SUPPLEMENTARY DATA

ANP32B-mediated repression of p53 contributes to maintenance of normal and CML stem cells

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Supplementary Methods

Mice

C57BL/6 CD45.2 mice were purchased from the Shanghai SLAC Laboratory Animal Co. Ltd. B-NDG® mice (NOD-*Prkdc*^{scid}*IL2rg*^{tm1}/Bcgen) were purchased from Beijing Biocytogen Co. Ltd. C57BL/6 CD45.1 mice (The Jackson Laboratory) were kindly provided by Dengli Hong at SJTU-SM. C57BL/6 *p53* knockout mice were kindly provided by Kewen Zhao at SJTU-SM. To specifically delete ANP32B in HSCs, C57BL/6 mice carrying the loxp-flanked *Anp32b* gene were established at the Shanghai Model Organisms Center and were further crossed with C57BL/6 *Scl-cre* mice. To induce Cre recombinase, Tamoxifen (Sigma, 10 mg/ml in corn oil) was administered at 50 μg/g body weight daily by intraperitoneal injection for 21 days. The primers used for genotyping were listed in Supplementary Table 1. All the animal experiments were approved by our institution and conducted according to the Guideline for Animal Care at Shanghai Jiao Tong University School of Medicine (SJTU-SM).

Flow cytometry

Bone marrow (BM) cells were freshly isolated and filtered through a 40 μm strainer to obtain single cell suspension. Peripheral blood (PB) cells were treated with ACK lysis buffer to remove red blood cells. The cells were surface-stained using fluorochrome-conjugated antibodies. Cell cycle was analyzed with Hoechst 33342 and Ki67-PE staining. Apoptosis was analyzed with Annexin V-PE and 7-AAD according to the manufacturer's instructions. Antibodies and dyes used in these experiments are listed in Supplementary Table 1.

Colony forming unit assays

BM cells from *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ mice were plated into 12-well plates containing MethoCult M3434 (StemCell Technologies) for CFU-GEMM colony formation analysis at 3×10^4 cells/well, MethoCult M3534 for CFU-GM colony formation analysis at 3×10^4 cells/well, MethoCult M3334 for CFU-E colony formation analysis at 3×10^5 cells/well, or MethoCult M3630 (StemCell Technologies) for CFU-Pre-B colony formation analysis at 3×10^5 cells/well according to the manufacturer's protocols. GFP$^+$LSKs sorting from primary CML recipients were then plated into 12-well plates containing complete MethoCult M3434 medium (StemCell Technologies) at 1000 cells/well. ANP32B-knockdown human HSCs and LICs were sorted and seeded into 12-well plates containing MethoCult H4436 (Stem Cell Technologies) at 200 cells/well. For some experiments, 5 μM *imatinib* or 2 μM Nutlin-3a was added to the medium for culture. The numbers of colonies were counted 7-10 days after plating.

Homing

1×10^7 BM cells from 8-10-week-old *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ mice were labeled with CFSE and injected into lethally irradiated CD45.1 mice. Total CFSE$^+$ cells in the BM, spleen, liver and CFSE$^+$LSKs, CFSE$^+$LT-HSCs, CFSE$^+$ST-HSCs, CFSE$^+$RLPs in BM (BM cells labeled with anti-lineage PerCP-Cy5.5, anti-Sca-1 PE-Cyanine7, anti-c-

Kit APC, anti-CD150 eFluor450 and anti-CD48 PE) were measured by flow cytometry 16h after transplantation.

Cell division tracing

BM cells from 8-10-week-old *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ mice were labeled with anti-lineage PerCP-Cy5.5, anti-Sca-1 PE-Cyanine7, anti-c-Kit APC, anti-CD150 eFluor450 and anti-CD48 PE, LT-HSCs were then sorted by flow cytometry and stained with 5 μM CFSE for 10 minutes in 37 ℃. Labeled cells were washed twice and cultured for 4 days. Harvest cells from culture wells and analyze directly by flow cytometry for cell division tracing. Cell divisions were multicolored and the CFSE^{max} proportion represents the first generation of LT-HSCs. For *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ mice injected with 5-FU (150 mg/kg) intraperitoneally, LT-HSCs were sorted at day 9. After staining with CFSE, cells were cultured for 15h and analyzed by flow cytometry.

For the analysis of CML, GFP^{+}LSK cells sorted from recipients receiving *BCR-ABL1*-transduced *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ Lin^{-} BM cells at 15 days post-transplantation were stained with 5 μM CellTrace™ Violet dye solution for 20 minutes in 37 ℃ and washed twice. After culturing for 24h, cells were harvested and analyzed by flow cytometry for cell division tracing. Multicolored plots refer to different generations and the Violet^{max} proportion represents the first generation (G1).

BrdU incorporation assay

Mice were injected with BrdU (100 mg/kg) intraperitoneally and then fed with BrdU-containing drinking water (1 mg/ml) for 2 days. BrdU incorporation was assessed via FACS analysis by using a FITC BrdU Flow Kit (BD) according to manufacturer's instructions.

5-Fluorouracil challenge

5-Fluorouracil (5-FU) was intraperitoneally administered to *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ mice at a dose of 150 mg/kg weekly for 3 weeks. The survival rates of the two groups were compared using a log-rank test.

CML patient samples and normal cord blood samples

Mononuclear bone marrow cells (BMMCs) were separated by density gradient centrifugation from 10 control bone marrow samples (non-leukemia) and 13 primary CML bone marrow samples. CML CD34^{+} cells were enriched from BMMCs by using EasySep™ Human CD34 Positive Selection Kit II (StemCell Technologies). All patient samples were collected at diagnosis before therapy. All CML patients were diagnosed in chronic phase and *BCR-ABL1* positive. The detailed information of control and CML patients were showed in Supplementary Table 2.

