Supplemental Information

MLL fusion proteins link transcriptional coactivators to previously active CpG-rich promoters

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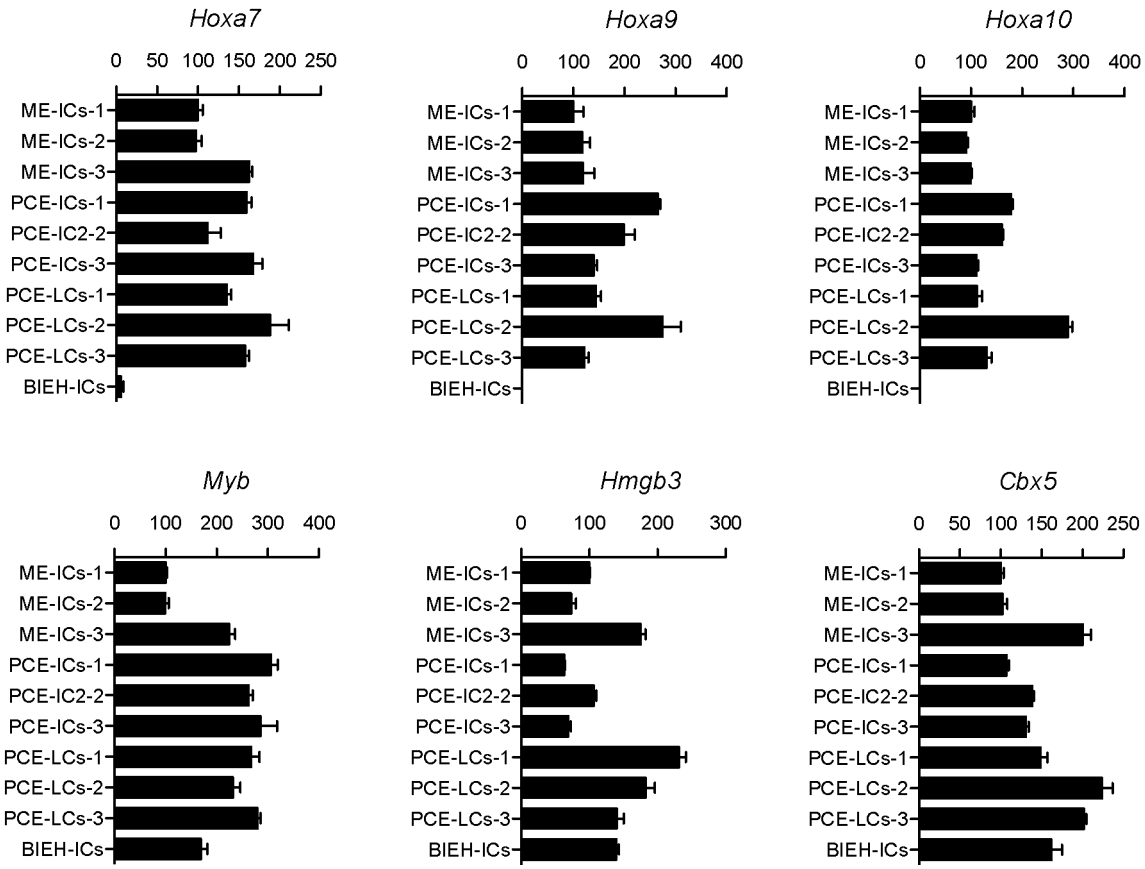

Figure S1. Expression profiles of PCE-leukemic cells

Expression profiles of leukemia-associated genes in PCE-leukemia cells (PCE-LCs). Gene expression in three independent PCE-induced leukemia cell lines was analyzed by RT-qPCR for the indicated genes. MLL-ENL-immortalized cells (ME-ICs), PCE-ICs, and BiEH-ICs are included for comparison. The expression levels are expressed relative to ME-ICs-1 arbitrarily set as 100.

