JQ1 affects BRD2-dependent and independent transcription regulation without disrupting H4-hyperacetylated chromatin states

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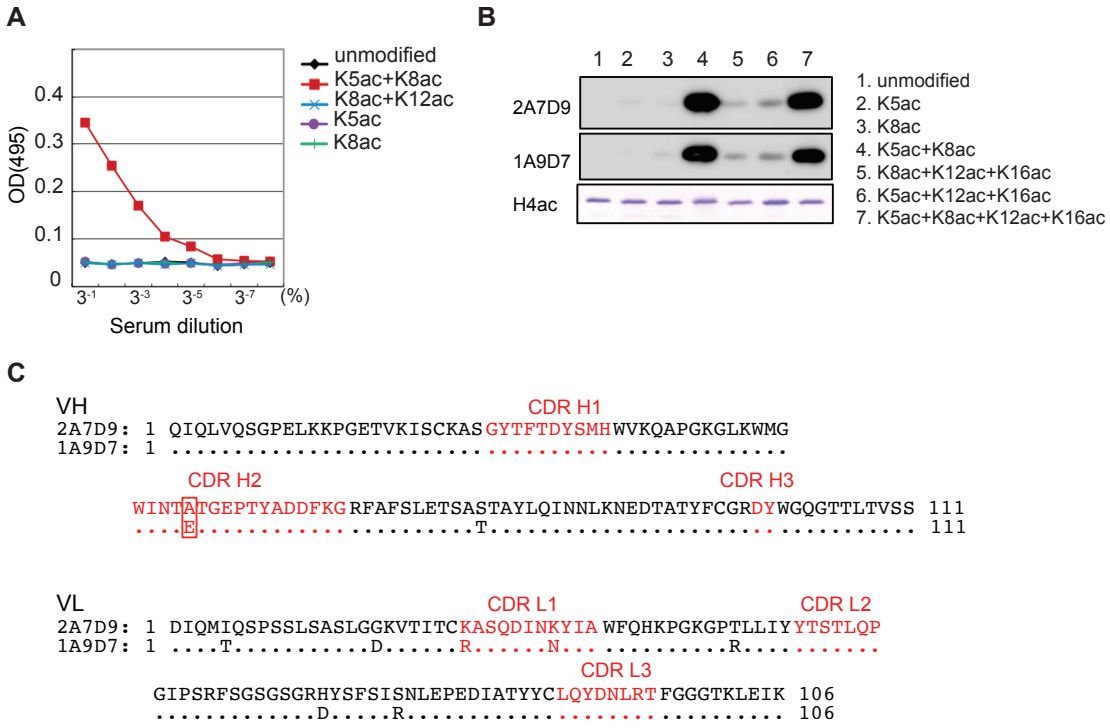


Figure S1. Validation of H4K5acK8ac antibodies by ELISA and Western blot. (**A**) ELISA assay for 1A9D7 antibody. Histone H4 protein containing indicated site-specific acetyllysine(s) are used as antigens. (**B**) Western blot analysis for 2A7D9 and 1A9D7 antibodies to acetylated H4 proteins. Lanes are as follows: Lane 1, Non-acetylated H4; lane 2, H4 with K5ac; lane 3, H4 with K8ac; lane 4, H4 with K5ac and K8ac; lane 5, H4 with K8ac, K12ac and K16ac; lane 6, H4 with K5ac, K12ac and K16ac; and lane 7, H4 with K5ac, K8ac, K12ac and K16ac. The bottom panel shows Coomassie Brilliant Blue (CBB)-stained H4 proteins used for western blotting. (**C**) Sequence alignment of 2A7D9 (top) and 1A9D7 (bottom). In the alignment, the conserved (identical) amino acid is denoted by a dot. Complementarity-determining regions (CDRs) are highlighted in red. The 54th amino acids of V_H are circled.

