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Supplemental Information

BIRC2/cIAP1 Is a Negative Regulator of HIV-1 Transcription and Can Be Targeted by Smac Mimetics to Promote Reversal of Viral Latency

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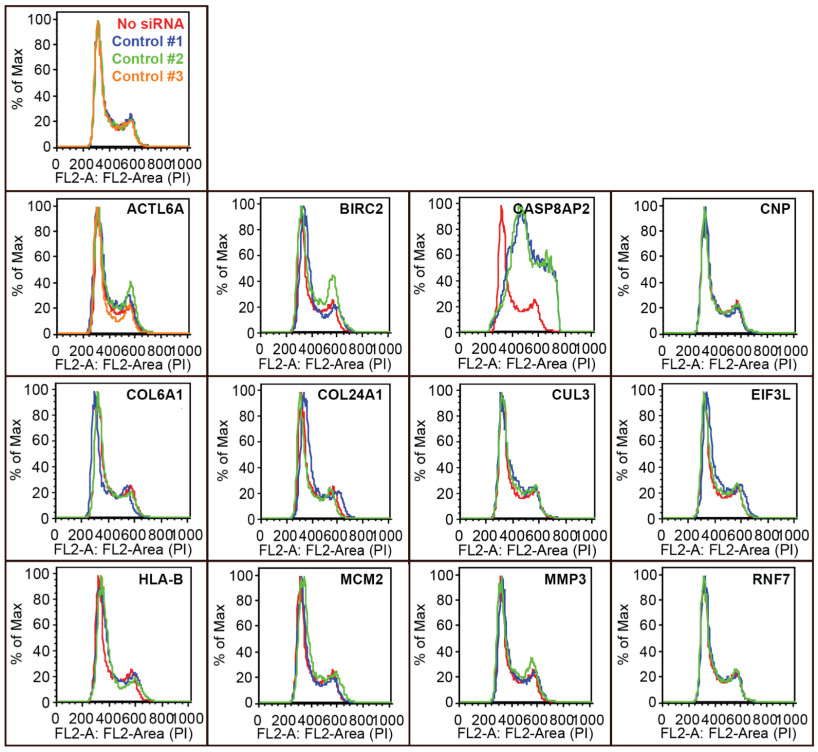
A


No siRNA
Control #1
Control #2
Control #3
% of Max
FL2-A: FL2-Area (PI)
ACTL6A
BIRC2
CASP8AP2
CNP
COL6A1
COL24A1
CUL3
EIF3L
HLA-B
MCM2
MMP3
RNF7

