SUPPLEMENTAL METHODS

Cell Culture

293T cells were cultured in DMEM (high glucose) containing 10% fetal bovine serum. THP-1, MONOMAC-6, NOMO-1, MV4-11, and NB-4 were cultured in RPMI1640 plus 10% fetal bovine serum. Human MA9.3 leukemic cells were cultured in IMDM plus 20% FBS with defined cytokines [1]. Enriched fresh hematopoietic stem and progenitor cells (HSPCs) were cultured *in vitro* in StemSpan (STEMCELL Technologies, Vancouver, BC, Canada) supplemented with 10 μg/ml heparin (Sigma, St Louis, MO, USA), 10 ng/ml murine SCF, 20 ng/ml murine TPO, 20 ng/ml human IGF-II, 10 ng/ml murine FGF-1, and 100 ng/ml human Angptl3 [2]. All recombinant proteins were purchased from PEPROTECH (Rock Hill, NJ, USA) or PROSPEC companies (Ness Ziona, Israel). All cell culture products were purchased from Life Technologies (Grand Island, NY, USA) unless otherwise specified in the text and figure legends.

Plasmids

MSCV-MLL-AF9-YFP was kindly provided by Dr. Zhijian Qian (University of Illinois at Chicago, USA). To construct shRNA targeting human SLUG, we cloned shRNA control and SLUG shRNA #1 and #2 into Age I and EcoR I sites of pLKO.1maxGFP2aPuro vector. All other shRNA constructs were purchased from Sigma and listed in Supplemental Table 4. Slc13a3 was amplified by PCR using Phusion™ high-Fidelity DNA polymerase (New England Biolabs), and then cloned into pMIG vector. To construct the Slc13a3-LUC luciferase reporter, ~1.4 kb of *Slc13a3* promoter region was amplified by PCR using specific primers and cloned in to pGL4.10 vector (Promega).

Retroviral and Lentiviral Transduction of Hematopoietic Cells

For generation of retrovirus, 293T cells were transduced with a mixture of DNA containing 1.5 μg of pCL-Eco (IMGENEX), 2.5 μg of pMIG vector only, or pMIG expression vectors by Fugene HD (Roche)

according to the manufacturer's instructions. For preparation of the lentiviruses, 293T cells were transfected with a mixture of DNA containing 2.5 μg of shRNA lentiviral vectors and 2.5 μg of the packaging mixture (Genecopoeia, Rockville, MD) by Fugene HD. All media containing retroviruses or lentiviruses were collected 24 hours after transfection, and filtered through a 0.45 μm pore-size filter. To transduce HSPCs, cells were enriched from BM of mice following 5-FU injection (150 mg/kg body weight). Enriched HSPCs or AML cells were cultured overnight and were spun at 200 X g. The supernatant was aspirated and replaced with retroviral- or lentiviral particle-containing supernatant supplemented with 5 μg/ml polybrene (Sigma) followed by centrifugation (900 X g for 45 min). After two days of transduction, lentivirus-transduced cells were selected with 1 μg/ml puromycin, and then were used for the further experiments.

BM Transplantation and NAC Administration

6-8-week-old C57BL/6.SJL (CD45.1) mice were given acidified antibiotic water (Sulfamethoxazole and Trimethoprim) for 7 days, and then lethally or sublethally-irradiated before transplantation. HSCs were transduced with retroviral or lentiviral particles for three days, and then transplanted into mice by retro-orbital injection. Recipient mice were continuously fed with acidified antibiotic water for 1 month to reduce the chance of spontaneous infection. For secondary transplantation, cells from the primary recipients were injected into sublethally-irradiated secondary recipient mice.

For NAC treatment, recipient mice were injected intraperitoneally (i.p.) once daily with NAC (Sigma-Aldrich) (100 mg/kg body weight) or PBS (control group) for ten consecutive days.

Colony-Forming Assay

Transduced HSPCs were plated in methylcellulose (R&D systems) with 50 ng/ml murine SCF, 10 ng/ml murine IL-3, and 10 ng/ml murine IL-6, and counted and passaged after 6-7 days. Values represent colony-forming unit frequencies, calculated as number of colonies per input cells (%).

