

# B-*myb* is an essential regulator of hematopoietic stem cell and myeloid progenitor cell development

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**The B-*myb* (MYBL2) gene is a member of the MYB family of transcription factors and is involved in cell cycle regulation, DNA replication, and maintenance of genomic integrity. However, its function during adult development and hematopoiesis is unknown. We show here that conditional inactivation of B-*myb* in vivo results in depletion of the hematopoietic stem cell (HSC) pool, leading to profound reductions in mature lymphoid, erythroid, and myeloid cells. This defect is autonomous to the bone marrow and is first evident in stem cells, which accumulate in the S and G<sub>2</sub>/M phases. B-*myb* inactivation also causes defects in the myeloid progenitor compartment, consisting of depletion of common myeloid progenitors but relative sparing of granulocyte–macrophage progenitors. Microarray studies indicate that B-*myb*-null LSK<sup>+</sup> cells differentially express genes that direct myeloid lineage development and commitment, suggesting that B-*myb* is a key player in controlling cell fate. Collectively, these studies demonstrate that B-*myb* is essential for HSC and progenitor maintenance and survival during hematopoiesis.**

myeloipiasis | myelodysplastic syndrome

Hematopoiesis is maintained by the renewal of multipotent hematopoietic stem cells (HSCs) that give rise to lineage-committed cells. HSCs are maintained in constant numbers in the bone marrow (BM), where they reside in a quiescent state (1, 2). According to the stochastic model of hematopoiesis, long-term HSCs (LT-HSCs), which can undergo extensive self-renewal, begin to differentiate into short-term HSCs (ST-HSCs) with limited self-renewing potential. ST-HSCs further differentiate into multipotent progenitors (MPPs) that unlike HSCs do not self-renew, but retain the ability to commit to multiple lineages. Lineage commitment begins to occur at the level of the common lymphoid and myeloid progenitors (CLPs and CMPs), which are thought to arise from MPPs. Whereas CLPs give rise to lymphoid cells, CMPs further differentiate into megakaryocyte–erythroid progenitors (MEPs) and granulocyte–monocyte progenitors (GMPs) (1, 2). More recent studies have identified additional intermediates, such as lymphoid-primed MPPs (LMPPs), in this developmental pathway (3).

The MYB family of transcription factors has three members: A-*myb* (MYBL1), B-*myb* (MYBL2), and c-*myb* (MYB). Disruption of the c-*myb* locus in mice results in embryonic lethality at embryonic day 15, primarily due to defective erythropoiesis in the fetal liver (4). A role for c-*myb* in adult hematopoiesis has been shown more recently using several conditional knockout (KO) and mutant mouse models. In adult thymocytes and B lymphocytes, disruption of c-*myb* blocks development at the DN3 and prepro B stages, respectively (5–8). c-*myb* expression is also required for erythropoiesis, myelopoiesis, and the development and maintenance of HSCs (9–12), underscoring the importance of this gene within the entire hematopoietic compartment.

B-*myb*-null mutant mice die in utero at days 4–6.5 of gestation due to defects in inner cell mass formation (13), which precludes the study of this gene in hematopoiesis. In this paper,

we describe the effects of conditional disruption of B-*myb* in adult hematopoietic cells. Loss of B-*myb* leads to depletion of the HSC pool, resulting in dramatic losses of mature cells in multiple lineages. The effect of B-*myb* deficiency is autonomous and is associated with defects in HSC cell cycle progression and increased levels of cell death in the myeloid progenitor compartment. Gene expression analysis indicates that B-*myb*-null LSK<sup>+</sup> cells differentially express genes that regulate myeloid lineage development and commitment, suggesting that B-*myb* plays a critical role in regulating HSC and progenitor cell maintenance.

## Results

**Loss of B-*myb* Expression Results in Defective Hematopoiesis.** In BM, B-*myb* mRNA is expressed at appreciable levels in the HSC and myeloid progenitor compartments, with the highest levels in the CMP and GMP populations. These levels are in stark contrast to those of mature, lineage<sup>+</sup> (Lin<sup>+</sup>) cells (Fig. 1A). To determine the role of B-*myb* in adult hematopoiesis, we generated a conditional B-*myb* floxed (B-*myb*<sup>F/F</sup>) mouse model (Fig. 1B–D) and crossed this mouse with the *Mx1*-cre strain (14). Administration of

## Significance

Hematopoiesis is a tightly regulated process by which hematopoietic stem cells (HSCs) give rise to mature cells. The balance between the propensity of HSCs to remain quiescent, to divide and generate more HSCs (self-renewal), or to divide and give rise to mature cells (differentiation) is essential for the long-term maintenance of blood cell formation. Mechanisms underlying cell fate decisions of HSCs are not completely understood. We observed that deletion of the B-*myb* gene leads to depletion of the HSC pool and losses of mature cells. Our further studies strongly suggest that these effects are due to defects in HSC proliferation and differentiation. We therefore identified B-*myb* as a critical component of the mechanism that balances self-renewal and differentiation of HSCs.

