SUPPLEMENTAL TABLES

Table S1. Specificity of inhibitory activities of largazole-thiol, TSA and SAHA against 11 HDAC enzymes in vitro.

Class	Enzyme	Largazole-thiol [M]	Trichostatin A [M]	SAHA [M]
Class I	HDAC-1	1.97E-10	7.48E-09	3.23E-07
	HDAC-2	4.15E-10	1.31E-08	9.19E-07
	HDAC-3	1.86E-10	3.04E-08	9.02E-07
	HDAC-8	1.21E-07	2.32E-07	8.98E-07
Class IIa	HDAC-4		6.51E-06	4.83E-05
	HDAC-5		2.49E-06	2.00E-05
	HDAC-7		2.45E-06	6.78E-05
	HDAC-9		2.45E-06	9.09E-05
Class IIb	HDAC-6	1.33E-08	1.09E-09	1.59E-08
	HDAC-10	4.22E-11	1.75E-08	1.09E-07
Class IV	HDAC-11	2.04E-10	1.57E-08	4.81E-07

Table S2. Growth inhibitory activity of largazole towards NCI 60 tumor cell lines.

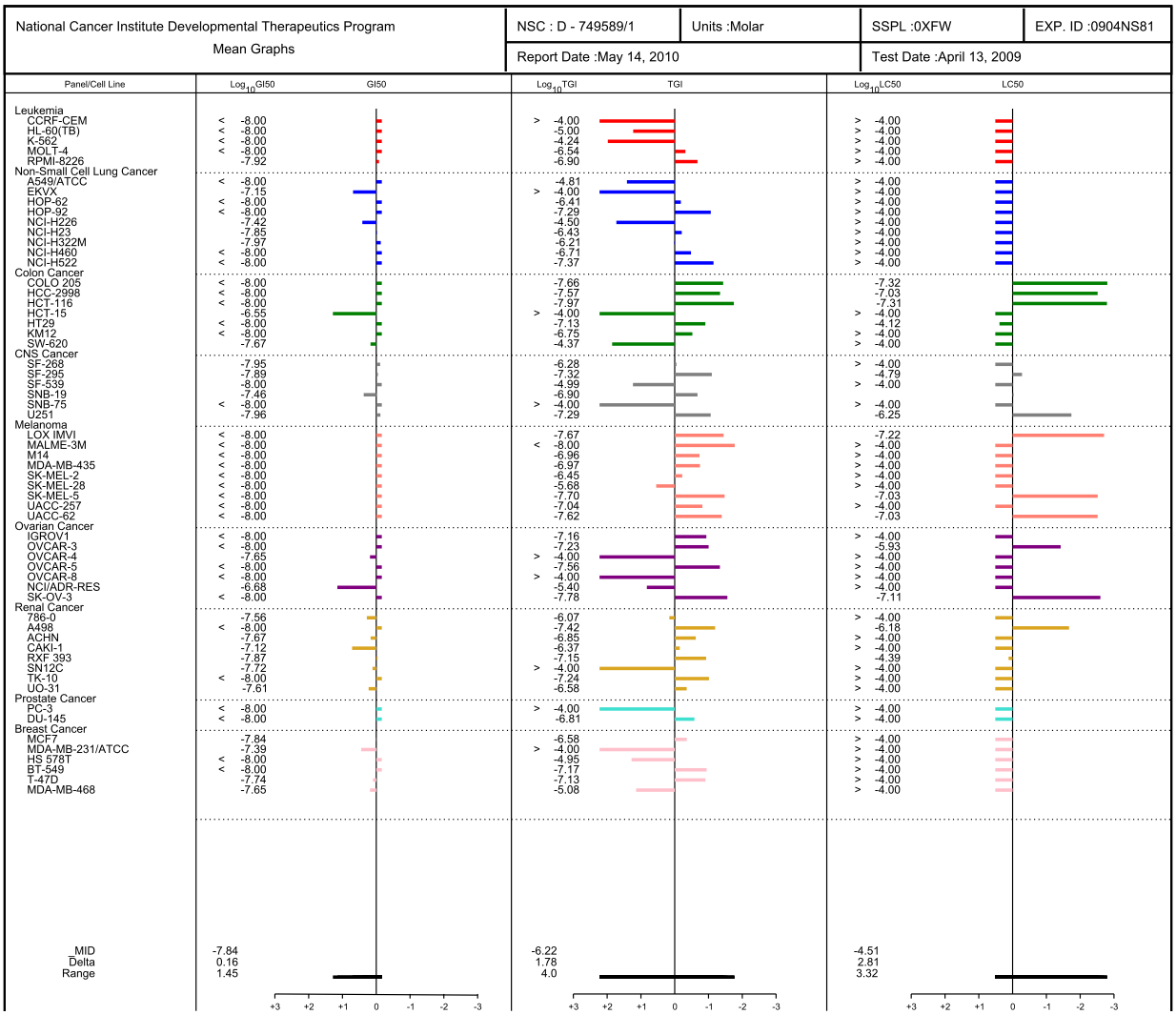


Table S3. FStitch-training genomic coordinates for H3K9ac analysis.

chromosome number	start	end	signal state
chr1	1	609	1
chr1	610	17779	0
chr1	17780	20366	1
chr1	20367	27926	0
chr1	27927	28863	0
chr1	28864	358752	0
chr1	359685	431168	0
chr1	431169	432700	1
chr1	432701	448575	0
chr1	448576	450082	0
chr1	450083	513397	0
chr1	513398	514090	0
chr1	514091	529796	0
chr1	529797	531292	1
chr1	531293	554227	0
chr1	554228	560352	1
chr1	560353	662915	0
chr1	664185	702501	0
chr1	848578	852147	1
chr1	887232	890566	0

Table S4. FStitch-training genomic coordinates for H3K27ac analysis.

chromosome number	start	end	signal state
chr1	1	526	1
chr1	527	17750	0
chr1	17751	21131	1
chr1	21132	27357	0
chr1	29318	79623	0
chr1	80395	431321	0
chr1	431322	432526	1
chr1	432527	530041	0
chr1	530042	531681	1
chr1	531682	554227	0
chr1	554228	560240	1
chr1	560241	702800	0
chr1	702801	705745	1
chr1	705746	750847	0
chr1	753988	829258	0
chr1	829259	832773	1
chr1	832774	845885	0
chr1	845886	852054	1
chr1	863540	868379	1

