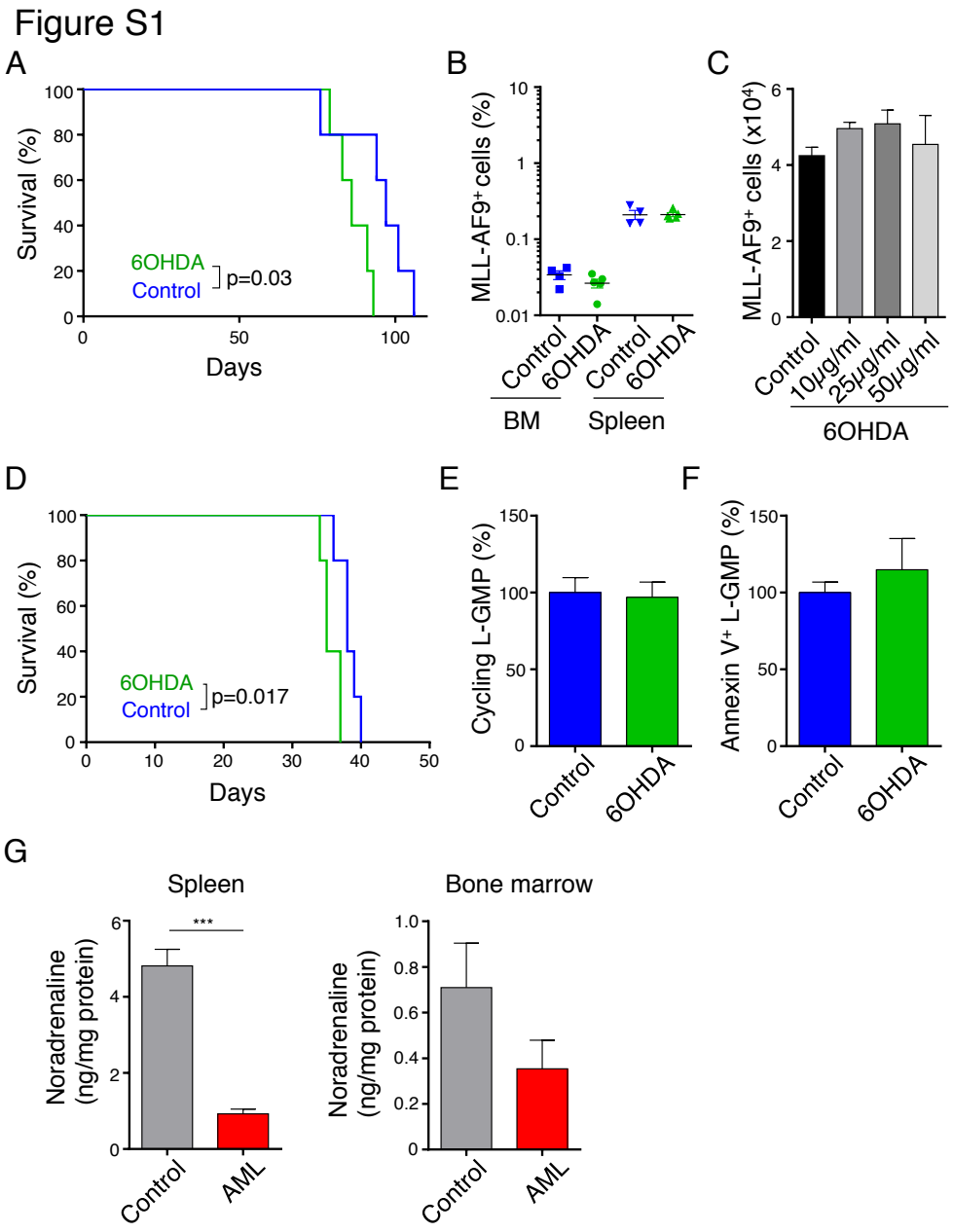

Figure S1, Related to Figure 1. Sympathetic neuropathy promotes leukemogenesis. (A) Survival curve of denervated and control mice after primary transplantation with preleukemic cells (n=5). (B) Homing assay; quantification of CFSE-labeled MLL-AF9 cells in bone marrow and spleen of denervated and control mice 2h after transplantation (n=4-5). (C) Quantification of MLL-AF9$^+$ cells after *in vitro* culture in increasing concentrations of 6-hydroxydopamine (6OHDA) (n=3). (D) Survival curve of control and denervated mice where 6OHDA was administered at day 10 and 12 after transplantation (n=5). (E) Quantification of cycling L-GMP from control and denervated mice (normalized to control, n=10). (F) Quantification of Annexin V$^+$ L-GMP from control and denervated mice (normalized to control, n=5). (G) Noradrenaline concentration in spleen (left, n=4) and bone marrow extracellular fluid (right, n=4-5). ***P<0.001 determined by Student's *t* test. Data are shown as mean ± s.e.m.

