Supporting Information

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SI Materials and Methods.

Peptides. GATA1 peptides were produced by the Rockefeller University peptide synthesis facility or Peptide 2.0. An N-terminal cysteine was added to allow coupling to Sulfo-link resin (Pierce).

Peptide Affinity Pulldown Assays. Peptides were coupled to Sulfolink resin according to manufacturer's instructions. Approximately 7.5 mg of nuclear extracts from mouse erythroleukemia (MEL) cells (1) were diluted to 150 mM NaCl and incubated with 200 ng immobilized peptide overnight at 4 °C. Beads were washed five times with buffer containing 450 mM NaCl, 50 mM Tris pH 7.5, and 0.5% Igepal and eluted by boiling in sodium dodecyl sulfate (SDS) sample buffer. Mass spectrometry was performed by the Proteomics Core Facility of the Genomics Institute at the University of Pennsylvania. For all small-scale binding studies, 25–50 ng immobilized peptide were incubated with extracts prepared from 10 million cells, or [^{35}S] methionine labeled protein generated from the rabbit reticulocyte system (TNT, Promega). All affinity assays were performed in the presence of 10 mM sodium butyrate, 1 mM PMSF, and protease inhibitor cocktail (Sigma).

Transfections and Coimmunoprecipitation. Transient transfections of 293T cells were performed using polyethylenimine (PEI) at a ratio of PEI to DNA of 3:1. Cells were harvested 48 h after transfection and nuclear extracts were prepared as described above. Extracts from 8 to 10 million cells were added to 5 μg antibody prebound to 20 μL protein G agarose. To reduce nonspecific binding, antibody-bound beads were incubated with extract prepared from untransfected cells prior to immunoprecipitation. After extensive washing, beads were boiled in SDS sample buffer, and bound proteins were analyzed by Western blotting. For coimmunoprecipitations of GATA1-ER and endogenous Brd3, G1E-ER4 cells were treated with the histone deacetylase inhibitors sodium butyrate and trichostatin A for 2 h prior to harvesting to preserve protein acetylation.

GST Pulldown Assays. GST fusion proteins were prepared as described. Equal amounts of GST, GST-BD1, GST-BD1 Y72F, and GST-BD2 were immobilized on glutathione sepharose beads and incubated with nuclear extracts from transiently transfected 293T cells as described above. Where indicated, chromatin was extracted by hypotonic lysis of cells in Buffer A (10 mM Hepes, 1.5 mM $MgCl_2$ 10 mM KCl, 10 mM sodium butyrate/protease inhibitor cocktail/50 μg/mL PMSF, pH 7.9), followed by centrifugation. The resulting nuclei were lysed in buffer containing 50 mM Tris, 10 mM EDTA, 1% SDS, 10 mM sodium butyrate, protease inhibitor cocktail, 50 μg/ml PMSF, pH 8.1) and the lysate was sonicated. Soluble chromatin was diluted with immunoprecipitation buffer (20 mM Tris, 150 mM NaCl, 2 mM EDTA, 0.01% SDS, 1% Triton X-100, 10 mM sodium butyrate, protease inhibitors, 50 μg/mL PMSF, pH 8.1) followed by overnight incubation at 4 °C with rotation.

In Vitro Acetylation of GATA1. Acetylation of the zinc finger region of GATA1 was performed as previously described (2). Mass spectrometry to identify GATA1 acetylation sites was performed at the Taplin facility at Harvard University.

Cell Culture. G1E cells were maintained as described (3). Where indicated, cells were treated with 100 nM estradiol (E2) for 21–24 h. MEL and 293T cells were cultured in Dulbecco modified Eagle medium with 10% fetal bovine serum, 2% penicillin-streptomycin, 1% glutamine, and 1% Na pyruvate. MEL differentiation was performed by adding 1.5% DMSO to the growth medium for 72 h. The murine megakaryocyte line L8057 was maintained in F-12 nutrient mixture (Invitrogen) with 100 U/mL penicillin, 100 U/mL streptomycin, 2 mM L-glutamine, and 10% fetal bovine serum.

Plasmids. The GATA1 expression plasmids pXM-GATA1 and the mutant derivatives pXM-GATA1 NC(A), pXM-GATA1 N(A), and pXM-GATA1 C(A) were described previously (2, 4). The GST-GATA1 zinc finger construct has been described (5, 6). The CREB binding protein (CBP) expression plasmid pCMV5-CBP and the GATA2 expression plasmid pXM-GATA2 have been described previously (7, 8). Lysine to arginine substitutions were introduced into the N-terminal (RPRRRMI) or C-terminal (SGRGRRRRGS) acetylation motifs of GATA1 and cloned into the mammalian expression vector pCDNA3.

