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Supplemental Data

Insights into GATA-1-Mediated Gene Activation versus Repression via Genome-wide Chromatin Occupancy Analysis

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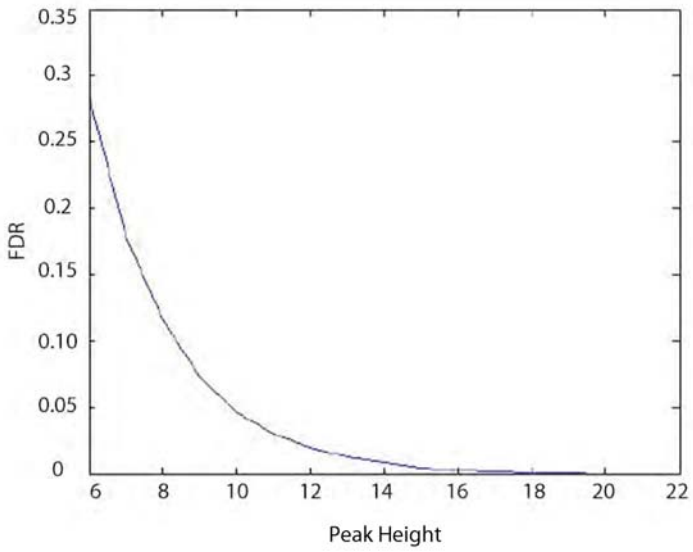

Figure S1. Relationship between peak height and false discovery rate (FDR). The number of sequence reads for a given peak call is plotted on the x-axis.