B

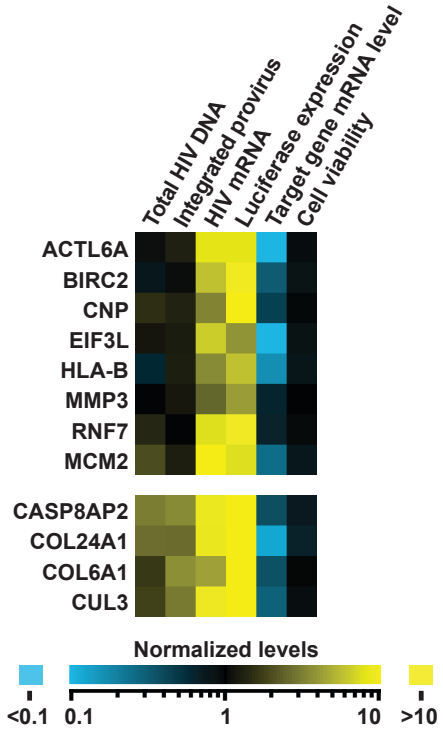
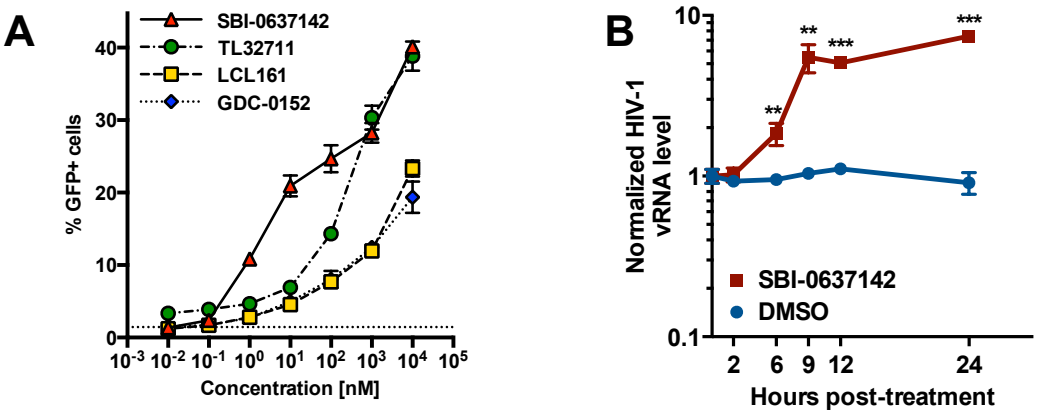
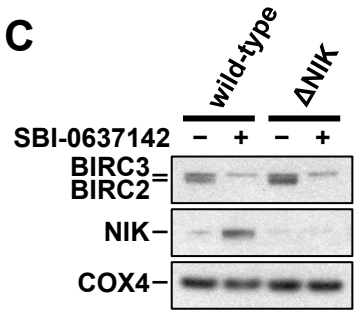
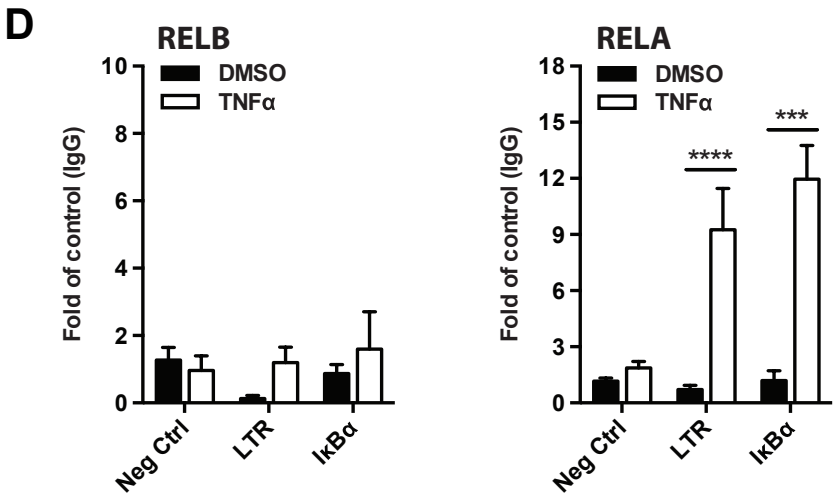

Total HIV DNA
Integrated provirus
HIV mRNA
Luciferase expression
Target gene mRNA level
Cell viability
ACTL6A
BIRC2
CNP
EIF3L
HLA-B
MMP3
RNF7
MCM2
CASP8AP2
COL24A1
COL6A1
CUL3
Normalized levels
<0.1
0.1
1
10
>10

Figure S1


A
SBI-0637142
TL32711
LCL161
GDC-0152
% GFP+ cells
Concentration [nM]
B
Normalized HIV-1 vRNA level
SBI-0637142
DMSO
Hours post-treatment


C
wild-type
ΔNIK
SBI-0637142
BIRC3
BIRC2
NIK
COX4


D
RELB
RELA
DMSO
TNFα
Fold of control (IgG)
Neg Ctrl
LTR
IκBα

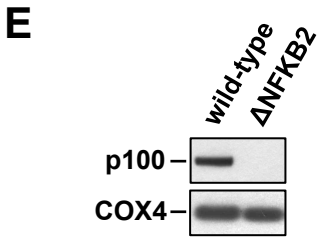
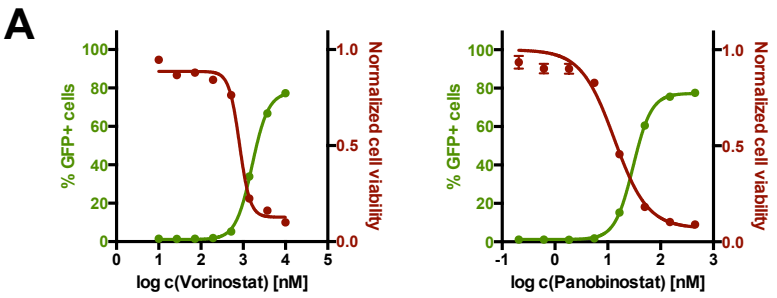
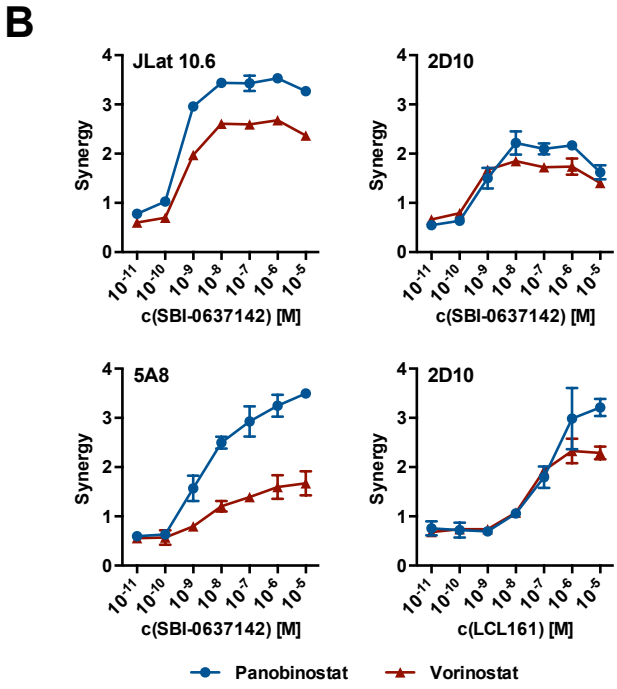