Murine HA-Brd3 isoform 2 and HA-Brd4 isoform 2 were amplified from a MEL cell cDNA library and inserted into pcDNA3 and the retroviral vector MigR1. Overlapping PCR was used to generate ΔBD1 (Δ33-144), ΔBD2 (Δ306-418), and ΔBD1+2 (Δ33-144; Δ306-418), followed by insertion into pcDNA3 and MigR1. Brd3 Y72F, Y347, and Y72F/Y347F were generated by the QuikChange site-directed mutagenesis kit per the manufacturer's instructions (Stratagene) and inserted into MigR1. BD1 (aa24-aa146) and BD2 (aa306-418) of Brd3 were isolated by PCR and inserted into pGEX 6P-1. A 5′ NLS derived from the SV40 Large T antigen and the 3' UTR from *Hbb-b1* were added for nuclear localization and increased mRNA stability, respectively, prior to insertion of BD1 and BD2 into MigR1.

Retroviral Infections. Retroviral infections were done as previously described (9).

Antibodies. Rabbit anti-Brd3 sera were generated using two independent peptide antigens corresponding amino acids (aa) 484–501 and aa 656–673 of mouse Brd3. Rabbit anti-Brd4 serum was raised against a peptide spanning aa 280–297 (Cocalico Biologicals, Inc). Specificity of the sera was tested by Western blot, immunoprecipitation, and ChIP analyses (Fig. 3 and Fig. S2). Additional antibodies used in this study were anti-GATA1 (sc-265; Santa Cruz), anti-GATA2 (sc-9008; Santa Cruz), anti-FOG1 (sc-9361; Santa Cruz), antiacetyl histone H3 (17-615; Upstate), antiacetyl histone H4 (06-866; Upstate), anti-acetyl-H4K5 (07-327; Upstate), anti-acetyl-H4K12 (07-595; Upstate), anti-HA (12CA5), and anti-β-actin conjugated to horseradish peroxidase (A3854; Sigma).

ChIP Followed by Massively Parallel Sequencing Analysis. Anti-Brd3 ChIP material from G1E or induced G1E-ER4 cells was sequenced on a HeliScope Single Molecule Sequencer (Helicos). Alignment to the mouse genome (mm9 RefSeq assembly) yielded 21.4e6 and 11.3e6 aligned reads in G1E and G1E-ER4 cells, respectively. Aligned reads were placed into 4.1e6 genomic bins of 500 bp each and normalized to adjust for differences in sequencing depth. Most genomic analyses described below were carried out using Galaxy (10); http://galaxy.psu.edu), and analysis histories are available on request. To generate Brd3 binding data for Fig. 5, the number of normalized Brd3 read counts within the genomic bin at the center of GATA1 peak intervals was queried for GATA1 binding sites derived from a published dataset that

was generated in G1E-ER4 cells (11). To categorize GATA1 sites into likely activating or repressive binding sites, each site was first assigned to the closest RefSeq gene's transcription start site. A published expression microarray dataset (11) (GEO accession number GSE18042) was used to define the subsets of GATA1 peaks associated with the top 5% induced (activating) and the top 5% repressed genes after activation of GATA1 in G1E-ER4 cells (repressive sites). Descriptive and inferential statistical analysis was performed using R (http://www.r-project.org). The built-in boxplot function was used to summarize each dataset to indicating the median, the upper and lower quartiles, and the range excluding outliers. The nonparametric Mann–Whitney *U* test was employed to test the statistical significance of the differences between pairs of normalized Brd3 read count distributions. Because the number of data points varied significantly among the different datasets, an equally sized random sample ($n = 400$) from each dataset was used for significance testing.

For Fig. S7, the genes ranking within the top 1%, 1% to 5%, and 5% to 25% expression level and the genomic coordinates of their transcribed regions (RefSeq) were determined. A combination of custom-written Ruby scripts and the MySQL database system was employed to determine mean normalized Brd3 read counts at 3,000 equally spaced positions one full gene length upstream of the transcription start site to one full gene length downstream of the transcription end site. For comparison, the genome-wide average Brd3 signal was plotted as well.

ChIP Realtime PCR Primers (5′ to 3′)

All primers correspond to the murine genomic sequence.