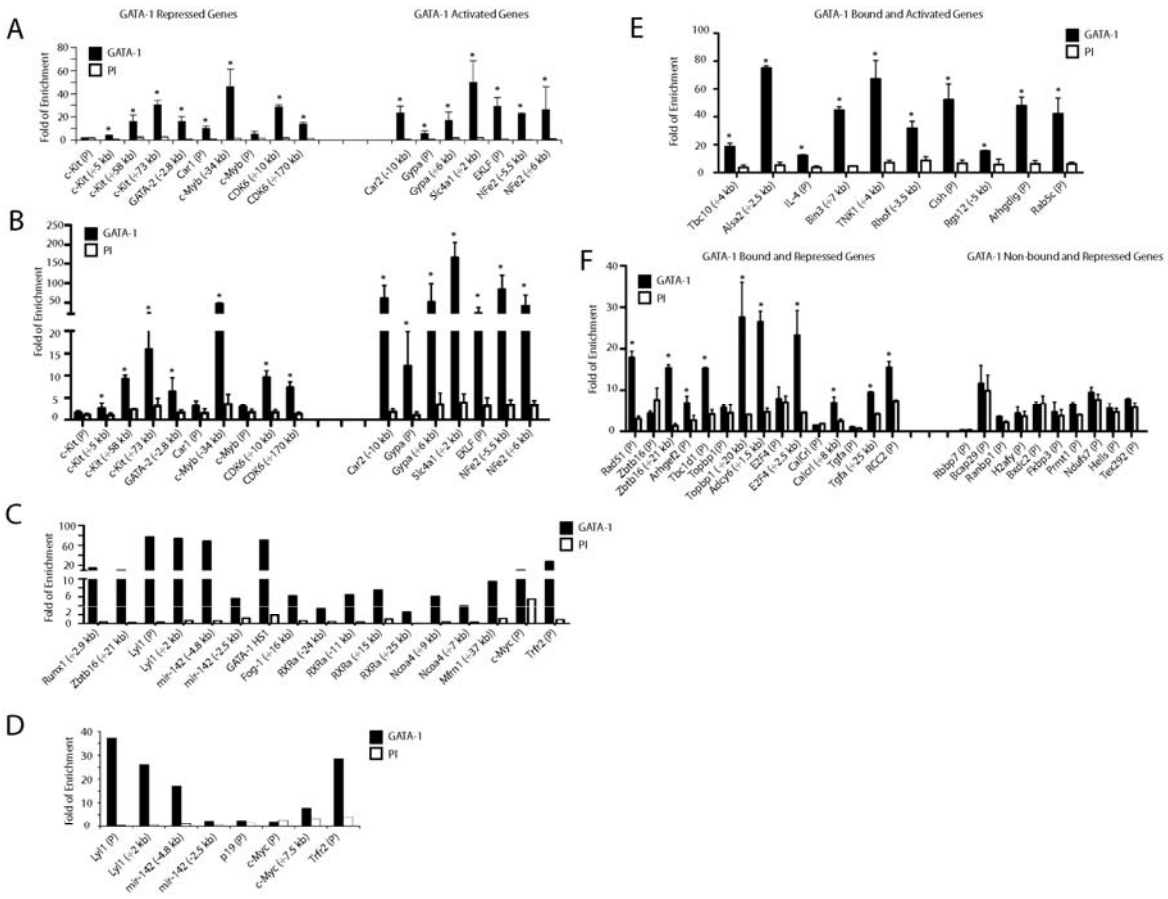

Fig. S2. Validation of GATA-1 enrichment peaks in induced MEL cells (A and C) or unsorted e13.5 murine fetal liver cells (B, D, E, and F). Fold of enrichment is expressed relative to a negative control region located 2 kb 5' to the *GATA-1* HS1 enhancer. For panels A, B, E and F, the mean of 3 independent ChIP assays is shown -/+ SEM. Enrichment differences between specific and control antibodies ≥ 2-fold and with p-values ≤ 0.05 (Student's t-test) are indicated with an asterisk. For panels C and D, the results from a single ChIP assay are shown. The promoters of c-Kit, c-Myb, Zbtb16, Topbp1, E2F4, Calcrl, and Tgfa were negative in the ChIP-seq data set, but were included here because of GATA-1 binding to other regulatory elements of these genes.

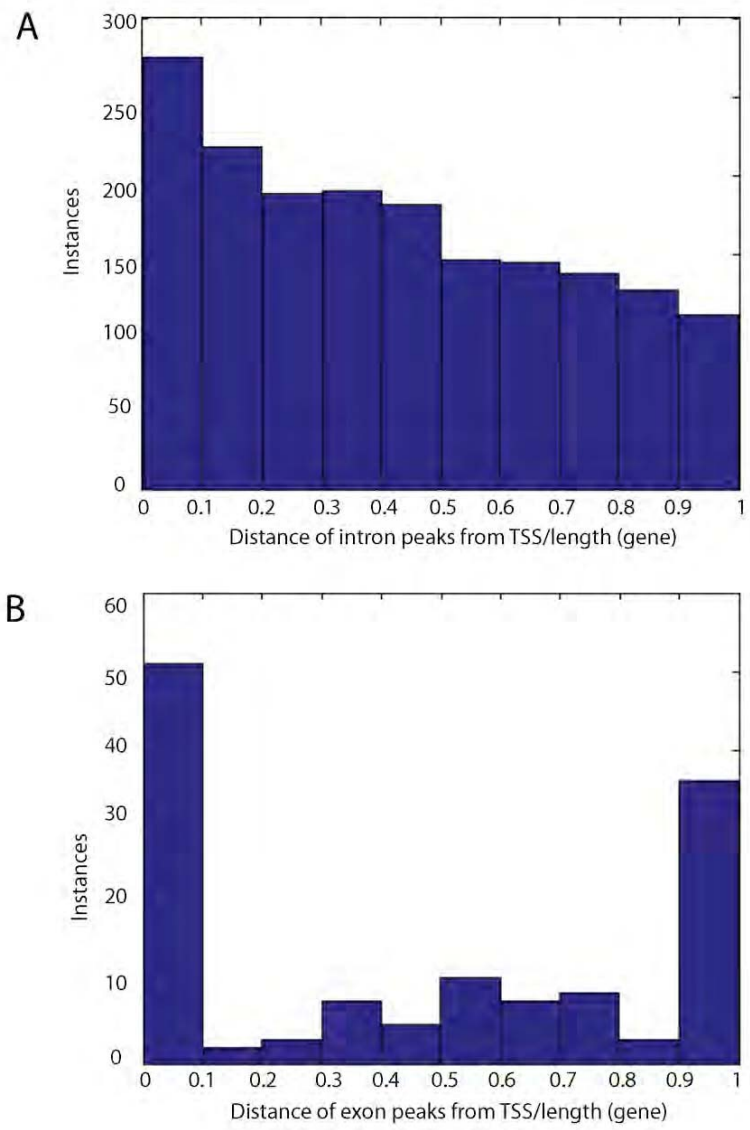

Fig. S3. Location of GATA-1 enrichment peaks relative to gene structure.