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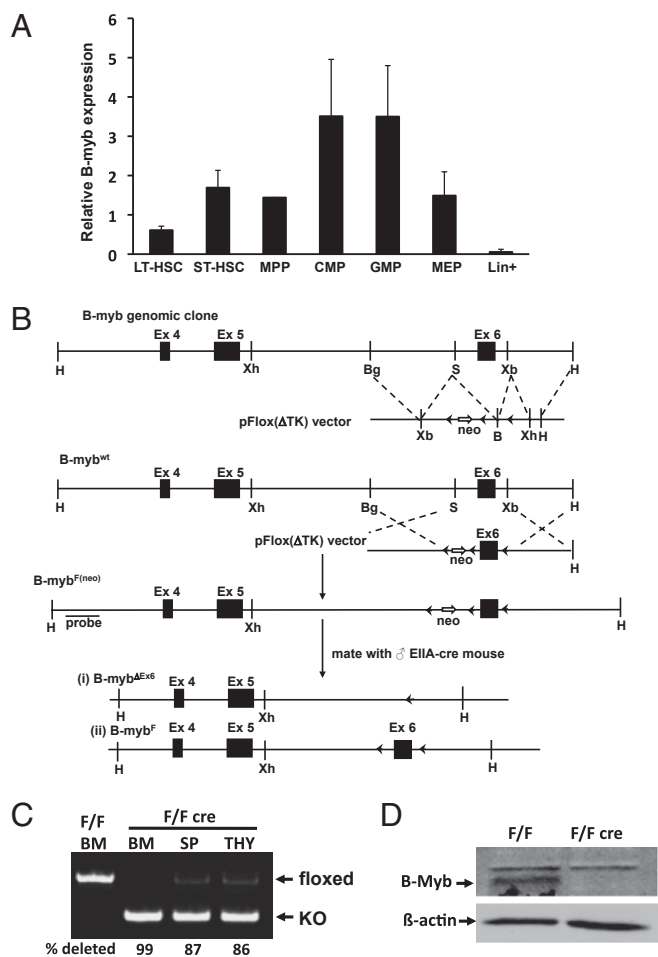
Data deposition: The microarray data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, [www.ncbi.nlm.nih.gov/geo](http://www.ncbi.nlm.nih.gov/geo) (accession no. GSE53875).

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**Fig. 1.** Murine *B-myb* genomic structure and target production. (A) Expression of *B-myb* in HSCs and progenitor cells. (All mRNA levels shown are normalized to that of  $\beta$ -actin.) All values represent mean  $\pm$  SEM. (B) Schematic representation of the mouse *B-myb* genomic clone. (Note: not drawn to scale.) LoxP sites are depicted as black arrowheads. Homologous recombination will generate the *B-myb*<sup>F(neo)</sup> allele that will be used for subsequent recombination by Cre recombinase. ES cells with either a type I or II deletion (*B-myb* <sup>$\Delta$ Ex6</sup> and *B-myb*<sup>F</sup>) will be produced following Cre expression. The probe that can be used to determine homologous recombination is indicated. Restriction enzyme sites: B, BamHI; Bg, BglII; H, HindIII; S, SmaI; Xb, XbaI; Xh, XhoI. Deletion of exon 6 produces a B-MYB protein without a DNA-binding domain and a shift in reading frame. (C) Representative semi-quantitative PCR analysis of genomic DNA isolated from pIpC-treated control and *B-myb* floxed mice showing the presence of floxed and deleted alleles in the BM, spleen (SP) and thymus (THY). The percentage of deletion is shown. (D) Western blot analysis of B-MYB protein (arrow) in sorted Lin<sup>-</sup> BM cells derived from pIpC-treated control (F/F) and floxed/Mx1Cre<sup>+</sup> (F/Fcre) mice.  $\beta$ -Actin is shown as a loading control.

polyinosinic:polycytidylic acid (pIpC) to *B-myb*F/F-Cre mice (designated “F/Fcre”) resulted in a dramatic loss of cellularity in the BM and spleen (Fig. 2 A and B) compared with identically treated littermate control (F/F or +/F) animals. Notably, we observed significant decreases in the number of B- and T-lymphoid, erythroid, and myeloid lineage cells, with the myeloid compartment being most profoundly affected in both tissues (Fig. 2 C and D). Statistically significant reductions in the number of platelets, total white blood cells, lymphocytes, and neutrophils were also observed in the peripheral blood (PB) of *B-myb*-deficient F/Fcre animals (Fig. S1). These differences resulted in changes in the frequencies of cell types in the spleen and BM (Fig. S2), as the percentages of B, erythroid, and myeloid cells were altered in the absence of *B-myb*

