Cell Reports, Volume *27*

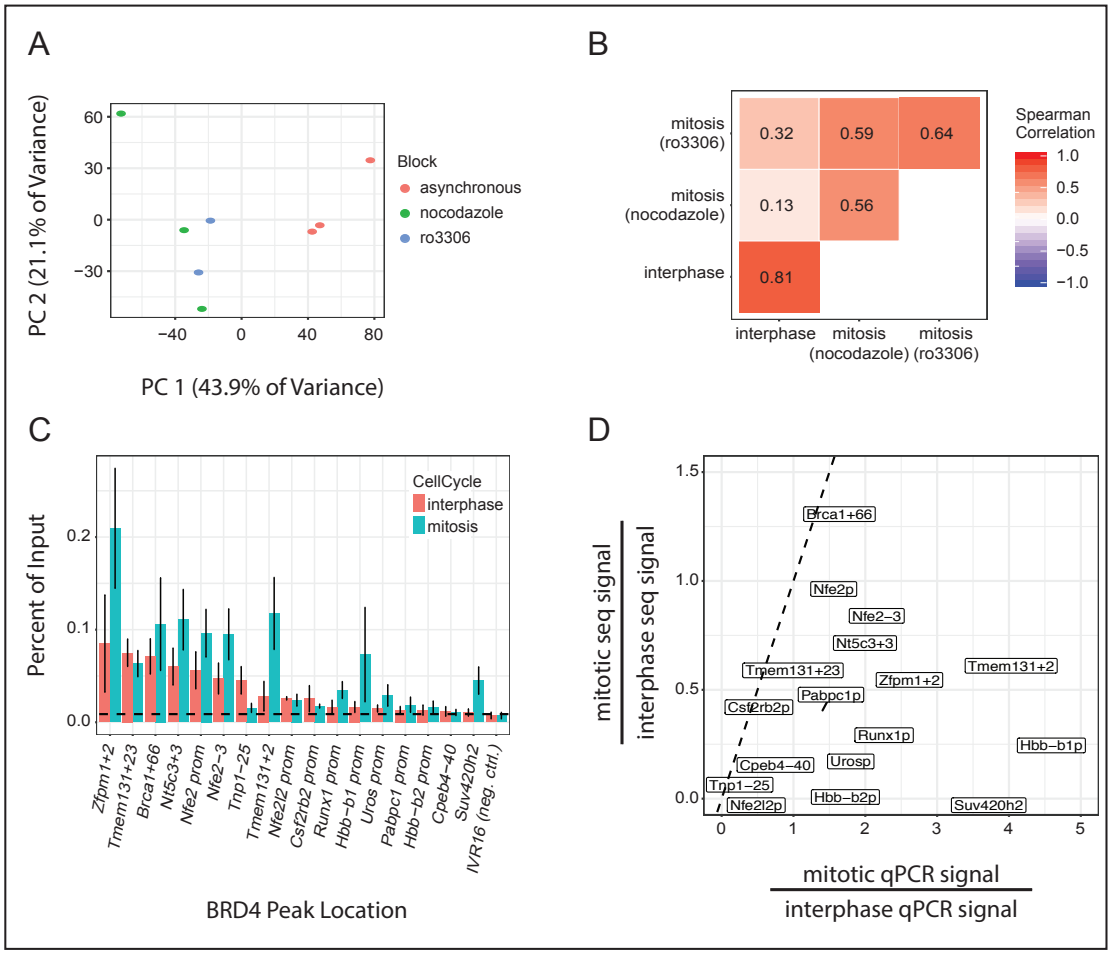
Supplemental Information

Interrogating Histone Acetylation and BRD4 as Mitotic Bookmarks of Transcription

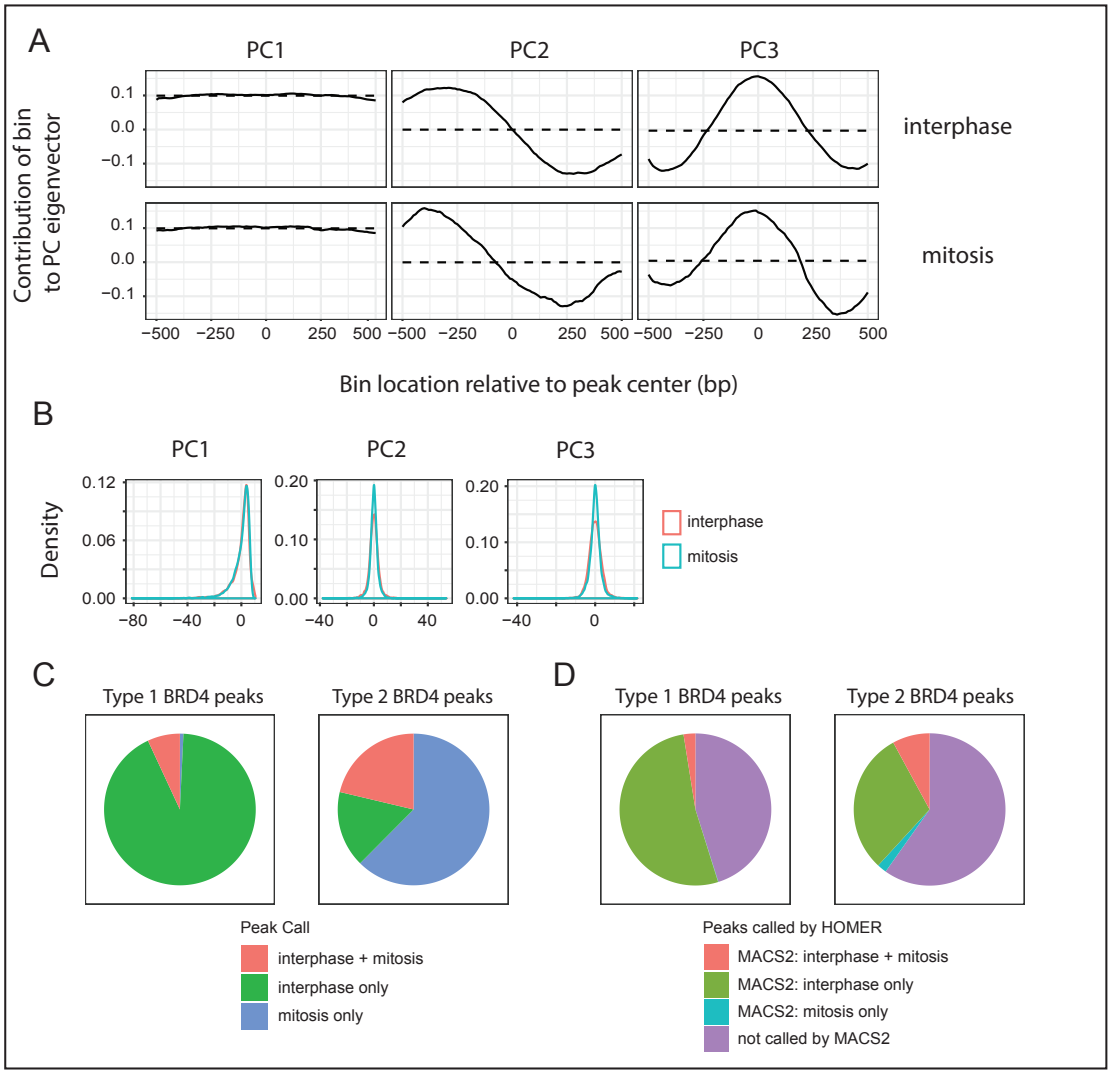
Vivek Behera, Aaron J. Stonestrom, Nicole Hamagami, Chris C. Hsiung, Cheryl A. Keller, Belinda Giardine, Simone Sidoli, Zuo-Fei Yuan, Natarajan V. Bhanu, Michael T. Werner, Hongxin Wang, Benjamin A. Garcia, Ross C. Hardison, and Gerd A. Blobel