β globin locus

HS4
Forward GAGATCCTGCCAAGACTCTGATAATACTA
Reverse CCTGGCTTCCTGGTCCAGTAG

Intervening Region (IVR) 4/3B
Forward CACGAGTTTCCTTTCATGCTTATG
Reverse CCAGCCCTTCTGGAACTTCTC

HS3
Forward CTAGGGACTGAGAGAGGCTGCTT
Reverse ATGGGACCTCTGATAGACACATCTT

HS2
Forward GGGTGTGTGGCCAGATGTTT
Reverse CACCTTCCCTGTGGACTTCCT

HS1
Forward CAGATCCTCAAACACTCTCCCATAA
Reverse TGCCTTCTTTGTCCCATCATT

Ey
Forward ATGACCTGGCTCCACCCAT
Reverse TCTTTGAAGCCATTGGTCAGC

IVR1
Forward TGTGCTAGCCTCAAGCTCACA
Reverse TCCCAGCACTCAGAAGAAGGA

BH1
Forward AGGTCCAGGGTGAAGAATAAAAGG
Reverse ATCTCAAGTGTGTGCAAAAGCCAGA

IVR2
Forward GTATGCTCAATTCAAATGTACCTTATTTTAA
Reverse TTACCTCTTTATTTCACTTTTACACATAGCTAA

Hbb-b1 promoter
Forward CAGGGAGAAATATGCT
Reverse GTGAGCAGATTGGCCCTTACC

Hbb-b1 intron 2
Forward CTTCTCTCTCTCCTCTCTCTTTCTCTAATC
Reverse AATGAACTGAGGGAAAGGAAAGG

IVR3
Forward ATAGGAAAGAAAATGCACACATAGATTC
Reverse CCCACGCCTCATTTATACTTTCAG

Hbb-b2 promoter
Forward GAGCCAGCATTGGGTATATAAAGC
Reverse ACAGACTCAGAAGCAAACGTAAGAAG

IVR4
Forward TGGCCATTTTTACTATGTTAATTTTGC
Reverse TAGACTTGTCATGGTTATGGATTGG

Kit locus

−232 kb
Forward TGCACTTGGCAAACTTCCTG
Reverse AGGGATGTGTCTCACCGTGTC

−114 kb
Forward GCACACAGGACCTGACTCCA
Reverse GTTCTGAGATGCGGTTGCTG

−86 kb
Forward CGGAGCACCGAAGAGTCTTG
Reverse GCAGCTCCGGGTAAAATAGAAAA

−51 kb
Forward CCACGGATAATGCCCTCTGT
Reverse GGGTCCCCGATGATACACTCT

promoter
Forward CACCTCCACCATAAGCCGAAT
Reverse CTCCTAGACAATAAAGGACAACCA

+5 kb
Forward GGCTGGAAACCACTGCCTTA
Reverse AGCCTTGCCTGTGCTTAAAGC

+17 kb
Forward GGCTGCCAAGGCTTGTCA
Reverse AAATCTGCCTGGCTTTTACACCTA

+58 kb
Forward GGAGGAGTTAGGGAATATGTCGATAG
Reverse GCAGTTCTCCAGGTTGAGTCAGA

+65 kb
Forward GACGCAACTTCCTTATGATCACAA
Reverse CCTGCCTCACACTGACCAAA

+73 kb
Forward AACTGAAGCGAGTACAGCATTCC
Reverse TGCTTTTGCTTGTGTACTGTTAACTG

+76 kb
Forward AAGAGGCAAGGTCAGAGTAAGCTT
Reverse GCGCACAGTTAGTGCTTATGTCA

Hba-a1 HS-31
Forward TTCTGACCTCACCTCAGCTAAGC

Reverse TGTGTGGGCAGAGGACACA

Hba-a1 HS-12
Forward ACCCTGACTCAAAACAACAAAGTAA
Reverse GGTTTCTGAGTTTCCTTATCTGCAA

Klf1 promoter
Forward TCTGCTCAAGGAGGAACAGAGCTA
Reverse GGCTCCCTTTCAGGCATTATCAGA

Eraf promoter
Forward TGCCTGCGTCTCGCTTAGT
Reverse GCTGAGCCCGCCTCATC

Eraf transcribed region (TR)
Forward TTAAAGGGTCTGGGCATCATG
Reverse CAACATCTTGGGAGAACGGTC

