Supporting Information

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SI Materials and Methods

Mice. To generate of B-*myb* floxed mice, a genomic fragment encoding exons 4–6 of the murine *B-myb* locus was isolated from a mouse 129sv/J library and subcloned into a modified pFlox gene-targeting vector that does not contain the thymidine kinase marker and permits cre-mediated deletion to occur in vivo (1). This plasmid was transfected into RW4 ES cells and euploid, homologous recombinant clones were injected into blastocysts using standard techniques. Germ-line transmission of the neo-floxed *b-myb* allele (B-*myb*^{Fneo}) was obtained and mice harboring this allele were then mated with EIIa-cre and C57BL6/J mice to obtain progeny that harbored a floxed B-*myb* allele (B-*myb*^{F}). All mice used for breeding and transplantation assays were purchased from The Jackson Laboratory. Experiments were performed under protocols approved by the Icahn School of Medicine at Mount Sinai's Institutional Animal Care and Use Committee according to federal and institutional guidelines and regulations.

Polyinosinic:polycytidylic acid (pIpC) was resuspended at a concentration of 1 mg·mL^{-1} in sterile Dulbecco's PBS (D-PBS). Mice were injected intraperitoneally (i.p.) at a dose of 10 mg·kg^{-1} body weight every other day over the course of 5 d (for a total of three injections). Tissues were harvested at the times indicated. Complete blood counts were measured using a Hemavet 950 multispecies hematology system (Drew Scientific Group).

Bone Marrow Transplantation. For transplantation assays, B-*myb* floxed mice were backcrossed for 10 generations onto a C57BL6/J background (CD45.2). B6.SJL-Ptprca Pep3b/BoyJ (CD45.1) or B-myb F/F and F/Fcre (CD45.2) adult recipient mice were lethally irradiated at a dose of 1,200 rads (split into two attenuated doses of 700 and 500 rads, 3 h apart), 18–24 h before transplantation. For noncompetitive transplants, 2×10^6 donor cells were injected into the retroorbital venous sinus of anesthetized recipient animals. For competitive, long-term repopulation assays, 1×10^6 donor and competitor cells were mixed in a 1:1 ratio (total of 2×10^6 cells) and injected as described above. All experiments were performed in triplicate using three to five recipients per donor for each experiment. Engraftment was monitored via FACS using peripheral blood samples obtained by submandibular vein bleeding at the indicated times (and up to 26 wk for competitive transplants) using antibodies directed against Gr1 (RB6-8C5) (PE), CD11b (M1/70), (PE-Cy7) CD3 (17A2) (FITC), B220 (RA3-6B2) (APC-AF750), CD45.1 (A20) (APC or Pacific Blue), and CD45.2 (104) (Pacific Blue, APC, or PerCPCy5.5).

Methylcellulose Assays. Whole bone marrow (BM) cells (1×10^4) were plated in 1.5 mL of Methocult M3434 (Stemcell Technologies) in a 35-mm dish in duplicate. Cells were cultured for 12–14 d before colonies were scored.

Immunoblot Analysis. Whole BM was harvested from by flushing the tibias and femurs of 8- to 12-wk-old animals with D-PBS supplemented with 2% (vol/vol) heat-inactivated FBS. Sorted Lin$^-$ cells were obtained as described in *Flow Cytometry*. Cells were lysed in lysis buffer (20 mM Tris·Cl, pH 7.5/150 mM NaCl/1% Nonidet P-40/0.5 mM EDTA) supplemented with protease inhibitor mixture (Roche Diagnostics) and 0.5 mM sodium orthovanadate as described previously by us (2–4). Twenty-five micrograms of clarified total cell lysate were separated by SDS/PAGE (8%), transferred to a nitrocellulose membrane, and probed with antibodies against B-MYB (Abcam) and β-actin (Sigma) according to the manufacturers' instructions. Proteins were visualized using an Odyssey imaging system (LI-COR Biosciences).