SUPPLEMENTAL MATERIAL

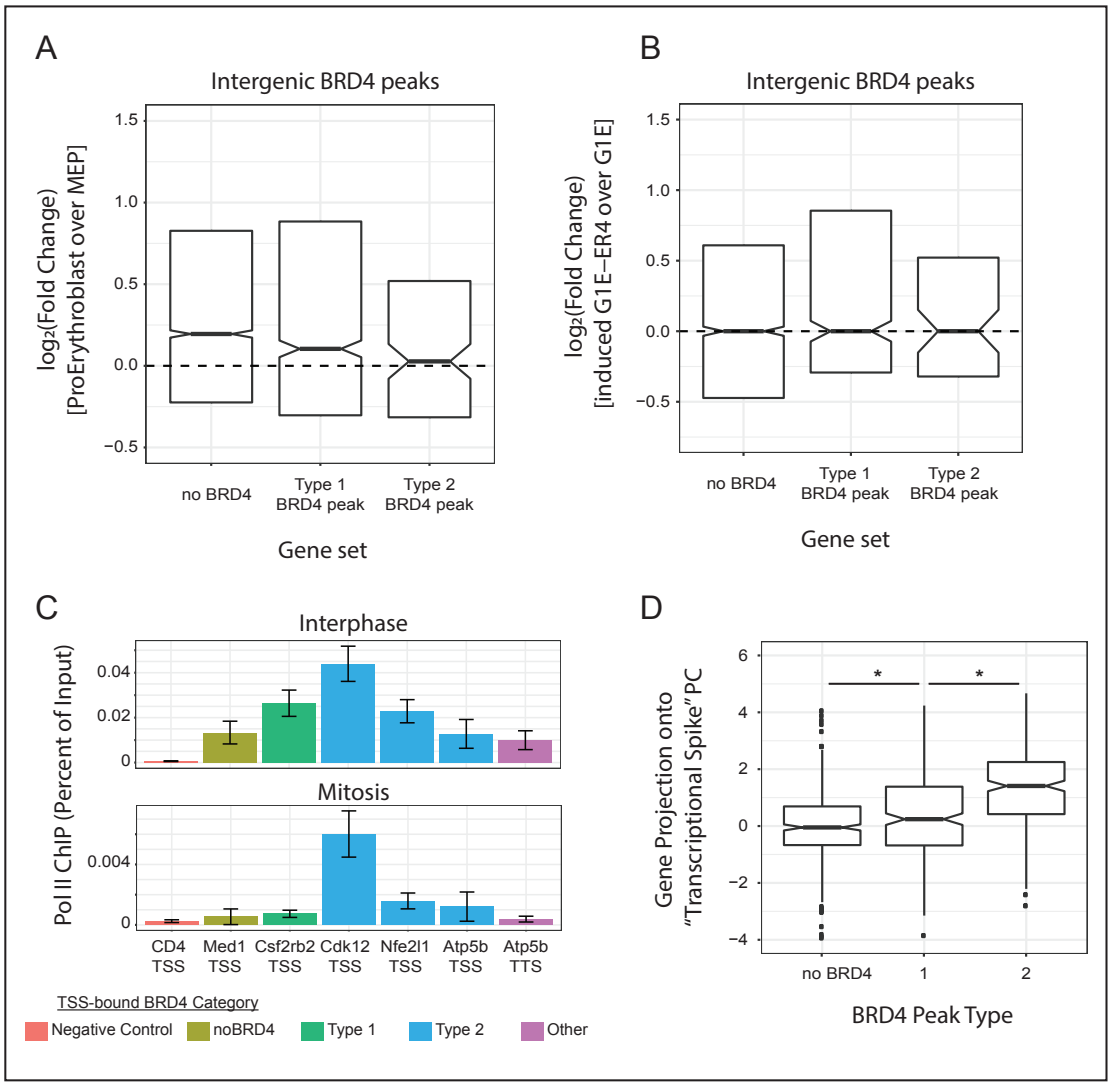
Supplemental Figures S1-S7



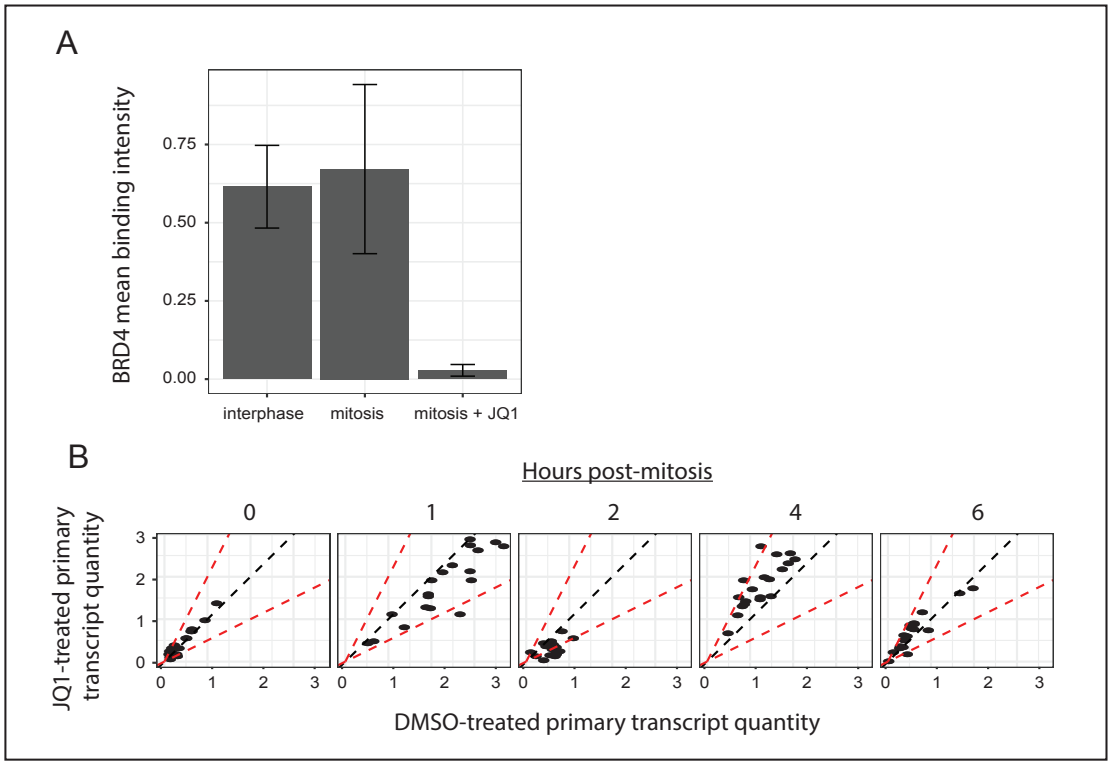
Supplementary Figure S1 (related to Main Figure 1): Comparison of interphase and mitotic BRD4 ChIP-seq requires normalization between datasets. (a) Principal component analysis (PCA) of qPCR-scaled BRD4 peak intensities from each ChIP-seq replicate. Replicates are either unsynchronized (red) or synchronized in mitosis using nocodazole (green) or ro3306 (blue). (b) Heatmap depicting mean Spearman correlation r comparing BRD4 peak intensities in interphase, nocodazole-synchronized mitosis, or ro3306-synchronized mitosis. Correlations are done for all replicates separately; depicted values are mean correlation coefficient for a given comparison. (c) BRD4 ChIP-qPCR in interphase (n = 3) or mitosis (n = 5) at 17 BRD4 interphase peaks. (d) Ratio of mitotic to interphase binding of BRD4 at 17 interphase BRD4 peaks measured either by ChIP-qPCR or ChIP-sequencing (library-size normalized). Dotted line represents y = x.



