA (shNT)	Iván D'Orso	Created in this study
Jurkat 2B2D (IPTG) KAP1 shRNA (shKAP1)	Iván D'Orso	Created in this study
U2 OS	ATCC HTB-96	Purchased
U2 OS NT shRNA (shNT)	Iván D'Orso	Created in this study
U2 OS KAP1 shRNA (shKAP1)	Iván D'Orso	Created in this study
HEK 293T/17	ATCC CRL-11268	Purchased
HEK 293FT	Thermo Fisher 70007	Purchased
SUPT1	ATCC CRL-1942	Purchased
Jurkat E6.1	ATCC TIB-152	Purchased

Table S1. Cell lines used and created in this study. Related to STAR Methods and Figures 2, 3, 4, 5 and 7

Target gene	Vector / Restriction sites	Primer numbers / sequences (5'-3') to generate shRNA vectors
NT	pLVTHM/ ClaI-MluI	**1342**/CGCGTCCCCCAACAAGATGAAGAGCACCAATTCAAGAGATTGGTGCTCTTCATCTTGTTGTTTTTGGAAAT **1343**/CGATTTTCAAAAACAACAAGATGAAGAGCACCAATCTCTTGAATTGGTGCTCTTCATCTTGTTGGGGGA
KAP1	pLVTHM / ClaI-MluI	**1338**/CGCGTCCCCCTGAGACCAAACCTGTGCTTATTCAAGAGATAAGCACAGGTTTGGTCTCAGTTTTTGGAAAT **1339**/CGATTTCCAAAAACTGAGACCAAACCTGTGCTTATCTCTTGAATAAGCACAGGTTTGGTCTCAGGGGGA
NELF-E	pLVTHM / ClaI-MluI	**1340**/CGCGTCCCCCTGGATTCCTTGTGCCTCATATTCAAGAGATATGAGGCACAAGGAATCCAGTTTTTGAAAAT **1341**/CGATTTTCAAAAACTGGATTCCTTGTGCCTCATATCTCTTGAATATGAGGCACAAGGAATCCAGGGGGA
NT	pLKO.1 / AgeI-EcoRI	SHC002 (Sigma)
KAP1	pLKO.1 / AgeI-EcoRI	TRCN0000017998 (Sigma) CCGGCCTGGCTCTGTTCTCTGTCCTCTCGAGAGGACAGAGAACAGAGCCAGGTTTTT
NT	pLKO.1-IPTG-3xLacO / AgeI-EcoRI	SHC332 (Sigma) CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTGTTGTTTTTG
KAP1	pLKO.1-IPTG-3xLacO / AgeI-EcoRI	TRCN0000017998 (Sigma) CCGGCCTGGCTCTGTTCTCTGTCCTCTCGAGAGGACAGAGAACAGAGCCAGGTTTTT

Table S2. shRNA vectors used in this study. Related to STAR Methods and Figures 2, 4, 5 and 7

Amplicon*	Primer Number / Sequence (5'-3')	Figure (Assay)
-353	**1093** / AAGGCTACTTCCCTGAT **1094** / TAGCACCATCCAAAGGTC	3E, 5F (ChIP)
-69	**1360** / CTTGCTACAAGGGACTT **1361** / AGGGCTCGCCACTCC	3E, 5F (ChIP)
-37	**1364** / CTTTCTACAAGGGACTTTCCGCTG **1365** / CTCCCAGGCTCAGATCTGGTC	3E, 5F (ChIP)
+141 5'-LTR specific	**1111** / GCTTAAGCCTCAATAAAGCTTGCCTTGAG **1112** / GTCCTGCGTCGAGAGATCTCCTCTG	3E, 5F (ChIP) 4C, 4E, S1D, S1F, S1G, S1I (RT-qPCR)
+2627 (+7232)**	**1358** / TGAGGGACAATTGGAGAAGTGA **1359** / TCTGCACCACTCTTCTCTTTGC	3E, 5F (ChIP) 3C, 4D, 4F, 5E, 7E, S1E, S1F, S1H, S1I, S4B, S4C, S4D, S4E, S4F, S4G (RT-qPCR)
+4230 (+9553)*** 3'-LTR specific	**1368** / ACAAGAGGAGGAAGAGGTGGGT **1369** / GCCCTGGTGTGTAGTTCTGCCA	3E, 5F (ChIP)
ACTB	**1256** / GATGATGATATCGCCGCGCT **1257** / CTTCTCGCGGTTGGCCTTGG	All RT-qPCR experiments

Table S3. DNA oligonucleotides used in this study. Related to STAR Methods and Figures 3, 4, 5 and 7

*The number of the amplicons used in real-time PCR quantification of the ChIP-enriched DNA represents the midpoint of the two primers respective to the transcription start site (TSS), upstream the TSS (-) and downstream the TSS (+).

