Supplemental data

Figure S1

c-Myb forms a complex with menin and MLL in SEM-K2 leukemia and normal hematopoietic cells. (**A**) Interaction of in vitro-translated, ^{35}S-methionine-labeled MLL-N fragment (indicates on the left) with F-menin. (**B** and **C**) Immunoprecipitations carried out with anti-menin antibody on nuclear extracts prepared from SEM-K2 cells (**B**) or human primary T lymphocytes stimulated with Phytohemagglutinin (PHA) (1μg/ml) for 24 hours, followed by IL-2 (30 ng/ml) for 6 days (**C**). IP samples were separated by SDS-PAGE and subjected to immunoblot with antibodies against to c-Myb, MLL, or menin.

Figure S2. c-Myb forms a complex with menin and MLL, regulates H3K4 methylation and the localizaton of MLL and menin on *Hoxa9* gene in KCL 22 cells. (**A**) c-Myb and MLL co-immunoprecipitated with menin in KCL 22 cells. (**B** and **C**) KCL-22 cells were nucleofected with control siRNA or *c-myb* siRNA. Whole-cell extracts were prepared 3 days after the initial nucleofection according to the cell number counts and probed by immunoblotting with α-c-Myb , α-menin, α-MLL^{C}, α-RbBP5, α-ASH2L, α-WDR5 α-β-actin (**B**), α-H3K4(Me)$_{1-3}$, and α-H3 (**C**) antibodies. (**D**) ChIP assay was performed on KCL-22 cells using α-c-Myb, α-MLL, and α-menin antibodies. c-Myb, MLL and menin were specifically detectable at *Hoxa9* promoter region. (**E**) ChIP asnalysis of KCL-22 cells with *c-myb* silencing was carried out using antibodies indicated at the top. No Ab or α-histone H3 antibody served as negative or positive control for ChIP.

Figure S3

c-Myb recruits menin and MLL to *Hoxa9* locus through canonical Myb binding sites. (**A**) Schematic representation of the canonical Myb binding sites and primer sets used on *Hoxa9* promoter, exon 1 and 2 locus. The black ovals represent c-Myb binding sites. Arrows show primer sets for ChIP assay. (**B**, **C** and **D)** ChIP assays were carried out with c-Myb, MLL, and menin antibodies and mouse IgG (mIgG) in KCL-22 cells. The precipitated DNA fractions were analyzed by quantitative Real-Time PCR with each specific primer set that was showed in **A.** (**E**). ChIP assays were carried out with c-Myb antibody in KCL-22 cells and the precipitated DNA fractions were analyzed by standard PCR with prime set P4. Primer sets for qPCR or standard PCR are: P1-F: CCGCAGGGATTATTTACAGG, P1-R: CAAATCGCATTGTCGCTCTA; P2-F: TGCCACCAAGTTGTTACATGA, P2-R: CGACCCACGGAAATTATGAA; P3-F: CCACGCTTGACACTCACACT, TCGCTGGGTTGTTTTTCTCT; P4-F: TTTCTCTTC CCCGCAGATAAC, P4-R: GGGCACCGCTTTTTCCG.

Figure S4

Relative expression of *c-myb* and *Hoxa9* in AML patients treated with *c-myb* targeted AS ODN. RNA samples were prepared from cells isolated from patients on the Day (D) indicated, and c-Myb and *Hoxa9* mRNA levels were quantitated by qRT-PCR as described in the legend of Figure 8B. Results from Patient #995 are shown in A and B, while results from Patient #1207 are shown in C and D.

