Supplemental Figure legends

Supplemental Figure 1.
ASXL1 transcript was detected in human leukemia cell lines with or without *ASXL1* mutations.
Relative expression levels of *ASXL1* were examined by qRT-PCR in leukemia cell lines. *ASXL1* genes of these cell lines harbor mutations as follows: K562, heterozygous *ASXL1* Y591Y/X; MEG-01, homozygous *ASXL1* G646WfsX12; TS9:22, homozygous *ASXL1* R693X; KBM5 homozygous *ASXL1* G710X; NOMO1 homozygous *ASXL1* R639X. The values were normalized by GAPDH mRNA levels. All data with error bars are presented as mean ± SEM of two independent experiments.

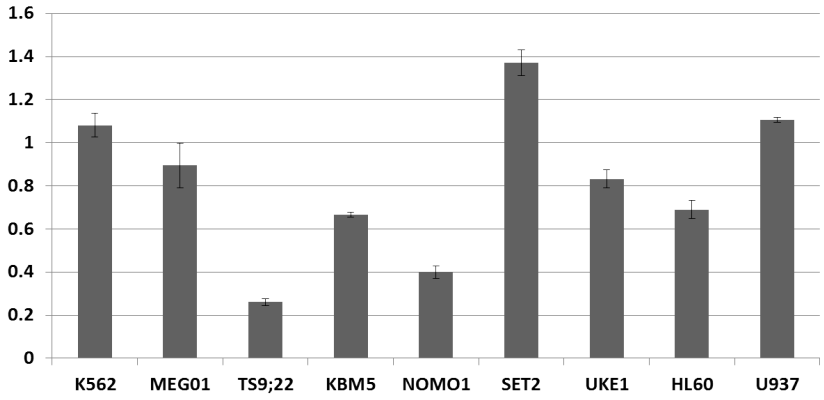
Supplemental Figure 2.
ASXL1 mutations inhibited myeloid differentiation, but have no impact on growth rate.
(A) Surface expression of CD11b in HL60 or FDC-P1 transduced with the indicated plasmid after incubation with 10^{-6}M ATRA (left) or 10 ng/mL GM-CSF (right), respectively, for three or six days was analyzed by flow cytometry. Red histograms indicate control (IgG). (left) blue, day3; (right) blue, day3; orange, day6.
(B) The relative growth rate of 32Dcl3 cells transduced with pMYs-IG (Mock), pMYs-FLAG–tagged ASXL1-MT1-IG (MT1), pMYs-FLAG–tagged ASXL1-MT2-IG (MT2), or pMYs-FLAG–tagged ASXL1-WT-IG (WT) in the presence of 1 ng/mL IL-3 (mean ± SEM).

Supplemental Figure 3.
ASXL1 mutations reduced the expression of Clec5a, an important gene for myeloid differentiation.
(A) Overexpression of *Clec5a* promoted the differentiation of 32Dcl3 cells. (left) Morphology of 32Dcl3 cells expressing pMYs-IP (Mock, top) and pMYs-Clec5a-IP (Clec5a, bottom) after incubation with 50ng/ml G-CSF for five days. Bars, 20 μm. (right) The proportion of differentiated and segmented cells is shown.
(B) Efficient knocked down of *Clec5a* expression with 2 different anti-*Clec5a* shRNAs compared to control in 32Dcl3 cells as shown by qRT-PCR. These

data were normalized by *Gapdh* mRNA. Control indicates pMXs-U6-Puro.
(C) Two shRNAs efficiently inhibited G-CSF induced surface expression of *Clec5a* in Flow cytometric analysis. Filled histograms show control (IgG).
(D) Related to Figure 3A, mean fluorescence intensities (MFIs) of CD11b are shown. 32Dcl3 cells transduced with indicated plasmids were incubated with 50 ng/mL G-CSF for 6 days.