Slc4a1 promoter
Forward CTGAGCAGTCAAGCCTTAGTTCAC
Reverse CCTGTCCAGTCCCTAAGGTCTTT

Zpfm1 intron 1
Forward TGCAAGTCCCATCCTGATAAGA
Reverse GCACGCCAGATAAGATCACAATT

Zpfm1 upstream
Forward GGCAGATGTTCACTGTGGCA
Reverse GGGAGGAGCCAGAGGTCA G

Lyl1 promoter
Forward TCAGCATTGCTTCTTATCAGCC
Reverse CGCAGAGGCCAGAGGATG

Gapdh promoter
Forward TCCCCTCCCCCTATCAGTTC
Reverse GACCCGCCTCATTTTTGAAA

Gapdh +2 kb
Forward GGGCAAGCAATCACCTCTTG
Reverse GGCCTGGCAGGGCTTTTA

CD4 5′ transcribed region
Forward CCAGAACATTCCGGCACATT
Reverse GGTAAGAGGGACGTGTTCAACTTT

GPIb promoter
Forward TGGTGGCTAGTAGCTGCAAAGTC
Reverse TTATCAGCTCTCTGCACAGCATTC

PF4 promoter
Forward GCTGCTGGCCTGCACTTAAG
Reverse GCCACTGGACCCAAAGATAAAG

cMpl promoter
Forward CTGCCAACAGAAGGCTCATG
Reverse CTGTCAGATACAGCCCCACGT

IIb promoter
Forward GCCATGAGCTCCAGTCTGATAA
Reverse AGCTCTTTCCCTTTCCCTGAA

IIb −3 kb
Forward AAATAGATGTCAAGTTGGCATAAACCT
Reverse TGCCAGCGTTCAAGTACAAAA

RT-PCR Primers

Pabpc1
Forward AACACAGACAATGGGTCCAC
Reverse GGACTCCCGCAGCATATTTA

Gapdh
Forward GATGCCCCCATGTTTGTGAT
Reverse GGTCATGAGCCCTTCCACAAT

Hbb-b1
Forward AACGATGGCCTGAATCACTTG
Reverse AGCCTGAAGTTCTCAGGATCC

Eraf
Forward GCCATGACAGAATTCCAGCAA
Reverse TTTGGACTTCAGAAAGGTCCTGTAT

Slc4a1
Forward TGGAGGCCTGATCCGTGATA
Reverse AGCGCATCGGTGATGTCA

Kit
Forward AGCAGATCTCGGACAGCACC
Reverse TGCAGTTTGCCAAGTTGGAG

Brd3
Forward GGACTCAAACCCAGACGAGATT
Reverse TGTTGACAATGGTTTCCTCTGC

1. Blobel GA, Nakajima T, Eckner R, Montminy M, Orkin SH (1998) CREB-binding protein cooperates with transcription factor GATA-1 and is required for erythroid differentiation. *Proc Natl Acad Sci USA* 95:2061–2066.
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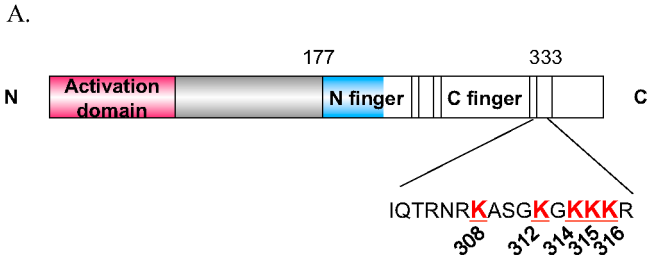
11. Cheng Y, et al. (2009) Erythroid GATA1 function revealed by genome-wide analysis of transcription factor occupancy, histone modifications, and mRNA expression. *Genome Res* 19:2172–2184.



B. Trypsin Digestion

Acetylated C-motif peptides	Acetylated residues
RKASGK@GK@K@KR	312, 314, 315
RK@ASGK@GKKK	308, 312
RK@ASGK@GK@KKR	308, 312, 314
RK@ASGK@GK@KK	308, 312, 314
RK@ASGK@GKK	308, 312
RK@ASGK@GK@K@K@RG	308, 312, 214, 315, 316
RK@ASGK@GK@K@KR	310, 312, 314, 315
KASGK@GK@K@KR	312, 314, 315
KASGK@GK@KK	312, 314

Fig. S1. Mass spectrometry (MS) of the zinc finger region of GATA1 acetylated by CBP in vitro. The zinc finger domain of GATA1 fused to GST [GST-f(GATA1)] was acetylated in vitro by purified recombinant acetyltransferase domain of CBP. MS analysis of trypsin digested products produced the listed acetylated GATA1 peptides. Acetylated lysine residues within the cluster are highlighted in red. Di-, tri-, tetra-, and penta-acetyl peptides are identified.

