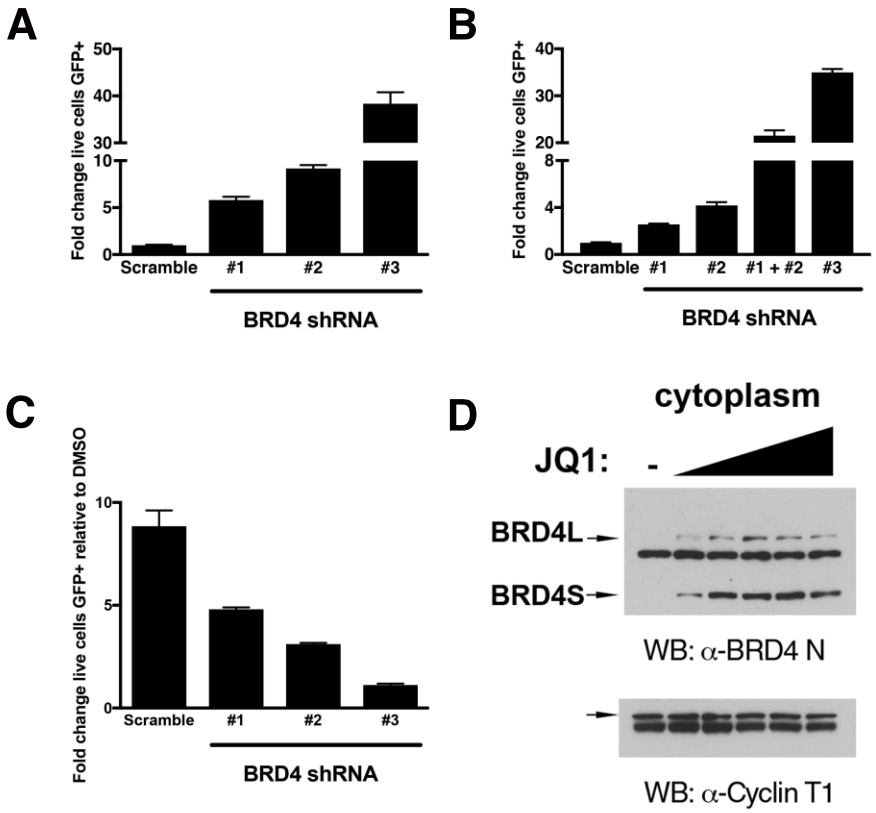

Figure S1. Related to Figure 1. Short BRD4 Promotes HIV-1 latency

(A) J-Lat A2 cells were transduced with lentiviral shRNAs targeting BRD4 as in Figure 1 or scramble non-targeting shRNAs as controls. Flow cytometry of GFP indicating HIV transcriptional activity (mean of three independent experiments analyzed in triplicate ± SEM).

(B) J-Lat A2 cells were transduced with indicated BRD4 shRNAs, either alone or in combination, and GFP levels were analyzed via flow cytometry. Mean (±SD) of one experiment performed in triplicate is shown.

(C) Flow cytometry of J-Lat A2 cells transduced with shRNAs described in Figure 1 treated with JQ1 (625nM for 18h). Means of three independent experiments analyzed in triplicate ± SEM is shown.

(D) Western blotting of cytoplasmic fractions from J-Lat A72 cells treated with increasing concentrations of JQ1, as in Figure 1D, using BRD4 and Cyclin T1 antibodies.