Figure S1

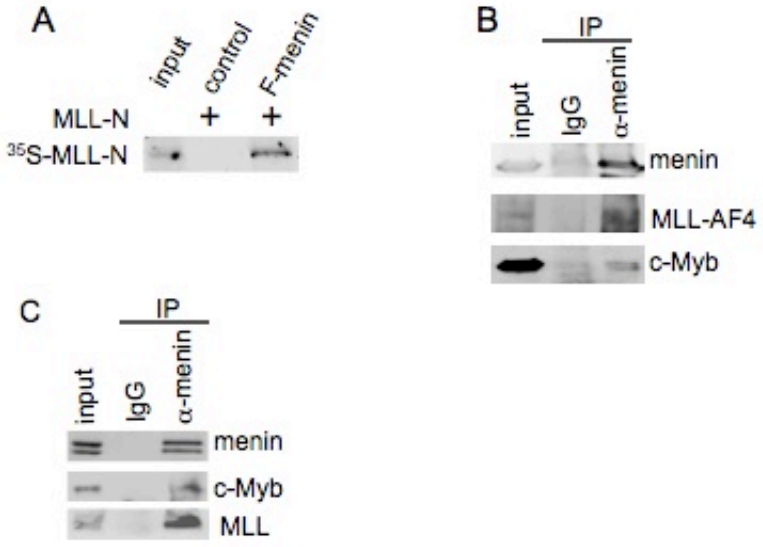

A
input
control
F-menin
MLL-N
+
+
35S-MLL-N
B
IP
input
IgG
α-menin
menin
MLL-AF4
c-Myb
C
IP
input
IgG