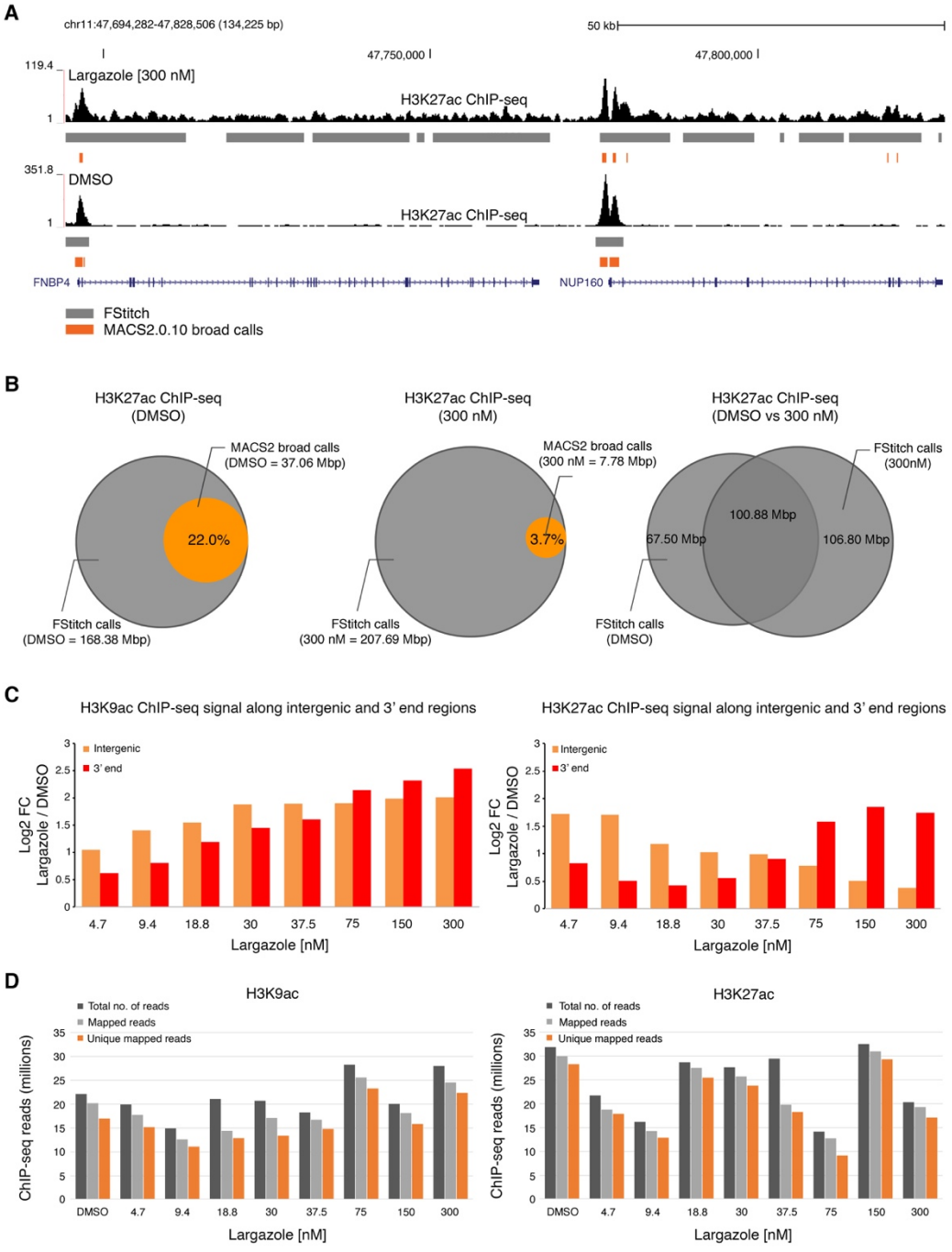


Figure S1. Systemic identification of genome wide acetylated histone marks with FStitch. (**A**) comparison of two ChIP-seq signal calling algorithms (FStitch and MACS2). A screen shot from Genome Browser (UCSC) showing H3K27ac ChIP-seq data in HCT116 cells untreated (bottom) or treated with 300 nM largazole (top). The 125 kb genomic window illustrates the statistical significant regions called by FStitch (grey) and MACS2.0.10 broad calls (orange) using their default signal thresholds. (**B**) Overlap between total genomic distances called by each algorithm. Venn diagram shows ~78% of the peaks called by FStitch (grey) using DMSO data were not detected by MACS2 (orange) and ~96.3% when a similar comparison was made using

the 300 nM ChIP-seq data. (**C**) The log2 fold change ratio for H3K9ac and H3K27ac enrichment in the 3' ends and intergenic territories with increasing doses of largazole (nM). (**D**) Number of mapped reads from individual ChIP-seq experiment targeting H3K9ac and H3K27ac.

