Regulation of MYB by distal enhancer elements in human myeloid leukemia

Mengjia Li1,2, Penglei Jiang1,2, Kai Cheng1,2, Zehui Zhang1,2, Shuyu Lan1,2, Xiaoxia Li1,2, Lirong Zhao1,2, Yucheng Wang1,2, Xiang Wang4, Jing Chen1,2, Tao Ji1,2, Bingshe Han1,2 and Junfang Zhang1,3

Abstract
MYB plays vital roles in regulating proliferation and differentiation of hematopoietic progenitor cells. Dysregulation of MYB has been implicated in the pathogenesis of leukemia. Although the transcription of MYB has been well studied, its detailed underlying regulatory mechanisms still remain elusive. Here, we detected the long-range interaction between the upstream regions, −34k and −88k, and the MYB promoter in K562, U937, and HL-60 cells using circularized chromosome conformation capture (4C) assay, which declined when MYB was downregulated during chemical-induced differentiation. The enrichment of enhancer markers, H3K4me1 and H3K27ac, and enhancer activity at the −34k and −88k regions were confirmed by ChIP-qPCR and luciferase assay respectively. ChIP-qPCR showed the dynamic binding of GATA1, TAL1, and CCAAT/enhancer-binding protein (C/EBP) at −34k and −88k during differentiation of K562 cells. Epigenome editing by a CRISPR-Cas9-based method showed that H3K27ac at −34k enhanced TF binding and MYB expression, while DNA methylation inhibited MYB expression. Taken together, our data revealed that enhancer elements at −34k are required for MYB expression, TF binding, and epigenetic modification are closely involved in this process in human myeloid leukemia cells.

Introduction
The transcription factor MYB is a key regulator for hematopoiesis1,2. Dysregulation of MYB often associates with various hematological disorders including acute myeloid leukemia (AML), chronic myeloid leukemia (CML), and acute lymphoblastic leukemia (ALL)3–5. Aberrant expression of MYB has been also reported in malignant solid tumors including colon cancer, breast cancer, adenoid cystic carcinoma, and brain cancer6–9. Recurrent chromosomal translocation, genomic duplication, C-terminal truncation, and N-terminal truncation contribute to MYB have been reported in human leukemia10–13.

The expression of MYB is precisely regulated under physiological conditions. Previous studies indicated that MYB transcription is mainly regulated through a transcriptional attenuation site within the first intron14,15. miRNAs including miR-150 and miR-17-92 can target MYB mRNA in a stage-specific manner16,17. PU.1 negatively regulates the c-myb promoter during granulocytic differentiation18. MYB is also an essential downstream target of Hoxa9/Meis1 in hematopoietic cells19. Recently increasing studies support that distal regulatory elements play vital roles in MYB regulation. Transgene insertion 77 kb upstream of c-myb markedly decreases c-myb expression in mouse20. Multiple distal regions 36, 61, 68, 81, and 109 kb upstream of c-myb are involved in c-myb regulation in erythroid differentiation in mouse21. Retroviral insertions upstream and downstream of c-myb lead to upregulation of c-myb in murine and feline cell lines22,23. And our previous study identified three murine leukemia virus integration regions (located at −25k, −56k, and −70k), which interact with c-myb...
through DNA looping and facilitate the integrated virus to activate c-myb expression in murine myeloid progenitor M1 cells. We further demonstrated that the regulatory element at the −28k region has an essential role in c-myb regulation during IL-6 induced differentiation in M1 cells. Distal enhancer elements have been also identified upstream and downstream of MYB in human. The −84k and −71k regions of MYB can regulate MYB and fetal hemoglobin in primary human erythroid progenitors (HEPs). An enhancer −140 kb downstream of MYB was recently identified with improved experimental and computational parameters from single-cell enhancer screens. Above observations support multiple distal and computational parameters from single-cell enhancer modiﬁcations at the enhancer features.

