Methylation of a CGATA element inhibits binding and regulation by GATA-1

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Alterations in DNA methylation occur during development, but the mechanisms by which they influence gene expression remain uncertain. There are few examples where modification of a single CpG dinucleotide directly affects transcription factor binding and regulation of a target gene in vivo. Here, we show that the erythroid transcription factor GATA-1 — that typically binds T/AGATA sites — can also recognise CGATA elements, but only if the CpG dinucleotide is unmethylated. We focus on a single CGATA site in the c-Kit gene which progressively becomes unmethylated during haematopoiesis. We observe that methylation attenuates GATA-1 binding and gene regulation in cell lines. In mice, converting the CGATA element to a TGATA site that cannot be methylated leads to accumulation of megakaryocyte-erythroid progenitors. Thus, the CpG dinucleotide is essential for normal erythropoiesis and this study illustrates how a single methylated CpG can directly affect transcription factor binding and cellular regulation.

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DNA methylation is an important epigenetic modification that is essential to mammalian development. Coordinated changes in DNA methylation have been documented during cellular differentiation, for example, in hematopoiesis, where many gene promoters undergo demethylation as early progenitors differentiate into granulocyte-macrophage progenitors, and genome-wide demethylation occurs during the terminal stages of erythropoiesis. However, the mechanisms by which the DNA methylation influences gene expression and differentiation are still not fully understood.

There are numerous examples where DNA methylation at multiple CpGs, for example in CpG islands in promoters and enhancers, is accompanied by a loss of transcription factor binding. For example, NFR1 is a DNA methylation-sensitive transcription factor whose binding is abrogated when a broad region encompassing its recognition motif is hypermethylated.

In this study, we explored how DNA methylation at a single CpG dinucleotide could interfere with binding and regulation by GATA-1, a critical transcription factor that modulates the expression of most if not all erythroid-specific genes. We show that methylation of a CGATA element reduces GATA-1 binding and gene regulation in cell lines. We extend these observations by showing that a single point mutation that converts the CGATA element to a TGATA site in a regulatory region of c-Kit—which can still be bound by GATA-1—but that is no longer sensitive to methylation—interferes with normal hematopoiesis in mice.

Results

GATA-1 binds to CGATA motifs and is blocked by methylation. The previously defined GATA-1 recognition motif contains either AGATA or TGATA within a A/TGATAA/G consensus sequence. However, in vitro GATA-1 can also bind CGATA elements. Using an electrophoretic mobility shift assay (EMSA) we confirmed that COS cell overexpressed and MEL cell endogenous GATA-1 is able to bind to CGATA, AGATA and TGATA, but less well to GGATA motifs, as previously reported. We then focussed on the CGATA element, since this motif contains a CpG element that may be subject to methylation. Chemically synthesised probes containing unmethylated, methylated or hemi-methylated CGATA motifs were tested in EMSAs. This revealed that both full methylation and hemi-methylation of the CGATA motif inhibits GATA-1 binding. Probe cold competition assays support the hypothesis that GATA-1 preferentially binds to unmethylated CGATA probes. In comparison with GATA-1, the related protein GATA-2 binds only weakly to probes containing CGATA motifs but this binding is also reduced by DNA methylation.

Methylation levels change at GATA-1 bound CGATA sites. We interrogated existing GATA-1 ChIP-seq data from G1E-ER4, murine erythroleukemia (MEL) cells and erythroblasts. This identified 38799 AGATA motifs, 24126 TGATA motifs and 2708 CGATA motifs that lay near the centre of GATA-1 ChIP-seq peaks reflecting in vivo binding (Supplementary Figure 3). Among all CGATA sites, there were 1139 CGATA sites which co-occurred in the same peak with (A/T)GATA motifs. We then integrated these data with genome-wide bisulphite sequencing information to identify individual CGATA sites where methylation levels changed during blood cell differentiation.

