Supplementary Material

Transactivation of *Sytl1/Slp1* by Meis1 promotes CXCL12/CXCR4 signaling and myeloid leukemogenesis *in vivo*

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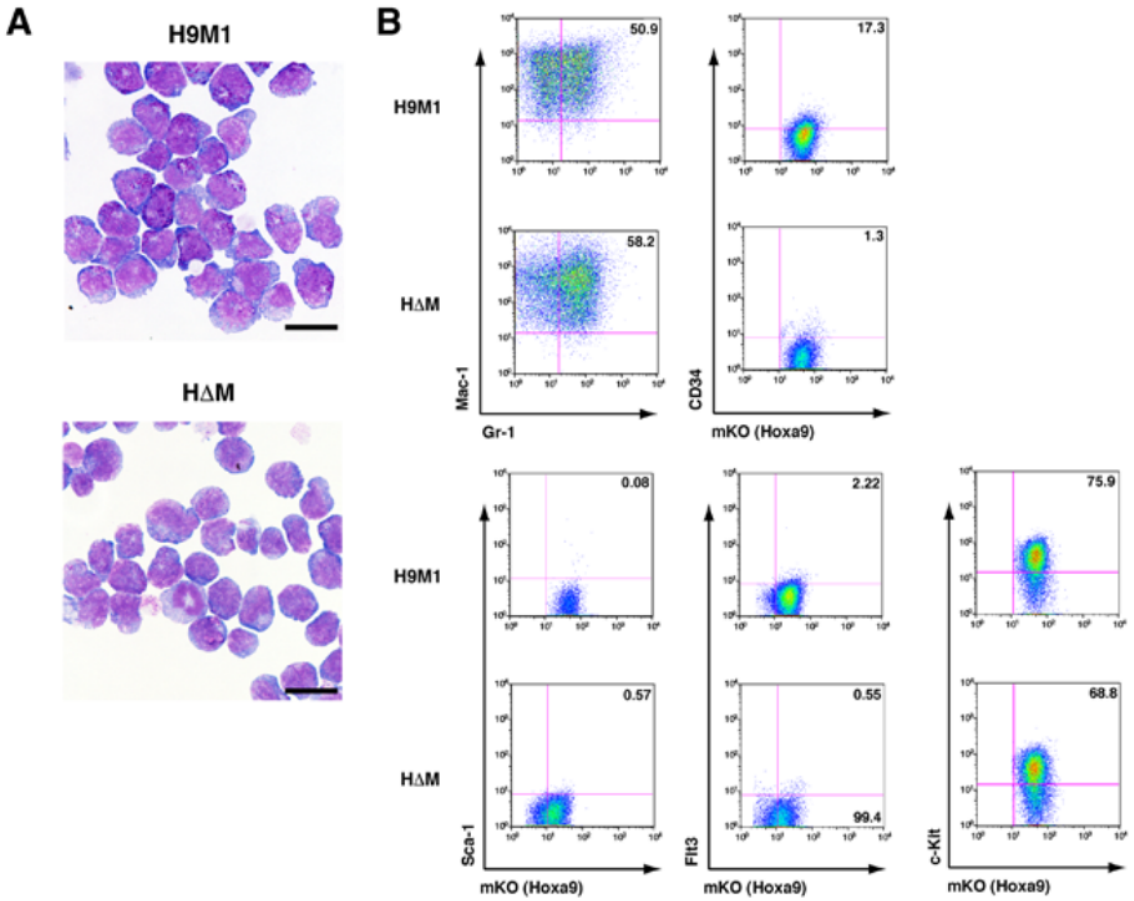
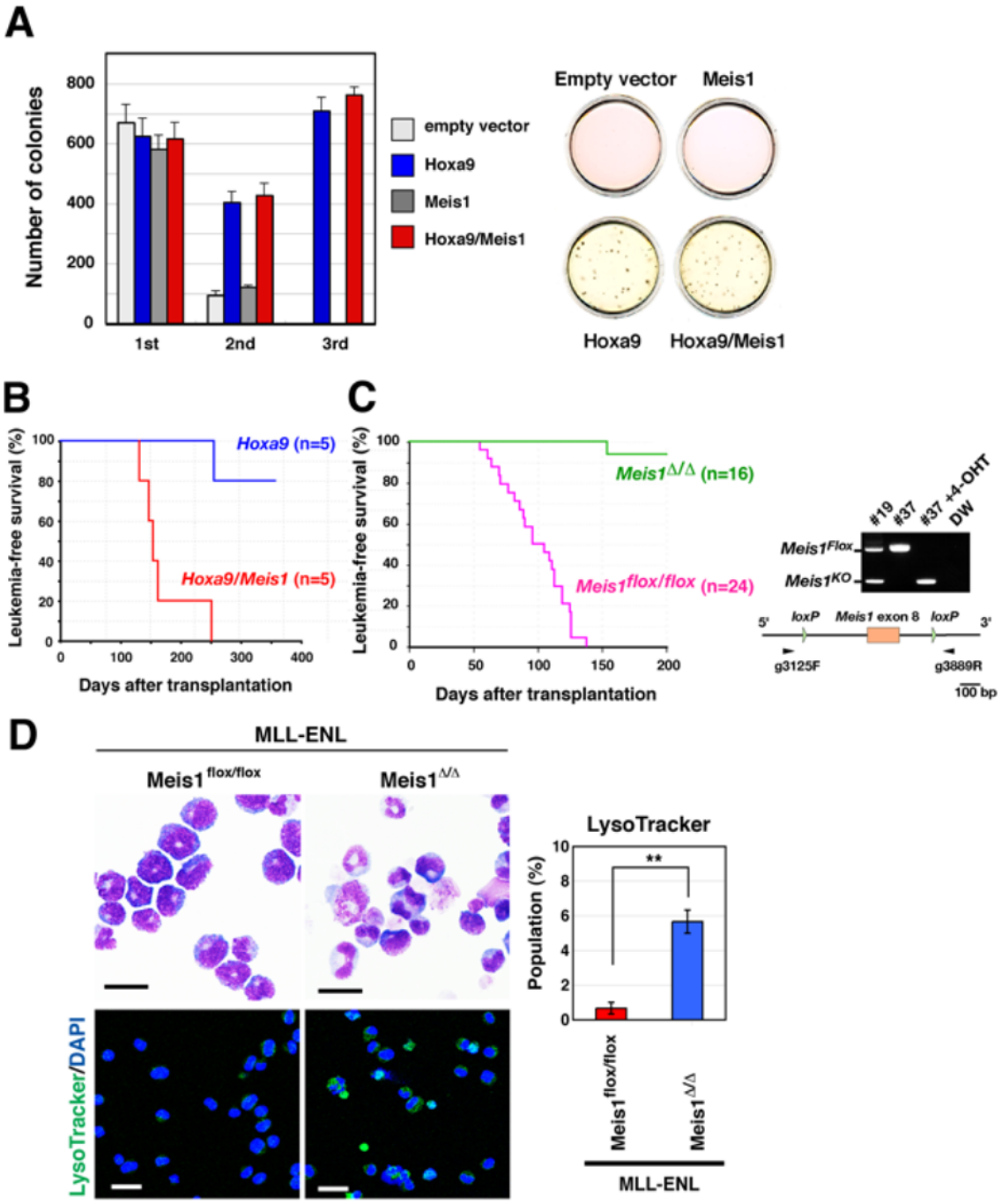
Ten Supplemental Figures