The −34k and −88k regions of MYB are enriched for enhancer features
H3K4me1 and H3K27ac are two commonly used hallmarks to identify putative genome-wide enhancers (Fig. 2A). ChIP-seq data from ENCODE (Encyclopedia of DNA Elements) show strong enrichment of H3K4me1 and H3K27ac at −34k, while strong enrichment of only H3K4me1 was detected at −88k. The enrichment of H3K4me1 (Fig. 2B) and H3K27ac (Fig. 2C) at −34k and −88k was further conﬁrmed using ChIP-qPCR. The DNA fragments representing H3K4me1 peaks, named −34k (1034 bp), −34k (1055 bp), and −88k (1388 bp), respectively (Fig. 2A), were cloned and inserted upstream of the MYB promoter controlling a ﬁreﬂy luciferase reporter gene (Fig. 2D), a −53k fragment without H3K4me1 enrichment was used as control. The constructs were transfected into HeLa (Fig. 2E) and K562 cells (Fig. 2F), respectively. Compared with the −53k fragment, the −34k, −34k, and −88k fragments all showed signiﬁcantly increased luciferase activity (Fig. 2E, F). The −34k fragment showed the highest enhancer activity. Taken together, these data indicate that these distal regions contain enhancers for MYB transcription.

Dynamic long-range interaction of the MYB locus during differentiation of human leukemia cells
We treated K562, U937 and HL-60 cells with hemin, 12-O-Tetradecanoylphorbol 13-acetate (TPA) or all-trans-retinoic acid (ATRA) to induce erythroid, monocytic and granulocytic differentiation, respectively. MYB mRNA and protein levels reduced remarkably after treatment in all three cell lines (Fig. 3A–C), which is consistent with previous studies that MYB is highly expressed in immature proliferating haematopoietic cells, and strongly downregulated during terminally differentiation. 4C assay was subsequently carried out using the MYB promoter as the bait fragment (Fig. 3D–F). In untreated cells high frequency long-range interaction between the MYB promoter and distal regions was observed, however the frequency of most long-range interactions strikingly diminished upon differentiation, especially at the −34k and −88k regions. Meanwhile, the intra-chromosomal interactions with the MYB promoter signiﬁcantly altered during differentiation in all tested cell lines (Supplemental Fig. 1A–C). The potential inter-chromosomal interaction
with the MYB promoter also showed dramatic changes during differentiation in all tested cell lines (Supplementary Tables 3–5). This finding is consistent with previous study that gene-regulatory chromatin interactions were altered upon ATRA induction in HL-60 cells. Thus, we concluded that downregulation of MYB upon differentiation is accompanied by a loss of communication between the MYB promoter and above distal enhancers.

**Binding of transcription factors at distal enhancers of MYB during differentiation**

We further investigated the roles of TFs at above enhancers in MYB regulation. Public ChIP-seq data of histone marks, DNase I hypersensitivity (DNase HS) and TF profiles were generated by the ENCODE project in the HBS1L-MYB region. DNase HS and the enrichment of GATA1, TAL1, C/EBPβ, c-Jun, and PU.1 were observed at the −34ka and/or −34kb regions (Fig. 4A). The enrichment of GATA1, TAL1, and c-Jun were observed at the −88k region (Fig. 4A). Strong enrichment of CTCF and Rad21, which participate in long-range chromatin interactions in the vicinity of 4C interaction sites, was observed mainly near the −34k region (Fig. 4A). The enrichment of GATA1, TAL1, and C/EBPβ was further confirmed by ChIP-qPCR in K562 cells (Supplemental Fig. 2A–C).

We investigated the binding of selected TFs at −34k and −88k regions during differentiation. Western blot showed that GATA1 decreased significantly, while TAL1, C/EBPβ, c-Jun, and PU.1 increased during hemin-induced differentiation in K562 cells (Fig. 4B). After hemin treatment, the binding of GATA1, TAL1, c-Jun decreased at −34kb, PU.1, and C/EBPβ binding increased at −34kb
and promoter; while the GATA1 binding decreased and PU.1 binding increased at −88k (Fig. 4C–G).