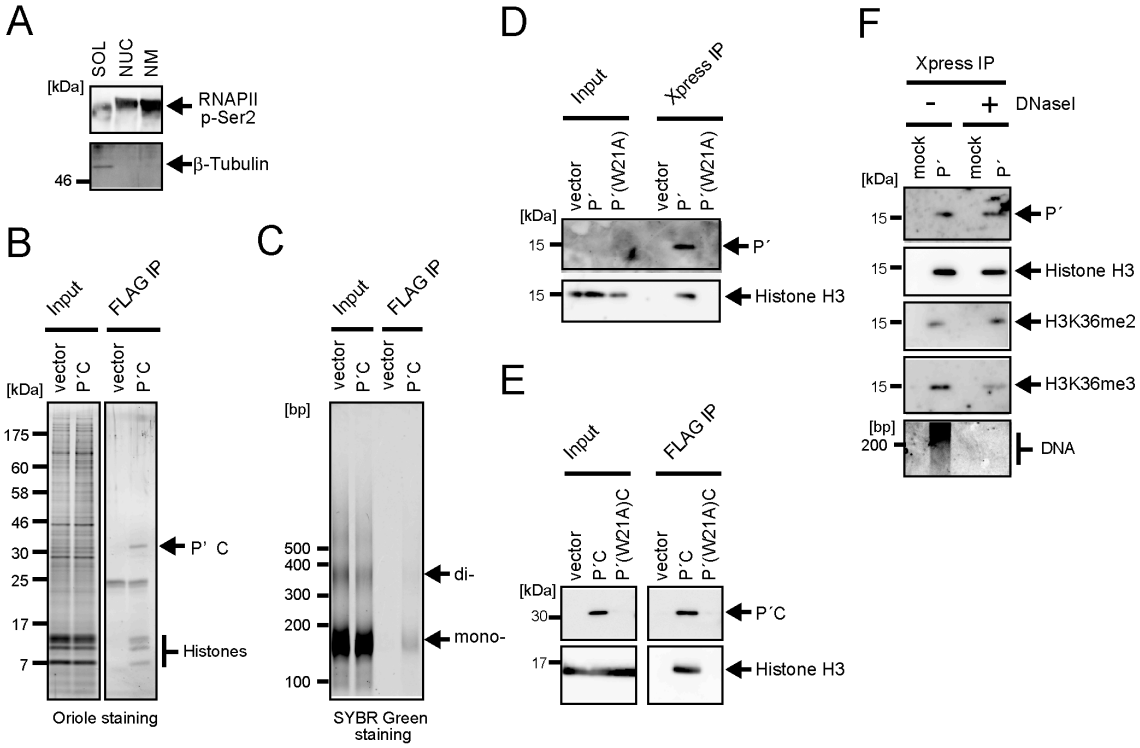

Figure S2. Characteristics of the nucleosomes coprecipitated with P´ and P´C mutants.

A) Distribution of endogenous RNAPII (phosphorylated at the Ser2 residue in its CTD) and β-tubulin proteins in the three subfractions. 293T cells were subfractionated into the soluble fraction (SOL), the nucleosome fraction (NUC), and the nuclear matrix fraction (NM) as in Figure 2E.

B) Proteins in the P′C-nucleosome complex. Proteins coprecipitated with P′C were visualized by Oriole staining.

C) DNAs in the P′C-nucleosome complex. DNAs coprecipitated with P′C were visualized by SYBR Green staining.

D) P´(W21A) does not coprecipitate nucleosomes. The nucleosome fractions of P´- or P´(W21A)-expressing cells were analyzed as in Figure 2I. P´ proteins were precipitated using anti-Xpress antibody. Nucleosomes were visualized by anti--histone H3 antibody.

E) P´(W21A)C does not coprecipitate nucleosomes. The nucleosome fractions of P´C- or P´(W21A)C-expressing cells were analyzed as in Figure 2I. P´C proteins were precipitated using anti-FLAG antibody. Nucleosomes were visualized by anti-histone H3 antibody.

F) The role of DNAs in P´-nucleosome interaction. The precipitates prepared as in Figure 2I were treated with DNase I (300 kunits/mL) for 10 min and washed to remove unbound material. Nucleosomes were visualized by antibodies specific for H3K36me2 and H3K36me3 modification or by anti-histone-H3 antibodies. DNAs coprecipitated with P′ were visualized by SYBR Green staining.

