Supplemental Methods

ChIP Protocol

Cells were fixed in 5 ml of 1% paraformaldehyde in media for 10 minutes at 37°C. After fixation, cells were diluted with 45 ml of media, pelleted, and washed once with PBS. The pellet was then subjected to a freeze/thaw and resuspended in ChIP lysis buffer (Upstate Biotechnology, Charlottesville, VA, catalog # 20-163) at a concentration of 5 X 10^6 cells/ 100ul lysis buffer, and then passaged three times through a 27G needle. Sonication was performed with a Branson Sonifier model 250 at 3 X 10 seconds on hold, at 50% duty cycle, 4.5 output. These sonication conditions produced a range of DNA fragment sizes from 200 to 800 bp's. As assessed by agarose gel electrophoresis, the bulk of the DNA fragments were 400-500 bp in length. 100 ul of sonicated chromatin was diluted up to 1 ml with dilution buffer (Upstate Biotechnology, Charlottesville, VA, ChIP kit, catalog# 17-295). The rest of the protocol was followed as suggested by the manufacturer (Upstate Biotech ChIP kit, catalog# 17-295) with one additional modification for mouse monoclonal antibodies. Mouse antibodies were incubated overnight, then incubated with 2 ug anti-mouse IgG for 7 hours, then incubated with agarose A for 4 hours, all at 4 degrees. ChIP was quantified relative to inputs using TaqMan™ Real Time PCR (Applied Biosystems, Foster City, CA) as described below.

Real Time PCR quantification

Total RNA was isolated using RNeasy columns (Qiagen, Valencia, CA), treated with DNase and reverse transcribed (SuperScript Synthesis System, Invitrogen, Carlsbad, CA). Real-time PCR quantification of gene expression was performed in

triplicate with the TaqMan™ primer/probe sets described below and an ABI Prism 7700 instrument (Applied Biosystems, Foster City, CA) using a standard curve and the relative quantitation method as described in ABI User Bulletin #2. Briefly, standard curves were generated using 2 to 10-fold dilutions of a standard cDNA sample in which all target genes of interest are expressed. Threshold values were chosen in the linear range of the PCR reactions and the C_T of each primer/probe reaction set was quantified against the standard curve and then normalized to the appropriate quantified internal control. Two different internal controls were used: *Gapdh* and *β–Actin*, each gave equivalent results. To compare the relative expression at different time points, +4-OHT (or +dimerizer where appropriate) samples were arbitrarily set to 1.0 and -4-OHT (or -dimerizer) sample values standardized relative to this. Error was calculated as outlined in the ABI User Bulletin #2 using standard deviations.

ChIP experiments were quantified relative to inputs using the TaqMan™ Real Time PCR (Applied Biosystems, Foster City, CA) primer/probe sets listed below as described before (27). In brief, the amount of genomic DNA coprecipitated with antibody was calculated as a % of total input the following way: $\Delta\Delta C_T = \Delta C_T$(input) T ΔC_T(Chromatin IP), % total = $2^{\Delta CT}$ x 5.0% (A 50ul aliquot was taken from each of 1ml of sonicated, diluted chromatin before antibody incubation, thus signal from input samples represent 5% of the total chromatin used in each ChIP). C_T values were determined by choosing threshold values in the linear range of each PCR reaction, usually at a threshold of 0.1. Each data point in each figure is the average of 6 separate Real Time PCR reactions. Error was calculated as described in ABI user bulletin #2 in the section on relative quantitation using the comparative C_T method. Briefly, an error range for each

value was calculated using the expression $2^{-\Delta\Delta CT}$ with $\Delta\Delta C_T$ + s and $\Delta\Delta C_T$ - s where s = the standard deviation of the $\Delta\Delta C$ value. The calculated + and - error range was then represented by error bars for each data point. Values calculated by this method are labeled as relative units on all the figures.

Although the amount of immunoprecipitated DNA is related to the absolute amount of protein present, signal is also dependent on other factors such as the kinetics of antibody-epitope recognition. Therefore quantified signal is not a direct measure of absolute amounts of protein present and different antibodies can give different quantified signal strengths. ChIP experiments generate background signals even in the absence of antibody controls. In our ChIP experiments performed either with no antibody, with a non-specific antibody (such as rabbit anti-mouse IgG), or with specific antibodies in knock-out cell lines where the target protein is not present (for example MLL ChIP in *Mll-/-* cells) consistently generated signal in the range of 0.01 to 0.08% of input.