Human cord blood CD34^{+} cells were enriched from mononuclear cells separated from healthy people' fresh cord blood samples by using EasySep™ Human CD34 Positive Selection Kit II (StemCell Technologies).

Viral infection of human CD34^{+} cells and murine xenografts

Human cord blood CD34^{+} cells and CML BM CD34^{+} cells were cultured in StemSpan™ SFEM medium supplemented with 50 ng/ml human SCF and 10 ng/ml human TPO. To induce the knockdown of ANP32B in human CD34^{+} cells, cells were transduced by two rounds of spin infection (1200 g, 32 °C, 90 min) in lentiviral shRNA supernatant. After the second round of infection, cells were collected and sorted for GFP-positive cells to do experiments like colony-forming assay, proliferation culture

and mice transplantation. 1×10^5 $CD34^+$ cells were transplanted into sub-lethally irradiated B-NDG mice via tail-vein injection.

Immunoprecipitation and mass spectrometry analysis

Immunoprecipitation of Flag-tagged proteins or endogenous proteins and Nano-LC−MS/MS with electrospray ionization used to identify interacting proteins were performed as previously described.[1, 2] The antibodies used in immunoprecipitation were listed in Supplementary Table 1. The interacting proteins identified were listed in Supplementary Table 3.

Recombinant expression and GST/S-tag pulldown

Recombinant ANP32B, p53 and derivates with N-terminal His-tag and S-tag were expressed using the PET-32a vector expression system and purified by Ni-NTA-μSphere chelated with Ni2SO4. Recombinant ANP32B was digested to remove the His-tag and S-tag, while recombinant p53 was digested to remove the His-tag but not S-tag. Recombinant proteins were kept in 20 mM Tris-HCl, pH 7.8, 500 mM NaCl, 1 mM DTT. GST and GST-tagged p53 proteins were purified using the pGEX-4T3 vector expression system and purified using GST Beads (Merck Millipore). For the GST/S-tag Pulldown assay, GST beads binding with GST or GST-tagged p53, S protein agarose (Merck Millipore) binding with S or S-tagged p53 were separately mixed with recombinant ANP32B in binding buffer (0.1% Triton X-100/PBS) for 4 h at 4 °C and subsequently washed five times with the binding buffer.

Immunofluorescence

Details of immunofluorescence have previously been described.[3] Primary

antibodies used are shown in Supplementary Table 1.

Luciferase assay

The relative luciferase activity was measured according to dual-luciferase assay protocol (Promega, E1910).

Quantitative RT-PCR

Total RNA was prepared using Trizol (Invitrogen) and subjected to reverse transcription to synthesize cDNA using random primers (Takara) and M-MLV Reverse Transcriptase (Promega) following the manufacturer's instructions. Quantitative RT-PCR was performed using the SYBR Green PCR Master Mix (Applied Biosystems). The primers used are listed in Supplementary Table 1.

ChIP-qRT-PCR

ChIP was performed with 5×10^5 cells per reaction. *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ LSK cells and CML GFP$^+$LSK cells were sorted and crosslinked in 1% formaldehyde, quenched in 0.125 M glycine, and lysed in Cell Lysis Buffer (50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 1% SDS). Lysates were sonicated with an EpiShear Probe Sonicator (Active Motif) and diluted 10 folds with Dilution Buffer (20 mM Tris-HCl (pH 8.0), 2 mM EDTA, 150 mM NaCl, 1% Triton X-100). Sonicated chromatin was incubated with 3 μg antibody at 4 °C. After overnight incubation, ChIP-Grade Protein G Agarose Beads (Cell Signaling Technology) were added to the ChIP reactions and incubated for 4 h at 4 °C to collect the immunoprecipitated chromatin. Subsequently, Protein G Agarose Beads were washed three times with 1ml ChIP Buffer (Cell Signaling Technology), once with 1 ml ChIP Buffer with 0.35 M NaCl. The chromatin was eluted in 1× ChIP

Elution Buffer (Cell Signaling Technology) followed by reverse crosslinking at 65 °C for at least 4 h. ChIP DNA was purified using QIAquick Spin Columns (Qiagen). The purified ChIP DNA was quantified by qRT-PCR. The primers used are listed in Supplementary Table 1.

RNA-Seq and gene set enrichment analysis

RNA-Seq was performed by NovelBio. The clean reads were aligned to mouse genome (version: GRCm38 NCBI) or human genome (version: GRCh38 NCBI) using the HISAT2. HTSeq was used to count gene and RPKM method was used to determine the gene expression. Differential expression analysis was performed using DESeq2 algorithm. For GSEA analysis, the p53 regulated gene set used in RNA-seq data from *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ LSK cells was created with differentially expressed genes analyzed from RNA-seq data of three biologically independent *p53*$^{+/+}$ and *p53*$^{-/-}$ HSPCs (FDR < 0.05, Log_2FoldChange > 1, *p53*$^{-/-}$ versus *p53*$^{+/+}$) obtained from the GEO (accession code GSE137126).[4] Another p53 regulated gene set used in RNA-seq data from KU812 cells was created with differentially expressed genes analyzed from RNA-seq data of CML CD34$^+$ cells treated with RITA or not (FDR < 0.05, Log_2FoldChange > 1 or < −1, RITA versus NDC) obtained from the European Nucleotide Archive under accession number PRJEB9942.[5] GSEA analysis was performed as described.[6]

Electrophoretic mobility shift assay

For the extraction of the nuclear fraction from 293T cells, NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific) was used according to

manufacturer's instructions. LightShift™ Chemiluminescent EMSA Kit and 0.45 μm Biodyne™ B Nylon Membrane (Thermo Fisher Scientific) were used to do the EMSA experiment. Briefly, nuclear fraction was incubated with a biotin-labelled p53 probe (30 bp, Beyotime Biotechnology) containing the p53-binding element of the p21 promoter in 1× binding buffer with 50 ng/μl poly(dI·dC) for 20 min at room temperature (RT), with or without highly purified ANP32B, control GST protein, or the free p53 probe. The complex was analyzed by 4% Tris-Borate-EDTA buffer–polyacrylamide gel electrophoresis (TBE-PAGE) and visualized by autoradiography according to manufacturer's instructions.