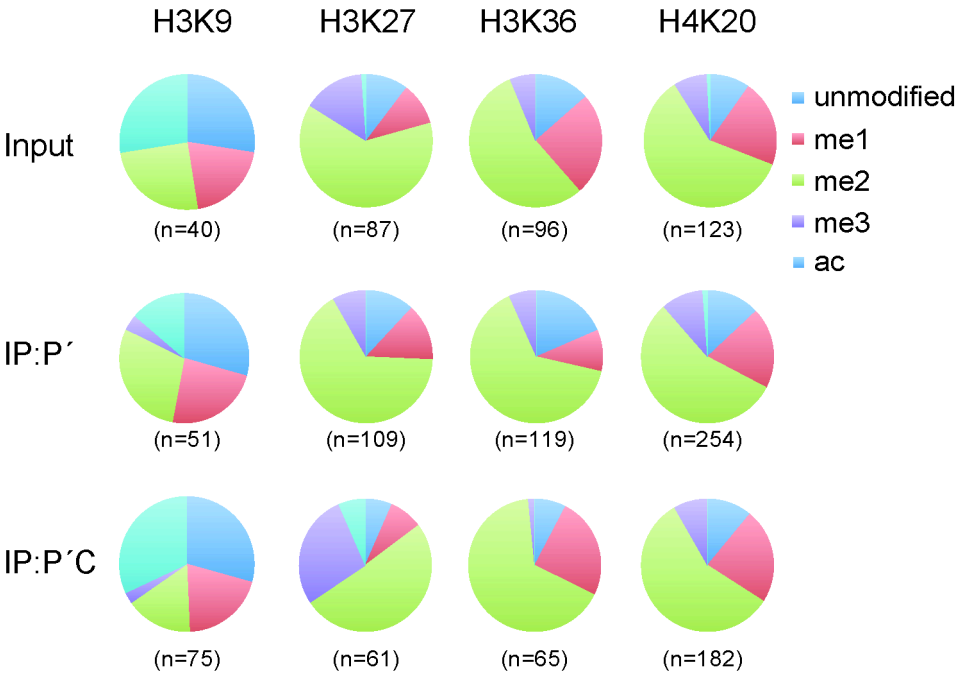

Figure S3. Frequencies of each histone modification in P´- and P´C-bound nucleosomes.

The histone proteins coprecipitated with P´(IP:P´) and P´C (IP:P´C) were analyzed by mass spectrometry. Bulk histones (Input) were also analyzed for comparison. The frequencies of different modification status detected by mass spectrometry analysis were expressed in the pie charts. n, number of peptides analyzed. It should be noted that modifications of histones may affect the digestion efficiency by trypsin in the sample preparation procedure and therefore the frequencies shown above may not faithfully reflect the actual ratio of each modification.

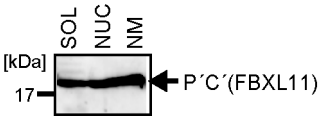

Figure S4. Subcellular distribution of the **P′C′(FBXL11) mutant**

Experiment was performed as in Figure 2E. The P′C′ (FBXL11) mutant was visualized by anti-FLAG antibody.

