Supplemental Inventory

Figure S1: contains gene expression data and additional ChIP data that enhances and clarifies data in Figure 1 but could not fit into the actually figure.

Figure S2: contains control data (such as protein expression levels) and additional data relating to recruitment and menin interaction.

Figure S3: contains important complex purification data for Figure 3 and additional immunofluorescence data.

Figure S4: immunoprecipitation data makes the important point that the protein-protein interaction does not depend on DNA but does not easily fit into Figure 4 where it is most appropriate. It also shows an additional CXXC domain that does not interact with PAF1 and is an additional important control for Figure 4, and we also show PHD finger sequence alignment that is important for some of our reasoning in the paper.

Figure S5: shows additional ChIP experiments requested by the reviewers and related to data in Figure 5.

Figure S6: contains control data (such as protein expression levels) and additional data requested by the reviewers and related to data in Figure 6.

Figure S7: contains important control data that could not fit in Figure7.

Details such as primer/probe sequences, antibodies used and mass spec techniques are also listed as these are important experimental details but don't fit in the main body of the text.

SUPPLEMENTAL FIGURES, FIGURE LEGENDS AND EXPERIMENTAL PROCEDURES

Figure S1 (Related to Figure 1)

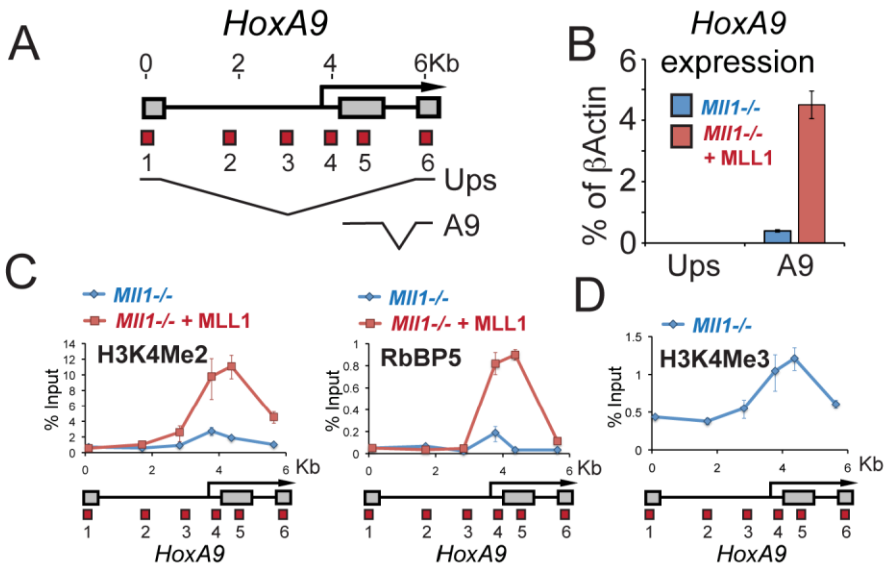


Figure S2 (Related to Figure 2)