Western blot assay and capillary electrophoresis-based immunoassay

Western blot assay was performed as previously described.[3] For the detection of ANP32B, p53 and β-actin in *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ LSK cells, the proteins were separated and detected using an automated capillary electrophoresis system Jess (ProteinSimple) with capillary cartridges of 12-230 kDa (ProteinSimple). Signals were detected with an HRP-conjugated secondary anti-rabbit or anti-mouse antibody and were visualized using the Proteinsimple Compass software.

Study approval

BM mononuclear cells of CML patients and human cord blood cells were kindly provided by the Department of Hematology at Ren-Ji Hospital, International Peace Maternity and Child Health Hospital, Shanghai Jiao Tong University School of Medicine. Written informed consent was obtained from all the patients, and all the procedures were approved by the Ethics Committee for Medical Research (IRB) at

Shanghai Jiao Tong University School of Medicine.

Supplementary Tables

Supplementary Table 1. Reagents, primers and plasmids used

Supplementary Table 2. Patients' information

Supplementary Table 3. Mass spectrometry data

Supplementary Figures

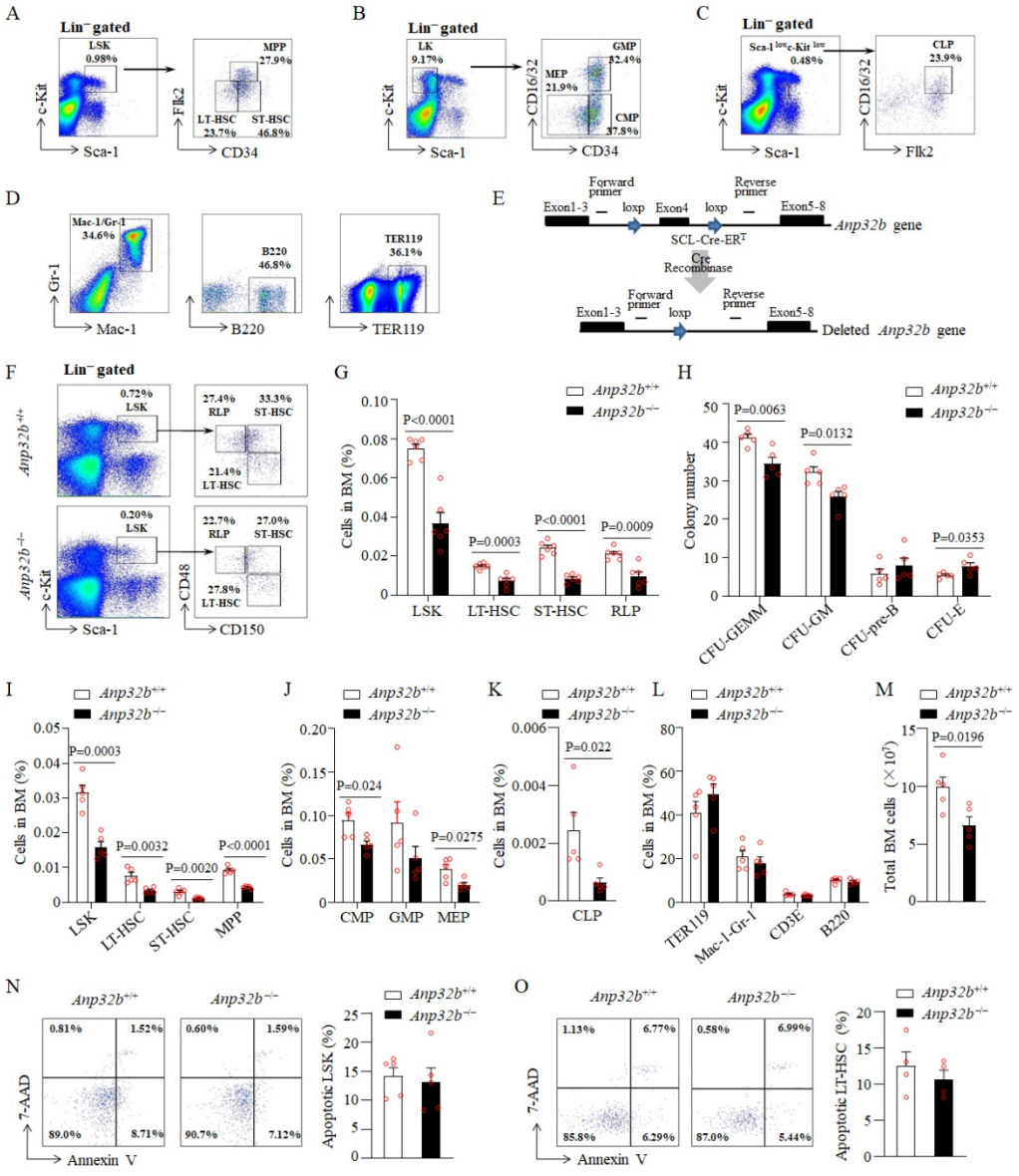

Figure S1. Deletion of *Anp32b* reduces HSC numbers by promoting quiescence.

