Supporting Information

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SI Materials and Methods

Cell Culture. To generate the HoxB4/AML-ETO progenitor cell line, mice were injected i.p. with 4 mg 5′-fluorouracil. At day 4 after treatment, bone marrow cells were harvested and cultured in SF medium Iscove's modified Dulbecco's medium (IMDM), L-glutamin, 25 mM Hepes, 2% FCS, 300 μg/mL Primatone RL (Sigma), 5 mg/mL insulin, 1× penicillin-streptomycin, 1× nonessential amino acids (NEAA) in minimum essential medium (MEM) containing 20 ng/mL stem cell factor (SCF), 2% IL6, and 1% IL3 final (supernatant). Cells were cultured for 48 h and thereafter infected with retroviral HoxB4 and AML1-ETO9a. Erythroid differentiation was induced by administration of 3 units/mL erythropoietin in SF the abbreviation SF was introduced above medium. Mouse erythroid leukemia (MEL) cells were cultivated in DMEM supplemented with 10% FCS, 1× penicillin-streptomycin (Invitrogen), and 10 μL/500 mL monothioglycerol (Sigma). MEL cell differentiation was induced by adding DMSO to a final concentration of 2%.

Methylcellulose Colony Assays. To investigate colony-forming unit erythrocyte (CFU-E) and burst-forming unit erythrocyte (BFU-E) capacity, Lin^- (CD4, CD8, CD11b, CD19, CD45R, Gr-1) $c\text{-}Kit^+$ bone marrow cells were plated at a density of 1×10^4/500 μL methylcellulose per 12-well plate (M3334; Stem Cell Technologies). CFU-E assays were supplemented with 2 units erythropoietin (Epo)/mL and incubated over 2 d at 37 °C with 5% CO_2. BFU assays were performed by adding 2 units Epo/mL and 1% IL3 (supernatant) over 8 d at 37 °C with 5% CO_2. The cultures were stained with benzidine (12% acetic acid containing 0.4% benzidine and 0.3% H_2O_2) to score the number of erythroid colonies.

Chromatin Immunoprecipitation Assays. Antibodies used were as follows: GATA-1, N6 (Santa Cruz Biotechnology); TRAP220/Med1, M-255 (Santa Cruz Biotechnology); Med26/CRSP70, H-228 (Santa Cruz Biotechnology); Pol II, N20 (Santa Cruz Biotechnology); TFIIB, C-18 (Santa Cruz Biotechnology); and acetylation-histone3, acH3K9 (Abcam). Precipitated DNA fragments were detected by real-time PCR using the Roche probe library and the following primers (5′-3′) for the β-globin promoter: (forward: CTGAGACTCCTAAGCCAGTG; reverse: GGAATCAGGCTTCGGTGAT; probe #89) and for hypersensitive site 2 of the LCR (forward: AAC ATT TCC TGA ATG ACT GTT AAG C; reverse: ATC ATA GAT GGA AGC TGG AAA AA; probe #110). All primers and probes were used at a concentration of 100 nM, and values were normalized to values of input DNA.

Quantitative Real-Time PCR. RNA purification and reverse transcription into cDNA were performed using the RNAqueous Microkit (Ambion) and the SuperScript II kit (Invitrogen), respectively. The 7500 Fast Real-time PCR System of Applied Biosystems was used to quantify mRNA/cDNA levels using the Roche probe library. Primers and probes were used at and 100 nM, and values were normalized to expression levels of *TATA binding protein* (*TBP*). Primers (5′-3′) and probes used for real-time PCR were the following: GATA-1 F, TCC CAG TCC TTT CTT CTC TCC; GATA-1 R, CAC ACA CTC TCT GGC CTC AC; probe #66; β-major globin F, TGT GCT GAG GAC TTG GTT CA; β-major globin R, TCC TTC CAC GCT TTT GAA TTA; probe #27; EKLF F, CAA GAG CTC GCA CCT CAA G; EKLF R, GAG CGA ACC TCC AGT CAC A; probe #68; NF-E2 F, TCC TCA GCA GAA CAG GAA CA; NF-E2 R, TGT CTC ACT TGG AAC ATT TAG ACC; probe #27; c-myb F, CAA CAG AGA ACG AGC TGA; c-myb R, GCT GCA AGT GTG GTT CTG TG; probe #40; GATA-2 F, CAC CCC TAA GCA GAG AAG CA; GATA-2 R, TGG CAC CAC AGT TGA CAC A; probe #15; c-Kit F, GAT CTG CTC TGC GTC CTG TT; c-Kit R, CTT GCA GAT GGC TGA GAC G; probe #15; Med1 F, GGA CCT TTC TAA AAT GGC TAT TAT GT; Med1 R, CGG GGT GAG ATA ACC AAC AC; probe #97; TBP F, GGG GAG CTG TGA TGT GAA GT; and TBP R, CCA GGA AAT AAT TCT GGC TCA T; probe #97.