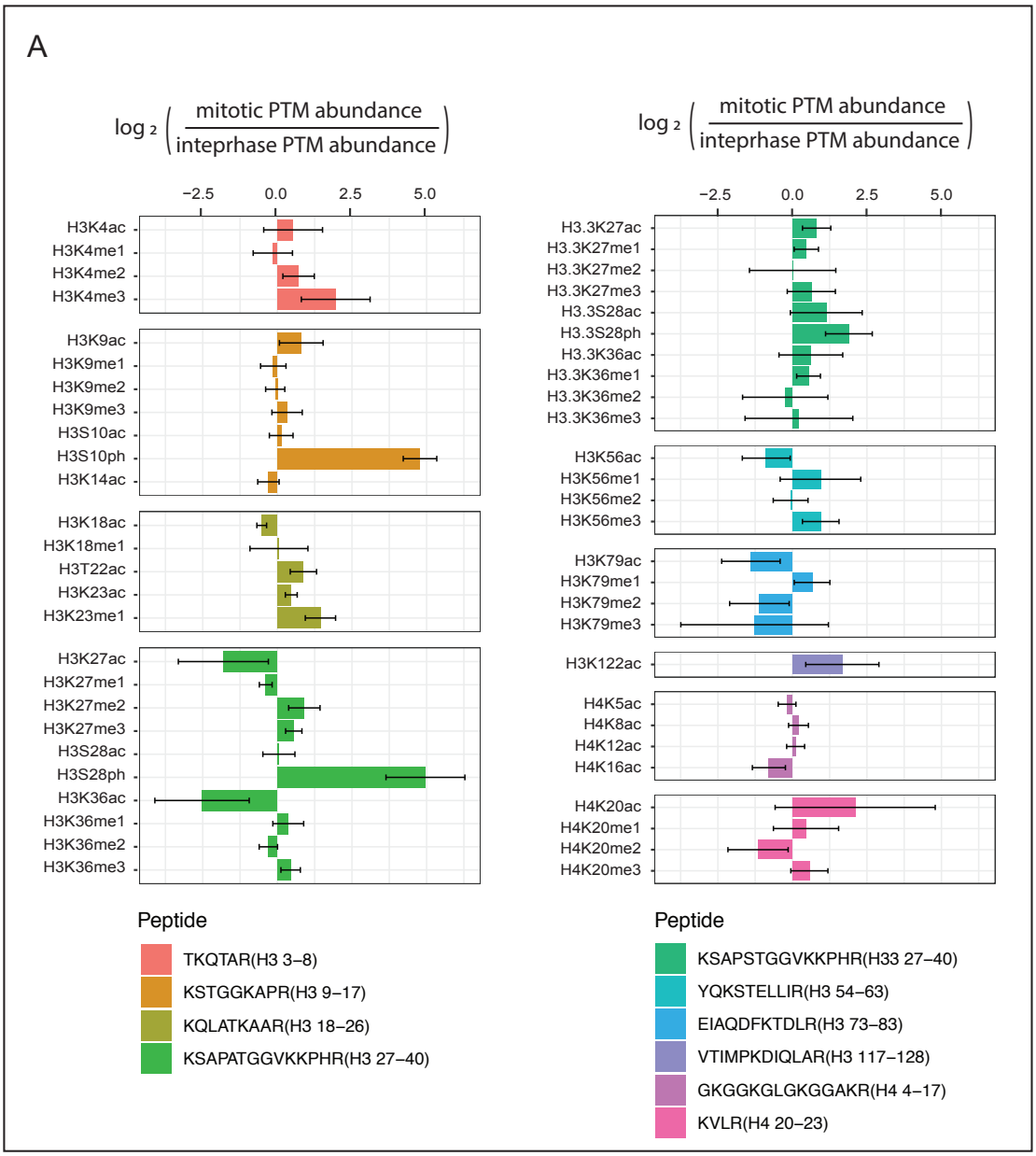
<u>Supplementary Figure S2 (related to Main Figure 2): Validation of PCA for identifying independent changes in binding characteristics.</u> (a) PC eigenvector profiles derived from either interphase or mitotic BRD4 data. (b) Density plot of peak projection values onto PC 1/2/3 during either interphase or mitosis. (c) Distribution of Type 1 or Type 2 peaks called by HOMER during interphase only (green), mitosis only (blue), or during both phases (red). (d) Distribution of Type 1 or Type 2 peaks called HOMER that were detected by MACS2 during interphase only (green), mitosis only (blue), during both phases (red), or not detected by MACS2 (purple).