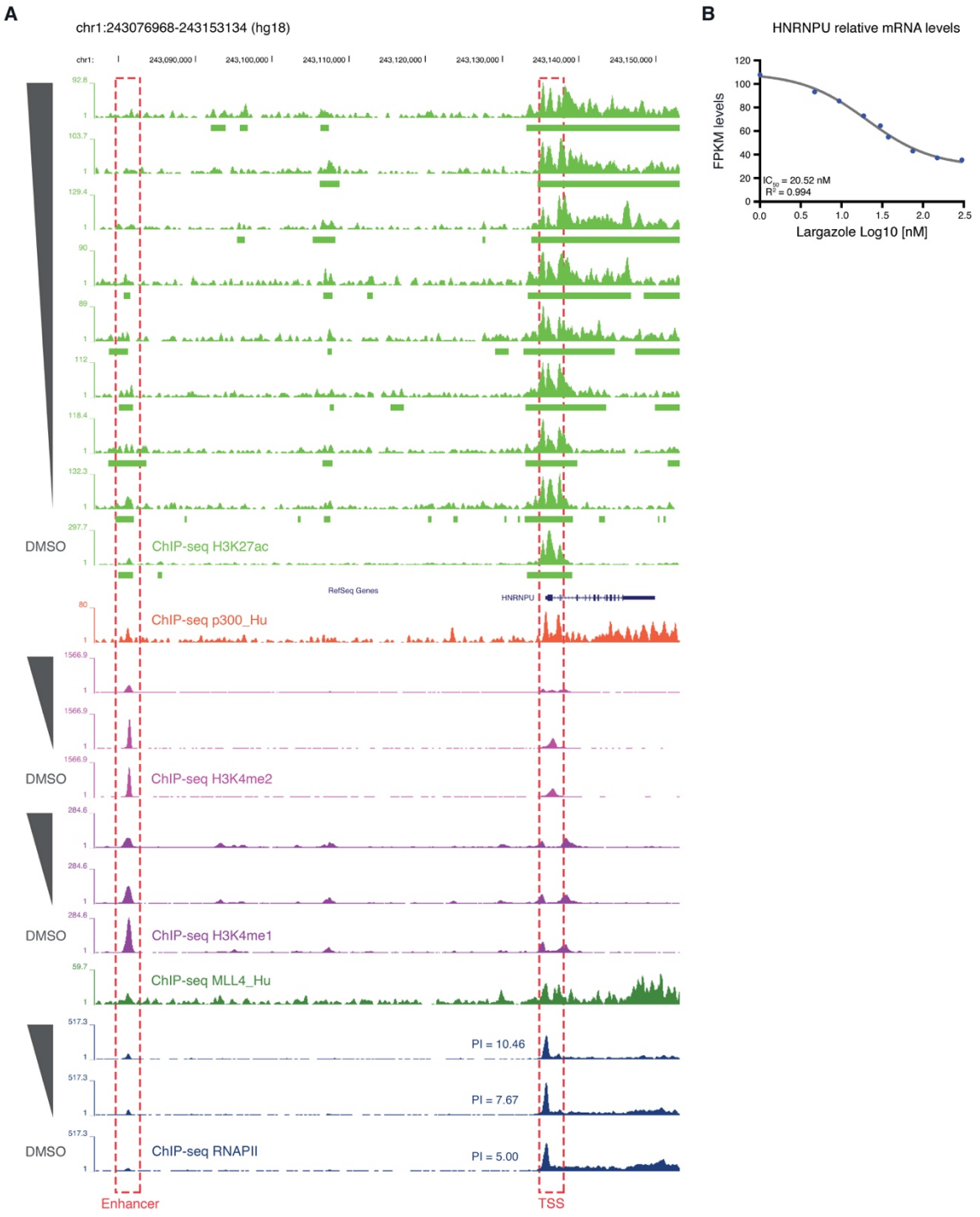


Figure S2. Inactivation of the HNRNPU associated transcriptional enhancer. (**A**) Screenshot of Genome Browser (UCSC) along the HNRNPU locus showing H3K27ac ChIP-seq (light green) and the associated signal determined by FStitch (light green rectangles) from HCT116 cells starting with untreated cells (DMSO) at the bottom and followed by eight increasing largazole dose treatments on top (4.7 nM to 300 nM). ChIP-seq signal accumulation for total RNAPII (blue), H3K4me1 (dark purple), and H3K4me2 (light purple) are shown for untreated (DMSO) HCT116 cells and for those treated with either 75 nM or 300 nM largazole concentrations.

ChIP-seq signal for p300 (orange) and MLL4 (dark green) were gathered from unstimulated HCT116 cells (Hu et al., 2013b). The transcriptional start site of HNRNPU gene and the associated upstream enhancer are denoted with red dotted rectangles. (**B**) Concentration inhibition profile of largazole towards the HNRNPU coding mRNA as determined by RNA-seq.