Supplementary Figure 2

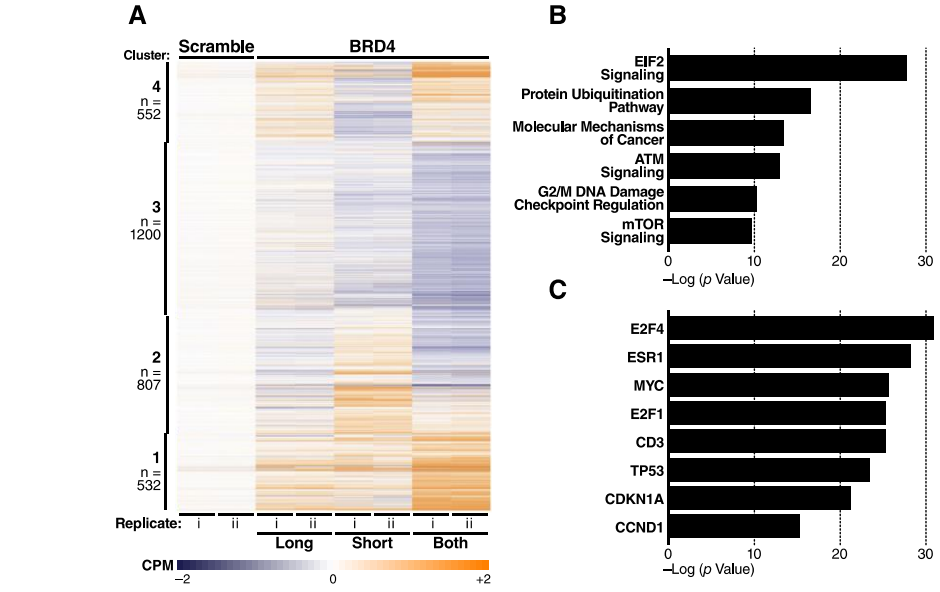

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Figure S2. Related to Figure 1. Transcriptomic disruption upon knockdown of BRD4 isoforms

(A) Heatmap of binned RNA-Seq data from J-Lat A72 cells transduced with shRNAs as in Figure 1. Unsupervised non-hierarchical clustering of differentially expressed genes is shown to the left. Bottom scale is -2 (blue) to 2 (orange) counts per million (CPM) normalized to the respective scramble control.

(B) Canonical pathways enriched in total differentially expressed genes as revealed by Ingenuity Pathway Analysis (Qiagen).

(C) Upstream regulators enriched in total differentially expressed genes as revealed by Ingenuity Pathway Analysis (Qiagen).

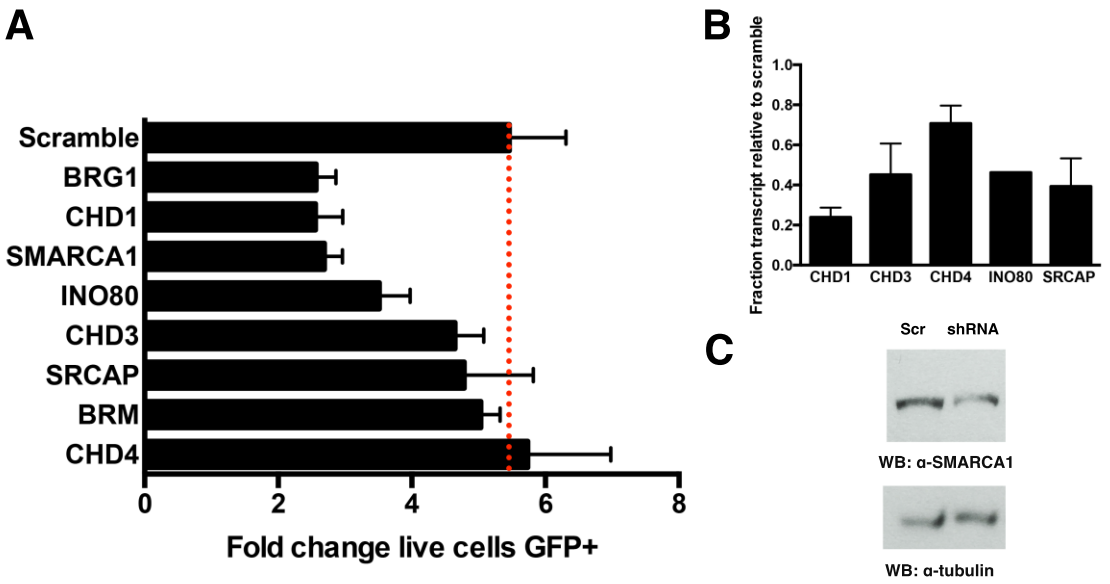

Figure S3. Related to Figure 3. Targeted Screen of Chromatin Remodeling ATPases Reveals BRG1 as Necessary for JQ1-Mediated Reversal of HIV-1 Latency

(A) Flow cytometry measuring HIV reactivation in the presence 625nM JQ1 for 18h in J-Lat A72 cells transduced with shRNAs targeting indicated factors.

(B, C) Knockdown was assessed via qPCR (relative to RPL13A) for all ATPases (B) with the exception of BRG1 (Figure 3A), BRM (Figure 3A), and SMARCA1 (C). Screen flow cytometry data represent the average of three independent experiments normalized to respective DMSO controls ±SEM, and the knockdown assessment is representative.