E
wild-type
ΔNFKB2
p100
COX4

Figure S2


A
% GFP+ cells
Normalized cell viability
log c(Vorinostat) [nM]
log c(Panobinostat) [nM]


B
JLat 10.6
2D10
5A8
2D10
Synergy
c(SBI-0637142) [M]
c(LCL161) [M]
Panobinostat
Vorinostat

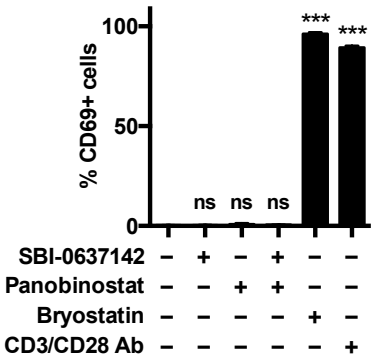

C
% CD69+ cells
ns
***
SBI-0637142
Panobinostat
Bryostatin
CD3/CD28 Ab

Figure S3

SUPPLEMENTAL FIGURE LEGENDS

Figure S1, related to Figure 1.

(A) Analysis of cell cycle progression upon gene knockdown. Cell cycle progression of HEK293T cells treated with individual siRNAs was evaluated by staining of the cells with propidium iodide and subsequent analysis by FACS. Transfection with non-targeting siRNAs (controls) was compared to untransfected cells. Non-targeting control siRNA #1 (red) and 2 or 3 targeting siRNAs (green, blue, orange) are shown for each indicated gene.

(B) Heatmap showing the effects of identified genes on specific steps of HIV-1 replication. HEK293T cells were treated with individual siRNAs targeting the indicated genes. Following infection with HIV-1(VSVg) the levels of HIV-1 total DNA, integrated provirus, and HIV-1 mRNA were determined by qPCR. Also shown are luciferase expression levels, target gene knockdown levels, and cell viability upon target gene knockdown. All values are normalized to non-targeting control siRNAs.

Figure S2, related to Figure 3.

(A) Smac mimetics act as latency reversing agents. 2D10 cells were treated with SBI-0637142, TL32711 (Birinapant), LCL161, or GDC-0152 for 36 hours at the indicated concentrations. Reversal of HIV latency was evaluated by measuring GFP expression using flow cytometry. Data are represented as mean ± SD of three biological replicates. Dotted line represents values of untreated cells.

(B) Kinetics of viral RNA expression in latently infected Jurkat cells upon Smac mimetic treatment. 2D10 cells were treated with 1μM SBI-0637142 or DMSO as negative control. Intracellular viral RNA levels were determined by qPCR at the time points indicated. Data was normalized to GAPDH. Graph shows mean ± SD of three biological replicates. ** indicates $p < 0.01$, *** indicates $p < 0.001$, determined by unpaired t-test.

(C) Knockout of *NIK* in 2D10 cells. Wild-type 2D10 cells and a clone with a knockout of *NIK* were treated with DMSO or 1μM SBI-0637142 and analyzed by Western blot for NIK expression levels.

(D) Impact of TNFα treatment on RELA and RELB interaction with the HIV-1 LTR. 2D10 cells were treated with 20ng/ml TNFα for 2 hours prior to ChIP analysis using antibodies against RELA, RELB, or IgG as control. RELA and RELB specific association with the HIV-1 LTR and the IκBα gene promoter region, or an intergenic region upstream of the *PABPC1* gene unknown to contain NF-κB binding sites as negative control, was detected by qPCR using specific primers and is shown as fold enrichment over IgG control. Data are represented as mean ± SEM of at least three experiments. *** indicates $p \leq 0.001$, **** indicates $p \leq 0.0001$, determined by unpaired t-test.

(E) Knockout of *NFKB2* in 2D10 cells. Wild-type 2D10 cells and a clone with a knockout of *NFKB2* were analyzed by Western blot for p100 expression levels.