To further assess the effect of TF binding to these distal regions, we determined the luciferase activity of the −34k construct after GATA1 overexpression in 293T cells (Fig. 4H). Unexpectedly, a 3-fold increase of luciferase activity of the −34ka construct was observed after GATA1 overexpression, while only a moderate increase was observed for luciferase activity of the −34kb construct and the construct contains only the MYB promoter, indicating that GATA1 binding is required for enhancer activity of the −34k region. Meanwhile, we overexpressed and knocked down GATA1 in K562 cells via lentiviral transduction. Our results showed that overexpression of GATA1 increased
MYB expression (Fig. 4I), while GATA1 knockdown reduced MYB expression (Fig. 4J), indicating GATA1 plays an important role in MYB expression, and corroborating a previous study that GATA1 overexpression leads to failure to repress MYB during erythroid differentiation of K562 cells. Together, our data showed that TF binding at the −34k enhancer elements play a critical role in MYB expression in K562 cells.

**Epigenetic modification of distal enhancers affects MYB expression**

Epigenetic modification can affect enhancer activity, probably via regulation of chromatin structure and TF binding. To test the effect of epigenetic modification on the function of above enhancers, here we applied a dCas9-based epigenome editing method. After co-transfection into K562 cells, a dCas9p300 Core fusion protein with a Flag tag was co-expressed with 2 gRNAs, which target one specific site, then MYB expression was examined (Fig. 5A). As shown in Fig. 5B, dCas9p300 Core was expressed in K562 cells after transfection. ChIP-qPCR using an antibody against the Flag epitope showed that dCas9p300 Core was recruited to the targeted sites by the gRNAs (Fig. 5C) and upregulated H3K27ac level at these sites specifically (Fig. 5D). And MYB expression was increased by elevated H3K27ac at the promoter, −34k and −88k regions (Fig. 5E), indicating that H3K27ac at these sites upregulates MYB expression.
Fig. 4 (See legend on next page.)
Meanwhile, the effect of local DNA methylation at these sites on MYB transcription was also investigated using a previously reported dCas9-DNMT3A fusion protein. Figure SF showed that dCas9-DNMT3A was expressed in K562 cells after transfection. ChIP-qPCR using a Flag epitope antibody showed that dCas9-DNMT3A was recruited to the targeted sites by the gRNAs (Fig. 5G), and upregulated 5-methylcytosine (5-mC) level at these sites specifically (Fig. 5H). And MYB expression was decreased by elevated 5-mC level at the promoter, −34k and −88k regions (Fig. 5I), indicating that DNA methylation can inhibit the function of these DNA elements.

Epigenetic modification of distal enhancers affects TFs binding and cell differentiation

To further understand the roles of epigenetic modification at above sites in MYB regulation, we examined the effect of H3K27ac on TF binding and cell differentiation in K562 cells. ChIP-qPCR showed that H3K27ac enrichment significantly reduced at the MYB promoter, −34k and −88k enhancer regions during hemin induced differentiation (Fig. 6A). dCas93000-Core targeting enhanced GATA1 binding at promoter, −34k and −88k (Fig. 6B), while dCas93000-Core targeting enhanced TAL1 binding at promoter and −34k (Fig. 6C), indicating that H3K27ac helped binding of these two TFs at above DNA elements. Furthermore, dCas93000-Core targeting at −34k could counter downregulation of MYB during early stages of hemin treatment (Fig. 6D), but failed to efficiently block hemin-induced repression of MYB eventually. As represented in our model (Fig. 6E), these data suggest that epigenetic modification at the MYB distal enhancers could affect TF binding and MYB expression during differentiation in K562 cells.

