SUPPLEMENTARY APPENDIX

Niche TWIST1 is critical for maintaining normal hematopoiesis and impeding leukemia progression

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Supplemental methods

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Supplemental Methods

Antibody

The following antibodies were used to stain HSPC or L-GMP: anti-CD150 (clone TC15-12F12.2, Biolegend, San Diego, CA, USA), anti-CD48 (clone HM48-1, BD Biosciences), anti-Sca-1 (clone D7), anti-c-Kit (clone 2B8), anti-CD16/32 (clone 93), anti-CD135 (clone A2F10), anti-CD34 (clone RAM34), anti-IL-7R (clone A7R34), lineage markers (anti-Ter119 (clone TER119), anti-B220 (clone RA3-6B2), anti-Gr-1 (clone RB6-8C5), anti-CD4 (clone GK1.5), anti-CD3a (clone 145-2C11), anti-Mac-1 (clone M1/70) and anti-CD8a (clone 53-6.7)). For detection of engraftment and chimerism, blood cells and BM cells were stained with antibodies against CD45.1 (clone A20, BD Biosciences). The following antibodies were used to stain stromal cells: anti-CD45 (clone 30-F11, BD Biosciences), anti-CD31 (clone 390, BD Biosciences), anti-Ter119 (clone TER-119, BD Biosciences), anti-CD140a (PDGFRα, clone APA5), anti-CD51 (clone RMV-7), anti-Sca-1 (clone D7) and anti-CD166 (clone eBioALC48). All antibodies were from eBioscience (San Diego, CA, USA) except special instruction.

RNA isolation and qRT-PCR

The expression of genes in BM stromal cells or leukemia cells was measured by qRT-PCR. MSCs, OLCs and ECs were sorted by FACS Aria II as described above. Leukemic stem cell was also sorted by FACS Aria II after staining. Cells were collected in lysis buffer, and RNA isolation was performed using the Rneasy Mini Kit (Qiagen, Hilden, Germany), and cDNA was prepared with Superscript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA). qRT-PCR was performed with SYBR Green PCR Kit (TaKaRa Bio Inc, Otsu, Shiga, Japan) and analyzed in an ABI Stepone Sequence Detection System. The primers used for the PCR reaction were shown in Table S1. PCR of the cDNA was performed using 40 cycles for all genes. Values for each gene were normalized to the relative quantity of *Gapdh* mRNA in each sample.

Micro-computed thomography (MicroCT)

Microarchitecture of cancellous bone was analyzed in femurs by microCT (resolution 10.5 μm, SIEMENS, Wittelsbacherplatz, Munich, Germany). Bones were scanned at energy level of 55 kVp, and intensity of 150 μA. Trabecular bone parameters were measured in the proximal metaphysis of the femurs, from 0.15 mm proximal to the distal growth plate and extending proximally 2.2 mm. Using Invon Research workplace software, bone microarchitectural parameters were assessed in trabecular of all mice.

Immuofluorescence microscopy of BM sections

Femurs of killed mice from each genotype were fixed for 12 h in 4% paraformaldehyde (PFA). Samples were then incubated in 30% sucrose for cryoprotection and snap frozen in super cryoembedding medium (SECM) (Section-lab co.ltd, Japan) at -80°C. Half-bone longitudinal femoral cryosections were obtained using the CryoJane tape transfer system (Leica Microsystems, Wetzlar, Germany). For microvessel observation, slides were incubated with antibodies Alexa Fluor 488 anti-mouse endoglin (clone MJ7/18, Biolegend), Alexa Fluor 647 anti-mouse Sca-1 (clone E13-161.7, Biolegend) for two days, followed by incubating with 4,6-diamidino-2-phenylindole (DAPI) for 12 h. Images were acquired using the FV1200MPE confocal microscope (Olympus, Tokyo, Japan) and FV10-ASW Version 04.00 software (Olympus).

Fibroblast colony-forming unit assay

To measure the frequency of MSCs in BM, the fibroblast colony-forming unit (CFU-F) assay was performed. Briefly, 4 × 10^6/mL BM mononuclear cells (BMMNCs) were plated into 6-well tissue-culture plates in triplicate. Cells were grown in DMEM/F12 medium supplemented with 5% FBS, 10 ng/mL murine platelet-derived growth factor BB (mPDGF-BB, PeproTech, Rocky Hill, NJ, USA), 10 ng/mL murine epidermal growth factor (mEGF, PeproTech) and 1× insulin transferrin selenium (ITS, Gibco, Carlsbad, CA, USA) at 37°C, 5% O_2, 5% CO_2 and humidified incubator for expansion. After 10 days, medium was

removed, and each well was washed with PBS, stained with 0.5% Crystal Violet in ethanol for 5-10 minutes at room temperature and scanned by Epson perfection V600 Photo.

Osteogenic differentiation of MSCs

MSCs were replated in six-well culture plates in triplicate at a density of 1.0 × 10^4 cells/cm^2 for osteogenic differentiation at 37°C in a 5% CO_2 humidified incubator. The osteogenic induction medium consisted of DF12 supplemented with 10% FBS, 10 mmo1/L β-glycerophosphate, 0.2 mmol/L ascorbic acid-2-phosphate and 10^{-8} mmo1/L dexamethasone. Leukocyte Alkaline Phosphatase Kit (based on naphthol AS-MX phosphate and fast blue RR salt with citrate, ALP) was used to stain ALP activity in osteogenic differentiation after 1 week. Osteogenic differentiation was also stained by Alizarin Red S after 3 weeks.