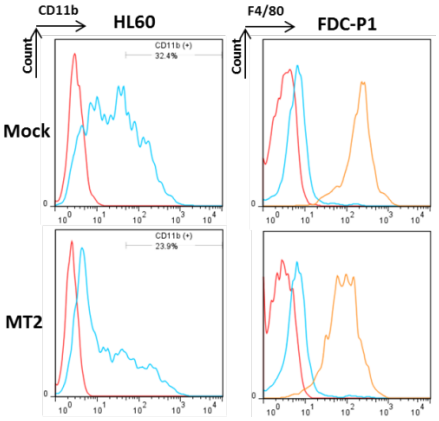
Supplemental Figure 4.
ASXL1 mutations induced MDS-like symptoms in a mouse BMT model.
Kaplan-Meier analysis for the survival of mice that received transplants of BM cells transduced with pMYs-IG (Mock, n=13, blue line) and pMYs-FLAG-ASXL1-MT1-IG (ASXL1-MT1, n=10, red line). *P*-values were calculated using log-rank test.

Supplemental Figure 5.
Mutant ASXL1 resulted in derepression of miR-125a.
Overexpression of ASXL1-MT, not ASXL1-WT, induced the expression of miR-125a. qRT-PCR for miR-125a in 32Dcl3 cells transduced with pMYs-IG, pMYs-FLAG-ASXL1-MT2-IG, pMYs-FLAG-ASXL1-WT. Relative expression levels normalized by RNU6B (RNA, U6 small nuclear 2) were shown.



Supplemental Figure 1

A



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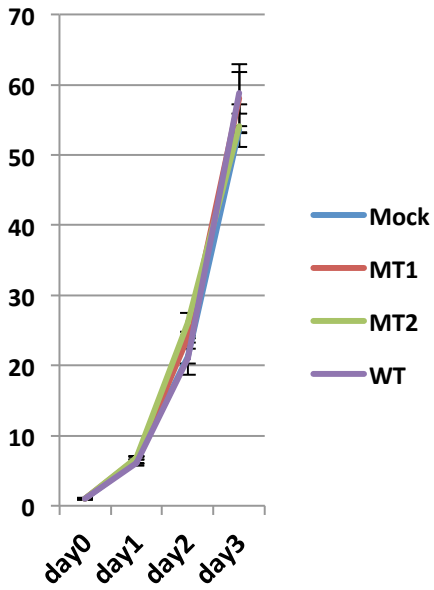
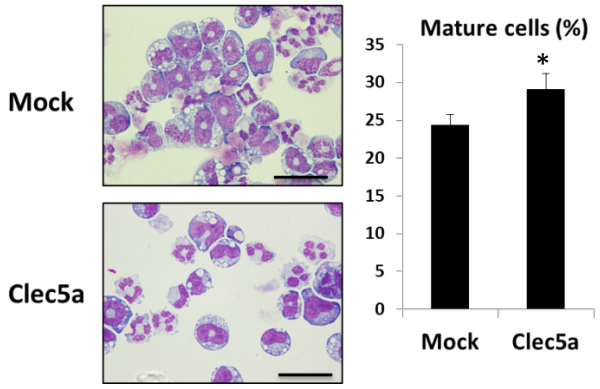


Supplemental Figure 2

A



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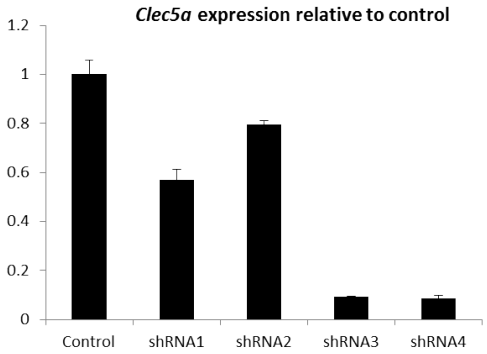