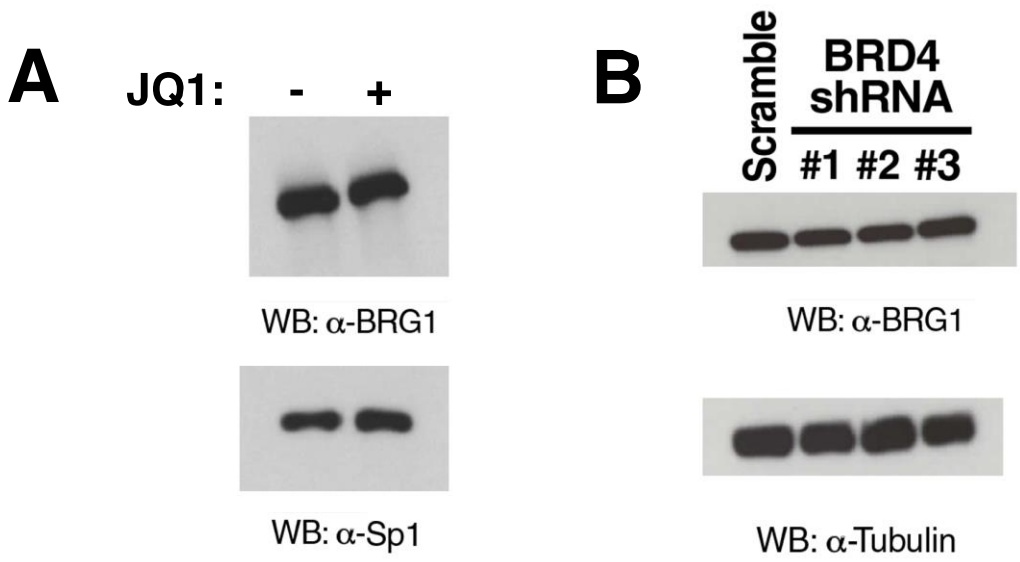

Figure S4. Related to Figure 5. BET Inhibition and BRD4 Knockdown Do Not Alter BRG1 Protein Levels

(A) Western blotting of J-Lat A72 nuclear/chromatin extracts from cells treated with DMSO or 625nM for 18h.

(B) Western blotting of J-Lat A72 nuclear/chromatin extracts from cells transduced with BRD4 shRNAs (see Figure 5).

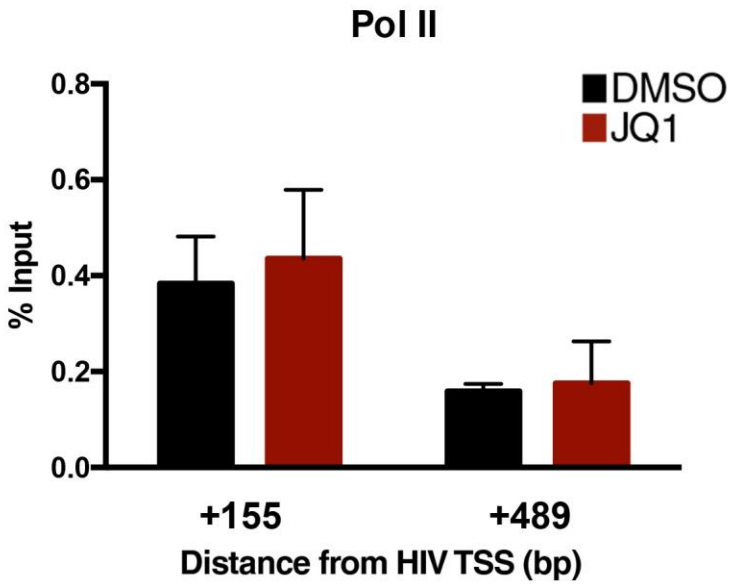

Figure S5. Related to Figure 5. JQ1 Minimally Induces Pol II Recruitment at the HIV Promoter

Pol II ChIP-qPCR data from J-Lat A72 cells treated with DMSO or 625nM for 18h. X-axis numbers represent nt distance relative to the HIV-1 TSS. Data are the mean of three independent experiments normalized to non-specific IgG values ±SEM.