Figure S2

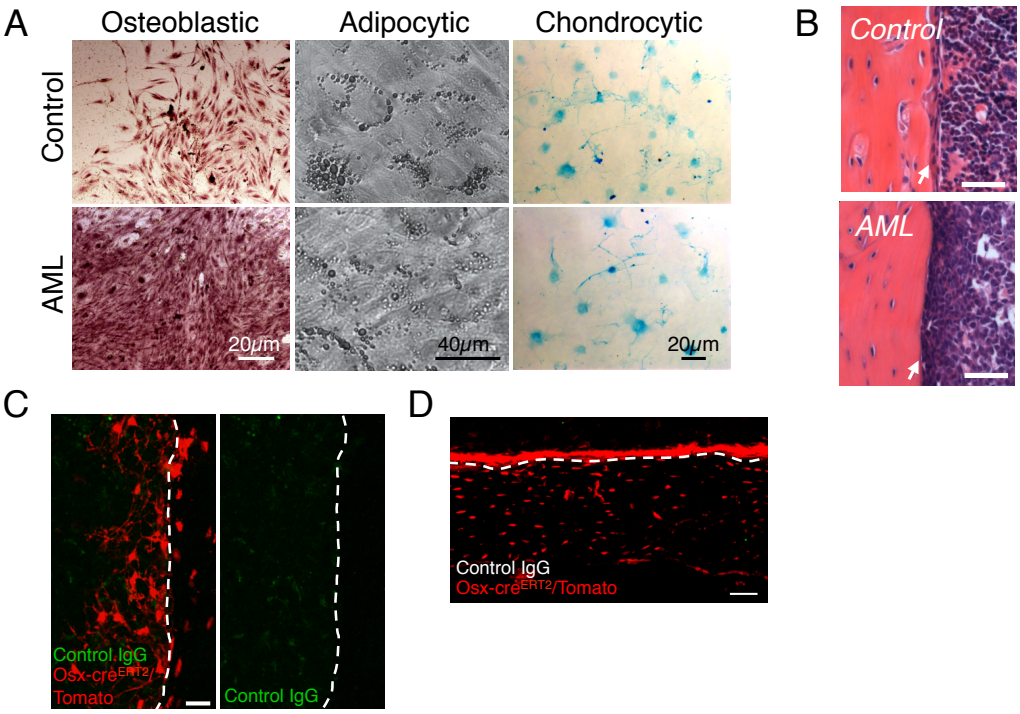

Figure S2, Related to Figure 3. Bone marrow *Nes*-GFP$^+$ MSPCs have differentiated toward the osteoblastic lineage. (A) Multilineage differentiation capacity of *Nes*-GFP$^+$ bone marrow cells were evaluated by plating equal numbers of sorted *Nes*-GFP$^+$ bone marrow stroma cells in corresponding differentiation conditions. Representative images for osteogenic differentiation indicated by Alizarin Red S staining, adipocytes identified by the characteristic production of lipid droplets, chondrocytic differentiation revealed by Alcian Blue staining. (B) Representative H&E images showing the lack of mature cuboidal osteoblasts in leukemia compared to healthy controls (arrows). Scale bar: 50µm. (C) Isotype control for LepR staining shown in Figure 3G and 3H. Scale bar: 20µm. (D) Isotype control for osteocalcin staining shown in Figure 3I. Scale bar: 50µm.