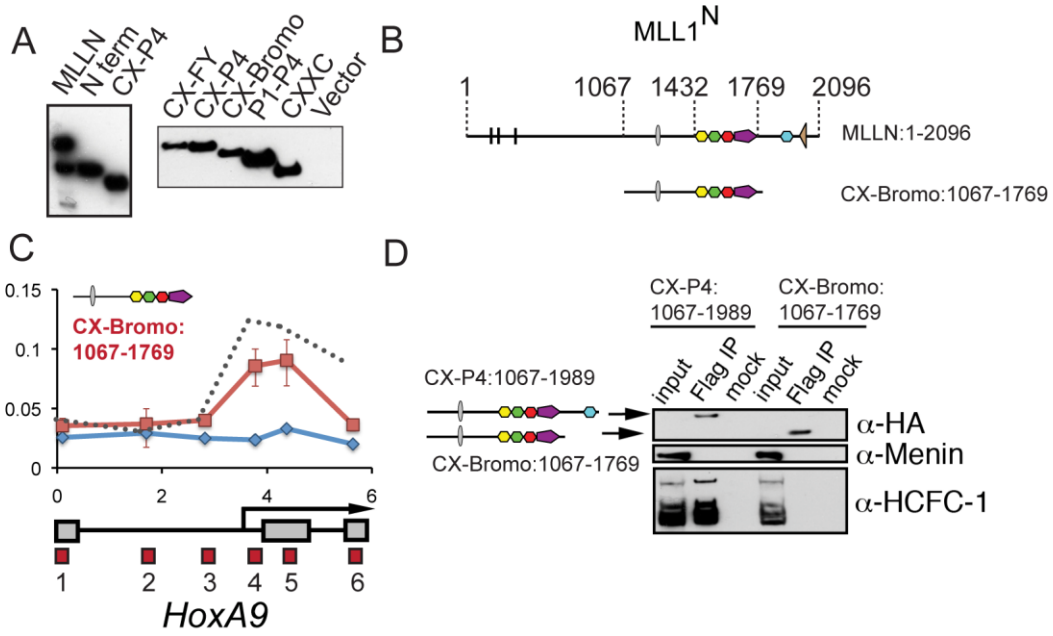
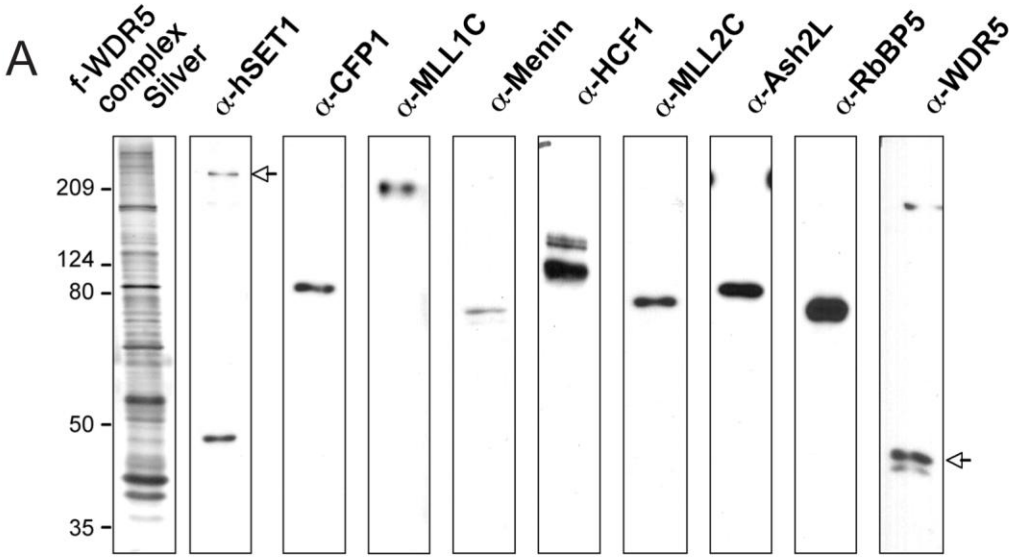
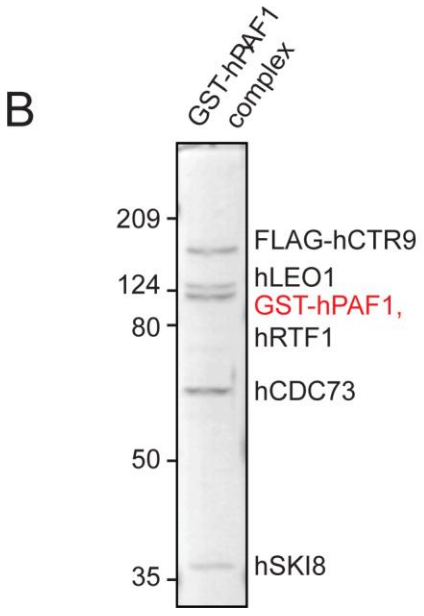


Figure S3 (Related to Figure 3)