BMEC isolation and tube formation assay

Femur and tibia and were isolated, crushed using a mortar and pestle and enzymatically disassociated in Hanks Balanced Salt Solution. BMECs were immunopurified from resulting cell suspensions using CD31 MicroBeads (MiltenyiBiotec, Bergisch-Gladbach, Germany). BMECs were cultured in low glucose DMEM (Life Technologies, Grand Island, NY, USA) and Ham's F-12 (CellGro, Manassas, VA, USA) (1:1 ratio), supplemented with 20% heat-inactivated FBS, antibiotic-antimycotic (CellGro), non-essential amino acids

(CellGro), 10 mM HEPES (CellGro), 100 mg/ml heparin (Sigma-Aldrich) and 50 mg/ml endothelial mitogen (Biomedical Technologies, Stoughton, MA, USA), in humidified incubators at 37°C under 5% CO_2. BMECs at passage 2 were used in tube formation assay. A 96-well plate was coated with Matrigel. Ten thousand BMECs were added to each well and incubated at 37°C. Twelve hours later, tube formation was analyzed.

In vivo homing assays

We purified 5 × 10^6 c-Kit$^+$ WT cells from B6.SJL mice (CD45.1), retro-orbitally injected them into *ER-Cre;Twist1*$^{+/+}$ or *ER-Cre;Twist1*$^{fl/fl}$ mice (CD45.2). Sixteen hours after transplantation, BM cells were harvested from the mice and the number of CD45.1$^+$LSK cells was determined by flow cytometry.

HSC mobilization and blood collection

Recombinant human G-CSF (Kyowa-kirin, Shanghai, China) was administered subcutaneously at 125 μg/kg of TBW every 12 h for eight consecutive injections. At 12 h after the last G-CSF administration, mice were killed and PB samples were obtained by cardiac puncture with a 25-gauge needle and 1 ml syringe containing ethylene diamine tetraacetic acid (EDTA).

Colony-forming cell assay (CFC)

BM cells (2 × 10^4 cells/ml), spleen cells (8 × 10^4 cells/ml) or PB mononuclear

cells (1×10^5 cells/ml) were mixed with semisolid methylcellulose medium M3434 (Stemcell, Vancouver, BC, Canada). The cultures were incubated in a humidified incubator with 5% CO_2 in air at 37°C for 7 days. The numbers of CFU colonies were counted using standard criteria.

Cell cycle analysis and Annexin V staining

For BrdU incorporation assays in vivo, mice were injected intraperitoneally with 50 mg/kg BrdU 16 h prior to cell harvest. Incorporated BrdU was processed with APC BrdU Flow Kit (BD Biosciences). To detect quiescence of HSC or LSC, cells were fixed and processed for Ki67-APC (BD Biosciences) and Hoechst 33342 (Invitrogen) staining according to manufacturer's instructions. For apoptosis analysis, cells were processed according to manufacturer's instructions (Annexin V Apoptosis detection kit, BD Biosciences).

ELISAs

BM cells were flushed from femurs and tibias into 1.5 ml PBS. BM supernatant samples were prepared by taking the top fraction after centrifugation at 1,000 x g for 10 min at 4°C. ELISAs were performed using kits from HCB (Vancouver B.C. Canada). Briefly, supernatant fluid was added to separate microplates containing specific Abs. Enzyme Conjugate was dispensed into each well and the plate was incubated at 37°C for 60 minutes. Then the plate was washed 5 times. Subsequently, Chromogen A and Chromogen B reagent were dispensed

into each well and the plate was incubated at room temperature in the dark for 20 minutes. Finally, the reaction was stopped by adding Stop Solution, and the absorbance at 450 nm was determined using a microplate reader.

Western blot analysis

LSCs ($GFP^+c\text{-}Kit^+Gr\text{-}1^-$) of chimeric Ctrl or KO recipients were sorted and 20 micrograms of total protein was used for Western blot analysis. The samples were separated by 10–15% SDS–PAGE gel. These proteins were then transferred to polyvinylidenedifluoride (PVDF) membranes. After blocking with 5% nonfat milk, the PVDF membranes were incubated with primary antibodies in a Tris-buffered saline (TBS) buffer overnight at 4 °C. These primary antibodies were anti-GAPDH or anti-cleaved Notch1 (Cell Signaling Technology, Danvers, MA, USA). On the following day, PVDF membranes were incubated with appropriate secondary antibodies for 2 h at room temperature. After the membranes had been soaked in an enhanced chemiluminescence reagent (Thermo Scientific) for 5 min, the blots were visualized using X-ray film.