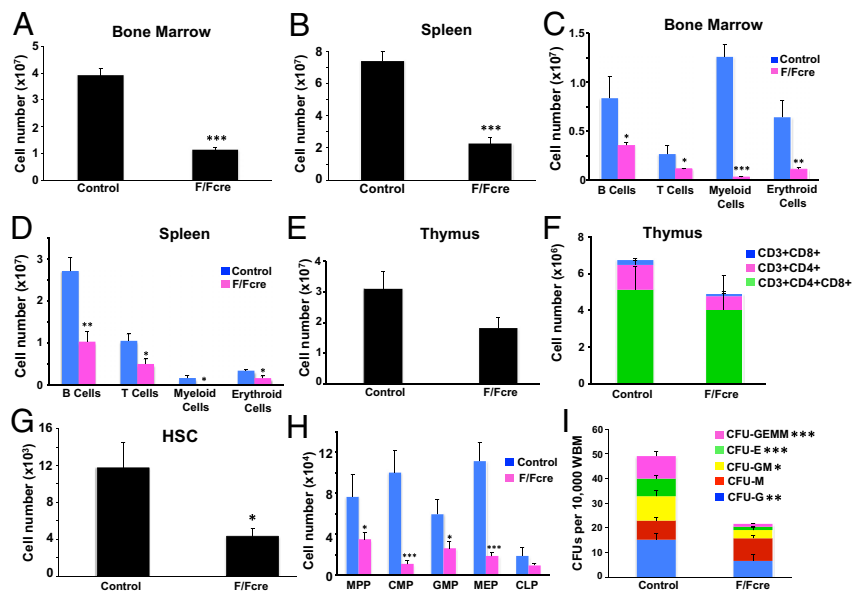
expression. Although the number of T-lineage (CD3<sup>+</sup>) cells were also decreased in both the BM and spleen, thymocytes and the CD3<sup>+</sup> cells in the thymus were not significantly depleted (Fig. 2 C–F).

A more detailed examination of the HSC and progenitor compartments showed that *B-myb*-deficient mice have marked decreases in the absolute number of HSCs (Lin<sup>-</sup>Sca1<sup>+</sup>cKit<sup>+</sup>CD150<sup>+</sup>CD48<sup>-</sup>), MPPs (Lin<sup>-</sup>Sca1<sup>+</sup>cKit<sup>+</sup>CD150<sup>+</sup>CD48<sup>-</sup>) (15) as well as CMPs (Lin<sup>-</sup>Sca1<sup>-</sup>cKit<sup>+</sup>CD34<sup>+</sup>CD16/32[Fc $\gamma$ R]<sup>mid</sup>), GMPs (Lin<sup>-</sup>Sca1<sup>-</sup>cKit<sup>+</sup>CD34<sup>+</sup>CD16/32[Fc $\gamma$ R]<sup>hi</sup>) and MEPs (Lin<sup>-</sup>Sca1<sup>-</sup>cKit<sup>+</sup>CD34<sup>lo</sup>CD16/32[Fc $\gamma$ R]<sup>lo</sup>) (Fig. 2 G and H) (16). Although we also observed a decrease in the number of CLPs (Lin<sup>-</sup>/IL7R $\alpha$ <sup>+</sup>/Sca1<sup>lo</sup>/cKit<sup>lo</sup>) (Fig. 2H) (17), this difference was not significant. Consistent with the reductions in myeloid progenitors, *B-myb*-deficient BM also formed fewer colonies in in vitro colony-forming unit (cfu) assays (Fig. 2I). All of the myeloid lineage colonies that were scored, with the exception of CFU-M, were reduced in number in cultures derived from *B-myb*-deficient BM. The increased number of CFU-M colonies, although not statistically significant, mirrors the CD11b<sup>+</sup>Gr1<sup>lo</sup> monocyte-containing population whose frequency is increased in *B-myb* F/Fcre mice (Fig. S24). Taken together, these results show that *B-myb* expression is required for adult hematopoiesis and for the maintenance of HSCs and myeloid progenitor cells.

