SUPPLEMENTAL DATA

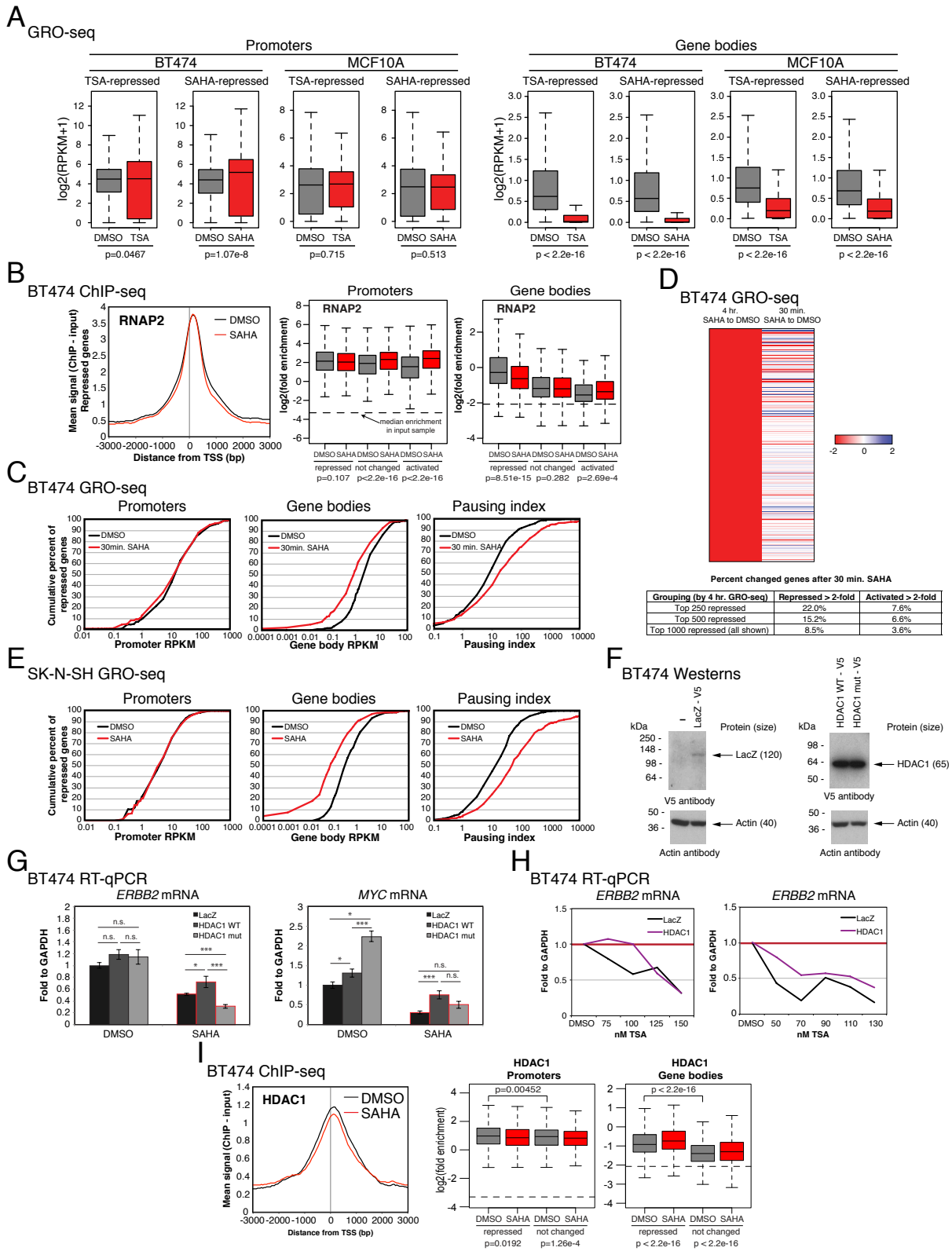

Figure S1. Elongation repression by HDACIs, related to Figure 1.