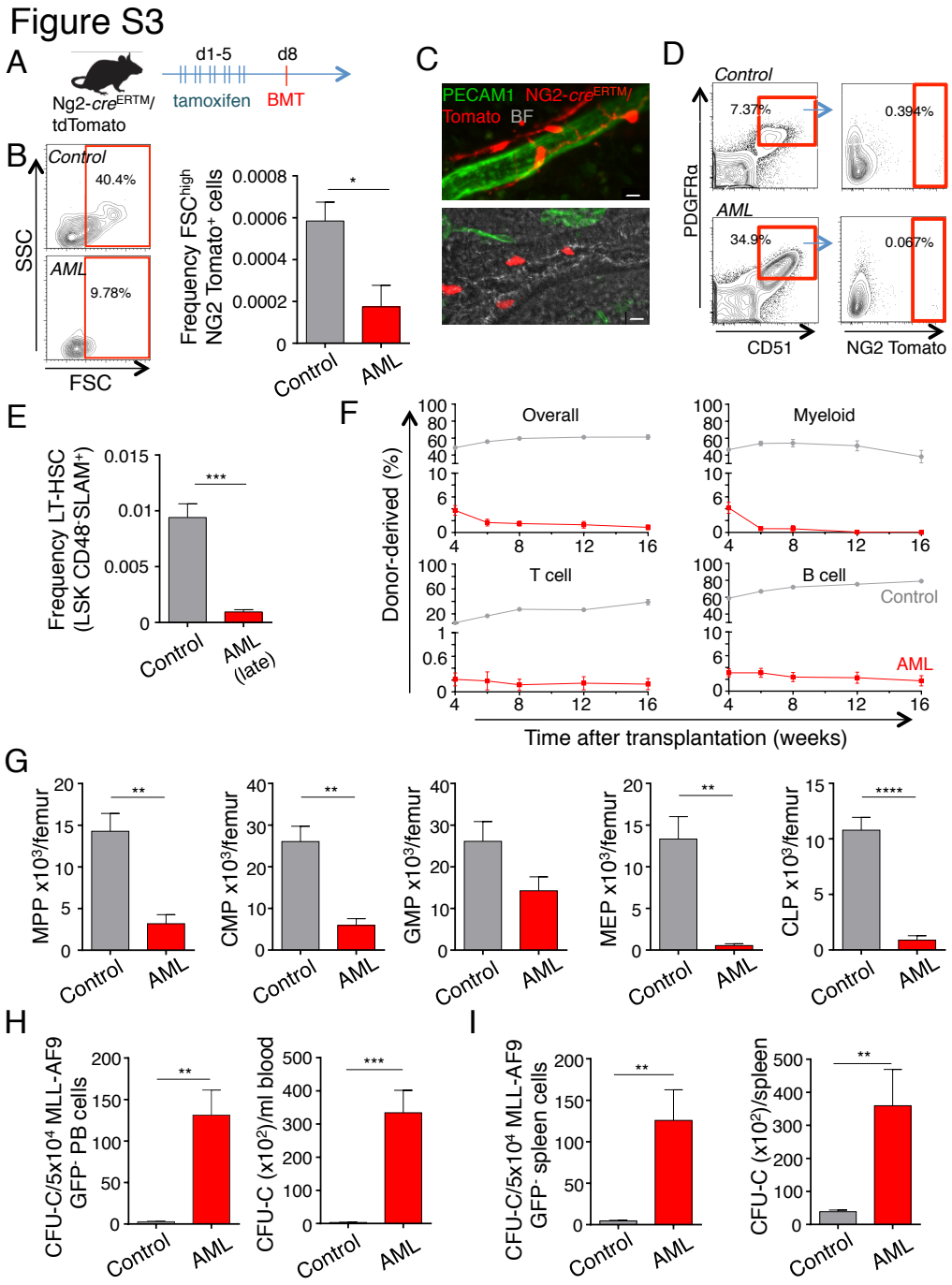

Figure S3, Related to Figure 4A-4H. Leukemic bone marrow niche has impaired HSC regulating capacity. (A) Experimental design to determine the lineage contribution of NG2$^+$ cells to the MSPC population. (B) Representative flow cytometry plots, gated on stromal (CD45$^-$Ter119$^-$ CD31$^-$) NG2-tomato$^+$ bone marrow cells and quantification showing a significant reduction in frequency of FSC^{high} NG2-tomato$^+$ cells in leukemic mice (n=3). (C) Z-stack confocal images of thick bone sections of *NG2-cre^{ERTM}/loxp-tdTomato* control mice stained with anti-PECAM1 antibody. Top image shows pericytic NG2$^+$ cells wrapping arterioles; bottom image shows small NG2$^+$ cells in the trabecular bone. Scale bar: 10µm. (D) Representative flow cytometry plots,

gated on stromal ($CD45^-Ter119^-CD31^-$) bone marrow cells showing that the majority of PDGFRα and CD51 double-positive cells are not labeled by tdTomato. (E) Frequency of phenotypic $Lineage^-Sca\text{-}1^+c\text{-}kit^+CD48^-CD150^+$ long-term repopulating HSCs in leukemic mice with >95% bone marrow infiltration (late) compared to matched control mice (n=4-5). (F) Quantification of long-term reconstituting HSCs by competitive reconstitution assay up to 16 weeks (control n=4-5; AML n=2-3). Number of transplanted bone marrow GFP^- CD45.2 cells adjusted to the actual proportion of GFP^- cells found in leukemic and control mice along with $1x10^6$ CD45.1 competitor cells. (G) HSPC analyses of leukemic and matched control mice: absolute numbers of multipotent progenitor cells (MPP), common myeloid progenitor cells (CMP), granulocyte-macrophage progenitor cells (GMP), megakaryocyte-erythroid progenitor cell (MEP) and common lymphoid progenitor (CLP) (n=4-5). (H) Left, CFU-C from $5x10^4$ sorted MLL-AF9 GFP^- peripheral blood (PB) cells. Right, CFU-C numbers per ml blood calculated with the frequency of MLL-AF9 GFP^- cells in the blood (n=4-5). (I) Left, CFU-C from $5x10^4$ sorted MLL-AF9 GFP^- spleen cells. Right, CFU-C numbers per spleen, calculated with the frequency of MLL-AF9 GFP^- cells in the spleen (n=4-6). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 determined by Student's *t* test. Data are shown as mean ± s.e.m.