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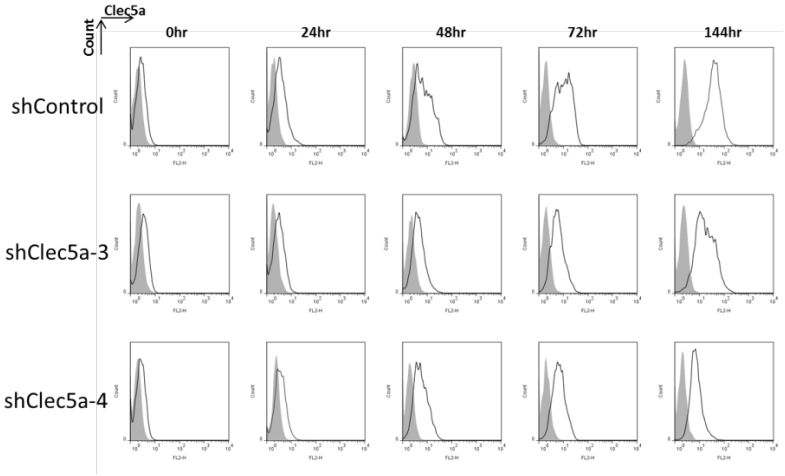

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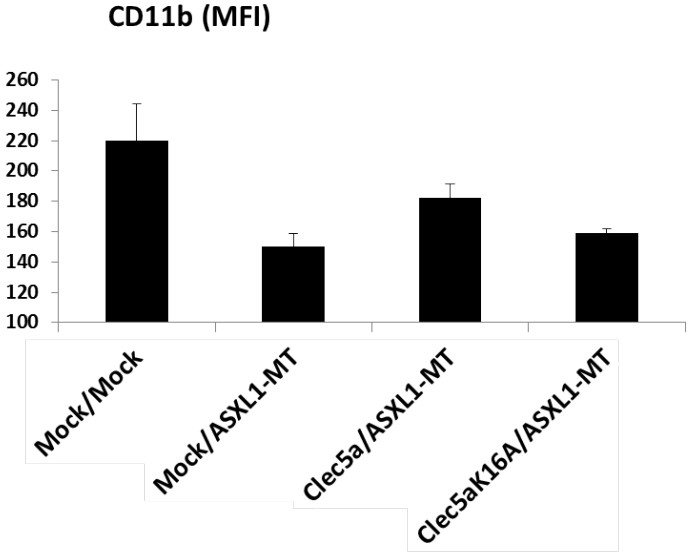
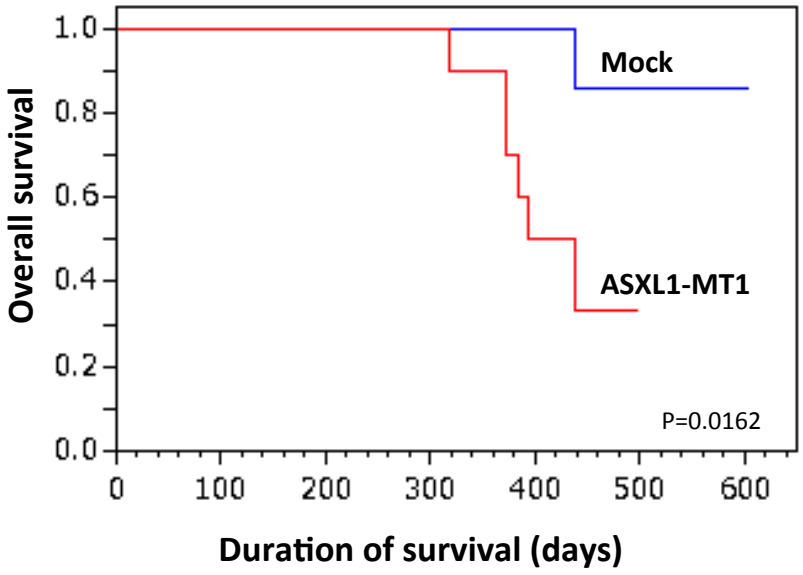
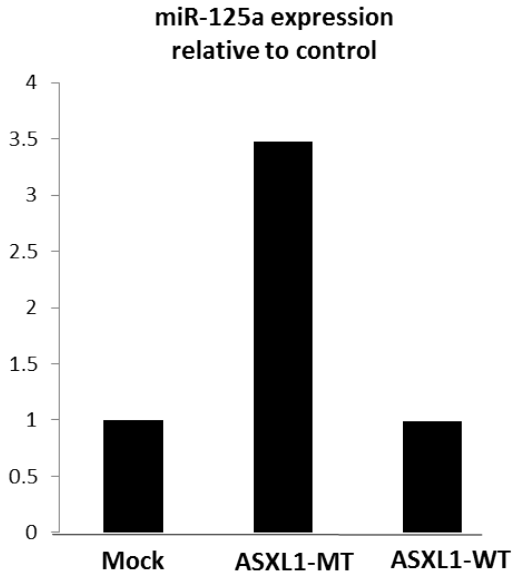


Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5

Supplemental Methods

Expression analysis

BM cells derived from mock-transplanted mice and ASXL1-MT transplanted mice were incubated with biotinylated antibodies for CD3e, B220, and TER-119, followed by incubation with streptavidin Micro Beads (Miltenyi Biotec). The marker-negative fraction was separated with LS Columns (Miltenyi Biotec). Using 32Dcl3 cells transduced with pMYs-IG and pMYs-FLAG-ASXL1-MT-IG and the sorted-BM cells, total RNA was extracted by Trizol reagent (Invitrogen) according to the manufacturer's protocol. Double-stranded cDNA was synthesized from 5 μg of total RNA with oligo (dT)24 T7 primer, amplified with T7 RNA polymerase up to approximately 50 μg of cRNA, and hybridized to Affymetrix Mouse Expression array 430A, which contains 45000 probe sets for 39000 transcripts and variants from over 34000 well-characterized mouse genes (Affymetrix). After washing and staining, the arrays were scanned on the GeneChip system confocal scanner (Affymetrix). The intensity for each feature of the array was captured with Affymetrix Microarray Suite (MAS) Version 5.0 software. Gene set enrichment analysis was performed by using Gene Ontology gene sets from the Molecular Signatures Database (http://www.broad.mit.edu/gsea/msigdb/) (1). miRNA expression analysis was performed as described previously (2). All RNA labeling reactions and hybridizations were carried out following the manufacture's prototype protocol (Agilent miRNA Microarray system, Version 0.3, early access). Briefly, 100 ng of total RNA including fraction of small mature miRNA was dephosphorylated by calf intestine alkaline phosphatase (p/n E2250Y, Amersham Biosciences) for 30 minutes at 37°C and denatured by adding DMSO (p/n D8418, Sigma) for 8 minutes at 100°C. Ligation was then carried out with T4 RNA ligase (p/n E2050Y, Amersham Biosciences) and pCp-Cy3 (p/n 5190-0408, Agilent) for 2 hours at 16°C that allowed us to perform a quantitative direct labeling method (3). The labeled miRNAs were desalted with Micro Bio-Spin 6 column (p/n 732-6221, Bio-Rad) and combined with Agilent 10×GE Blocking Agent and 2×Hybridization Buffer (p/n 5190-0408). The mixture was heated for 5 minutes at 100°C and immediately cooled to 0°C. Each sample was hybridized to the Agilent early access Human 8×15 K microRNA microarrays covered 470 miRNAs (AMADID 015508, early access)

for 20 hours at 55°C (20 r.p.m.). Array slides were washed with 6× SSC/0.005% Triton X-100 for 10 minutes, then 0.1× SSC/0.005% Triton X-100 for 5 minutes, both at room temperature. Slides were scanned using the Agilent DNA Microarray scanner with 5 µm resolution and the eXtended Dynamic range setting (XDR Hi 100%, Low 5%) to avoid saturated features. The data were extracted by Agilent Feature Extraction Software v9.5 using the miRNA_120106 protocol which extracts intensities of multiple probes with multiple features per probe and reports the measurements and errors as the TotalGeneSignal and TotalGeneSignalError for each of the miRNAs. These values were imported to the GeneSpring GX version 7.3.1 without applying any normalization algorithm. The miRNA profiles generated on the Agilent platform were prior normalized to the amount of input total RNA in which 100 ng of total RNA were equally used for each assay and all of the labeled targets were loaded on each array.