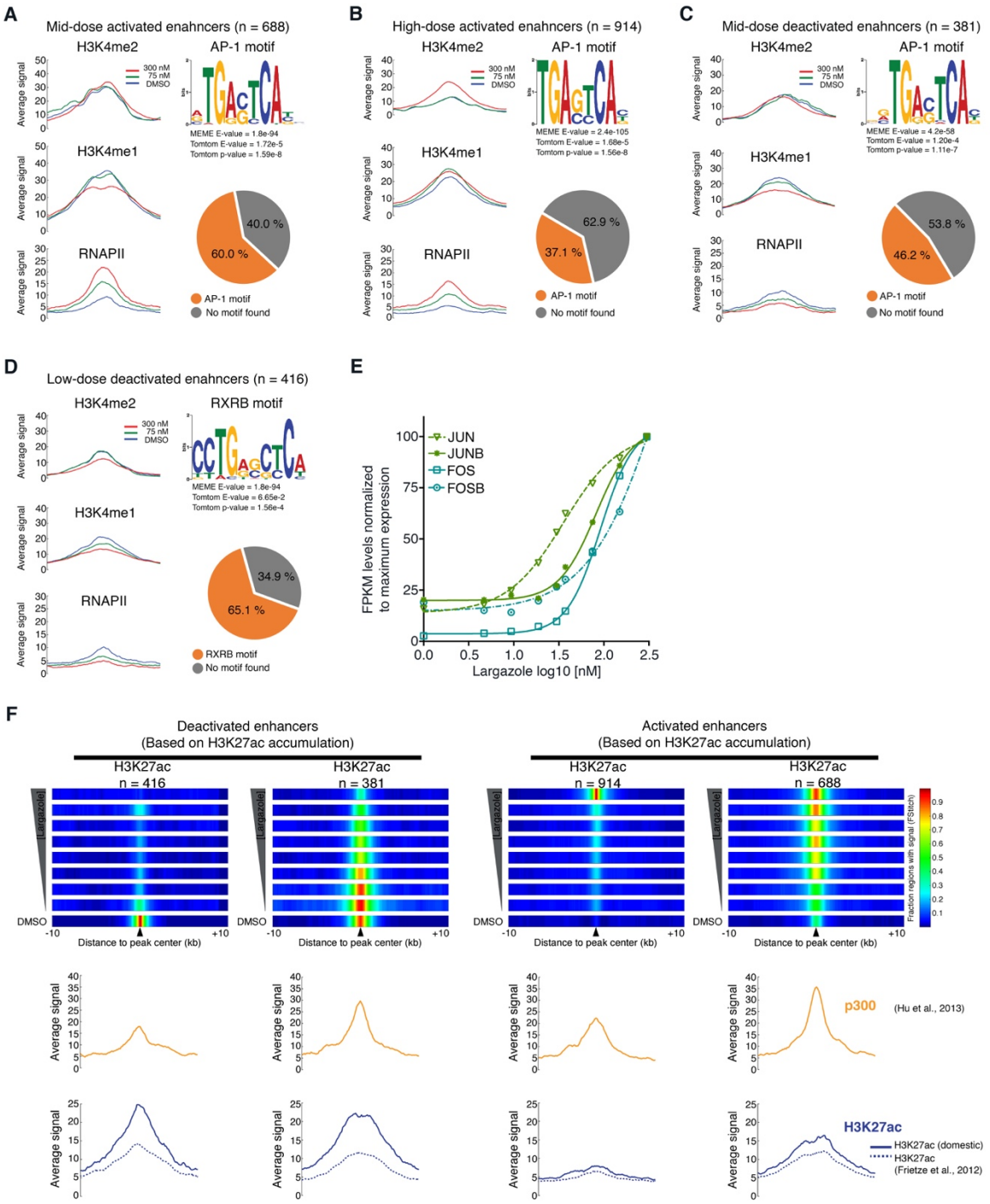


Figure S3. Meta-analysis of histone modification changes, RNAPII occupancy and motif enrichment for remodeled enhancers. (**A, B, C, D**) Average normalized densities of ChIP-seq reads for RNAPII, H3K4me1, and H3K4me2 along a +/- 1 kb distance centered on isolated enhancer (IE) regions presented in Figure 5C. Data from three ChIP-seq experiments are shown; DMSO (blue), largazole 75 nM (green), and largazole 300 nM (red). Sequence motif associated with the corresponding cluster of isolated poised (A,B) or isolated canonical (C,D) enhancers. Shown are the determined E-values from the MEME de novo motif finding algorithm