Figure S4

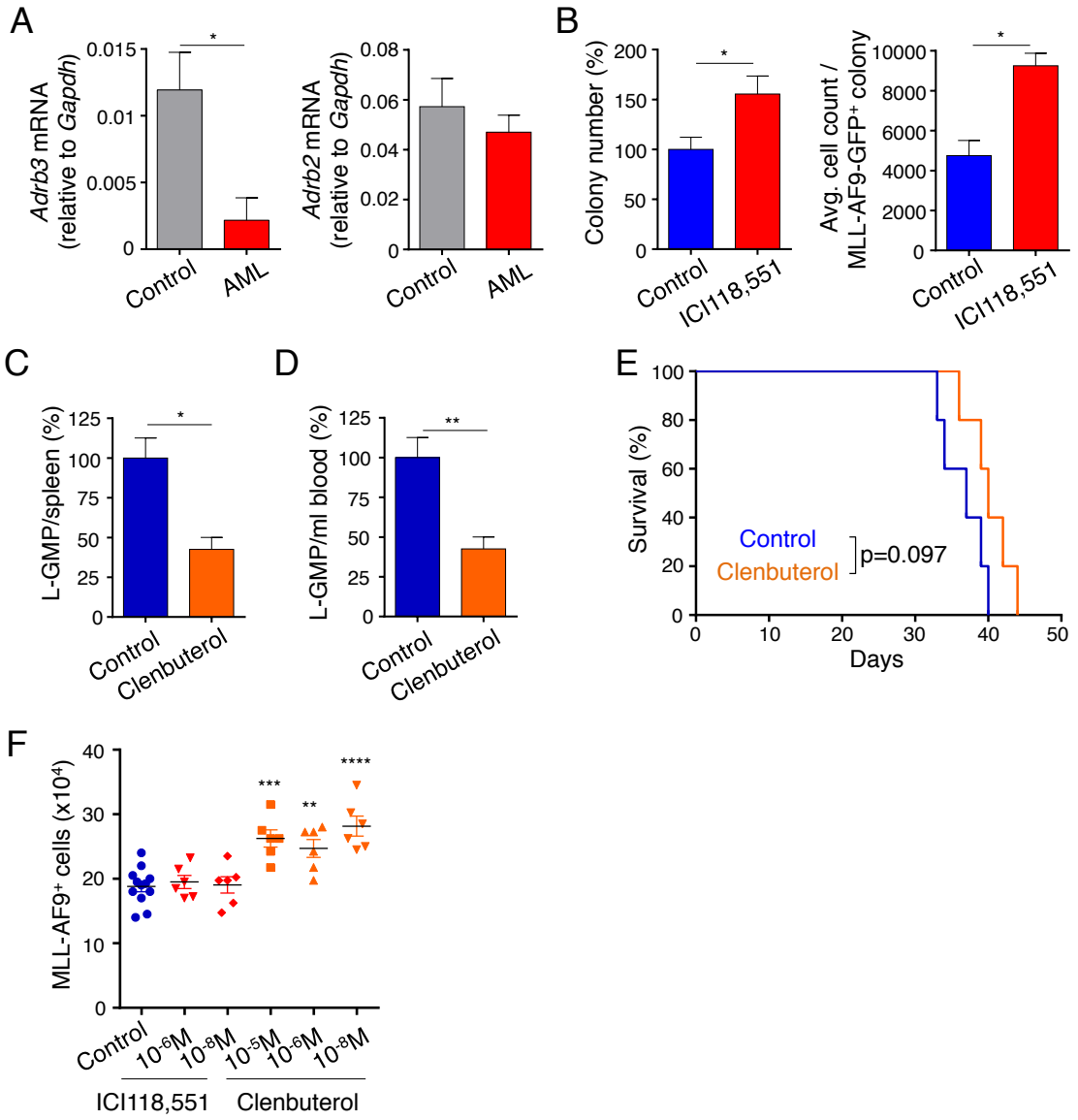

Figure S4, Related to Figure 4I-4L. Leukemic bone marrow niche supports AML progression through β2-adrenergic receptor. (A) Gene expression analysis of *Adrβ3* and *Adrβ2* in sorted bone marrow PDGFRα^{+}/CD51^{+} stromal cells by real-time PCR (n=5-8). (B) Left, primary colony formation from 1000 sorted MLL-AF9 GFP^{+} bone marrow cells from mice treated with the Adrβ2-inhibitor (ICI118,551) and control leukemic mice (normalized to control, n=4,5). Right, average cell count per MLL-AF9-GFP^{+} colony (average of 10 colonies, n=3). (C and D) Absolute numbers of L-GMP per spleen (C) and ml blood (D) in mice treated with the Adrβ2-agonist (Clenbuterol hydrochloride) and control leukemic mice, 19 days after transplantation (normalized to control, n=7-8). (E) Survival curve of mice treated with the Adrβ2-agonist (Clenbuterol hydrochloride) and control leukemic mice (n=5). (F) Quantification of MLL-AF9^{+} cells after *in vitro* culture in increasing concentrations of the Adrβ2-inhibitor (ICI118,551) and the Adrβ2-agonist (Clenbuterol hydrochloride) (n=6-12). *P<0.05, **P<0.01, ***P<0.001, ****P<0.001 determined by Student's *t* test. Data are shown as mean ± s.e.m.