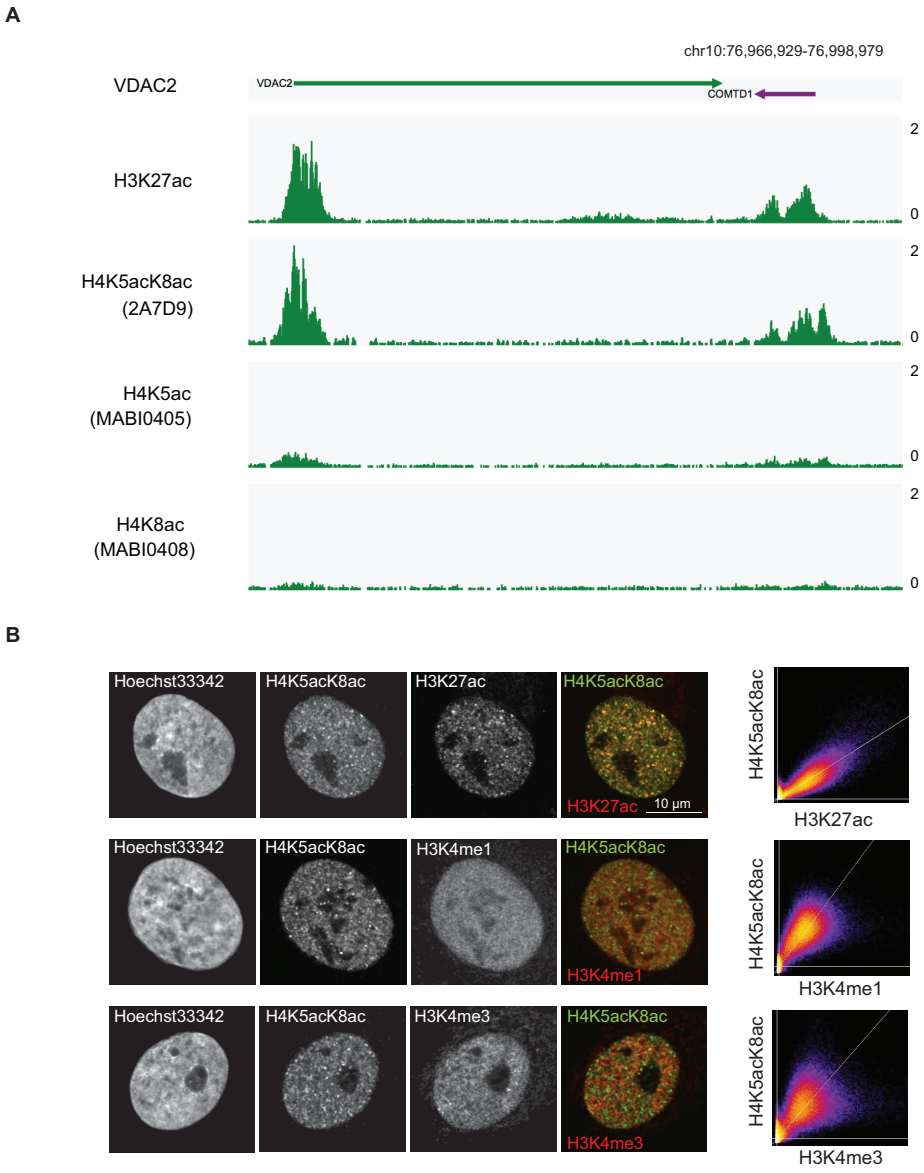


Figure S2. Validation of H4K5acK8ac antibody by ChIP-seq and Immunostaining. (**A**) Genome browser view of VDAC2 region showing high quality ChIP grade of H4K5acK8ac antibody especially in comparison to the commercially available mono-acetyl (H4K5ac and H4K8ac) antibodies. (**B**) Co-localization of H4K5acK8ac with H3K27ac (upper), H3K4me1 (middle), and H3K4me3 (lower) as validated by immunofluorescence (IF). Hoechst33342 (left) represents nuclei staining. Scale bar, 10 µm. The most right panel indicates correlation between IF signals derived from the immunostaining of the indicated antibodies.

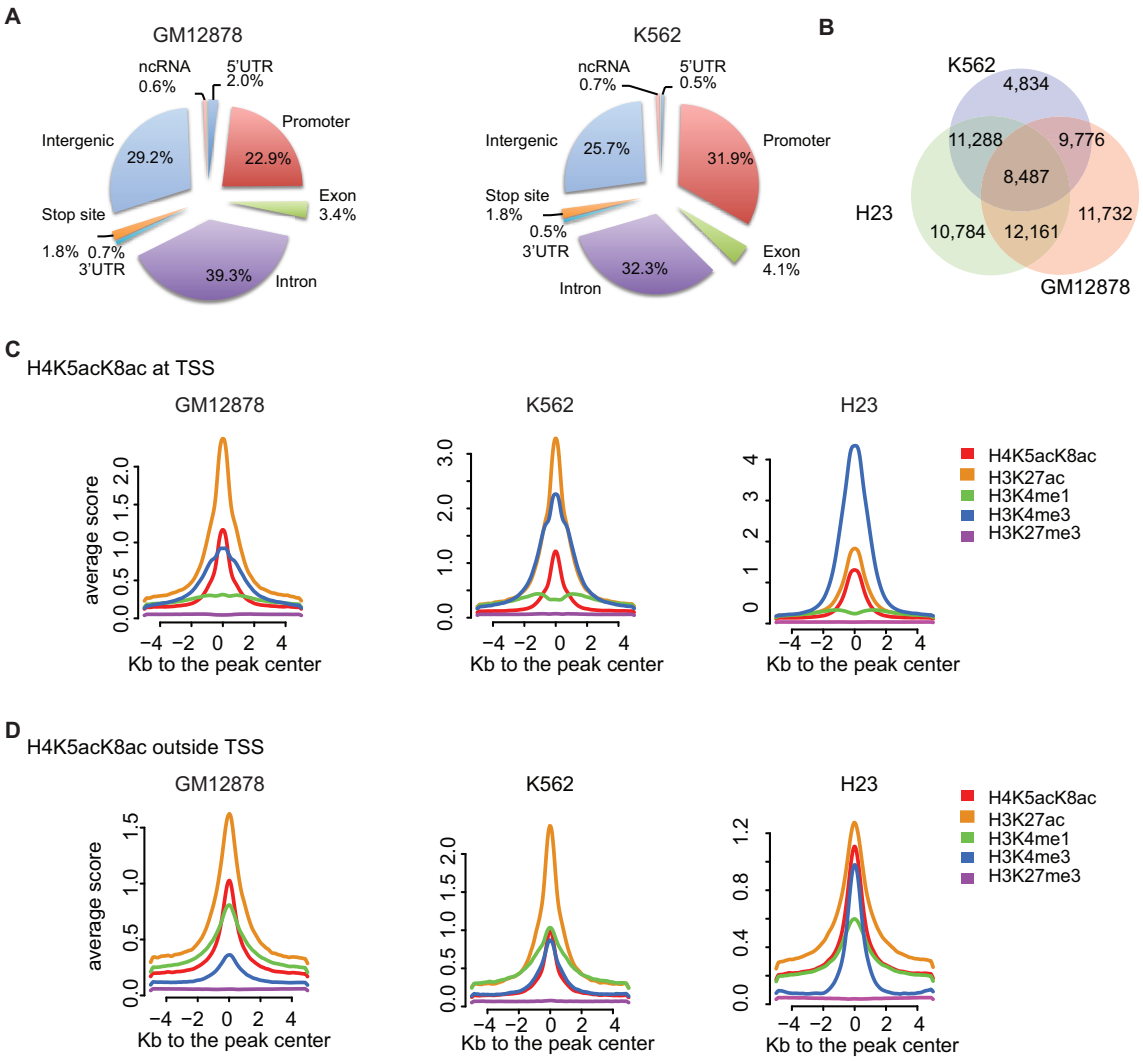


Figure S3. Genome-wide H4K5acK8ac ChIP-seq in GM12878 and K562. (**A**) Genomic localization of H4K5acK8ac peaks in GM12878 and K562 cell lines. Promoter: ±1 kb from TSS (RefSeq). Intergenic region: 1 kb upstream of TSS or 1 kb downstream from transcription stop site. (**B**) Venn diagram showing an overlap of H4K5acK8ac peaks among three different cell lines indicated. (**C-D**) Enrichment of four histone modification marks around H4K5acK8ac peaks located at TSS (**C**) and outside TSS (**D**) in GM12878, K562, and H23. Average profiles (average RPM) of histone modification marks are plotted within ± 5 kb from the center of the H4K5acK8ac peaks.

