Supplemental Figures

Figure S1

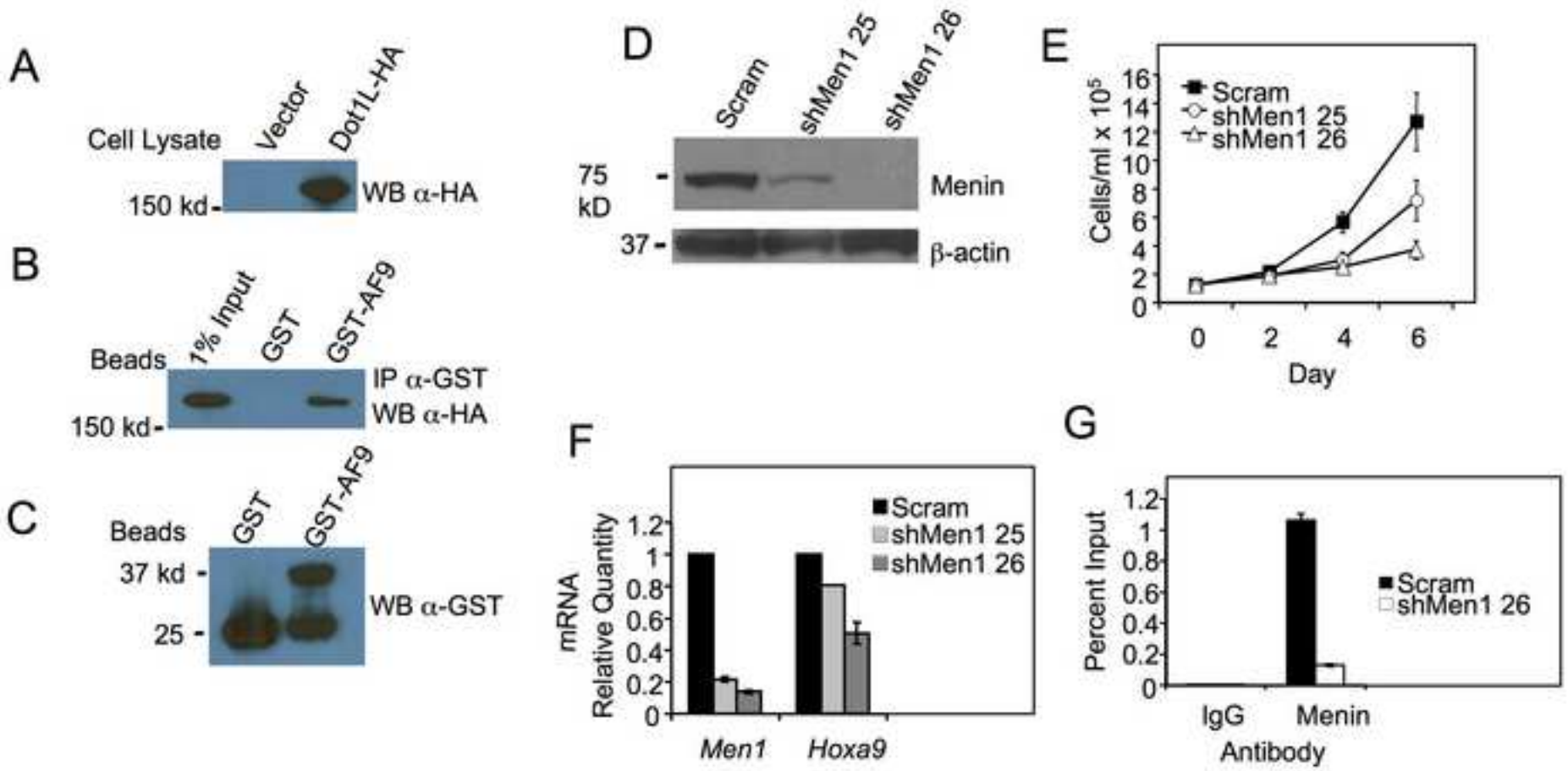
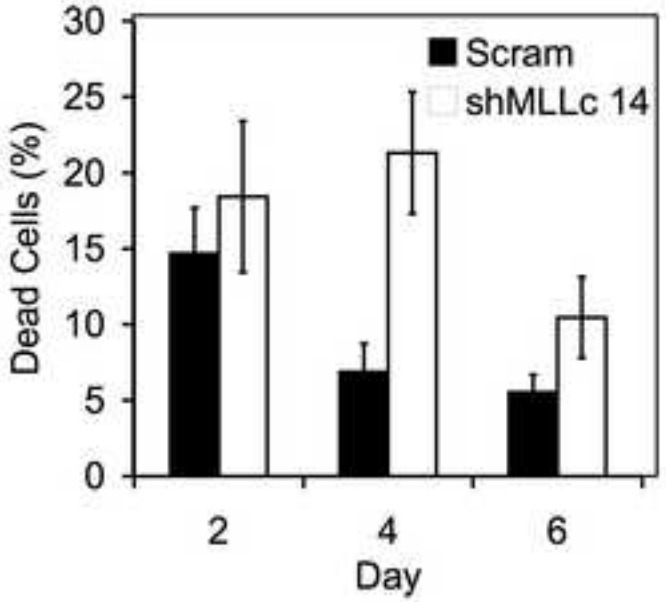