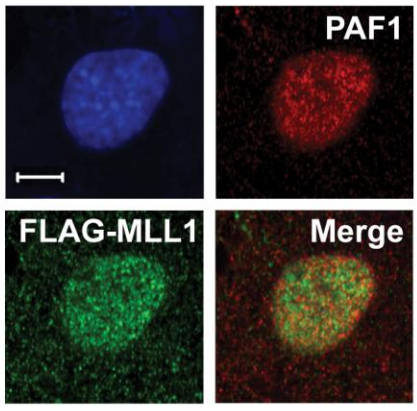
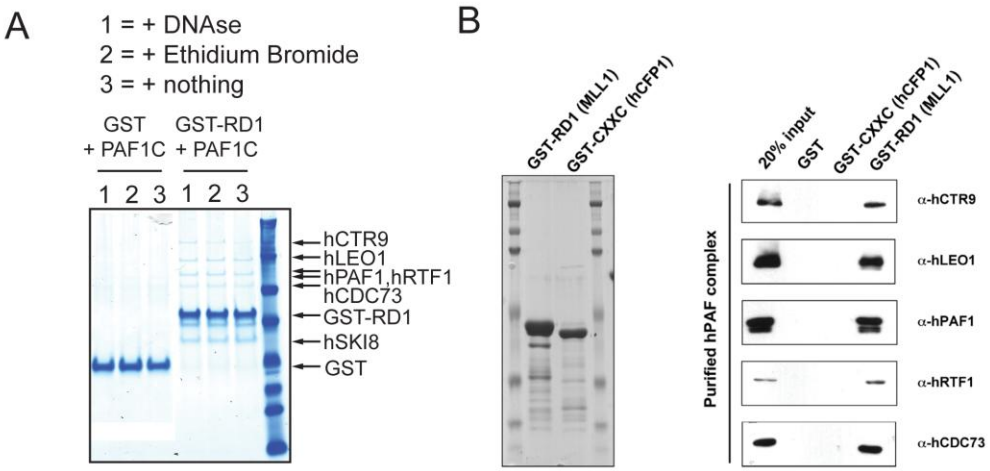


Figure S4 (Related to Figure 4)



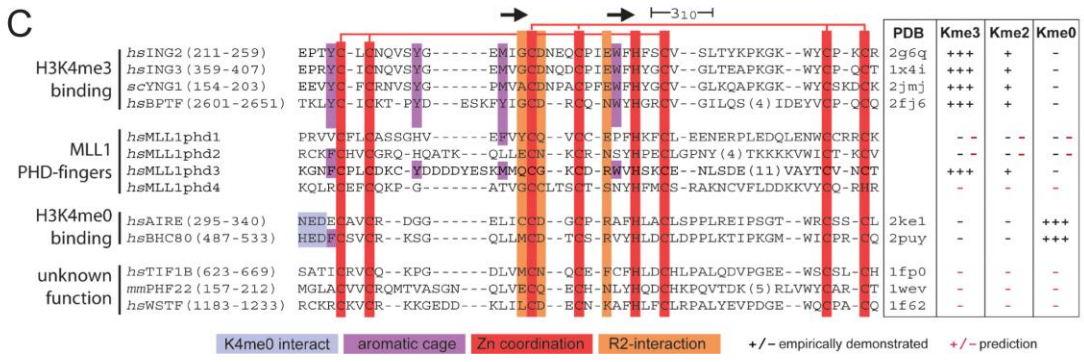


Figure S5 (Related to Figure 5)