(A) Boxplots of GRO-seq read density for promoter-proximal and gene body RPKM. The promoter is defined as TSS to + 300 bp, gene body is +300 bp to end for GRO-seq data analysis. Statistics are from Wilcoxon rank sum tests.

(B) Average RNAP2 ChIP-seq read density from two biological replicates around the TSSs of SAHA-repressed genes (as defined by significant gene body density reduction in two GRO-seq experiments). 968 repressed genes were analyzed. As indicated, the horizontal dashed line shows the median signal from chromatin input control. For ChIP-seq fold enrichment in different gene regions, promoters were defined as -100 to +500 base pairs relative to TSSs, and gene bodies were defined as +1500 downstream of TSSs to gene end. Genes were a minimum of 2000 base pairs. *P*-values reported in box plots are from Wilcoxon rank sum tests.

(C) Effect of a 30 min. SAHA treatment on BT474 is shown with cumulative percent plots for significantly (log-likelihood ratio $P < 10^{-16}$) repressed genes. Pausing index is promoter RPKM divided by gene body RPKM. There were 400 significantly repressed genes.

(D) Heatmap that represents the top 1000 most repressed genes sorted by magnitude of repression after 4 hr. treatment, and the corresponding genes after a 30 min. treatment with SAHA. Red indicates repression, blue activation. The percentage of genes changed by greater than 2-fold is shown in a table below. The percentages of the 1000, 500, and 250 top-most repressed genes from the 4 hr. treatment are reported.

(E) Cumulative percent plots of significantly (log-likelihood ratio $P < 10^{-16}$) repressed genes in SK-N-SH cells treated with SAHA. There were 376 significantly repressed genes.

(F) Cell lysates from BT474 that were uninfected (-) or infected with overexpression constructs delivered with lentiviruses were isolated and western blotted with V5 antibody. Actin loading control was also blotted. Mass is indicated next to the labels of the bands in kilodaltons.

(G) After overexpression of indicated proteins, the response to SAHA applied for 24 hr. RT-qPCR after overexpression of LacZ, or HDAC1 WT or mut with DMSO or 3.5μM SAHA. n = 8 to 11 from at least 3 biological replicates. Bars indicate standard error.

(H) Dose response of TSA after overexpression of LacZ or HDAC1 WT. Each point represents one sample. The two panels represent two independent biological replicates.

(I) Distribution of binding of HDAC1 after DMSO or SAHA treatment. Average of two ChIP-seq experiments.

Overlap of genes in gene body expression change categories from GRO-seq experiments

	Category	# of genes this GRO-seq	# of genes 2013 GRO-seq	# overlapping	# expected to overlap
BT474 SAHA	repressed	7389	1558	968	323
	not changed	12586	24789	10302	8744
	activated	4245	1677	596	200
BT474 TSA	repressed	6354	1609	998	287
	not changed	6399	15308	4087	2745
	activated	3450	800	365	77
MCF10A TSA	repressed	3866	1042	689	113
	not changed	9623	14408	6166	3886
	activated	5101	758	569	108

Table S1. Agreement between GRO-seq data sets, related to Figure 1**.**

The number expected to overlap represents what would be obtained given a random selection of the number of genes in each category from each experiment for the 35682 genes in the annotation used.

Median fold change in promoters

median(SAHA/DMSO)

Factor	repressed	not changed	activated
BRD4	0.737	0.877	1.071
H3K27Ac	0.974	1.218	1.626
H3Ac	0.653	0.779	0.986
H4Ac	0.699	0.806	1.030
HDAC1	0.944	0.949	0.982
RNAP2	0.938	1.277	1.674

Table S2. Promoter fold change, related to Figure 1.

Density of ChIP-seq enrichment in SAHA treatment was divided by density in DMSO treatment for each gene in their promoter regions, and the median value is reported.