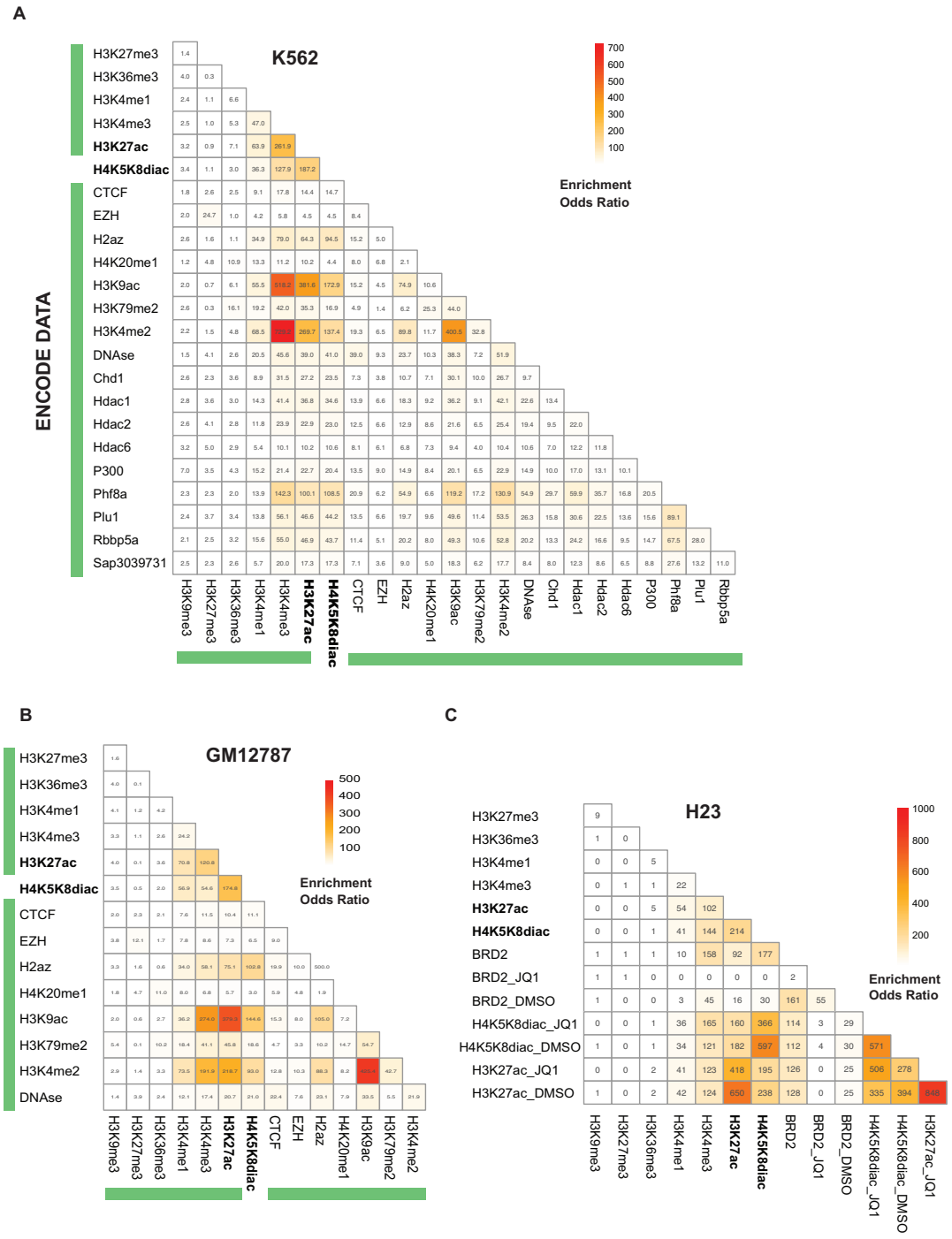


Figure S4. Genome-wide co-occurrence of different histone modifications in K562, GM12878, and H23 cell lines. For each histone mark, the genome was divided into 200 bp windows and binarized to 0 (no modification) and 1 (modification detected above background) using BinarizeBam function from ChromHMM suite. The enrichment/co-occurrence for each combination of histone marks was calculated using Fisher Exact test and the odds ratios were then plotted. High odd ratios indicate high co-occurrence of given histone mark combination across the genome.

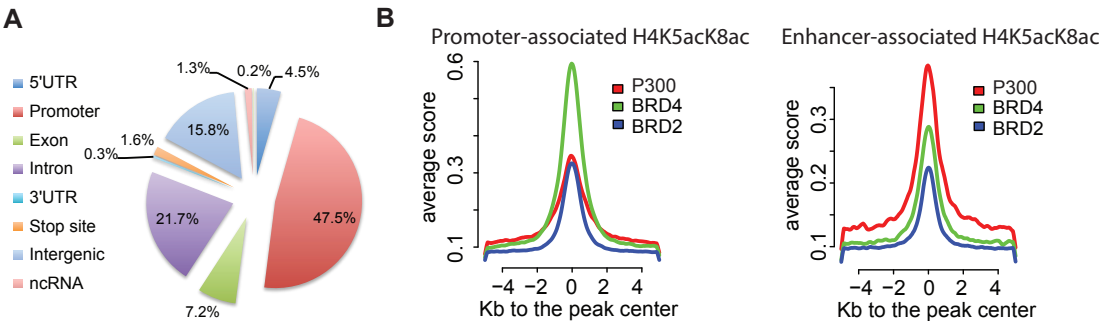


Figure S5. Association of BRD2 with H4K5acK8ac. (**A**) Genomic localization of BRD2 binding sites. (**B**) BRD2, BRD4, and EP300 are enriched at promoter- and enhancer-associated H4K5acK8ac peaks (left and right, respectively). Promoter-associated H4K5acK8ac are defined as peaks located within ± 5 kb from TSS, and enhancer-associated H4K5acK8ac as ± 5 kb outside TSS. Publicly available BRD4 ChIP-seq from SCLC cell line H2171 and EP300 ChIP-seq A549 were used.

