Supporting Information

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SI Materials and Methods

Mice and Bone Marrow Transplantation. *Gabpa*$^{fl/fl}$-Mx1Cre mice, genotyping, polyinosine-cytosine (pIC) injection, and bone marrow transplantation were previously described (1), and mice were bred to Rosa26-loxP-STOP-loxP-YFP mice (Jackson Labs). The chronic myelogenous leukemia (CML) mouse model was previously described (2).

Analysis of Peripheral Blood and Mouse Tissues. Complete blood counts were performed with Hemavet 950 (Drew Scientific). Preparation of bone marrow cells was previously described (1). Bone was fixed with formalin and decalcified with DeCal solution (Sigma-Aldrich). Tissue sections were prepared by the pathology core facility of University of Massachusetts Medical School, and examined with an Eclipse E600 bright-field microscope (Nikon).

Flow Cytometry, Cell Sorting, and Cell Cycle Analysis. Flow cytometry and cell sorting used LSRII and MoFlow (BD Biosciences), and staining of bone marrow and peripheral blood cells was previously described (1). For cell cycle analysis, cells were fixed with ethanol and stained with propidium iodide (Sigma-Aldrich) or with Ki-67 (BD Biosciences).

Reverse Transcription, Real-Time Quantitative PCR, and Immunoblotting. Reverse transcription, quantitative real-time PCR, and immunoblotting were performed as previously described (1). Primary antibodies included β-actin (I-19; Santa Cruz Biotechnology) and PRKD1, PRKD2, and phospho-PRKD (Cell Signaling Technology).

Cell Culture, Chemicals, and Retroviral Gene Transfer. Culture of NIH/3T3, HEK293 (American Type Culture Collection) cells and mouse bone marrow cells was performed as previously described (1). Human K562 cells were grown in RPMI/10% (wt/vol) FBS and CRT0066101 (Cancer Research Technology Discovery Laboratories, Wolfson Institute for Biomedical Research, London WC1E 6BT, United Kingdom) dissolved in DMSO. MTT assay was performed in a microplate reader (Victor 3 multilabel count; Perkin-Elmer). In vitro colony-forming assays were performed per manufacturer protocol (StemCell Technologies). Primary human normal or CML cells were cultured in Iscove modified Dulbecco medium (Sigma-Aldrich) supplemented with a serum substitute BIT, 40 μg/mL low-density lipoproteins, 100 ng/mL recombinant human Flt3-ligand, 100 ng/mL stem cell factor, 20 ng/mL recombinant human IL-3, 20 ng/mL IL-6, and 20 ng/mL granulocyte colony-stimulating factor (StemCell Technologies).

Data Analysis. Kaplan–Meier survival curves were generated with Prism 5 (GraphPad). Flow cytometry data were analyzed with Diva (BD Biosciences) and FlowJo software (Tree Star). DNA chromatin immunoprecipitation followed by high-throughput DNA sequencing (ChIP-Seq) data were analyzed with ChIP-Seeqer 2.0 software (3). DNA microarray data were analyzed with SAS 9.1 (SAS Institute). Annotation and demonstration of ChIP-Seq and microarray data were generated with BioGPS (4), University of California, Santa Cruz, Genome Browser (5), and Galaxy (https://main.g2.bx.psu.edu/).

1. Yang ZF, et al. (2011) GABP transcription factor is required for myeloid differentiation, in part, through its control of Gfi-1 expression. *Blood* 118(8):2243–2253.
2. Peng C, et al. (2010) PTEN is a tumor suppressor in CML stem cells and BCR-ABL-induced leukemias in mice. *Blood* 115(3):626–635.
3. Giannopoulou EG, Elemento O (2011) An integrated ChIP-seq analysis platform with customizable workflows. *BMC Bioinformatics* 12:277.
4. Wu C, et al. (2009) BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources. *Genome Biol* 10(11):R130.
5. Kent WJ, et al. (2002) The human genome browser at UCSC. *Genome Res* 12(6): 996–1006.