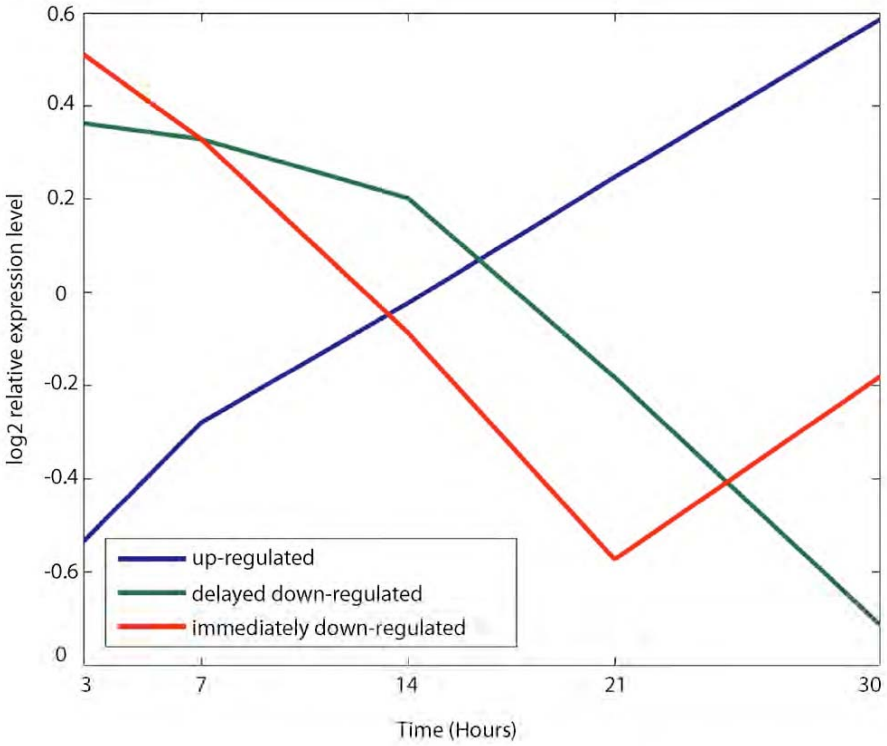

Fig. S4. Expression patterns of differentially expressed gene clusters. Three cluster of gene expression patterns are shown based on cDNA microarray analysis of induced G1ER cells, (A) up regulated genes, (B) delayed repressed genes, and (C) immediately repressed genes.