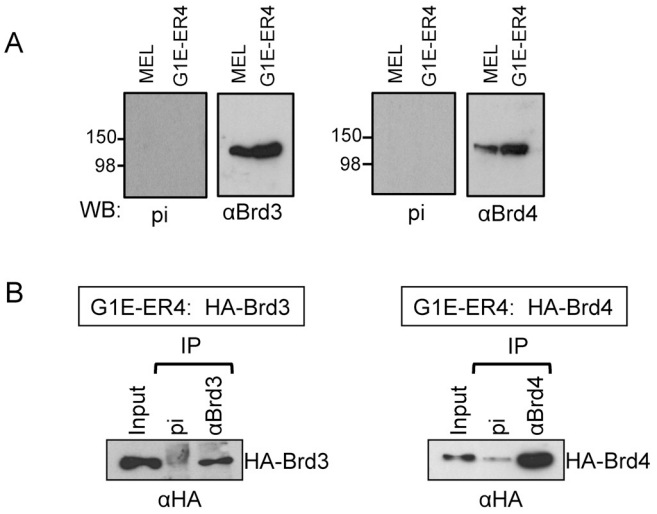


Fig. S2. Characterization of Brd3 and Brd4 antibodies. (*A*) Western blots of nuclear extracts from G1E-ER4 and MEL cells with Brd3 and Brd4 antibodies or preimmune (pi) sera. (*B*) Nuclear extracts from HA-Brd3 or HA-Brd4 expressing G1E-ER4 cells were immunoprecipitated with anti-Brd3, anti-Brd4, or preimmune sera. Bound material was analyzed by Western blot using HA antibodies.

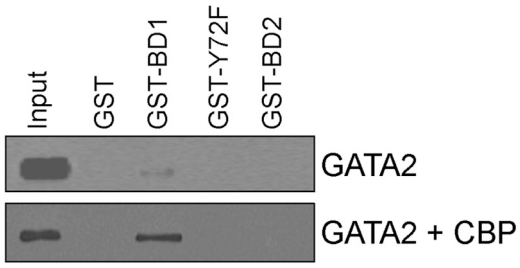


Fig. S3. GATA2 associates with BD1 in a CBP-dependent manner. GST-BD1, -BD2, and -Y72F were immobilized on glutathione sepharose and exposed to nuclear extracts from 293T cells transiently transfected with GATA2 alone or with CBP. Bound proteins were analyzed by Western blot with antibodies against GATA2. Input equals 2.5%.

