Supplemental Materials

8 Supplemental Figures and 3 Supplemental Tables

Supplemental Figure S1.

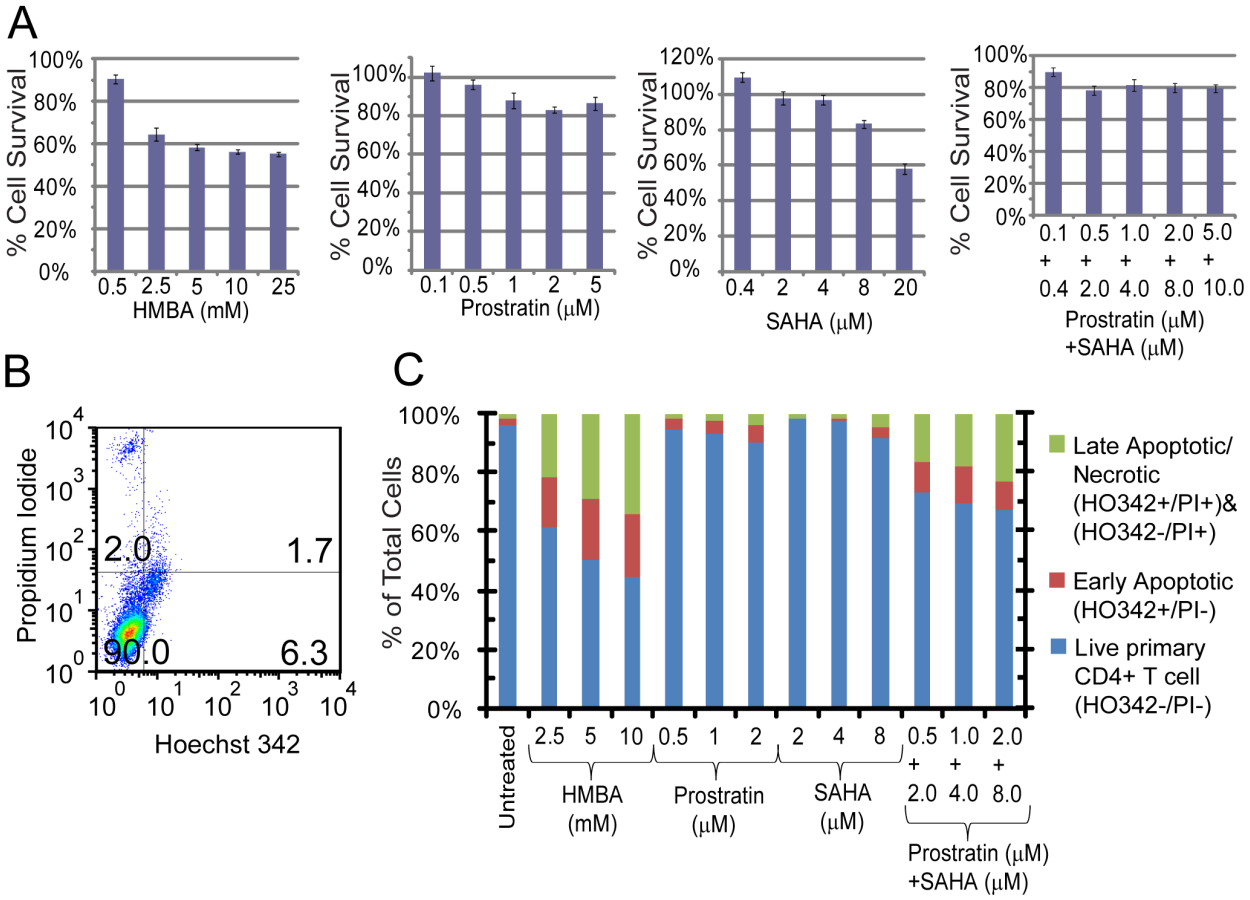

Figure S1. Cell viability after perturbation with anti-latency drugs

(A) Jurkat-based *LGIT* subtype B Off sorts were stimulated with latency reactivating drugs for 24 hours and analyzed by for cell viability using the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (MTS assay, Promega BioScience, Madison, WI). In summary cells were treated with five different concentrations of HMBA (0.5, 2.5, 5.0, 10.0, and 25.0 mM), prostratin (0.1, 0.5, 1.0, 2.0, and 5.0 μM), SAHA (0.4, 2.0, 4.0, 8.0, and 20.0 μM), or the combination of prostratin+SAHA (0.1 μM prostratin + 0.4 μM SAHA, 0.5 μM prostratin + 2.0 μM SAHA, 1.0 μM prostratin + 4.0 μM SAHA, 2.0 μM prostratin + 8.0 μM SAHA, and 5.0 μM prostratin + 10.0 μM SAHA). The MTS assay was performed 24 hours after drug treatment

and spectrophotometry (Victor3 1420 Multi-label Counter, Perkin Elmer, Waltham, MA) was used for absorbance measurements. Sample measurements were normalized by negative (vehicle) controls. Presented data are averages of biological triplicate measurements and error bars are standard deviations. The following drug concentrations were used for all experiments in the manuscript: 5 mM HMBA, 1.0 μM prostratin, 4.0 μM SAHA, and 1.0 μM prostratin + 4.0 μM SAHA.