Figure S3, related to Figure 4.

(A) Dose response of JLat 10.6 cells treated with HDACi. Following treatment with panobinostat or vorinostat for 36 hours JLat 10.6 cells were evaluated for reactivation of latent provirus by measuring GFP expression using flow cytometry. Cell viability was assessed by determining cellular ATP levels.

(B) Level of drug synergy between Smac mimetic and HDACi. The level of synergy between HDACi and Smac mimetics, when used in combination (see Fig. 4A), was evaluated using the Bliss independence model. Data are represented as mean ± SD of biological triplicates.

(C) Activation of resting CD4+ T cells upon LRA treatment. Resting CD4+ T cells from three healthy donors were treated with 10μM SBI-0637142, 100nM Panobinostat, 30nM Bryostatin, CD3/CD28 antibody beads, or a combination thereof, for 48 hours prior to analysis of CD69 surface expression by FACS. Data is represented as mean ± SEM of three donors. *** indicates $p < 0.001$ determined by unpaired t-test.

Table S1, related to Figure 1B:
139 genes identified by siRNA screen in HIV-1(VSVg)-infected HIK293T cells.

Gene symbol	Normalized luciferase activity [fold change] - siRNA A	Normalized luciferase activity [fold change] - siRNA B
CYLD	16.4357	8.0944
BAF53A	10.1565	7.0609
MCM2	9.7744	4.0175
RNF7	5.8401	4.9686
DCLRE1C	12.3409	2.2605
CASP8AP2	5.7124	4.5880
SPTAN1	10.7234	2.3932
PPP2CA	8.8763	2.6937
MMP3	5.3252	4.1427
CNP	5.3847	3.7683
BCL2	12.6254	1.5834
SEC3L1	9.9753	1.8056
NFKBIA	9.9132	1.7373
PCNA	4.1222	3.8537
ABCG1	5.7841	2.6632
SOM	5.9403	2.3594
BIRC2	4.1112	3.3360
KIAA0648	4.9677	2.6731
TNFRSF1B	4.1992	3.0572
USP46	3.8782	3.2180
BAT4	3.7861	3.0525
CUL3	5.0121	2.0836
POLA	3.7217	2.7096
SST	3.3463	2.9389
MARK2	3.9664	2.3973
PHTF2	4.8546	1.8507
COL6A1	3.1367	2.8366
MAPK10	3.5729	2.4794
CREB1	5.3028	1.5919
COL24A1	2.9147	2.8661
CDC2	3.1238	2.6416
CXCL13	3.5870	2.1635
RNPS1	3.7430	2.0510
UBE2A	3.5199	2.1686
APOC2	3.9817	1.8456
RPA1	2.9917	2.4401
IGSF9	3.0338	2.3950
ASC	4.8232	1.5042
H1FX	3.1979	2.2524
ARF1	3.0811	2.2672
LOC147991	2.9412	2.3601
GNB2L1	2.6431	2.5699
NEUROG3	2.6075	2.5936
KIAA0543	3.0436	2.1472
SMAP-1	4.0219	1.6200
RRM2	3.1676	2.0481
COPG	2.8100	2.2975
MOV10	2.7541	2.3392
FLJ34389	3.9919	1.5913
INCENP	3.4727	1.7849
DDIT4L	3.3447	1.8339
DSP	3.0084	2.0064
SPG7	2.5784	2.3379
HB-1	3.1201	1.8974
TMED2	3.0156	1.9556
TRIP	3.1244	1.8852
WNT9B	2.7210	2.1483
APLP1	3.3806	1.6463
CALR	2.6426	2.1059
LOC124245	2.8733	1.9365
BYSL	2.5379	2.1728
TOLLIP	2.9194	1.8134
SARDH	2.5755	2.0454
NUDT3	2.7249	1.9146
SKP1A	2.6008	1.9888