Median fold change in gene bodies

median(SAHA/DMSO)

Factor	repressed	not changed	activated
BRD4	1.112	1.085	1.068
H3K27Ac	4.229	4.325	4.094
H3Ac	2.552	2.622	2.642
H4Ac	3.817	4.075	4.110
HDAC1	1.119	1.053	1.020
RNAP2	0.804	0.980	1.103

Table S3. Gene body fold change, related to Figure 1.

Density of ChIP-seq enrichment in SAHA treatment was divided by density in DMSO treatment for each gene in their gene body regions, and the median value is reported.

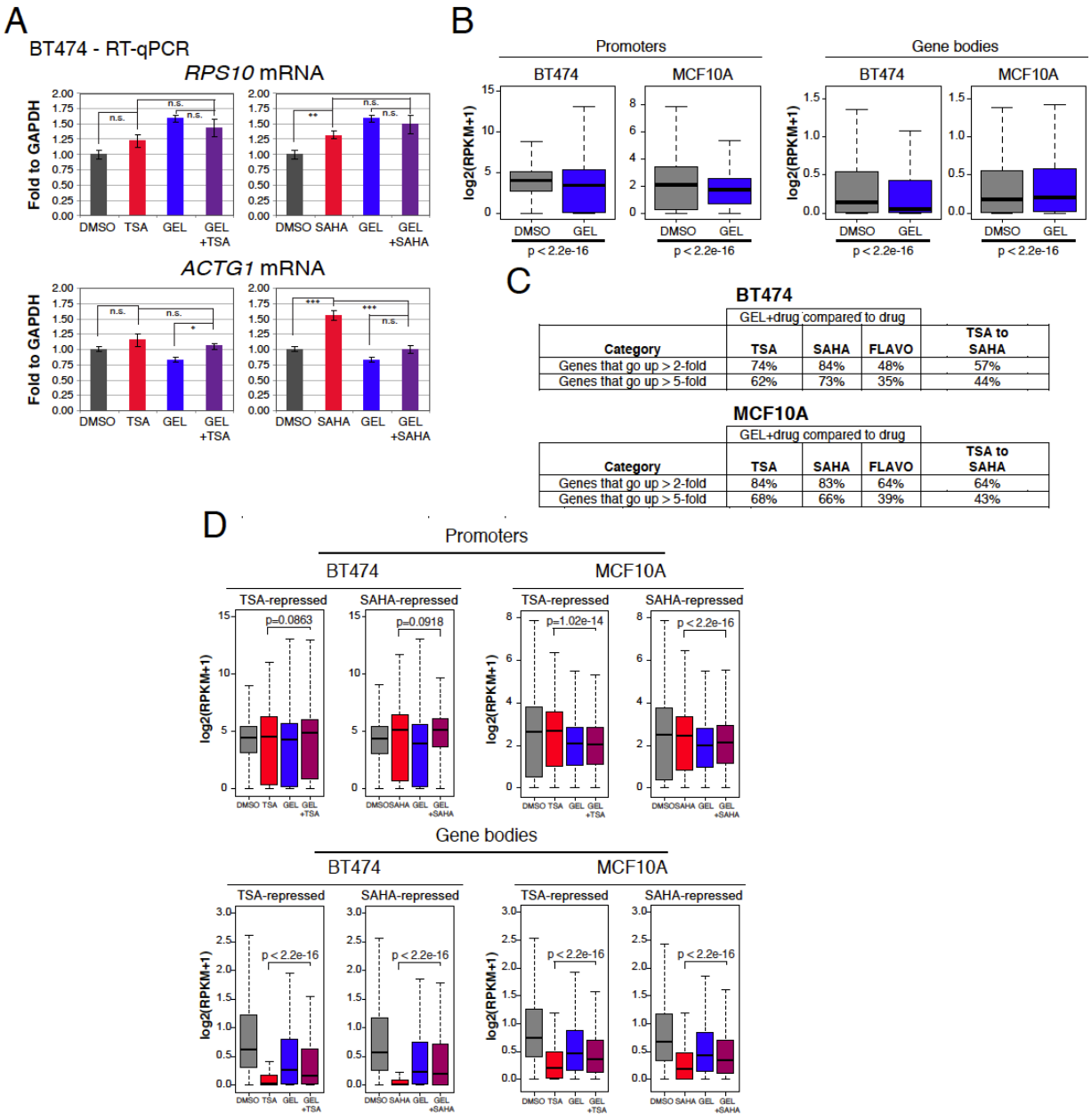

Figure S2. HSP90 inhibition antagonizes HDACIs, related to Figure 2.