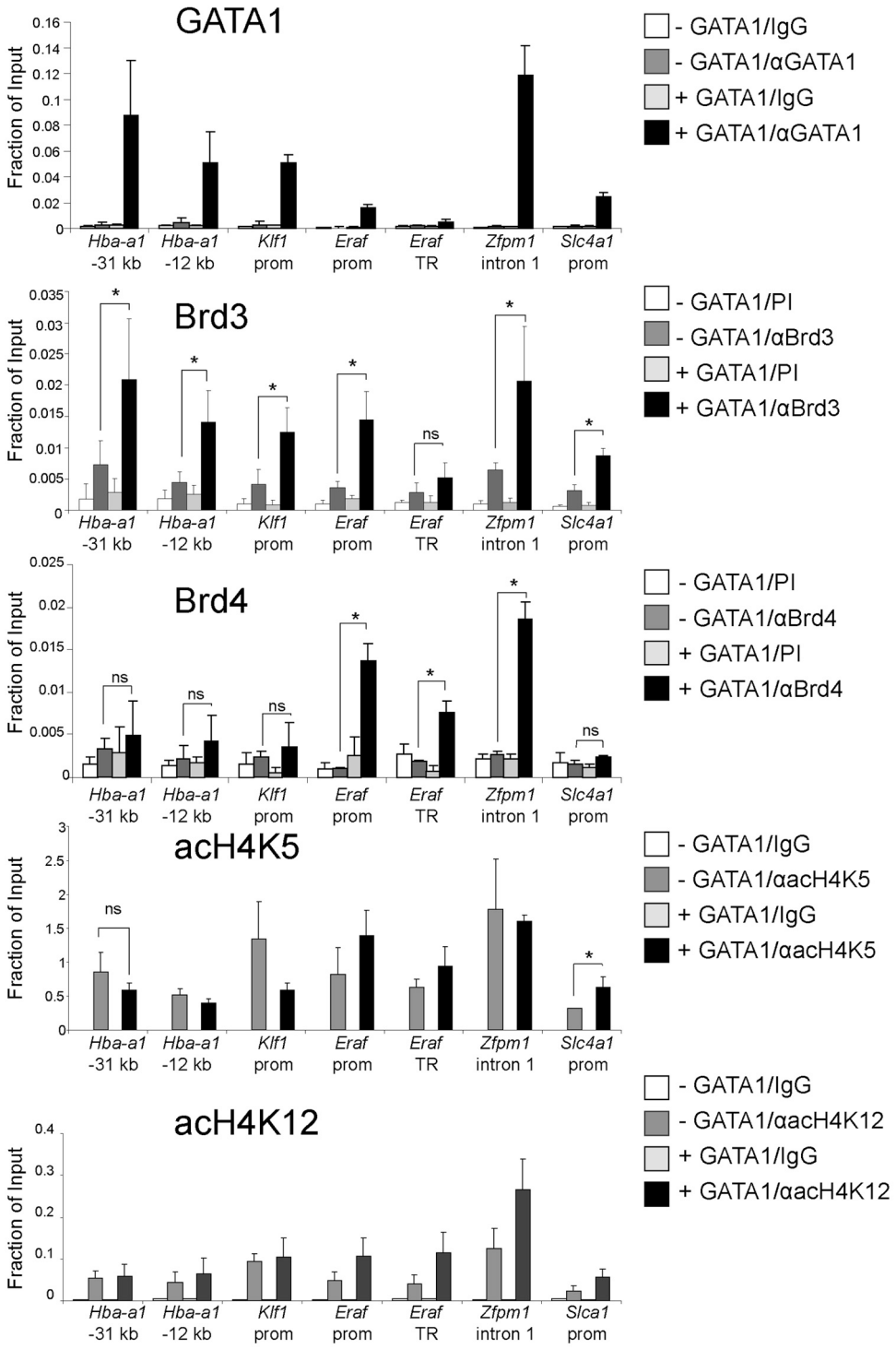


Fig. S4. Cooccupancy of Brd3 and GATA1 at active target genes. ChIP-qPCR with the indicated antibodies or control IgG in G1E (+GATA1) and estradiol-treated G1E-ER4 (+GATA1) using primers specific for indicated loci. The data shown are the averages of three to six experiments. Asterisks indicate $p < 0.05$. ns, not significant. Error bars represent standard deviation.

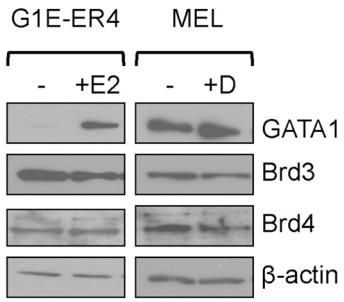


Fig. S5. Brd3 and Brd4 protein levels are unchanged following erythroid maturation. Anti-Brd3, -Brd4, and -GATA1 Western blots of nuclear extracts from G1E-ER4 and MEL cells before and after estradiol (+E2) and DMSO (+D) treatment, respectively. β-actin served as loading control.