and from TOMTOM describing the certainty of the match between the identified motif and the transcription factor database position weight matrices. Pie charts illustrate the percentage of enhancer elements positive for the corresponding identified consensus motif. (**E**) Largazole stimulated transcriptional activation of genes coding for protein members of the AP-1 complex. mRNA accumulation levels from HCT116 cells treated for 16 h with the indicated largazole concentration. (**F**) Four clusters of isolated enhancers based on their largazole-induced functional state and dose-response: largazole-deactivated enhancers at low-dose (n = 416), mid-dose (n = 381), and largazole-activated enhancers at high-dose (n = 914) and mid-dose (n = 688). Top panel, shown are the fraction of enhancer regions with H3K27ac signal as described in Figure 5. Bottom panel, average normalized density of ChIP-seq reads from unstimulated HCT116 cells for p300 (orange) (Hu et al., 2013b) and H3K27ac (solid blue from domestic and dotted blue from (Frietze et al., 2012)) along a +/- 1 kb distance centered on enhancer regions shown on top.

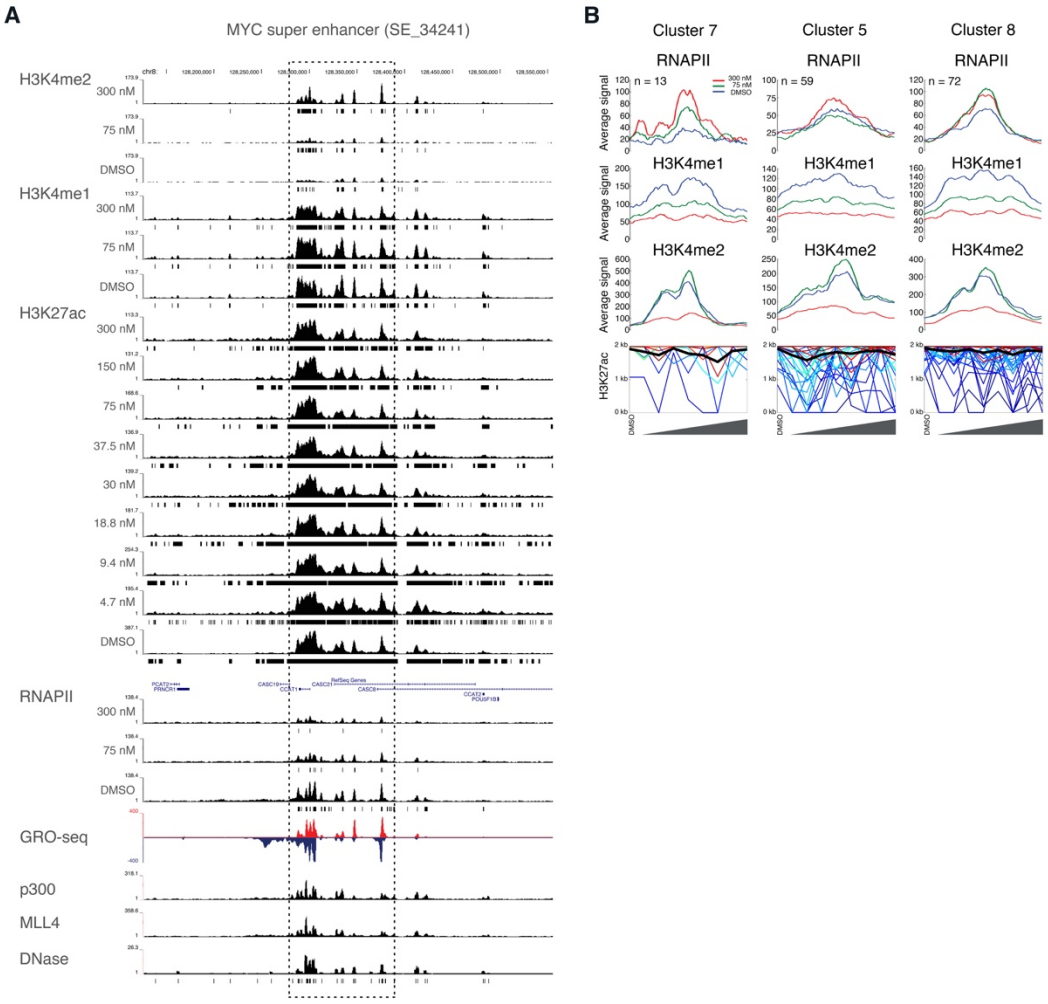
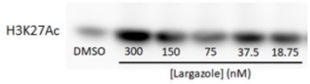
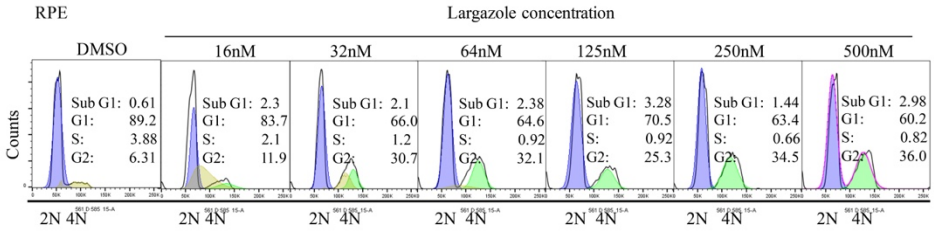