(A) RT-qPCR of genes not repressed by HDACIs. Quantitation of *RPS10* and *ACTG1* mRNA relative to *GAPDH* in BT474 after single and combined treatments is shown. Statistical significance was determined with a two-tailed t-test where *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, and n.s. = not significant ($p > 0.05$). Bars represent standard error. n = 6 from 2 biological replicates. GEL was administered at 20 μM.

(B) GRO-seq RPKM of reads in promoters and gene bodies in BT474 and MCF10A after GEL treatment.

(C) The percentage of genes antagonized by GEL co-treatment in GRO-seq with two different thresholds. An elongation drug not antagonized by HSP90 inhibition (flavopiridol, or FLAVO) is also shown. The percent of genes differentially repressed by two related drugs, TSA and SAHA, which should not display recovery, is shown for comparison.
(D) GRO-seq read densities in promoters and gene bodies of TSA and SAHA repressed genes in BT474 and MCF10A. *P*-values were determined with Wilcoxon rank sum tests.

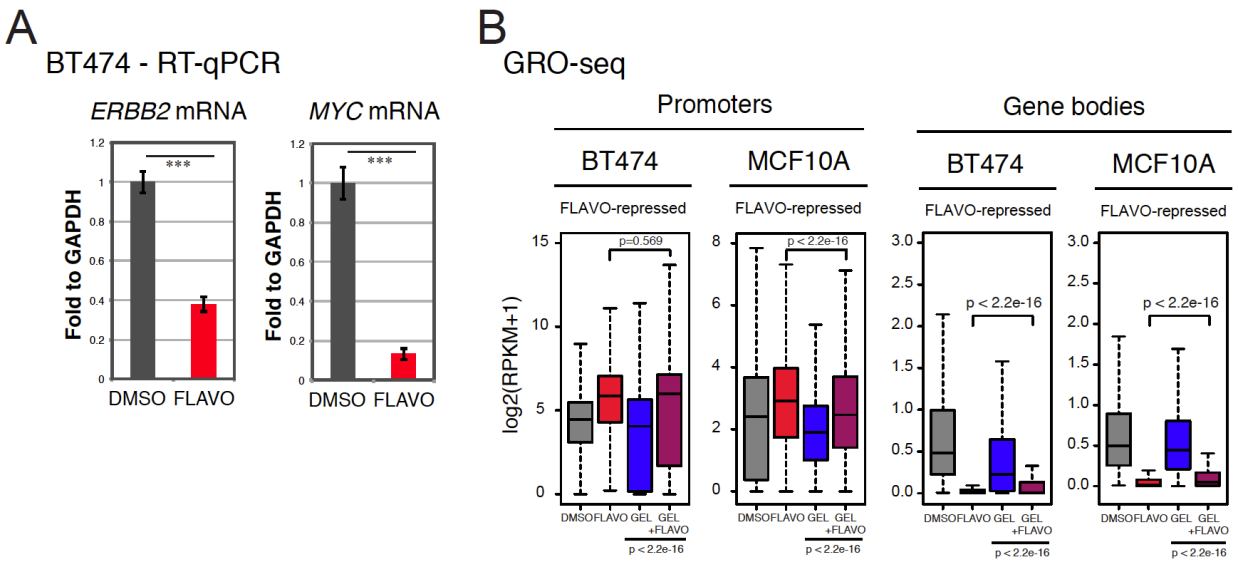

Figure S3. Effect of P-TEFb inhibition on elongation, related to Figure 4.