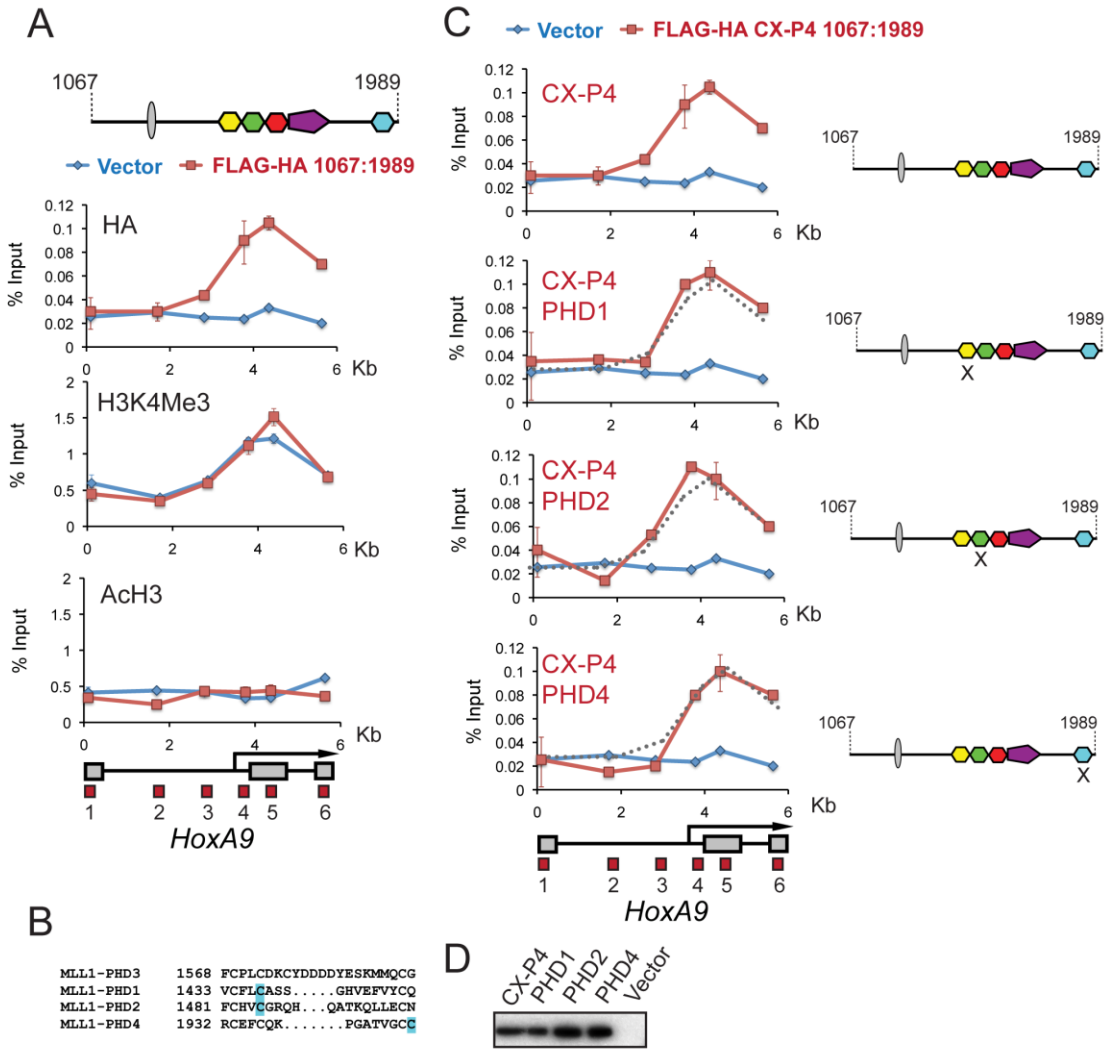
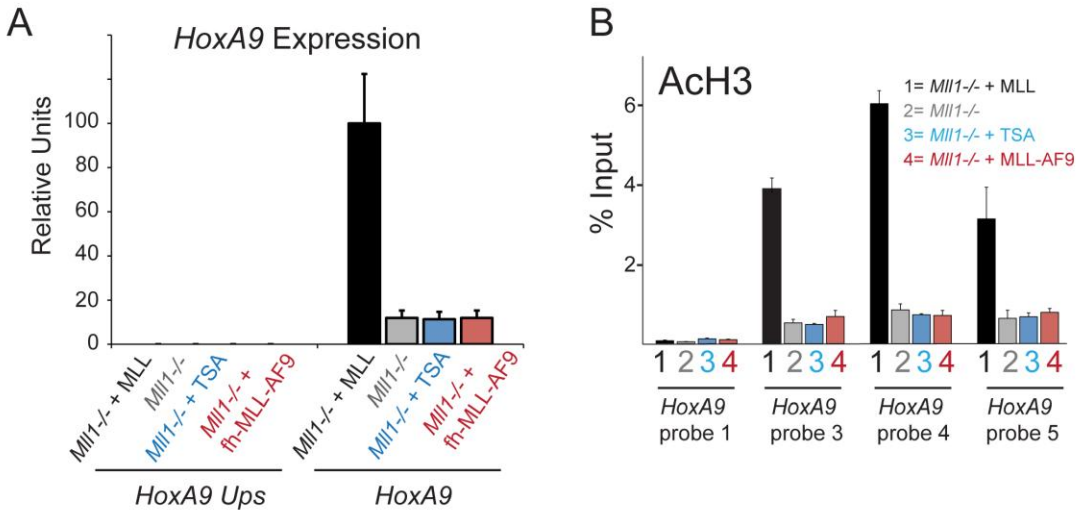
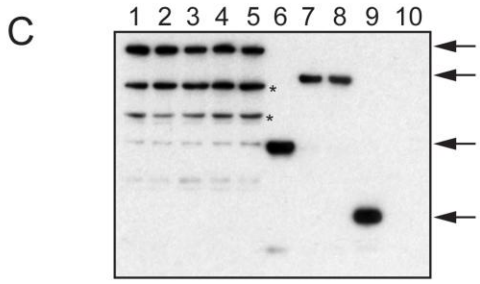