**Note that +2627 and +7232 are the same amplicon. +2627 is the position of the amplicon respective to the TSS in the HIV Tat+ (E4) and HIV Tat- (2B2D) cell-based models and +7232 is the position of the amplicon respective to the TSS in the HIV Tat+ (10.6, 6.3, 8.4, and 9.2) cell-based models and in infection experiments with HIV-1_{NL4-3}.

***Note that the +4230 and +9553 are the same amplicon. +4230 is the position of the amplicon respective to the TSS in the HIV Tat+ (E4) and HIV Tat- (2B2D) cell-based models and +9553 is the position of the amplicon respective to the TSS in the HIV Tat+ (10.6, 6.3, 8.4, and 9.2) cell-based models and in infection experiments with HIV-1_{NL4-3}.

Target	Company	Catalogue Number	Assay (Dilution used)
β-actin (C4)	Santa Cruz	sc-47778	Western blot (1:5000)
NELF-E (H-140)	Santa Cruz	sc-32912	Western blot (1:2000)
KAP1 (20C1)	Abcam	ab22553	Western blot (1:5000) ChIP (5 μg / 20 million cells)
RNA polymerase II (N-20)	Santa Cruz	sc-899X	ChIP (5 μg / 20 million cells)
Cdk9 (C-20)	Santa Cruz	sc-484	ChIP (5 μg / 20 million cells)
NF-κB p65 (C-20)	Santa Cruz	sc-372	Western blot (1:5000)
CXCR4 (PE conjugated)	BD Biosciences	555974	Flow cytometry (1:500)
CD4 (APC conjugated)	Thermo Fisher	MHCD0405	Flow cytometry (1:1000)
p24 (FITC conjugated)	Beckman Coulter	6604665	Flow cytometry (1:500)
IL4	PeproTech	500-P24	Primary CD4 T cell Polarization (2 μg/ 2 mL/ 10 million cells)
IL12	PeproTech	500-P154	Primary CD4 T cell Polarization (2 μg/ 2 mL/ 10 million cells)
Normal Mouse IgG	Santa Cruz	sc-2025	ChIP (5 μg/ 20 million cells)
Donkey anti-rabbit IgG-HRP	Santa Cruz	sc-2313	Western blot (1:10.000)
Goat anti-mouse IgG-HRP	Santa Cruz	sc-2005	Western blot (1:10.000)

Table S4. Antibodies used in this study. Related to STAR Methods and Figures 2, 3, 4, 5 and 7

Insert	Vector / tag	Restriction sites / Reference
FFL LUC	pTRIP	SpeI-XhoI / (Schoggins et al., 2011)
Tat	pTRIP / STREP	SpeI-XhoI / This study
Tat C22G	pTRIP / STREP	SpeI-XhoI / This study
KAP1 shRNA	pLVTHM	ClaI-MluI / This study
NELF-E shRNA	pLVTHM	ClaI-MluI / This study
Non Target (NT) shRNA	pLVTHM	ClaI-MluI / This study
KAP1 shRNA	pLKO.1	AgeI-EcoRI / This study
Non Target (NT) shRNA	pLKO.1	AgeI-EcoRI / This study
KAP1 shRNA	pLKO.1-IPTG-3xLacO	Proprietary Sigma
Non Target (NT) shRNA	pLKO.1-IPTG-3xLacO	Proprietary Sigma
GAL4	pcDNA4TO	HindIII-EcoRI / This study
GAL4–CycT1	pcDNA4TO	EcoRI-XhoI / This study
GAL4–CDK9	pcDNA4TO	EcoRI-XhoI / This study
GAL4–CDK9 T186A	pcDNA4TO	EcoRI-XhoI / This study
HIV LTR – FFL – LUC	pcDNA3.1+	(D'Orso et al., 2012)
HIV LTR 5xGal4 – FFL LUC	pcDNA3.1+	(D'Orso et al., 2012)
CMV – RL LUC	pCMV	(D'Orso et al., 2012)