(B) Human primary CD4+ T cells were analyzed by dual laser flow cytometry for cellular apoptosis and necrosis. Twenty-four hours after incubation with each anti-latency drug, 1×10^6 PBS-BSA-washed cells were first incubated for 7 minutes at 37°C with 1 μg Hoechst 342 and then for 10 minutes on ice with 1 μg propidium iodide. Cells were analyzed with the CyAn ADP 9color flow cytometer (Dako, Carpinteria, CA). Two-parameter histograms were generated to determine the percentage of cells that are characterized as "live" (HO342-/PI-), "early apoptotic" (HO342+/PI-), or "late apoptotic and necrotic" (HO342+/PI+ and HO342-/PI+).

(C) Same as in (B) for the following drug conditions of human primary CD4+ T cells: untreated (column 1), HMBA (2.5, 5.0, 10.0 mM, columns 2-4), prostratin (0.5, 1.0, and 2.0 μM, columns 5-7), SAHA (2.0, 4.0, and 8.0 μM, columns 8-10), or the combination of prostratin+SAHA (0.5 μM prostratin + 2.0 μM SAHA, 1.0 μM prostratin + 4.0 μM SAHA, and 2.0 μM prostratin + 8.0 μM SAHA, columns 11-13). Data are representative of the 2-D histograms from (B).

Supplemental Figure S2.

127 isolates	Sp1 site I (3 mutant isolates, 2.4%)									
Site position	1	2	3	4	5	6	7	8	9	10
LTR position	-55	-54	-53	-52	-51	-50	-49	-48	-47	-46
A	3	1	0	0	127	1	0	0	1	0
G	121	126	127	127	0	125	1	126	125	0
C	0	0	0	0	0	0	0	0	1	127
T	3	0	0	0	0	1	126	1	0	0

127 isolates	Sp1 site II (2 mutant isolates, 1.6%)									
Site position	1	2	3	4	5	6	7	8	9	10
LTR position	-66	-65	-64	-63	-62	-61	-60	-59	-58	-57
A	0	0	0	0	0	0	0	2	127	0
G	1	127	127	126	0	126	127	125	0	0
C	0	0	0	1	126	1	0	0	0	127
T	126	0	0	0	1	0	0	0	0	0

127 isolates	Sp1 site III (13 mutant isolates, 10.2%)									
Site position	1	2	3	4	5	6	7	8	9	10
LTR position	-77	-76	-75	-74	-73	-72	-71	-70	-69	-68
A	4	125	5	1	1	0	0	1	6	1
G	123	2	122	126	0	126	0	125	121	0
C	0	0	0	0	74	0	6	1	0	122
T	0	0	0	0	52	1	121	0	0	4

127 isolates	κB site I (3 mutant isolates, 2.4%)									
Site position	1	2	3	4	5	6	7	8	9	10
LTR position	-90	-89	-88	-87	-86	-85	-84	-83	-82	-81
A	0	0	0	125	2	0	0	0	1	0
G	127	127	127	2	0	0	0	0	0	1
C	0	0	0	0	125	0	0	0	126	126
T	0	0	0	0	0	127	127	127	0	0

127 isolates	κB site II (0 mutant isolates 0.0%)									
Site position	1	2	3	4	5	6	7	8	9	10
LTR position	-104	-103	-102	-101	-100	-99	-98	-97	-96	-95
A	1	0	0	127	0	0	0	0	0	0
G	126	127	127	0	0	0	0	0	0	0
C	0	0	0	0	127	0	0	0	127	127
T	0	0	0	0	0	127	127	127	0	0

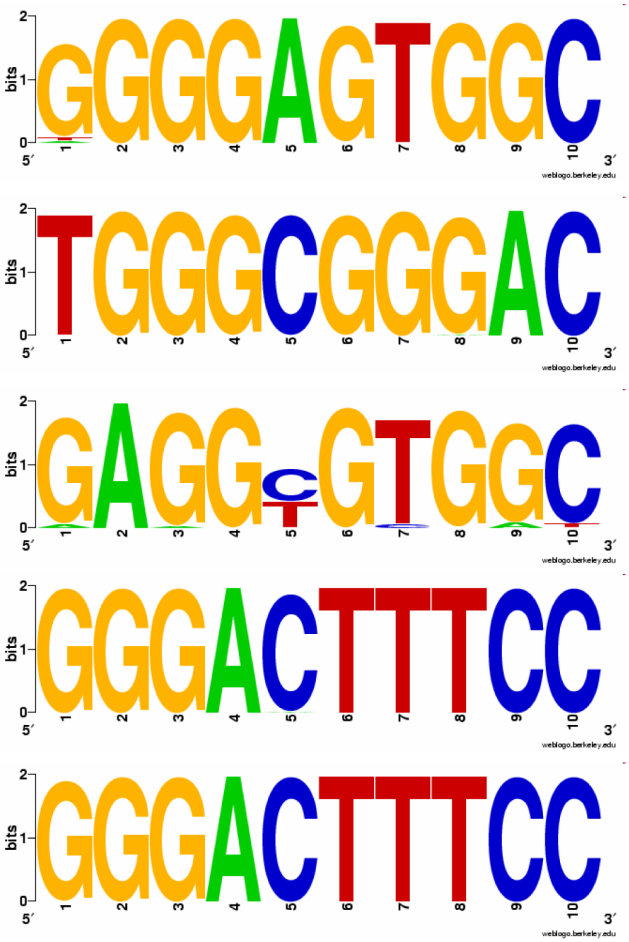

Figure S2. Analysis of Sp1 and κB polymorphisms in subtype B isolates from the LANL HIV-1 genome database