RNA sequencing of in vivo mouse samples

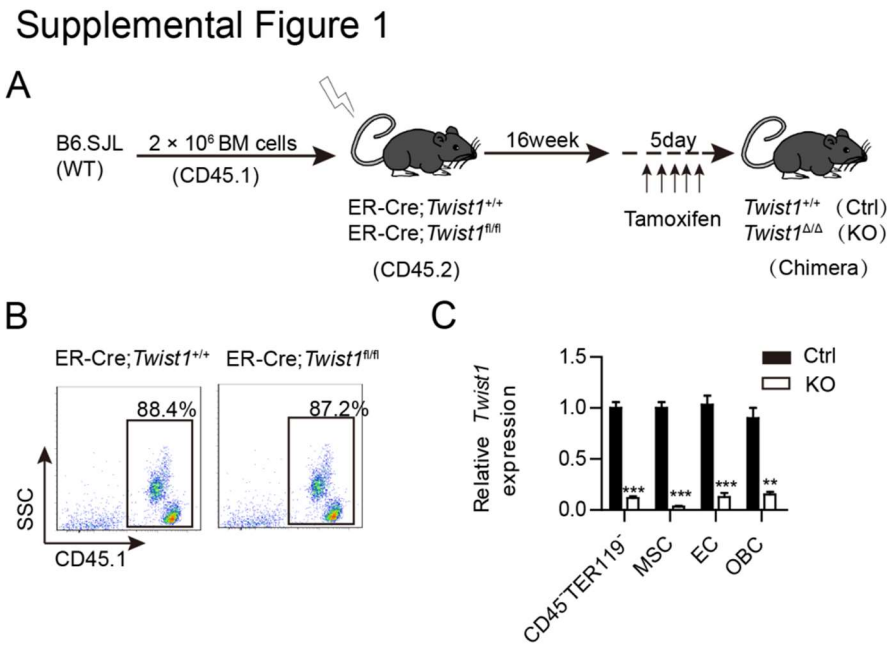
For next-generation sequencing, total RNA was isolated using Qiagen RNeasy Mini Kit from FACS sorted $CD45^-Ter119^-$ cells (1×10^5), obtained from the BM of *Twist1*-deleted mice and control mice 2 weeks after tamoxifen treatment. Each sample was a pool from 4 different animals. The RNA sequencing library

was prepared with the NEBNext®UltraTM RNA Library Prep Kit to construct index-tagged cDNA. The library quality was assessed on the Agilent Bioanalyzer 2100 system. Libraries were sequenced on an IllumiaHiseq platform following the standard RNA sequencing protocol with the TruSeq PE Cluster Kit v3-cBot-HS (Illumina, Inc, Hayward, California, USA). Expression data was compared between both samples by the analysis of individual selected genes for differential expression. Data is available from GEO (GSE107814).

Retrovirus Production and Transduction

DNMAML1 expressing vector was constructed by inserting a DNMAML1 cDNA, which was a gift from Professor Hudan Liu, into pMSCV-PGK-BFP. Retroviral vectors were transfected into 293T cells using Lipofectamine 2000 (Invitrogen). Supernatant containing retrovirus was collected and concentrated 48 and 72 h later and was used for the transduction. $GFP^+c\text{-}Kit^+$ leukemic cells from MLL-AF9-induced leukemic mice were cultured 12 h prior to virus infection in Iscove's modified Dulbecco's medium, supplemented with 10% FBS, 50 ng/ml murine stem cell factor (PeproTech), 10 ng/ml murine IL-3 (PeproTech), 10 ng/ml murine IL-6 (PeproTech). After prestimulation, leukemic cells were resuspended with the virus-containing medium plus 8 μg/ml polybrene (Sigma-Aldrich) and transduced by spin infection for 90 min at 2,000 rpm. The procedure was repeated on the following day. Forty-eight hours after beginning

the transduction, leukemic cells were used for in *vivo* experiments.



Supplemental Figure 1. Generation of *Twist1* knockout chimeric mice.

(A) Experimental schematic for the generation of microenvironment *Twist1*-deleted and control chimeric mice. "*Twist1*$^{\Delta/\Delta}$" denotes mice with microenvironment knockout (KO) of *Twist1*, whereas "*Twist1*$^{+/+}$" denotes mice without *Twist1* deletion as the experimental control (Ctrl).

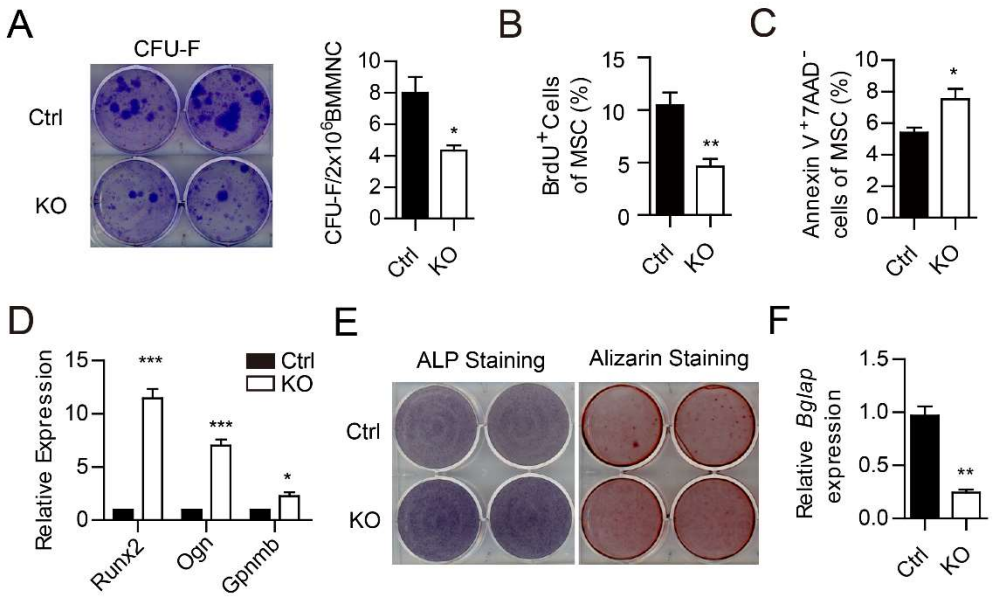
(B) Representative FACS profiles of donor-derived peripheral blood (PB) cells in chimeric mice 16 weeks after transplantation.