Peripheral Blood Analysis

Blood samples were collected into EDTA-coated tubes via lateral tail vein incision and analyzed on a Hemavet 950 analyzer (Drew Scientific). PB smears were stained with Wright-Giemsa staining according to the manufacturer's instructions (BioScientific).

Flow Cytometric Analysis

BM mononuclear cells (MNCs) were isolated from mouse BM and purified by density gradient centrifugation through Ficoll. For analysis of LSCs, one million of BM MNCs or splenocytes were stained with a mixture of biotinlylated antibodies against mouse CD11b, CD3e, CD45R (B220), Ly-6G (Gr-1), and TER-119. Subsequently, the cells were co-stained with streptavidin-Prep-Cy5.5, anti-c-Kit-PE/Cy7, anti-CD34-APC, and anti-CD16/32-APC/Cy7. For cell-cycle analysis, cells were stained with Hoechst 33342 and Pyronin Y (Sigma) in HBSS containing 10% fetal bovine serum. For apoptosis analysis, cells were stained with Annexin V (Biolegend) in Annexin V buffer according to the manufacturer's instructions. All antibodies, unless otherwise stated, were purchased from Biolegend and eBioScience. Flow cytometry was performed on a BD LSRFortesa or FACAria, and all flow cytometric data were analyzed with FlowJo Software (TreeStar).

ChIP assay

ChIP assay was performed with a ChIP-IT-Express Enzymatic kit (Active Motif, Carlsbad, CA, USA) according to the manufacturer's instructions. Genomic DNA was pulled down by anti-Flag or IgG control antibodies (Thermo Fisher Scientific, Rockford, IL, USA). Immunoprecipitated DNA fragments were amplified by using the pair of primers listed in Supplemental Table 3.

RNA Extraction, Reverse Transcription-PCR, and Real-time PCR

Total RNA samples were extracted using Quick-RNA MicroPrep Kit (Zymo Research). For real-time PCR, 50 ng of total RNA was reverse-transcribed using Superscript III^{TM} cCNA Synthesis Kit (Invitrogen). Hprt or Gapdh expression were used as internal controls to normalize relative expression of each gene. The list of primers is included in the supplemental Table 1.

Luciferase Reporter Assay

293T cells were cultured in 24-well plate overnight, and then transfected with 50 ng of Slc13a3 luciferase reporter, 400 ng of pMig or pMig-Slug, and 50 ng of pCMV-LacZ using Fugene HD. After 72 hours of transfection, cells were lysed in 250 µl of the passive lysis buffer (Promega) and assayed with a luciferase assay kit (Promega) as directed by the manufacturer. Luciferase activities were expressed as relative luciferase/LacZ activities and normalized to those of control transfections in each experiment.

Limiting Dilution Assays

LSCs were isolated from BM of *Slug*$^{+/+}$ and *Slug*$^{-/-}$ recipient mice that developed full-blown leukemia were co-stained with LSC antibodies, sorted, and injected into sublethally irradiated C57BL/6 mice with three different doses of donor cells for each group. The number of recipient mice developed leukemia was counted for each group with each dose of donor cells. ELDA software [3] was used to estimate the frequency of LSCs/LICs.

Statistical analyses

Unpaired Student's t-test was used to statistically analyze all the two experimental groups. A log-rank (Mantel-Cox) test was used to determine p values for all Kaplan-Meier survival analyses. Microarray data were analyzed by analysis of variance (ANOVA) testing. Differences of $P < 0.05$ were considered statistically significant and are denoted as * $P < 0.05$; ** $P < 0.01$. All qPCR and PCR results were repeated at least three times.

1. Wei J, Wunderlich M, Fox C, Alvarez S, Cigudosa JC, Wilhelm JS*, et al.* Microenvironment determines lineage fate in a human model of MLL-AF9 leukemia. *Cancer Cell* 2008 Jun; **13**(6)**:** 483-495.

2. Zhang CC, Kaba M, Ge G, Xie K, Tong W, Hug C*, et al.* Angiopoietin-like proteins stimulate ex vivo expansion of hematopoietic stem cells. *Nat Med* 2006 Feb; **12**(2)**:** 240-245.

3. Hu Y, Smyth GK. ELDA: extreme limiting dilution analysis for comparing depleted and enriched populations in stem cell and other assays. *J Immunol Methods* 2009 Aug 15; **347**(1-2)**:** 70-78.