Using the LANL database of subtype B HIV-1 genome isolates, 127 sequences were analyzed to determine the variability in Sp1 and κB sites within the U3 region of the 5' LTR. Sequences which contained Sp1 polymorphisms in positions 1 (G, T, or A), 2 (G or A), 5 (G, C, or T), 9 (G or A), and 10 (C or T) were ignored. Likewise, polymorphisms in κB sites 1 (G or A) and 4 (A or G) were ignored. All other polymorphisms were considered as detrimental mutations. Nucleotide positions are denoted as white (neutral polymorphisms), gray (detrimental polymorphisms), or black (the polymorphisms created for Sp1 and κB mutants, Figure 2). Some

genome isolates contained more than one point mutation within a single κB or Sp1 site, but were counted as only a single mutant.

Supplemental Figure S3.

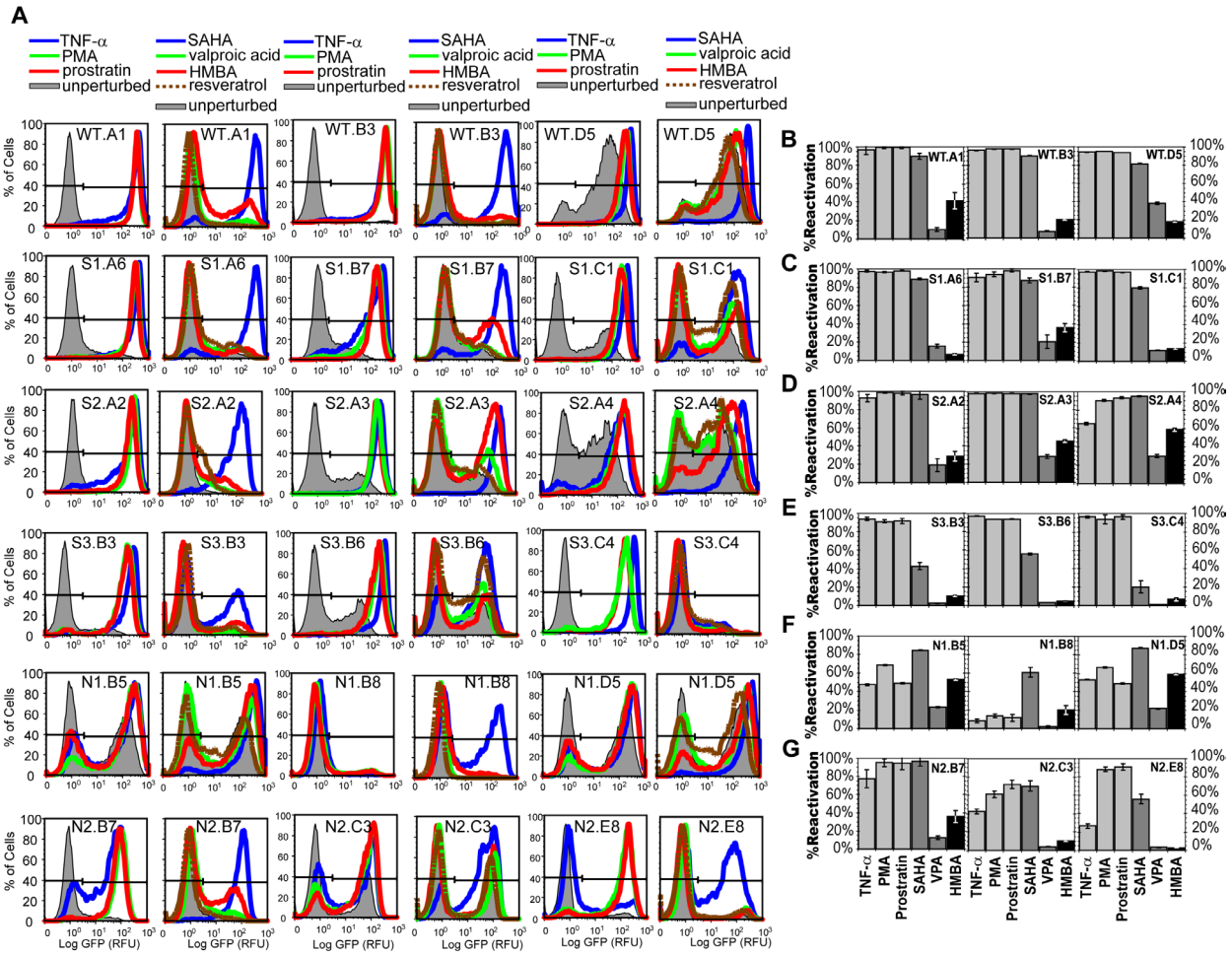

Figure S3. GFP histograms of *LGIT* PheB clones with reactivation

(A) WT and mutant *LGIT* PheB clones, isolated as in Figure 1A, panel 6A, were treated with pharmacological agents to reactivate the Off population. The following PheB clones were selected for reactivation: WT *LGIT* clones (WT.A1, WT.B3, and WT.D5), *mutI Sp1 LGIT* clones (S1.A6, S1.B7, and S1.C1), *mutII Sp1 LGIT* clones (S2.A2, S2.A3, S2.A4), *mutIII Sp1 LGIT* clones (S3.B3, S3.B6, and S3.C4), *mutI NF-κB LGIT* clones (N1.B5, N1.B8, and N1.D5), and *mutII NF-κB LGIT* clones (N2.B7, N2.C3, and N2.E8). For each clone, two histograms (left and right panels) include overlays of each pharmacological treatment, including TNF-α (blue outline, left panel), PMA (green outline, left panel), prostratin (red outline, left panel), SAHA (blue

outline, right panel), valproic acid (green outline, right panel), HMBA (red outline, right panel), and resveratrol (brown dotted outline, right panel). All drug treatments were performed in biological triplicate, and each histogram is a representative replicate.