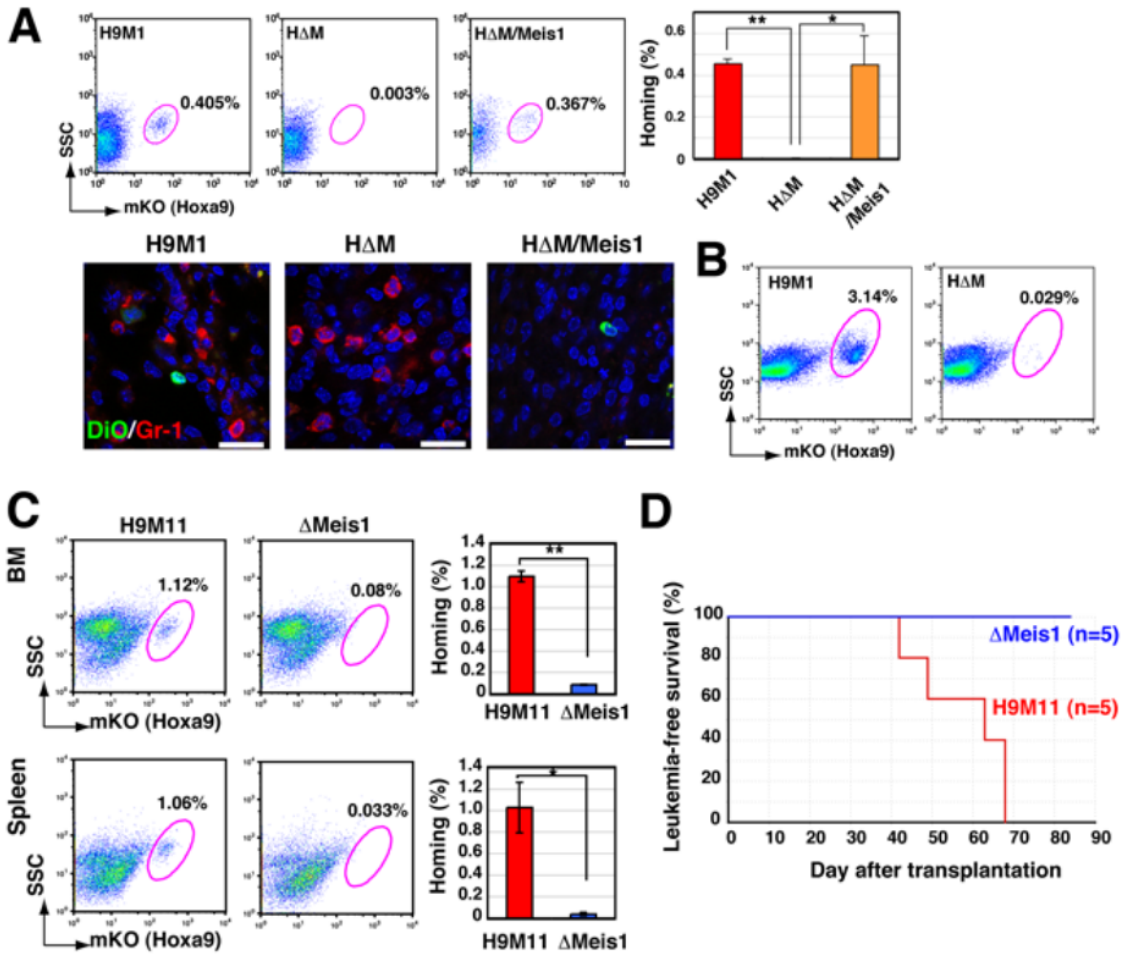


Supplemental Figure 1. Characterization of H9M1 cells and the effect of *Meis1* knockout. (*A*) Giemsa staining of H9M1 and HΔM cells. Scale bar: 20 μm. (*B*) Surface marker expression observed in H9M1 and HΔM cells. *Meis1* KO induced a mild increase of Gr-1-positive, Sca-1 positive fractions and a decrease of the CD34-positive fraction. Data are representative of 2 independent experiments.