HUWE1	2.5555	2.0209
HIST1H2BG	3.1588	1.6244
RPS28	2.8781	1.7753
PTP4A1	2.2823	2.2267
LOC56902	2.6710	1.8952
ARL6IP	2.2796	2.2148
WWP1	3.1838	1.5402
FOXF1	2.2049	2.2021
FLJ10652	2.2817	2.1052
TRAF3	2.5508	1.8490
HNRPD	1.9816	2.3622
PCDHGC5	2.4096	1.9204
FKBP9	2.7999	1.6354
GFPT1	2.8429	1.5990
FYN	2.2625	1.9757
RFC3	2.4740	1.7898
RPS19	2.4816	1.7757
TCEB1	2.0571	2.1322
COL25A1	2.2629	1.9360
ARL10B	2.1091	2.0735
N4BP2	2.3102	1.8737
ARID5A	2.6680	1.6122
ELAVL1	2.4303	1.7147
PSIP1	2.3450	1.7684
A1BG	2.4770	1.6692
FBXO39	2.4382	1.6781
C10ORF58	2.2061	1.8450
HLA-B	2.2398	1.8131
MRE11A	2.4244	1.6656
MAPK6	2.3082	1.7492
CUL2	2.5064	1.5954
DUSP6	2.0443	1.9428
NFIB	2.1595	1.8185
RRM1	2.0690	1.8970
CUTL1	1.9807	1.9483
LRRC4B	1.9675	1.9338
VSNL1	2.2678	1.6776
CUL1	2.4034	1.5814
SMOC2	2.2119	1.7071
TMEM42	2.2623	1.6625
VCP	2.0463	1.8119
CIRBP	1.9906	1.8221
CAMK2G	2.0364	1.7797
LOC155054	2.0052	1.7965
RNF31	2.2811	1.5740
PDGFA	1.8798	1.8720
UGT2A1	2.0627	1.6740
TUSC2	2.1243	1.6231
CDT1	1.9329	1.7759
TBCD	1.8974	1.7942
OGT	2.0277	1.6737
SMARCB1	2.1654	1.5227
BIRC5	1.8156	1.8095
XPO1	1.8288	1.7945
NUDT10	1.9933	1.6150
FLJ20512	1.9537	1.5846
MUS81	1.7882	1.7270
GOLPH3	1.8379	1.6636
FLJ38984	1.8912	1.5810
BG1	1.7766	1.6733
RLN2	1.7620	1.6737
WNT9A	1.7755	1.6558
EIF3L	1.8948	1.5380
ZDHHC8	1.7427	1.6609
HIST1H1C	1.8701	1.5416
REPS1	1.7222	1.6691
RFP2	1.7119	1.6743
ZNF142	1.7932	1.5650
GMDS	1.8369	1.5154
LOC90693	1.7829	1.5568
UBE2L3	1.6680	1.6261
PASK	1.7468	1.5258
MEGF11	1.6399	1.6026
DNCLI1	1.7097	1.5180

Table S2, related to Figure 1D: Results of validated siRNAs in HIV-1(VSVg) infection assay

Entrez Gene ID	NCBI Gene Symbol	siRNA used for life cycle mapping	siRNA target sequence	Normalized luciferase expression [fold increase]	Normalized cell viability [%]	Normalized target gene expression level [%]
86	ACTL6A	*	AAAGCTTTAACTGGCTCTATA	8.1	92.2	7.0
86	ACTL6A		CACAATGTTAGGAGTCAGTCA	8.1	67.4	17.3
329	BIRC2	*	TCCCAGGTCCCTCGTATCAAA	9.5	84.1	31.7
329	BIRC2		CTAGGAGACAGTCCTATTCAA	7.1	71.2	39.3
9994	CASP8AP2		CAGCTGATGTGCGGAAGTCAA	10.8	70.9	37.5
9994	CASP8AP2	*	CAGTCTGATCTCAATAAGGAA	21.4	73.0	37.3
1267	CNP		CAGGGCCTTGCTAATCGGGTT	11.9	104.2	48.6
1267	CNP	*	CGCCGGGACATCAGAATTCTT	11.3	95.7	43.7
255631	COL24A1		AACACTCTACTTGAACCTAAA	4.5	80.1	33.5
255631	COL24A1	*	CTGAACTACCTTAGCAATTTA	14.9	65.4	11.9
1291	COL6A1		CACCATCGTGGACATGATCAA	10.3	87.8	22.3
1291	COL6A1	*	CCCGGGTTTGACGGCATTCAA	11.3	102.2	36.4
8452	CUL3		AACAACTTTCTTCAAACGCTA	26.3	77.1	37.5
8452	CUL3	*	TCGAGATCAAGTTGTACGTTA	41.2	90.7	29.3
51386	EIF3L	*	TACGCTTATCCCAGCGACTAT	3.8	86.8	7.9
51386	EIF3L		AAGCGAGGCAGCCATGTCTTA	2.6	92.2	10.8
3106	HLA-B	*	TCCTGGAATTTGAGAGAGCAA	5.8	78.8	16.4
3106	HLA-B		CGGGCATGACCAGTACGCCTA	4.0	91.7	15.8
4171	MCM2	*	CTGCGGGACTATGTGATCGAA	7.6	76.6	25.4
4171	MCM2		CTCATTGGAGATGGCATGGAA	4.7	89.5	13.0
4314	MMP3	*	AGGGATTGACTCAAAGATTGA	4.1	98.8	62.1
4314	MMP3		GACAGTGGTCCTGTTGTTAAA	2.9	96.6	39.7
9616	RNF7	*	CTGTTCAATCATTGAGTGGTA	9.3	94.3	64.9
9616	RNF7		ATGCTTATGGTTGATCAGTTA	7.3	80.0	42.5

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

siRNA transfections. Individual siRNAs were transfected into HEK293T cells at a concentration of 12.5 nM using RNAiMAX Transfection Reagent (Life Technologies) following a reverse transfection protocol as previously described (König et al., 2007). Cells were infected with HIV-1(VSVg) 48 hours post transfection (König et al., 2008).