(B) Summary of data from (A) for WT *LGIT* PheB clones (WT.A1, WT.B3, and WT.D5). Flow cytometry measurements were performed 18 hours after stimulation to measure the change in the fraction of Off (latent) cells, as indicated by % Reactivation (see Methods and Materials for further discussion). All data are averages of biological triplicates, and error bars are standard deviations.

(C-G) Same as in (B) for three PheB clones from *mutI Sp1 LGIT* (S1.A6, S1.B7, S1.C1); *mutII Sp1 LGIT* (S2.A2, S2.A3, S2.A4); *mutIII Sp1 LGIT* (S3.B3, S3.B6, and S3.C4); *mutI NF-κB LGIT* (N1.B5, N1.B8, and N1.D5); and *mutII NF-κB LGIT* (N2.B7, N2.C3, and N2.E8).

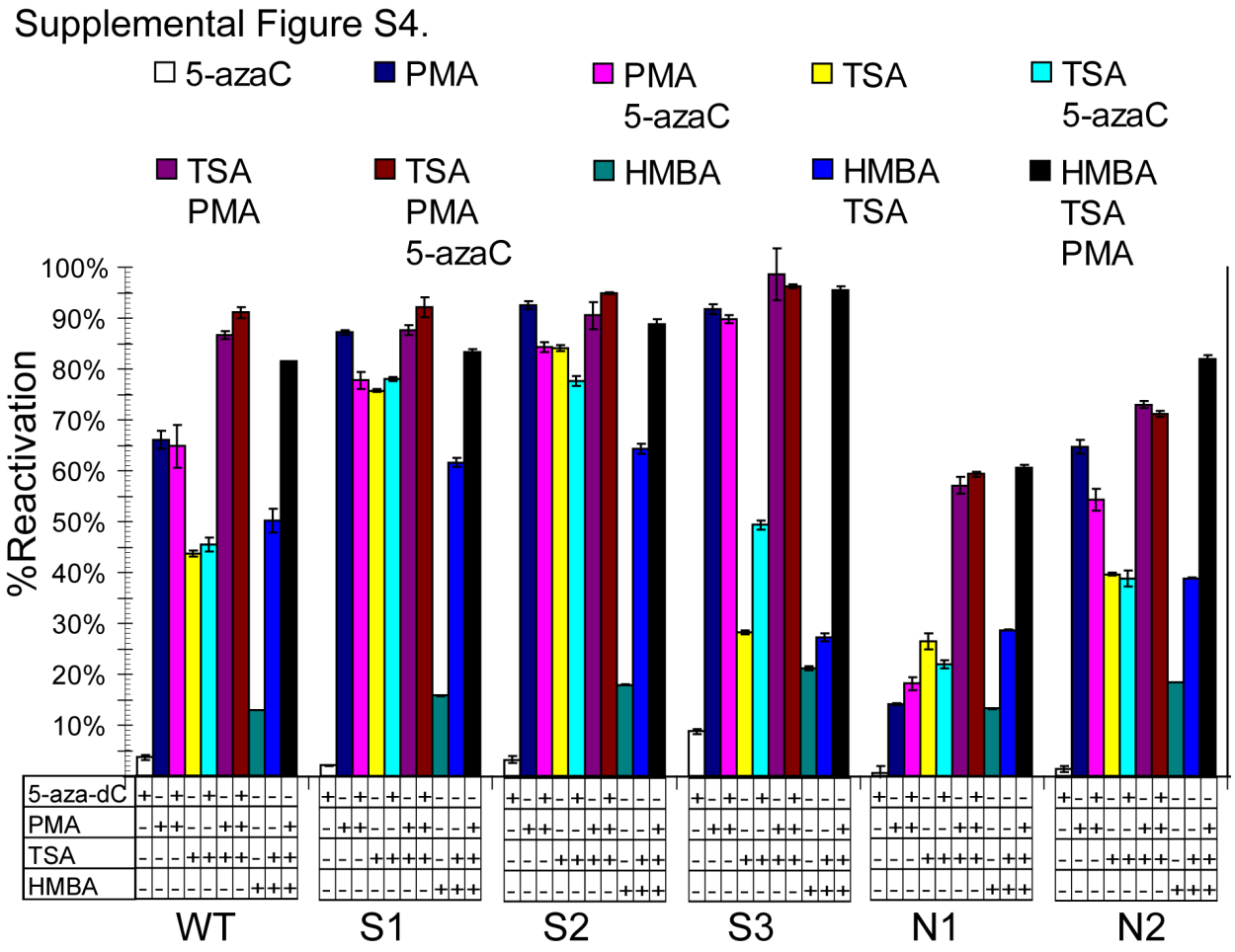

Figure S4. Single and combinatorial treatment with DNA methylase inhibitor 5-aza-deoxycytidine

Polyclonal Off sorts for WT and Sp1/κB *LGIT* mutants were treated with 5-aza-dC 48 hours prior to analysis with flow cytometry. For combinatorial treatments, other drugs (PMA, TSA, or HMBA) were administered 24 hours prior to flow cytometry analysis. All drug treatments and flow cytometry analyses were performed in biological triplicate and error bars represent the standard deviation. Drug concentrations are provided in the main text Methods and Materials section.