Discussion

Mounting evidence indicates that the MYB gene is under regulation by distal enhancers. Multiple distal upstream and downstream regulatory elements have been reported from −25k to −250k regions of c-myb in mouse, which are involved in c-myb regulation in erythroid differentiation and leukemogenesis. In human cells, distal enhancer elements have been identified at regions 84 kb, 71 kb upstream, and 140 kb downstream of MYB. Above studies indicate that the MYB locus is under control of a complex regulatory network involving multiple upstream and downstream regulatory elements. However, how these enhancers coordinate in MYB regulation is unclear so far.

Using 4C assay showed that the −34k, −88k upstream regions interact with the MYB promoter, and binding sites of CTCF and Rad21 were identified near the −34k, −88k regions using public ChIP-seq data. The enhancer activity of the −34k and −88k regions was further confirmed by enrichment of H3K4me1 and H3K27ac and luciferase reporter assay (Figs. 1 and 2). Meanwhile, the binding of TFs (GATA1, TAL1, C/EBPβ, c-Jun, and PU.1) at −34k and −88k during differentiation was confirmed by ChIP-qPCR. Above data showed that the enhancer elements at the −34k, −88k regions interact with the MYB promoter in human K562, U937, and HL-60 leukemia cells. However, in human erythroid progenitors, long-range interactions with the MYB promoter were detected at multiple upstream sites ranging from −63k to −92k, which affect MYB expression. Above evidence indicates that long-range interactions between distal enhancers and the MYB promoter may perform in a cell-specific manner.

By further analysis of the 4C-assay data, additional long-range interaction with the MYB promoter in K562, U937, and HL-60 cells was detected from more sites, including −107k and +140k regions (Supplementary Table 6), along chromosome 6 at much lower frequency than the −34k and −88k regions. Our results are consistent with a recent report that an enhancer +140k downstream of MYB can regulate MYB expression in K562 cells via DNA-looping, supporting that the enhancer at +140k is required for MYB regulation. Meanwhile, many other intrachromosome and interchromosome sites showed interaction with the MYB promoter in our analysis, the potential roles of these interactions are unknown so far.

Distal enhancers contribute to the activation of gene transcription via conformational loops that bring them
Fig. 5 (See legend on next page.)
physically close to gene promoters. Lineage-specific dynamic and enhancer–promoter contacts cooperate in terminal differentiation. We observed a loss of long-range interaction frequency from −34k and −88k during differentiation of human leukemia cells, accompanied by MYB downregulation. And a significant decline of long-range interaction from other intrachromosome and interchromosome sites was also observed. The loss of chromatin looping between distal enhancers and the MYB promoter during differentiation was also reported in MEL cells. Above data showed the dynamic long-range interaction between the MYB promoter and the −34k/−88k regions during hemin induced differentiation in K562 cells.

TFs are required for enhancer function and involved in establishing and stabilizing long-range chromatin interactions. We first showed that GATA1 binds to the −34k and −88k enhancer regions and upregulates MYB expression (Fig. 4). GATA1 is considered as the “master” transcription factor in erythropoiesis. And it has been reported that GATA1 and TAL1 bind to the −71k and −84k enhancer regions of the MYB locus and positively regulate MYB expression along erythroid cell differentiation. c-Jun binding at the promoter and −34k regions decreased during differentiation, our results support the idea that c-Jun principally binds to distal enhancers, and promoters and is considered a pioneer factor in modulating chromatin structure of distal enhancers in K562 cells. PU.1 has been reported to suppress MYB expression through direct binding to the MYB promoter and recruitment corepressors HDAC1 and/or DNMT3a/b. Here, we found that PU.1 binding at the promoter and −34k regions increased during differentiation, indicating that PU.1 in the MYB enhancer regions can also downregulate MYB expression. Furthermore, C/EBPβ binding at the promoter and −34k regions increased during differentiation, and the expression of C/EBPβ was indeed changed during myeloid differentiation. C/EBPβ acts as a transcription repressor for genes of liver proliferation. Further studies will be required to determine whether C/EBPβ has a specific function in MYB control during myeloid differentiation.