***B-myb*-Mediated Defects in the BM Are Autonomous.** We next performed noncompetitive BM transplantation assays to determine whether the defect in hematopoiesis was intrinsic to BM. Whole BM was isolated from CD45.2<sup>+</sup> control or *B-myb*-deficient mice and transplanted into lethally irradiated CD45.1<sup>+</sup> recipients (Fig. S3A). pIpC was administered once engraftment was stable (8–10 wk posttransplant) and ~90% of the PB in the recipients was reconstituted from CD45.2<sup>+</sup> donor-derived cells (Fig. 3A). Mice transplanted with *B-myb*-deficient BM and treated with pIpC became pancytopenic (Fig. 3 B and C) and showed profound decreases in the numbers of LT-HSCs and myeloid progenitors (Fig. 3 D and E) as well as reduced PB counts (Fig. S4). On the other hand, reciprocal, noncompetitive BM transplants, whereby wild-type BM (CD45.1<sup>+</sup>) was transplanted into lethally irradiated recipient (CD45.1<sup>+</sup>) mice (Fig. S3B). Engraftment was monitored every 4 wk to determine the contribution of CD45.1<sup>+</sup> and CD45.2<sup>+</sup> cells. The control CD45.2<sup>+</sup> cells reconstituted the myeloid and B- and T-lineage cells in the PB at levels comparable to the competitor for the 16-wk duration of the experiment (Fig. S6). In contrast, multilineage reconstitution by *B-myb*-deficient BM was strongly impaired at 4 wk posttransplant, and decreased progressively over time, demonstrating that *B-myb* is required for the maintenance and self-renewal of HSCs.

***B-myb*-Deficient HSCs Fail to Long-Term Repopulate.** Because loss of *B-myb* caused a dramatic decrease in the number of HSCs, we performed competitive repopulation studies to determine whether *B-myb* KO HSCs were capable of long-term reconstitution in vivo. Whole BM was isolated from pIpC-treated control and *B-myb* F/Fcre animals (CD45.2<sup>+</sup>), mixed in a 1:1 ratio with competitor wild-type (CD45.1<sup>+</sup>) BM and transplanted into lethally irradiated recipient (CD45.1<sup>+</sup>) mice (Fig. S3B). Engraftment was monitored every 4 wk to determine the contribution of CD45.1<sup>+</sup> and CD45.2<sup>+</sup> cells. The control CD45.2<sup>+</sup> cells reconstituted the myeloid and B- and T-lineage cells in the PB at levels comparable to the competitor for the 16-wk duration of the experiment (Fig. S6). In contrast, multilineage reconstitution by *B-myb*-deficient BM was strongly impaired at 4 wk posttransplant, and decreased progressively over time, demonstrating that *B-myb* is required for the maintenance and self-renewal of HSCs.

***B-myb* Disruption Leads to Aberrant Cell Cycle Progression of HSCs.** Loss of HSC renewal is often associated with a defect in proliferative control. In their steady-state, HSCs are largely quiescent and enter the cell cycle in response to stimuli that trigger differentiation and lineage commitment (18). *B-myb* has been shown to play a role in the cell cycle progression of multiple cell types and is involved in regulating progression through the S and G<sub>2</sub>/M phases (19–25). To determine whether loss of *B-myb*

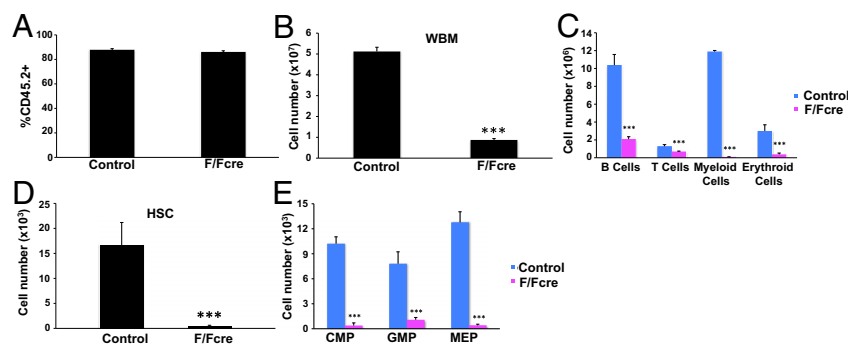


**Fig. 2.** *B-myb* is required for hematopoiesis. *B-myb*-deficient (*F/Fcre*; *Mx1-cre-B-myb<sup>F/F</sup>*) and control (*B-myb<sup>+F</sup>* or *B-myb<sup>F/F</sup>*) mice were treated with plpC every other day over a 5-d period. Tissues were harvested on day 21 posttreatment. Total number of cells isolated from BM (A) and spleen (B) of control and *B-myb*-deficient mice. Analysis of mature lineage cells present in the BM (C) and spleen (D) of control and *B-myb*-deficient mice. Erythroid, B-, T-, and myeloid lineage cells are defined as TER119<sup>+</sup>, B220<sup>+</sup>, CD3<sup>+</sup>, and CD11b<sup>+</sup>Gr1<sup>+</sup>, respectively. Total number of thymocytes (E) and mature populations (F) in the thymuses of control and *B-myb* *F/Fcre* mice. (G) Total number of HSCs in the BM of control and *B-myb*-deficient mice. (H) Total number of MPPs, myeloid progenitors (CMPs, GMPs, and MEPs), and lymphoid progenitors (CLPs) in the BM of control and *B-myb*-deficient mice. (I) Colony-forming assay showing the frequency of whole BM cells that formed megakaryocyte (GEMM), erythroid (E), granulocyte–monocyte (GM), monocyte (M), or granulocyte (G) colonies in culture. All values represent mean  $\pm$  SEM.  $n \geq 3$  per population for each genotype. \* $P \leq 0.05$ , \*\* $P \leq 0.005$ , \*\*\* $P \leq 0.0005$ .