Flow Cytometry. Single-cell suspensions were prepared in phenol red-free RPMI supplemented with 2% heat-inactivated FBS. Defined numbers of cells were stained on ice for 30 min (or 90 min for CD34), washed, and then subjected to flow cytometric analysis. Fluorochrome-conjugated antibodies used for staining are as follows: lineage mixture [Gr1 (RB6-8C5), CD11b (M1/70), CD3 (17A2), B220 (RA3-6B2)] conjugated to either FITC, PE, or PerCP-Cy5.5; Sca-1 (D7) conjugated to either PE-Cy7, PerCP-Cy5.5 or Alexa Fluor 700; APC-H7 c-kit (2B8); CD34 (RAM34) conjugated to either APC, Alexa Fluor 647, or Pacific Blue; CD16/32 (93) conjugated to PerCP-Cy5.5, Alexa Fluor 647, or APC, PECy7 CD48 (BCMI); CD150 PerCPCy5.5 or APC (TC15-12F12.2); PE CD127 (A7R34); CD45.1 (A20) conjugated to either APC, PE, or PerCP-Cy5.5; CD45.2 (104) conjugated to either Pacific Blue, PE, or PerCP-Cy5.5. All antibodies were purchased from eBioscience, BioLegend, or BD Biosciences. Flow cytometric analysis and sorting was performed at the Flow Cytometry Shared Resource Facility at the Icahn School of Medicine at Mount Sinai. Data for analytical purposes were acquired using a LSRII (BD Biosciences). Sorted cell populations were obtained using Influx (BD Biosciences), Arial II (BD Biosciences), and MoFlo (Beckman Coulter) high-speed sorters. All data were analyzed using FlowJo software (Treestar). Cell cycle progression was measured using the FITC BrdU Flow Kit (BD Biosciences) according to the manufacturer's instructions except that DAPI was substituted at a concentration of 1 mg·mL^{-1} to measure DNA content. Mice received a single i.p. injection of BrdU (2 mg) 2 h before harvesting cells for flow cytometry. Annexin V staining was measured using the FITC Annexin V Apoptosis Detection Kit I (BD Biosciences) according to the instructions of the manufacturer except that DAPI was substituted at a concentration of 1 mg·mL^{-1} to measure DNA content.

In Vitro Deletion of B-*myb*. Common myeloid and granulocyte–monocyte progenitors (CMPs and GMPs) were sorted from control and B-*myb*F/Fcre mice as described previously (4, 5). Ten thousand cells of each sorted population were plated in Iscove's modified Dulbecco's medium supplemented with 10% heat-inactivated FBS and 50 ng/mL GM-CSF (Peprotech) in the presence and absence of 1,000 U·mL^{-1} recombinant IFN-α (PBL Assay Science). Cells were counted by trypan-blue exclusion and subjected to flow cytometric analysis 72 h postplating.

Microarray Analysis. Total RNA was extracted from FACS sorted LSK$^+$ populations isolated from pIpC-treated mice (1 wk post-treatment) using the ARCTURUS Picopure RNA isolation kit (Life Technologies). Three to five mice were pooled per sample. Gene expression profiling of two biological replicates for each population was performed using the Illumina Mouse WG-6 Version 2.0 Expression Beadchip kit at the Icahn School of Medicine at Mount Sinai's Genomics Core Facility. Data have been deposited in the Gene Expression Omnibus database (accession no. GSE53875).

Pathway Analysis. Illumina raw data were quantile normalized so that the distributions of samples could be directly compared. *t* tests where then applied with SciPy (http://scipy.org) to compare the control and B-myb–deficient LSK$^+$ datasets. A cutoff

P value of 0.01 was applied using Benjamini–Hochberg false discovery rate to include enough genes for enrichment analysis. Finally, the up and down gene lists were analyzed using the ChIP Enrichment Analysis database within ENRICHR (6, 7).