Figure S2



Supplemental Figure

Figure S3

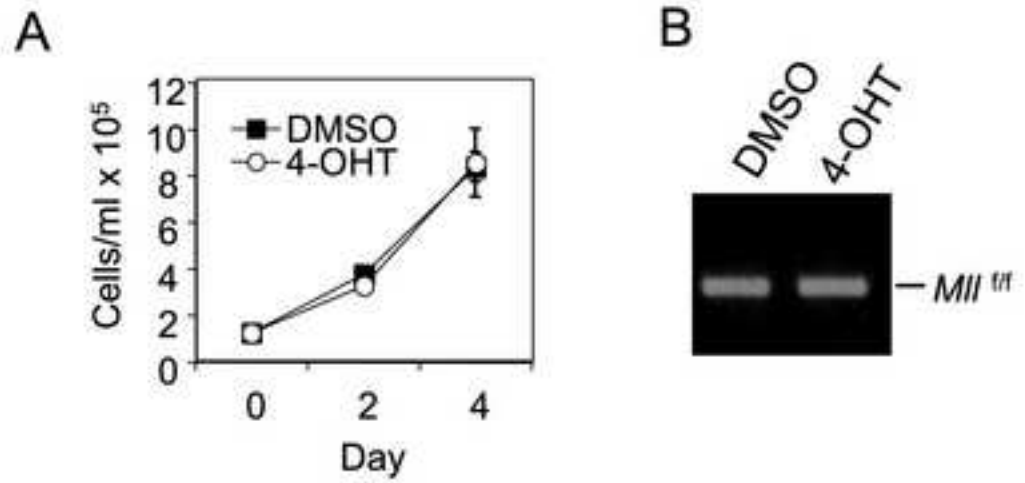