Related to Figure 1.

(A-D) Representative FACS sorting profiles analyzed in figure 1A.

(E) Schematic of the Anp32b floxed allele showing the deletion of floxed exon 4 following Cre recombinase activity. Use of *Scl-Cre-ER*^{T} results in specific deletion in HSCs following tamoxifen treatment.

(F-G) Representative FACS profiles (F) and frequencies (G) of LSK cells, LT-HSCs ($Lin^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}CD150^{+}CD48^{-}$), ST-HSCs ($Lin^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}CD150^{+}CD48^{+}$) and restricted lineage progenitors (RLPs, $Lin^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}CD150^{-}CD48^{+}$) in BM from 8-10-week-old *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ mice ($n = 6$).

(H) Total BM cells from 8-10-week-old *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ mice were seeded in indicated methylcellulose medium for the quantification of CFU-GEMM, CFU-GM, CFU-pre-B and CFU-E colonies ($n = 5$).

(I) Frequencies of LSK cells, LT-HSCs, ST-HSCs and MPPs in BM from 14-month-old *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ mice ($n = 5$).

(J-L) Frequencies of CMPs, GMPs, MEPs (J), CLPs (K), erythroid cells, myeloid cells, T and B cells (L) in BM from 14-month-old *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ mice ($n = 5$).

(M) Total number of BM cells was calculated in 14-month-old *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ mice ($n = 5$).

(N-O) Apoptosis analysis of LSK cells ($n = 5$) (N) and LT-HSCs ($n = 4$) (O) in BM from 8-10-week-old *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ mice. Representative FACS profiles (left) and percentages of apoptotic cells (right) were shown.

Error bars denote mean ± SEM. Statistical significance was determined by two-tailed unpaired *t*-test (G-O). All animal experiments were repeated at least twice with similar results, and the results of one representative experiment are shown.

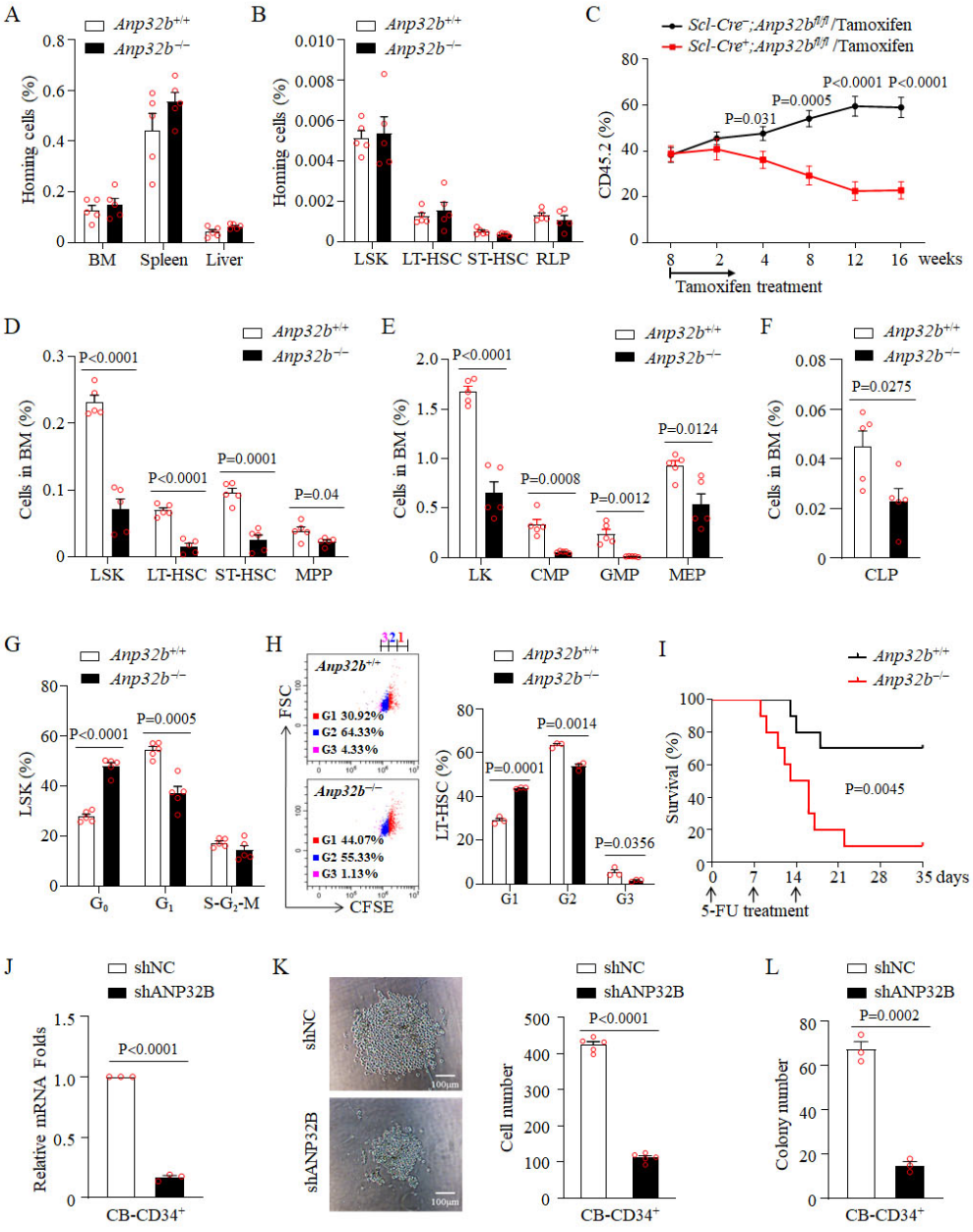

Figure S2. *Anp32b* deficiency reduces long-term repopulation capacity of HSCs. Related to Figure 2.