Recent studies typically suggest that active enhancers display lower 5-mC levels than poised or silent enhancers, along with TF binding as well as the presence of active histone marks H3K4me1 and H3K27ac. Here, we showed that the induction of histone acetylation at the −34k and −88k regions enhanced MYB transcription and TF binding (Figs. 5 and 6). Conversely, DNA methylation at the −34k and −88k regions leads to downregulation of MYB. Our results coincide with that hypermethylation at enhancers is generally associated with reduced chromatin accessibility and decreased TFs binding. DNA methylation of enhancers can influence cell-type specific gene expression, and regulate relevant genes in acute myeloid leukemia and chronic myeloproliferative neoplasms. And histone acetylation is required for enhancer function. Cooperation of TFs with epigenetic modifications of chromatin contributes to the activation of regulatory elements including promoters and enhancers, TFs must gain access to their binding sites, and binding of TFs also modify the chromatin landscape. Meanwhile, enhanced histone acetylation at the −34k region alone only delayed but failed to block hemin-induced repression of MYB (Fig. 6D), which is consistent with the relatively minor decrease in H3K27ac at this region upon hemin-induced differentiation (Fig. 6A), indicating that H3K27ac at this region is of secondary importance in hemin induced downregulation of MYB.

In the present study, we identified enhancer element at the −34k and −88k regions, which play roles in regulation of MYB in human leukemia cells. Our data will help understanding the mechanisms of regulation/dysregulation of MYB under physiological and pathological conditions in human.

Materials and methods

Cell culture and treatment

K562 (CCL-243, ATCC, Manassas, VA), U937 (CRL-15932, ATCC), and HL-60 (CCL-240, ATCC) cells were
maintained in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (10099141, Gibco, Auckland, New Zealand). HeLa cell line (CCL-2, ATCC) was cultured in DMEM (10569044, Gibco, USA) supplemented with 10% FBS. Authentication of these cell lines were conducted by short tandem repeat
(STR) markers, and no mycoplasma contamination was detected. All cell lines were supplemented with 1% penicillin-streptomycin-glutamine solution (SV30082.01, HyClone, Utah, USA) and cultured at 37 °C in a humidified atmosphere containing 5% CO₂. For treatment, K562, U937, or HL-60 cells were seeded at a density of 1 × 10⁵ cells/ml, then cultured for 72 h with 30 μM Hemin (S1280, Sigma-Aldrich, Missouri, USA), 2 μM 12-O-Tetradecanoylphorbol 13-acetate (TPA) (S1819, Beyotime, Shanghai, China) or 0.16 μM all-trans-retinoic acid (ATRA) (R2625, Sigma-Aldrich) to induce erythroid, granulocytic, and monocytic differentiation, respectively.

**Antibodies**

The following antibodies were used in this study: anti-H3K4me1 (ab8895, Abcam), anti-H3K27ac (ab4729, Abcam), anti-5-methylcytosine (ab10805, Abcam), anti-H3K4me1 (ab8895, Abcam), anti-H3K27ac (ab4729, Abcam), anti-5-methylcytosine (ab10805, Abcam), anti-GATA1 (ab11852, Abcam), anti-CEBP/β (ab15050, Abcam), anti-c-Jun (G-4) (sc-74543X, Santa Cruz), and anti-Flag (M2) (F1804, Sigma-Aldrich). Anti-mouse IgG HRP-linked antibodies (G-21234, Invitrogen) and anti-rabbit IgG HRP-linked antibodies (G-21040, Invitrogen) were used to detect protein expression using the Western blot analysis.