(C) Quantitative real-time PCR (qRT-PCR) analysis was conducted to confirm deletion of *Twist1* in total stromal cells (CD45$^-$Ter119$^-$), mesenchymal stem cells (MSCs, CD140a$^+$CD51$^+$CD45/Ter119/CD31$^-$), endothelial cells (EC, CD45$^-$Ter119$^-$CD31$^+$) and osteolineage cells (OLCs, Sca-1$^-$CD166$^+$CD45/Ter119/CD31$^-$) from chimeric Ctrl and KO mice after tamoxifen injection.

Data represent mean ± SD from three independent experiments. **, P< 0.01,

***, $P < 0.001$ (Student's *t* test).

Supplemental Figure 2



Supplemental Figure 2. *Twist1* deletion leads to inhibited mesenchymal stem cell (MSC) growth, enhanced osteogenic commitment, and reduced terminal differentiation.

(A) Clonogenic capacity of bone marrow (BM)-MSCs from chimeric control (Ctrl) and knockout (KO) mice. Representative crystal violet staining of BM-MSC-CFU are shown at left, and CFU number are shown at right (*n* = 4, 2 independent experiments).

(B) Proliferation analysis of MSCs in chimeric Ctrl and KO mice (*n* = 4, 2 independent experiments).

(C) Apoptosis analysis of MSCs in chimeric Ctrl and KO mice (*n* = 4, 2 independent experiments).

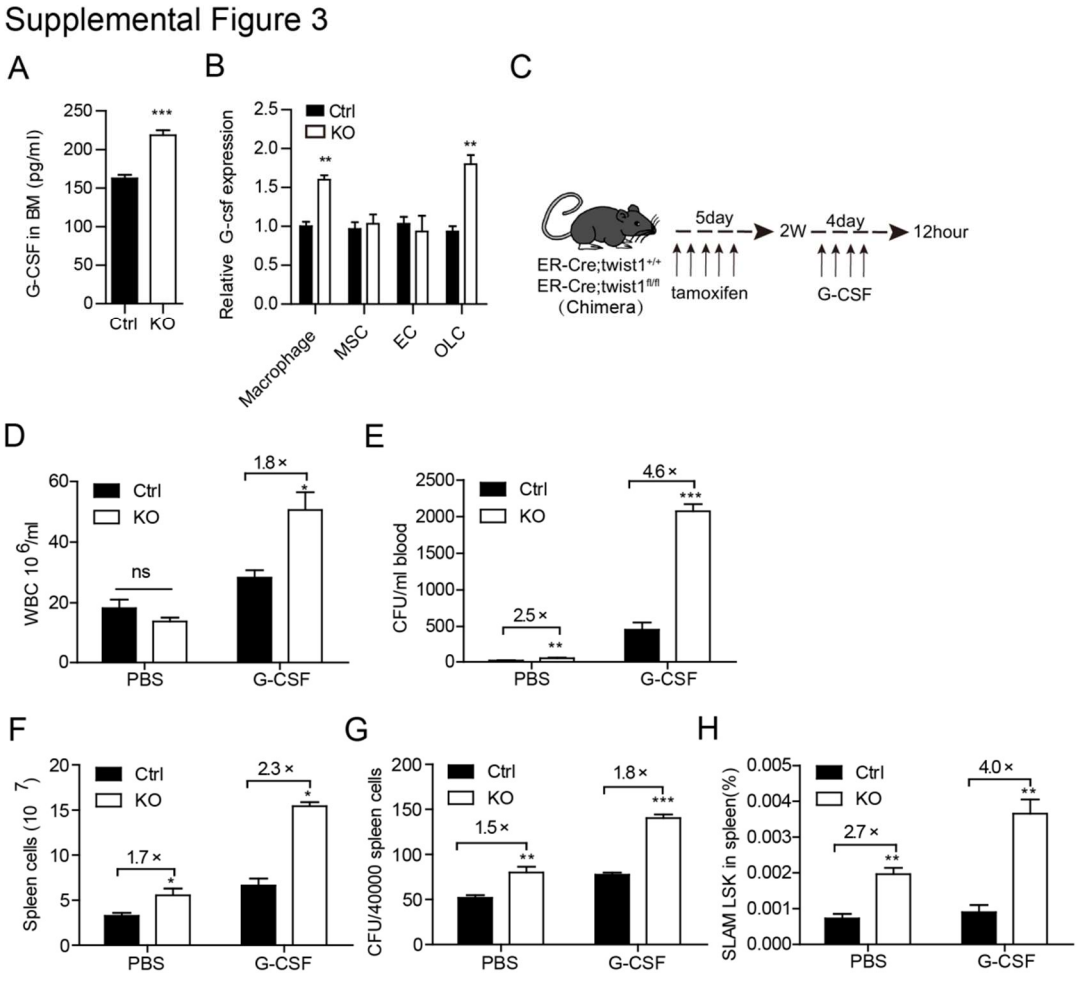
(D) Quantitative real-time PCR (qRT-PCR) analysis of early osteoblastic differentiation relative genes (*Runx2*, *Ogn* and *Gpnmb*) in MSCs of chimeric

Ctrl and KO mice. Data represent mean ± SD from three independent experiments.

(E) Functional analysis showing that *Twist1* deletion increased alkaline phosphatase (ALP) staining and matrix mineralization compared to Ctrl (n = 3, 2 independent experiments).