(A-B) BM cells from *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ mice were stained with CFSE and injected into lethally irradiated recipients. Percentages of CFSE^{+} cells in BM, spleen, and liver (A) as well as CFSE^{+}LSK cells, CFSE^{+}LT-HSCs, CFSE^{+}ST-HSCs, CFSE^{+}RLPs in BM (B) were analyzed at 16h post-transplantation (n = 5).

(C) Competitive transplantation assay was conducted with *Scl-Cre*$^{-}$*;Anp32b*$^{fl/fl}$ or *Scl-Cre*$^{+}$*;Anp32b*$^{fl/f}$ BM cells (6×10^5) along with 6×10^5 BM cells from CD45.1 competitor. Inducible deletion of *Anp32b* was performed with tamoxifen at 8 weeks post-transplantation for 21days. Percentages of donor-derived cells in PB were analyzed at the indicated time points ($n = 6$).

(D-F) Frequencies of LSK cells, LT-HSCs, ST-HSCs and MPPs (D), LK cells, CMPs, GMPs, MEPs (E) and CLPs (F) in BM were analyzed at day 18 after 5-FU treatment ($n = 5$). 5-FU was intraperitoneally administered to 8-10-week-old *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ mice at a dose of 150 mg/kg.

(G) Cell-cycle analysis of LSK cells in BM from 8-10-week-old *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ mice at day 18 after 5-FU treatment ($n = 5$).

(H) Cell division tracing of LT-HSCs from *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ mice treated with 5-FU (150 mg/kg) intraperitoneally. LT-HSCs were sorted at day 9 and stained with CFSE. After culturing for 15 hours, percentages of cells in each generation were calculated ($n = 3$). G, generation.

(I) *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ mice were intraperitoneally administered 5-FU (150 mg/kg) weekly for 3 times (arrows), and the survival rates were analyzed ($n = 10$).

(J) Knockdown efficiency of ANP32B in human CD34^{+} cells infected with shNC or shANP32B by quantitative RT-PCR.

(K) Representative proliferation images of human CD34^{+} cells infected with shNC or shANP32B. 200 cells were seeded in the U-bottom 96-well plate and the cell numbers in each well were evaluated after culturing for 10 days ($n = 5$).

(L) Colony-forming assay for human $CD34^+$ cells infected with shNC or shANP32B Colony numbers were evaluated at day 10 ($n = 3$).

Error bars denote mean ± SEM. Statistical significance was determined by two-tailed unpaired *t*-test (A-H, and J-L) and log-rank test (I). The experiments in J-L were repeated three times independently with similar results, and the results of one representative experiment are shown. All animal experiments were repeated at least twice with similar results, and the results of one representative experiment are shown.