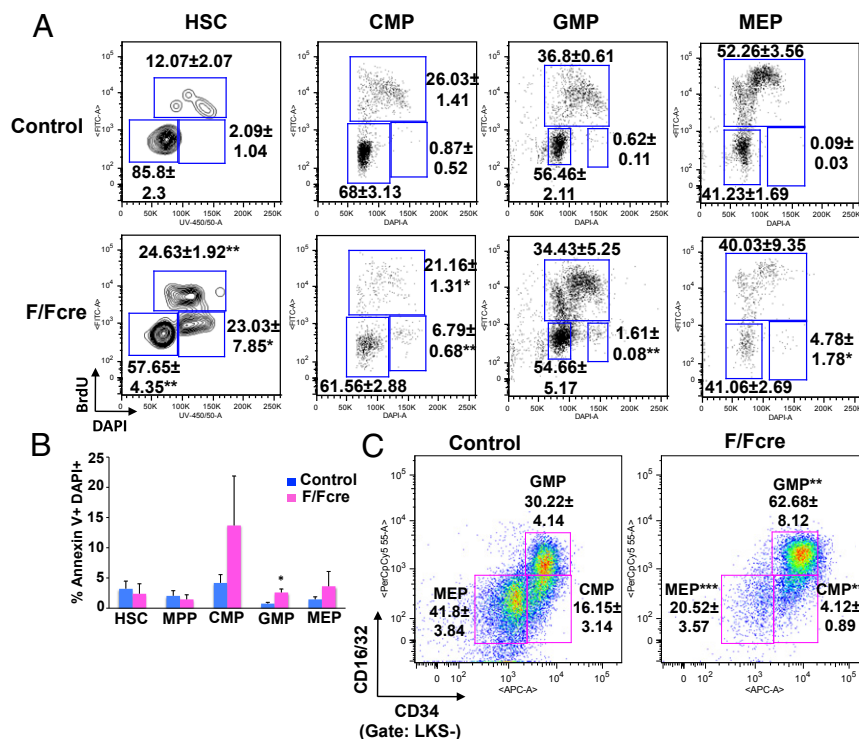
expression led to altered cell cycle kinetics in HSCs, plpC-treated control and *B-myb* *F/Fcre* mice were injected with BrdU and BM was harvested 2 h posttreatment. BrdU incorporation was significantly higher in *B-myb*-deficient HSCs compared with those of the control, reflecting increases in the fraction of cells in the S and G<sub>2</sub>/M phases (Fig. 4A). In the myeloid compartments, more subtle but statistically significant increases in the frequencies of cells in the G<sub>2</sub>/M phase were observed as a consequence of *B-myb* deletion. These studies indicate that loss of *B-myb* expression alters cell cycle progression predominantly in HSCs, and to a lesser extent, in myeloid progenitors.

**Loss of *B-myb* Increases Cell Death of Myeloid Progenitors.** We next determined whether altered cell cycle kinetics in *B-myb* KO mice

resulted in differentiation-associated cell death, which might account for the depletion of HSCs and progenitors, by staining BM from plpC-treated control and *B-myb* KO mice with Annexin V. *B-myb* KO mice did not have increased percentages of apoptotic (Annexin V<sup>+</sup> DAPI<sup>+</sup>) HSCs or MPPs (Fig. 4B). However, the fraction of apoptotic CMPs, GMPs, and MEPs was higher in *B-myb*-deficient animals, with the difference in cell death being significant in the GMP population. Interestingly, despite the fact that *B-myb*-deficient mice have a profound reduction in the number of myeloid progenitors and mature myeloid cells (Fig. 2), the relative frequency of GMPs was actually increased in these animals (Fig. 4C). To determine whether the higher percentage of GMPs in the *B-myb* KO mice resulted from increased differentiation of CMPs and/or proliferation of GMPs, we sorted



**Fig. 3.** Cell-autonomous role for *B-myb* in hematopoiesis. Wild-type mice (CD45.1) were transplanted with control or *B-myb**F/Fcre* (CD45.2) whole BM (WBM). (A) Animals transplanted with BM of both genotypes showed nearly equal donor cell contribution at 8–10 wk posttransplant, before plpC administration. (B) Total number of control and *B-myb*-deficient CD45.2<sup>+</sup> cells in the WBM of recipient animals following treatment with plpC. (C) Total number of erythroid (TER119<sup>+</sup>), B- (B220<sup>+</sup>), T- (CD3<sup>+</sup>), and myeloid lineage (CD11b<sup>+</sup>Gr1<sup>+</sup>) CD45.2<sup>+</sup> cells in the BM of recipients transplanted with control and *B-myb*-deficient BM following plpC treatment. Total number of control and *B-myb*-deficient CD45.2<sup>+</sup> HSCs (D) and myeloid progenitors (CMPs, GMPs and MEPs) (E) in the BM of recipient animals following treatment with plpC. All values represent mean  $\pm$  SEM.  $n \geq 3$  per population for each genotype. \*\*\* $P \leq 0.0005$ .