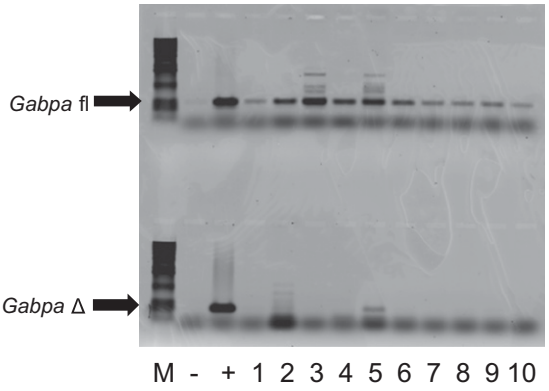

Fig. S1. Gabpα is essential for proliferation of bone marrow stem and progenitor cells. Bone marrow cells from *Gabpa*$^{fl/fl}$-Mx1Cre mice were harvested 21 d after pIC injection and subjected to in vitro colony forming assay. Genomic DNA were harvested from 100 individual colonies, 10 of which are illustrated here, and PCR was performed to amplify floxed (fl) and deleted (Δ) *Gabpa* alleles. M, DNA size marker, –, no DNA control; +, positive control DNA for indicated amplicons.

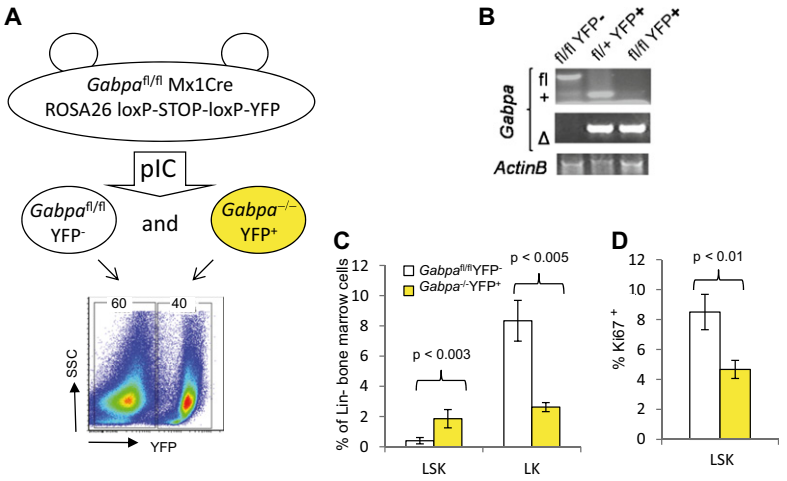

Fig. S2. Conditional deletion of *Gabpa* in mouse bone marrow reduces HSC cell cycle activity. (*A*) Schema for marking cells with yellow fluorescent protein (YFP) to distinguish YFP$^+$ Gabpa-null HSCs from YFP$^-$ replete (undeleted) HSCs, and flow cytometry of bone marrow 1 d after two pIC injections; numbers indicate the percentage of each population based on YFP expression. (*B*) PCR for *Gabpa* floxed (fl/fl), WT (+), or deleted (Δ) alleles, and *Actin B* control in sorted YFP$^+$ and YFP$^-$ bone marrow. (*C*) Percentage of HSC (LSK) and progenitor cells (LK) in the Lin$^-$ compartment of YFP$^+$ and YFP$^-$ cells, and (*D*) HSC cell cycle status based on Ki-67 expression.