Supplemental Experimental Procedures

***In vivo* treatments.** To generate leukemia cells, Lineage$^-$ Sca-1$^+$ c-Kit$^+$ were purified from C57BL/6 mice using flow cytometry, transduced with pMSCV-MLL-AF9-GFP, resorted as single cells in 96-well plate and grown in methylcellulose (M3234, Stem Cell Technologies) supplemented with Interleukin-3 for six passages as previously described (Krivtsov et al., 2006; Wang et al., 2010). For primary transplantation, mice were sublethally irradiated with 600 cGy in a Cesium Mark 1 irradiator (JL Shepperd&Associates) and transplanted retro-orbitally under isoflurane anesthesia with 5x10^5 preleukemic cells from sixth passage in culture. Mice undergoing serial transplantations were transplanted with the indicated number of leukemic cells with no prior irradiation. For xenotransplantation experiments NSG mice were treated with 100 mg/kg 6-OHDA on day 0 and 250 mg/kg 6-OHDA on day 2 and sub-lethally irradiated (200 cGy) on day 5 at least 4h before transplantation. Human mononuclear cells, derived from untreated patients with the initial diagnosis of AML, were depleted of CD3$^+$ and CD56$^+$ cells using the Dynabeads® magnetic separation technology (Invitrogen) and transplanted via the retro-orbital route in NSG mice. Human AML samples were obtained from the Department of Hematology, University Hospital Essen, Germany, with written consent according to institutional guidelines, approved by the Ethics Commission of the University of Essen-Duisburg. Protocols were approved by the Institutional Review Board.

Competitive Reconstitution. Competitive repopulation assays were performed using CD45.1/CD45.2 congenic system. The number of transplanted GFP$^-$ CD45.2 bone marrow cells was adjusted to the actual proportion of GFP$^-$ cells found in late-stage leukemic and control donor mice along with 1x10^6 CD45.1 competitor cells and transplanted into lethally irradiated

(12 Gy) CD45.1 recipient mice. CD45.1/CD45.2 chimerism of recipient's blood was analyzed up to 16 weeks after transplantation with anti-CD45.1, anti-CD45.2, anti-Gr-1, anti-CD11b, anti-B220 and anti-CD3.

Cell isolation. Bone marrow primary cells were isolated as previously described (Pinho et al., 2013). For analyses of endothelial cells 0.2% Dispase (Gibco) was added. For flow cytometry sorting, cells were enriched by immunomagnetic depletion using anti-CD45 magnetic beads (Miltenyi Biotec), following the manufacturer's recommendations. For osteolineage-enriched fraction quantification, bone marrow was flushed and discarded, compact bones were chopped to small fragments and incubated in 3 mg/ml collagenase type I (Sigma-Aldrich) in HBSS (Gibco) with 10% FBS (StemCell Technologies) for 90 min at 37°C and later filtered. Cells were sorted on a FACSAria (BD Biosciences) to > 95% purity.

Flow Cytometry. Fluorochrome-conjugated or biotinylated mAbs specific to mouse CD45 (clone 30-F11), Ter119 (clone Ter-119), PDGFRα (clone APA5), CD51 (clone RMV-7), c-Kit (clone 2B8), CD34 (clone RAM34), Sca-1 (clone D7), CD11b (clone M1/70), CD127 (clone A7R34), CD144 (clone BV13), CD16/32 (clone93), CD135 (clone A2F10), CD41 (clone MWReg30), CD48 (clone HM48-1), CD150 (clone TC15-12F12.2), CD45.1 (clone A20), CD45.2 (clone 104), Gr-1 (clone RB6-8C5), B220 (clone RA3-6B2), CD3 (clone 145-2C11), hematopoietic lineage cocktail and corresponding isotype controls were purchased from eBioscience. CD31 (clone MEC13.3) were from Biolegend. Anti-LepR from R&D systems. For the analyses of L-GMP CD3e, B220, Gr-1, Ter119, CD127, c-Kit, Sca-1, CD34 and CD16/32 were used. Fluorochrome-conjugated mAbs specific to human CD45 (clone HI30), CD33 (clone WM-53), CD3 (clone OKT3) and CD19 (clone

HIB19) were purchased from eBioscience. *Nes*-GFP positive staining was gated in reference to cells from wild-type mice without the GFP transgene and positive specific antibodies labeling were gated in reference to corresponding isotype control or fluorescence minus one (FMO) corresponding sample. Multiparameter analyses of stained cell suspensions were performed on a LSRII (BD Biosciences) and analyzed with FlowJo software (Tree Star). $DAPI^-$ single cells were evaluated for all the analyses.