**Fig. 4.** Loss of *B-myb* results in aberrant cell cycle progression in HSCs and increased cell death of myeloid progenitors. (A) BM was harvested from plpC-treated control and *B-myb* F/Fcre mice 2 h following injection with BrdU. The cells were then subjected to flow cytometric analysis to determine the percentage of cells in the indicated populations that are in the G<sub>0</sub>/G<sub>1</sub> (lower left gate), S (upper gate), and G<sub>2</sub>/M (lower right gate) phases of the cell cycle. (B) Frequency of Annexin V<sup>+</sup>DAPI<sup>+</sup> HSCs and myeloid progenitors of plpC-treated control and *B-myb*-deficient mice. (C) Frequencies of myeloid progenitors in the BM of plpC-treated control and *B-myb*-deficient mice. Representative plots are shown. All numerical values represent mean ± SEM for three independent experiments. *n* ≥ 3 per population for each genotype. \**P* ≤ 0.05, \*\**P* ≤ 0.005, \*\*\**P* ≤ 0.0005.

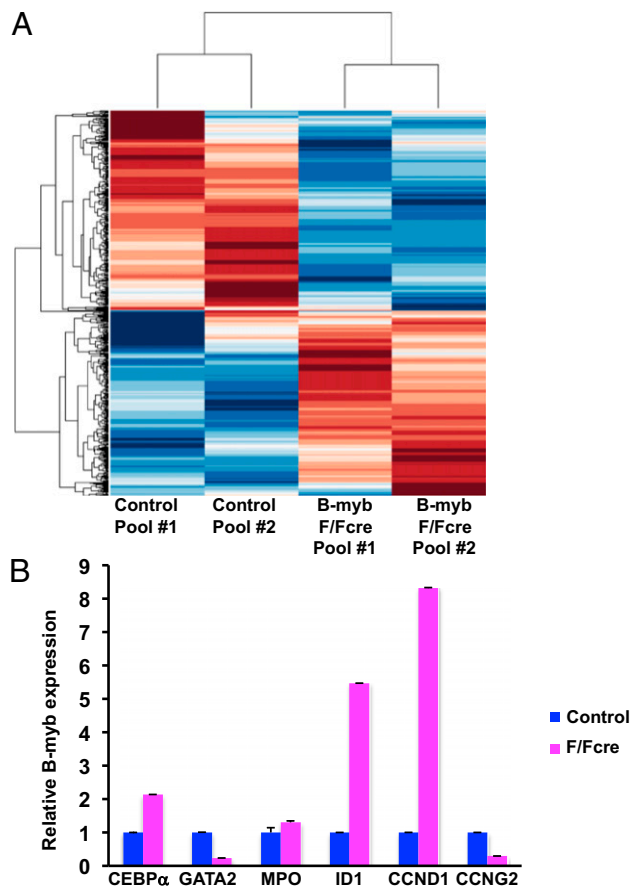
these populations from control and *B-myb* F/Fcre mice (16) and plated them in GM-CSF-containing medium in the presence of IFN-α to delete floxed sequences in vitro (12). Loss of *B-myb* resulted in lower cell numbers, reflecting an inhibition of both CMP and GMP proliferation (Fig. S7A). Furthermore, a sharp decrease in the frequency of CD11b<sup>+</sup> and Gr1<sup>+</sup> mature myeloid cells was observed in cultures of CMPs. The frequency of the CD11b<sup>+</sup>Gr1<sup>hi</sup> population in the *B-myb* F/Fcre GMP cultures was also significantly decreased (Fig. S7B), suggesting that loss of *B-myb* affects proliferation and differentiation along the myeloid lineage.