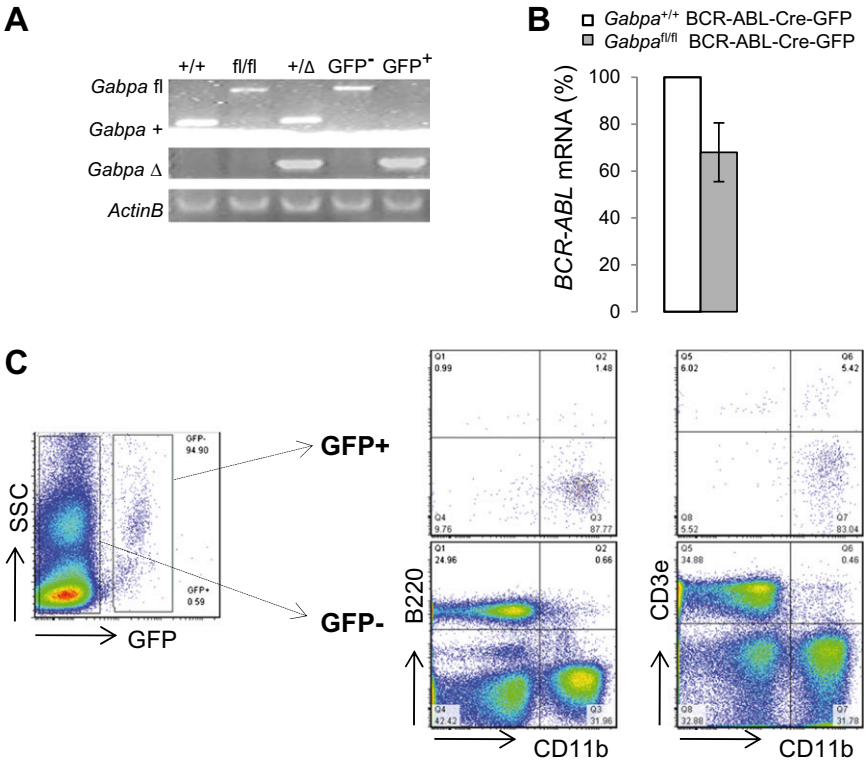

Fig. S3. PCR of genomic DNA demonstrates deletion of *Gabpa* in *Gabpa$^{fl/fl}$* bone marrow infected with breakpoint cluster region-ABL tyrosine kinase (BCR-ABL)-Cre-GFP retrovirus, and flow cytometry of peripheral blood from secondary recipient mice. (*A*) PCR for floxed (fl), WT (+), and deleted (Δ) *Gabpa* alleles, and *Actin B* control in sorted GFP$^-$ and GFP$^+$ peripheral blood granulocytes and in WT ($^{+/+}$), floxed (fl/fl), and heterozygous deleted (+/Δ) controls. (*B*) Quantitative RT-PCR for *BCR-ABL* expression in GFP$^+$ Gabpα-undeleted and GFP$^+$ Gabpα-null bone marrow cells. (*C*) Flow cytometry of peripheral blood for CD11b, B220, and CD3e expression on GFP$^-$ and GFP$^+$ cells 90 d after transplantation of the secondary recipient mice with bone marrow from primary recipient mice transplanted with BCR-ABL-Cre-GFP–infected *Gabpa$^{fl/fl}$* (fl/fl) cells; percentages of cells in relevant quadrants are indicated.