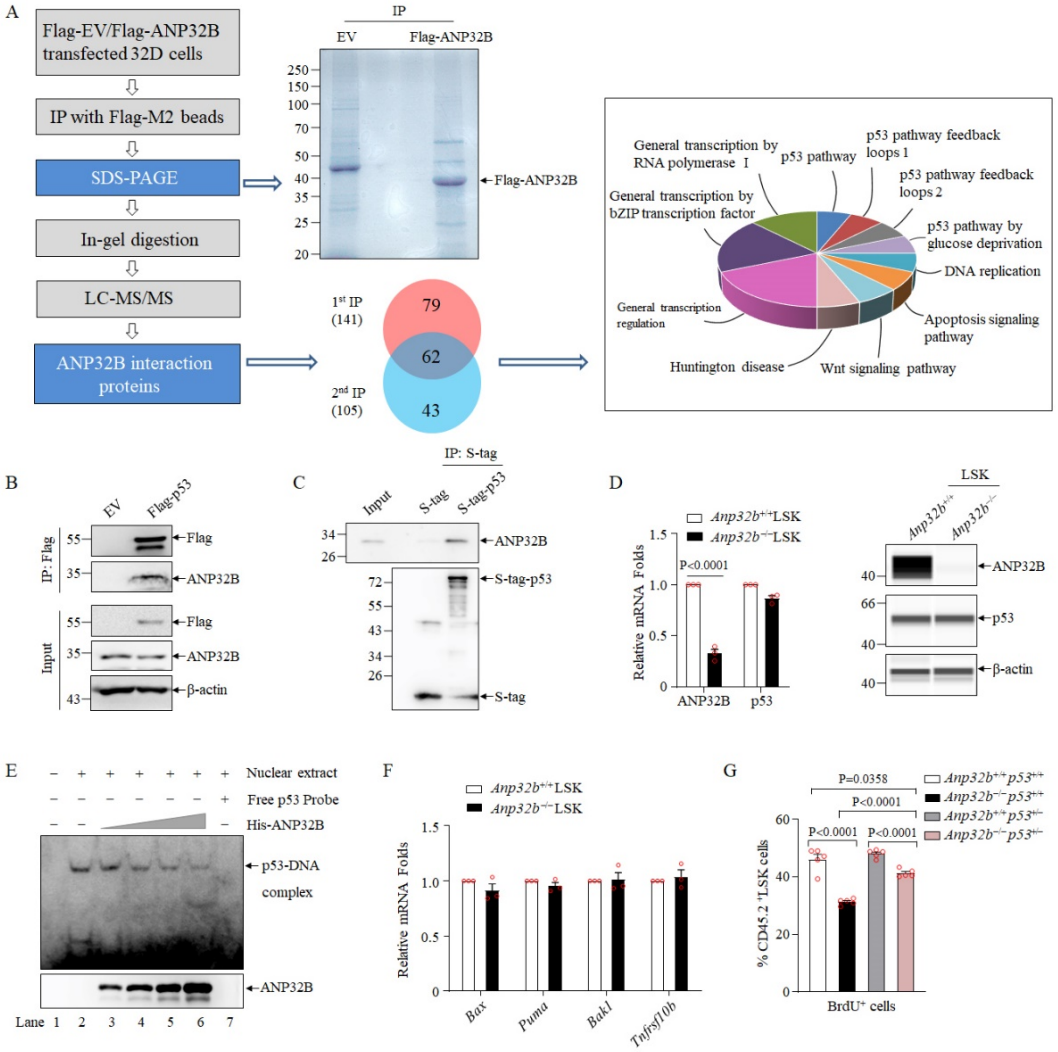

Figure S3. ANP32B interacts with and inhibits the transcriptional activity of p53 to maintain the function of HSCs. Related to Figure 3.

(A) The workflow for identifying ANP32B-interacting proteins in 32D cells and GO pathway analysis of 62 overlapping proteins identified in two independent experiments.

(B) Western blot analysis of indicated proteins in the inputs and immunoprecipitates of Flag-tagged p53 transfected 32D cells.

(C) Bacterially expressed ANP32B was incubated with S-tag or S-tagged p53, followed by S-tag pulldown and western blot analysis of indicated proteins.

(D) ANP32B and p53 expression in *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ LSK cells were evaluated

by quantitative RT-PCR and capillary electrophoresis-based chemiluminescent blot.

(E) Electrophoretic mobility shift assay (EMSA) was performed to analyze the effect of ANP32B on the DNA binding activity of p53. For lane 2-7, nuclear fraction of 293T cells (containing p53 protein) and the biotin-labelled p53 probe were incubated in each reaction to form p53-DNA complex. Specificity of the shift was confirmed by the complete competition with free p53 probe (lane 7). ANP32B expressions were examined by western blot.

(F) Relative mRNA expression of indicated genes in *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ LSK cells were evaluated by quantitative RT-PCR.

(G) Proliferation assay of CD45.2^{+}LSK cells from the recipients of competitive transplantation assay conducted with *Anp32b*$^{+/+}$*p53*$^{+/+}$, *Anp32b*$^{-/-}$*p53*$^{+/+}$, *Anp32b*$^{+/+}$*p53*$^{+/-}$, and *Anp32b*$^{-/-}$*p53*$^{+/-}$ BM cells as donor. The recipients were injected with BrdU intraperitoneally and fed with BrdU-containing drinking water for 2 days at 16 weeks post-transplantation. Percentages of BrdU^{+} cells in CD45.2^{+}LSK cells were shown ($n = 5$).

Error bars denote mean ± SEM. Statistical significance was determined by two-tailed unpaired *t*-test (D, F and G). The experiments in B-F were repeated three times independently with similar results, the experiments in G were repeated twice, and the results of one representative experiment are shown.

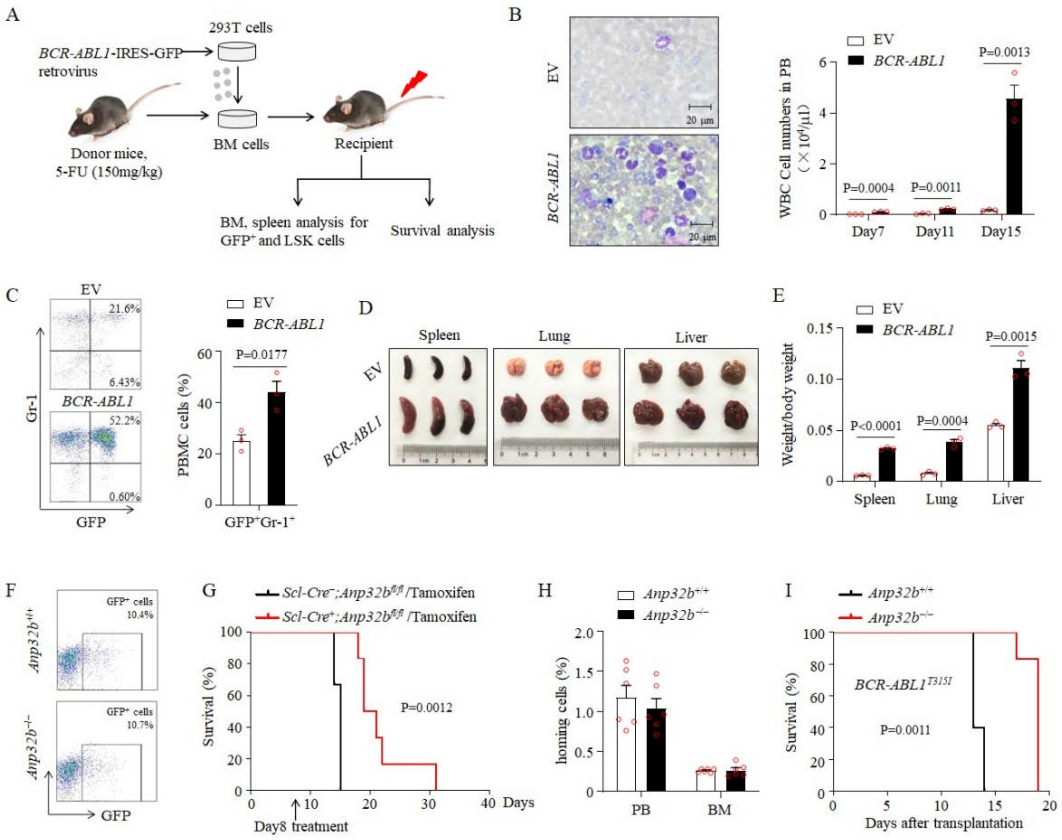

Figure S4. Loss of *Anp32b* impairs *in vivo* progression of CML and maintenance of LSCs. Related to Figure 4.