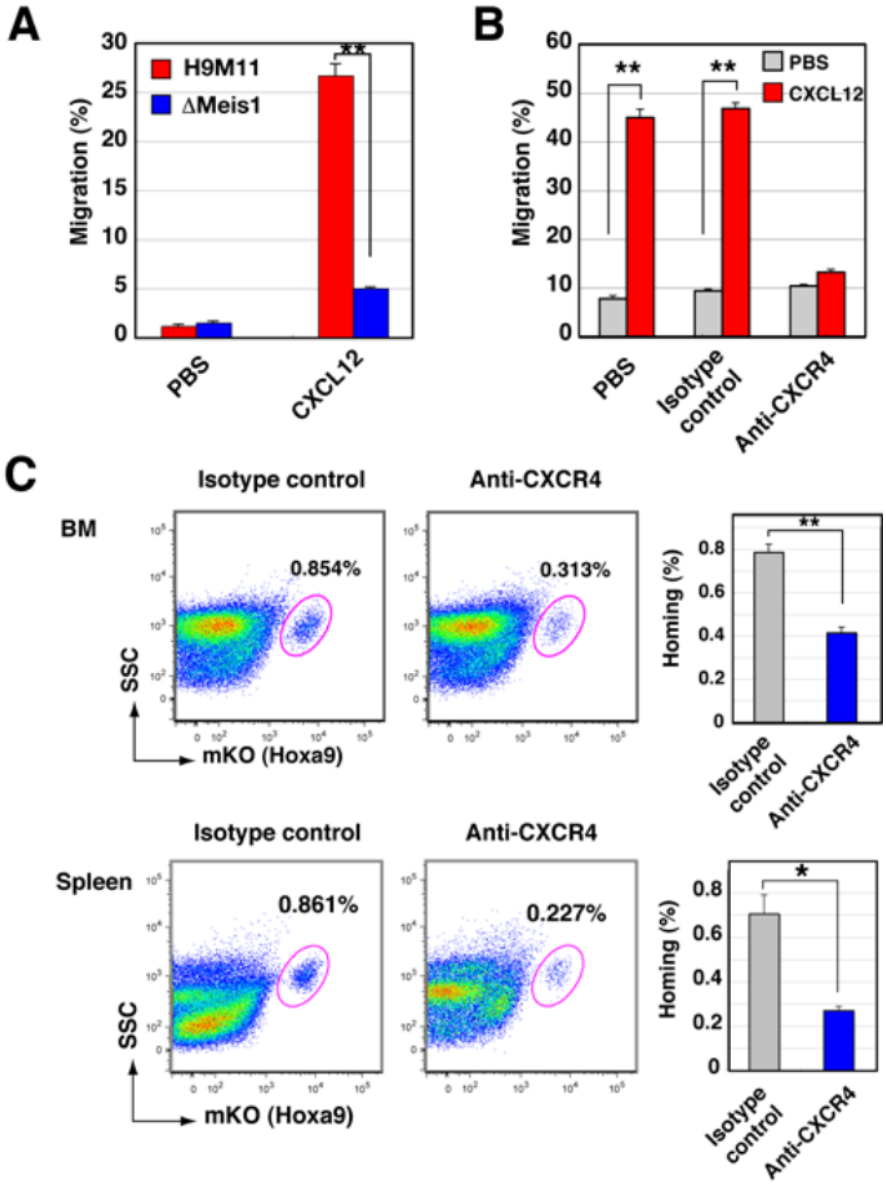


Supplemental Figure 2. Meis1 functions in leukemogenesis, leukemic cell homing and engraftment. (*A*) Replating assays using primary murine bone marrow cells infected with indicated retroviruses. The colony numbers in methylcellulose culture during three serial replating experiments were measured. Data represent means ± SEM from 3 independent experiments (left) and representative cultures at the third replating (right) are shown. (*B*) Leukemia-free survival of lethally irradiated recipient mice transplanted with bone marrow cells transduced with *Hoxa9* and *Meis1*, or with *Hoxa9* only. (*C*) Leukemia-free survival of lethally irradiated recipient mice transplanted with bone marrow cells transduced with the *MLL-ENL* retrovirus. *Meis1* KO was achieved by post-transplantation tamoxifen

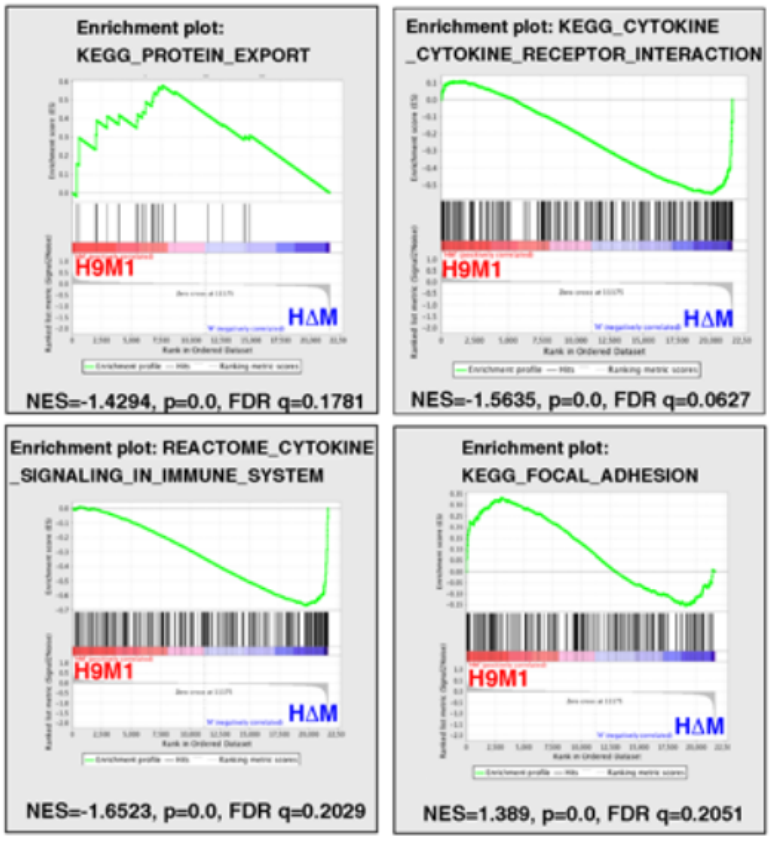
administration for two weeks twice at five-day intervals (top). One of sixteen mice administered tamoxifen developed AML due to heterozygous preservation of *Meis1* through an acquired mutation of the *loxP* site. Genomic PCR of the *Meis1* locus in *MLL-ENL*-induced AML using bone marrow cells derived from *Meis1* conditional knockout mice (*left*). $Meis1^{flox/flox}$ (#37), $Meis1^{ko/flox}$ (#19) and $Meis1^{ko/ko}$ (#37 with 4-OHT treatment). The map and the position of primers (g3125F: 5'-GCATCTTCTTACCAGTTGACG-3' and g3889R: 5'- CACATGGACTCCCGAGCC-3') of the *Meis1* flox allele are shown (bottom). (*D*) Differentiation of *MLL-ENL*-induced AML cells by *Meis1* knockout was demonstrated by the increased frequency of granulocytes (top, Giemsa staining) or increased content of lysosomes (bottom, LysoTracker staining). Representative pictures of 3 independent experiments. Scale bar: 20 μm. Quantification of LysoTracker-positive cells is indicated (right). Data are mean ± SEM from 3 independent experiments (**, $P < 0.01$ by 2-tailed Student's *t* test).