(F) MSCs from chimeric Ctrl and KO mice were induced for osteogenic differentiation in vitro, and cells were collected after 1 week, late osteoblastic differentiation relative gene *Bglap* in these cells was analyzed. Data represent mean ± SD from three independent experiments.

*, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ (Student's *t* test).



Supplemental Figure 3. *Twist1* deletion induces granulocyte colony-stimulating factor (G-CSF) secretion and enhances hematopoietic stem/progenitor cell (HSPC) response to exogenous G-CSF-induced mobilization.

(A) Protein concentration of G-CSF in bone marrow (BM) supernatant analyzed by ELISA (*n* = 4, 2 independent experiments).

(B) Quantitative real-time PCR (qRT-PCR) analysis of G-CSF in macrophages, mesenchymal stem cells (MSCs), endothelial cells (ECs) and osteolineage cells (OLCs) of chimeric control (Ctrl) and knockout (KO) mice. Data represent mean ± SD from three independent experiments.

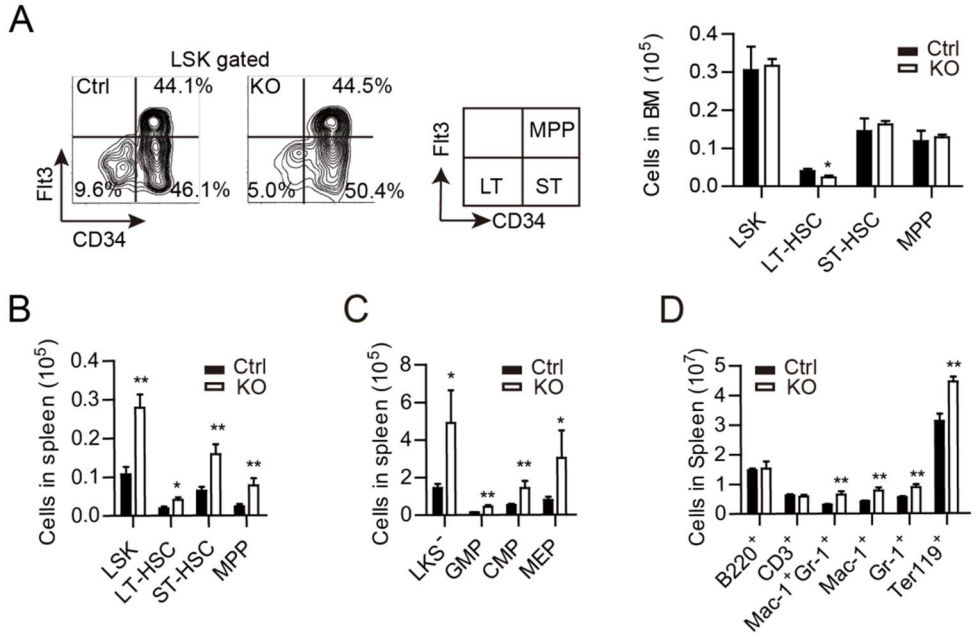
(C) Experimental schematic for HSPC mobilization by G-CSF or vehicle (phosphate buffered saline, PBS) in chimeric Ctrl and KO mice.

(D-E) The white blood cell (WBC) count (D) and the number of colony-forming unit (CFU) (E) in peripheral blood (PB) of chimeric Ctrl and KO mice after PBS or G-CSF injection (n = 3-6).

(F-H) Number of spleen cells (F), number of CFU (G), and frequency of SLAM LSK cells ($CD150^{+}CD48^{-}$ LSK) in spleen (H) of chimeric Ctrl and KO mice after PBS or G-CSF injection (n = 3-5).

Column plots show the mean ± SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant (Student's *t* test).

Supplemental Figure 4



Supplemental Figure 4. *Twist1* deletion reduces hematopoietic stem cell (HSC) in bone marrow (BM) and promotes extramedullary hematopoiesis.

(A) FACS analysis of long-term HSCs (LT-HSCs), short-term HSCs (ST-HSCs), and multipotent progenitors (MPPs) of BM cells in chimeric control (Ctrl) and knockout (KO) mice. Representative FACS profiles are shown at left, and cell number are shown at right (*n* = 4, 3 independent experiments).

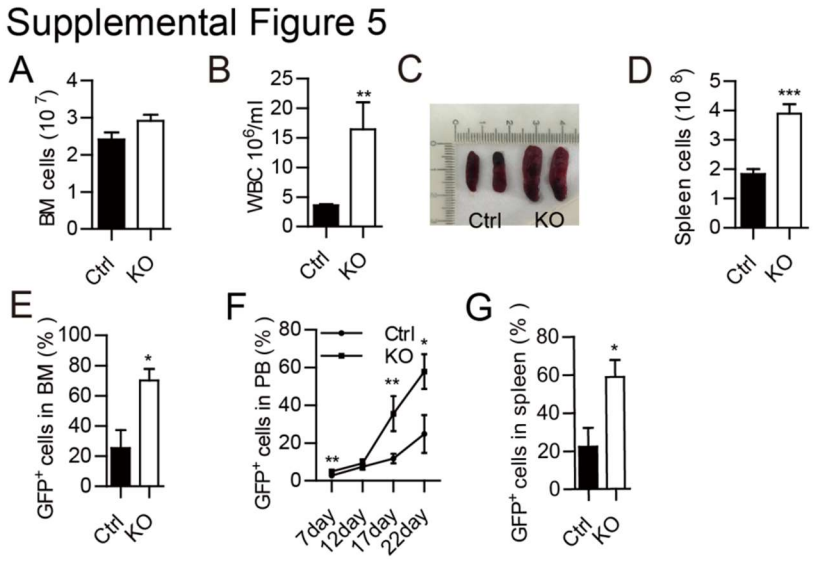
(B) Number of LSK ($Lin^-Sca\text{-}1^+c\text{-}Kit^+$), LT-HSCs, ST-HSCs, and MPPs in the spleen of chimeric Ctrl and KO mice (*n* = 4, 3 independent experiments).

