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Supplemental information

Recruitment of MLL1 complex is essential for SETBP1 to induce myeloid transformation

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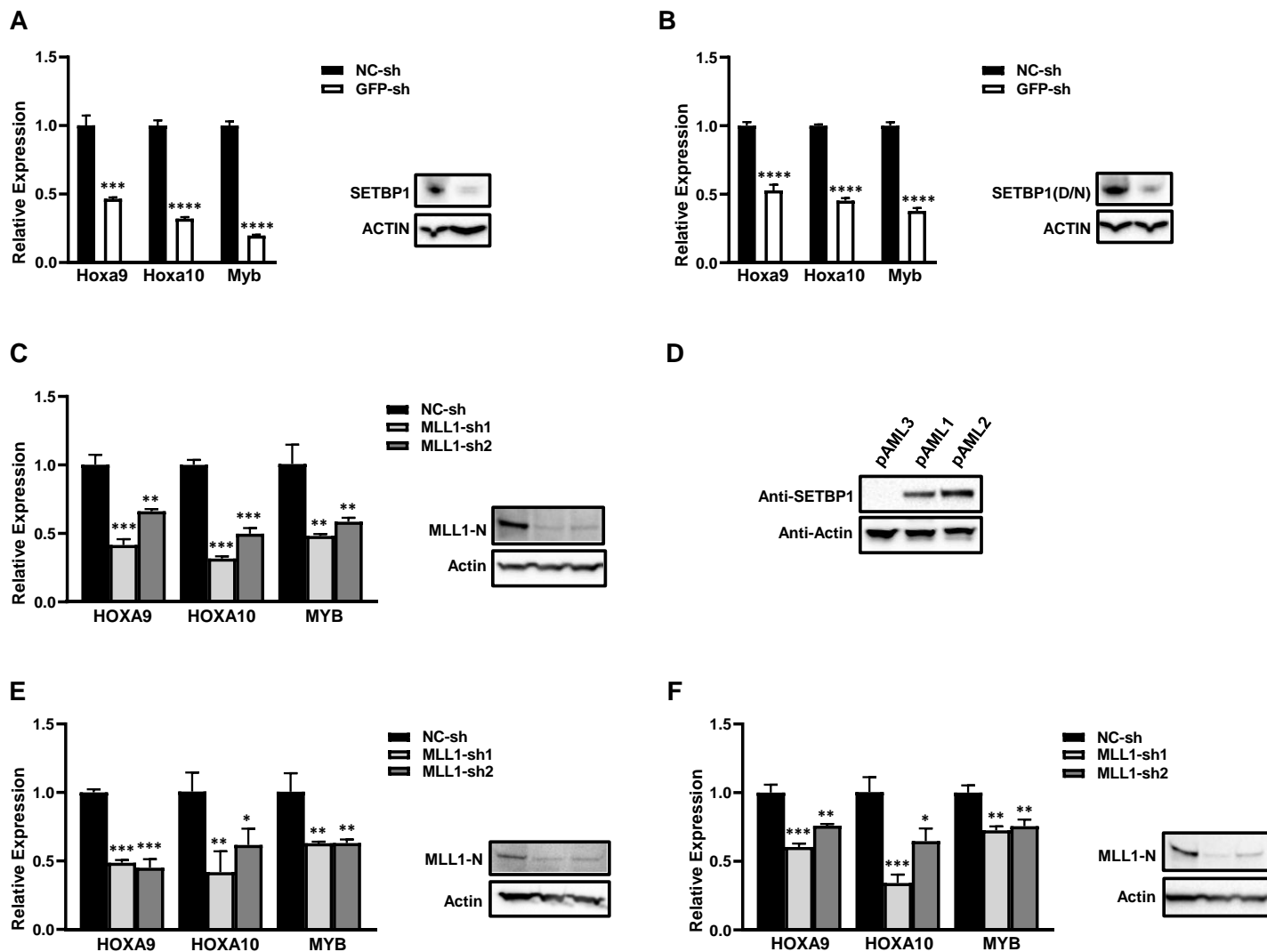


Figure S1, related to Figure 1.