Viral infections with luciferase reporter virus. VSV-G-pseudotyped lentiviral supernatant (HIV-1(VSVg)) was generated using the pNL43-Luc-E-R+ (HIV-1 wild-type Δenv, encoding firefly luciferase GL3) vector as previously described (König et al., 2007). Following a 24 hour incubation of cells with HIV-1(VSVg), luciferase expression levels were determined using the Bright-Glo Luciferase Assay System (Perkin Elmer). A second set of siRNA-transfected cells was mock infected and cell viability was determined 72 hours post transfection using the ATPlite cell viability assay (Perkin Elmer).

Infection with LTR mutant viruses. HEK293T cells transfected with siRNA or treated with compounds were infected with VSV-G-pseudotyped HIV-1 containing either wild-type or mutant NF-κB binding sites in the LTR (Bosque and Planelles, 2009). After 24 hours of infection HIV-1 mRNA levels were analyzed by qPCR as described below.

Mapping to viral life cycle stages. HEK293T cells were transfected with individual siRNAs and infected with HIV-1(VSVg) as described above. DNA was isolated using the DNeasy 96 Blood & Tissue kit (Qiagen) and quantitated using Quant-iT PicoGreen R dsDNA Assay Kit (Life Technologies). Proviral DNA content was measured using Alu-PCR and total HIV DNA using internal PCR primers as described in (Butler et al., 2001; König et al., 2008; O'Doherty et al., 2002) using an Applied Biosystems 7500 Fast Real Time PCR system. All tested siRNAs were compared to a set of three scrambled negative control siRNAs analyzed in parallel. All siRNAs were tested in three independent biological replicates, and each biological replicate was assayed twice. DNA oligonucleotides used are summarized in a table below.

Oligonucleotides used for viral DNA quantification.

Primer	Oligonucleotide sequence	Position
HIV LTR (R) Forward	GCCTCAATAAAGCTTGCCTTGA	522:543 in R
HIV LTR (U5) Reverse	TCCACACTGACTAAAAGGGTCTGA	599:622 in U5
Alu Forward	GCCTCCCAAAGTGCTGGGATTACAG	in human genome
HIV Gag Reverse	GCTCTCGCACCCATCTCTCTCC	782:803 bridges beginning of gag
LTR Molecular Beacon	FAM-GCGAGTGCCCGTCT GTTGTGTGACTCTGGTAACTAGCTCGC-Dabcyl	

To quantify HIV mRNA levels samples were prepared and analyzed by qPCR using the Fast SYBR Green Cells-to-Ct Kit (Life Technologies). The primer pair 5'-TGTGTGCCCGTCTGTTGTGT-3' and 5'-GAGTCCTGCGTCGAGAGATC-3' was used to detect HIV-1 late RT product. mRNA levels were normalized to the cellular gene *GAPDH* using the primer pair 5'-CATGAGAAGTATGACAACAGCCT-3' and 5'-AGTCCTTCCACGATACCAAAGT-3'.

Cell cycle analysis. HEK293T cells were transfected with siRNAs, collected three days post transfection and fixed in absolute ethanol at -20°C overnight. Cells were washed twice with DPBS and resuspended in 3.8 mM sodium citrate, 40 µg/ml propidium iodide, and 0.5 µg/ml RNase A in DPBS. After incubating at 4°C for 3 hours, flow cytometry was performed on a BD FACScan (BD Biosciences).

Chemical compounds. Synthesis of SBI-0637142 was performed as previously described (Vamos et al., 2013). LCL161 (Weisberg et al., 2010) and TL32711 (Birinapant) (Condon et al., 2014) were obtained from ChemieTek. GDC-0152 (Flygare et al., 2012) was obtained from Santa Cruz Biotechnology. Bryostatin was purchased from Sigma-Aldrich. Vorinostat (suberanilohydroxamic acid, SAHA) and panobinostat were purchased from LC Laboratories and Fisher Scientific, respectively. All compounds were dissolved in dimethyl sulfoxide (DMSO, Fisher Scientific). Equal concentrations of DMSO were used as negative control.