(C) Number of ($Lin^-c\text{-}Kit^+Sca\text{-}1^-$), common myeloid progenitors (CMPs), granulocyte/macrophage progenitors (GMPs), and megakaryocyte/erythroid progenitors (MEPs) in the spleen of chimeric Ctrl and KO mice (*n* = 4, 3 independent experiments).

(D) Number of B cells ($B220^+$), T cells ($CD3^+$), myeloid cells ($Mac\text{-}1^+$ and $Gr\text{-}1^+$)

and erythrocytes ($Ter119^+$) in the spleen of chimeric Ctrl and KO mice (n = 4, 3 independent experiments).

Column plots show the mean ± SD. *, $P < 0.05$; **, $P < 0.01$ (Student's t test).



Supplemental Figure 5. *MLL-AF9*-induced acute myeloid leukemia (AML) is accelerated in *Twist1*-deficient recipients.

(A) Bone marrow (BM) cells of control (Ctrl) and knockout (KO) recipients 22 days after transplantation (*n* = 5, 2 independent experiments).

(B) White blood cell (WBC) count in peripheral blood (PB) 14 days after transplantation in Ctrl and KO recipients (*n* = 5, 2 independent experiments).

(C) Representative image of spleens in Ctrl and KO recipients 22 days after transplantation.

(D) Spleen cells of Ctrl and KO recipients 22 days after transplantation (*n* = 5, 2 independent experiments).

(E) Percentage of GFP$^+$ leukemic cells in BM of Ctrl and KO recipients 22 days after transplantation (*n* = 4, 2 independent experiments).

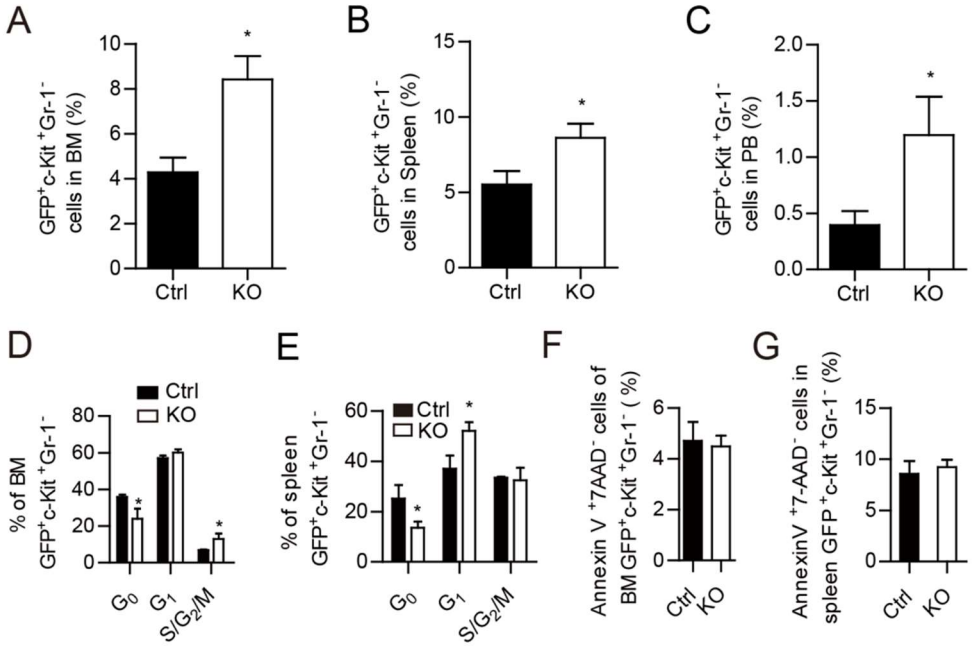
(F) Percentage of GFP$^+$ leukemic cells in PB at the indicated time points after transplantation in Ctrl and KO recipients (*n* = 5, 2 independent experiments).

(G) Percentage of GFP$^+$ leukemic cells in spleen of Ctrl and KO recipients 22

days after transplantation (n = 5, 2 independent experiments).

Column plots show the mean ± SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (Student's t test).

Supplemental Figure 6



Supplemental Figure 6. Enhanced proliferation of leukemic stem cell (LSC) in *Twist1*-deficient recipients.