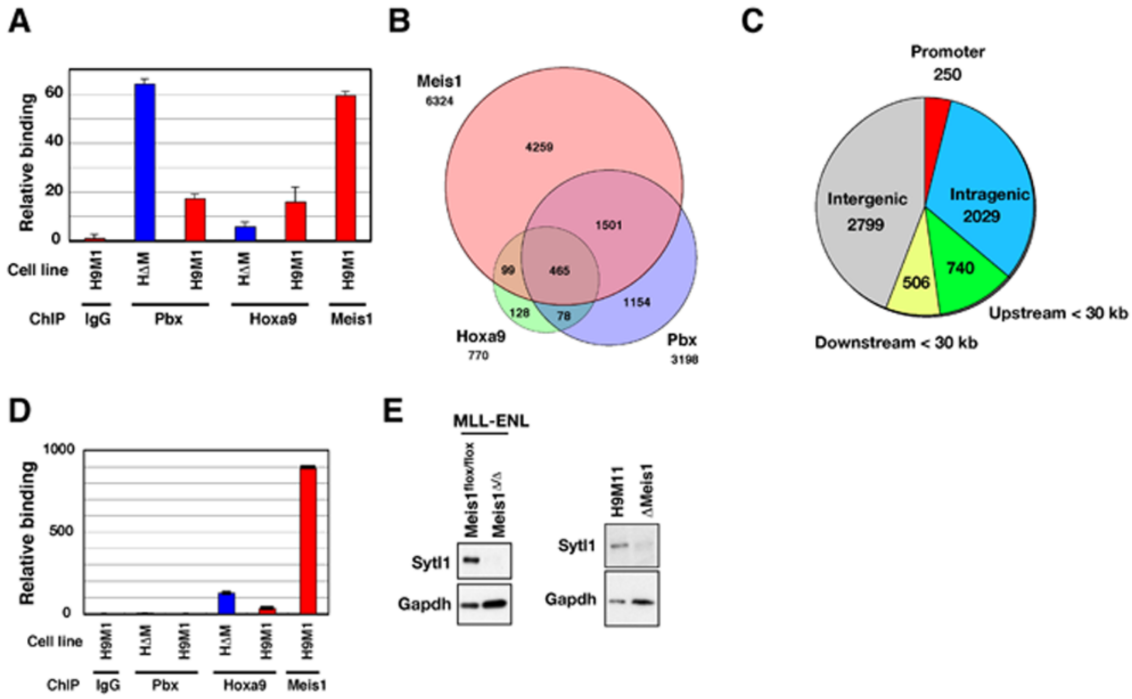
Supplemental Figure 3. Meis1 functions in leukemogenesis and leukemic cell homing. (*A*) H9M1 cells in the spleen 48 hours after transplantation were detected as an mKO-positive fraction by flow cytometry. Representative data of 3 independent experiments (top left). A significant reduction of H9M1 cells in the spleen was observed in animals transplanted with *Meis1* knockout cells. The frequency was restored by re-introduction of *Meis1*. Frequencies of mKO-positive cells in the spleen are indicated as the means ± SEM of 3 independent experiments (*, $P < 0.05$, **, $P < 0.01$ by 1-way ANOVA with Dunnett's multiple comparison test) (top right). DiO-stained H9M1 cells were detected in frozen sections of the spleen (bottom), absent in *Meis1* knockouts (center) and were observed after *Meis1* re-introduction into HΔM (right). Gr-1 is indicated by red fluoro-dye and nuclei were counterstained with DAPI. Representative pictures of 3 independent experiments. Scale bar: 20 μm. (*B*) H9M1 cells in the spleen two weeks after transplantation were detected as in (*A*). (*C*) H9M11 cells in bone marrow (top) or the spleen (bottom) 48 hours after transplantation were detected as an mKO-positive fraction by flow cytometry. Representative data of 3 independent experiments (left). A significant reduction of H9M11 cells was observed after transplantation of *Meis1* knockout cells. Frequencies of mKO-positive cells in spleen are indicated as the means ± SEM from 3 independent experiments (*, $P < 0.05$, **, $P < 0.01$ by 2-tailed Student's *t* test) (right). (*D*) Survival curve of the mice transplanted with H9M11 cells with or without *Meis1* knockout.