**Plasmid construction for expression of dCas9-effector proteins and gRNAs**

The constructs, pcDNA-dCas9<sup>300</sup> Core (61357, Addgene),<sup>38</sup> pcDNA-dCas9<sup>300</sup> Core (D13999Y) (61358, Addgene),<sup>38</sup> pdCas9<sup>DNTM3ΔA</sup> (71666, Addgene),<sup>39</sup> and pdCas9<sup>DNTM3A</sup> (71685, Addgene)<sup>39</sup> were from Addgene. gRNAs targeting the MYB promoter and enhancer regions were designed using Feng Zhang lab’s Target Finder software (http://crispr.mit.edu). Best guides, with highest score of the inverted likelihood of off-target binding, were selected, and the gRNA sequences are shown in supplementary Table 1. Expression plasmids for gRNAs were constructed by cloning annealed oligos into pSpgRNA (#47108 Addgene)<sup>34</sup> using BbsI (R0539, NEB) and T4 ligase (M0202, NEB). Then these plasmids were transfected into K562 cells using Lipofectamine 3000 (L3000015, Invitrogen) according to the manufacturer’s instructions. Forty-eight hours later, cells were harvested for analysis.

**Lentivirus production and cell transduction**

For creation of the shGATA1 vectors, oligonucleotides for shGATA1 (sequences are shown in supplementary Table 1) were annealed and ligated into the digested pLKO.1-puro vector at the EcoR I and AgeI sites. For creation of the GATA1 overexpression vector, GATA1 PCR product was cloned into pLX-IRES-NEO at the EcoRI and XbaI sites. In all, packaging plasmids pCMV-VP26, pCMV-DR8.91, and the relevant lentiviral transfer vectors in a 3:8:10 mass ratio were cotransfected into 293T cells using TurboFect Transfection Reagent (R0531, ThermoFisher). The media containing lentivirus particles were collected after 48 h. And used to infect K562 cells immediately in the presence of 8 μg/ml hexadimethrine bromide (H9268, Sigma-Aldrich). Cells were collected for analysis after 72 h.

**Quantitative real-time PCR analysis**

Total RNA was isolated using TRIzol reagent (15596-018, Invitrogen). And 1 μg of total RNA was reverse-transcribed with PrimeScript™ RT reagent Kit with gDNA Eraser (RR047A, TaKaRa, Beijing, China). The levels of specific RNAs were measured using a Light Cycler 480II real-time PCR machine and the iTaql™ Universal SYBR Green Supermix (1725124, Bio-Rad, Hercules, CA, USA) according to the manufacturer instructions. All samples were assayed in triplicate. Data were normalized to a human Gapdh (glyceraldehyde-3-phosphate dehydrogenase) control. Relative quantitation was carried out by the comparative threshold cycle (CT) method. Statistical analysis was performed using the GraphPad Prism 8 software. The primer sequences are listed in supplementary Table 1.

**Western blot analysis**

Western blot analysis was performed as previously described.<sup>25</sup> Proteins were isolated from cells and protein concentration was determined by a bicinchoninic acid

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**Fig. 6** Epigenetic modification of distal enhancers affects TFs binding and cell differentiation. A. H3K27ac ChIP-qPCR enrichment at the MYB promoter and enhancer regions in K562 cells treated with hemin to induce erythroid differentiation. B, C. GATA1 (B) and TAL1 (C) ChIP-qPCR enrichment at the MYB promoter and enhancers in K562 cells co-transfected with the indicated dCas9<sup>300</sup> Core and two gRNAs targeted to the each MYB promoter and enhancer region. dCas9<sup>300</sup> Core (D13999Y) contained a nonfunctional residue substitution at the acetyltransferase domain and was used as a negative control. D. K562 cells co-transfected with the indicated dCas9<sup>300</sup> Core fusion protein and two gRNAs targeted to the −34k region were treated with hemin for indicated times, then MYB mRNA levels were determined using RT-qPCR. E. Model of the dynamic long-range interaction during differentiating human leukemia K562 cells. Distal cis-regulatory elements (−34k and −88k regions) enriched for activating epigenetic modification H3K4me1 and H3K27ac, and transcription factors containing GATA1 (orange ovals), TAL1 (violet rectangles) and c-Jun (green diamonds), allowing for high-level expression of MYB. During differentiation, intergenic transcription factor occupancy decreases at the cis-regulatory elements, leading to a destabilization of the DNA-looping and a dramatic decrease of MYB transcription, allowing cells to terminally differentiate. Data are represented as mean ± SD of three independent experiments, and P values are calculated using Student’s t-test (*P < 0.05; **P < 0.01; ***P < 0.001) in A–D.
and ligation. Primers for the to a second round of digestions with a 4-base cutter DpnII. After precipitation, chromatin was further subjected at 37 °C overnight with shaking followed by diluted ligation.