Figure S6 (related to Figure 6)





1= fh-MLL-AF9 in *Mll1-/-* cells
2= fh-MLL-AF9 in *Mll1-/-* + MLL1 cells
3= fh-MLL-AF9 R1153A in *Mll1-/-* + MLL1 cells
4= fh-MLL-AF9 K1176A in *Mll1-/-* + MLL1 cells
5= fh-MLL-AF9 R1153A/K1176A in *Mll1-/-* + MLL1 cells
6= fh-CXXC 1067:1432 in *Mll1-/-* + MLL1 cells
7= fh-CX-P4 1067:1989 in *Mll1-/-* + MLL1 cells
8= fh-CX-P4 W1954E in *Mll1-/-* + MLL1 cells
9= fh-CXXC RD1 1067:1250 in *Mll1-/-* + MLL1 cells
10= empty vector in *Mll1-/-* + MLL1 cells
* degradation products

Figure S7 (Related to Figure 7)

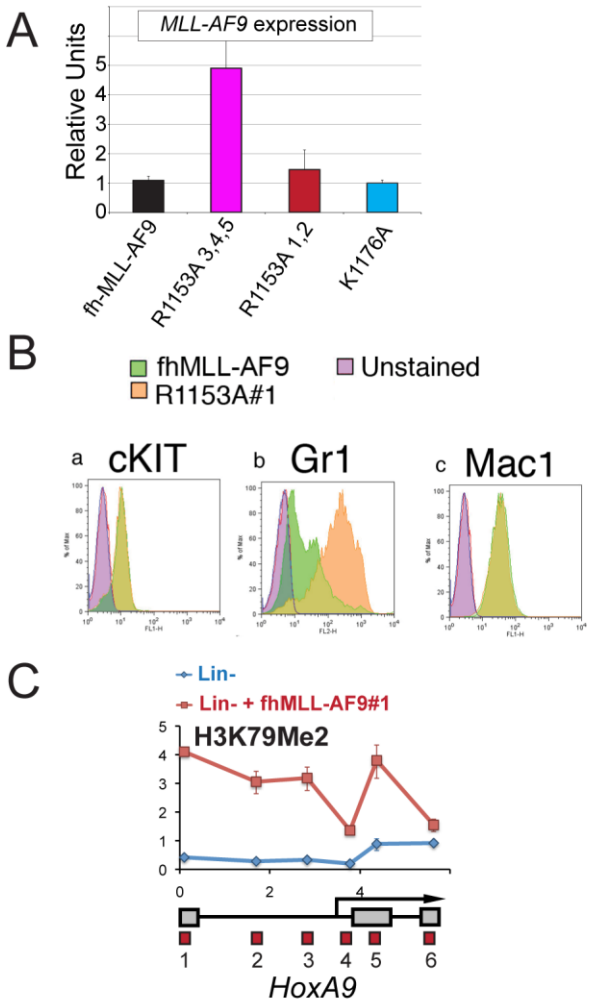


Figure S1 (related to Figure 1). (A) a schematic of the *HoxA9* locus showing an upstream (Ups) and the major canonical *HoxA9* (A9) transcripts. (B) Expression of the canonical *HoxA9* transcript is increased in *Mll1-/-* MEFs reconstituted with MLL1 (black bars) versus *Mll1-/-* MEFs (grey bars). The Ups transcript is not expressed. Error bars represent standard deviation from three separate PCR reactions (C) and (D) Chromatin immunoprecipitation (ChIP) experiments at *HoxA9* in *Mll1-/-* (blue line) versus *Mll1-/-* reconstituted with MLL1 (red line) MEF cells using the antibodies indicated. ChIP results

shown are typical for at least two independent experiments. Error bars represent standard deviation of three separate PCR reactions.

Figure S2 (related to Figure 2). (A) A western blot showing expression of the constructs from Figure 2C probed with an anti-HA antibody. The fh-MLLN fragment is very large (~300KDa) relative to the smallest constructs and for efficient separation and blotting they had to be run on a different % gels. Expression of the CX-P4 construct was run on both gels for comparison purposes. (B) and (C) a minimal fragment lacking PHD4 can still bind across *HoxA9*. ChIP results shown are typical for at least two independent experiments. Error bars represent standard deviation of three separate PCR reactions. (D) Menin and HCFC1 do not interact with a minimal MLL1 fragment that can be recruited to *HoxA9 in vivo*. FLAG and HA double tagged CXXC-PHD4 and CXXC-Bromo constructs were expressed in 293T cells. Extracts were immunoprecipitated with FLAG M2 beads and western blots were probed with HA, Menin and HCFC1 antibodies as shown.