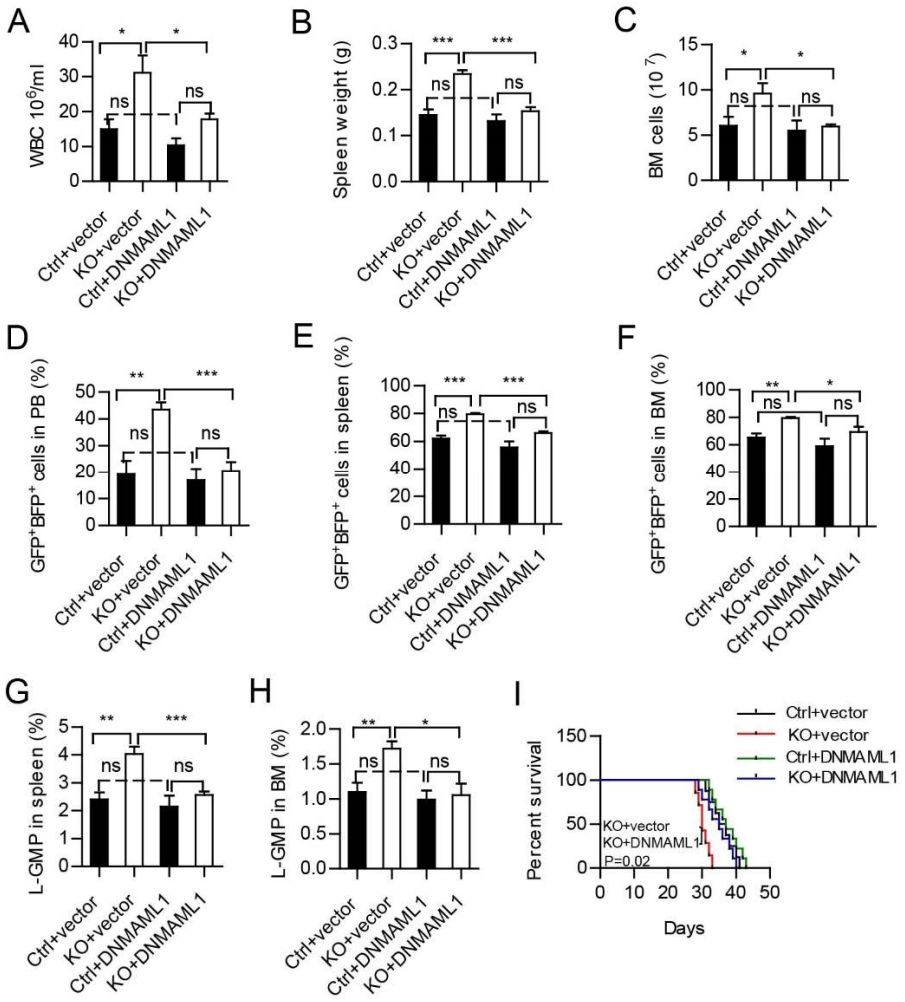
(A-C) Frequency of GFP$^+$c-Kit$^+$Gr-1$^-$ cells in bone marrow (BM) (A), spleen (B) and peripheral blood (PB) (C) of control (Ctrl) and knockout (KO) recipients (*n* = 3-5, 2 independent experiments).

(D-E) Percentage of the cell cycle distribution of BM (D) and spleen (E) GFP$^+$c-Kit$^+$Gr-1$^-$ cells in Ctrl and KO recipients (*n* = 4, 2 independent experiments).

(F-G) Apoptosis analysis of BM (F) and spleen (G) GFP$^+$c-Kit$^+$Gr-1$^-$ cells in Ctrl and KO recipients (*n* = 4, 2 independent experiments).

Column plots show the mean ± SD. *, *P*< 0.05 (Student's *t* test).

Supplemental Figure 7



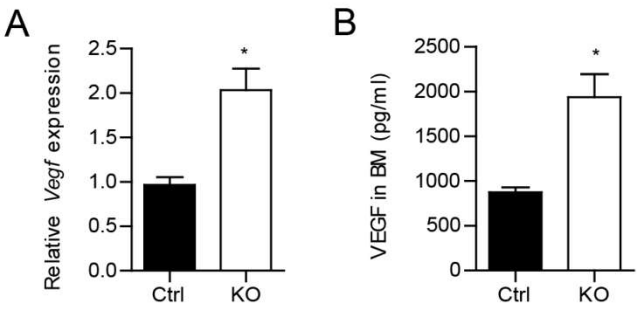
Supplemental Figure 7. Blockade of Notch with dominant-negative MAML1 attenuates acute myeloid leukemia (AML) development in *Twist1*-deficient mice.

(A-H) GFP$^+$c-Kit$^+$ leukemic cells were infected with MSCV-PGK-BFP retrovirus carrying DNMAML1 or an empty vector. Forty-eight hours after infection, cells were intravenously injected into sub-lethally irradiated wild-type recipient mice. Two weeks later, GFP$^+$BFP$^+$ cells were obtained and transplanted into chimeric control (Ctrl) and knockout (KO) recipient mice. White blood cell (WBC) count (A), spleen weight (B), bone marrow (BM) cells (C), leukemic cell infiltration (D-

F), and leukemic stem cell (LSC) in spleen (G) and BM (H) were detected 22 days after transplantation (n = 4-6, 2 independent experiments, Column plots show the mean ± SD. *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$; ns, not significant, Student's t test).

(I) Survival curve of chimeric Ctrl and KO recipients transplanted with DNMAML1 or control leukemic cells (n = 7-8, log-rank test).