Supplemental Figure S5.

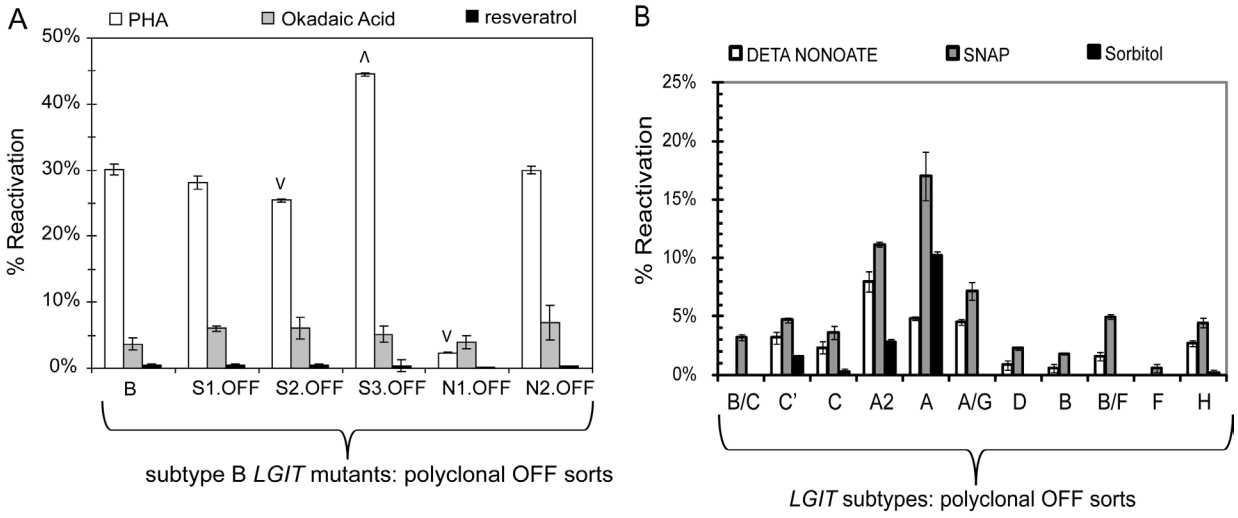

Figure S5. Reactivation of *LGIT* Off-sorts harboring mutations in Sp1 or κB elements

(A) Jurkat polyclonal Off sorts for subtype B Sp1 and κB *LGIT* mutants (Figure 1B, panel 6c) were treated with 30 nM okadaic acid (Sigma-Adrich) or 20 μg/ml phytohemagluttinin (PHA, Sigma-Aldrich) for 24 hours or with 30 μM resveratrol (Sigma-Aldrich) for 48 hours prior to analysis with flow cytometry. All drug treatments and flow cytometry analyses were performed in biological triplicate and error bars represent the standard deviation. Upward (Λ) and downward (V) arrows indicate increases and decreases compared to WT *LGIT*, respectively ($p<0.05$).

(B) Jurkat polyclonal Off sorts for *LGIT* subtype variants (B/C, C, C', A2, A, A/G, D, B, B/F, F, and H, Figure 1B, panel 6c) were reactivated with stimulants of the of latent infections by YY1 and AP-1 pathways. Cells were treated with 425 μM DETA-NONOate (Cayman Chemical), 500 μM (±)-S-Nitroso-N-acetylpenicillamine (SNAP, Calbiochem), and 500 mM sorbitol (Sigma-Aldrich). Filled circles (•) indicate at least 5% reactivation of latent infections. Flow cytometry measurements were performed 18 hours after stimulation to measure the change

in the fraction of Off (latent) cells, as indicated by % Reactivation (see Methods and Materials for further discussion). All data are averages of biological triplicates, and error bars are standard deviations.

Supplemental Figure S6.