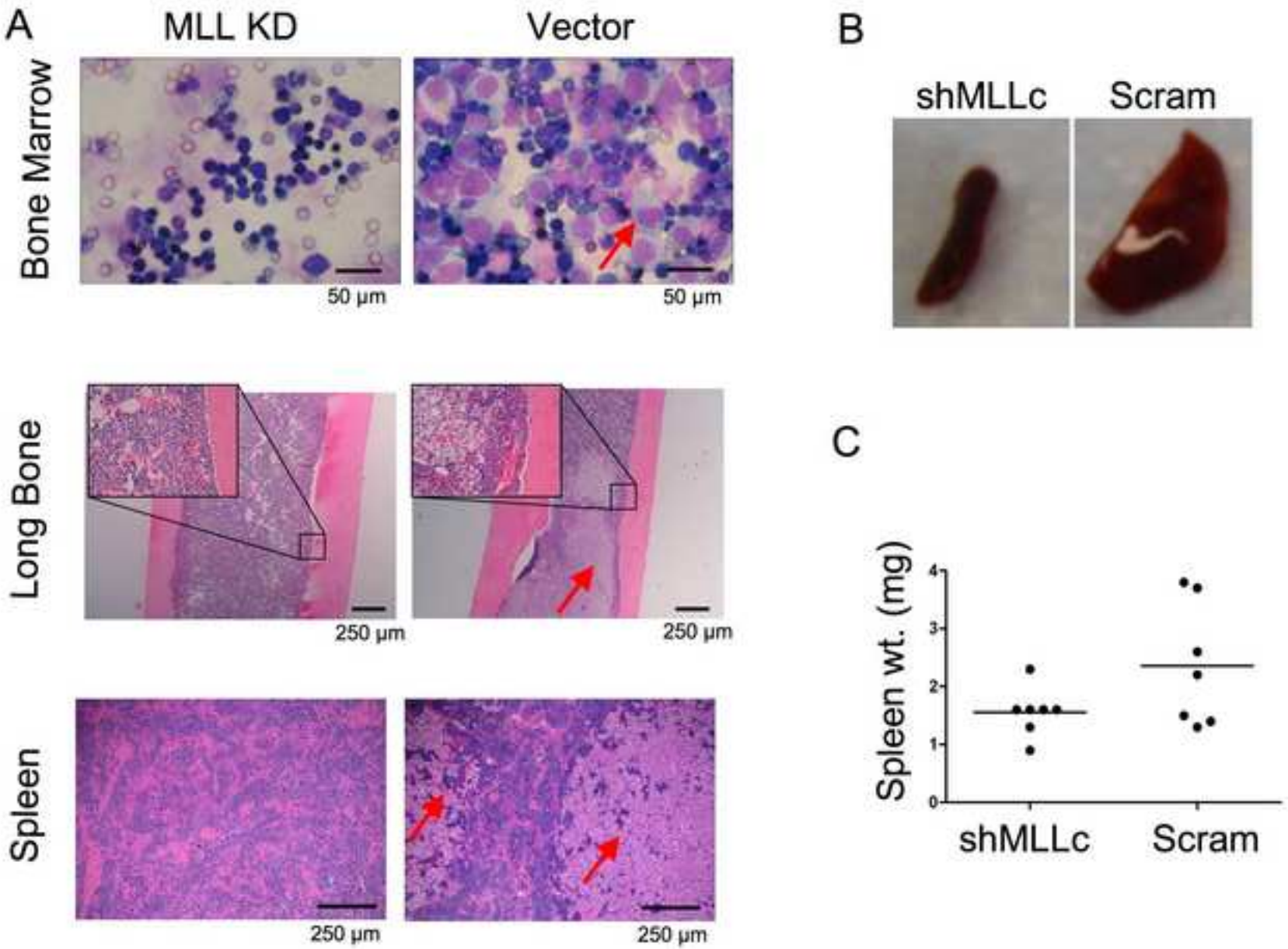
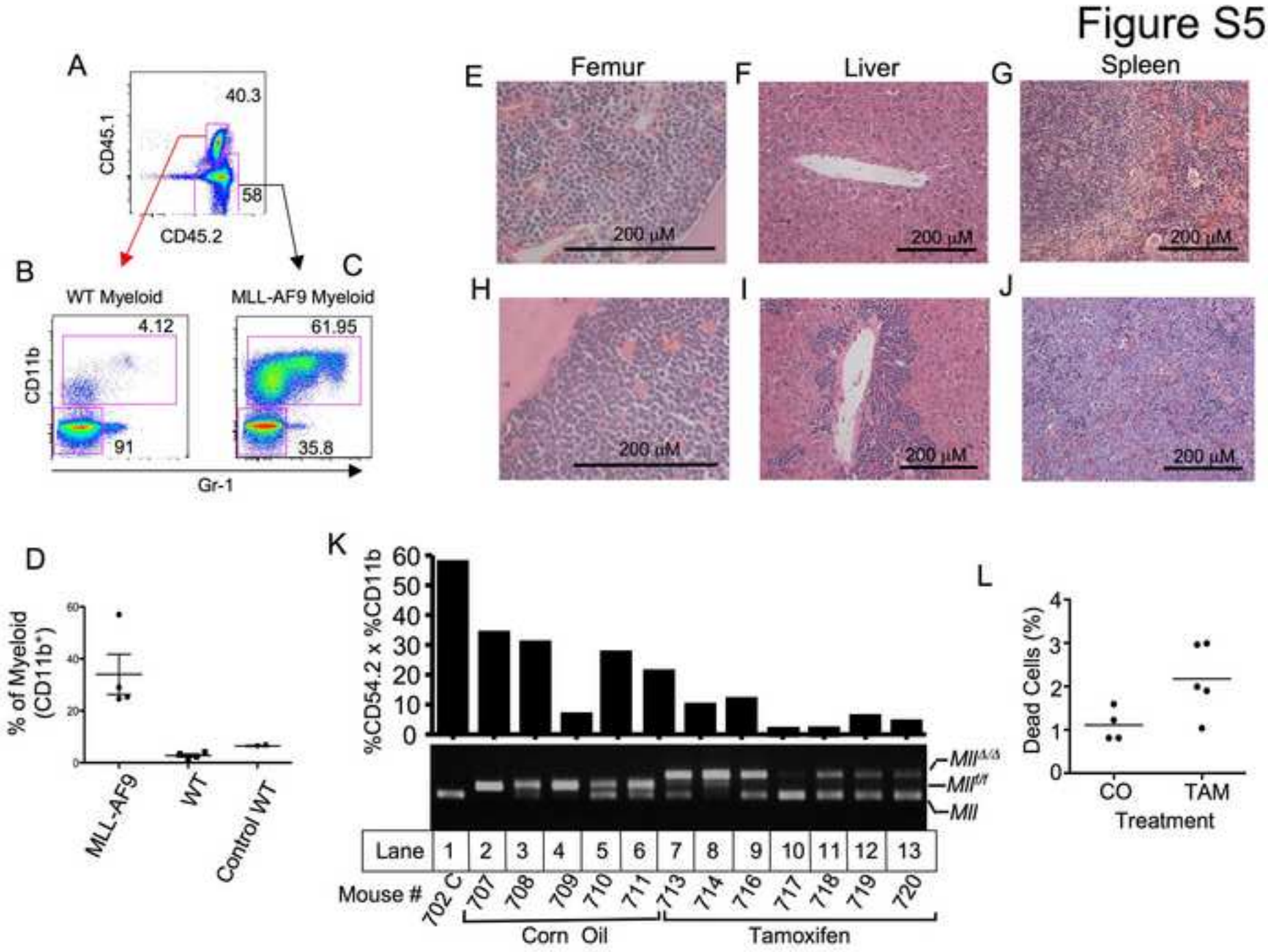