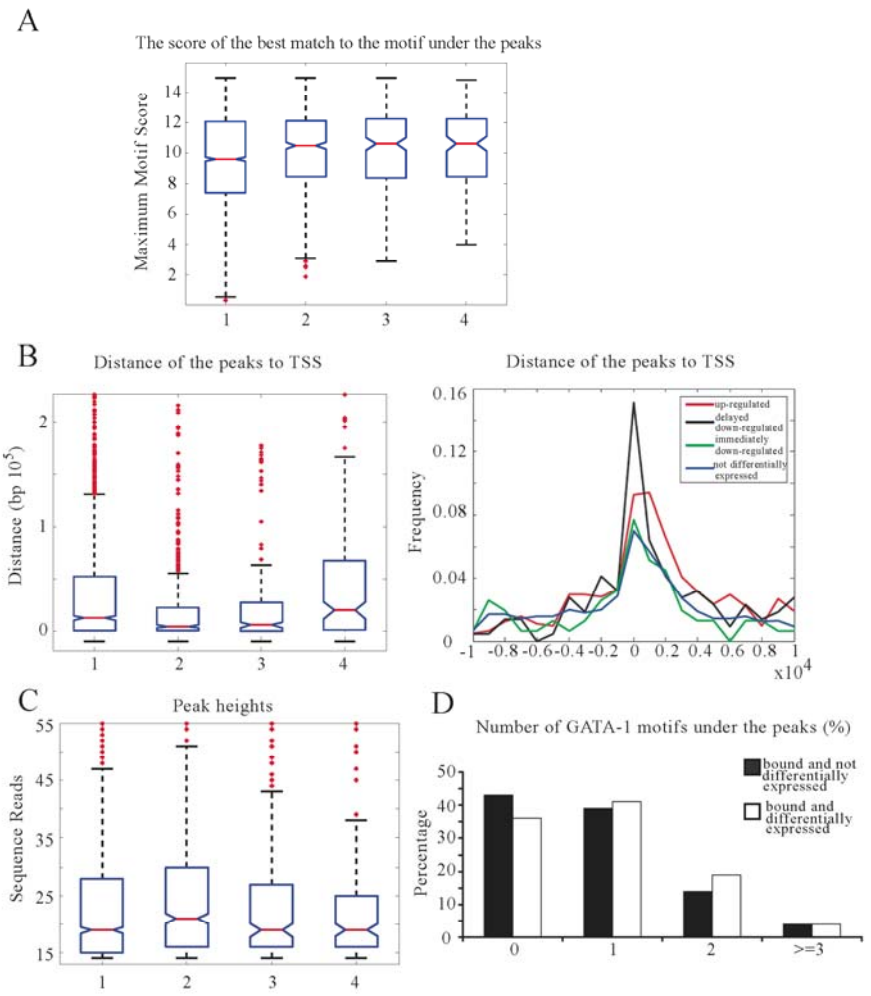

Fig. S5. Scale of the differences for four characteristics of the peaks between genes that are bound, but not differentially expressed and genes that are bound and differentially expressed: (A) the score of the best match to the motif under the peaks; (B) the distances of the peaks to the TSS; (C) the peak height; and (D) the number of GATA-1 motifs under the peaks. These four peak characteristics are evaluated for bound and not differentially expressed genes, bound and up regulated genes, bound and delayed down regulated genes, and bound and immediately down regulated genes. For panels A, B and C, the scale of the differences is represented as a box plot for each class of genes under investigation. The boxes have lines at the lower quartile, median, and upper quartile values. The values on the x-axis represent: (1) bound and not differentially expressed genes; (2) bound and up regulated genes; (3) bound and delayed down regulated genes, and (4) the bound and immediately down regulated genes.

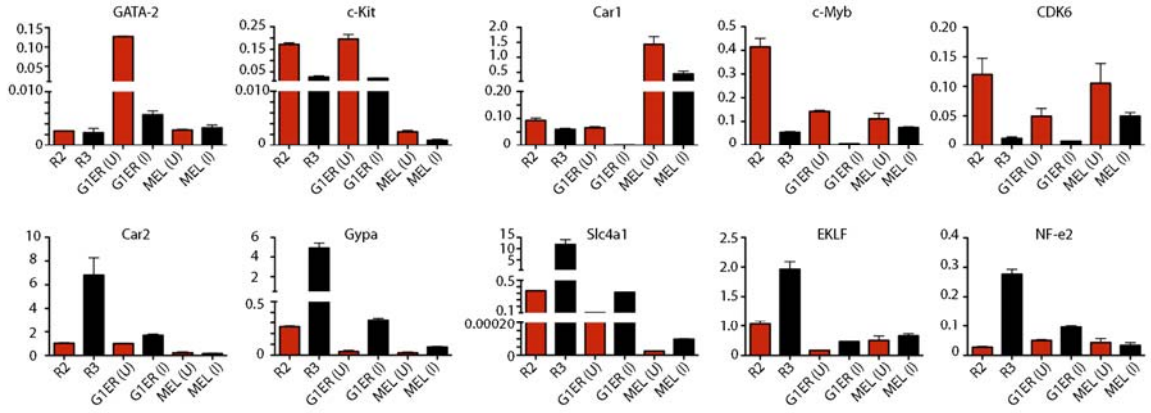

Fig. S6. Expression profiles of the five GATA-1 bound and repressed genes and the five GATA-1 bound and activated genes in murine fetal-liver derived e14.5 primary R2 and R3 erythroid cells, un-induced (U) and induced (I) G1ER and MEL cells. Data of earlier developmental stage of primary cells and un-induced cell lines are presented as red, and data of later developmental stage of primary cells and induced cell lines are presented as black. Numbers on Y-axis indicate mRNA transcript levels for the different genes relative to GAPDH mRNA transcript levels.