α-menin
menin
c-Myb
MLL

Figure S2

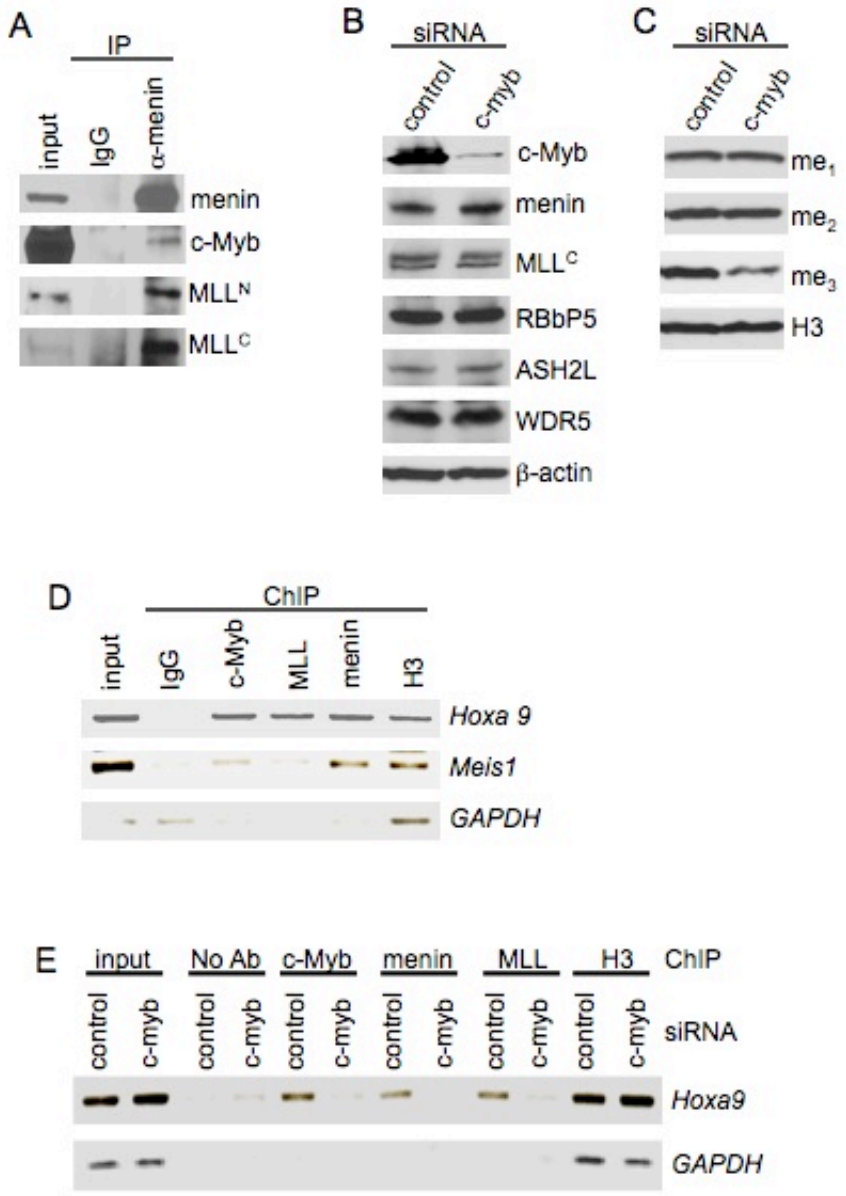
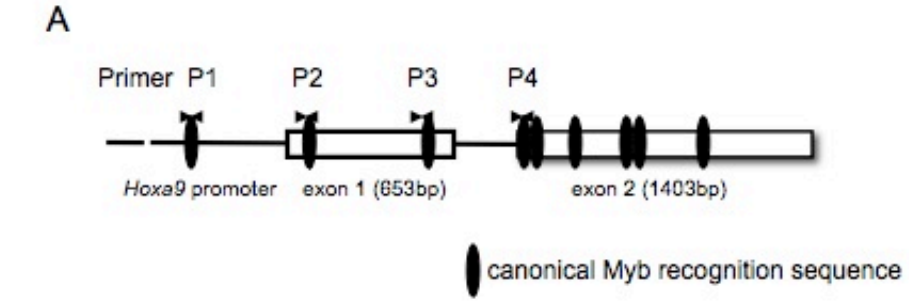
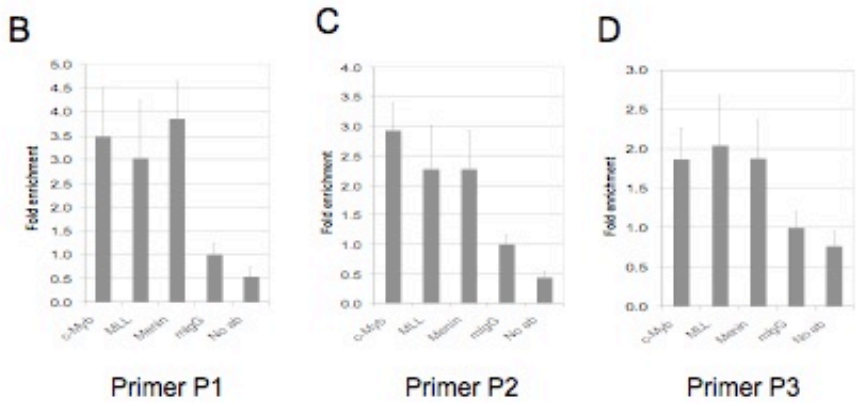