Among these genes, c-Kit was repressed by GATA-1 while Zfpml and Rhd were activated by GATA-1 (Supplementary Figure 5). The remaining genes, Rnf220, Abat, Ctdp1, Pbx1, Ulk4, Scrt1, Nicor2 and Camsap111, did not show evidence of being regulated by GATA-1 in this system (Supplementary Figure 5). We focussed our attention on a single CGATA element within an enhancer/silencer in intron 2 of the c-Kit gene, a gene that encodes an important cell surface receptor for the hematopoietic growth factor, stem cell factor.

c-Kit is a GATA-1 target gene with a regulatory CGATA site. c-Kit is broadly expressed in hematopoietic stem cells and progenitors, and its expression is downregulated as cell differentiation proceeds. High expression of c-Kit in hematopoietic stem cells and progenitors is essential for their self-renewal and proliferation, and the ultimate repression of c-Kit in the erythroid lineage is mediated in part via GATA-1. Importantly, we noted that the intron 2 CGATA element resides in a small region that in erythroid and related cells is not only notable for its strong GATA-1 ChIP-Seq peak, but is also accessible to ATAC sequencing and DNase-I mapping, and carries histone marks consistent with it being part of an functional distal regulatory element (e.g. an enhancer and/or silencer) (Fig. 2d).

We first compared the levels of methylation at this element, in purified murine haematopoietic stem cells and cells that had differentiated down the erythroid lineage, to assess whether methylation declined as expected. Haematopoietic stem cells (LSK; Lineage−, Sca1+, c-Kit+) and erythroblasts (ERY, Ter119+, CD71+) were collected through flow cytometry cell sorting and subjected to pyrosequencing (Fig. 2e). Consistent with previous genome-wide bisulphite sequencing data, the site is highly methylated in LSKs (~80%) but hypomethylated in the erythroid lineage (~10%) (Fig. 2e).

We next moved to cellular models to investigate whether GATA-1 could bind and regulate c-Kit expression from this element in living cells, and whether mutation of this motif, or alteration in its methylation status affected GATA-1 binding and c-Kit regulation. It should be noted that while GATA-1 can serve as an activator or repressor, it is known to repress c-Kit in maturing erythroid cells through multiple elements. Therefore, we anticipated that disrupting the CGATA motif in intron 2 might de-repress c-Kit in erythroid cell lines.

To test whether the CGATA motif in c-Kit intron 2 is regulatory, we utilised MEL cells in which the CGATA element is essentially unmethylated (Supplementary Figure 6) and in which we therefore expected to observe GATA-1 binding (Fig. 3a). ChIP assays confirmed that GATA-1 binds this site in MEL cells (Fig. 3d). We then used CRISPR-mediated editing in MEL cells to mutate the c-Kit +33 kb CGATA element to TTATA to disrupt the motif (Fig. 3b) and tested whether this prevented GATA-1 binding. As expected, this mutation disrupted GATA-1 binding without affecting GATA-1 binding to the positive control site or at other bound GATA sites within the c-Kit gene locus (Fig. 3d). We also tested the impact of this mutation on c-Kit expression and saw a clear de-repression (Fig. 3c), consistent with the view that GATA-1 can bind and repress c-Kit at least in part via binding the intron 2 CGATA element that is unmethylated and able to be bound in mature erythroid cells.

Increasing c-Kit CGATA site methylation alters regulation. To investigate if DNA methylation of the CGATA element could block GATA-1 binding and whether it influenced the functional repression of c-Kit by GATA-1, we employed another murine...
erythroid cell line, G1E-ER4. This line has become a useful model for functional studies as it expresses an estrogen inducible GATA-1-ER fusion. We tested for methylation of the CGATA element in G1E-ER4 cells and found the site was largely unmethylated. Reasoning that transient methylation might be being erased by demethylases of the Tet family, we checked the expression of Tet1, Tet2 and Tet3. We found that Tet2 was highly expressed and used CRISPR-mediated gene editing to knock out Tet2 in G1E-ER4 cells. This disruption was associated with ensuing methylation of the CGATA element. GATA-1 is inducible and leads to G1E-ER4 cell differentiation. Tet2 knock out did not influence G1E-ER4 cell survival or proliferation.