Figure S3 (related to Figure 3). (A) A silver stain and western blots of the f-WDR5 complex show the presence of multiple MLL family methyltransferase complexes. (B) The purified GST-PAF1 complex. (C) FLAG (MLL1) and PAF immunofluorescence in *Mll1-/-* + MLL1 cells.

Figure S4 (related to Figure 4). (A) Binding of the PAF complex to MLL-GST-RD1 in the presence of DNAse (lane 1) or ethidium bromide (lane 2). (B) A region containing the CXXC domain of CFP1, equivalent to the RD1 region of MLL1, was GST tagged and purified. GST pulldowns with GST, GST-RD1 (MLL1) or GST-CXXC (CFP1) in the presence of the PAF1 complex. Results were blotted and probed with the antibodies

indicated. (C) A comparison of the 4 PHD fingers of MLL1 and predictions of binding to H3K4Me3, 2 or 0. Zinc coordination residues are colored in red, the aromatic cage residues necessary for H3K4Me3 recognition are colored in purple. MLL1 PHD1, 2 and 4 don't possess the hydrophobic residues necessary for methylated lysine recognition.

Figure S5 (related to Figure 5). (A) *Mll1-/-* cells transfected with the MLL1 CX-P4 (1067:1989) recruitment domain have no effect on H3K4Me3 or AcH3 (red line) compared to empty vector control cells (blue line). ChIP results shown are typical for at least three independent experiments. Error bars represent standard deviation of three separate PCR reactions. (B) Blue highlight = sites of C-A point mutations in PHD fingers 1,2 and 4. ChIP results shown are from a single experiment. Error bars represent standard deviation of three separate PCR reactions. (C) Point mutations that disrupt a zinc finger in each of PHD fingers 1,2 and 4 (see B) don't affect recruitment to *HoxA9* in *Mll1-/-* cells. (D) Anti-HA western blots of the constructs in C.