Supplemental Figure 8

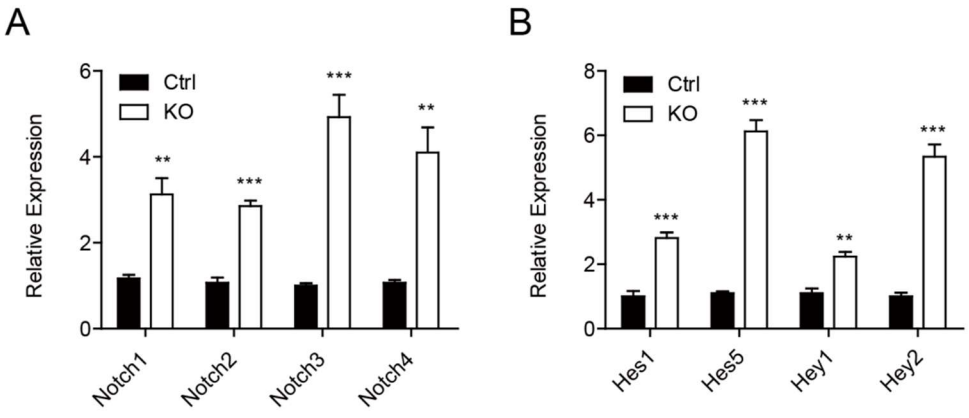


Supplemental Figure 8. Increased expression of vascular endothelial growth factor (VEGF) in *Twist1*-deficient bone marrow (BM).

(A) Quantitative real-time PCR (qRT-PCR) analysis of the expression of *Vegf* of freshly sorted BM mononuclear cell from control (Ctrl) and knockout (KO) mice. Data represent mean ± SD from three independent experiments.

(B) Protein concentration of VEGF in BM supernatant analyzed by enzyme-linked immunosorbent assay (ELISA) (n = 4, 2 independent experiments). Column plots show the mean ± SD. *, $P < 0.05$ (Student's t test).

Supplemental Figure 9



Supplemental Figure 9. Expression of Notch receptors and target genes in normal hematopoietic stem cell (HSC).

(A-B) Analysis of Notch receptors (A) and target genes regulated by Notch pathway (B) in freshly sorted CD34$^-$LSK (Lin$^-$Sca-1$^+$c-Kit$^+$) from chimeric control (Ctrl) and knockout (KO) mice by quantitative real-time PCR (qRT-PCR). Data represent mean ± SD from three independent experiments. **, $P < 0.01$; ***, $P < 0.001$ (Student's *t* test).

Supplemental Table 1. Primers used for the amplification of mouse transcripts by qRT-PCR.

Gene	Primer sequence	
	Sense (5'-3')	Antisense (5'-3')
Twist1	CGGGTCATGGCTAACGTG	CAGCTTGCCATCTTGGAGTC
Twist2	CGCTACAGCAAGAAATCGAGC	GCTGAGCTTGTCAGAGGGG
Cxcl12	CGCCAAGGTCGTCGCCG	TTGGCTCTGGCGATGTGGC
Vcam1	GACCTGTTCCAGCGAGGGTCTA	CTTCCATCCTCATAGCAATTAAGGTG
Scf	TTGTTACCTTCGCACAGTGG	AATTCAGTGCAGGGTTCACA
m220 Scf	CCCGAGAAAGGGAAAGCCG	ATTCTCTCTCTTTCTGTTGCAACATACTT
Angpt1	CTCGTCAGACATTCATCATCCAG	CACCTTCTTTAGTGCAAAGGCT
Opn	TCCCTCGATGTCATCCCTGTTG	GGCACTCTCCTGGCTCTCTTTG
Runx2	TTACCTACACCCCGCCAGTC	TGCTGGTCTGGAAGGGTCC
Ogn	ACCATAACGACCTGGAATCTGT	AACGAGTGTCATTAGCCTTGC

Gpnmb	CCCCAAGCACAGACTTTTGAG	GCTTTCTGCATCTCCAGCCT
DLL1	CTGCAGGAGTTCGTCAACAA	ATACGCGAAAGAAGGTCCTG
DLL3	CGAGCTACAAATTCATTCTTTCG	ACGCTCGTGCTCAGTGCT
DLL4	GCTGCAGGAGTTCGTCAAC	GAAGGAGTTGGTGCCCAATA
Jagged-1	TTCTCACTCAGGCATGATAAACC	CATCTCTGGGACGACAGAACT
Jagged-2	CGTGTGCCTTAAGGAGTACCA	GCGGCAGGTAGAAGGAGTT
Notch1	CAGTGCCTGCCCTTTGAGT	GCGATAGGAGCCGATCTCAT
Nothc2	TCACAGGGCAGAAGTGTGAA	GGCACATAAGGGCTGTCAC
Notch3	ACCTGGCTACCAGGGTCAA	CACTACGGGGTTCTCACACA
Notch4	GTGTGCCTGGCCACATACC	ACTGGTAGGAACCCAAGGTG
Hes1	GAGAGGCTGCCAAGGTTTTT	ACATGGAGTCCGAAGTGAGC
Hes5	CCGGTGGTGGAGAAGATG	CTTTGCTGTGTTTCAGGTAGC
Hey1	CACTGCAGGAGGGAAAGG	CCCCAAACTCCGATAGTCCAT

	TTAT	
Hey2	AAGCGCCCTTGTGAGGAA A	TCGCTCCCCACGTCGAT
Vegf	ACATCTTCAAGCCGTCCTG TGT	CGCATGATCTGCATGGTGAT
Bglap	GGGCAATAAGGTAGTGAAC AG	GCAGCACAGGTCCTAAATAGT
Dtx	ATCAGTTCCGGCAAGACAC AG	CGATGAGAGGTCGAGCCAC
G-csf	ATGGCTCAACTTTCTGCCC AG	CTGACAGTGACCAGGGGAAC
Gapdh	GACCCCTTCATTGACCTCA AC	CTTCTCCATGGTGGTGAAGA