qRT-PCR primers for mRNA expression

	Gene	Forward Primer Sequence	Reverse Primer Sequence
Human	***HOXA9***	CCACGCTTGACACTCACACT	CAGTTCCAGGGTCTGGTGTT
	CLEC5A	TCTTGGAATGAAAGCAGGGACTT	TTCAGTTTCTCTGGCGTGTTGACA
	ASXL1	GCCTCGAGTTGTCCTGACTC	TCTGTTGCGCTTCATTTGAC
	GAPDH	GAGCTGAACGGGAAGCTCACTGG	CAACTGTGAGGAGGGGAGATTCAG
Mouse	***Clec5a***	CCTTGGAAAGACAGCATGGATTA	ACTTCAGTTTCTCTGGAGTGTTGACA
	Hoxa5	CTCATTTTGCGGTCGCTATCC	ATCCATGCCATTGTAGCCGTA
	Hoxa9	GTAAGGGCATCGCTTCTTCC	ACAATGCCGAGAATGAGAGC
	Hoxa10	CCTGCCGCGAACTCCTTTT	GGCGCTTCATTACGCTTGC
	Gapdh	GCATTGTGGAAGGGCTCATG	TTGCTGTTGAAGTCGCAGGAG

qPCR primers for ChIP-qPCR studies

Gene	Forward Primer Sequence	Reverse Primer Sequence
Hoxa5	TCGAGTCCGACTGAACGGCG	GTTGTCCAGTCGTAAATCCTGC
Hoxa10	AAACTCTGGCTCGGGATTGG	GAGCATGACATTGTTGTGGGAT
Hoxa9	GGAGGGAGGGGAGTAACAAA	AGTTTCTGGGCCCTGTATGA
Clec5a	CAGAAGTAGAAGTTCCCTTCCTCT	CTGCTCGGTAATGTCCTTGTT
miR-125a-1	GCACACAAGCTCGTGTCTGT	TCCTCAACTATACAACCTCCTAC
miR-125a-2	GTGCTGTGAATGTATCTCTGTG	GGTGGACCAGAAAAACCCTT
miR-125a-3	GACCCTTTAACCTGTGAGGA	GAGTTTCAAATGATGGTCAAGC

Sequence of shRNA for *Clec5a*

sh1 top	GATCCGGAACAGTCTGTCCCAGAAACTTCAAGAGAGTTTCTGGGACAGACTGTTCCTTTTTTGGAAAG
sh1 bottom	AATTCTTTCCAAAAAAGGAACAGTCTGTCCCAGAAACTCTCTTGAAGTTTCTGGGACAGACTGTTCCG
sh2 top	GATCCGGATTATTGTGCAACACAAGGTTCAAGAGACCTTGTGTTGCACAATAATCCTTTTTTGGAAAG
sh2 bottom	AATTCTTTCCAAAAAAGGATTATTGTGCAACACAAGGTCTCTTGAACCTTGTGTTGCACAATAATCCG
sh3 top	GATCCGGATCAACAACTCTGTGTTCATTCAAGAGATGAACACAGAGTTGTTGATCCTTTTTTGGAAAG
sh3 bottom	AATTCTTTCCAAAAAAGGATCAACAACTCTGTGTTCATCTCTTGAATGAACACAGAGTTGTTGATCCG
sh4 top	GATCCGGACATAGCTGGTATTGAGAATTCAAGAGATTCTCAATACCAGCTATGTCCTTTTTTGGAAAG
sh4 bottom	AATTCTTTCCAAAAAAGGACATAGCTGGTATTGAGAATCTCTTGAATTCTCAATACCAGCTATGTCCG

Reference

1. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A.* 2005;102(43):15545-50.
2. Yamamoto Y, Yoshioka Y, Minoura K, Takahashi RU, Takeshita F, Taya T, Horii R, Fukuoka Y, Kato T, Kosaka N, et al. An integrative genomic analysis revealed the relevance of microRNA and gene expression for drug-resistance in human breast cancer cells. *Mol Cancer.* 2011;10:135.
3. Wang H, Ach RA, and Curry B. Direct and sensitive miRNA profiling from low-input total RNA. *RNA.* 2007;13(1):151-9.

Supplemental Table 1

Systematic Name	Fold change ([ASXL1-MT] vs [Mock])
mmu-miR-671-3p	5.53
mmu-miR-125a-5p	4.08
mmu-miR-714	3.22
mmu-miR-18b-3p	3.15
mmu-miR-129-1-3p	2.88
mmu-miR-3107-5p	2.69