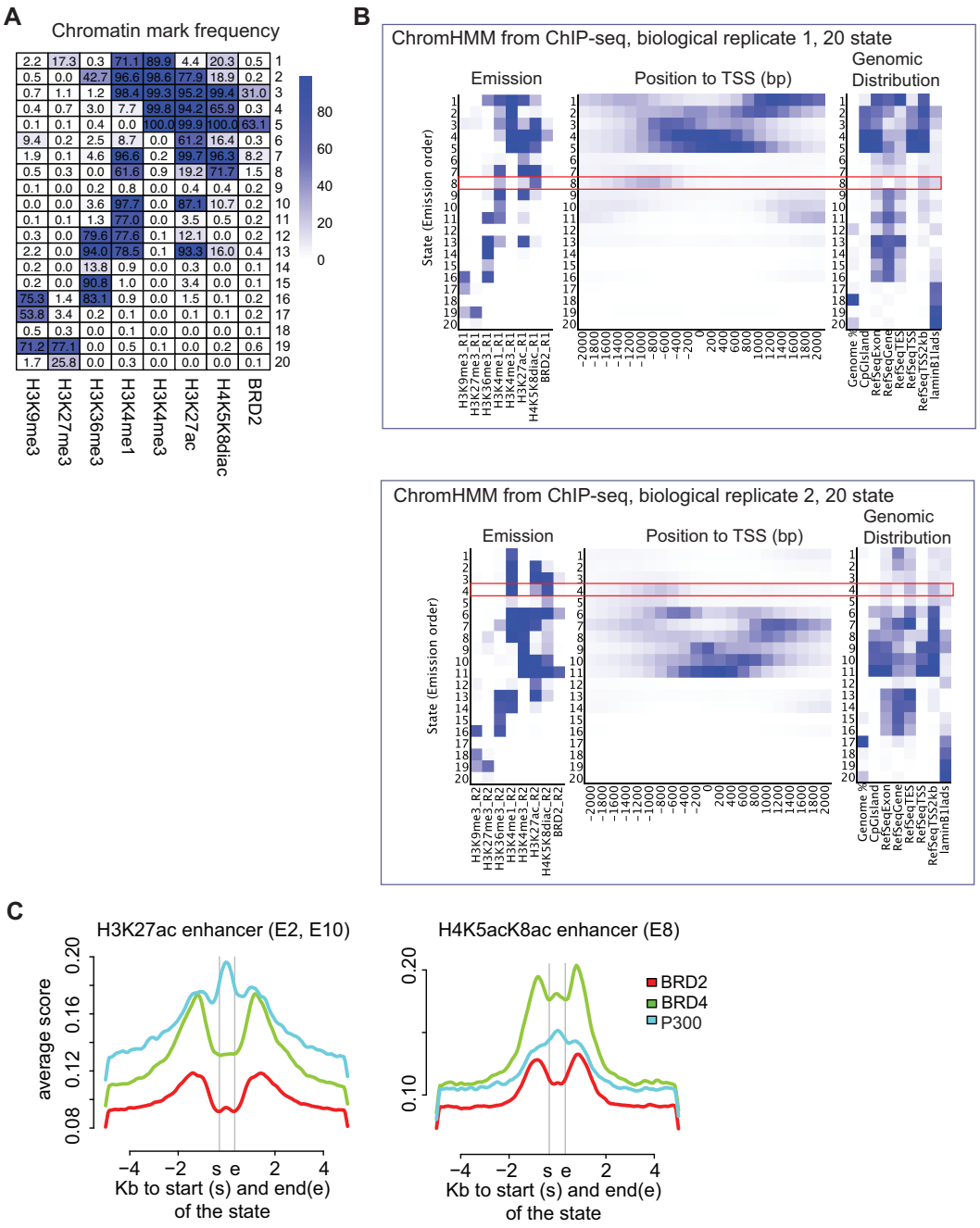


Figure S6. **Global chromatin states by ChromHMM**. (**A**) Frequency of each histone modification mark or transcription factor BRD2 in each state. The frequency ratio is used to predict the chromatin states shown in Fig. 3C. The color density indicates enrichment. (**B**) ChromHMM using ChIP-seq data set derived from the biological replicate 1 (upper) and biological replicate 2 (lower). The same types of chromatin states were identified from both biological replicates and from the pooled ChIP-seq data set shown in Fig. 3C. (**C**) Normalized average plots showing enrichment of BRD2, BRD4 (H2171) and EP300 (A450) ChIP-seq intensities in the H3K27ac enhancer states (left, E2 and E10) and H4K5acK8ac enhancer state (right, E8).