TGTTGGATATATTGC-3′
AGTATTAATTTGCCTTGTCC-3′

analyzed visualization via an R package 4C-ker. Reads amplify sample libraries. Multiplexed sequencing was performed. Chromatin immunoprecipitation (ChIP) was carried out to shear the chromatin. Immunoprecipitation of cross-linked chromatin was performed overnight at 4 °C with antibodies. An equal amount of isotype immunoglobulin G (IgG) was used as background control. Primers for antibodies. An equal amount of isotype immunoglobulin G (IgG) was used as background control. Primers for ChIP-qPCR are shown in supplementary Table 1.

Circularized chromosome conformation capture (4C) assay
4C assay was performed as previously described. In brief, 1 × 10⁷ cells were fixed in 1% formaldehyde for 10 min at room temperature and sonicated to shear the chromatin. Immuno precipitation of cross-linked chromatin was performed overnight at 4 °C with antibodies. An equal amount of isotype immunoglobulin G (IgG) was used as background control. Primers for ChIP-qPCR are shown in supplementary Table 1.

Dual-luciferase reporter assay
The MYB promoter (chr6:135 501 805–135 502 522, hg19) was amplified, digested with XhoI and BglII and cloned into the pGL4 luciferase reporter vector (Promega). The upstream regions –34ka (chr6:135 467 317-135 468 351, hg19), –34kb (chr6:135 468 624-135 469 679, hg19), –53k (chr6:135 448 094-135 448 922, hg19) and -88k (chr6:135 414 242-135 415 630, hg19) were amplified and cloned into pGL4.10-MYB-promoter mentioned above via KpnI/NheI digestion. Then these reporter vectors were transfected into K562 or HeLa cells using Lipofectamine 3000 (L3000015, Invitrogen) according to the manufacturer’s instructions. Luciferase activity was measured using the Dual-Luciferase Reporter Assay System (E1960, Promega) on a FlexStation 3 multimode microplate reader. All assays were performed in triplicate and repeated at least three times.

Bioinformatics and statistical analysis
The ChIP-seq datasets were obtained from the ENCODE project were visualized with the WashU Epigenome Browser (https://epigenomewidget.wustl.edu/) . 4C-seq data were analyzed via the R package 4C-ker (https://github.com/rr1859/R.4Cker) . Statistical significance ( P < 0.05) for RT-qPCR, ChIP-qPCR, and luciferase reporter assay experiments was assessed by Student’s two-tailed t-test. Data were obtained from at least three independent experiments and are expressed as the means ± standard deviation (SD).

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Author details
1Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, Shanghai Ocean University, Shanghai, China. 2National Demonstration Center for Experimental Fisheries Science Education, Shanghai Ocean University, Shanghai, China. 3International Research Center for Marine Biosciences, Ministry of Science and Technology, Shanghai Ocean University, Shanghai, China. 4Department of Hematology/Oncology, Shanghai Children’s Medical Center (SCMC), Shanghai Jiao Tong University School of Medicine, Shanghai, China

Author contributions
B.H. and J.Z. conceived the study and designed the experiments. B.H., J.Z., M.L., P.J. performed development of methodology and writing, review and revision of the paper. M.L., P.J., K.C., Z.Z., S.L., X.L., L.Z., and Y.W. provided acquisition, analysis and interpretation of data, and statistical analysis. J.C., T.J., and X.W. provided technical and material support. All authors read and approved the final manuscript.