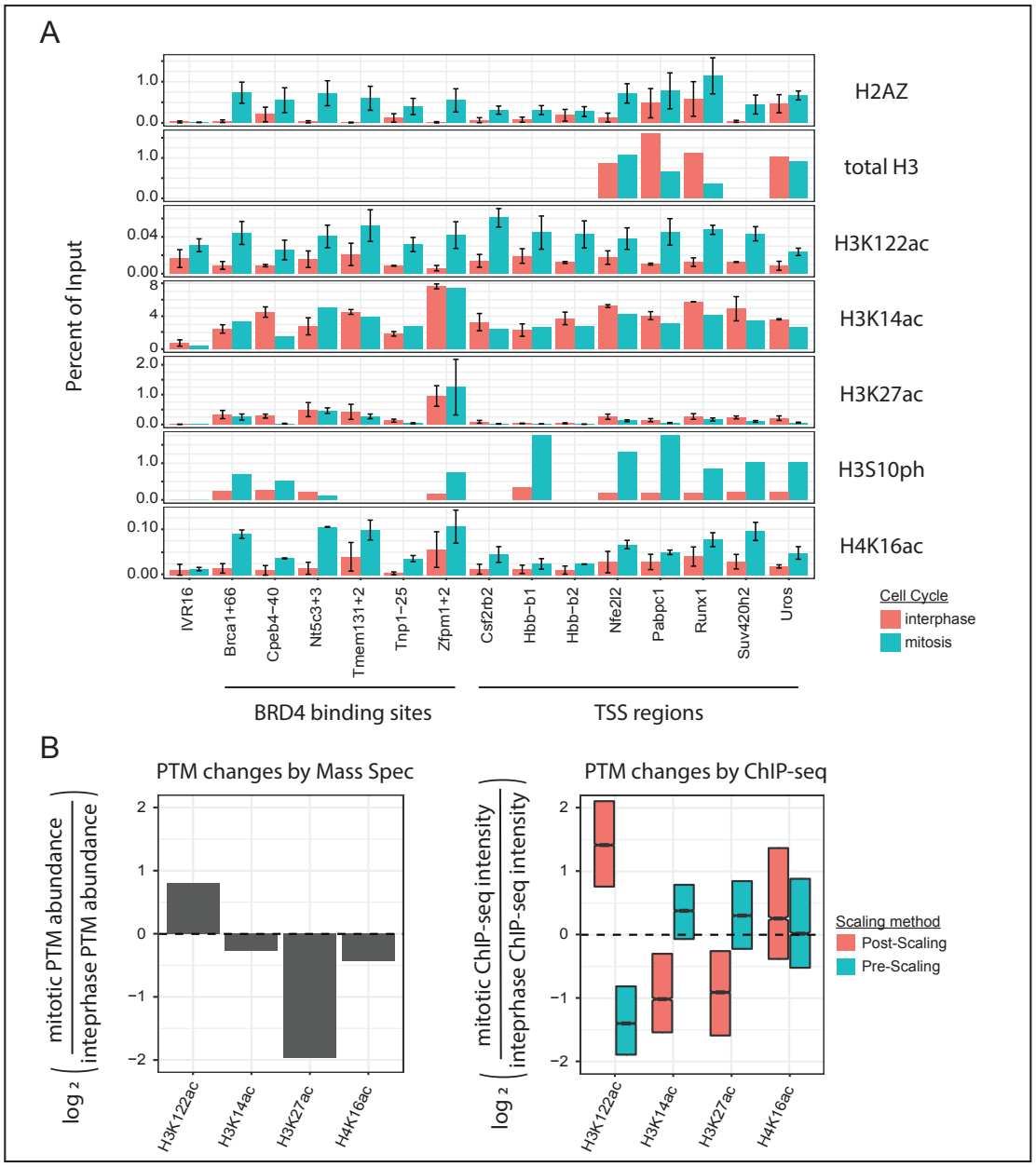
<u>Supplementary Figure S3 (related to Main Figure 3): BRD4 mitotic chromatin occupancy displays multiple features consistent with a bookmarking protein.</u> RNA-seq gene expression changes during either (a) primary cell differentiation from MEP to proerythroblast or (b) GATA1-mediated differentiation of the G1E-ER4 proerythroblast cell line. Genes are categorized by containing either an intergenic Type 1 or Type 2 BRD4 binding peak or as having no BRD4 binding. Boxplot center is median, hinges are 25% and 75% percentile. (c) Pol II ChIP-qPCR in asynchronous or nocodazole-synchronized mitotic cells, assessed in gene TSS-proximal or TTS-proximal (Atp5b gene) regions. All values are mean +/- SEM, n = 3. (d) Projection onto PC1 ("Transcriptional Spike" PC) (Hsiung et al., 2016) for genes proximal to either a Type 1 or Type 2 BRD4 binding peak or having no nearby BRD4 binding. Genes were subsampled to match asynchronous Pol II binding intensity between groups. Boxplot center is median, hinges are 25% and 75% percentile, * Wilcoxon $p < 0.01$.