Figure S4


A
MLL KD
Vector
Bone Marrow
50 µm
50 µm
Long Bone
250 µm
250 µm
Spleen
250 µm
250 µm
B
shMLLc
Scram
C
Spleen wt. (mg)
0
1
2
3
4
shMLLc
Scram

Supplemental Figures



Supplemental Figure Legends

Figure S1. **Related to Figure 2, *Men1* knockdown (KD) in THP-1 cells reduces *HOXA9* expression and cell growth.** (A-C) Human 293T cells were transfected with either control vector or the Dot1L-HA plasmid to express Dot1L (A, lane 2), and the resulting cell lysates were incubated with glutathione sepharose beads conjugated to either GST or GST-AF9 (containing the last 90 amino acid residues of human AF9). Washed beads were separated on SDS-PAGE and probed with an anti-HA antibody to determine the presence of Dot1L-HA (B). As a control, the beads were also probed with an anti-GST antibody to determine the amounts of GST or GST-AF9 bound to the beads (C). (D) THP-1 cells were transduced with either control scrambled or human *Men1* shRNAs, followed by puromycin selection (2 μg/ml) and immunoblotting analysis with an anti-menin antibody. (E) A growth curve of the control and *MEN1* shRNA-transduced cells (+/- SD). (F) The mRNA levels of *MEN1* and *HOXA9* genes in the control and *MEN1* knockdown cells (+/- SD). (G) ChIP assay for menin binding to the *HOXA9* locus in the control and *MEN1* shRNA-transduced cells (+/- SD).

Figure S2. Related to Figure 3, Wild-type MLL knockdown increases the percentage of dead cells. THP-1 cells were transduced with either scrambled vector or MLL-C shRNA-14. Puromycin-selected cells were seeded on day 0, and counted for trypan blue-stained dead cells and trypan blue-excluded live cells on the indicated days. The percentage of dead cells was calculated by dividing the number of dead cells with the sum of the live and dead cells. Error bars denote +/- SD.

Figure 3. Related to Figure 4, 4-hydroxyl tamoxifen (4-OHT) does not affect growth of MA9-transformed BM cells that harbor the floxed *Mll* alleles but not the *Cre-ER* transgene. MA9 retroviruses were transduced into $Mll^{f/f}$ BM lacking Cre-ER, and the transformed cells were

treated with either DMSO or 4-OHT and monitored for cell growth (A) and *Mll* excision by genotyping (B). Error bars denote +/- SD.