(A) Experimental strategy for generation of a mouse model of CML.

(B) Wright-Giemsa staining of PB smears (left panels) and total number of WBC in PB were conducted at the indicated time points (right panels) from the recipients of EV or *BCR-ABL1*-transduced Lin$^-$ BM cells (n = 3).

(C-E) Representative FACS profiles and the percentage of leukemia cells (GFP$^+$Gr1$^+$) in PB (C), gross pathology (D) and relative weights (E) of the spleens, lungs, and livers from the recipients of EV or *BCR-ABL1*-transduced Lin$^-$ BM cells (n = 3).

(F) The percentage of *BCR-ABL1*-transduced GFP$^+$ cells from *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ donor mice before first transplantation.

(G) Survival curves for recipients receiving *BCR-ABL1*-transduced *Scl-Cre*$^-$*;Anp32b*$^{fl/fl}$

and *Scl-Cre*$^{+}$*;Anp32b*$^{fl/fl}$ Lin^{-} BM cells followed by tamoxifen treatment for 3 weeks at 8 days post-transplantation (n = 6).

(H) *BCR-ABL1*-transduced *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ Lin^{-} BM cells were stained with CFSE and injected into lethally irradiated recipients. Percentages of CFSE^{+} cells in PB and BM, were analyzed by flow cytometry at 18h post-transplantation (n = 6).

(I) Survival curves for recipients transplanted with *BCR-ABL1*^{T315I}-transduced *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ Lin^{-} BM cells (n = 5 for *Anp32b*$^{+/+}$ and n = 6 for *Anp32b*$^{-/-}$ recipients).

Error bars denote mean ± SEM. Statistical significance was determined by two-tailed unpaired *t*-test (B, C, E and H) or log-rank test (G and I). All animal experiments were repeated at least twice with similar results, and the results of one representative experiment are shown.

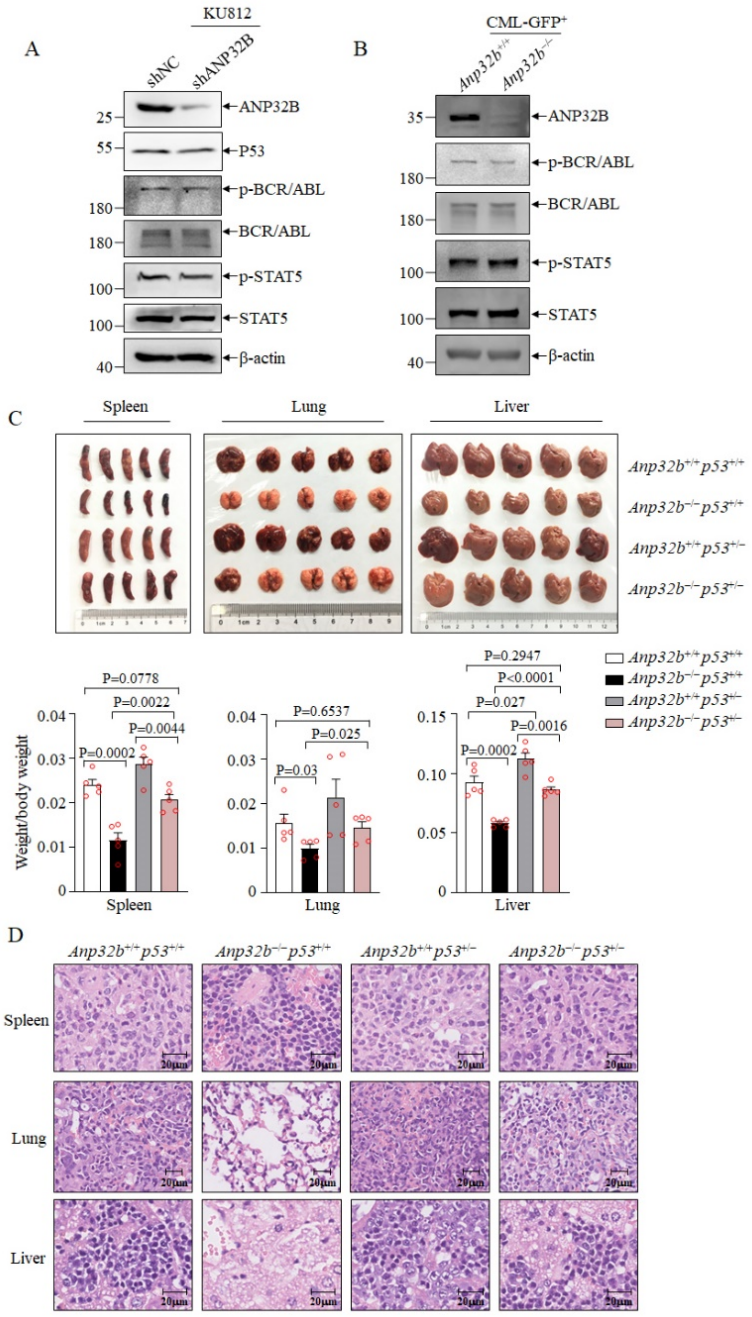

Figure S5. ANP32B-mediated transcriptional repression of p53 enables maintenance of CML LSCs. Related to Figure 5.