We tested the binding of GATA-1 to the CGATA element using ChIP assays in wildtype and Tet2 knockout lines and as expected found reduced GATA-1 binding to the CGATA +33 kb site in the c-Kit promoter in knockout cells where methylation levels are higher. Interestingly, GATA-1 binding was also reduced at intron 4 of the c-Kit gene, suggesting the that binding to this site might be also influenced by DNA methylation possibly indirectly via chromatin conformation or some other mechanism since this is not a CGATA element. Global hypermethylation did not affect the binding of GATA-1 at a positive control region or other GATA sites in c-Kit gene locus. This in vivo result is consistent with the in vitro EMSA assays (Fig. 1), suggesting that methylation at CGATA sites blocks GATA-1 binding. We then considered function. Importantly, the overall levels of c-Kit were comparable in the wildtype and the Tet2 knockout lines before

**Fig. 1 DNA methylation inhibits the binding of GATA-1 in vitro.** a GATA-1 canonical consensus motif from JASPAR. Electrophoretic mobility shift assays (EMSAs) assessing the ability of full-length mouse GATA-1 overexpressed in COS cells to bind to probes containing CGATA, AGATA, TGATA and CGATA motifs. b, unmethylated/methylated CGATA probes c or unmethylated/hemi-methylated CGATA probes d. Untransfected COS cells are shown as a control in each case. In a, c a GATA-1 antibody has been used to super-shift the GATA-1-probe complex. The gel shifts shown in b-d were repeated three times independently with similar results. Arrows indicate migration of super-shifted GATA1, GATA-1-DNA complexes and free probes respectively. Source data are provided as a Source data file.
GATA-1 induction with tamoxifen, suggesting that no major perturbation of the chromatin configuration at the locus had occurred (Fig. 4c). When GATA-1 is induced in the wildtype line, c-Kit expression diminishes, consistent with previous results. In Tet2 knockout cells, where the CGATA site is more heavily methylated, GATA-1-mediated repression was attenuated (Fig. 4c). In summary, our results confirm that methylation modulates GATA-1 binding in cell lines and suggest that c-Kit regulation is also attenuated in vivo.

Methylation at the c-Kit CGATA site is important in mice. To definitively determine whether this single CpG dinucleotide is required for c-Kit regulation during hematopoiesis in vivo, we tested the importance of this site using CRISPR gene editing in...
mice. Rather than simply disrupting the site, we introduced a C>T mutation, to convert the CGATA element to a TGATA site (Fig. 5a). We reasoned that this site would now be bound constitutively, rather than only after the onset of de-methylation during haematopoiesis. Bone marrow samples were collected from wild type and homozygous mutant mice (Supplementary Fig. 11a). The overt phenotype of the mutant mice and blood counts were not significantly different from wildtype (Supplementary Fig. 11b). We investigated the proportion and absolute number of bone marrow precursors and proportion of erythroid populations using flow cytometry. There was no change in the proportion of hematopoietic stem cells, multipotent progenitor populations or erythroid populations between wild type mice and mutant mice (Supplementary Fig. 12). However, we detected a clear accumulation of megakaryocyte-erythroid progenitors (MEPs) in mutant mice (Fig. 5b). In addition, in mutant mice, the overall expression level of c-Kit in MEPs was significantly reduced in comparison to that of wild type mice (Supplementary Fig. 13). This result is consistent with this single CpG dinucleotide playing a role in haematopoietic differentiation in vivo.

Discussion

In vivo GATA-1 has been implicated in binding primarily to AGATA or TGATA elements, and not to CGATA sites, but this may be a consequence of genomic ChIP-Seq analyses under-appreciating the importance of CGATA sites, due to the rarity of CpG dinucleotides in mammalian genomes. In addition, since CpG dinucleotides are often methylated the subset of CGATA elements bound by GATA-1 will be further limited. Nevertheless, the fact that CGATA elements can be subjected to methylation,
and that this influences binding of GATA-1, means that such elements may be instrumental in mediating the effects of alterations in methylation that have been observed to occur during haematopoietic differentiation.

Our work provides a clear illustration that at least one CpG dinucleotide within a CGATA site, that is progressively demethylated during haematopoiesis, is involved in c-Kit regulation in vivo. Methylation of the site inhibits GATA-1 binding in vitro and in cellular assays, and modestly impairs repression by GATA-1. A single point mutation that converts the CGATA element to a TGATA site—which can still be bound by GATA-1 but that can no longer provide a methylation sensitive binding site—interferes with normal haematopoiesis in mice (Fig. 6). These results suggest that DNA methylation at the binding site inhibits GATA-1 binding and show that a single residue’s ability to be methylated can influence gene regulation in vivo.