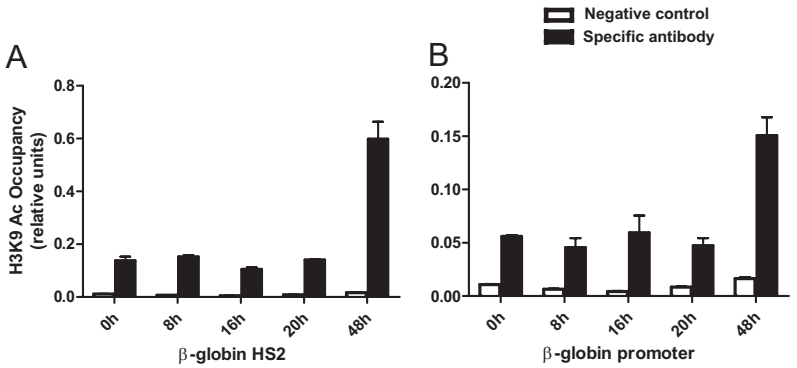

Fig. S1. Chromatin immunoprecipitation analysis of the active histone mark H3K9ac at the β-globin locus in differentiating MEL cells. Acetylated H3K9 is already present at both the β-globin locus control region (HS2) (*A*) and the β-major promoter (*B*) before induction of MEL cells by DMSO. The *y* axis represents the absolute enrichment (mean ± SD, two independent experiments) normalized to input. Background is defined as the unspecific binding to an unrelated control sequence (−106 kb upstream of GATA-1 locus).

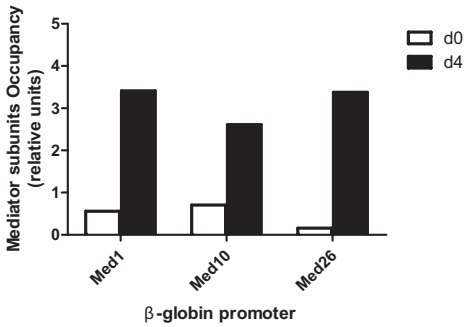

Fig. S2. Mediator subunits Med1, Med10, and Med26 are recruited to the β-major promoter in differentiating MEL cells. Chromatin immunoprecipitation was performed with HA or Med26 antibody at the β-major promoter in HA-Med1–transduced, HA-Med10–transduced, or wild-type MEL cells noninduced or 96 h after DMSO induction. The *y* axis represents the relative enrichment normalized to the negative control.