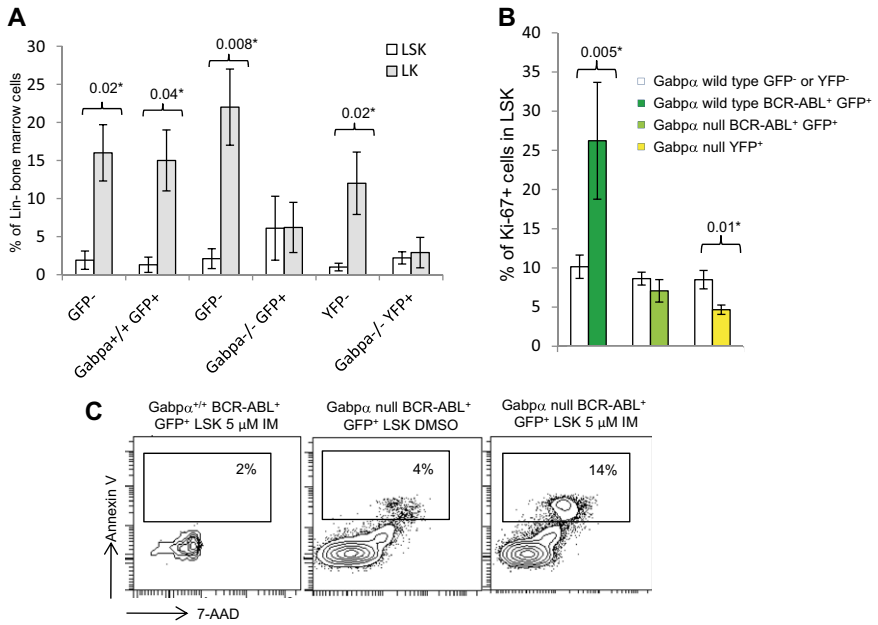

Fig. S4. *Gabpa* deletion causes loss of bone marrow progenitor cells, reduced cell cycle activity of HSCs, and increased sensitivity to imatinib for apoptosis. Flow cytometry analysis of bone marrow stem and progenitor cells from mice transplanted with BCR-ABL-Cre-GFP–infected *Gabpa*$^{+/+}$ or *Gabpa*$^{fl/fl}$ cells, or cells from *Gabpa*$^{fl/fl}$Mx1CreYFP mice after treatment with polyI:C. Bars represent the percentages of LK and LSK cells in Lin^{-} bone marrow (*A*) and the percentage of Ki-67^{+} cells in HSCs (*B*). Asterisks and *P* values of Student *t* test indicate statistical significance. (*C*) Bone marrow cells from mice transplanted with BCR-ABL-Cre-GFP–infected *Gabpa*$^{+/+}$ or *Gabpa*$^{fl/fl}$ cells were grown with 0.1% DMSO or 5.0 μM imatinib mesylate (IM) for 24 h, stained with annexin V and 7-AAD, and analyzed by flow cytometry.