**Fig. 4 DNA methylation blocks the binding of GATA-1 to the c-Kit CGATA site and attenuates repression in G1E-ER4 cells.**

a) CRISPR/Cas9 was used to knock out Tet2 in G1E-ER4 cells. Western blot using an antibody to Tet2 identifies clones where Tet2 has been knocked out. A blot using an antibody to actin is shown as a loading control. Three Tet2 knock out clones and clonal wild-type controls were selected for further investigation. The experiment was repeated 3 times independently with similar results. b) Methylation level by pyrosequencing of the c-Kit Intron 2 CGATA site in three clonal G1E WT and G1E Tet2 knock out cells (n = 3 biologically independent cells), mean ± standard deviation. c) The expression level of c-Kit in G1E WT and G1E Tet2 KO cells by qPCR. Expressed relative to 18S expression, n = 3 biologically independent cells, mean ± standard deviation. Cells were induced with the addition of tamoxifen for 24 h to restore GATA-1-ER fusion proteins in cell nucleus (TAM). Cells incubated with ethanol were used as mock control (NC). d) GATA-1 ChIP-qPCR in wild type and G1E Tet2 cells after 24 h of GATA-1 induction by incubating with tamoxifen. KLF1 promoter region was used a positive control (+ve). An upstream region distal to the KLF3 gene was used as a negative control (−ve), the CGATA site at c-Kit intron 2 is the +33 site, n = 3 biologically independent cells, mean ± standard deviation, ChIP-qPCR was repeated two times independently with similar results. Source data are provided as a Source data file.
**Methods**

**EMSA.** EMSA were performed. The sense oligonucleotide was labelled with \(^{32}\)P triphosphate before annealing with the antisense strand via slow cooling from 100 °C to room temperature. Labelled duplex DNA oligonucleotides were purified with Quick Spin Columns (Sigma-Aldrich, #11273922001). In all, 5 μg pMT3-GATA-1 (full length) was transfected into COS-7 cells (gift from Stu Orkin, Harvard Medical School Boston, USA) for 48 h using Fugene6 (Promega, #E2691).

Nuclear extracts were harvested from COS-7 cells. Nuclear extracts were incubated with radiolabelled probes at 4 °C for 10 min. The protein-probe mixtures were run at 4 °C on a 6% native polyacrylamide gel in TBE buffer (45 mM Tris, 45 mM borate acid, 1 mM EDTA). COS empty lane was included as control to show any background binding to the probe. Super-shifting of GATA-1 was achieved using an anti-GATA-1 antibody (Santa Cruz technology, #sc-256, 1:500 dilution). Gels were run for 1 h and 30 min, at 250 volts. After running, gels were dried under

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**Fig. 5** Introduction of a single C > T mutation in the CGATA site in c-Kit intron 2 in a mouse model demonstrates that the CpG at this site plays a role in haematopoiesis. **a** A (C > T)GATA mutation was introduced at c-Kit intron 2 in C57BL/6J mice using CRISPR/Cas9. **b** Flow cytometry analysis of progenitor populations in mouse bone marrow from WT and homozygous (C > T)GATA mutant mouse littermates. CD16/32 low and CD34 negative cells are megakaryocyte-erythroid progenitors (MEPs). CD16/32 medium and CD34 positive cells are common-myeloid progenitors (CMPs). CD16/32 high and CD34 positive cells are granulocyte-monocyte progenitors (GMPs). All progenitors were derived from lineage negative, Scal negative, c-Kit positive (LSK) populations. **c**, **d** The percentage and absolute cell number of different populations in each mouse have been summarised in the histogram, n = 4 biologically independent animals, mean ± standard deviation. Mann–Whitney U, two-tailed statistical test, P-values shown. Measurements were taken from distinct samples (animals). Source data are provided as a Source data file.
Chromatin immunoprecipitation. Chromatin immunoprecipitation (ChIP) experiments were performed. In all, 7 × 10^9–1 × 10^10 cells were used for each ChIP. Cells were crosslinked with 1% formaldehyde for 10 min and the reaction was quenched by addition of 2.5 mM glycine. Fixed cells were sonicated at high voltage for 20 min (30 on, 30 seconds off) using Bioruptor® (Diagenode) to obtain 200–300 bp DNA fragments. The fragmented chromatin was pulled down at 4°C overnight using an antibody against GATA-1 (Santa Cruz Biotechnology, sc-256, 1:5000 dilution). Chromatin cross-linking was then reversed at 65°C overnight followed by DNA purification. Real-time qPCR was performed on ChIP DNA on an Applied Biosystems Viia7 Real-time PCR System (Applied Biosystems). Primers used for ChIP-qPCR are listed in Supplementary Table 5.

