Plasmids
pFMLL-AF9 was created by cloning the last 276 bp of human AF9 into the *Pml1* and *Xho1* sites of MSCV-pFMLL-polyL (gift from Robert Slany described previously[1]). pFMLL-PHD#1-AF9, pfMLL-PHD#2-AF9, pFMLL-PHD#3-AF9, pFMLL-PHDB-AF9 and pFMLL-PHD#4-AF9 were generated by cloning the above AF9 sequence into *Nsi1* and *Xho1* sites of MSCV-pFMLL-polyL followed by cloning MLL PHD fingers into *Pml1* and *Nsi1* sites.

Western Blotting
Cells were lysed in BC-300 (20mM Tri-HCL [pH8.0], 10% glycerol, 300mM KCl, 1mM EDTA, 0.1% NP-40) separated on a 6% Tris-Glycine gel (Invitrogen, Carlsbad, CA) and transferred to nitrocellulose. Membranes were probed with polyclonal anti-flag produced in rabbit (Sigma, St. Louis, MO).

qRT-PCR
RNA was extracted from bone marrow cells using TRIzol™ reagent (Invitrogen). cDNA was generated using Superscript III™ Reverse Transcriptase (Invitrogen) according to manufacturer's instructions. Relative quantitation of real time PCR product was performed using comparative ΔΔCt method (described in ABI Prism 7700 Sequence Detection System User Bulletin No. 2) and SYBR green fluorescent labeling and ABI 7500 PCR Detection System. Primer sequences are as follows: Flag-F 5′-GGACTACAAGGACGACGATGA-3′, Flag-R 5′-ACAGCTGTGCGCCATGTT-3′, Gapdh-f 5′-CCTTCCGTGTTCCTACCC-3′, Gapdh-R 5′-CTGCTTCACCACCTTCTTG-3′. *Hoxa9* and *Gapdh* were detected using Taqman™ primer probe sets from Applied Biosystems (Foster City, CA).

Retroviral transduction and colony assays
Six week old C57Bl/6 female mice were obtained from Taconic Farms (Hudson, NY). Intraperitoneal (IP) injections were performed on mice with 150mg/kg 5-Fluorouracil (5-FU) (Pharmacia Kalamazoo, MI) 5 days before bone marrow harvest. Bone marrow is harvested, treated with NH4Cl and spin-infected with viral supernatant and polybrene on consecutive days. Cells were plated in methocult M3234 (Stem Cell Technologies, Vancouver, BC) with 15% FBS (Stem Cell Technologies), IL-3 (10ng/ml), IL-6 (10ng/ml), GM-CSF (10ng/ml), SCF (100ng/ml) (R&D Systems, Minneapolis, MN), Pen/Strep 1%, and G418 (1mg/ml) according to manufacturer protocol. Colonies were counted and plates were flooded with PBS and replated after 7-10 days of culture using the above procedure. All animal studies were approved by the University of Michigan Committee on Use and Care of Animals and Unit for Laboratory Medicine.

Morphology Analysis and Microscopy
Liquid cultured cells were cytospun and stained with Hema 3 Stain Kit (Thermo Fisher Scientific, Waltham, MA). Images were acquired using a 100× lens and Olympus BX-51 microscope with Olympus DP controller software (Olympus, Center Valley, PA).

Co-Immunoprecipitation
292 cells were transiently transfected with MSCV fusion plasmids using FuGene 6 (Roche, Basel, Switzerland) following manufacturer's instructions. Cells were lysed in BC-300 (described above) and incubated with M2 beads (Sigma) at 4 degrees overnight. Beads were washed three

times with BC-300 lysis buffer and proteins were eluted in loading buffer supplemented with β-mercaptoethanol. Lysate was run on 6% or 4-20% Tris-Glycine gels (Invitrogen) and transferred to nitrocellulose. Blots were probed with polyclonal anti-Flag produced in rabbit (Sigma) or anti-Menin (Bethyl Laboratories, Montgomery, TX).

Chromatin Immunoprecipitation

ChIP experiments were performed as described previously.[2] Briefly, 293 cells were transiently transfected as described above. Cells were fixed with 1% formaldehyde exposed to one cycle of freeze/thaw. Cells were then lysed in ChIP lysis buffer (Upstate, Temecula, CA) supplemented with protease inhibitors. Cells were sonicated for 30 minutes using 30 sec on-off pulses with a Bioruptor (Diagenode, Sparta, NJ). Supernatant was diluted ten fold with ChIP dilution buffer (Upstate), and pre-cleared with protein-G Dynabeads (Invitrogen). Lysate was then incubated with protein-G Dynabeads conjugated with polyclonal anti-Flag antibody (Sigma) or normal rabbit IgG (Santa Cruz, Santa Cruz, CA) at four degrees for 4 hours. Beads were washed with low salt, high salt and LiCl wash buffers (Upstate) followed by a TE wash. Protein-DNA complexes were eluted by addition of elution buffer (1%SDS, 8mg/ml NaHCO3) and incubation at 42 degrees for thirty minutes. Cross links were reversed by addition of 200mM NaCl and incubation at 65 degrees overnight. RNase A (10 mg/ml) was added and DNA fragments purified using Qiaquick PCR purification kit (Qiagen, Valencia, CA). qPCR was performed as described above with the following primer sets: P1-F: CAAATCGCATTGTCGCTCTA, P1-R: CCGCAGGGATTATTTACAGG.

Luciferase Assays

293 cells were transiently transfected with MSCV, CMV-Renilla, and Hoxa9-LUC constructs using FuGene 6 (Roche) according to manufacturer's instructions. Cells were then serum starved in 0.5% FBS in OPTI-MEM media for 48 hours. Luciferase assays were performed using the Dual Luciferase assay kit (Promega, Madison WI) according to manufacturer's instructions. Emission was detected using a Monolight 3010 (BD Biosciences, San Jose, CA).

REFERENCES

1. Slany RK, Lavau C, Cleary ML. The oncogenic capacity of HRX-ENL requires the transcriptional transactivation activity of ENL and the DNA binding motifs of HRX. *Mol Cell Biol*. 1998;18:122-129.

2. Milne TA, Dou Y, Martin ME, Brock HW, Roeder RG, Hess JL. MLL associates specifically with a subset of transcriptionally active target genes. *Proc Natl Acad Sci U S A*. 2005;102:14765-14770.