PCR. Genotyping of the B-*myb* locus using genomic DNA extracts was performed using the following primers: FWD: 5′-GTTCTCCATCCTGCCTTGAGT-3′ and REV: 5′-GCTCACGTCGCCTGAAAGAG-3′. For semiquantitative PCR, the number of amplification cycles was reduced to 25. Quantitation was performed using ImageJ software (http://rsbweb.nih.gov/ij/). RT-PCR conditions and primer sequences used to assess B-*myb* and β-actin expression levels in sorted BM populations have been previously described by us (8). For quantitative PCR (qPCR) of sorted LSK^+ cells, total RNA was extracted as described for the microarray analysis and subjected to reverse transcription using SuperScript III (Life Technologies). qPCR of the resulting cDNA templates was performed using the FastStart SYBR Green Master kit (Roche Diagnostics). Expression levels of individual genes were normalized to β-actin. Sequences of primers used for qPCR validation of the microarray data and quantitation of other previously published B-myb target genes are as follows: CCND1(F): 5′-ACTTCCTCTCCTGCTACCG-3′ and CCND1(R): 5′-AGGGCTTCAATCTGTTCCTG-3′; CCNG2(F): 5′-GAACAGAGATACCAACCTCGG-3′ and CCNG2(R): 5′-GTTTCAGTGCCAGATCCAAAG-3′; CEBPα (F): 5′-CATGCCGGGAGAACTCTAAC-3′ and CEBPα (R): 5′-CTGGAGGTGACTGCTCATC-3′; GATA2(F): 5′-CCCCTAAGCAGAGAAGCAAG-3′ and GATA2(R): 5′-ATTCATCTTGTGGTAGAGCCC-3′; ID1(F): 5′-GCTGAACTCGGAGTCTGAAG-3′ and ID1(R): 5′-GCCTCAGCGACACAAGATG-3′; myeloperoxidase (MPO)(F): 5′-CCCGCATTCCTTGTTTTCTG-3′ and MPO(R): 5′-GCTTCTCCCCATTCCATCG-3′; C-MYC(F): 5′-GCTGTTTGAAGGCTGGATTTC-3′ and C-MYC(R): 5′-GATGAAATAGGGCTGTACGGAG-3′; PLK1(F): 5′-TGTAGTTTTGGAGCTCTGTCG-3′ and PLK1(R): 5′-TCCCTGTGAATGACCTGATTG-3′; FOXM1(F): 5′-GCCATGATACAGTTTGCCATC-3′ and FOXM1(R): 5′-AGAGAAAGGTTGTGACGAATAGAG-3′; CDC25B(F): 5′-TGTGAATTCTCGTCTGAGCG-3′ and CDC25B(R): 5′-AAGTTCGGATGCTGTGGG-3′; AURKB(F): 5′-AGGGAGAACTGAAGATTGCAG-3′ and AURKB(R): 5′-CCCGATGCACCATAGATCTAC-3′; CENPF(F): CTTACCCAGGAGTTACAGCAAG-3′ and CDNPF(R): 5′-TGCCTGAAGAGCTTGTTCTG-3′; BIRC5(F): 5′-CGATTTGAATCCTGCGTTTGAG-3′ and BIRC5(R): 5′-AGTTCTTGAAGGTGGCGATG-3′; CCN2A(F): 5′-GTCCTTGCTTTTGACTTGGC-3′ and CCN2A(R) 5′-ACGGGTCAGCATCTATCAAAC-3′; CDC2A(F): 5′-TGCAGGACTACAAGAACACC-3′ and CDC2A(R): 5′-GCCATTTTGCCAGAGATTCG-3′; CENPE(F): 5′-TGGAAAGTAGAGAAAAGGCTGAG-3′ and CENPE(R): 5′-TCCTTCAATCGCACACCTTC-3′; CCNB1(F): 5′-CTGACCCAAACCTCTGTAGTG-3′ and CCNB1(R): 5′-CCTGTATTAGCCAGTCAATGAGG-3′; and β-actin(F): 5′-ACCTTCTACAATGAGCTGCG-3′ and β-actin(R): 5′-CTGGATGGCTACGTACATGG-3′.

Statistical Analysis. Statistical analysis was performed using a standard, unpaired, two-tailed Student *t* test. Data are graphed as mean ± SEM. Results were considered significant as $P \leq 0.05$.

1. Holzenberger M, et al. (2000) Cre-mediated germline mosaicism: A method allowing rapid generation of several alleles of a target gene. *Nucleic Acids Res* 28(21):E92.
2. Lieu YK, Kumar A, Pajerowski AG, Rogers TJ, Reddy EP (2004) Requirement of c-myb in T cell development and in mature T cell function. *Proc Natl Acad Sci USA* 101(41): 14853–14858.
3. Baker SJ, Kumar A, Reddy EP (2010) p89c-Myb is not required for fetal or adult hematopoiesis. *Genesis* 48(5):309–316.
4. Lieu YK, Reddy EP (2012) Impaired adult myeloid progenitor CMP and GMP cell function in conditional c-myb-knockout mice. *Cell Cycle* 11(18):3504–3512.
5. Akashi K, Traver D, Miyamoto T, Weissman IL (2000) A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature* 404(6774):193–197.
6. Chen EY, et al. (2013) Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics* 14:128.
7. Lachmann A, et al. (2010) ChEA: Transcription factor regulation inferred from integrating genome-wide ChIP-X experiments. *Bioinformatics* 26(19):2438–2444.
8. Lieu YK, Reddy EP (2009) Conditional c-myb knockout in adult hematopoietic stem cells leads to loss of self-renewal due to impaired proliferation and accelerated differentiation. *Proc Natl Acad Sci USA* 106(51):21689–21694.