(A) FLAVO treatment effect on *ERBB2* and *MYC* transcript levels as measured by RT-qPCR. n = 12 from 5 independent experiments. *** represents $P < 0.001$ based on a two-tailed student's t-test. Bars represent standard error.

(B) GRO-seq read densities of FLAVO-repressed genes in BT474 and MCF10A with indicated treatments of drugs. *P*-values were determined with Wilcoxon rank sum tests.

A

Overlap of top 1000 most repressed genes - GRO-seq

		TSA	SAHA	JQ1	FLAVO
BT474	TSA	100%	-	-	-
	SAHA	20.1%	100%	-	-
	JQ1	23.9%	20.3%	100%	-
	FLAVO	16.8%	17.7%	15.6%	100%
MCF10A	TSA	100%	-	-	-
	SAHA	30.9%	100%	-	-
	JQ1	28.1%	29.8%	100%	-
	FLAVO	19.2%	18.7%	23.0%	100%

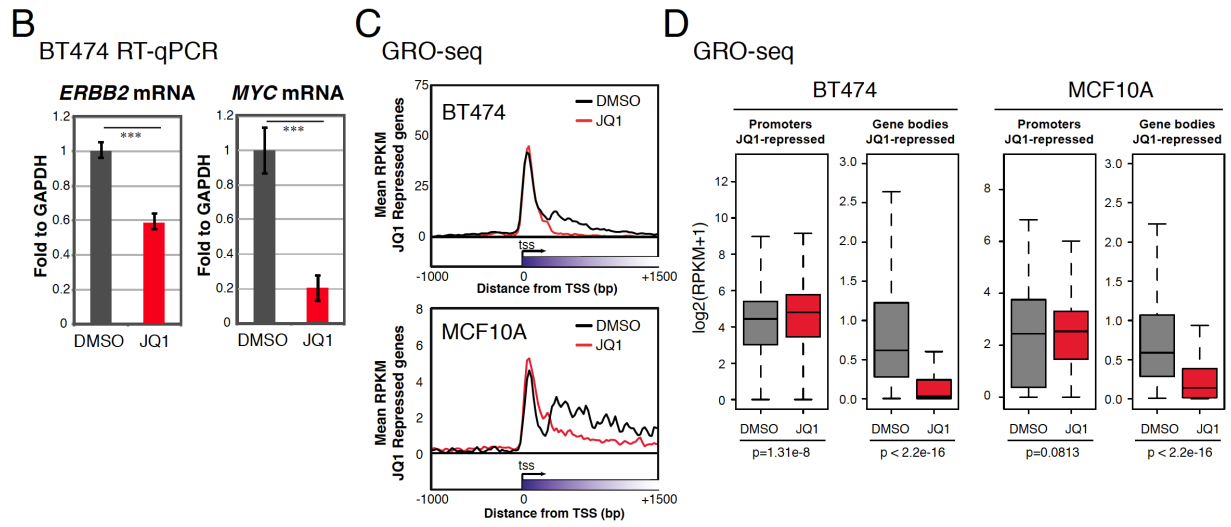

Figure S4. Magnitude of binding changes of different factors at intergenic BRD4 binding sites, and effects of JQ1 on transcription, related to Figure 6.

(A) Comparative overlap of the top 1000 most repressed genes by the different drugs in GRO-seq.

(B) JQ1 repression of *ERBB2* and *MYC* transcript levels after 24 hr. using RT-qPCR. n = 12 samples from 4 biological replicates. *** represents $P < 0.001$ from a two-tailed student's t-test. Bars represent standard error.

(C) Elongation pattern of DMSO control or JQ1 treatment are shown with metagenes of JQ1-repressed genes in BT474 and MCF10A. There were 5083 significantly repressed genes in BT474 and 4577 in MCF10A.

(D) Quantitation of density in promoters and gene bodies is shown. *P*-values on box plots are from Wilcoxon rank sum tests.