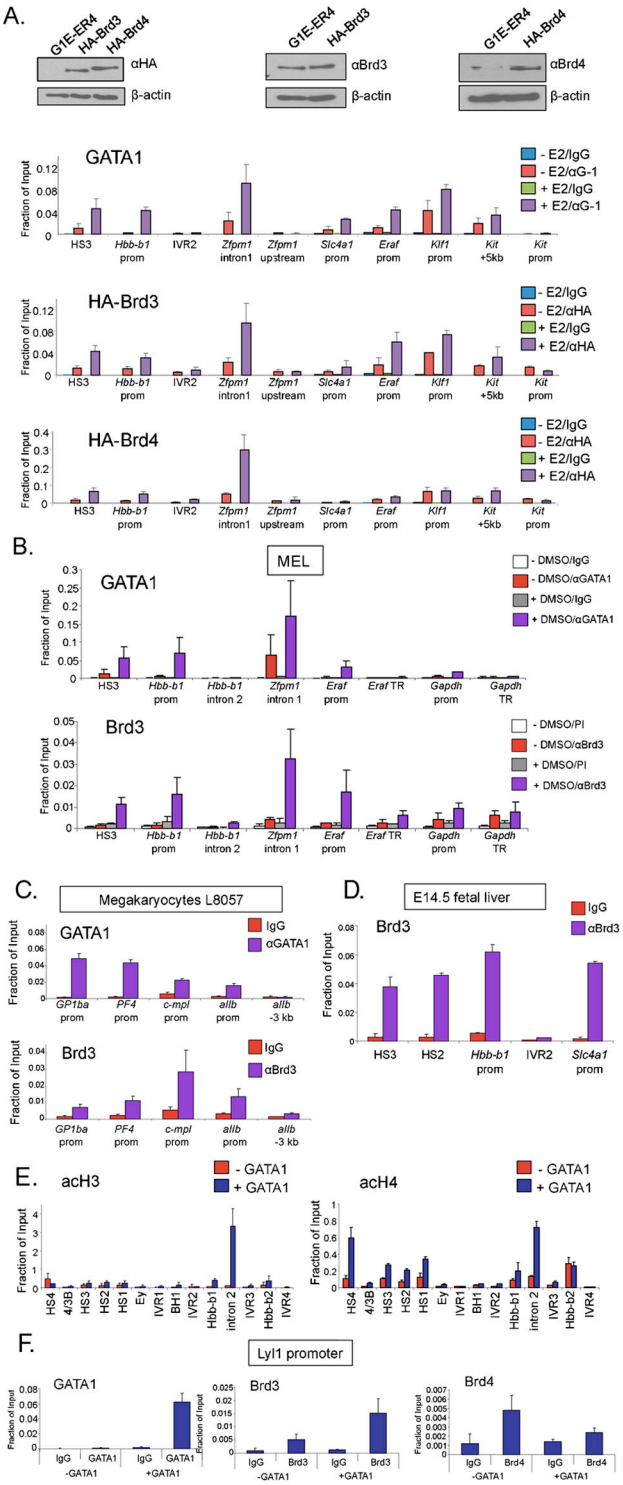


Fig. S6. Brd3 occupancy correlates with GATA1 and not histone acetylation in numerous hematopoietic cell lines and primary fetal livers. (*A*) Anti-HA ChIP assays before and after estradiol treatment revealed that the overall distribution of HA-Brd3 is similar to that of endogenous Brd3, correlating well with that of GATA1. Of note, Brd4 overexpression led to occupancy at sites not bound by endogenous Brd4 (e.g., HS3, Kit +5). This highlights the potential for artifacts from overexpression of chromatin binding proteins. ChIP-qPCR was performed using GATA1 and Brd3 antibodies or isotype matched controls in MEL cells before and after DMSO-induced differentiation (*B*), megakaryocytic L8057 cells (*C*), and E14.5 fetal liver erythroblasts (*D*) ChIP-qPCR across the *Hbb-b1* locus (*E*) was performed in G1E (+GATA1) and E2-treated G1E-ER4 (+GATA1) cells using antibodies recognizing di-acH3 (K9/14) and tetra-acH4 (K5/8/12/16). (*F*) As in *E* at the repressed Lyl1 gene using antibodies specific for GATA1, Brd3, or Brd4. Results are the averages of two to four independent experiments. Error bars represent standard deviation.

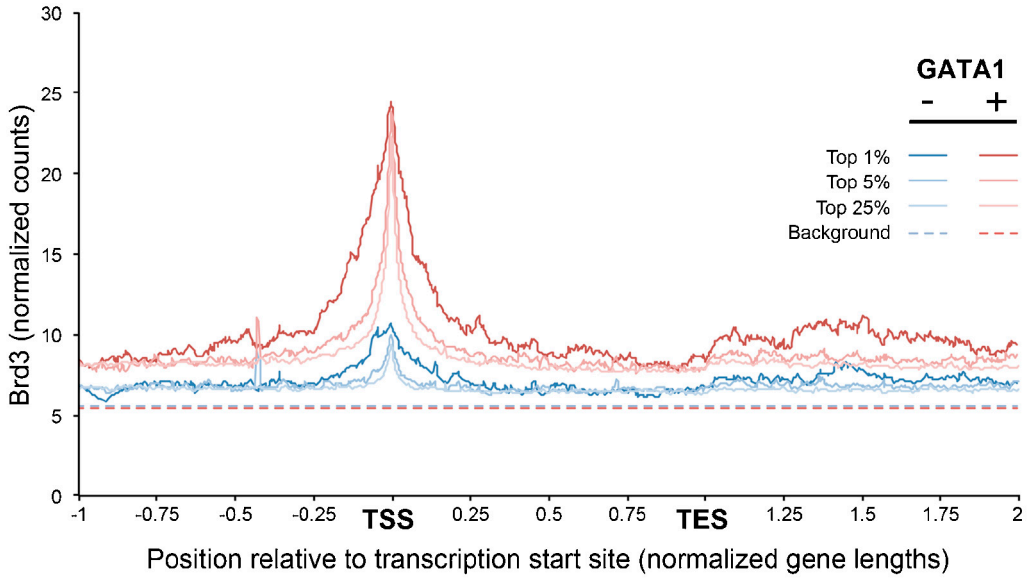


Fig. S7. Composite profiles of mean normalized Brd3 binding signals of top 1%, 5%, or 25% transcribed genes in cells with or without active GATA1. The plots extend one full gene length 5′ and 3′ of the transcribed regions. TSS, transcription start site; TES, transcription end site; Background, mean Brd3 signal genome-wide.