TaqMan™ Probe and Primer Sequences

All TaqMan™ probes are 5'FAM/3'TAMRA probes special ordered from ABI (catalog #450024). The Forward and Reverse primers are not designed to be used in the absence of the Taqman™ probes and will not give accurate results when used with CYBR green or as conventional PCR primers. Probes and primers for gene expression: *Hoxa9a* Forward - GCACCTGGCCCGGAG, Probe - CTCGGAAGAAGCGATGCCCTTACACAAA, Reverse - CTCCAGTTCCAGCGTCTGGT; *Hoxa9b* Forward - CTATGCTTGTGGTTCTCCTCCAG, Probe - CCCCATCGATCCCAATAACCCAGC,

Reverse - GAGCGAGCATGTAGCCAGTTG; *Hoxa9T* Forward - GCGCCGGACGGTAGTTG, Probe - AGAAAAACAACCCAGCGAAGGCGC, Reverse - CGGCATTGTTTTCGGAGAA; *Hoxc8* Forward - ACAGTAGCGAAGGACAAGGC, Probe - AAATCAGAACTCGTCTCCCAGCCTCA, Reverse - TTCCAAGGTCTGATACCG; *Meis1* Forward - GCATGCAGCCAGGTCCAT, Probe - ACCGGTCCACCACCTGAACCACG, Reverse - TAAAGCGTCATTGACCGAGGA; *Hoxa7* Forward - CGGCCGAAGCCAGTTTC, Probe - CGCAGTTCAGGACCCGACAGGAA, Reverse - GCGCCGCGTCAGGTAG; *Hoxa1* Forward - CGAGCTTACCCCTCTGACCAT, Probe - AACTTTCCAATCCTGCGCGGTCAGT, Reverse - CCGCCGCAGCTGTTG;

Probes and Primers for quantification of ChIP results: *Hoxa9*-1 (at 1Kb) Forward - AGCCACAGGACCCGGC, Probe - CTTCGTTGCTGTCCCCTGCGTTTTC, Reverse - CCAAACAGAGCCTCCCTGG; *Hoxa9*-2 (at 1.5 Kb) Forward - GCCATCAAGGCCTAATCGTG, Probe - CCTGCGGTGGCAACCTCAGATCC, Reverse - AAGACCCGAAGCTCCTCCTG; *Hoxa9*-3 (at 2.4 Kb) Forward - CACCCGCGGCGTCTT, Probe - CCCACATCGAGGGCAGGAAACACT, Reverse - CGAACCAATGGATCTGGCA; *Hoxa9*-4 (at 3.2 Kb) Forward - TAGACTCACAAGGACAATATCTCCTTTT, Probe - TGAATTTTCCCCCTTTTGGGCCAC, Reverse - AGGTACTGAGTATTAAGCAGCTGTTTACA; *Hoxa9*-5 (at 4.3 Kb) Forward - TGACCCCTCAGCAAGACAAAC, Probe - CCTCTTGATGTTGACTGGCGATTTTCCC, Reverse - TCCCGCTCCCCAGACTG; *Hoxa9*-6 (at 4.5 Kb) Forward - AGGGTGATCTGGCCGATGT, Probe -

AAGCGCCTGGCTGGCTTTCCA, Reverse - AAAATGGGCTACCGACCCTAGT; *Hoxa9*-7 (at 4.8 Kb) Forward - CACAGCGAGGCAAACGAAT, Probe - TGTTGGTCGCTCCTGACTTTCCACC, Reverse - TTATTGTTTCGGAAGCCACACA; *Hoxa9*-8 (at 5.2 Kb) Forward - CGCGATCCCTTTGCATAAAA, Probe - ATTATGACTGCAAAACACCGGGCCATT, Reverse - CGTAAATCACTCCGCACGCT; *Hoxa9*-9 (at 5.6 Kb) Forward - CAGCTCTGGCCGAACACC, Probe - CTTCAGTCCTTGCAGCTTCCAGTCCAA, Reverse - TTCCACGAGGCACCAAACA; *Hoxa9*-10 (at 5.8 Kb) Forward - GGTGCGCTCTCCTTCGC, Probe - TACCCTCCAGCCGGCCTTATGGC, Reverse - GCATAGTCAGTCAGGGACAAAGTGT; *Hoxa9*-11 (at 7.1 Kb) Forward - TCTCTCTCCCTCCGCAGATAAC, Probe - CCAACTGGCTACATGCTCGCTCCA, Reverse - GGGCATCGCTTCTTCCG; *Meis1* same as for gene expression (see above); *Hoxa1* same as for gene expression (see above); *Hoxc8* Forward - TCTTCATGTCGTGGATTGATGAA, Probe - AATCGCGTGTAAGCGCCGCC, Reverse - GCTCTCACTTAGCTCTTTCCTCTAACA.

Supplemental Figure Legends

Supplemental Figure 1. Histone H3 lysine 79 dimethylation at *Hox a9* in MLL-AF9 cells (black line) is comparable to levels seen in MLL-ENL-ER cells. Lysine 79 dimethylation is absent in neutrophils (white line), where *Hox a9* is not expressed.

Supplemental Figure 2. The apoptosis marker caspase-3 is not present in its active cleaved form in MLL-ENL-ER cells grown without 4-OHT for 12 days. Cells grown

either with (+, lane 2 and 4) or without (-, lane 1 and 3) 4-OHT were subjected to western blot analysis with an antibody that recognizes both cleaved and full length forms of caspase-3 (lane 1 and 2) or recognizes just the cleaved products (lane 3 and 4). Full length caspase-3 is visible (black arrowhead) but there are no bands for the expected cleaved product sizes (white arrowheads) indicating that caspase-3 is not active in these cells.