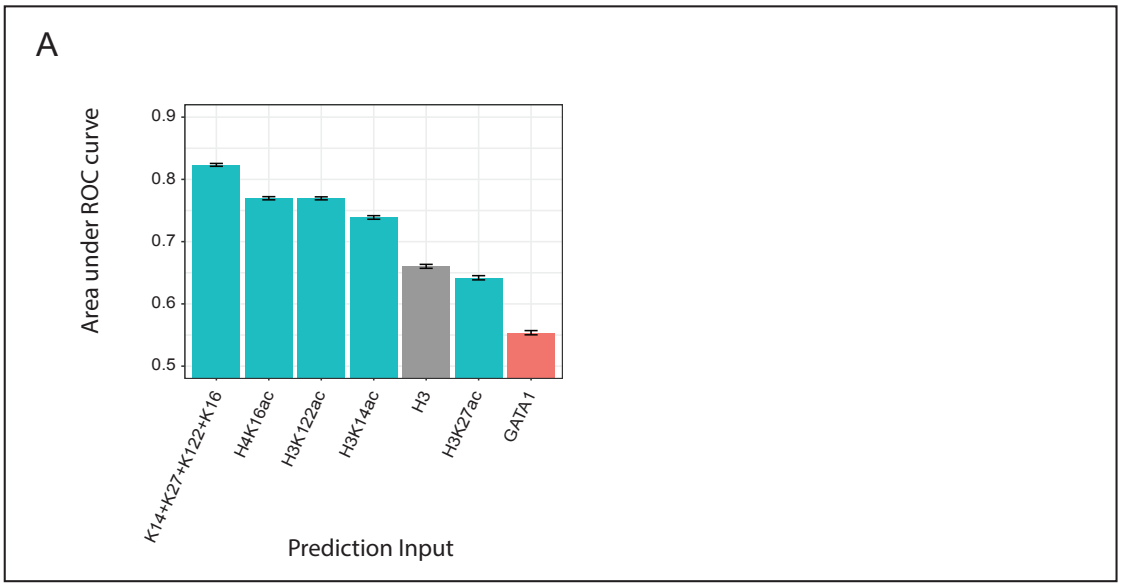
Supplementary Figure S4 (related to Main Figure 4): JQ1 disrupts BRD4 binding but not transcriptional reactivation following mitosis. (a) BRD4 binding intensity at TSS regions (1 kb, centered on TSS) of the 16 erythroid genes tested in Figure 4b and 4b, during interphase, mitosis, or during mitosis treated with 1 hr 250 nM JQ1, mean +/- 95% CI. (b) Comparison of RT-qPCR quantities for 16 primary transcripts after treatment with either 1 uM JQ1 or DMSO during mitosis. Dotted black line represents equal levels; dotted red lines show 2-fold difference. Data are normalized to GAPDH mature transcript and to mean quantity across all time points.