Figure S4. **Related to Figure 5, Wt *Mll* KD in THP-1 cells reduces engraftment of THP-1 cells in immunodeficient mice**. (A) Scrambled shRNA (Vector) or MLL-C shRNA 14-transduced (KD) THP-1 cells ($1.5x10^6$/mouse) were injected into NOD-SCID mice (n=7 in each group) and sacrificed at the end of the 6th week, to detect leukemic infiltration in the bone marrow (smear) by Giemsa staining (top panel) and in long bones (middle panel) and spleens (bottom panel) by H&E staining. The arrowhead denotes the infiltrated THP-1 cells. (*B*) A representative pair of spleens from mice receiving either vector-transduced THP-1 cells (right panel) or MLL KD THP-1 cells (left panel). (*C*) The distribution of the spleen weight for each group of recipient mice.

Figure S5. Related to Figure 8, Tamoxifen-induced WT *Mll* excision in MLL-AF9 recipient mice. (A) Peripheral blood (PB) white blood cells (WBCs) from MA9-BM-transplanted mice were isolated seven weeks after transplantation via Ficoll gradient and stained with various distinct fluorescence-labeled antibodies, as indicated. The donor cells were only CD45.2+ (lower right quadrant), while recipient or normal co-transplanted BM-derived cells were CD45.1+/2+ (upper right quadrant). A small percentage (4%) of cells in wt recipient PB WBCs were myeloid (CD11b+/Gr1+) (B), but a large percentage (~62%) of the MA9-transformed BM-derived cells were CD11b+ (C). (D) Comparison of the percentages of myeloid cells derived from MA9-transduced BM, the co-transplanted normal BM, and BM from normal WT mice without BM transplantation (+/- SEM). (E) MA9-transformed BM and control normal BM cells were transplanted into irradiated recipient mice, as outlined in Figure 8A. Nine weeks after transplantation, the femur, liver, and spleen were harvested and processed for H&E staining.

MA9-transduced BM transplanted mice (H-J), in contrast to the normal BM transplanted mice (E-G), displayed leukemia cell infiltrations in femur (H), liver (I), and spleen (J). (K) Mice were transplanted with MA9-transduced primary BM as described in Figure 8A, and fed with either corn oil or TAM three weeks after transplantation. Peripheral blood was collected and processed for flow cytometry analysis or genomic PCR. Top: Percentage of CD11b+ cells from the MA9-transduced donor cells (CD45.2+). Bottom: Detection of the $Mll^{\Delta/\Delta}$, $Mll^{f/f}$, and wt *Mll* alleles from peripheral WBCs. (L) Increase in number of dead cells from TAM-fed mice that were transplanted with primary MA9 leukemia cells (harboring $Mll^{f/f}$*;Cre-ER*) ($p<0.05$). Splenocytes, which were from primary MA9 leukemia cell-transplanted mice a week after feeding with either corn oil or TAM, were stained with a Live/Dead Fixable Dead Cell Stain Kit (Invitrogen).

Supplemental Experimental Procedures

Antibodies for Western blotting, immunoprecipitation, ChIP assay, and flow cytometry analysis

Antibodies purchased from Abcam (Cambridge, MA) were: α-H3K4m3, H3K4m2, H3K79m2, cyclin A2, cyclin E2, and α–HA; from Bethyl Laboratories (Montgomery, TX): α-menin, AF9-C, Dot1L, MLL-N, and MLL-C; from Sigma (St. Louis, MO): α-ß-actin; from Pharmingen (San Diego, CA): anti-CD45.2 (104), c-Kit (2B8), CD34 (RAM34), B220 (RA3-6B2), Gr1 (RB6-8C5), CD4 (RM4-5), and CD8 (53-6.7); from Zymed (San Francisco, CA): α–GST; from eBioscience (San Diego, CA): CD45.1 (A20), Sca-1 (E13-161.7), and CD11b (M1/70). Biotinylated antibodies were revealed with Streptavidin-Pacific Blue (Molecular Probes, Eugene, OR) or PE-Texas Red (Caltag, Burlingame, CA). Lineage$^+$ cells were defined with α-Gr1, TER119, B220, CD19, CD8, CD4, CD3, and CD127 (IL-7R).