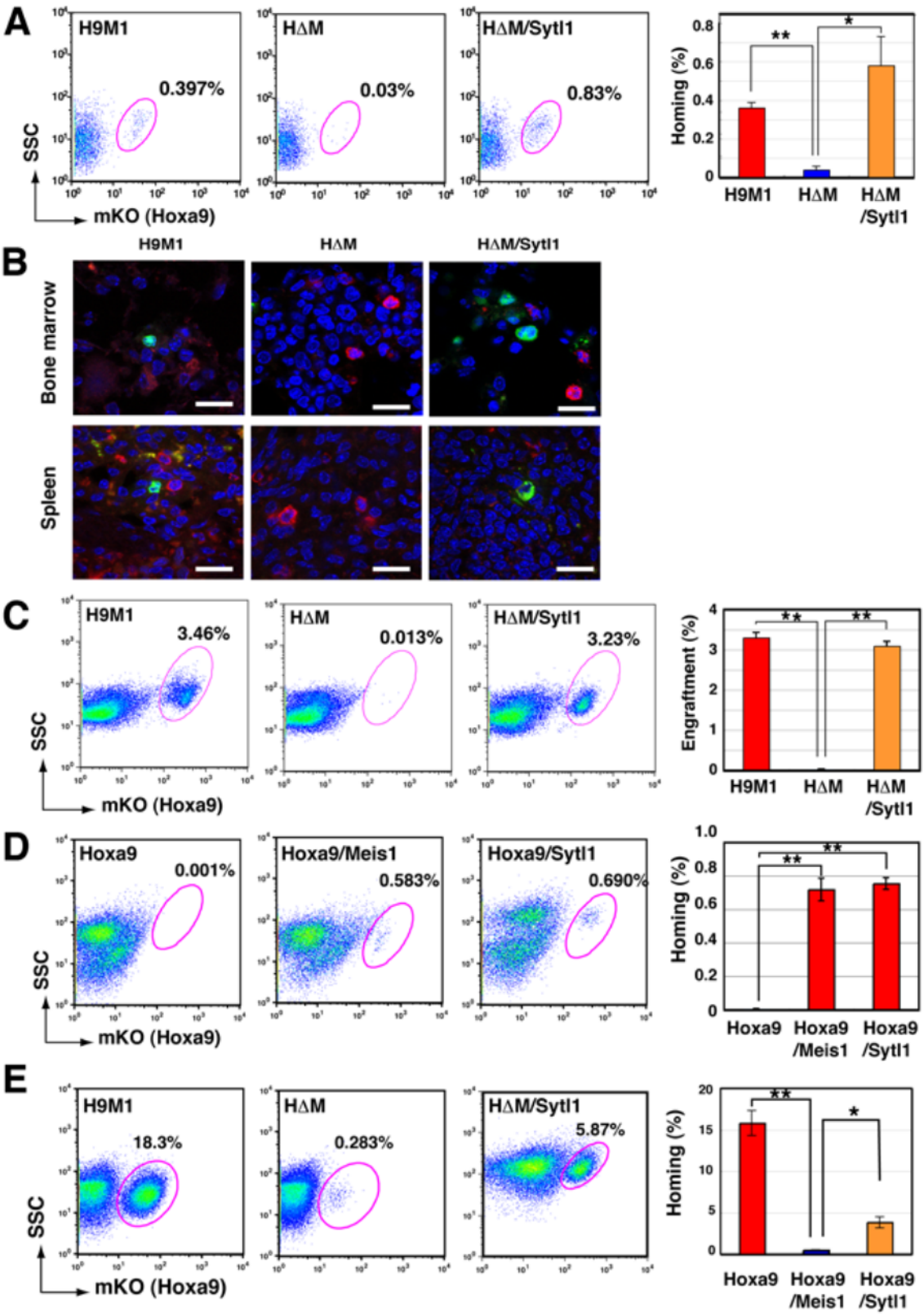
Supplemental Figure 4. Association of the CXCL12-induced cell migratory activity with Meis1. (*A*) Cell migration assay of the H9M11 cell line. CXCL12 significantly enhanced cell migration of H9M11 cells but not in Meis1-deleted H9M11. Results represent mean frequencies of migratory cells/5 x 10^5 cells ± SEM (n = 3, **, P < 0.01 by 2-tailed Student's t test). (*B*) Suppression of cell migration by an anti-CXCR4 antibody. H9M1 cells were treated with an anti-CXCR4 or an isotype control antibody in the presence or absence of CXCL12, and the frequencies of cell migration are indicated (n = 3, **, P < 0.01 by 2-tailed Student's t test). (*C*) Inhibition of cell homing by anti-CXCR4. H9M1 cells in bone marrow 48 hours after transplantation was detected by flow cytometry and gating on the mKO-positive fraction. A significant reduction of H9M1 cells in bone marrow (top) and spleen (bottom) was observed as a result of anti-CXCR4 treatment. Representative data of 3 independent experiments. Frequencies of mKO-positive cells in bone marrow and the spleen are indicated as the means ± SEM of 3 independent experiments (*, P < 0.05, **, P < 0.01 by 2-tailed Student's t test) (right).



Supplemental Figure 5. The data sets of gene expression differences resulting from *Meis1* knockout were used for GSEA. Enrichment plots are shown for selected sets identified by GSEA. Normalized enrichment scores (NES), nominal *p*-values and FDR *q*-values are indicated.