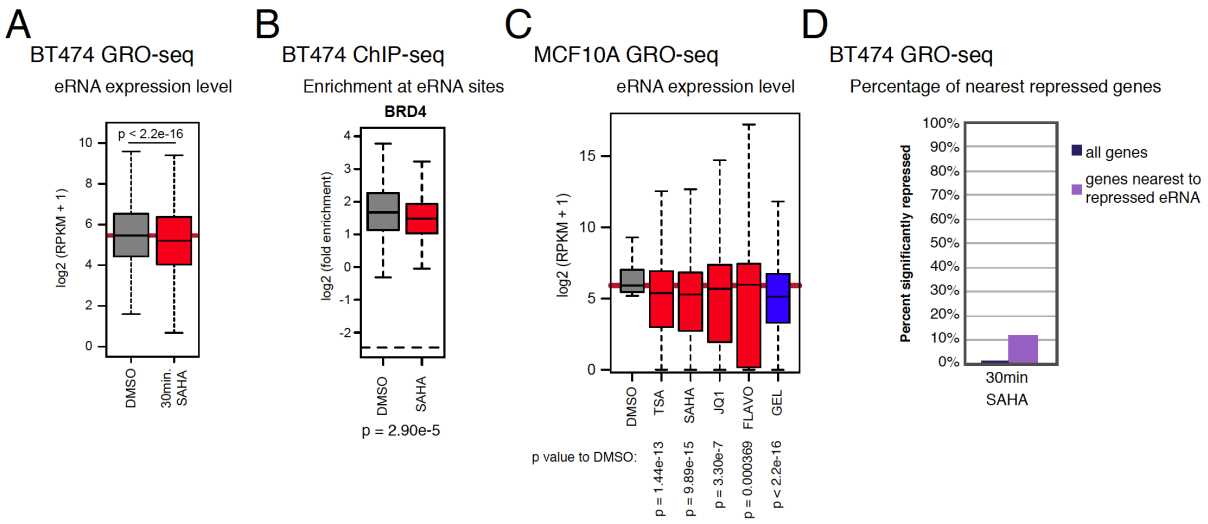

Figure S5. eRNA expression changes after elongation inhibitor treatment, and BRD4 binding after SAHA treatment, related to Figure 7.

(A) eRNA expression after 30 min. SAHA. The same set of eRNAs defined in Figure 7 were analyzed. Horizontal red line is drawn at the median signal of DMSO.

(B) ChIP-seq signal of BRD4 at predicted enhancer sites. Horizontal dashed line is median signal from input at these same sites. All *P*-values reported are from Wilcoxon rank sum tests.

(C) Expression of top 400 eRNAs in MCF10A predicted based on nascent transcripts detected with GRO-seq analyzed in HOMER, due to the lack of BRD4 ChIP-seq data for MCF10A. Horizontal red line is the median signal of DMSO. All *P*-values reported are from Wilcoxon rank sum tests.

(D) Analysis of the total percentage of genes repressed by a 30 min. SAHA treatment compared to the percentage of genes nearest the top 100 most repressed eRNA production sites.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Overexpression Constructs

Constructs were cloned into pLenti6.3/V5-TOPO vector (K5315-20) from Invitrogen. Sequences were confirmed by digestion and sequencing with CMV forward and V5 reverse primers. LacZ control virus was made as previously described (Kim et al., 2011).

Cloning

HDAC1 primers were 5'- GCCACCATGGCGCAGACGCAGGGCAC -3' and 5'- GCGGCCAACTTGACCTCCTCCTTG -3'. The template for HDAC1 WT was from Addgene #13820 (Emiliani et al., 1998), and the HDAC1 mut template with H141A was from S. Schreiber in the pBJ5 vector.

Virus titering and treatment

Viruses for protein overexpression were titered in HT1080 with 6 μg/mL blasticidin (Fisher Scientific) with multiple dilutions of the virus. HDAC1 viruses were infected at MOI 10, and LacZ at MOI 5. 40,000 cells were infected with 1x polybrene (Millipore) for 3 days. At this time, drugs were applied for 24 hr., and then harvested for RT-qPCR analysis.