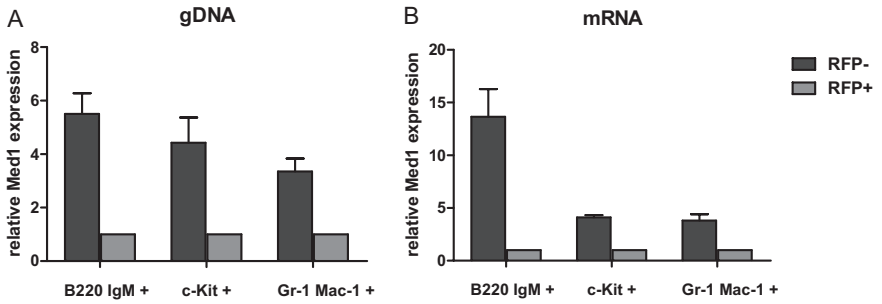

Fig. S3. Med1 is efficiently deleted in lymphoid, myeloid, and progenitor cells in Mx-Cre–mediated Med1 conditional knockout mice. Lymphoid ($B220^+/IgM^+$ splenic B cells), myeloid ($Gr\text{-}1^+/Mac\text{-}1^+$ bone marrow cells), and progenitor cells ($lin\text{-}/c\text{-}Kit^+$ bone marrow cells) were sorted by FACS out of RFP– and RFP+ populations, and Med1 deletion was verified at both the genomic level (*A*) and the transcription level (*B*) by RT-PCR.