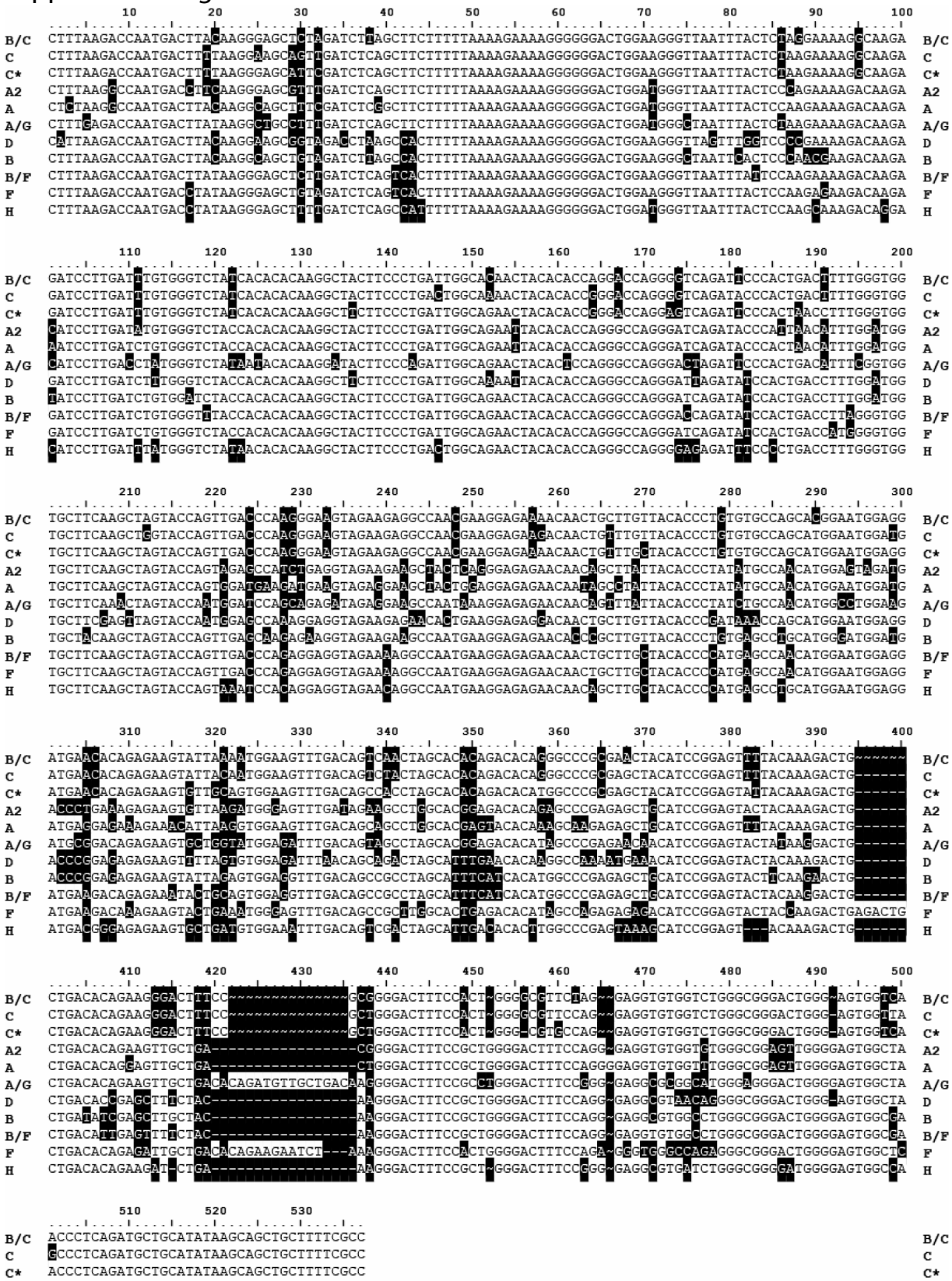

Figure S6. Sequence alignments of U3 regions for global subtypes and circulating recombinant forms (CRFs)

The complete U3 regions of the following subtypes and CRFs were cloned into the *LGIT* lentivirus and used to construct unique latency models: B/C, C, C', A2, A, A/G, D, B, B/F, F, and H. Refer to Table S1 for details of each isolate. See Figure 1B for identification of various cis-binding elements (e.g., Sp1, κB, YY1, AP-1, etc.).

Supplemental Figure S7.