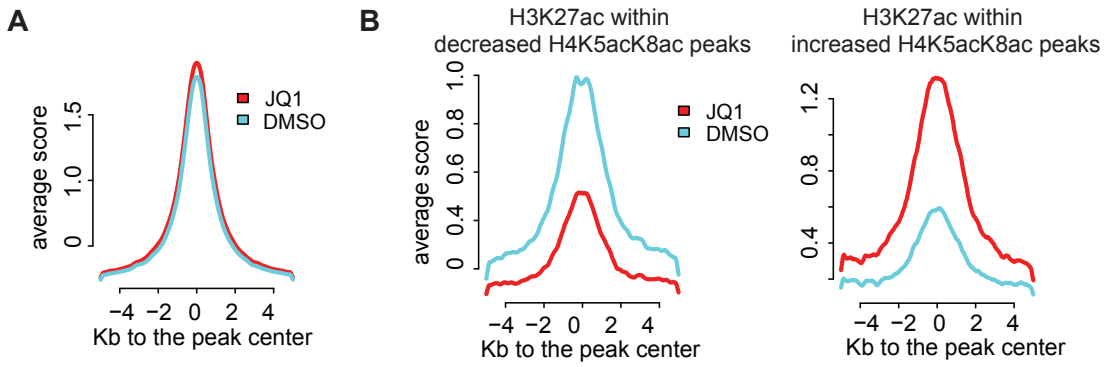
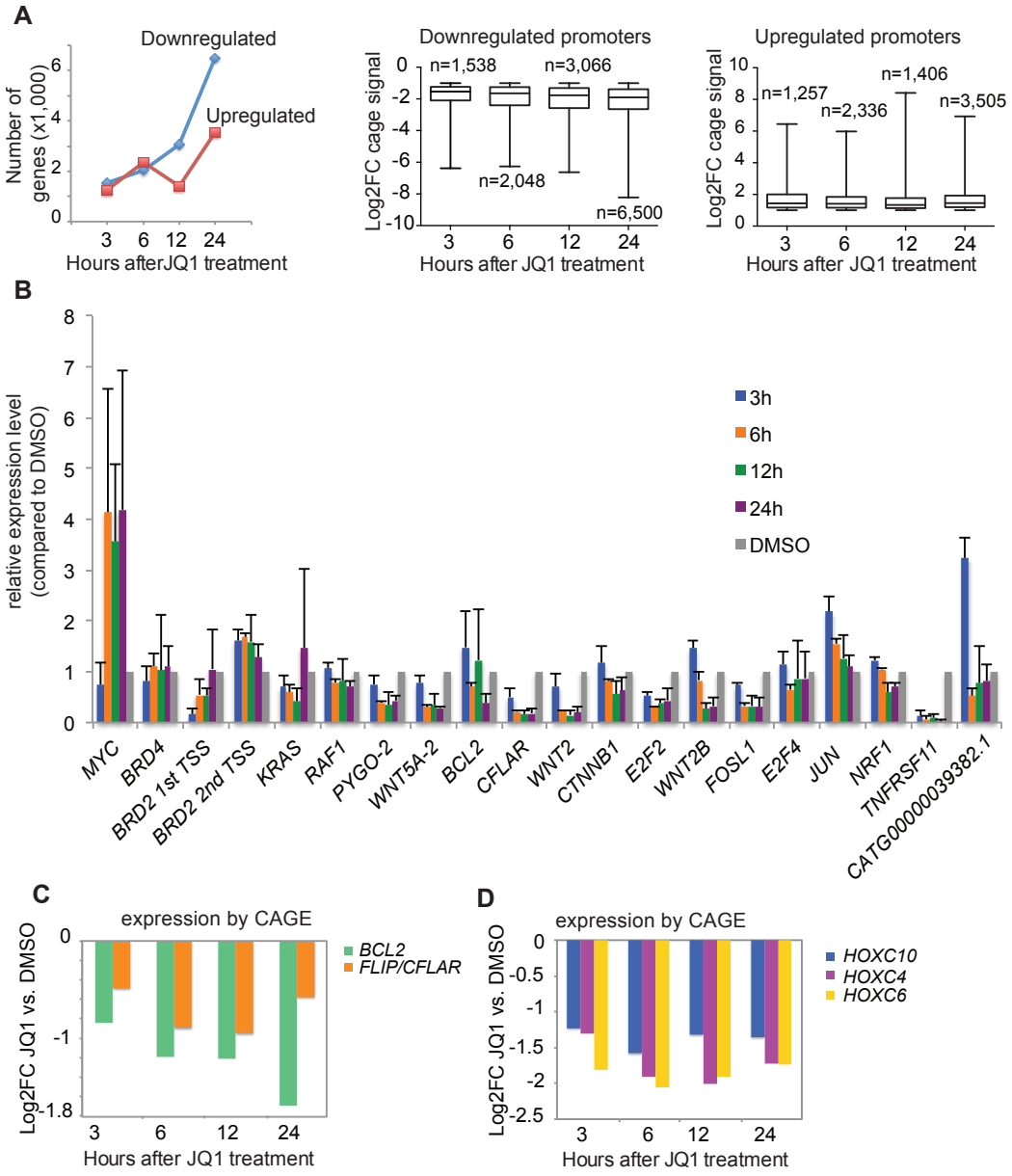


Figure S7. JQ1 effect on H3K27ac peaks. (**A**) 500 nM JQ1 treatment for 24 hrs did not affect the global H3K27ac sites, as indicated by normalized average plot. H3K27ac ChIP-seq signals from JQ1 (red) or DMSO (blue) treated cells were plotted into the robust H3K27ac ChIP-seq peaks from the untreated cells (± 5 kb from the peak center). (**B**) Change of H3K27ac intensities upon JQ1 treatment. Left: decrease of H3K27ac intensities detected in the JQ1-decreased H4K5acK8ac peaks upon JQ1 treatment. Right: Increase of H3K27ac intensities in the JQ1-increased H4K5acK8ac peaks upon JQ1 treatment. Cells were treated with JQ1 (red) or DMSO (blue).


A
Downregulated
Upregulated
Number of genes (x1,000)
Hours afterJQ1 treatment
Downregulated promoters
Log2FC cage signal
n=1,538
n=3,066
n=2,048
n=6,500
Hours after JQ1 treatment
Upregulated promoters
n=1,257
n=2,336
n=1,406
n=3,505
B
relative expression level (compared to DMSO)
3h
6h
12h
24h
DMSO
MYC
BRD4
BRD2 1st TSS
BRD2 2nd TSS
KRAS
RAF1
PYGO-2
WNT5A-2
BCL2
CFLAR
WNT2
CTNNB1
E2F2
WNT2B
FOSL1
E2F4
JUN
NRF1
TNFRSF11
CATG00000039382.1
C
expression by CAGE
Log2FC JQ1 vs. DMSO
BCL2
FLIP/CFLAR
Hours after JQ1 treatment
D
expression by CAGE
Log2FC JQ1 vs. DMSO
HOXC10
HOXC4
HOXC6
Hours after JQ1 treatment