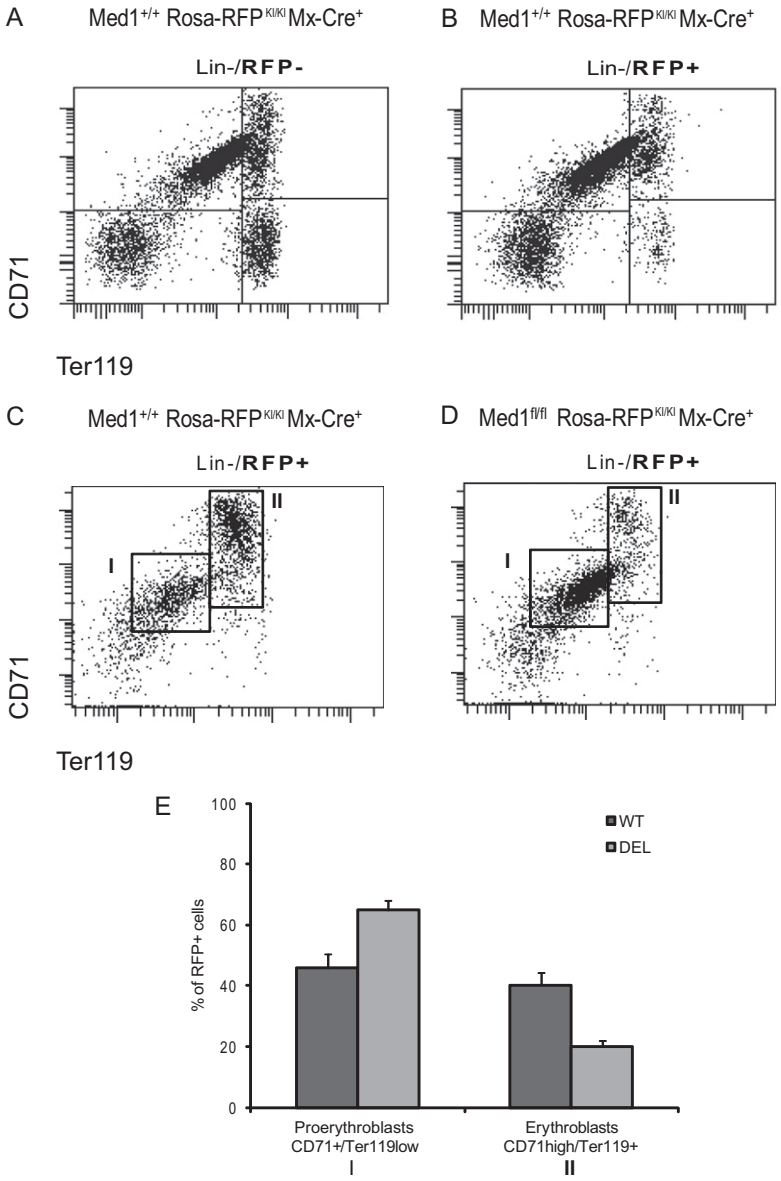

Fig. S4. Med1-deficient mice have an increased proportion of proerythroblasts and reduced numbers of erythroblasts. (*A* and *B*) Mature erythrocytes (CD71^{-}/Ter119^{+}) but not early progenitor (CD71low/Ter119-) and erythroblast (CD71^{+}/Ter119low, CD71 high/Ter119^{+}) stages lack expression of the red fluorescent protein (RFP) reporter gene. (*C* and *D*) Flow cytometry analysis of erythroid subsets, using the markers CD71 and Ter119, revealed that Med1 deficiency leads to a partial block in erythroid maturation reflected by an increased proerythroblast (I) to erythroblast (II) ratio (3.6:1) compared with wild-type littermates (1.2:1). (*E*) Quantification of the flow cytometry data comparing Med1 wild-type and Med1-deficient erythroid cell populations, displayed as the percentage of RFP^{+} cells.

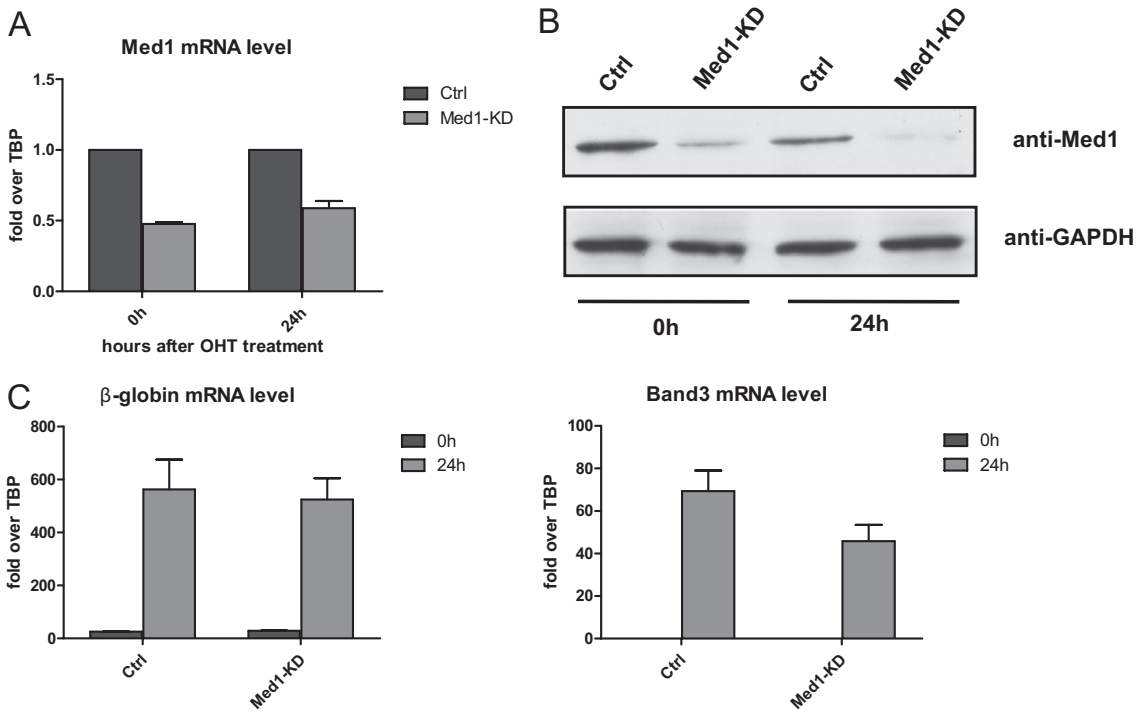

Fig. S5. Med1 knockdown in the G1E-ER-GATA1 system has no effects on β-globin and Band3 induction. (*A* and *B*) Med1 knockdown efficiency was checked by RT-PCR (*A*) and Western blot (*B*) in the wild-type (Ctrl) and Med1-shRNA–transduced (Med1-KD) G1E-ER-GATA1 cell line untreated and 24 h after tamoxifen treatment. (*C*) Expression of β-globin and Band3 was determined by RT-PCR.