Figure S6 (related to Figure 6). (A) *HoxA9* upstream (Ups) and canonical (A9) transcript (as in Figure S1A) expression in *Mll1-/-* + MLL (Black bar), *Mll1-/-* (Grey bar), *Mll1-/-* + Trichostatin A (Blue bar, TSA, an HDAC inhibitor) and *Mll1-/-* + fh-MLL-AF9 (Red bar) cells. (B) AcH3 ChIP in *Mll1-/-* + MLL (Black bar), *Mll1-/-* (Grey bar), *Mll1-/-* + TSA (Blue bar) and *Mll1-/-* + fh-MLL-AF9 (Red bar) cells. (C) anti-HA Western blot of construct expression Figure 6C-J. All gene expression and ChIP experiments are from a single experiment. Error bars represent the standard deviation of three separate PCR reactions.

Figure S7 (related to Figure 7). (A) MLL-AF9 construct expression is roughly equal in several fh-MLLAF9 (Black Bar), R1153A lines 1 and 2 (red bar) and K1176A (blues bar)

lines, while MLL-AF9 is over expressed in R1153A lines 3,4 and 5. Gene expression was averaged across 5 lines for wild type and K1176A and across the R1153A lines as indicated. Error bars represent the standard deviation. (B) fhMLL-AF9 #1 and fhMLL-AF9 R1153A#3 cells were subjected to FACs analysis and they are equally cKit (a) and Mac1(c) positive, but R1153A#3 cells stain much more positively with Gr1 (b). (C) H3K79Me2 is increased across HoxA9 in the presence of MLL-AF9 (red line) relative to progenitor enriched (blue line) cells.

Supplemental Procedures

Antibodies

α-MLL1^{C} was a generous gift from the Roeder lab. α-Menin (A300-105A), α-MLL2 (A300-113A), α-MLL1N (A300-087A), α-HCFC1 (A300-399A), α-RbBP5 (A300-109A), α-ASH2L (A300-107A), α-BMI1 (A300-694A), α-CTR9 (A301-395A), α-LEO1 (A300-175A), α-RTF1 (A300-179A) α-PAF1 (A300-172A) α-CDC73 (A300-170A), α-SET1 are all available from Bethyl Laboratories. Other antibodies used include: α-HA (Roche, 11583816001), α-M2 FLAG (Sigma, F3165), α-GST (Santa Cruz, sc-138), α-HDAC1 clone 2E10 (Millipore, 05-614), α-WDR5 (Millipore, 07-706), α-HP1 clone 2HP-2G9 (Millipore, MAB3446), α-βActin (Sigma, A5316), α-MLL1C (Millipore, 05-765) and α-HOXA9 (Millipore, 07-178). Histone antibodies used: α-H3K4me3 (Active Motif, 39159), α-H3K4me2 (Millipore, 07-030), α-H3K4me2 (Abcam, ab7766), α-H3 (Abcam, ab1791). FACs antibodies: Gr-1 (Ly-6G, clone RB6-8C5), Mac-1, (CD11B, clone M1/70), and c-Kit (clone 2B8) were all from BD PharMingen.

Immunofluorescence staining

Immunofluorescence staining of 293 cells and MEFs was performed using a standard protocol. Briefly, cells were fixed with 3.7% paraformaldehyde in PBST (PBS, 0.1% Triton-X-100), washed three times with PBST and blocked for at least 30 min in PBST containing 2% BSA at RT. The cells were stained overnight at 4°C with primary antibodies diluted in PBST containing 2% BSA and 3% normal goat serum using the following dilutions: PAF1 1:500 (Bethyl), MLL1 1:100 (Millipore) or Flag-M2 1:1000 (Sigma-Aldrich). Primary antibodies were detected with appropriate Alexa 488 and Rhodamine Red-conjugated secondary antibodies (Molecular Probes-Invitrogen and Jackson Immuno Research Lab Inc.). Slides were counterstained with TO-PRO-3 iodide (Invitrogen) for 5 min and mounted with ProLong Gold antifade reagent (Invitrogen). Stainings were analyzed using an upright Zeiss LSM 510 confocal laser scanning microscope. Images were obtained using the Zeiss LSM software, and Photoshop Illustrator was used to prepare figures.