<u>Supplementary Figure S5 (related to Main Figure 5): Changes in histone PTM abundance between interphase and mitosis as determined by mass spectrometry.</u> (a) Log2-fold changes in individual histone PTM abundance between interphase and mitosis. Marks are grouped by histone peptide post-trypsin digestion, bars represent mean +/- SEM, n = 7.



Supplementary Figure S6 (related to Main Figure 6): Comparison of histone PTM abundance between interphase and mitosis. (a) Interphase and mitotic ChIP-qPCR for histone PTMs at a set of BRD4 binding peaks and gene TSS regions. Bars are mean +/- SEM, n = 2 if error bar is present, n = 1 otherwise. (b) Comparison of histone acetylation abundance changes detected by mass spectrometry and ChIP-seq. Left, median changes in abundance of particular BRD4-binding histone PTMs between interphase and mitosis. Right, boxplots of changes in histone mark intensity found by ChIP-seq across each mark's respective peaks. Boxplots are colored by whether qPCR-scaling was used for data normalization, boxplot center is median intensity change, hinges are 25% and 75% percentiles.



Supplementary Figure S7 (related to Main Figure 7): Quantification of predictive modeling of BRD4 mitotic occupancy. (a) Area under the ROC curves in Figure 7e, mean +/- SEM of independent runs.

MARK	TYPE	PEPTIDE	FAI	FAM	RATIO
H3K4AC	Acetyl	TKQTAR(H3 3-8)	1.37E-03	8.59E-04	6.26E-01
H3K4ME1	Methyl	TKQTAR(H3 3-8)	8.27E-02	1.44E-01	1.74E+00
H3K4ME2	Methyl	TKQTAR(H3 3-8)	2.07E-03	2.77E-03	1.34E+00
H3K4ME3	Methyl	TKQTAR(H3 3-8)	2.38E-04	1.07E-03	4.52E+00
H3K9AC	Acetyl	KSTGGKAPR(H3 9-17)	1.51E-02	2.37E-02	1.57E+00
H3K9ME1	Methyl	KSTGGKAPR(H3 9-17)	1.58E-01	2.16E-01	1.36E+00
H3K9ME2	Methyl	KSTGGKAPR(H3 9-17)	2.04E-01	2.18E-01	1.07E+00
H3K9ME3	Methyl	KSTGGKAPR(H3 9-17)	1.88E-01	2.16E-01	1.15E+00
H3S10AC	Acetyl	KSTGGKAPR(H3 9-17)	7.61E-02	7.11E-02	9.34E-01
H3S10PH	Phospho	KSTGGKAPR(H3 9-17)	2.06E-02	3.76E-01	1.82E+01
H3K14AC	Acetyl	KSTGGKAPR(H3 9-17)	3.13E-01	3.37E-01	1.08E+00
H3K18AC	Acetyl	KQLATKAAR(H3 18-26)	3.14E-02	2.17E-02	6.90E-01
H3K18ME1	Methyl	KQLATKAAR(H3 18-26)	6.09E-03	8.52E-03	1.40E+00
H3T22AC	Acetyl	KQLATKAAR(H3 18-26)	8.38E-04	1.21E-03	1.44E+00
H3K23AC	Acetyl	KQLATKAAR(H3 18-26)	1.57E-01	2.48E-01	1.58E+00
H3K23ME1	Methyl	KQLATKAAR(H3 18-26)	3.09E-03	4.93E-03	1.59E+00
H3K27AC	Acetyl	KSAPATGGVKKPHR(H3 27-40)	6.94E-04	1.62E-04	2.34E-01
H3K27ME1	Methyl	KSAPATGGVKKPHR(H3 27-40)	4.68E-01	2.61E-01	5.57E-01
H3K27ME2	Methyl	KSAPATGGVKKPHR(H3 27-40)	2.24E-01	2.22E-01	9.89E-01
H3K27ME3	Methyl	KSAPATGGVKKPHR(H3 27-40)	9.23E-02	1.10E-01	1.20E+00
H3S28AC	Acetyl	KSAPATGGVKKPHR(H3 27-40)	1.85E-02	1.61E-02	8.70E-01
H3S28PH	Phospho	KSAPATGGVKKPHR(H3 27-40)	1.05E-03	4.74E-02	4.53E+01
H3K36AC	Acetyl	KSAPATGGVKKPHR(H3 27-40)	3.94E-03	9.37E-04	2.38E-01
H3K36ME1	Methyl	KSAPATGGVKKPHR(H3 27-40)	1.71E-01	1.35E-01	7.89E-01
H3K36ME2	Methyl	KSAPATGGVKKPHR(H3 27-40)	1.47E-01	1.78E-01	1.21E+00
H3K36ME3	Methyl	KSAPATGGVKKPHR(H3 27-40)	7.38E-02	9.70E-02	1.31E+00
H3.3K27AC	Acetyl	KSAPSTGGVKKPHR(H33 27-40)	6.11E-04	1.45E-03	2.37E+00
H3.3K27ME1	Methyl	KSAPSTGGVKKPHR(H33 27-40)	2.92E-01	4.06E-01	1.39E+00
H3.3K27ME2	Methyl	KSAPSTGGVKKPHR(H33 27-40)	5.93E-02	1.15E-01	1.94E+00
H3.3K27ME3	Methyl	KSAPSTGGVKKPHR(H33 27-40)	1.04E-01	8.32E-02	8.03E-01
H3.3S28AC	Acetyl	KSAPSTGGVKKPHR(H33 27-40)	3.57E-02	1.01E-01	2.83E+00
H3.3S28PH	Phospho	KSAPSTGGVKKPHR(H33 27-40)	4.77E-03	6.75E-02	1.41E+01
H3.3K36AC	Acetyl	KSAPSTGGVKKPHR(H33 27-40)	8.17E-03	1.65E-02	2.02E+00
H3.3K36ME1	Methyl	KSAPSTGGVKKPHR(H33 27-40)	1.11E-01	2.23E-01	2.01E+00