(A) Left panel, real-time RT-PCR analysis of relative *Hoxa9/Hoxa10/Myb* mRNA levels in *Setbp1*-immortalized cells at 72 hours after transduction with *GFP*-specific shRNA (*GFP-sh*) or a non-targeting control shRNA (*NC-sh*). Data are represented as mean \pm SD ($n=3$). ***, $p < 0.001$; ****, $p < 0.0001$ (two-tailed Student's *t* test). Right panel, Western blotting analysis of the same transduced cells using indicated antibodies. (B) Same analyses as in (A) performed in *Setbp1(D/N)*-immortalized cells. Data are represented as mean \pm SD ($n=3$). ****, $p < 0.0001$ (two-tailed Student's *t* test). (C) Left panel, real-time RT-PCR analysis of relative *HOXA9/HOXA10/MYB* mRNA levels in another sAML patient bone marrow cells with *SETBP1* missense mutation (p.I871T) at 72 hours after transduction with indicated negative control or *MLL1*-specific shRNAs. Data are represented as mean \pm SD ($n=3$). **, $p < 0.01$; ***, $p < 0.001$ (two-tailed Student's *t* test). Right panel, Western blotting analysis of same transduced cells using indicated antibodies. (D) Western blotting analysis showing significantly increased *SETBP1* protein levels in two primary AML patient bone marrow samples with overexpression of wild-type *SETBP1* (pAML1 and pAML2) compared to a control primary AML sample (pAML3) without *SETBP1* overexpression. (E) & (F) Same analyses as in (C) performed in pAML1 and pAML2 cells. Data are represented as mean \pm SD ($n=3$). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (two-tailed Student's *t* test).

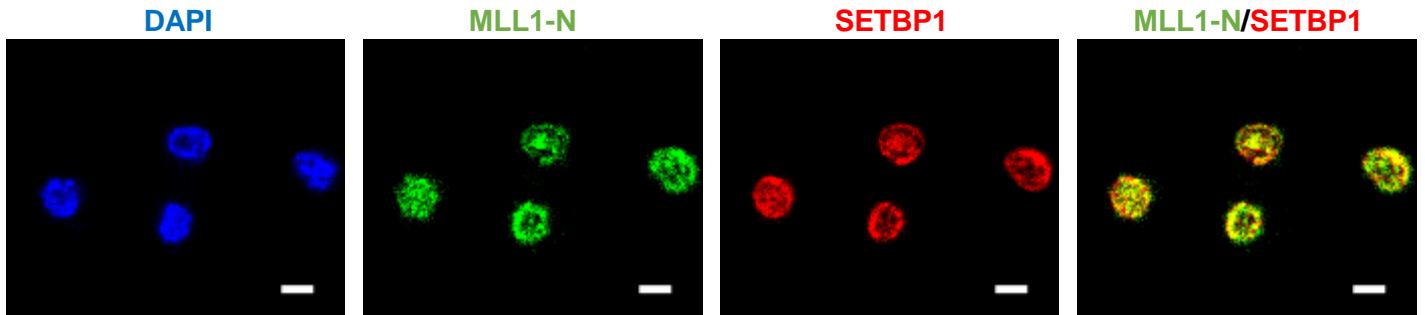
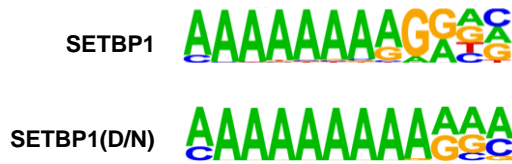


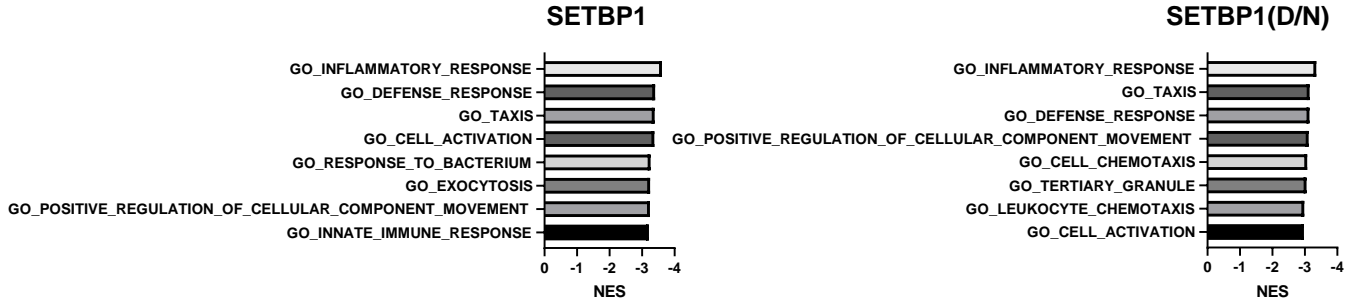
Figure S2, related to Figure 2.