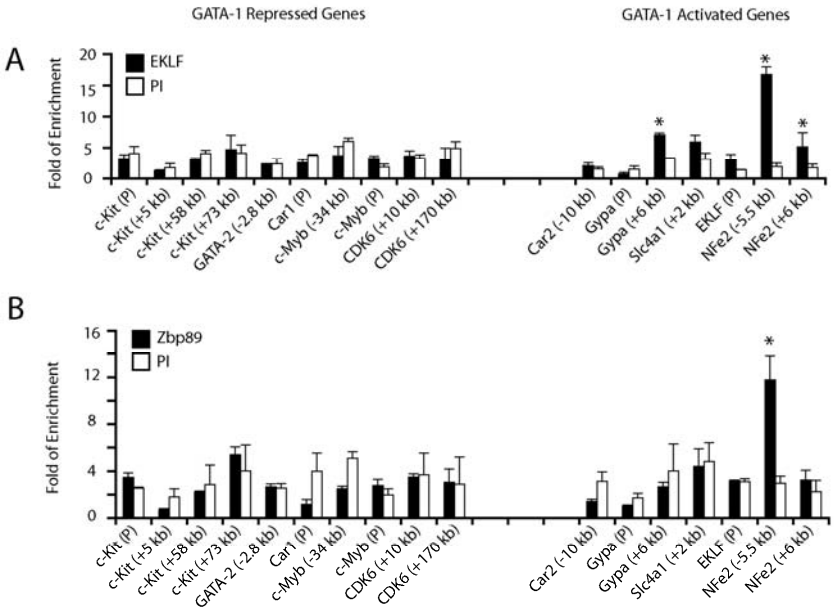

Fig. S7. Chromatin occupancy by EKLF and ZBP-89 at GATA-1 bound sites of selected repressed and activated genes in CD71$^{+/low}$ Ter119^{+} primary fetal liver erythroid cells. Fold of enrichment is expressed relative to a negative control region located 2 kb 5' to the *GATA-1* HS1 enhancer. The mean of 3 independent ChIP assays is shown -/+ SEM. Enrichment differences between specific and control antibodies ≥ 2-fold and with p-values ≤ 0.05 (Student's t-test) are indicated with an asterisk.

Supplemental Tables

Supplemental Tables S1, S2, S3, S4, S5, S7, S8 are included as separate Excel files.

Table S6. Comparison of ChIP-seq enrichment peak calls to previously validated GATA-1 occupancy sites within a 66 Mb region of mouse Chromosome 7 in G1-ER4 cells induced with 10^{-7} mol/L beta-estradiol for 24 hrs (Cheng et al., 2008).

Threshold	Total number of enrichment peaks	FDR	Number of enrichment peaks in 66 Mb region of chromosome 7 studied by Cheng et al.	Overlap with 63 previously validated peaks within this region[a].	% Overlap	Number of False Negative Calls (i.e., no overlap with 63 validated peaks)	Overlap with 59 original peaks not able to be validated in Cheng et al.[b]
14	4199	0.01	83	21	33	42	0
12	5533	0.02	108	25	40	38	0
10	7959	0.05	148	26	41	37	0
8	12837	0.12	246	30	48	33	1

[a] Enrichment peaks detected by ChIP-chip and validated by independent ChIP assay (Cheng et al., 2008).
[b] Enrichment peaks on chromosome 7 interval detected by ChIP-chip, but not able to be subsequently validated (Cheng et al., 2008).

Supplemental Experimental Procedures

Antibodies and Reagents

GATA-1 (sc-265), GATA-1 (sc-1234), Fog-1 (sc-9362), HEB (sc-357), E47 (sc-349), EKLF (sc-17194), Zbtb7a (sc-33683), Pol II (sc-899), Gfi-1b (sc-8559), Suz12 (sc-46264), normal rabbit IgG (sc-2027), normal mouse IgG (sc-2025), normal rat IgG (sc-2026), normal goat IgG (sc-2028), and normal Armenian hamster IgG (sc-3886) were purchased from Santa Cruz Biotechnology. Suz12 antibody (3737S) and Ezh2 antibody (3147) were purchased from Cell Signaling Technology, H3K4me3 antibody (ab8580) was purchased from Abcam, and H3K27me3 antibody (07-449) was purchased from Upstate. The SCL antibody (258C) was kindly provided by Dr. Richard Baer (Columbia University), Mi-2β antibody was kindly provided by Dr. Stephen T. Smale (UCLA), and additional Zbtb7a antibody was kindly provided by Dr. Pier Paolo Pandolfi (Harvard Medical School).

GATA-1 antibody sc-265 was used for both co-IP and ChIP. GATA-1 antibody sc-1234 and Ezh2 antibody 3147 were used for Western blot only. Suz12 antibody 3737S was used for co-IP only. Suz12 antibody sc-46264, Fog-1 antibody sc-9362, and Gfi-1b antibody sc-8559 were used for both ChIP and Western blot. The rest of these antibodies were used for ChIP only. All chemicals were purchased from Sigma unless stated otherwise.

Preparation of ChIP-seq DNA library

Libraries were prepared from 1-10 ng of ChIP DNA according to Illumina's instructions accompanying the DNA Sample Kit (Part# 0801-0303) with some modifications.