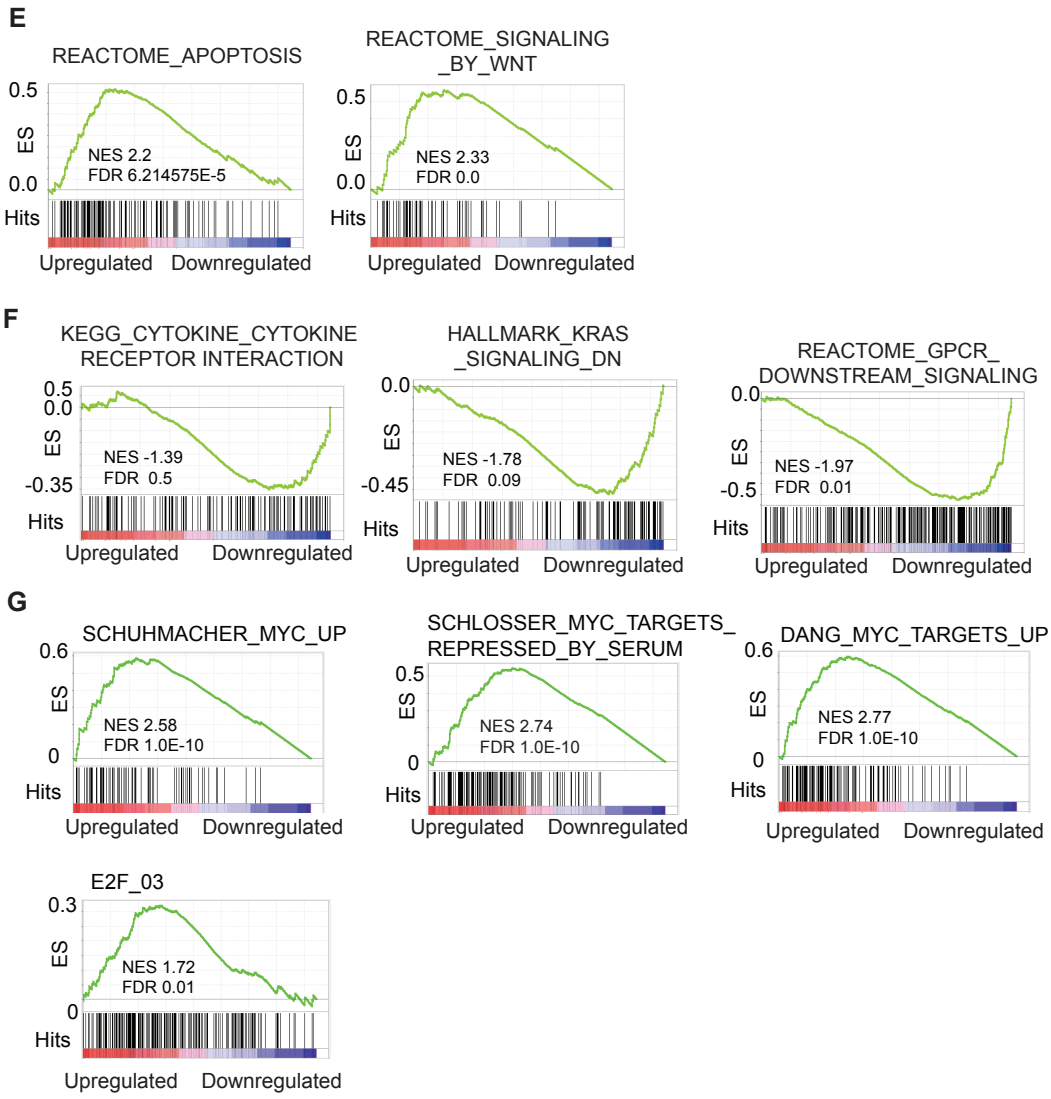


Figure S8. JQ1 effects on the gene expression in H23 cells. (**A**) Number of genes that were downregulated (blue) or upregulated (red) increased with the prolonged JQ1 treatment (left panel); the middle panel shows the number of downregulated gene time (log_2FC of CAGE signal ≤ -1) increased with the incubation; the right panel: the number of upregulated genes (log_2FC of CAGE signal ≥ 1). (**B**) Validation of the expression of 20 genes upon JQ1 treatment at 3, 6, 12, and 24 hrs. The gene expression level was determined from the JQ1- and DMSO-treated cells and normalized against the housekeeping gene *GAPDH*. (**C**) Bar plot showing the decrease of *BCL2* and *CFLAR* expression as upon JQ1 treatment. Log_2FC of CAGE signals calculated from the JQ1- and DMSO-treated cells. (**D**) Bar plot showing the decrease of *HOXC10, HOXC6,* and *HOXC4* expression as upon JQ1 treatment. Log_2FC of CAGE signals calculated from the JQ1- and DMSO-treated cells. (**E–G**) GSEA of differentially expression genes

showing the enrichment of: (**E**) Apoptosis pathway and signaling by Wnt in the upregulated genes; (**F**) Cytokine–cytokine receptor interaction, target genes of KRAS signaling, and G-coupled protein receptor (GPCR) in the downregulated genes; (**G**) MYC target genes from multiple datasets: Schumacher, Schlosser, Dang (upper), and E2F (lower) target genes in the upregulated genes. ES: enrichment score; NES: normalized enrichment score; FDR: false discovery rate; Hits: upregulated genes (red) and downregulated genes (blue).

Figure S9. Genome browser view of the genes whose promoter Is not bound by BRD2. *FOS* (upper) and *IL6* (lower).