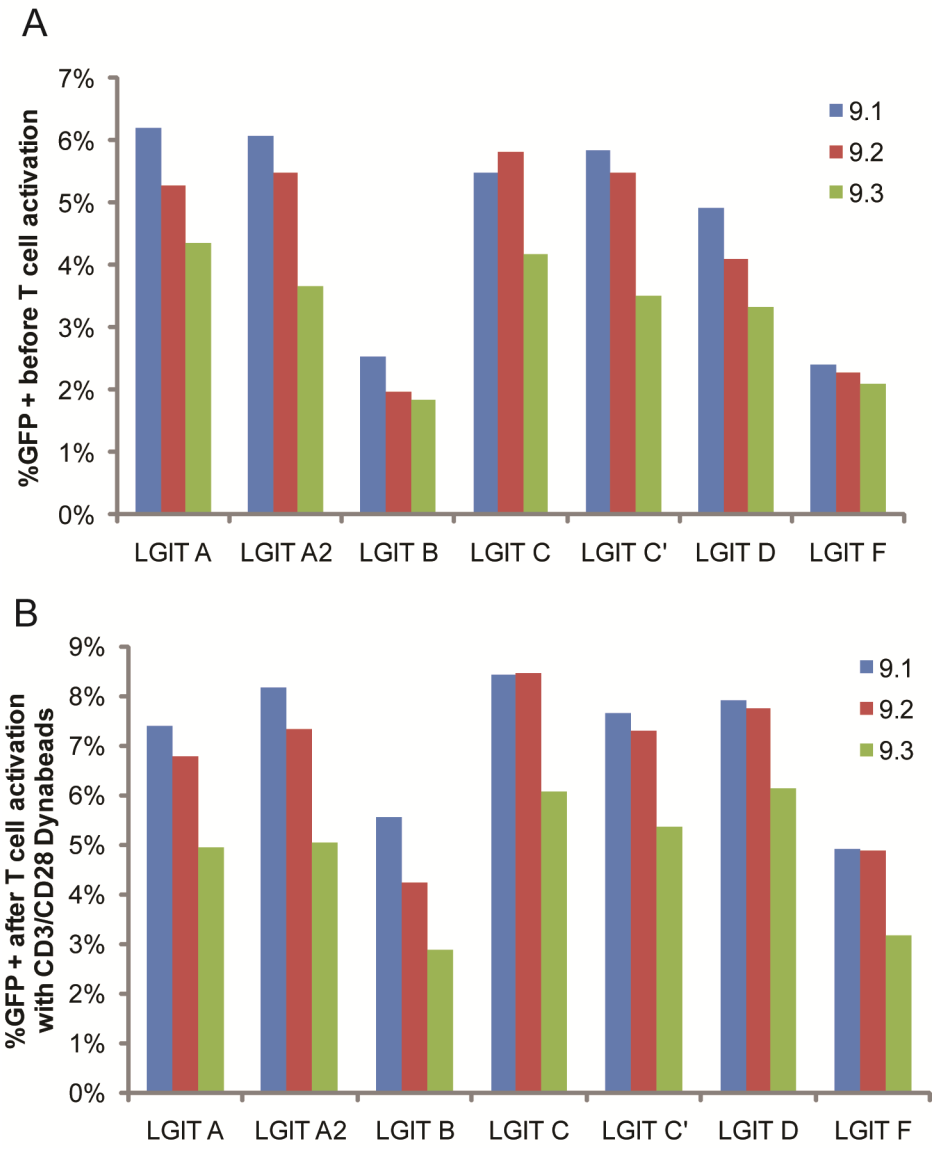

Figure S7. GFP expression before and after T cell activation for primary cells

(A) Flow cytometry was used to measure GFP expression in primary CD4+ T cells seven days after the infection with the *LGIT* subtype variants (14 days post isolation). Cells from each of three donors was infected at low MOI (<0.10), thus the percentage of cells expression GFP is less than 10%.

(B) Same as in (A) for cells treated with CD3/CD28 Dynabeads in order to activate T cells. The percentages of GFP+ cells for all donor and subtype pairs were used as baselines to normalize the latency reactivation experiments in Figure 4A.

Supplemental Figure S8.

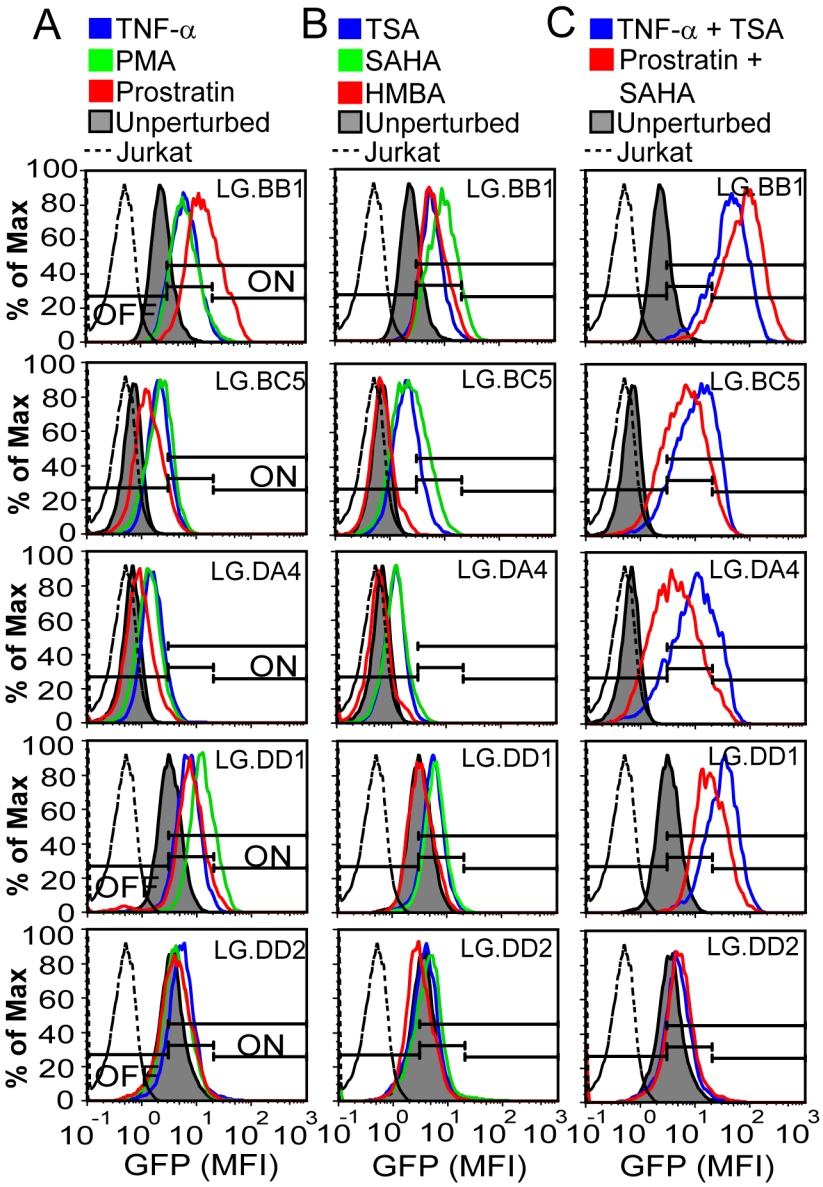

Figure S8. Latency reactivation of *LG* clones with subtype B LTR

(A) Five WT *LG* clones (BB1, BC5, DA4, DD1, and DD2) were isolated to examine latency reactivation for Tat-deficient lentivirus (Figure 1A, panel 4b). For each clone, histogram overlays of stimulation with NF-κB/PKC activators, including TNF-α (blue outline), PMA (green outline), prostratin (red outline), unperturbed clone (shaded gray), and uninfected Jurkat

(dotted black outline). The position of the Off gate is set for uninfected Jurkats (GFP-), whereas the On gate indicates GFP+ cells.