Cell-cycle analyses and Annexin V staining. For cell-cycle analyses of niche components, digested and antibody stained bone marrow cells were fixed in 2% paraformaldehyde (PFA) in PBS, permeabilized with 0.1% Triton X-100 and stained with Ki-67 (eBioscience, clone SolA15) and Hoechst 33342 (Molecular Probes) at 1 μg/ml for 30min. For cell-cycle analyses of L-GMP, cells were incubated in 2% FCS, 10 mM Hepes and 5 μg/ml Hoechst 33342 containing DMEM (Gibco) for 45 min at 37°C, and then processed as described above. For apoptosis analyses, cells were processed according to manufacturer's instructions (Annexin V-PE Apoptosis detection kit, BD Biosciences).

CFU-F/CFU-OB assay. $1x10^3$ sorted cells were seeded per well in a 6-well adherent tissue culture plate using phenol-red free α-MEM (Gibco) supplemented with 20% FBS (Hyclone), 10% MesenCult stimulatory supplement (StemCell Technologies) and 0.5% penicillin-streptomycin. One-half of the media was replaced after 7 days and at day 14 cells were stained with Giemsa staining solution (EMD Chemicals). For CFU-OB culture 1 mM ascorbic acid-2-phosphate was added, with one-half media change every week for 28 days. Staining for alkaline phosphatase

was performed according to manufacturer's recommendations (Sigma Aldrich), von Kossa staining was done with 2.5% weight/volume $AgNO_3$ incubation for 30 min.

Cell differentiation. For osteogenic, adipogenic and chondrogenic differentiation, mouse *Nes*-GFP^+ cells, isolated from CFU-F cultures of control or leukemic mice, were treated with stemXvivo osteogenic, adipogenic or chondrogenic differentiation media, according to manufacturer's instructions (R&D Systems). All cultures were maintained with 5% CO_2 in a water-jacketed incubator at 37°C for 4-6 weeks and half-medium changes were performed bi-weekly. Osteogenic differentiation indicated by mineralization of extracellular matrix and calcium deposits was revealed by Alizarin Red S staining. Cells were washed twice with PBS and fixed with 4% PFA for 30 min. After rinsing in distilled water, cells were stained with 40 mM Alizarin Red S (Sigma-Aldrich) solution at pH 4.2, rinsed in distilled water, and washed in Tris-buffered saline for 15 min to remove nonspecific staining. Adipocytes were identified by the characteristic production of lipid droplets. Chondrocytic differentiation was revealed by Alcian Blue staining of the mucopolysaccharides. Cells were washed twice with PBS and fixed with 10% formalin for 60 min, rinsed in distilled water and stained with 1% Alcian Blue 8GX solution in 3% acetic acid (Sigma Aldrich) overnight. To remove nonspecific staining, cells were incubated with a 6:4 dilution of ethanol:acetic acid for 20 min and finally washed with PBS.

LTC-IC assay. To determine LTC-IC frequencies, serial dilutions of sorted GFP^- bone marrow cells isolated from control and AML mice were plated on single-well stromal cultures; 4 weeks later each individual well was assayed for the presence of CFU-C as described (Miller et al., 2008).

LTC-IC frequencies were determined by using the ELDA software (http://bioinf.wehi.edu.au/software/elda/).

Colony formation. Sorted MLL-AF9 GFP$^-$ or MLL-AF9 GFP$^+$ cells were plated in MethoCult GF M3434 (for MLL-AF9 GFP$^-$ cells) or MethoCult M3234 supplemented with 20 ng/ml mSCF, 10 ng/ml IL-3, 10 ng/ml IL-6 (Peprotech) (for MLL-AF9 GFP$^+$ cells, in technical duplicates) according to the manufacturer's recommendation (Stem Cell Technologies). Colonies were assigned scores after 6–7 days.

RNA isolation and quantitative real-time PCR. Sorted cells were collected in lysis buffer and RNA isolation was performed using the Dynabeads® mRNA DIRECT™ Micro Kit (Invitrogen). Reverse transcription was performed using the RNA to cDNA EcoDry™ Premix system (Clontech), following the manufacturer's recommendations. Quantitative real-time PCR was performed as previously described (Mendez-Ferrer et al., 2010). The relative mRNA abundance was calculated using the ΔCt method. Gene expression data was normalized to *Gapdh*. Primer sequences are included below.