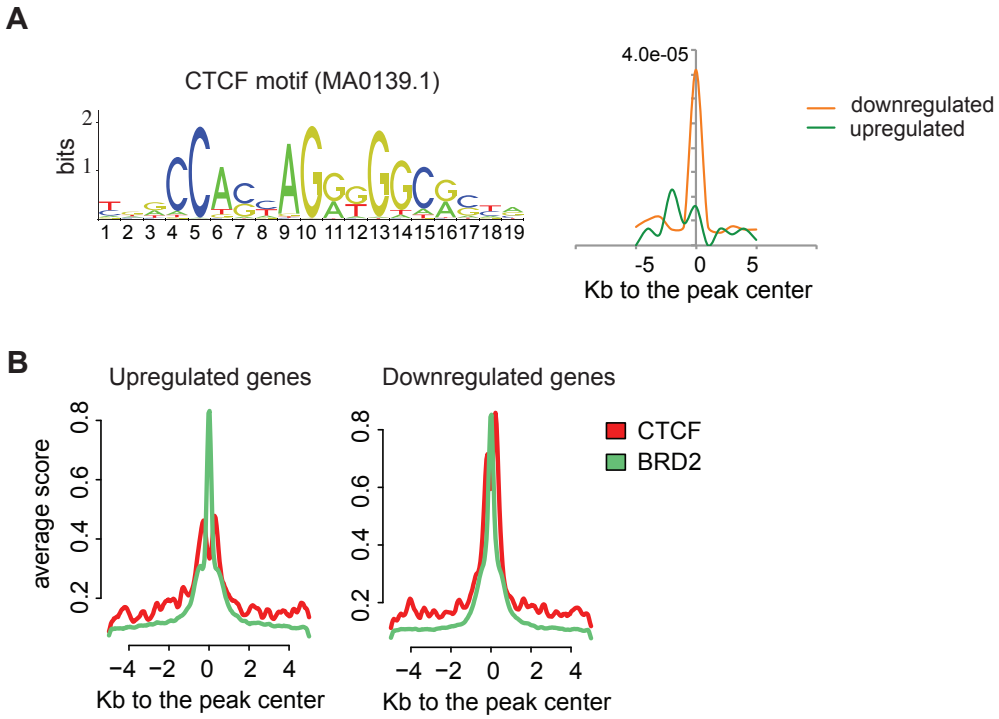
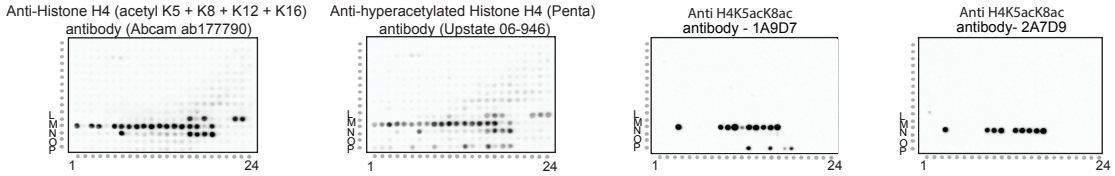


Figure S10. Enrichment of CTCF in the BRD2 peaks. (**A**) CTCF-binding motif enrichment in the BRD2 peaks. Motif enrichment analysis was performed using HOMER with CTCF motif from Jaspar (left panel). The right panel: y-axis: motif per base-pair per peak within ± 5 kb from the peak summit (x-axis) in two different sets of BRD2 peaks at the downregulated (orange) and upregulated (green) genes. (**B**) Enrichment of CTCF ChIP-seq signals (ENCODE ChIP-seq in A549) within the BRD2 peaks associated with the upregulated (left) and downregulated (right) genes. Red: CTCF, green: BRD2 signals.



Modification number	Location	Sequence	Name	Mod1	Mod2	Mod 3	Mod 4	N-terminus	Antibody			
									ab177790	Upstate 06-946	1A9D7	2A7D9
275	L11	R K S A P A T G G V Kac K P H R Y R P G	H3 26-45	K36ac				acetylated		✔		
276	L12	S G R G K G G K G L G K G G A K R H R	H4 1-19	unmod				free				
277	L13	pS G R G K G G K G L G K G G A K R H R	H4 1-19	S1P				free				
278	L14	S G Rme2s G K G G K G L G K G G A K R H R	H4 1-19	R3me2s				free				
279	L15	S G Rme2a G K G G K G L G K G G A K R H R	H4 1-19	R3me2a				free				
280	L16	S G R G Kac G G K G L G K G G A K R H R	H4 1-19	K5ac				free	✔	✔		
281	L17	S G R G K G G Kac G L G K G G A K R H R	H4 1-19	K8ac				free		✔		
282	L18	S G R G K G G K G L G Kac G G A K R H R	H4 1-19	K12ac				free	✔	✔		
286	L22	pS G R G Kac G G K G L G K G G A K R H R	H4 1-19	S1P	K5ac			free	✔	✔		
287	L23	S G Rme2s G Kac G G K G L G K G G A K R H R	H4 1-19	R3me2s	K5ac			free	✔	✔		
288	L24	S G Rme2s G K G G Kac G L G K G G A K R H R	H4 1-19	R3me2s	K8ac			free		✔		
289	M 1	S G Rme2a G Kac G G K G L G K G G A K R H R	H4 1-19	R3me2a	K5ac			free	✔	✔		
290	M 2	S G Rme2a G K G G Kac G L G K G G A K R H R	H4 1-19	R3me2a	K8ac			free		✔		
291	M 3	S G R G Kac G G Kac G L G K G G A K R H R	H4 1-19	K5ac	K8ac			free	✔	✔	✔	✔
292	M 4	S G R G K G G Kac G L G Kac G G A K R H R	H4 1-19	K8ac	K12ac			free	✔	✔		
293	M 5	S G R G K G G Kac G L G K G G A Kac R H R	H4 1-19	K8ac	K16ac			free		✔		
294	M 6	S G R G K G G K G L G Kac G G A Kac R H R	H4 1-19	K12ac	K16ac			free	✔	✔		
295	M 7	pS G Rme2s G Kac G G K G L G K G G A K R H R	H4 1-19	S1P	R3me2s	K5ac		free	✔	✔		
296	M 8	pS G Rme2a G Kac G G K G L G K G G A K R H R	H4 1-19	S1P	R3me2a	K5ac		free	✔	✔		
297	M 9	S G Rme2s G Kac G G Kac G L G K G G A K R H R	H4 1-19	R3me2s	K5ac	K8ac		free	✔	✔	✔	✔
298	M10	S G Rme2a G Kac G G Kac G L G K G G A K R H R	H4 1-19	R3me2a	K5ac	K8ac		free	✔	✔	✔	✔
299	M11	S G R G Kac G G Kac G L G Kac G G A K R H R	H4 1-19	K5ac	K8ac	K12ac		free	✔	✔	✔	✔
300	M12	S G R G K G G Kac G L G Kac G G A Kac R H R	H4 1-19	K8ac	K12ac	K16ac		free	✔	✔	✔ weak	
301	M13	pS G Rme2s G Kac G G Kac G L G K G G A K R H R	H4 1-19	S1P	R3me2s	K5ac	K8ac	free	✔	✔	✔	✔
302	M14	pS G Rme2a G Kac G G Kac G L G K G G A K R H R	H4 1-19	S1P	R3me2a	K5ac	K8ac	free	✔	✔	✔	✔
303	M15	S G Rme2s G Kac G G Kac G L G Kac G G A K R H R	H4 1-19	R3me2s	K5ac	K8ac	K12ac	free	✔	✔	✔	✔
304	M16	S G Rme2a G Kac G G Kac G L G Kac G G A K R H R	H4 1-19	R3me2a	K5ac	K8ac	K12ac	free	✔	✔	✔	✔
305	M17	S G R G Kac G G Kac G L G Kac G G A Kac R H R	H4 1-19	K5ac	K8ac	K12ac	K16ac	free	✔	✔	✔	✔
306	M18	G K G G A K R H R K V L R D N I Q G I T	H4 11-30	unmod				acetylated				
307	M19	G Kac G G A K R H R K V L R D N I Q G I T	H4 11-30	K12ac				acetylated	✔	✔		
308	M20	G K G G A Kac R H R K V L R D N I Q G I T	H4 11-30	K16ac				acetylated				
309	M21	G K G G A K Rme2s H R K V L R D N I Q G I T	H4 11-30	R17me2s				acetylated				
310	M22	G K G G A K Rme2a H R K V L R D N I Q G I T	H4 11-30	R17me2a				acetylated				
311	M23	G K G G A K R H Rme2s K V L R D N I Q G I T	H4 11-30	R19me2s				acetylated				
312	M24	G K G G A K R H Rme2a K V L R D N I Q G I T	H4 11-30	R19me2a				acetylated				
319	N 7	G Kac G G A Kac R H R K V L R D N I Q G I T	H4 11-30	K12ac	K16ac			acetylated	✔	✔		
328	N16	G Kac G G A Kac R H R Kme1 V L R D N I Q G I T	H4 11-30	K12ac	K16ac	K20me1		acetylated	✔	✔		
329	N17	G Kac G G A Kac R H R Kme2 V L R D N I Q G I T	H4 11-30	K12ac	K16ac	K20me2		acetylated	✔	✔		
330	N18	G Kac G G A Kac R H R Kme3 V L R D N I Q G I T	H4 11-30	K12ac	K16ac	K20me3		acetylated	✔	✔		
331	N19	G Kac G G A Kac R H R Kac V L R D N I Q G I T	H4 11-30	K12ac	K16ac	K20ac		acetylated	✔	✔		
366	P 6	P D P A K S A P A P K Kac G S K K A V T	H2B 1-19	K12ac				free		✔ weak		
369	P 9	P D P A Kac S A P A P K Kac G S K K A V T	H2B 1-19	K5ac	K12ac			free		✔ weak		
373	P13	P D P A K S A P A P K Kac G S Kac K A V T	H2B 1-19	K12ac	K15ac			free		✔	✔	
376	P16	P D P A Kac S A P A P K Kac G S Kac K A V T	H2B 1-19	K5ac	K12ac	K15ac		free		✔	✔	
377	P17	P D P A Kac S A P A P K K G pS Kac K A V T	H2B 1-19	K5ac	S14P	K15ac		free		✔ weak		
378	P18	P D P A K S A P A P K Kac G pS Kac K A V T	H2B 1-19	K12ac	S14P	K15ac		free		✔ weak	✔ weak	
379	P19	P D P A Kac S A P A P K Kac G pS Kac K A V T	H2B 1-19	K5ac	K12ac	S14P	K15ac	free		✔ weak	✔ weak	