Immunofluorescence staining of mouse LSK cells using a SETBP1-specific antibody together with a MLL1-N-specific antibody. Nuclei were counterstained with DAPI. Scale bar, 5μm.

A



B



C

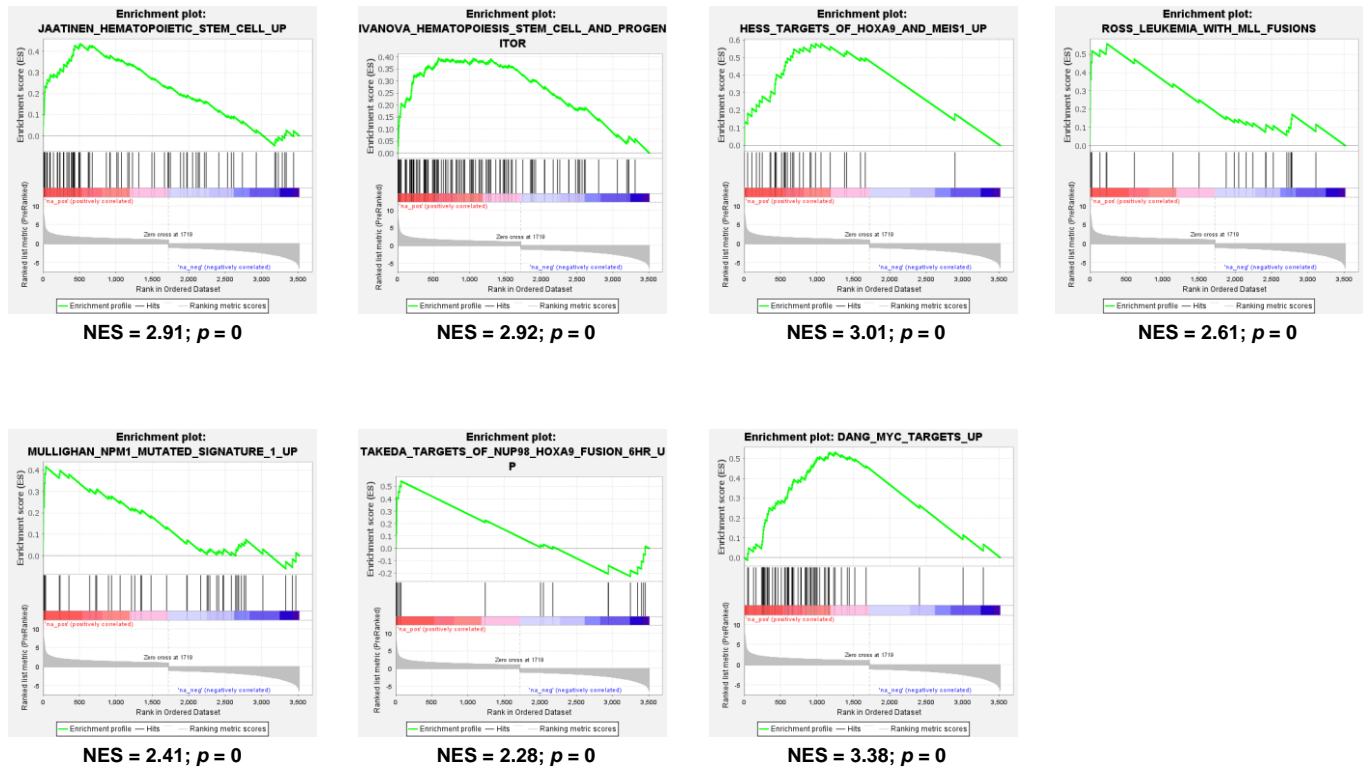


Figure S3, related to Figure 4.