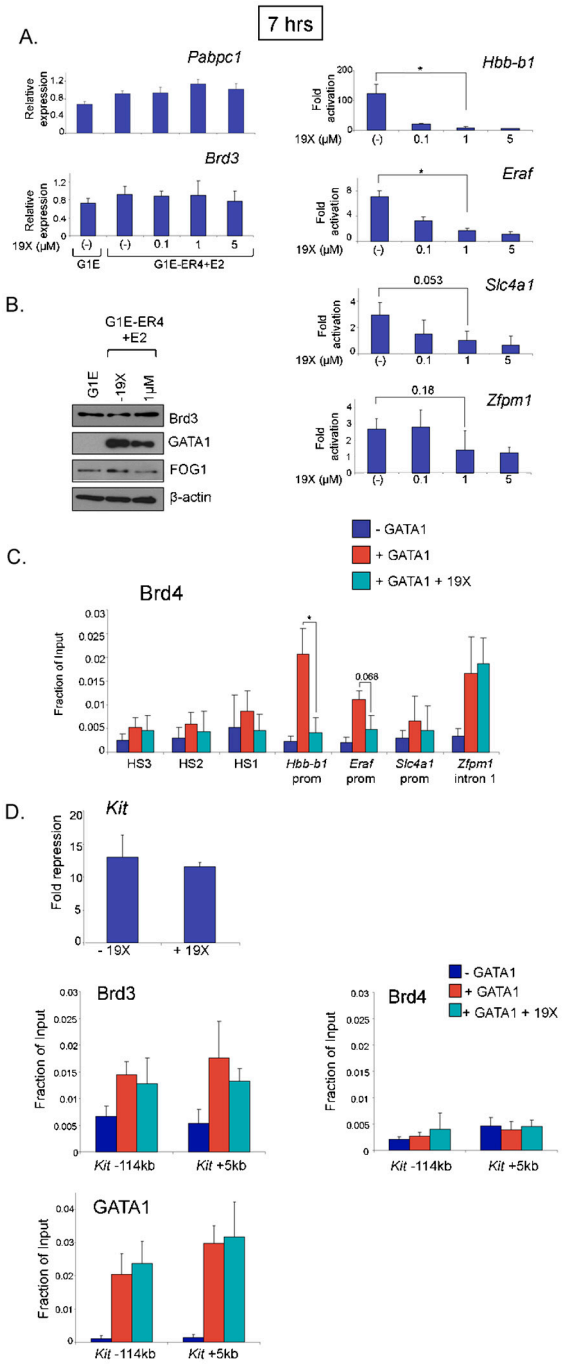


Fig. S8. (*A*) 19X blocks erythroid maturation without affecting GATA1 protein levels. qRT-PCR measuring mRNA levels of GATA1 target genes (*Right*) and nontarget genes (*Left*) in G1E or estradiol (E2)-treated G1E-ER4 cells grown in the presence of varying concentrations of 19X for 7 h. (*B*) Western blots showing protein levels of GATA1, Brd3, and FOG1 in G1E and estradiol-treated G1E-ER4 cells before and after treatment with 1 μM 19X for 24 h. β-actin served as loading control. (*C*) 19X displaces Brd4 from several GATA1 targets. Brd4 ChIP in G1E and estradiol-treated G1E-ER4 cells grown in the presence or absence of 1 μM 19X compound for 24 h. (*D*) *Kit* repression by GATA1 is unaffected by 19X. (*D*, *Top*) qRT-PCR using primers for *Kit* show the same level of repression in the presence or absence of 19X. (*Middle* and *Bottom*) Anti-Brd3, -Brd4, or -GATA1 CHIP at the *Kit* locus of G1E and estradiol-treated G1E-ER4 grown in the presence or absence of 1 μM 19X for 24 h. Data are the average of five independent experiments. Asterisks indicate $p < 0.05$. Error bars denote standard deviation.