Pyrosequencing. Genomic DNA was extracted and purified from cells using a BioRobotics Genomic DNA Mini Kit, according to the manufacturer’s instructions (Life Technologies, #K1820-02). Briefly, harvested cells were lysed at 55°C for 10 min and then gDNA was precipitated by adding 100% ethanol and purified through the provided column. Genomic DNA was treated with bisulfite and purified following using an EpiTect Fast Bisulfite Conversion Kit, following manufacturer’s instructions (Qiagen, #59824). Target regions were amplified from bisulfite converted gDNA using MyTag HS DNA polymerase (Bioline, #BIO-25045) with Biotin-labelled primers. Pyrosequencing of samples was performed by Australia Genome Research Facility (AGRF) using the PyroMark Q24 platform. All primers used for pyrosequencing are listed in Supplementary Table 6.

CRISPR/Cas9 genome editing in cell lines. Single guide RNAs (sgRNAs) were designed using the website [https://www.benchling.com/crispr/](https://www.benchling.com/crispr/) and guides with low off-target scores were selected. sgRNAs were cloned into pSpCas9(BB)–2A-GFP (px458) plasmid (Addgene, #48138) [41], Tet2 knock out cells were generated by CRISPR/Cas9 using non-homologous end joining (NHEJ). For site specific mutations, a DNA donor template was used to drive homology-directed repair (HDR). In all, 150 bp DNA donor templates for genomic modification were synthesised by Integrated DNA Technologies (IDT), containing the mutation in the middle of the sequence. sgRNA plasmid and DNA donor were co-transfected into cells using Neon transfection system (ThermoFisher). In all, 48 h after transfection, cells were sorted with BD FACSaria III flow cytometer for those positive for GFP (indicating that they had taken up the pSpCas9(BB)–2A-GFP plasmid) and negative for the live-dead marker 7-AAD (Bio-RAD, #1351102, one drop per sample). After a further 48 h, GFP and 7-AAD negative cells were single sorted into 96-well plates. After a week, clonal populations derived from single cells were screened through Sanger sequencing and western blot. Primers and donors used for genome editing are listed in Supplementary Table 7.

Brdu cell proliferation assay. The proliferation of G1E-ER4 cells was assessed by using a BrdU cell proliferation assay kit, according to the manufacturer’s instructions (Cell Signaling Technology, #6813). We plated 1 × 10^4 G1E-ER4 cells into wells of a 96-well plate, added 10 μl 1x BrdU into each well and incubated for 4, 8 or 24 h. After the incubation, the cells were fixed and denatured for 30 min, then 100 μl/well BrdU detection antibody solution (Cell Signaling Technology, #94079, 1:10000 dilution) was added and incubated at room temperature for 1 h. After the incubation, each well was washed three times with 100 μl wash buffer. Next 100 μl/well HRP-conjugated secondary antibody solution (Cell Signaling Technology, #40709, 1:10000 dilution) was added and incubated at room temperature for 30 min. After the incubation, the cells were washed three times with 100 μl wash buffer. TMB substrate (100 μl) was added and incubated for 30 min before adding stopping buffer. After adding the stopping buffer, absorbance was read at 450 nm to detect cell proliferation.