Briefly, DNA was end-repaired with a combination of T4 DNA polymerase, *E. coli* DNA Pol I large fragment (Klenow polymerase) and T4 polynucleotide kinase using End-It DNA End-Repair Kit (Epicenter Cat. No. ER0720). The blunt, phosphorylated ends were treated with Klenow fragment (32 to 52 exo minus) and dATP to yield a protruding 3- 'A' base for ligation of Illumina's adapters which have a single 'T' base overhang at the 3' end. Products of ~275-750 bp (insert plus adaptor and PCR primer sequences) were band isolated from an agarose gel, purified using Gel Extraction kit (Qiagen) and PCR amplified with Illumina primers for 18 cycles. Amplified fragments were then purified using QIAquick PCR purification Kit (Qiagen). The purified DNA was captured on an Illumina flow cell for cluster generation. Libraries were sequenced on the Genome Analyzer following the manufacturer's protocols. Raw ChIP-Seq data were processed using the Illumina software pipeline. ChIP-Seq reads were aligned to the reference mouse genome (UCSC, mm8).

Flow Cytometric Analysis and Cell Sorting

For flow cytometric analysis, fetal liver single cell suspensions were stained with APC-conjugated anti-CD71 antibody (Pharmingen, cat. no. 341029) and Phycoerythrin (PE)-conjugated anti-Ter119 antibody (Pharmingen, cat. no. 55367) following standard procedures, and analyzed on a FACScalibur™ flow cytometry instrument (BD Biosciences).

For erythroid precursor cell isolation, Lin^- (excluding the Ter119 antibody) $CD71^+Ter119^+$ cells were sorted from e13.5 and e14.5 mouse fetal liver cells. Mouse lineage Panel (Cat. no. 559971), PE–conjugated anti-CD71 antibody (Cat. no. 553267),

PE-Cy7 conjugated anti-Ter119 antibody (Cat. no. 5578530), and fluorescein isothiocyanate (FITC)–conjugated anti-CD71 antibody (Cat. no. 553266) were purchased from BD Pharmingen. Propidium iodide (PI) was added to exclude dead cells. For cell sorting from wild-type C57BL6 fetal liver cells, FITC-CD71 and PE-Ter119 antibodies were used; for cell sorting from EED knockout fetal liver cells, PE-CD71 and PE-Cy7-Ter119 antibodies were used. Standard flow cytometry staining procedures were performed. The cells were sorted using an Aria multicolor high-speed cell sorter (BD Biosciences).

Co-immunoprecipitation Assays

Nuclear extracts (NE) were prepared as previously described(Woo et al., 2008), diluted 2-3 fold by adding NE dilution buffer (31 mM Tris-HCl, pH 7.5, 1.6 mM EDTA, 0.16% NP-40 (v/v) protease inhibitor cocktail (1:100)). For co-IP using NE from MEL-^{bio}GATA-1 cells, Dynabeads M-280 Streptavidin were added to NE directly. For co-IP using antibodies, before adding the NE, antibodies were incubated with Dynabeads protein A or G for 3 hr, and crosslinked to beads by 25 mM DMA. 0.5 to 1 mg NE was used for each co-IP. After overnight incubation, beads were washed with IP washing buffer (equal volume of NE buffer C + NE dilution buffer) for 3 times. The protein complexes were eluted from beads by 50 mM glycine pH 2.8.

Quantitative RT-PCR

Total RNA were extract from primary erythoid cells, MEL and G1ER4 cells using RNeasy Plus Mini Kit (Qiagen), reverse transcribed using iScript cDNA Synthesis Kit

(BioRad), and analyzed by real-time PCR on a MyiQ real-time PCR instrument (BioRad). All measurements were made in duplicate (primary cells) or triplicate (G1-ER4 and MEL cells). Levels were calculated relative to GAPDH using the $2^{\Delta Ct}$ method. The primers used are listed in supplemental Table S8.

Supplemental References

Cheng, Y., King, D. C., Dore, L. C., Zhang, X., Zhou, Y., Zhang, Y., Dorman, C., Abebe, D., Kumar, S. A., Chiaromonte, F., *et al.* (2008). Transcriptional enhancement by GATA1-occupied DNA segments is strongly associated with evolutionary constraint on the binding site motif. Genome Res *18*, 1896-1905.

Woo, A. J., Moran, T. B., Schindler, Y., Choe, S. K., Langer, N. B., Sullivan, M. R., Fujiwara, Y., Paw, B. H., and Cantor, A. B. (2008). Identification of ZBP-89 as a Novel GATA-1 Associated Transcription Factor Involved in Megakaryocytic and Erythroid Development. Mol Cell Biol.