Western blot analysis. To analyze whole cell lysates, cells were lysed in radioimmunoprecipitation assay (RIPA) buffer. Cytoplasmic extracts were prepared by incubating cells in Buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitors (Sigma Aldrich)). The cells were incubated on ice for 15 min. Next, 10% Nonidet P-40 was added to a final concentration of 0.5% and the suspension was vortexed for 10 s. Cytoplasmic extracts were collected after pelleting nuclei by centrifugation. Nuclear extracts were prepared by washing isolated nuclei twice using Buffer A, followed by incubation in Buffer B (20 mM HEPES, pH 7.9, 400 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitors (Sigma Aldrich)) for 15 min on ice. After centrifugation at 20,000 rcf for 10 min the supernatants were collected as nuclear extracts. Protein concentrations of all extracts were determined using the Pierce BCA Protein Assay Kit (Life Technologies) and Western blot analysis was conducted following standard protocols. Primary antibodies against NIK (#4994), p100/p52 (#4882), IκBα (#9242), RELB (#4922), HSP90 (#4874), COX4 (#4850), and β-actin (#4970) were purchased from Cell Signaling. Primary antibodies against PARP1 (sc-8007) and RELA (sc-8008) were purchased from Santa Cruz Biotechnology. Antibodies recognizing BIRC2 (AF8181) and BIRC2/BIRC3 (MAB3400, clone 315301) were obtained from R&D Systems. An antibody against GAPDH (TA802519, clone 2D9) was purchased from OriGene. Secondary antibodies against mouse, rabbit, and goat IgG were purchased from LI-COR Biosciences and Bio-Rad. Western blots were analyzed by detection of chemiluminescence or using a LI-COR Odyssey CLx Imager.

HIV-1 infection of Human CD4+ T cells. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient centrifugation (Histopaque, Sigma Aldrich) from buffy coats of healthy human donors (San Diego Blood Bank). CD4+ T cells were subsequently isolated by negative selection using magnetic beads (CD4+ T cell isolation kit II; Miltenyi Biotec). CD4+ T cells were cultured in RPMI1640 supplemented with 10% FBS, 100 IU penicillin, 100 µg/ml streptomycin, 0.1 M HEPES, 2 mM L-glutamine, and 20 units/ml interleukin-2 (IL-2) (NIH AIDS Reagent Program). Cells were activated with 4 µg/ml phytohemagglutinin-P (PHA) (Sigma) for 48 hours. Activated CD4+ T cells were treated with SBI-0637142, LCL-161, DMSO, or mock treated for 24 hours prior to infection with HIV-1(VSVg). Luciferase expression levels were determined using Bright-Glo Luciferase Assay System (Perkin Elmer). Cell viability of treated cells was determined using ATPlite cell viability assay (Perkin Elmer). Values were normalized to mock-treated cells; average value of DMSO-treated cells was defined as 1.

cDNA overexpression. Complementary DNA of CD40 or LTβR in the expression vector pLX304 was transfected into HEK293T cells using Lipofectamine 2000 transfection reagent (Life Technologies) according to the manufacturer's instructions. 24 hours after transfection cells were infected with HIV-1(VSVg) for 24 hours prior to the analysis of luciferase expression levels using the Bright-Glo Luciferase Assay System (Perkin Elmer).

Chromatin Immunoprecipitation: 2D10 cells were stimulated with 1 µM SBI-0637142 or DMSO for 9 h or with 20 ng of human TNFα for 2 h as a control. Cross-linking was carried out using a two-step method as previously described (Nowak et al., 2005). Cells were then lysed in RIPA buffer supplemented with protease and phosphatase inhibitor cocktails (Sigma Aldrich). Total cell lysates were extensively sonicated using a Covaris S220 Focused Ultrasonicator. Lysates were then cleared by centrifugation and supernatants were diluted 10X in RIPA buffer without SDS. Immunoprecipitations were carried out as previously described (Carey et al., 2009). The diluted whole cell lysates were incubated with Dynabeads protein G (Life Technology) and anti-RELB or anti-RELA antibodies (sc-226 and sc-372, Santa Cruz Biotechnology), or a rabbit anti-human IgG control overnight at 4°C. IP samples were washed with four different buffers according to the protocol (Carey et al., 2009). To revert the cross-link, eluted samples were incubated overnight at 65°C in the presence of 0.3 M NaCl. DNA fragments were precipitated in phenol-chloroform and DNA pellets were reconstituted in water. Samples were prepared and analyzed by qPCR. Primer pairs were designed to span the NF-κB binding site-containing region of the HIV-1 LTR, the promoter region of IκBα, or an intergenic region upstream of the *PABPC1* gene unknown to contain NF-κB binding sites as negative control. The following primer sequences were used: 5'-AGGTTTGACAGCCGCCTA-3' and 5'-AGAGACCCAGTACAGGCAAAA-3' for the HIV-1 LTR, 5'-GACGACCCCAATTCAAATCG-3' and 5'-TCAGGCTCGGGGAATTTCC-3' for the IκBα promoter, and 5'-CATGGGTGGAGCTGGTCAAT-3' and 5'-TCCTAGCAGAGATCCATGCAGAT-3' for the negative control.