Generation of c-Kit (C > T)GATA site mutant mice. Generation of mice was done with approval of the UNSW Animal Care and Ethics Committee (Approval No. 16/19A). The CRISPR/Cas9 system was utilised for c-Kit (C > T)GATA mouse generation by injecting sgRNA and Cas9 protein (EnGen® SpyCas9 NLS) into fertilised oocytes [42]. Briefly, sgRNAs were designed using the website [https://www.benchling.com/crispr/](https://www.benchling.com/crispr/) [41]. A linearised DNA template for each sgRNA was generated using a non-cloning method by virtue of a T7-conjugated PCR. The forward primer contained the T7 promoter minimal sequence, upstream of the 20 bp sgRNA sequence and a sequence complimentary to the 5’ end of the sgRNA scaffold of pSPCas9(BB)–2A-GFP. The reverse primer is complimentary to the 3’ end of the
sgRNA scaffold of pSpCas9(BB)–2A-GFP. The linearised DNA was amplified within T7 forward primer and sgRNA scaffold reverse primer using Q5 polymerase (NEB, #E7820). The resulting linearised DNA was in vitro transcribed into sgRNA using a T7 Quick High Yield RNA synthesis kit, followed by the manufacturer’s instructions (NEB, #E2050S). sgRNAs were purified using NucAway Spin columns (ThermoFisher, #AM10070). The donor DNA template was synthesised from Integrated DNA Technologies (IDT). The sgRNA, Cas9 protein and DNA donor were microinjected into BL6 mouse oocytes at 3 days post-coitum (dpc) and immediately transferred to foster mothers (Swiss strain). The C57BL/6 J offspring were genotyped through Sanger sequencing. Briefly, mouse tails were digested in Direct PCR (tail) lysis reagent (Australian Biosearch, #AB-102-T) at 55 °C overnight and then heat inactivated at 85 °C for 45 min. In all, 1 μl of lysates were used as templates for a genotyping PCR using Q5 polymerase (NEB, #0491). PCR products were Sanger sequenced to check for the presence of the mutation. Mice that were heterozygous for the c-Ki (C > T)GATA mutation were backcrossed with wild type C57BL/6 J mice for five generations to eliminate potential off-target effects. Primers used for in vitro transcription and genotyping are listed in Supplementary Table 8.

Animal husbandry. All animal work was carried out in accordance with approval from the UNSW Animal Care and Ethics Committee (Approval Nos. 16/5B and 18/156B). Animals were housed in a specific pathogen-free environment, at a constant ambient temperature of 22 °C, on a 12 h dark-high cycle and with ad libitum access to standard chow and water.

Flow cytometry analysis of erythrocyte populations. Mouse bone marrow cells were stained with CD71 (Biolegend, #334104, 1:100 dilution), Ter119 (BD Bioscience, #553673, 1:200 dilution) and DAPI (Life Technologies, #62248) for 30 min. After staining, cells were washed with 3 ml FACS buffer and centrifuged at 300 x g for 5 min. Cells were resuspended in 500 μl FACS buffer. Stained samples were run on BD LSRII Fortessa SORP flow cytometer. Data was collected using BD FACSDiva software and analysed using FlowJo V10 software. Flow cytometry gating strategies are shown in Supplementary Fig. 1a/b.

Flow cytometry analysis of HSPCs. Haematopoietic stem cell and progenitor cells (HSPCs) were analysed by flow cytometry as described below. Mouse bone marrow was lysed with 9 ml RO water for 10 s followed by adding 1 ml 10x PBS to get rid of mature red blood cells since mature red blood cells do not have cell nucleus and will burst immediately under low osmotic pressure in water. Lysed bone marrow cells were stained with biotin-conjugated lineage marker antibody cocktail (CD3 monoclonal antibody (ThermoFisher Scientific, #13-0073-82, 1:200 dilution), CD4 monoclonal antibody (ThermoFisher Scientific, #13-0041-85, 1:100 dilution), CD11b monoclonal antibody (ThermoFisher Scientific, #13-0112-85, 1:100 dilution), CD5 monoclonal antibody (ThermoFisher Scientific, #13-0081-85, 1:200 dilution), CD45R (B220) monoclonal antibody (ThermoFisher Scientific, #13-0452-85, 1:200 dilution), Ly-6G monoclonal antibody (ThermoFisher Scientific, #13-5931-85, 1:200 dilution), CD117 (c-Kit) APC-H7 antibody (BD Bioscience, #561229, 1:200 dilution), CD52 monoclonal antibody (ThermoFisher Scientific, #13-5921-82, 1:200 dilution), at 4 °C for 30 min. Stained cells were washed with FACS buffer (1xPBS, 5% FCS, 2 mM EDTA) and centrifuged at 300 x g for 5 min. Cells were resuspended in 150 μl FACS buffer. Stained samples were run on BD LSRII Fortessa SORP flow cytometer. Data was collected using BD FACSDiva software and analysed using FlowJo V10 software. Flow cytometry gating strategies are shown in Supplementary Fig. 1a/b.