(B) As in (A), histogram overlays of stimulation with HDAC inhibitors, including TSA (blue outline), SAHA (green outline), HMBA (red outline), unperturbed clone (shaded gray), and uninfected Jurkat (dotted black outline).

(C) As in (A), histogram overlays of combinatorial stimulation, including TNF-α+TSA (blue outline), prostratin+SAHA (red outline), unperturbed clone (shaded gray), and uninfected Jurkat (dotted black outline).

Table S1. Primer Sequences for QPCR

Primer Sequence (5'→ 3')	Gene (mRNA target)	*LG* Clone
AGCAAAGACCCCAACGAGAA	*GFP*	All
CGTCCATGCCGAGAGTGAT	*GFP*	All
ACCTGACTGACTACCTCATGAAGATC	*β-Actin*	All
GTGGCCATCTCTTGCTCGAA	*β-Actin*	All
ACCCCAGGTCTCTAGCGTAAA	Upstream in *SPN* (spnUS_forward)	BB1
ATGCCTCGTGAGTTCTCATCT	Upstream in *SPN* (spnUS_reverse)	BB1
TGGTGAAGGATAACCATGTGGTG	Downstream in *QPRT* (qprtDS_forward)	BB1
CTGCTGCATTCCACTTCCA	Downstream in *QPRT* (qprtDS_reverse)	BB1
GGGGAGGAGTTCGATGCAG	Near upstream *ATXNL2* (atxUS_forward)	BC5
CTTTGGGGACATGCGGGAA	Near upstream *ATXNL2* (atxUS_forward)	BC5
GGAGCCGAAGGCATCTTGG	Downstream in *ATXNL2* (atxDS_foward)	BC5
TCTGGAATTGTTGTAGACGCC	Downstream in *ATXNL2* (atxDS_reverse)	BC5
AATACTGACGGGCGAGATATGA	Far downstream in *ZMYM2* (zmDS1_forward)	DA4
TCCAGTCTTGGTCTGTGAAGT	Far downstream in *ZMYM2* (zmDS1_reverse)	DA4
GGAGCCATATTGTTCAACTGCT	Near downstream in *ZMYM2* (zmDS2_forward)	DA4
GATGACTGCATACTTAGAGCCTG	Near downstream in *ZMYM2* (zmDS2_reverse)	DA4
GCGAGTTCAGTGGCGTGGGG	Upstream in *ZMYM2* (zmUS_forward)	DA4
AGGGGGTGGTGCTGAACCTGT	Upstream in *ZMYM2* (zmUS_reverse)	DA4

Table S2. Isolate and Origin of HIV-1 Subtype Variants

Isolate (Country of Isolation)	Subtype of Origin	Reference
pBR020.1 (Brazil)	F	(Gao et al., 1998)
p92UG037.1 (Uganda)	A	(Gao et al., 1998)
p93BR029.4 (Brazil)	B/F	(Gao et al., 1998)
p94UG114.1 (Uganda)	D	(Gao et al., 1998)
p92NG003.1 (Nigeria)	A/G	(Gao et al., 1998)
p90CF056.1 (Central African Rep.)	H	(Gao et al., 1998)
p93IN904 (India)	C	(Lole et al., 1999)
p93IN999 (India)	C (referred to as C')	(Lole et al., 1999)
p94CY017.41 (Cyprus)	A2	(Gao et al., 1998)
p98CN009.8 (China)	B/C	(Rodenburg et al., 2001)
pIIIB wildtype *LGIT* (USA)	B	(Simon et al., 1995)

1

Table S3. Summary of HIV-1 Latency Models

Model	Clonal or Polyclonal	Tat Dependent	Chromatin Silencing	Transcriptional Interference	Synergistic Reactivation (prostratin+SAHA)
LG WT subtype B, Jurkat	Clonal	No	Yes	Probable for clones BB1, BC5, and DA4	3 of 5 clones (BC5, DA4, and DD2)
LGIT WT subtype B, Jurkat	Both	Yes	Yes	Unknown, possible for polyclonal	1 of 1 polyclonal Off sorts
LGIT κB mutants, Jurkat	Both	Yes	Yes	Unknown, but possible for polyclonal	1 of 2 polyclonal Off sorts
LGIT Sp1 mutants, Jurkat	Both	Yes	Yes	Unknown, but possible for polyclonal	1 of 3 polyclonal Off sorts
LGIT subtype variants, Jurkat	Polyclonal	Yes	Yes	Unknown, but possible for polyclonal	6 of 11 polyclonal Off sorts
LGIT subtype variants, Human CD4+ primary cells	Polyclonal	Yes	Yes	Unknown, but possible for polyclonal	6 of 7 subtypes 10 of 21 total subtype/ donor combinations

2