**Disruption of *B-myb* Results in Altered Gene Expression in LSK<sup>+</sup> Cells.**

To gain mechanistic insight into the hematological defects in *B-myb*-deficient mice, we performed a oligonucleotide microarray analysis of the Lin<sup>-</sup>Scal<sup>+</sup>ckit<sup>+</sup> (LSK<sup>+</sup>) population because the defect in development originates in the HSCs. Comparative analysis and hierarchical clustering identified 438 and 470 genes that were significantly up- and down-regulated, respectively, in the *B-myb* KO cells (Fig. 5A). When these gene lists were further analyzed using the ChIP Enrichment Analysis (ChEA) database within ENRICHR (<http://amp.pharm.mssm.edu/Enrichr>) (26, 27), considerable overlap was observed with the promoters of genes that are occupied by transcription factors during the control of maintenance, development, and multipotency of HSCs and progenitor cells as well as embryonic stem cells (ESCs) (Dataset S1) (28–34). Of these transcription factors, C-MYC and E2F-1 are known to be intricately involved in B-MYB-dependent transcription (22, 25, 35–38), whereas others, such as JARID1A, RUNX1 (AML1), TAL1 (SCL), LMO1, and FLI1, are not as well defined with respect to B-MYB transcriptional activation and repression of its targets (25, 39). In addition to the transcription factors listed above, we also detected an overlap with

genes that are targets of C-MYB, which is known to regulate promoters that are both distinct from and common with other members of the MYB family (40, 41).

We next confirmed the pattern of expression of differentially expressed genes known to be expressed in HSCs that, based on the predicted level of expression in the absence of *B-myb*, might possibly account for the observed phenotype. Quantitative PCR (qPCR) revealed that the level of *cyclin D1* (*CCND1*), which promotes G<sub>1</sub>/S progression, is increased by approximately eight-fold in *B-myb*-deficient LSK<sup>+</sup> cells (Fig. 5B). We also detected a decrease in S/G<sub>2</sub> cyclin, *cyclin G2* (*CCNG2*) expression after *B-myb* deletion. The dramatic changes in the myeloid progenitor compartment of the *B-myb* KO mice also prompted us to assess the expression of select genes that govern myeloid lineage development. ID1, GATA2, and CEBPα are transcription factors that regulate HSC development as well as various stages of myeloid lineage commitment (42). *ID1* levels, which normally increase as cells transition to the GMP stage (43), were elevated in *B-myb* KO LSK<sup>+</sup> cells (Fig. 5B), along with those of *CEBPα* and to a lesser extent, myeloperoxidase (*MPO*). Of these, *CEBPα* is required for the development of CMPs to GMPs (44). *GATA2*, on the other hand, was decreased in the absence of *B-myb*. We also examined the expression pattern of genes that modulate S phase progression and G<sub>2</sub>/M transition, some of which have been implicated in the proliferative defects observed in *B-myb*-null ESCs (22). Of the genes analyzed in Fig. S8, only *PLK1* and *BIRC5* (*survivin*) were predicted to be differentially expressed. The expression pattern of these genes does not overlap with *B-myb* KO ESCs, which might suggest a differential use of target genes in LSK<sup>+</sup> cells, or, simply reflects a greater accumulation of *B-myb*-deficient LSK<sup>+</sup> cells in S phase compared with ESCs (22). Given that *B-myb*-null HSCs display a profound engraftment defect,



**Fig. 5.** Computational analysis of the *B-myb* LSK<sup>+</sup> transcriptome. (A) Clustering analysis of altered gene expression in microarrays of control and *B-myb*-depleted LSK<sup>+</sup> cells. (B) Quantitative mRNA expression (qPCR) of differentially expressed genes in control and *B-myb* KO LSK<sup>+</sup> cells. All results were normalized to  $\beta$ -actin expression and are graphed as the average fold-change ( $\pm$ SEM). PCR reactions were performed in triplicate using cDNAs isolated from at least two independent pools of sorted cells per genotype.

these studies suggest that loss of *B-myb* induces changes in gene expression that impair self-renewal and results in aberrant lineage commitment.

## Discussion

Here, we describe the effect of loss of *B-myb* during adult hematopoiesis. Our studies show that *B-myb*-deficient mice are pancytopenic, with dramatic losses in BM and splenic cellularity, have a profound cell-autonomous defect in HSC repopulation capacity, altered HSC (and to lesser extent of myeloid progenitor) cycling, as well as increased apoptosis of myeloid progenitors. Studies have shown that cycling HSCs and those that have recently undergone cell division have profound reductions in their engraftment potential, and that long-term repopulation is dependent on the ability of these cells, or at least a portion of them, to remain in a quiescent state (18). HSC quiescence is key to maintaining the balance between long-term self-renewal and differentiation. Accordingly, genetic disruption of genes that alter cell division cause BM failure due to HSC exhaustion (45). Analysis of *B-myb*-deficient HSCs revealed that significant percentages of cells are in the S and G<sub>2</sub>/M phases. *B-myb* KO myeloid progenitors also have increased frequencies of G<sub>2</sub>/M phase cells. In addition, CMPs and GMPs in which *B-myb* is disrupted in vitro proliferate more slowly. These results are consistent with previous reports documenting a role for *B-myb* in S/G<sub>2</sub>/M progression and checkpoint control (20, 22, 25,