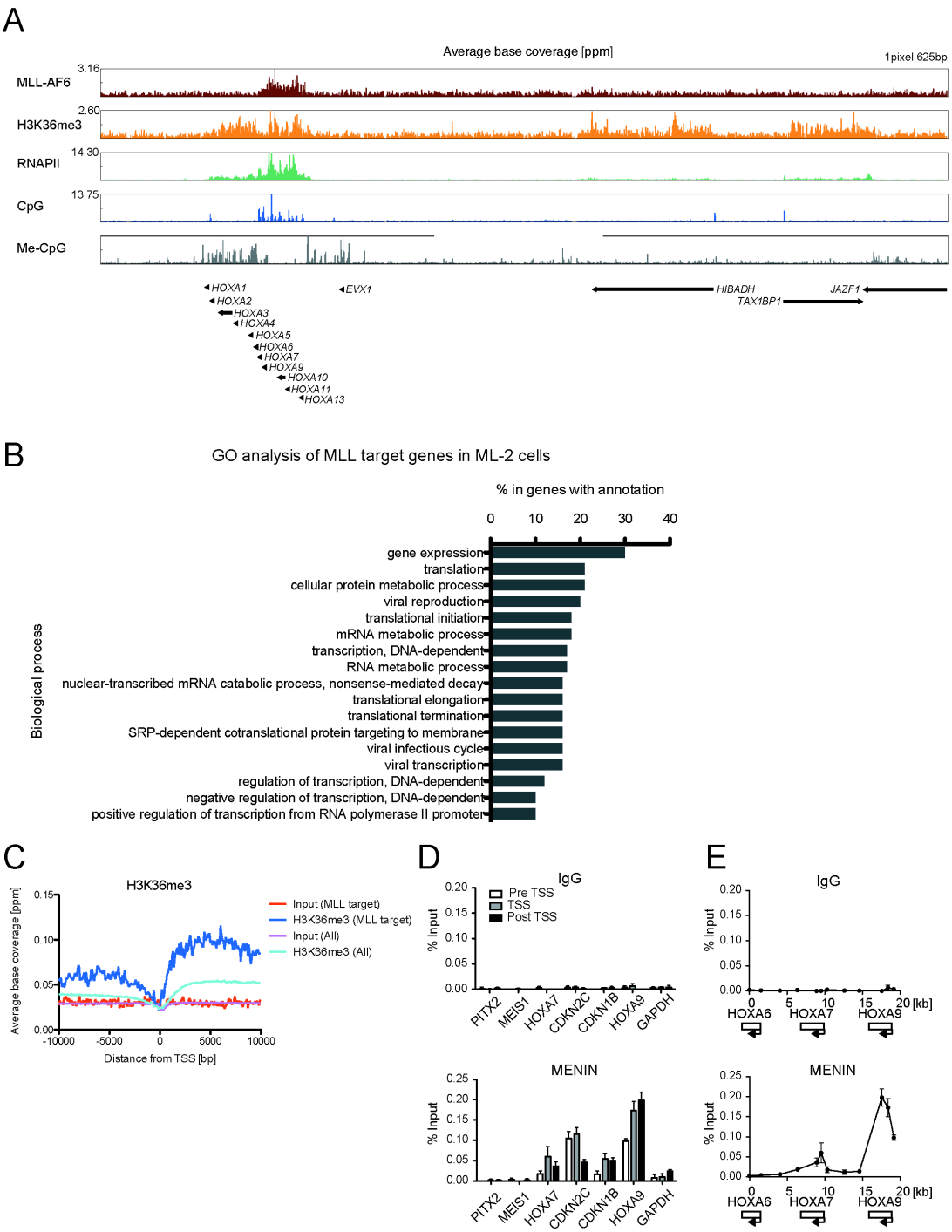

Figure S5. Profiles of the MLL target genes in ML-2 cells

A. Representative results of ChIP-seq for MLL-AF6, H3K36me3, and RNAPII and CIRA/MIRA-seq for non-methylated CpGs (CpG) and methylated CpGs (Me-CpG) at the *HOXA* cluster are shown with the locations of genes.

B. Gene ontology (GO) analysis of MLL-AF6 target genes in ML-2 cells. The GO file was downloaded from the GO web site (http://www.geneontology.org/), and the

biological process GO term enrichment in the MLL-AF6 target gene set with annotation was plotted.

C. Average distribution of H3K36me3 at the 154 MLL-AF6-occupied TSSs. The input data and all TSS data are included for comparison. Analysis was performed as in Figure 6A.

D. Genomic localization of menin in ML-2 cells at various gene loci. Analysis was performed as in Figure 6B. IgG control is shown for comparison.

E. Genomic localization of menin in ML-2 cells at the posterior *HOXA* loci. Analysis was performed as in Figure 6C. IgG control is shown for comparison.