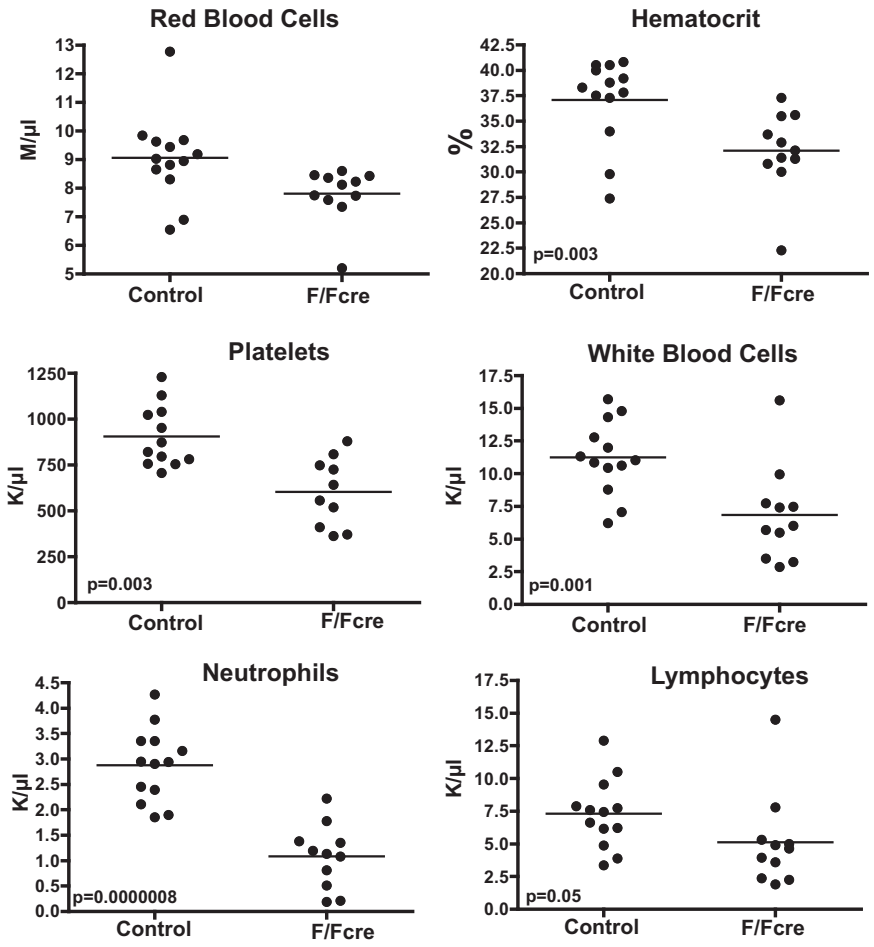

Fig. S1. Disruption of B-myb results in altered peripheral blood counts. Mean values are shown as horizontal lines in each column. n = 11 to 13 mice.