ChIP Primer and Probe sequences

All probes are Taqman FAM/TAMRA probes. Mouse *HoxA9* primer/probe sets used are the following:

1= Forward: GCCATCAAGGCCTAATCGTG, Reverse: AAGACCCGAAGCTCCTCCTG, Probe: CCTGCGGTGGCAACCTCAGATCC; 2= Forward: TAGACTCACAAGGACAATATCTCCTTTT, Reverse: AGGTACTGAGTATTAAGCAGCTGTTTACA, Probe: TGAATTTTCCCCCTTTTGGGCCAC; 3= Forward: TGACCCCTCAGCAAGACAAAC, Reverse: TCCCGCTCCCCAGACTG, Probe: CCTCTTGATGTTGACTGGCGATTTTCCC; 4= Forward: CGCGATCCCTTTGCATAAAA, Reverse: CGTAAATCACTCCGCACGCT, Probe: ATTATGACTGCAAAACACCGGGCCATT; 5= Forward: GGTGCGCTCTCCTTCGC,

Reverse: GCATAGTCAGTCAGGGACAAAGTG, Probe: TACCCTCCAGCCGGCCTTATGGC; 6= Forward: TCTCTCTCCCTCCGCAGATAAC, Reverse: GGGCATCGCTTCTTCCG, Probe: CCAACTGGCTACATGCTCGCTCCA. The mouse *Hoxc8* primer/probe set used is the following: Forward: GGTACATTTCCGTAGCCCAGAA, Reverse: TTTGTTCAGCCCCATTCAGAA, Probe: TTATGGCCCTGTTTGTCTCCCTGCTCT.

cDNA Primer and Probe sequences

All probes are Taqman FAM/TAMRA probes.

HoxA9 Ups (Figure S1): Forward: GCACCTGGCCCGGAG, Reverse: CTCCAGTTCCAGCGTCTGGT, Probe: CTCGGAAGAAGCGATGCCCTTACACAAA.

HoxA9 A9 (Figure S1 and Figure 7): Forward: GAATGAGAGCGGCGGAGAC, Reverse: GAGCGAGCATGTAGCCAGTTG, Probe: CCCCATCGATCCCAATAACCCAGC.

MLL-AF9 (Figure S7A): Forward: CAGATGGAGTCCACAGGATCAG, Reverse: TTTTCACTTCAAGGATCCCTAAGC, Probe: CCCACACATTTTCTGCTTCACAGTCTTCCTTA.

Protein Identification by LC-Tandem MS: Purified immunocomplexes were resolved on a SDS-PAGE and proteins visualized using Colloidal Coomassie stain. Unique bands were excised and destained with 30% methanol for 4 h. Upon reduction and alklylation of the cysteines, proteins were digested overnight with sequencing grade, modified trypsin (Promega). Resulting peptides were resolved on a nano-capillary reverse phase column (Picofrit column, New Objective) using a 1% acetic acid/acetonitrile gradient at 300 nl/min and directly introduced in to a linear ion-trap mass spectrometer (LTQ XL, ThermoFisher). Data-dependent MS/MS spectra on the 5 most intense ion from each full MS scan were collected (relative CE ~35%). Proteins were

identified by searching the data against Human IPI database (v 3.41) appended with decoy (reverse) sequences using X!Tandem/Trans-Proteomic Pipeline (TPP) software suite. All proteins with a ProteinProphet probability score of >0.8 (error rate <2%) were considered positive identifications and manually verified.