Knockout cell lines: *NIK* and *NFKB2* knockout cells were generated using the Cas9 nuclease system. sgRNAs oligos were designed using the CRISPR design tool (http://tools.genome-engineering.org) and were

cloned into the pSpCas9(BB) vector as previously described (Ran et al., 2013). Sorting of transfected cells by FACS was carried out by co-expressing pSpCas9(BB) containing the specific sgRNAs oligos with the pmCherry-C1 vector. The sgRNA sequences used to knock out *NIK* or *NFKB2* were: 5' – CGTCTACAAGCTTGAGGCCG – 3' specific for exon 2 of the *NIK* gene (NG_033823.1 – 31417 to 31436), and 5' – CTTCACAGCCATATCGAAAT – 3' specific for exon 5 of the *NFKB2* gene (NG_033874.1 – 7627 to 7646).

Jurkat HIV latency model. JLat 10.6 cells (Jordan et al., 2003) from Dr. Eric Verdin were obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH. 5A8 cells (Chan et al., 2013; Sakane et al., 2011; Spina et al., 2013) were obtained from Dr. Warner C. Greene (Gladstone Institutes), and 2D10 cells (Pearson et al., 2008) from Dr. Jonathan Karn (Case Western Reserve University). Latently infected Jurkat cells were treated with compounds and subsequently analyzed for GFP expression by flow cytometry using a BD LSRFortessa cell analyzer with high throughput sampler (BD Biosciences). Cell viability was determined using the ATPlite cell viability assay (Perkin Elmer).

Analysis of drug synergy. The Bliss independence score (Bliss, 1939) was used to calculate the expected level of latency reversal when combined treatment with drugs A and B is additive. The Bliss Score was calculated using $F_{AB} = F_A + F_B * (1 – F_A)$ where F_A and F_B represent the fraction of GFP+ cells reactivated upon treatment with drug A and drug B alone and F_{AB} represents the fraction of GFP+ cells reactivated upon combined treatment with drug A and drug B. Synergy was calculated as follows: Synergy = $Observed_{AB} / F_{AB}$ where $Observed_{AB}$ represents the fraction of GFP+ cells observed upon combined treatment with drug A and drug B.

Treatment of resting CD4+ T cells from aviremic HIV patients. LRA activity of compounds was evaluated using the recently described REVEAL (rapid *ex vivo* evaluation of anti-latency) assay (Spivak et al., 2015). Aviremic HIV-1 infected patients on ART were recruited for phlebotomy according to an approved institutional review board protocol at the University of Utah (Spivak et al., 2015). Inclusion criteria mandated viral suppression (less than 50 HIV-1 RNA copies/mL) for a minimum of six months, ART initiation during chronic HIV-1 infection (greater than six months since seroconversion) and compliance with a stable ART regimen for a minimum of twelve months per participant and provider report. Informed consent and phlebotomy were performed in the Center for Clinical and Translational Science Clinical Services Core at the University of Utah Medical Center.

Peripheral blood mononuclear cells were isolated from whole blood immediately after phlebotomy via density gradient centrifugation, followed by negative selection of resting CD4+ T cells using magnetic bead separation (Miltenyi Biotec and StemCell Technologies). Aliquots of $5x10^6$ resting CD4+ T cells were cultured under multiple conditions: culture medium and DMSO (solvent) alone (negative control), CD3/CD28 antibody-coated magnetic beads (positive control) and medium containing candidate latency reversing agents at concentrations previously shown to induce viral reactivation. At 48 hours culture supernatants were collected for real time quantitative polymerase chain reaction (qPCR). Supernatant from each sample was collected for

quantification of cell-free virions using a two-step qPCR that makes use of a primer and probe set for conserved regions of the 3' LTR of HIV-1 mRNA (Shan et al., 2013).

Analysis of resting CD4+ T cell activation. Resting CD4+ T cells isolated from the blood of healthy donors were treated with DMSO, 10 μM SBI-0637142, 100 nM panobinostat, 30 nM bryostatin, or CD3/CD28 antibody-coated magnetic beads for 48 hours. Cells were stained with PE-labeled anti-CD69 antibody sc-18880PE (Santa Cruz Biotechnology) and analyzed by FACS.

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