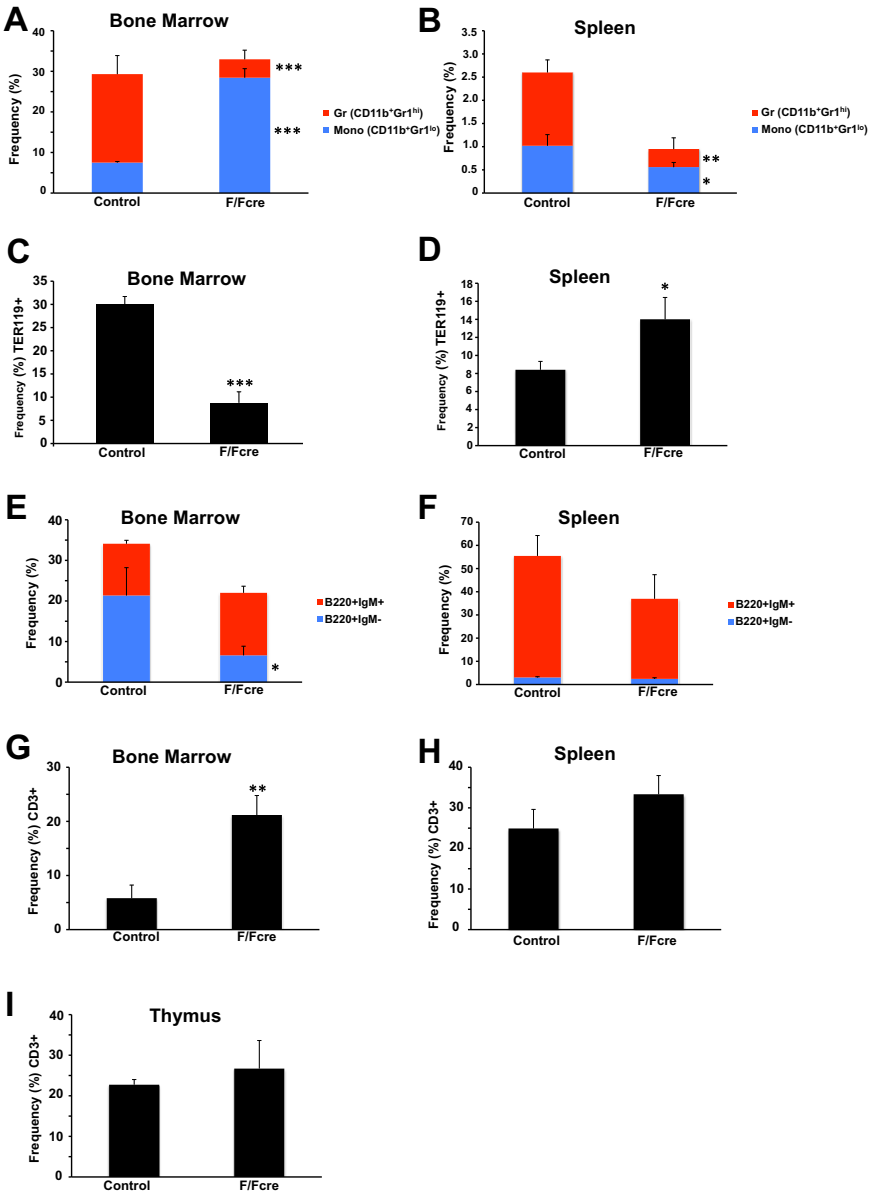

Fig. S2. Frequencies of myeloid (*A* and *B*), erythroid (*C* and *D*), B-(*E* and *F*), and T-lineage cells (*G* and *H*) in the BM, spleen, and thymus (*I*) of B-myb–deficient mice. All values represent mean ± SEM. $n \geq 3$ per population for each genotype. *$P \leq 0.05$, **$P \leq 0.005$, ***$P \leq 0.0005$.

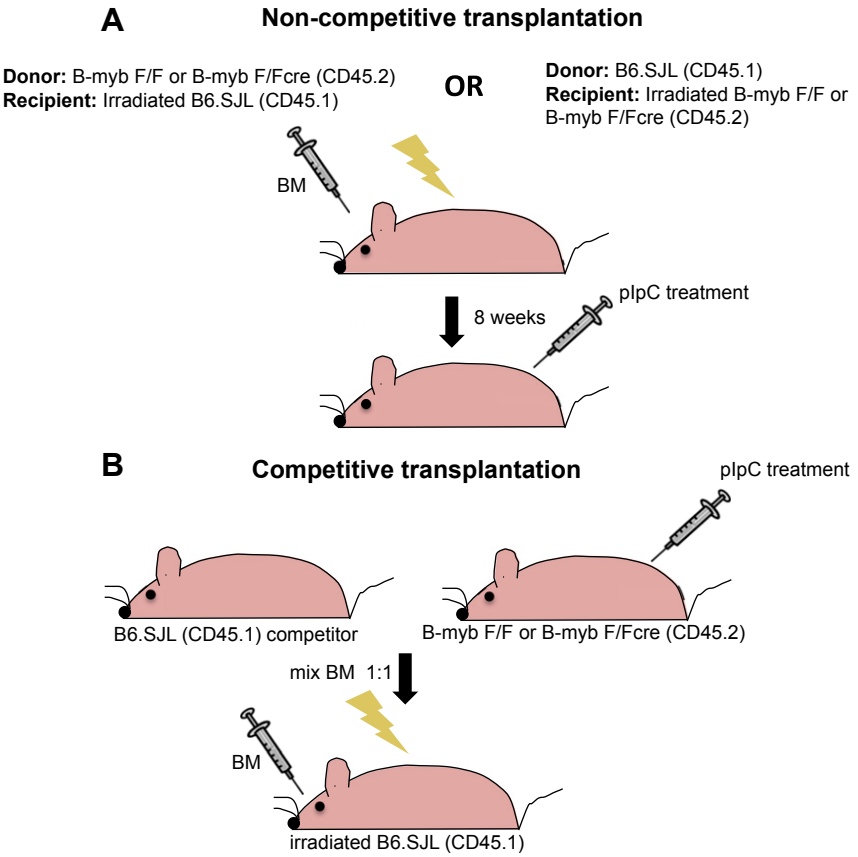

Fig. S3. Schematic representation of BM transplantation. (*A*) Schematic representation of noncompetitive transplants using BM isolated from B-mybF/F (control) or B-myb F/F MxCre mice (both CD45.2). Cells were transplanted into lethally irradiated recipient B6.SJL (CD45.1) mice. Alternatively, BM was isolated from wild-type B6.SJL (CD45.1) mice and transplanted into lethally irradiated B-mybF/F (control) or B-myb F/F MxCre mice (both CD45.2). pIpC was administered to all recipients 8 wk posttransplantation. (*B*) Schematic representation of competitive BM transplantation. BM isolated from B-mybF/F (control) or B-myb F/F MxCre mice (both CD45.2). pIpC-treated mice were mixed in a ratio of 1:1 with B6.SJL (CD45.1) competitor BM cells and transplanted into lethally irradiated CD45.1 recipient animals.