A
IP
input
IgG
α-menin
menin
c-Myb
MLL^N
MLL^C
B
siRNA
control
c-myb
c-Myb
menin
MLL^C
RBbP5
ASH2L
WDR5
β-actin
C
siRNA
control
c-myb
me1
me2
me3
H3
D
ChIP
input
IgG
c-Myb
MLL
menin
H3
Hoxa 9
Meis1
GAPDH
E
input
No Ab
c-Myb
menin
MLL
H3
ChIP
control
c-myb
siRNA
Hoxa9
GAPDH

Figure S3


A
Primer P1
P2
P3
P4
Hoxa9 promoter
exon 1 (653bp)
exon 2 (1403bp)
canonical Myb recognition sequence


B
C
D
Fold enrichment
c-Myb
MLL
Menin
mIgG
No ab
Primer P1
Primer P2
Primer P3

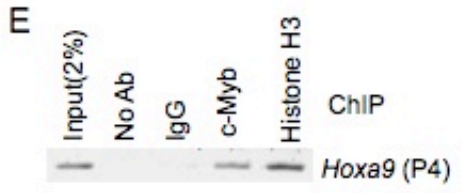

E
Input(2%)
No Ab
IgG
c-Myb
Histone H3
ChIP
Hoxa9 (P4)

Figure S4

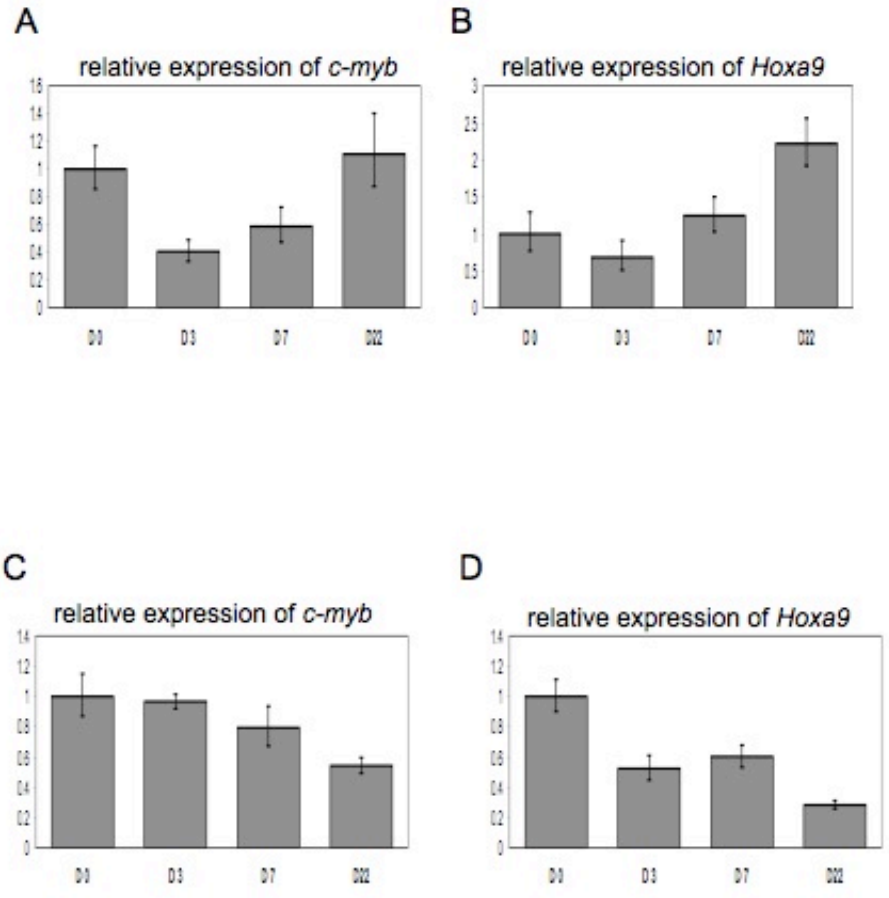

A
relative expression of c-myb
B
relative expression of Hoxa9
C
relative expression of c-myb
D
relative expression of Hoxa9

Table S1- Clinical Characteristics of Patients Providing Material for Study

Patient ID	Sex	Diagnosis	t(4;11) MLL-AF4	FLT3	Cytogenetics
970	F	AML; M2 without t(8;21)	negative	ITD+	46,XX; del(17)(p12)[8] /46,XX[17]RARA split neg. in 200, P53 deletion pos in 9/209
972	F	AML; Biphenotypic	negative	normal	45,XX,-7, del(17)t(7;17) (p11.2;p11.2)[19] /46,XX
973	M	AML-MLD without prior MDS or MPD	N/A	ITD+	N/A
866	M	AML; M4 (myelomonocytic)	N/A	N/A	N/A
995	F	AML with inversion (16) (p13; q22)	N/A	N/A	44,XX,der(4)t(4;15)(q35;q21),-15,inv(16)(p13.3q22), -22[cp2]/46,XX[9]11/13/07: 46,XX,add(4)(q35),inv(16)(p13.3q22)[16]
1207	F	AML; M4 (myelomonocytic)	N/A	N/A	43-45,XX,der(3)t(3;12)(p10;q10),inv(7)(q11.1q22), del(11)(q22q23),12,-12,der(14)t(12;14)(q13;24),del(17)(p12),der(17)t(?12;17)(p11.2;q11.2),+mar[cp25]

Table S2- Human Specific Primers Used for Quantitative Real Time PCR

Primers	Forward primer sequence	Reverse primer sequence
c-myb	5'-GAAGGTCGAACAGGAAGGTTATCT-3'	5'-GTAACGCTACAGGGTATGGAACA-3'
B-myb	5'-CAGAGCCCTTGGAGGAATT-3'	5'-CAGGCTCGTTTCTGGTGG-3'
Hoxa9	5'-AAAACAATGCTGAGAATGAGAGCG-3'	5'-TGGTGTTTTGTATAGGGGCAC-3'
Meis1	5'- TGACCGTCCATTACGAAACCT-3'	5'-CCAGTCCAACCGAGCAGTAAG-3'
GAPDH	5'-GACAGTCAGCCGCATCTCTT-3'	5'-CCAATACGACCAAATCCGTTGAC-3'

The probe for *c-myb* expression is 5'-TCAAAAGCCAGCCAGCCACAGTG-3'. The probe for *GAPDH* expression is 5'-CGTCGCCAGCCGAGCCACATCG-3'. The probes were modified with reporter dye 6-FAM at 5' end and quencher black hole at 3' end.