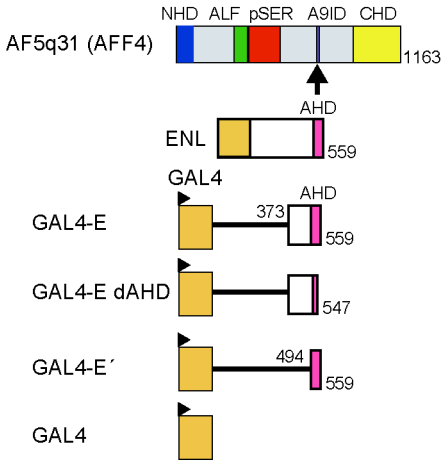

Figure S6. Schematic representation of the GAL4-ENL fusion proteins

Table S1. List of MLL-AF6 target genes in ML-2 cells

AIMP1	*CCDC103*	*FDFT1*	*HOXA9*	*PDLIM2*	*RPL8*	*TCF4*
ANP32E	*CCDC56*	*FOXD4L1*	*JMJD1C*	*POLR2A*	*RPL9*	*THAP9*
APOLD1	*CDC2L5*	*FOXP1*	*LOC100216545*	*PPIL5*	*RPLP1*	*TIMM13*
ATF4	*CDKN1B*	*FUS*	*LOC153684*	*PPP1R10*	*RPLP2*	*TMED1*
ATF5	*CDKN2C*	*GAS5*	*LOC401093*	*PSMG2*	*RPS15A*	*TOM1*
BAT1	*CENPL*	*GFI1*	*LZTR1*	*PTPN6*	*RPS18*	*TRPS1*
BAT2	*CEP76*	*GGA3*	*MBNL1*	*PTPRK*	*RPS23*	*TXN2*
BAT3	*CNTD1*	*GLMN*	*MED22*	*RASSF1*	*RPS3A*	*VPS52*
BAT4	*COMMD3*	*GNB2L1*	*MGAT2*	*REEP3*	*RUNX2*	*YWHAE*
BLCAP	*CTAGE5*	*HADHA*	*MLL5*	*RPAP2*	*SATB1*	*ZBTB4*
BRD2	*DACH1*	*HADHB*	*MLLT10*	*RPL10A*	*SEC31A*	*ZNF335*
BUD31	*DARS2*	*HEXIM1*	*MPO*	*RPL12*	*SERBP1*	
C10orf140	*DLX-AS*	*HNRNPA1*	*MRPS7*	*RPL21*	*SF3B14*	
C11orf58	*DLX5*	*HNRNPH1*	*MYC*	*RPL23*	*SFRS1*	
C19orf48	*DLX6*	*HNRNPK*	*NDUFA4*	*RPL31*	*SFRS7*	
C5orf39	*EFTUD2*	*HOXA10*	*NDUFA4L2*	*RPL36AL*	*SIN3A*	
C7orf55	*EIF3D*	*HOXA11*	*NUP62*	*RPL37*	*SLBP*	
C9orf64	*EIF4A1*	*HOXA11-AS*	*NUP85*	*RPL6*	*SNHG6*	
CALM2	*EMB*	*HOXA13*	*PCBP1*	*RPL7*	*SUPT3H*	
CBX5	*FAM169A*	*HOXA7*	*PDAP1*	*RPL7A*	*TBCK*	

Table S2. Antibodies used in this study

Epitope	Maker/Reference	ID/Product no.	Application
Histone H2B	Abcam	ab1790	WB
Histone H3	Abcam	ab1791	WB
Histone H3	Active Motif	39163	WB
H3K36me1	Abcam	ab9048	WB
H3K36me2	Cell Signaling	9758	WB
H3K36me2	Abcam	ab9049	ChIP
H3K36me3	Abcam	ab9050	ChIP, WB
H3K27me3	Millipore	07-449	WB
H3K27ac	Abcam	ab4179	WB
H3K4me2	Abcam	ab7766	ChIP, WB
FLAG	Sigma	F-7425	WB
FLAG	Sigma	M2	ChIP, IP,WB
Xpress	Santa Cruz Biotech.	M-21/sc-499	WB
Xpress	Santa Cruz Biotech.	D-8/sc-7270	IP
LEDGF	Bethyl laboratories	A300-848A	ChIP
RNAPII	Millipore	CTD4H8/05-623	WB, ChIP
RNAPII P-Ser2	Abcam	ab5095	WB
Tubulin, beta	Santa Cruz Biotech.	sc-9104	WB
ENL	Yokoyama et al. 2010	ENL-3.1	WB
MLL	Yokoyama et al. 2010	rpN1	ChIP
Menin	Bethyl laboratories	A300-105A	ChIP
AF5q31	Bethyl laboratories	A302-538A	WB
p300	Bethyl laboratories	A300-358A	WB
(Rabbit IgG)	Millipore	pp64B	ChIP