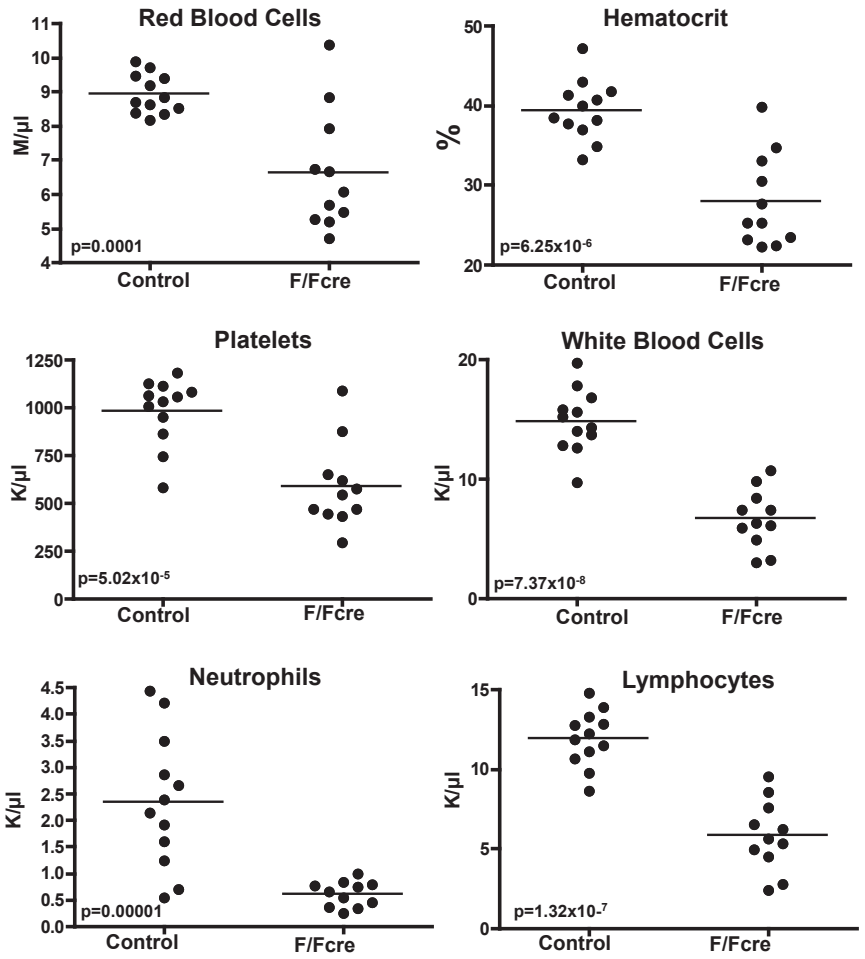

Fig. S4. Altered peripheral blood counts are conferred by the BM of B-myb–deficient mice. Lethally irradiated, wild-type mice (CD45.1) were transplanted with BM derived from control or B-mybF/Fcre mice (CD45.2). Animals were treated with pIpC at 8–10 wk posttransplant and peripheral blood counts were performed 21 d following pIpC administration. Mean values are shown as horizontal lines in each column.