(A) Top DNA motifs associated with SETBP1- and SETBP1(D/N)-bound peaks from ChIP-seq studies. The indicated motifs were found in 19.36% of SETBP1-bound peaks and 19.43% of SETBP1(D/N)-bound peaks respectively. (B) Top GO gene sets with negative enrichment from GSEA analysis of differentially expressed genes in mouse LSK cells induced by ectopic *Setbp1* and

Setbp1(D/N) expression. (C) GSEA analysis of differentially expressed genes in mouse LSK cells induced by *Setbp1(D/N)* expression using indicated gene sets.

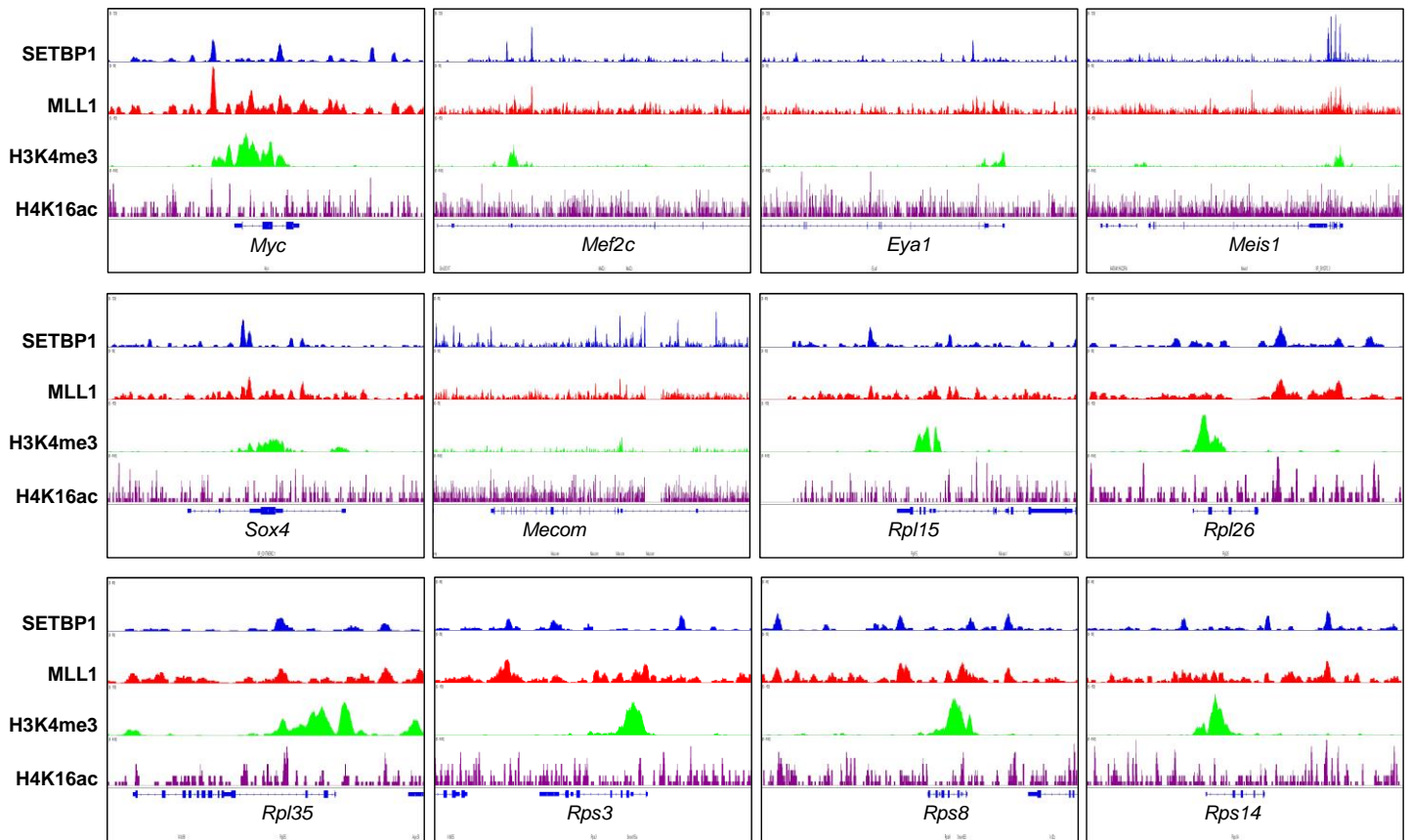
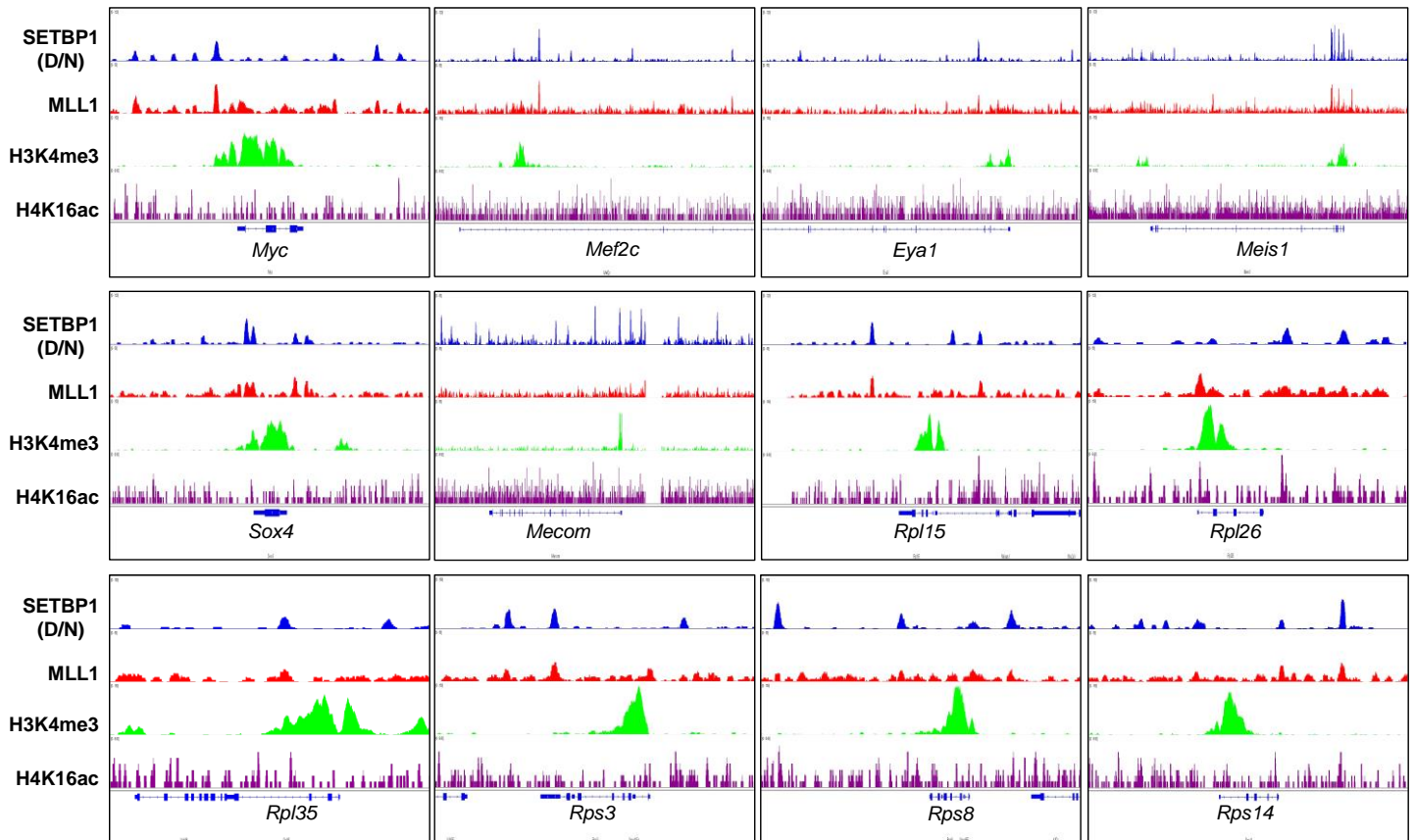
A**B**

Figure S4, related to Figure 4 and Figure 5.