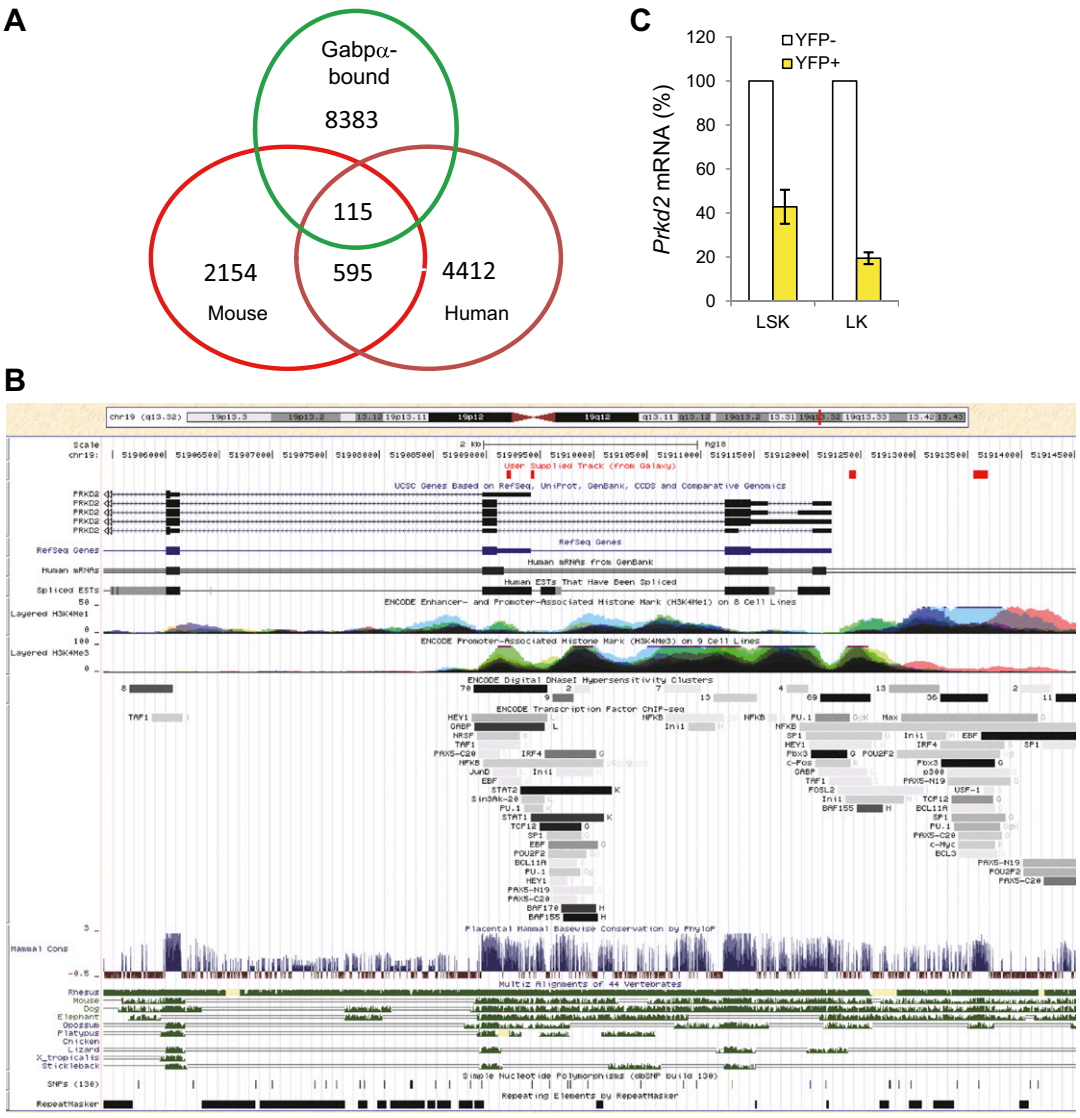

Fig. S5. Bioinformatic analysis of Gabpα-bound genes that are up-regulated in CML. (*A*) Venn diagram representing numbers of transcripts that are up-regulated at least twofold in mouse CML stem cells vs. normal HSCs, and in human CD34$^+$ CML cells vs. normal CD34$^+$ cells, overlapping with Gabp-bound mouse genes from ChIP-Seq. (*B*) Diagram of human PRKD2 chromatin binding by transcription factors, including GABPα. Red boxes at top indicate binding sites for GA binding protein (GABP) in human CD34$^+$ blood cells. Color peaks indicate promoter and enhancer locations of histone methylation at H3K4. Black and gray bars indicate binding sites for individual transcription factors reported with different cell lines. (*C*) Quantitative RT-PCR for Prkd2 expression in YFP$^+$ (Gabpα-null) and YFP$^-$ (undeleted) HSC (LSK) and progenitor cells (LK).