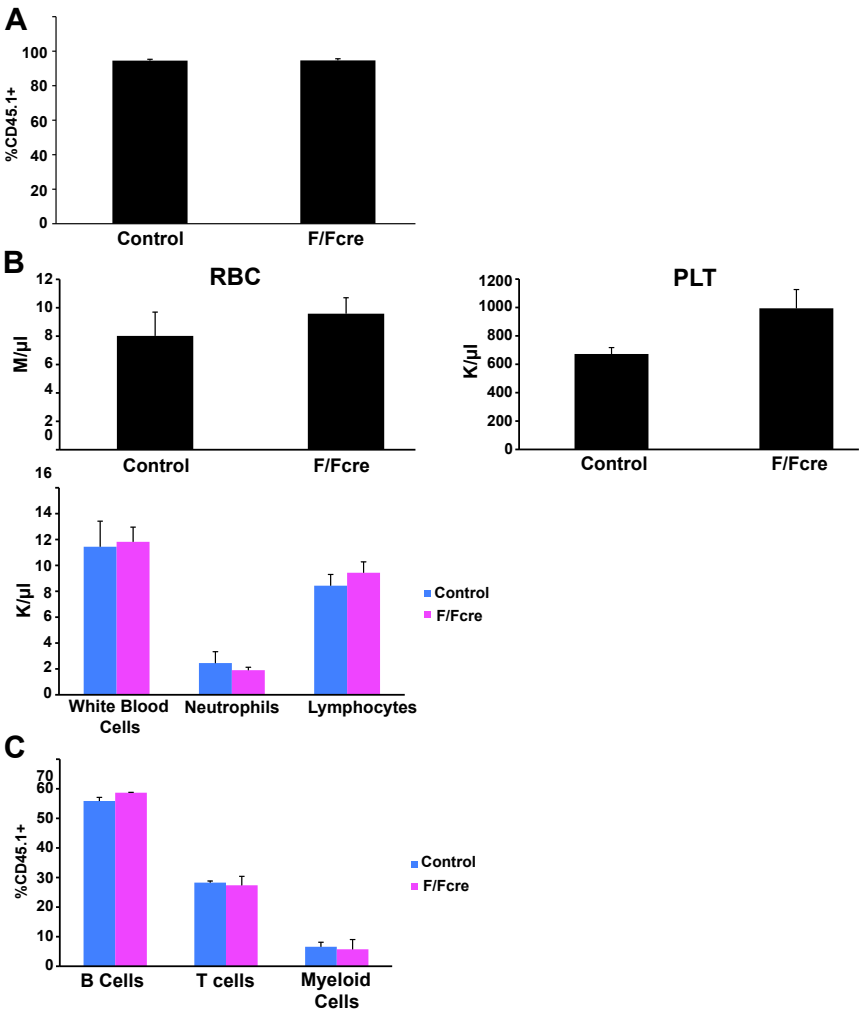

Fig. S5. A B-myb–deficient microenvironment does not confer abnormal hematopoeisis. Lethally irradiated control or B-mybF/Fcre animals (CD45.2) were transplanted with wild-type B6.SJL (CD45.1) BM. (*A*) Recipients of both genotypes showed comparable donor-cell reconstitution before pIpC administration (8–10 wk posttransplant). Complete blood counts (*B*) and total number of CD45.1$^+$ B-, T-, and myeloid lineage in the peripheral blood (*C*) of transplanted control or B-myb–deficient recipient animals. B cells, B220$^+$; T cells, CD3$^+$; myeloid cells, CD11b$^+$Gr1$^+$). All values represent mean ± SEM.