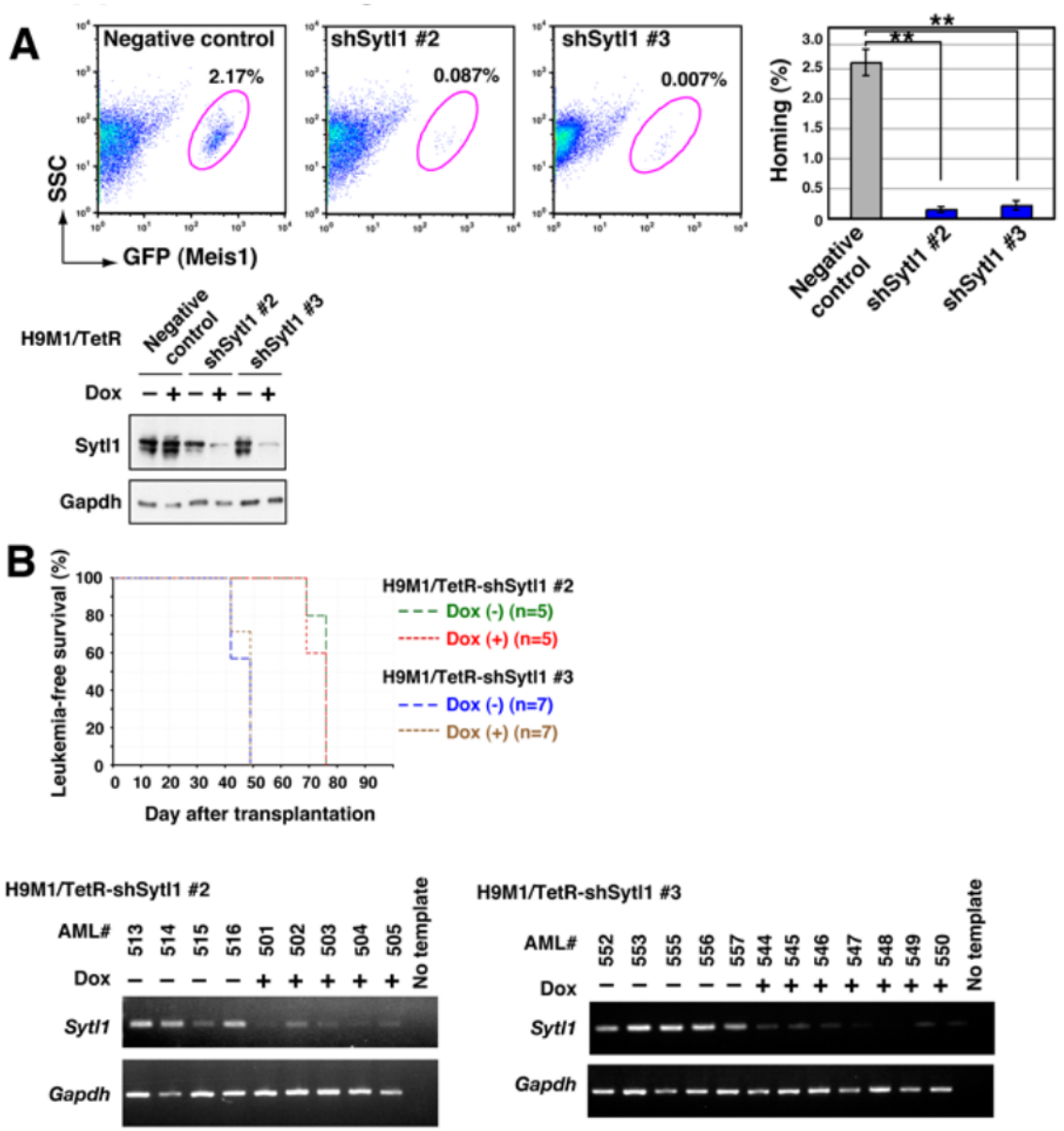


Supplemental Figure 6. ChIP-seq identified *Sytl1* as a transcriptional target of Meis1. (*A*) Chromatin immunoprecipitation of Meis1 was validated by quantitative PCR of the *Maf* locus. Immunoprecipitated DNA samples using indicated antibodies were amplified using *Maf* forward (5'- TGGCTGCGCCCCAGTAGC-3') and reverse (5'- GAGAGCGGCAGCGATTAGC-3') primers. Data are presented as the means ± SEM of 3 independent experiments. (*B*) Venn diagrams for Meis1, PBX and Hoxa9 binding sites in the whole genome. (*C*) Distribution of 6,324 Meis1-binding peaks in relation to known genes. Meis1 binding regions were divided into indicated categories. Promoter regions indicate < one kb from the transcription start site, and intergenic regions indicate > 30 kb from genes nearby. (*D*) Quantitative ChIP PCR for Meis1-binding at the *Sytl1* locus. Data are presented as the means ± SEM of 3 independent experiments. (*E*) Decreased Sytl1 protein expression in *MLL-ENL*-induced AML cells (left) and H9M11 cells (right) by *Meis1* KO, shown by immunoblotting using α-Sytl1; blots are representative of 2 independent experiments.

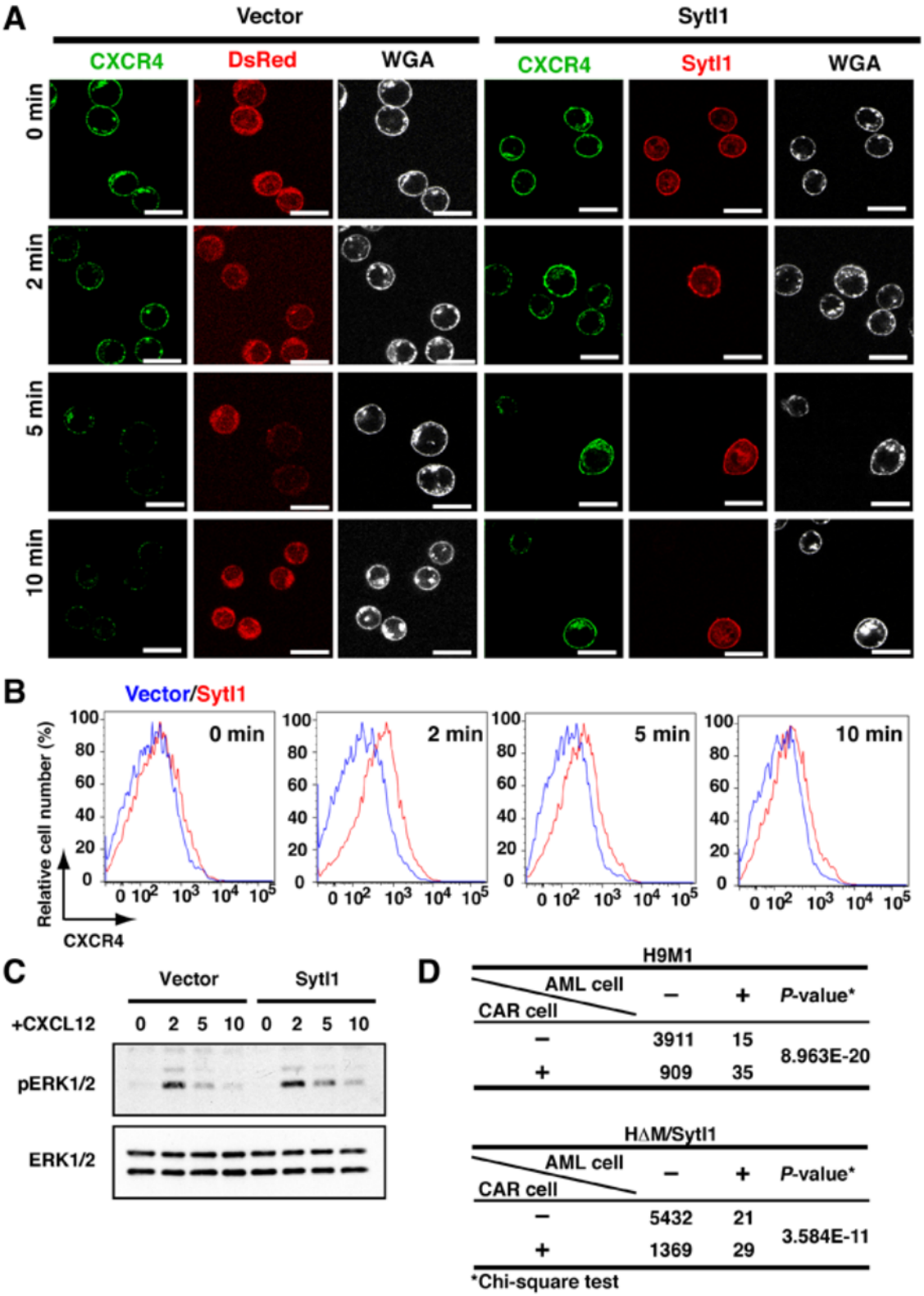


Supplemental Figure 7. Sytl1 promotes leukemic cell homing and engraftment. (*A*) *Sytl1* was retrovirally introduced into HΔM cells. Forty-eight hours after transplantation of the indicated cells, spleen samples were analyzed for