Table S1. Peptide array analysis using anti-H4K5acK8ac antibodies and two commercially available antibodies. The upper panel shows images of ECL detection of the MODified histone peptide array (Active Motif, 13001) containing 384 different patterns of histone tail modification from the indicated antibodies (from the left to right): anti-histone H4 (acetyl K5+K8+K12+K16) antibody (Abcam, ab177790); anti-hyperacetylated histone H4 (Penta) antibody (Upstate, 06-946); 1A9D7 antibody; and 2A7D9 antibody. The lower table is the list of the peptides, which were recognized by the tested antibodies.

	2A7D9 Fab + H4(1–12) K5acK8ac	1A9D7 Fab + H4(1–12) K5acK8ac
Data collection		
Space group	$P2_12_12_1$	$P2_1$
Cell dimensions		
a, *b*, *c* (Å)	39.95, 85.98, 122.85	39.35, 164.09, 73.70
α, β, γ (°)	90.00, 90.00, 90.00	90.00, 104.50, 90.00
Resolution (Å)	50.00–1.70 (1.73–1.70)*	50.00–1.80 (1.83–1.80)*
R_{sym}	0.070 (0.623)	0.053 (0.334)
$I / \sigma I$	26.8 (2.3)	28.3 (2.5)
Completeness (%)	98.8 (93.6)	99.0 (96.8)
Redundancy	6.7 (6.2)	3.5 (3.1)
Refinement		
Resolution (Å)	36.97–1.70	37.77–1.80
No. reflections	46,824	82,524
R_{work} / R_{free}	0.166 / 0.195	0.194 / 0.224
No. atoms		
Protein	3,315	6,599
Ligand / ion	-	26
Water	522	789
B-factors		
Protein	16.57	22.31
Ligand / ion	-	54.54
Water	30.61	34.32
R.m.s. deviations		
Bond lengths (Å)	0.006	0.004
Bond angles (°)	0.87	0.75
PDB ID	5YE3	5YE4

Table S2. **Crystallographic data collection and refinement statistics.** *Values in parentheses are for highest-resolution shell.

Table S3. Annotation of H4K5acK8ac, H3K27ac, and BRD2 peaks with the actively expressed CAGE signals based on FANTOMCAT. A. H4K5acK8ac peaks. B. H3K27ac peaks. C. BRD2 peaks. (XLSX)

Table S4. The list of H4K5acK8ac-based super-enhancers and the associated genes. (XLSX)

Table S5. Pathway analysis on the differentially expressed genes upon JQ1 treatment for 3h, 6h, 12h, and 24h (p-value < 0.01). A. Upregulated Pathways. B. Downregulated pathways. (XLSX)

Table S6. List of the primers for qPCR and ChIP-seq. (XLSX)

Table S7. List of the differentially expressed genes upon JQ1 treatment for 3h, 6h, 12h, and 24h (CAGE analysis). (XLSX)

Table S8. Pathway analysis on the upregulated or downregulated BRD2-bound genes. (XLSX)

Table S9. The list of TF-binding motifs identified by MARA using CAGE data set. (XLSX)