46, 47). A similar phenotype is also observed in the zebrafish *crash&burn* (*crb*) mutant (48), which harbors a loss of function mutation in *bmyb*. Cells in these embryos have delays in mitotic progression, genomic instability, and increased cell death, and it is thought that loss of *bmyb* during S phase impairs CYCLIN B1 expression, which is required for progression through G<sub>2</sub> (48, 49). Although we encountered difficulties in examining the cell cycle of LT-HSCs as a distinct population in *B-myb* KO mice due to their extremely low numbers, our data suggest that loss of *B-myb* pushes HSCs out of quiescence, but arrests them in the S and G<sub>2</sub>/M phases. As loss of quiescence is associated with a failure to maintain long-term self-renewal (45), these data provide a partial explanation for HSC depletion in these animals. *B-myb* KO LSK<sup>+</sup> cells express substantially higher levels of *CCND1* mRNA, which, in complex with CDK4/6, drives the cell through G<sub>1</sub> and into S-phase (50). It is interesting to note that differentiation of LT-HSCs into ST-HSCs and MPPs, but not HSC self-renewal, correlates with the induction of *CCND1* mRNA (18). As the *CCND1* promoter is bound by B-MYB in ChIP assays (25), this increase in *cyclin D1* mRNA is likely due to the absence of repression of the *CCND1* promoter by B-MYB. HSC differentiation is also accompanied by increases in *CCNG2*, whereas self-renewing HSCs do not exhibit such changes in gene expression (18). *CCNG2* is considered an atypical cyclin in that its up-regulation is associated with cell cycle inhibition, and studies have demonstrated the involvement of this gene in the induction and/or maintenance of cell cycle arrest (51). *CCNG2* mRNA levels in *B-myb*-deficient LSK<sup>+</sup> cells were decreased, and it is therefore possible that *B-myb* KO HSCs do enter the differentiation program but ultimately fail to complete it. It is interesting to note that even though the S and G<sub>2</sub>/M checkpoints in *B-myb*-null ESCs are defective, these cells still progress through the cell cycle, even with a loss of genomic integrity (20, 22). Hence, defects in replication stress responses could also lead to abnormalities in HSC function and differentiation in the absence of *B-myb* expression.

Because multiple lineages are affected by the loss of *B-myb*, we also examined the level of cell death in the stem and progenitor compartments. We did not detect increases in the frequency of Annexin V<sup>+</sup> HSCs or MPPs; however, we did observe increased cell death in the three populations of myeloid progenitors, which would additionally contribute to the depletion of mature myeloid cells. As previously stated, despite the fact that *B-myb* KO mice have a profound reduction in the number of myeloid progenitors and mature myeloid cells, the percentage of GMPs in these animals is actually increased compared to controls. Accordingly, the level of *ID1*, which is normally up-regulated as the cells transition from the LMPP to GMP stage (43), is higher in *B-myb*-deficient LSK<sup>+</sup> cells. In addition, we observed higher levels of *CEBP $\alpha$*  and lower levels of *GATA2* in *B-myb* KO LSK<sup>+</sup> cells. Previous studies have shown that GMP development is also favored if *CEBP $\alpha$*  is up-regulated before *GATA2*, and these cells will continue to give rise to neutrophils and monocytes if *CEBP $\alpha$*  expression is maintained (1).

When viewed from the perspective of increased GMP frequency and its relation to pancytopenia, the *B-myb* KO phenotype is remarkably similar to that seen in patients with high-risk myelodysplastic syndrome (MDS) (52, 53), a group of blood disorders that are characterized by cytopenias that arise due to ineffective hematopoiesis and often progress to acute myelogenous leukemia. *MYBL2* is expressed at 20–30% of normal levels in CD34<sup>+</sup> cells of ~65% of MDS cases, irrespective of karyotype (46, 54). Consistent with the notion that partial loss of *MYBL2* expression is associated with MDS, it has recently been shown that *B-myb* heterozygous mice develop MDS and other myeloproliferative neoplasms at 12–24 mo of age (54). Although the hematopoietic compartment of younger animals did not show significant changes, the phenotype of a subset of the aged animals, particularly those

subjected to transplant-induced replicative stress, is similar to that of *B-myb* KO mice immediately following pIpC administration. These data suggest that *B-myb* haploinsufficiency cooperates with other genetic lesions to cause disease, in contrast to that which occurs in the absence of *B-myb* expression or in cells expressing levels of *B-myb* that mirror those seen in CD34<sup>+</sup> MDS cells (approximately 20–30% of normal) (46). A better understanding of the role of *B-myb* in normal hematopoietic cell development will hopefully allow us to define its role in disease states that result in defective hematopoiesis, such as MDS.

## Materials and Methods

Detailed information on animals, bone marrow transplantation, and flow cytometry can be found in *SI Materials and Methods*. Microarray and RT-PCR analysis were performed as described in *SI Materials and Methods*. Primer sequences are provided in *SI Materials and Methods*.

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