RT-qPCR of genes not repressed by HDACIs

Primers for *RPS10* are *RPS10*-F 5'-TGCCCCCGGAGATTGTGCCT'3' and *RPS10*—R 5'-AGCCCCAGCCTCGGCTTTCT-3'. Primers for *ACTG1* are *ACTG1*-F 5'- AAGACCTGTACGCCAAC-3' and *ACTG1*-R 5'- CCGAGTACTTGCGCTCT-3'.

GRO-seq

Cells were washed, swelled, and lysed in lysis buffer with a reduced amount of IGEPAL detergent to minimize nuclei damage while still being able to lyse cells (composition: 10 mM

Tris-Cl pH 7.5, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 10% glycerol, 2 U/mL SUPERasin (Ambion), and 0.25% IGEPAL). Nuclear run-on reactions were performed on fresh nuclei (no freezing), and nascent RNA was isolated. Adaptors were ligated the same as before, however, TruSeq small RNA sample prep kit adapters and primers (Illumina) were used to make the samples suitable for multiplexing. The PCR reaction was run for 20-24 cycles for BT474, 21 cycles for MCF10A GRO-seq. The indexed libraries were purified by running on a 6% native polyacrylamide gel, and were cut out between 150 and 300 base pairs. The size profile of the isolated libraries was confirmed with a high-sensitivity DNA chip from Agilent according to manufacturer's protocol and libraries were mixed in equal quantities based on qPCR quantitation and sent for sequencing.

ChIP Antibodies

Antigen	Source	Company	Name	Cat #	Lot #
BRD4	rabbit	Bethyl		A301-985A50	2 or 4
H3Ac	rabbit	Upstate		06-599	25233
H3K27Ac	rabbit polyclonal	Abcam		ab4729	GR132150-3
H4Ac	rabbit	Upstate		06-866	26393 or 2459612
HDAC1	rabbit	Abcam		ab7028	676297
HSP90β	mouse monoclonal	Invitrogen	H9010	37-9400	1615278A
NELFA	goat	Santa Cruz	A-20	sc-23599	A2313 or L1013
RNAP2	rabbit	Santa Cruz	N-20	sc-899 X	I1514

Sequencing Data Alignment

Index sequences from multiplexed primers were used to identify treatment group and reads were demulitplexed. Sequencing data was aligned to hg18 using bowtie2 (Langmead and Salzberg, 2012). One sample for each treatment was conducted for GRO-seq. DMSO and SAHA treatments for ChIP-seq were done in duplicate.

Because HDACI treatment increased the amount of acetyl-histone immunoprecipitated and the data was normalized to the total number of reads for fold enrichment determination, the SAHA

treatments of acetyl ChIPs was multiplied by the yield of immunoprecipitated DNA at the end of the ChIP relative to DMSO. SAHA treated H3Ac was multiplied by a factor of 1.902, H3K27Ac by 2.817, and H4Ac by 3.367 to account for the increase in amount of available substrates for antibody binding after HDACI treatment. Peak calling was done without this regularization.

MCF10A eRNA annotation

HOMER (Heinz et al., 2010) was used to predict intergenic enrichment of GRO-seq signal using the GRO-seq data analysis module of the program. RefSeq annotated genes and 10kb downstream were excluded.

SUPPLEMENTAL REFERENCES

Emiliani, S., Fischle, W., Van Lint, C., Al-Abed, Y., and Verdin, E. (1998). Characterization of a human RPD3 ortholog, HDAC3. Proceedings of the National Academy of Sciences of the United States of America *95*, 2795-2800.

Heinz, S., Benner, C., Spann, N., Bertolino, E., Lin, Y.C., Laslo, P., Cheng, J.X., Murre, C., Singh, H., and Glass, C.K. (2010). Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Molecular cell *38*, 576-589.

Langmead, B., and Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. Nature methods *9*, 357-359.