H3.3K36ME2	Methyl	KSAPSTGGVKKPHR(H33 27-40)	5.24E-02	5.11E-02	9.74E-01
H3.3K36ME3	Methyl	KSAPSTGGVKKPHR(H33 27-40)	1.74E-02	5.22E-02	3.00E+00
H3K56AC	Acetyl	YQKSTELLIR(H3 54-63)	3.37E-04	5.12E-04	1.52E+00
H3K56ME1	Methyl	YQKSTELLIR(H3 54-63)	4.45E-04	5.62E-03	1.26E+01
H3K56ME2	Methyl	YQKSTELLIR(H3 54-63)	2.03E-02	6.32E-02	3.11E+00
H3K56ME3	Methyl	YQKSTELLIR(H3 54-63)	1.46E-03	1.67E-03	1.15E+00
H3K79AC	Acetyl	EIAQDFKTDLR(H3 73-83)	2.48E-03	9.50E-04	3.84E-01
H3K79ME1	Methyl	EIAQDFKTDLR(H3 73-83)	2.03E-02	2.66E-02	1.31E+00
H3K79ME2	Methyl	EIAQDFKTDLR(H3 73-83)	2.75E-02	1.73E-02	6.29E-01
H3K79ME3	Methyl	EIAQDFKTDLR(H3 73-83)	1.89E-02	4.92E-02	2.61E+00
H3K122AC	Acetyl	VTIMPKDIQLAR(H3 117-128)	3.37E-03	5.21E-03	1.55E+00
H4K5AC	Acetyl	GKGGKGLGKGGAKR(H4 4-17)	1.58E-02	1.69E-02	1.07E+00
H4K8AC	Acetyl	GKGGKGLGKGGAKR(H4 4-17)	1.42E-02	1.96E-02	1.38E+00
H4K12AC	Acetyl	GKGGKGLGKGGAKR(H4 4-17)	2.78E-02	3.79E-02	1.37E+00
H4K16AC	Acetyl	GKGGKGLGKGGAKR(H4 4-17)	2.55E-01	1.60E-01	6.27E-01
H4K20AC	Acetyl	KVLR(H4 20-23)	7.86E-05	1.93E-04	2.46E+00
H4K20ME1	Methyl	KVLR(H4 20-23)	1.97E-01	6.84E-01	3.47E+00
H4K20ME2	Methyl	KVLR(H4 20-23)	5.89E-01	2.17E-01	3.69E-01
H4K20ME3	Methyl	KVLR(H4 20-23)	1.78E-02	1.47E-02	8.27E-01

Supplementary Table S1 (related to Main Figure 5): Abundance of modified peptides during interphase and mitosis as revealed by mass spectrometry. Fractional abundance calculated as the ratio between the abundance of peptide containing a particular PTM to the abundance of total peptide. FAI = Fractional Abundance during Interphase. FAM = Fractional Abundance during Mitosis. RATIO = Abundance during mitosis divided by abundance during interphase