mKO-positive fractions by representative flow cytometry (3 experiments) (left). Frequencies of mKO-positive cells in the spleen are indicated as the means ± SEM from 3 independent experiments (*, $P < 0.05$; **, $P < 0.01$ by 1-way ANOVA with Dunnett's multiple comparison test) (right). (*B*) DiO-stained H9M1, HΔM and HΔM transfected with Sytl1 cells were detected in frozen sections of bone marrow (top panels) and spleen (bottom panels). Gr-1 is indicated by red fluoro-dye and nuclei were counterstained with DAPI. Representative pictures of 3 independent experiments. Scale bar: 20 μm. (*C*) Representative flow cytometric analysis as in (*A*) was performed two weeks after transplantation (3 experiments) (left). Frequencies of mKO-positive cells in bone marrow are indicated as the means ± SEM ($n = 3$, **, $P < 0.01$ by 1-way ANOVA with Dunnett's multiple comparison test) (right). (*D*) Homing of *Hoxa9*-transformed wild-type bone marrow cells was enhanced by both *Meis1* and *Sytl1*. Representative flow cytometry of 3 independent experiments (left). Frequencies of mKO-positive cells in bone marrow were measured 48 hours after transplantation and indicated as the means ± SEM ($n = 3$, **, $P < 0.01$ by 1-way ANOVA with Dunnett's multiple comparison test) (right). (*E*) Homing of H9M1, HΔM and HΔM/Sytl1 cells by intra-femoral injection. Forty-eight hours after transplantation of the indicated cells, spleen samples were analyzed for mKO-positive fractions by representative flow cytometry (5 experiments) (left). Frequencies of mKO-positive cells in the spleen are shown as the means ± SEM from 5 independent experiments (*, $P < 0.05$, **, $P < 0.01$ by 1-way ANOVA with Dunnett's multiple comparison test) (right).