Table S5. Plasmids used in this study. Related to STAR Methods and Figures 2, 3, 4, 5 and 7

Equation	Measured parameters	Dimension	Reference	Model Reference	Tat+ KAP1+	Tat- KAP1+	Tat+ KAP1-	Tat- KAP1-
dRNA/dt								
τ_RNA					0.2071	0.2071	0.2071	0.2071
μ_RNA	0.05	Transcript/sec	(Tay et al., 2010)	3	3.093	3.093	3.093	3.093
k_Mm	1E+5	Average number NF-κB per cell	(Tay et al., 2010)		120	120	120	120
k_synth(h)	n.d.				8.2E-6	8.2E-6	8.2E-6	8.2E-6
k_synth(v)	0.1	Transcript/sec	(Weinberger et al., 2005)	6	3.93E-4	3.93E-4	3.93E-4	3.93E-4
k_decay	0.2	1/hr	(Reddy and Yin, 1999)	0.003	0.0132	0.0132	0.0132	0.0132
dTat/dt								
k_trans	0.005 – 0.5	Protein/sec	(Weinberger et al., 2005)	0.3 – 30	0.5412*	0	0.5412*	0
μ_Tat	25 – 100	-	(Reddy and Yin, 1999)	60	35.4256	35.4256	35.4256	35.4256
k_MTat	n.d.				125	125	125	125
d_Tat	0.154	Protein/hr	(Reddy and Yin, 1999)	0.025	0.0194	0.0194	0.0194	0.0194
dNF-κB/dt								
β	2E-5 – 0.01	1/sec	(Tay et al., 2010)	0.0012 – 0.6	0.5	0.5	0.5	0.5
d_NF-κB	0.05	1/sec	(Tay et al., 2010)	3	3	3	3	3
dKAP1/dt								
d_KAP1	n.d.				0.006	0.006	0.006	0.006
ρ	n.d.				0.081*	0.081*	0.001	0.001
dTNF/dt								
d_TNF	0.0002	1/sec	(Tay et al., 2010)	0.012	0.012	0.012	0.012	0.012
dRNAbasal/dt								
α	1E-8	Transcript/sec	(Weinberger et al., 2005)	6E-7	0.008	0.008	0.008	0.008
d_RNAbasal	0.0122	Nucleotides/sec	(Reddy and Yin, 1999)	0.00244	0.00667	0.00667	0.00667	0.00667

Table S6. SDE Parameters Used in the Mathematical Modeling. Related to STAR Methods and Figure 6

Note: The asterisk (*) indicates the only two parameters that change in the four different scenarios. N.d. denotes not determined.

$$\frac{d[RNA]}{dt} = \mu_{RNA}\frac{[NF\kappa B]}{k_{Mm} + [NF\kappa B]} + \tau_{RNA}[KAP1][NF\kappa B] + k_{synth(h)}[KAP1][Tat] + k_{synth(v)}[Tat] - k_{decay}[RNA] \quad (1)$$

$$\frac{d[Tat]}{dt} = k_{trans}[RNA] + \mu_{Tat}\frac{[Tat]}{k_{MTat} + [Tat]} - d_{Tat}[Tat] \quad (2)$$

$$\frac{d[NF\kappa B]}{dt} = \beta[TNF] - d_{NF\kappa B}[NF\kappa B] \quad (3)$$

$$\frac{d[KAP1]}{dt} = \rho - d_{KAP1}[KAP1] \quad (4)$$

$$\frac{d[TNF]}{dt} = -d_{TNF}[TNF] \quad (5)$$

$$\frac{d[RNAbasal]}{dt} = \alpha - d_{RNAbasal}[RNAbasal] \quad (6)$$

$$[RNAtot] = [RNA] + [RNAbasal] \quad (7)$$

Table S7. System of ODEs Describing the Deterministic Approximation of the Mathematical Model. Related to STAR Methods and Figure 7