Sample	Total Tags	Mapped Tags
DMSO_BRD4.1	39787057	38402467
DMSO_BRD4.2	35118886	34290080
DMSO_BRD4.3	47073281	43519248
DMSO_BRG1.1	37494392	35522186
DMSO_BRG1.2	35160019	32726945
DMSO_ATAC.1	70258268	67469014
DMSO_input.1	30240996	25798593
DMSO_input.2	35835457	29793598
DMSO_input.3	32765315	26815133
DMSO_polII.1	52937018	43868906
DMSO_polII.2	39917419	38652036
JQ1_BRD4.1	41040011	39262978
JQ1_BRD4.2	30768417	25107028
JQ1_BRD4.3	51920881	41604201
JQ1_BRG1.1	45587840	44010500
JQ1_BRG1.2	46728098	45461766
JQ1_ATAC.1	100675510	96900178
JQ1_input.1	32474538	27561140
JQ1_input.2	39716940	33755427
JQ1_input.3	29760842	24868159
JQ1_polII.1	47759352	45997031
JQ1_polII.2	49831921	48331980
RNA_scr.1	48885572	36726146
RNA_scr.2	56828958	42848770
RNA_sh1.1	43746089	32355456
RNA_sh1.2	47885622	37034631
RNA_sh2.1	50331493	38305565
RNA_sh2.2	49194910	37757689
RNA_sh3.1	43094648	33146144
RNA_sh3.2	53506369	41328763

Table S1. Related to Figures 6, 7, S2. Mapping statistics for high throughput sequencing experiments.

Primer	Application	Forward (5′ to 3′)	Reverse (5′ to 3′)
AflII_HIV	AflII accessibility	TGGGAGCTCTCTG GCTAACTA	CTGGTTTCCCTTT CGCTTTC
LM-PCR_A	LM-PCR 1st strand synthesis	-	ATTTTTGGCGTAC TCACCAGTC
LM-PCR_linkers	LM-PCR ligation	GCGGTGACCCGG GAGATCTGAATTC	GAATTCAGATC
LM-PCR_B	LM-PCR amplification	GCGGTGACCCGG GAGATCTGAATTC	ATTTTTGGCGTAC TCACCAGTC
LM-PCR_C	LM-PCR labeling	-	6-FAM-TACTCACCAGTCG CCGCCCCTCGCCT CTTG
HIV_ChIP_155	ChIP-qPCR	AGTGTGTGCCCGT CTGTTGT	TTCGCTTTCAGGT CCCTGTT
HIV_ChIP_489	ChIP-qPCR	GCAAGCAGGGAG CTAGAACG	GGATGGTTGTAGC TGTCCCAGT
HIV_GFP	RT-qPCR	ATGGTGAGCAAG GGCGAGGAG	GTGGTGCAGATGA ACTTCAG
RPL13A	RT-qPCR	GCCCTACGACAAG AAAAAGCG	TACTTCCAGCCAA CCTCGTGA
CHD1	RT-qPCR	AAACAGTAGTGG AGAATCAAGCC	AGAACTCGAACC AGATCCAGAG
CHD3	RT-qPCR	CCGTCAGCATTGG GTGTGAA	TCTTGCGTTTTCG GGGTTTTC
CHD4	RT-qPCR	GGAGCCTAAATCA TCTGCTCAG	GTGAGGGTTCGAT AATCCTCCT
INO80	RT-qPCR	TGCGACAAACGTC AGCTATCT	CTGGGGCAATAAT GGATTACTGT
SRCAP	RT-qPCR	AACTGGCGCTATC TCATTCTGG	CTGTTCTGCAAGG GAGTTCCT
BDS	Cloning	CGACAAGCTTCC CCCAGAGACCTC	GACGGATCCCTAC TCAGGCTCGTCC
IDS	Cloning	CGACAAGCTTATG CCGGACGAGCCTG	GACGGATCCCTAC TTGCACTTGTCCT C
ET	Cloning	CGACAAGCTTTCG GAGGAAGAGGAC AAG	GACGGATCCCTAT TGAGGTTTCCTTT TCTTCCG
SV40_NLS_linker	Cloning	GACAAGCTTCCAA AGAAGAAGCGGA AGGTCAAGCTTGA T	ATCAAGCTTGACC TTCCGCTTCTTCTT TGGAAGCTTGTC

Table S2. Related to Figures 1–5, S3, S5. PCR primers used.