Figure S4. Depletion of active enhancer associated marks along super enhancers. (**A**) Screenshot of Genome Browser (UCSC) along the MYC super enhancer (SE #34241) (Khan and Zhang, 2016) showing normalized ChIP-seq data in untreated (DMSO) or the indicated largazole dose treatment of HCT116 cells targeting H3K4me2, H3K4me1, H3K27ac, RNAPII, GRO-seq (red and blue) from (Allen et al., 2014), p300 and MLL4 from and the associated DNAse I hypersensitivity peak clusters from ENCODE (www.encodeproject.org). (**B**) A total of 1534 individual enhancers clustered by largazole-induced RNAPII read density changes. Shown are the average normalized density of ChIP-seq reads for RNAPII, H3K4me1 and H3K4me2 along a +/- 1 kb distance centered on overlapping peak regions. Data from three ChIP-seq experiments are shown: DMSO (blue), 75 nM (green), and 300 nM (red). Accumulation of H3K27ac signal (FStitch calls) along the corresponding +/- 1 kb enhancer locations from nine ChIP-seq experiments starting with vehicle (DMSO) at the left and followed by increasing largazole dose treatments to the right (4.7 nM – 300 nM). Each line illustrates the accumulation of H3K27ac signal (FStitch) along an individual enhancer with centroids (means) indicated by black solid lines.

A



B



C

Number of enhancers per category under basal conditions.

Enhancer category	RPE	HCT116
H3K27ac + H3K4me1	28,299	41,017
H3K27ac + H3K4me1 + RNAPII	1,332	8,045
Super enhancers (SE)	162	538

D

Relative mRNA expression levels (FPKMs) of major histone remodeling enzymes across largazole treatments in HCT116 cells.

Gene	DMSO_FPKM	4.68nM_FPKM	9.37nM_FPKM	18.75nM_FPKM	30nM_FPKM	37.5nM_FPKM	75nM_FPKM	150nM_FPKM	300nM_FPKM
EP300	3.9	3.9	3.9	4.3	4.4	4.1	3.7	3.5	4.2
KAT2A (GCN5)	42.8	30.5	25.6	21.4	18.9	17.9	16.4	13.7	12.8
KAT2B (PCAF)	0.2	0.4	0.6	0.7	0.5	0.6	0.6	0.6	0.6
CREBBP	5.3	5.2	5.2	4.5	4.2	3.8	3.5	3.0	2.8
MLL	2.2	2.2	2.1	2.3	2.3	1.9	2.0	2.1	2.0
MLL2	3.8	5.0	4.1	3.6	2.8	2.6	1.8	1.9	1.4
MLL3	0.5	0.6	0.7	0.9	1.1	1.1	1.3	1.2	1.2
MLL4	11.0	9.8	8.0	7.4	6.2	6.5	5.5	4.8	4.3

Figure S5. Dose-dependent cell cycle, H3K27 acetylation, and transcript level changes in largazole treated RPE cells. (**A**) Global changes in H3K27ac induced in RPE cells by increasing concentrations of largazole exposure for 16 h as determined by immunoblotting. (**B**) Analysis of cell cycle state distribution by propidium iodide staining using flow cytometry in RPE cells treated with the indicated largazole concentration for 25 h. (**C**) Comparison of the total number of enhancers identified in RPE and HCT116 cells. (**D**) Expression profiles of a set of histone remodeling genes in HCT116 cells treated for 16 h with increasing concentrations of largazole.