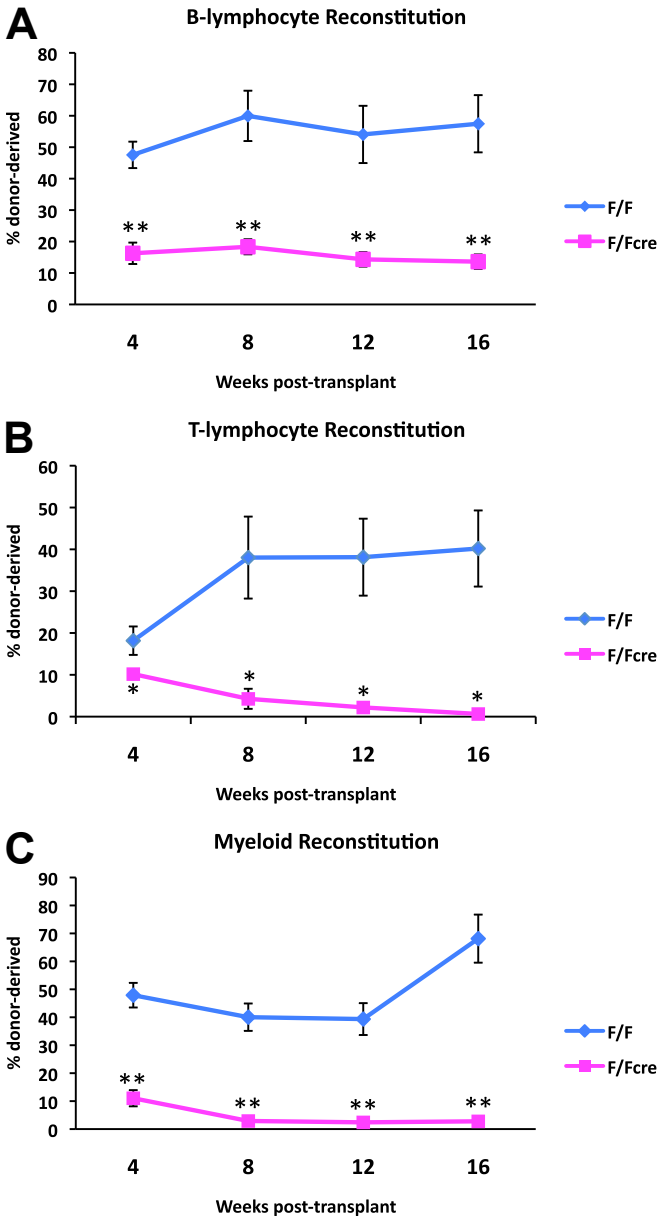

Fig. S6. Impaired BM reconstitution by B-myb–deficient HSCs. (*A*) B-, (*B*) T-, and (*C*) myeloid lineage reconstitution by donor cells derived from control B-myb floxed (F/F) and B-myb floxed-MxCre (F/Fcre) mice. Donor cells (1×10^6) were combined with 1×10^6 recipient cells and transplanted into irradiated mice. Transplants were performed 10 d after pIpC treatment. Reconstitution was monitored for a minimum period of 16 wk. Three independent experiments were performed. Data shown are from one representative experiment and all values represent mean ± SEM. $*P < 0.05$, $**P < 0.005$.

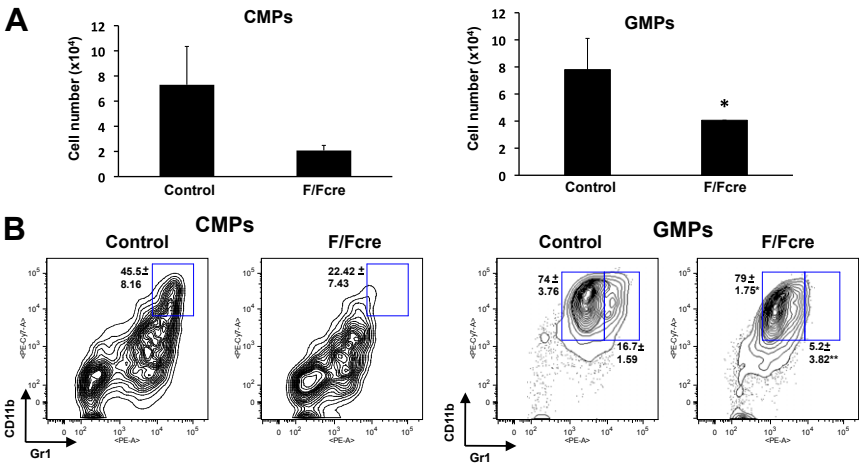

Fig. S7. Loss of B-myb impairs proliferation and differentiation of myeloid progenitors. Total number of viable cells (*A*) and frequencies of $CD11b^+$ and $Gr1^+$ cells (*B*) in control and B-myb F/Fcre CMPs and GMPs grown in the presence of GM-CSF and IFN-a for 72 h. Representative plots are shown. All numerical values represent mean ± SEM for three independent experiments. $n = 3$ per population for each genotype. $^*P \leq 0.05$, $^{**}P \leq 0.005$.

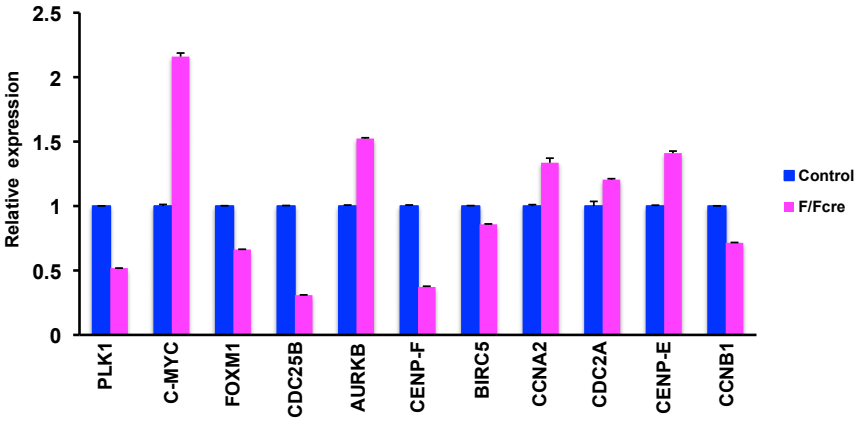

Fig. S8. Relative expression levels of genes that regulate S- and G_2/M-phase cell cycle progression in control and B-myb–deficient LSK^+ cells. All results were normalized to β-actin expression. PCR reactions were performed in triplicate and graphed as average fold-change ± SEM.

Other Supporting Information Files

Dataset S1 (XLS)