(A) Western blot analysis of indicated proteins in KU812 cells with shNC and shANP32B infection.

(B) Western blot analysis of indicated proteins in GFP^{+} cells sorted from BM of *Anp32b*$^{+/+}$ and *Anp32b*$^{-/-}$ CML recipients 12 days after first transplantation.

(C-D) Gross pathology (upper panels) and relative weights (lower panels) (C), hematoxylin-eosin staining (D) of the lungs, spleens, and livers at 14 days post-transplantation from recipients in figure 5H ($n = 5$).

Statistical significance was determined by two-tailed unpaired *t*-test (C). All experiments were repeated at least twice with similar results, and the results of one representative experiment are shown.

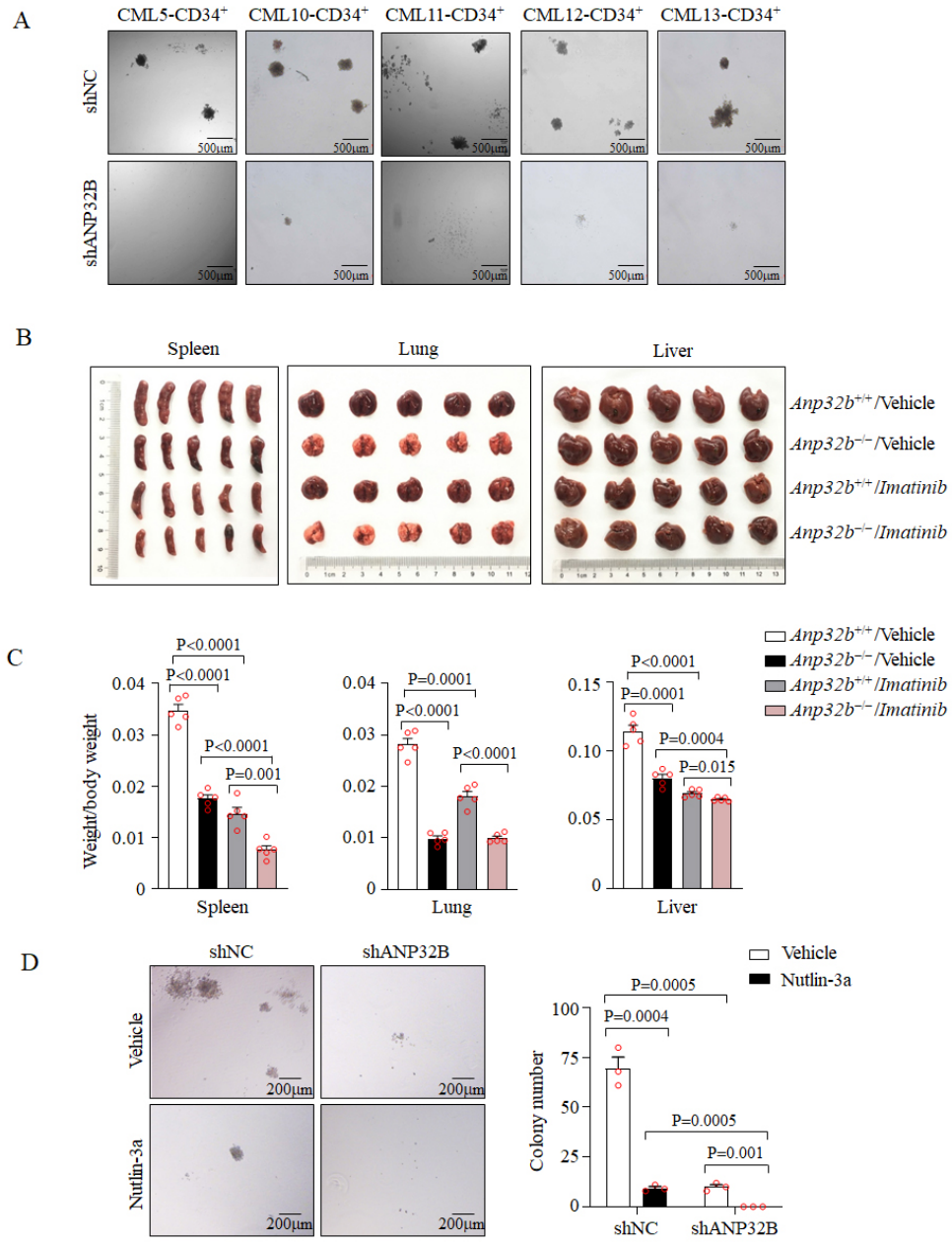

Figure S6. Targeting ANP32B impairs human CML development and synergizes with imatinib therapy to eradicate CML. Related to Figure 6.

(A) Colony-forming assay for human CML CD34$^+$ cells infected with shNC or shANP32B. Representative images were evaluated at day 10 (n = 3).

(B-C) Gross pathology (B) and relative weights (C) of the spleens, lungs, and livers from recipients in figure 6K (n = 5).

(D) Human CML CD34$^+$ cells (CML#12 patient) infected with shNC or shANP32B

were seeded into methylcellulose medium with 2 μM Nutlin-3a or DMSO as vehicle. Colony numbers were counted at day 10 after plating (n = 3).

Error bars denote mean ± SEM. Statistical significance was determined by two-tailed unpaired *t*-test (C and D). The experiments in B-C were repeated twice with similar results, and the results of one representative experiment are shown. The experiments in A and D are presented from an independent experiment.

Supplementary References

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