Immunoprecipitation and Western blotting

Human 293T cells were transfected with either control vector or the Dot1L-HA plasmid, and the resulting cells were lysed in RIPA buffer supplemented with 1x protease inhibitor cocktail (Sigma-Aldrich). The lysates were incubated with glutathione sepharose beads conjugated with either GST protein or GST-AF9 fusion protein, which was expressed and purified from DH5α cells. The precipitated proteins from the beads were resolved in SDS-PAGE and probed with the anti-HA antibody. For Western blot, cell lysates were separated through SDS-PAGE and processed for detection with ECL Western Blotting Detection Reagents (GE Healthcare Life Sciences), as previously described (Jin, et al., 2003).

Engraftment of human leukemia cells into NOG mice

NOD/Shi-*scid*/IL-2Rγ^{null} mice, 6-8 weeks old, were obtained at the Stem Cell Core Facility at the University of Pennsylvania and irradiated at 275 rad 24 hrs prior to tail injection with 1.5 x10^{6} scrambled vector control THP-1 cells or cells transduced with shRNA 14 targeting MLL-C. Mice were weighed weekly starting from the time of injection. The recipient mice were sacrificed 6 weeks after injection, and the long bones (femurs) and spleens were collected for histological analysis by H & E staining.

Analysis of Peripheral Blood Cells and Statistical Analysis

Blood samples collected from submandibular bleeds were stored in Microtainer tubes with EDTA (BD Biosciences, Franklin Lakes, NJ). The hematology profile of each mouse was analyzed immediately on a Hemavet blood cell counter (CDC Technologies, Oxford, CT). For flow cytometry or genotyping of peripheral white blood cells, mice were bled into 4% Sodium Citrate in FACS tubes and IMDM (1ml) with 2% FBS was added to the tube, followed by addition of an underlay of 1ml Ficoll solution (GE healthcare). The samples were centrifuged at 1,000 x g for 20 min, and the white layer was collected, washed once in 1x PBS, and used for further analysis.

ShRNA sequences

MEN1 shRNA sequences: #25 GCTGTACCTGAAAGGATCATA CTCGAG TATGATCCTTTCAGGTACAGC; #26 GTGCAGATGAAGAAGCAGAAA CTCGAG TTTCTGCTTCTTCATCTGCAC

Sequences of primers for RT-PCR

Primer sequences for mouse cDNAs:

mmMLLc:

(F) TGAGCCGTGAGGGTTCAAG, (R) GTGAACGGTTTGCGGATG

mmHoxa5:

(F) TTCCACTTCAACCGCTACCT, (R) CGGCCATACTCATGCTTTTC

mmHoxa9:

(F) CCCCGACTTCAGTCCTTGC, (R) GATGCACGTAGGGGTGGTG

mmCCNA2:

(F) GCCTTCACCATTCATGTGGAT

(R) TTGCTGCGGGTAAAGAGACAG

Primer sequences for human cDNAs:

hsMLLc:

(F) GGCCTGAATTTCTCCACAGA, (R) TTCGACAGACGCTGTAGGTG

hsHoxa9: (F) AGACCGAGCAAAAGACGAG, (R) CTGAGGTTTAGAGCCGCTTT

hsCCNA2: (F) CGCTGGCGGTACTGAAGTC, (R) AAGGAGGAACGGTGACATGC

hsCCNE2: (F) AAGTAGCCGTTTACAAGCTAAGC, (R) TGATGTTTCTTGGTGACCTCC

Primer sequences for murine genes for ChIP assay:

mmHoxa9 prom amp A:

(F) TGGAAGGCACAAAATTCACA, (R) AATTAACCCGGGAGGAACAC

mmHoxa9 prom amp B:

(F) CATCGATCCCAGTAAGTGTCTC, (R) CCGCCCCCTCACTGCAGCAGC

Primer sequences for human genes for ChIP assay:

hs*Hoxa9* promoter amp D:

(F) AGTGGCGGCGTAAATCCT, (R) TGATCACGTCTGTGGCTTATTTGAA

or

(F) CCGCCTTTATTCCTCTCTCC, (R) AGTGCAACAGAGTGCCC

hsCCNA2 promoter :

(F) CCAGCCAGTTTGTTTCTCCC, (R) GACCAATGAAAGCGCTCG