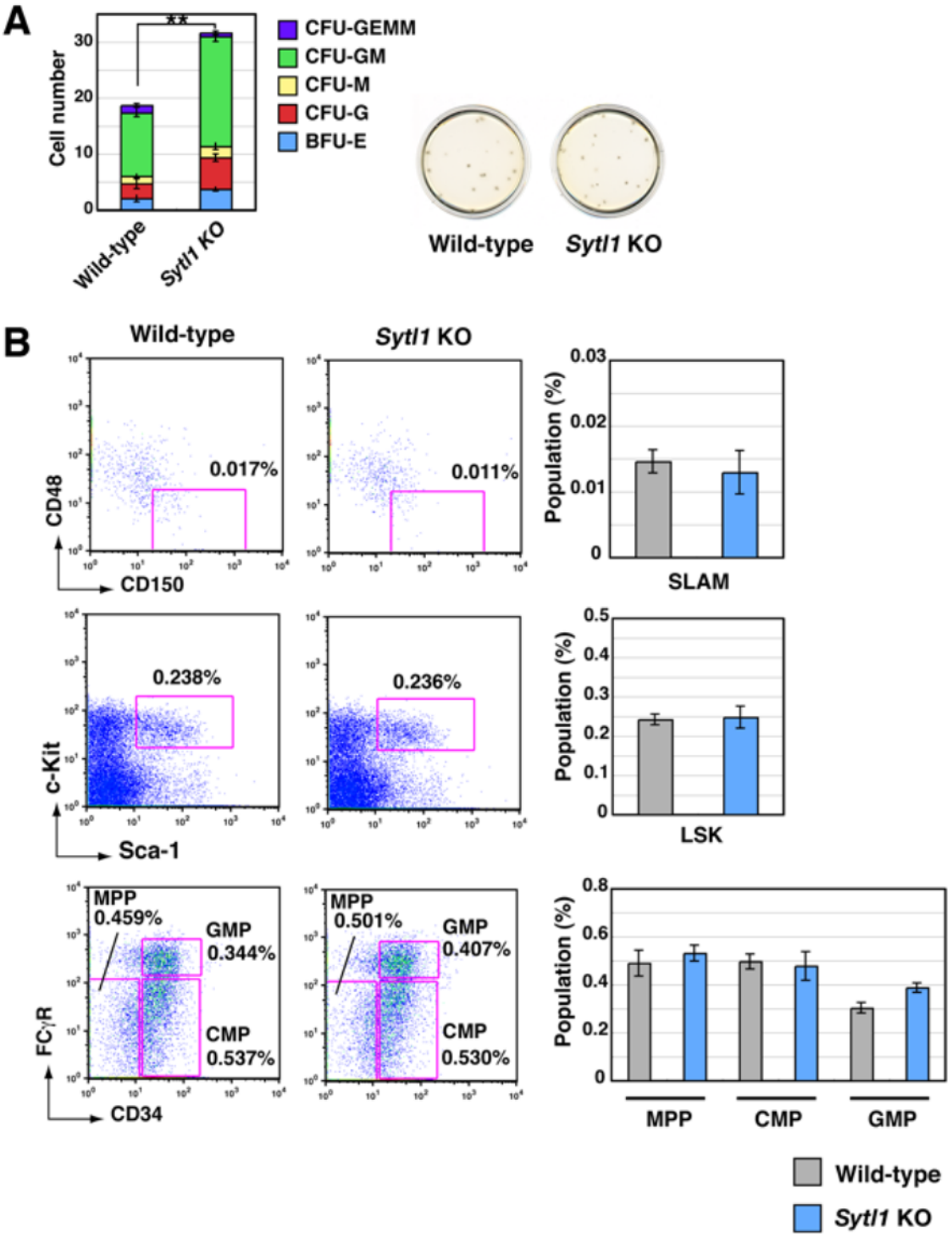


Supplemental Figure 8. Doxycycline-inducible *Sytl1* knockdown in H9M1 cells. (*A*) Homing of leukemia cells to bone marrow was suppressed by inducible Sytl1 knockdown. Representative of 3 experiments (top left). Frequencies of GFP-positive cells in bone marrow are measured 48 hours after transplantation and indicated as the means ± SEM (n = 3, **, P < 0.01 by 1-way ANOVA with Dunnett's multiple comparison test) (top right). Knockdown of *Sytl1* was confirmed by Western blotting; blots are representative of 2 experiments (bottom). (*B*) Doxycycline-inducible *Sytl1* knockdown did not suppress leukemia development. Kaplan-Meyer survival curves are shown in shSytl1 #2 and #3 cohorts with or without doxycycline administration (top). *Sytl1* knockdown was confirmed in leukemic cells sorted from peripheral blood of leukemic mice by RT-PCR. Representative of 2 experiments (bottom).



Supplemental Figure 9. Sytl1 promotes membrane trafficking of CXCR4 and activates downstream signaling. (*A*) Membrane localization of CXCR4 in 32Dcl3 cells was analyzed by immunofluorescence. Sytl1 expression was visualized as a DsRed-Sytl1 fusion protein. Alexa Fluor 350-conjugated wheat germ agglutinin (WGA) was used to detect plasma membrane. Representative pictures of 3

independent experiments. Scale bar: 20 μm. (*B*) Representative flow cytometric analysis of 32Dcl3 cells transfected with *Sytl1* (red) or empty vector (blue) (3 experiments). CXCR4 on the cellular surface was detected with an anti-CXCR4 antibody and flow cytometric analysis at the indicated time after CXCL12 stimulation. (*C*) ERK phosphorylation after CXCL12 stimulation in 32Dcl3 cells. Minutes after CXCL12 stimulation are indicated; blots are representative of 3 experiments. (*D*) Statistical evaluation of juxtaposition of CAR cells and AML cells. Confocal images of bone marrow frozen sections were obtained and the numbers of both CAR, H9M1 or HΔM/Sytl1 cells per 225 μm^2 in bone marrow were counted. CAR cells and AML cells on the same 225 μm^2 grid were judged as positive. The relationship between CAR cells and H9M1 cells, and CAR cells and HΔM/Sytl1 cells was significant by Chi-square test. *P* values are shown.



Supplemental Figure 10. Hematopoiesis of *Sytl1* KO mouse bone marrow. (*A*) Colony forming assay showed mild but significant increase of colony formation in *Sytl1* KO bone marrow (n = 3, **, $P < 0.01$ by 2-tailed Student's t test) (left). Representative figures for colony formation are shown (3 experiments) (right). (*B*) Compositions of SLAM cells (CD34- and CD48-negative and CD150-positive LSK cells) (top) and LSK cells (middle) and myeloid progenitors (bottom) are exhibited. Representative of 3 independent experiments.

Supplemental Video Legends

Supplemental Video 1. Motility of 32Dcl3 cells during CXCL12 stimulation. 32Dcl3 cells (2×10^4) transfected with an expression vector bearing the Cxcr4-GFP fusion gene were cultured on 35-mm glass-based dishes coated with 2 μg/mL recombinant mouse VCAM-1/CD106 Fc chimera with growth medium. After 24 hours cultivation, 2×10^4 CXCL12-coated beads were added to the dishes. After addition of CXCL12-coated beads, GFP images were obtained in a 5% CO_2 humidified atmosphere at 37˚C. Time-lapse images were taken at 30 seconds intervals over 30 minutes using an EMCCD camera. Movie is representative of 40 cells in 3 independent experiments.

Supplemental Video 2. Motility of 32Dcl3 cells expressing Sytl1 on CXCL12 stimulation. 32Dcl3 cells (2×10^4) transfected with expression vectors bearing the Cxcr4-GFP of Sytl1-DsRed fusion genes were cultured on 35-mm glass based dishes coated with 2 μg/mL recombinant mouse VCAM-1/CD106 Fc chimera with growth medium. After 24 h cultivation, 2×10^4 CXCL12-coated beads were added to the dishes. After addition of CXCL12-coated beads, GFP and DsRed images were obtained in a 5% CO_2 humidified atmosphere at 37˚C. Time-lapse images were taken at 30 seconds intervals over 30 minutes using an EMCCD camera. Movie is representative of 40 cells in 3 independent experiments.

Supplemental Table 5. Primer sequences

Genes	Primers	Sequences
Cxcl12	Forward primer	5'-GAGCCAACGTCAAGCATCTG-3'
	Reverse primer	5'-GGGTCAATGCACACTTGTC-3'
Gapdh	Forward primer	5'-TGTCGTGGAGTCTACTGGTGTCTTC-3'
	Reverse primer	5'-GGAGATGATGACCCTTTTGGCTC-3'
Sytl1	Forward primer	5'-TCTGATGTCCTCAAACGAGATGC-3'
	Reverse primer	5'-ACCAAAATGGGCGTGATGG-3'
Sytl1	Forward primer	5'-CAAGTGCTGGGATTTAGAGG-3'
(promoter)	Reverse primer	5'-GTCATACCTGCAACCGTGGC-3'
Meis1	Forward primer1	5'-GCATCTTCTTACCAGTTGACG-3'
	Forward primer2	5'-GACGATGATGACCCTGATAAG-3'
	Reverse primer	5'-CACATGGACTCCCGAGCC-3'
D3Mit159	Forward primer	5'-CTAACCCAGATAGGGCTTTGG-3'
	Reverse primer	5'-AGATTACCCTCTGGTGTTTACATACA-3'
c-Maf	Forward primer	5'- TGGCTGCGCCCCAGTAGC-3'
	Reverse primer	5'-GAGAGCGGCAGCGATTAGC-3'