Peripheral blood count. Blood was collected by cardiac puncture from wild type and c-Ki (C > T)GATA heterozygous mutant mice and added to 50 μl 100 μM heparin (Sigma, #H3149-250UK) in BD vacutainer blood collection tubes (BD Bioscience, #387839). Blood counts were measured by Sysmex XN-1000RF. Original numbers of cells were calculated using the following dilution factor: Correct number of cells = X μl of blood x 50 μl X μl of blood

Bioinformatic analysis. GATA-1 ChiP-Seq datasets from different cell types were downloaded from the ENCODE portal[25] [https://www.encodeproject.org]. Broad peaks were first split into narrow peaks using PeakSplitter function in PeakAnalyzer software[26] for improving individual subpeaks’ analysis. Different components from MEME SUITE was used to perform peak and motif analysis. Comprehensive motif analysis was performed using MEME CHIP[27] which was then used to scan the peaks containing ‘CGATA’ motifs. Finally peak annotation was performed using PeakAnnotator function in PeakAnalyzer[28]. Reduced representation bisulfite sequencing (RRBS) datasets from blood cells and foetal liver tissues were downloaded from GEO[29]. Single CpG methylation data from different stages of erythroid maturation was analysed using the Bioconductor package RnBeads[30]. DNA methylation levels of CGATA Peaks were measured by Bedtools[31] from RnBeads output. Heatmaps of DNA methylation changes of CGATA Peaks were drawn using ggplot library[32] in R. All dataset accession numbers are listed in Supplementary Table 9.

Statistical analysis. The mean and standard deviation (SD) are shown for the data in each figure, except where n < 3. Two-tailed Mann-Whitney U (non-parametric) tests were performed to assess the significance of differences of proportions of common-myeloid progenitors (CMPs) megakaryocyte-erythroid progenitors (MEPs) in bone marrow between wild type and (C > T)GATA mutant mice. The statistical analyses for ChiP-Seq and RRBS experiments were performed by various software programs, details are provided above.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. All ChiP-Seq, ATAC-Seq and DNA-Seq data were downloaded from Encyclopedia of DNA Elements Database (ENCODE) (Supplementary Table 9). All reduced representation bisulphite sequencing (RRBS) data were downloaded from Gene Expression Omnibus (GEO) database (Supplementary Table 9). Publicly available DNA methylation dynamics data were open sources online (http://www.medical-epigenomics.org/papers/broad_mirror/invmethylation/index.html). The source data underlying Figs. 1b–d, 2e, 3c, d, 4a–d and 5c, d and Supplementary Figs 1a, b, 2a, 5, 7, 10a–d, 11b, 12a–c and 13 are provided as a Source data file.

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Author contributions

L.Y., M.C. and K.G.R.Q designed the study and experiments. L.Y. performed and analyzed most experiments and data. Z.C. and M.R.W performed the bioinformatic analysis to identify CGATA sites that are bound by GATA-1 in existing ChIP-seq datasets and where there is a change in methylation levels during hematopoiesis. F.D and L.M.I. generated the CRISPR/Cas9 gene edited mouse line. E.S.S. assisted with the animal studies and optimisation of the haematopoietic flow cytometry panel. L.Y., M.C. and K.G.R.Q wrote the paper. K.G.R.Q and M.C. supervised the study. All authors have read and approved the contents of the paper.

Competing interests

The authors declare no competing interests.

Additional information

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