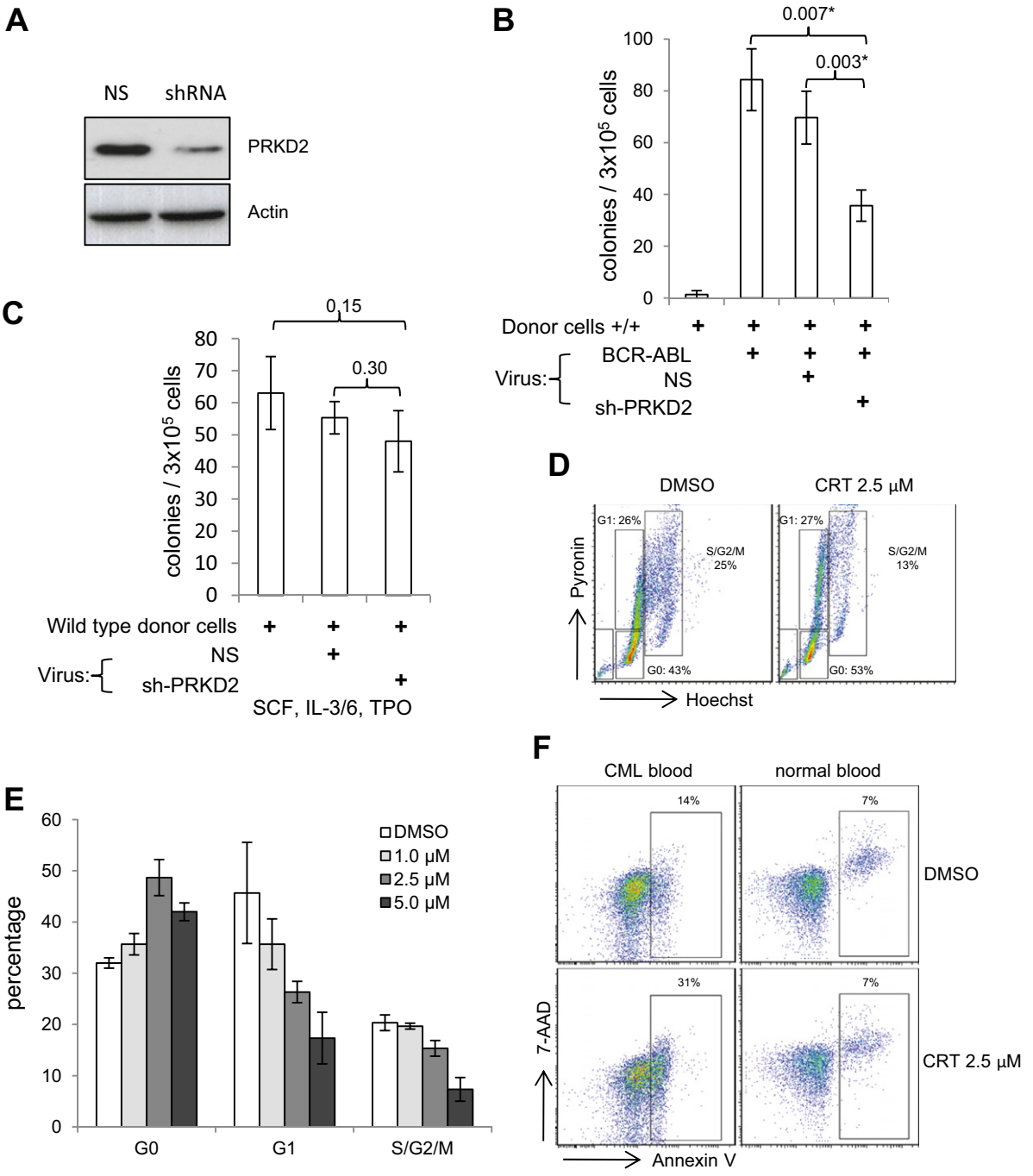

Fig. S6. PRKD2 mediates the effects of cell proliferation and apoptosis of CML cells. (*A*) Knockdown of Prkd2 by lentiviral infection of BCR-ABL–transduced WT bone marrow cells with scrambled (NS) or shRNAs directed against Prkd2, analyzed by immunoblotting with antibodies against PRKD2 or β-actin. (*B*) In vitro colony forming assay (in the absence of added cytokines) and (*C*) WT mouse bone marrow cells [in the presence of cytokines, including stem cell factor (SCF), IL-3, IL-6, and TPO] infected with the indicated viruses. (*D*) Cell cycle analysis of human primary CML blood cells treated with DMSO or 2.5 μM CRT0066101 for 48 h, followed by Hoechst and pyronin Y staining; and (*E*) summary of percentages of cells in G_0, G_1, and $S/G_2/M$ treated with indicated concentration of CRT0066101. (*F*) Flow cytometry of annexin V- and 7-AAD–stained human CML or normal blood cells after treatment with 2.5 μM CRT0066101 for 24 h.