Table S3. Custom-made probes used for ChIP- and CIRA-qPCR in this study

Position	Fwd primer seq	Rev primer seq	Reporter seq
CDKN1B TSS	GGGTCTGTGTCTTTTGGCT	GCCCGAACCCCTCTCG	CCAGCGACTGCCCTC
CDKN1B Pre TSS	GTCCCGAGGGTCCCTTC	GTGTGCCTACCTCATCTCATACG	CAGCTGTCACATTCTG
CDKN1B Post TSS	GCTTTGGGAGAGCTAACTTTATTGGT	CGGATCTTACCATCTCCAGTTTCTG	ACCTGGCCCACTGCTT
CDKN2C TSS	GGCGGCTGCCCTGT	CCCGGTGCCACTTTGC	CTGTGCCCCTTTGCTG
CDKN2C Pre TSS	CTCCACAACCGTCTTAAATAACAAACC	GCGGGCTTGAGTCTGTGA	CAGCTGCCCCAATTC
CDKN2C Post TSS	CTGTGGAGTCGTCAGAATTCTTCAT	CGATTCACACGTGATTATTCAGCAAA	CCTCGCCTCGCTTTT
EVI-1 Pre TSS	TCACCAGACAGTCATCAATCTCTCT	GAAGGGCGTGCAAAATTTTCAAAC	CCCGCCCAAACAGCAT
EVI-1 TSS	GCTGCGGAGGATCTGAAAGG	CTCCTTCCCAGTTCCAATGGG	CAGGAGGAGGAGAGTTT
EVI-1 Post TSS	CACCACCCTTCATCTCTTTAGCAT	TGGCAGCTTCCTGGAGATATAAAAG	AAGCTGAGATTTTCCC
GAPDH TSS	CCACATCGCTCAGACACCAT	GCGAACTCACCCGTTGACT	CCGACCTTCACCTTCC
GAPDH Pre TSS	CCCCTCCTAGGCCTTTGC	GCTGAGAGGCGGGAAAGTT	ACTACCGCAGAGCCTC
GAPDH Post TSS	GGCTCTCTCCCATCCCTTCT	AGGAGTGGGAGCACAGGTAA	CCCCACACACATGCAC
HOXA6 Pre TSS	AACTCGCACCCACGAATAGG	GCAGTGACAAAGGTGGCTTT	CCTGCCGGCCTGCACT
HOXA6 -2.5kb	GCCTATATGGCCGGGAAATCT	GTCCAGCTCCGGTCAGG	CCCCGCCTCGGCTAC
HOXA6 Post TSS	TTCCAGTGTCTCCCCAAAGC	GGAGATGAGGGAGGAGAGAGAAAA	CAGCCCCAGACTCAG
HOXA7 Pre TSS	GCCTTCCCCGTCTGGAT	ACTCTGCCCAAGTCTTCTCTCA	CAGGCCGGACTTAGAC
HOXA7 Post TSS	TGCCAGGGTCCATTTCAAGATG	CCCTCATCCCCAGGACCTT	CTCTGTCCTCATTCCC
HOXA7 -2.5kb	CTAGGGATCACTCACTCACTGGAT	AGGCAAATTGCTTCCTAAAGGTTTC	CACCCTTGCCTCTCTC
HOXA7 TSS	GACGCCTACGGCAACCT	GCCTTTGGCGAGGTCACT	CCCTGCGCCTCCTAC
HOXA7 +4kb	CCAGAGCTGATTCCGGATTCG	CCTCGCCTGGTCTGCA	CCCTGAACCCAGCCCC
HOXA9 Pre TSS	TGGCTGCTTTTTTATGGCTTCAATT	CCGCGTGCGAGTGC	CCCCTCACATAAAATT
HOXA9 TSS	TCACCACCACCCCTACGT	GCAAGCCCGCGAAGGA	CAGGAGCGCATGTACC
HOXA9 Post TSS	AGTGGCGGCGTAAATCCT	TGATCACGTCTGTGGCTTATTTGAA	CCCGCAGCCTCATC
HOXA9 +5kb	GGATATTCCACCAAAGCCCTTTCAT	GGCAATGCCAATAAAAGAGGTGTTT	CTGCCGCCGATAAAG
MEIS1 TSS	TTTGCTTCAGGTCCCGTAGAC	CCTTAACGTCTCCAGCAACGT	ACTGGTCCCAGATCTT
MEIS1 Pre TSS	CGGCGTTGATTCCCAATTTATTTCA	CACACAAACGCAGGCAGTAG	CCGCCAGCTTTATTTT
MEIS1 Post TSS	TCTCAGCGCCTCCAAATCTTG	TTTGTGTGTGTGAAATTTAGCTATTTAGGTTTT	CCAGGCAGTTATTTTC
PITX2 Pre TSS	CGGTGTCCCAGCTAAGCT	CGAACGAGCTAGGCTTGTCA	CAGCGTCCCGGCCTT
PITX2 TSS	AGAGAGAGTGCGAGACCGA	GCCACTGGCAGTTTCTTTCTG	CCTCTCCAGCTTTCTC