Data availability
The data used in this study has been deposited in NCBI’s Gene Expression Omnibus repository and are accessible through GEO accession number GSE140321.

Conflict of interest
The authors declare no competing interests.

Ethics approval and consent to participate
No ethical approval was required for this study.

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References

1. Ramsay, R. G. & Gonda, T. J. MYB function in normal and cancer cells. Nat. Rev. Cancer 8, 533–544 (2008).
2. Gregk, K. T., Caretta, S. & Nuss, S. L. Critical roles for c-Myc in hematopoietic progenitor cells. Semin. Immunol. 20, 247–256 (2008).
3. Nguyen, N. et al. Myb expression is critical for myeloid leukemia development induced by Setbp1 activation. Oncotarget 7, 86300–86312 (2016).
4. Negi, V. et al. Hoxa9 and Hoxa10 induce CML myeloid blast crisis development through activation of Myb expression. Oncotarget 8, 98853–98864 (2017).
5. Nakano, K., Uchimaru, K., Utsunomiya, A., Yamaguchi, K. & Watanabe, T. Dys-regulation of c-Myc pathway by aberrant expression of proto-oncogene MYB provides the basis for malignancy in adult T-cell leukemia/lymphoma cells. Clin. Cancer Res. 22, 5915–5928 (2016).
6. Qu, X. et al. c-Myc promotes growth and metastasis of colorectal cancer through c-fos-induced epithelial-mesenchymal transition. Cancer Sci. 110, 3183–3196 (2019).
7. Li, Y. et al. c-Myc enhances breast cancer invasion and metastasis through the Stathm-Axin2 pathway. Cancer Res. 76, 3364–3375 (2016).
8. Dner, Y. et al. An oncogenic MYB feedback loop drives alternate cell fates in adenoid cystic carcinoma. Nat. Genet. 45, 265–272 (2016).
9. Zhang, J. et al. Whole-genome sequencing identifies genetic alterations in pediatric low-grade gliomas. Nat. Genet. 45, 602–612 (2013).
10. Clapper, E. et al. The C-MYB locus is involved in chromosomal translocation and genomic duplications in human T-cell acute leukemia (T-ALL), the translocation defining a new T-ALL subtype in very young children. Blood 110, 1251–1261 (2007).
11. Lahortiga, I. et al. Duplication of the MYB oncogene in T cell acute lymphoblastic leukemia. Nat. Genet. 39, 593–595 (2007).
12. Tomita, A. et al. Truncated c-Myc expression in the human leukemia cell line TK-6. Leukemia 12, 1422–1429 (1998).
13. Fretich, C. A. et al. N-terminal truncated Myb with new transcriptional activity involved in the growth-promoting effects of MYB in human Ph-positive leukemia cells. Blood 92 (2003).
14. Xiao, C. et al. MiR-150 controls B cell differentiation by targeting the translational efficiency of the MYB promoter in the human leukemia cell line TK-6. Mol. Cell Biol. 35, 10364–10372 (2015).
15. Zhang, J. et al. Distal regulation of c-myc expression during IL-6-induced differentiation in murine myeloid progenitor M1 cells. Cell Death Dis. 7, e2364 (2016).
53. Brettingham-Moore, K. H., Taberlay, P. C. & Holloway, A. F. Interplay between transcription factors and the epigenome: insight from the role of RUNX1 in leukemia. *Front. Immunol.* **6**, 499 (2015).

54. Perez-Pinera, P. et al. RNA-guided gene activation by CRISPR-Cas9-based transcription factors. *Nat. Methods* **10**, 973–976 (2013).

55. Raviram, R. et al. 4C-ker: a method to reproducibly identify genome-wide interactions captured by 4C-Seq experiments. *PLoS Comput. Biol.* **12**, e1004780 (2016).

56. Li, D., Hsu, S., Purushotham, D., Sears, R. L. & Wang, T. WashU epigenome browser update 2019. *Nucleic Acids Res.* **47**, W158–W165 (2019).