Mouse primers		***Sequence 5'-3'***
Gapdh	*s*	TGTGTCCGTCGTGGATCTGA
	as	CCTGCTTCACCACCTTCTTGA
Cxcl12	*s*	CGCCAAGGTCGTCGCCG
	as	TTGGCTCTGGCGATGTGGC
Vcam1	*s*	GACCTGTTCCAGCGAGGGTCTA
	as	CTTCCATCCTCATAGCAATTAAGGTG

Angpt1	*s*	CTCGTCAGACATTCATCATCCAG
	as	CACCTTCTTTAGTGCAAAGGCT
Opn	*s*	TCCCTCGATGTCATCCCTGTTG
	as	GGCACTCTCCTGGCTCTCTTTG
Scf	*s*	CCCTGAAGACTCGGGCCTA
	as	CAATTACAAGCGAAATGAGAGCC
Adrb2	*s*	ATCTGAAGGAAGATTCCACGCCCA
	as	AGAGGGTGAATGTGCCCATGATGA
Adrb3	*s*	TGCGCACCTTAGGTCTCATTATGG
	as	AAACTCCGCTGGGAACTAGAGAGG

Immunofluorescence staining. Tissues were fixed with 4% PFA via perfusion, and femoral or tibial bone tissues were further fixed with 4% PFA for 30 min at 4°C, and incubated in 10%, 20%, and 30% sucrose each for 1h at 4°C for cryoprotection and embedded in 5% carboxymethyl cellulose (SECTION-LAB). Sections, 20-30 µm thick, were prepared using Kawamoto's film method (Kawamoto and Shimizu, 2000). Anti-mouse osteocalcin antibody (R21C-01A, Takara) and anti-perilipin antibody (D1D8, Cell Signaling) were used. Images were acquired using a laser-scanning confocal microscope (SP5 AOBS, Leica), Leica LAS-AF software (Leica), and image J (Schneider et al., 2012). For staining of sympathetic nerve fibers, sections were incubated with anti-tyrosine hydroxylase antibody (AB152, Millipore) for 72h. Images were acquired using a ZEISS AXIO examiner D1 microscope (Zeiss) with a confocal scanner unit, CSUX1CU (Yokogawa) and reconstructed in three dimensions with Slide Book software (Intelligent Imaging

Innovations). For whole-mount tissue preparation of the sternum, sternal bones were collected and transected with a surgical blade into 2–3 fragments. The fragments were bisected sagittally to expose the bone marrow cavity, fixed in 4% PFA and stained as previously described (Kunisaki et al., 2013). Whole-mount tissue preparation of the cremaster muscle was performed as previously described (Scheiermann et al., 2012).

Histology and Immunohistochemistry. Cleaned bones were fixed in 10% neutral buffered formalin for 24h and decalcified in 10% EDTA. Tissues were then processed and embedded in paraffin. 5-µm thick sections were cut and used for H&E or tartrate-resistant acid phosphatase (TRAP) staining.

Noradrenaline measurements. Spleens and BMEF were rapidly harvested and frozen in liquid nitrogen. Tissues were homogenized in HPLC solvent. Noradrenaline levels were determined by HPLC at the Neurochemistry Core Laboratory, Vanderbilt University's Center for Molecular Neuroscience Research (Nashville, TN).

MicroCT analysis. Bones were fixed in 10% neutral buffered formalin for 24h and then kept in 70% ethanol at 4°C. The bone samples were scanned using a high-resolution SkyScan micro-CT system. Images were acquired using a 10 MP digital detector, 10 W power energy (100 kV and 100 µA) and a 0.5 mm aluminum filter. X-ray projections were generated from the sample each 0.3 degrees, obtaining 297 consecutive slices with a 6.82 µm image pixel size. Five exposures by projection (1767 ms exposure time) were used to produce high-contrast low-noise images. Trabecular and cortical regions were defined as positions along the long axis of the femur relative to the growth plate reference. The cortical region of interest was selected as ± 1.0 mm

in length from the mid-diaphysis of the bone. Trabecular bone parameters were determined 350 μm below the bottom edge of the growth plate, going 2 mm toward the diaphysis.

Supplemental References

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