Genome browser tracks showing co-localization of SETBP1 (A) or SETBP1(D/N) (B) with MLL1, H3K4me3, and H4K16ac at indicated loci of oncogenic transcription factor gene targets and ribosomal protein gene targets.

A

	Rpl141	✓	✓
	Rpl140	✓	✓
	Rpl139	✓	✓
	Rpl138	✓	✓
	Rpl137a		
	Rpl137		
	Rpl136a1	✓	✓
	Rpl136a	✓	✓
	Rpl136	✓	✓
	Rpl135a	✓	✓
	Rpl135	✓	✓
	Rpl134	✓	✓
	Rpl132	✓	✓
	Rpl131	✓	✓
	Rpl130		
	Rpl129		
	Rpl128		
	Rpl127a	✓	✓
	Rpl127	✓	✓
	Rpl126	✓	✓
	Rpl124		
	Rpl123a		
	Rpl123		
	Rpl122	✓	✓
	Rpl121		
	Rpl119	✓	✓
	Rpl118a	✓	✓
	Rpl118	✓	✓
	Rpl117		
	Rpl115	✓	✓
	Rpl114	✓	✓
	Rpl113a	✓	✓
	Rpl113	✓	✓
	Rpl112	✓	✓
	Rpl111	✓	✓
	Rpl10a	✓	✓
	Rpl10		
	Rpl9		
	Rpl8		
	Rpl7a		
	Rpl711		
	Rpl7		
	Rpl6		
	Rpl5	✓	✓
	Rpl4		
	Rplp2	✓	✓
	Rplp1	✓	✓
	Rplp0		

	Rps30		
	Rps29	✓	✓
	Rps28		
	Rps271	✓	✓
	Rps27a	✓	✓
	Rps27		
	Rps26	✓	✓
	Rps25		
	Rps24		
	Rps23	✓	✓
	Rps21		
	Rps20		
	Rps19		
	Rps18	✓	✓
	Rps17		
	Rps16		
	Rps15a		
	Rps15	✓	✓
	Rps14	✓	✓
	Rps13		
	Rps12	✓	✓
	Rps11		
	Rps10		
	Rps9		
	Rps8	✓	✓
	Rps7		
	Rps6		
	Rps5	✓	✓
	Rps4x		
	Rps3a		
	Rps3		
	Rps2	✓	✓
	Rpsa		

B

	Rpl141	✓	✓
	Rpl140	✓	✓
	Rpl139	✓	✓
	Rpl138	✓	✓
	Rpl137a	✓	✓
	Rpl137		
	Rpl136a1	✓	✓
	Rpl136a	✓	✓
	Rpl136	✓	✓
	Rpl135a	✓	✓
	Rpl135	✓	✓
	Rpl134	✓	✓
	Rpl132	✓	✓
	Rpl131	✓	✓
	Rpl130		
	Rpl129	✓	✓
	Rpl128	✓	✓
	Rpl127a	✓	✓
	Rpl127	✓	✓
	Rpl126	✓	✓
	Rpl124	✓	✓
	Rpl123a	✓	✓
	Rpl123	✓	✓
	Rpl122	✓	✓
	Rpl121	✓	✓
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	Rpl113a	✓	✓
	Rpl113	✓	✓
	Rpl112	✓	✓
	Rpl111	✓	✓
	Rpl10a	✓	✓
	Rpl10		
	Rpl9		
	Rpl8	✓	✓
	Rpl7a		
	Rpl711		
	Rpl7	✓	✓
	Rpl6		
	Rpl5	✓	✓
	Rpl4	✓	✓
	Rplp2	✓	✓
	Rplp1	✓	✓
	Rplp0		

	Rps30		
	Rps29	✓	✓
	Rps28		
	Rps271	✓	✓
	Rps27a	✓	✓
	Rps27	✓	✓
	Rps26	✓	✓
	Rps25	✓	✓
	Rps24	✓	✓
	Rps23	✓	✓
	Rps21	✓	✓
	Rps20	✓	✓
	Rps19	✓	✓
	Rps18	✓	✓
	Rps17	✓	✓
	Rps16		
	Rps15a	✓	✓
	Rps15	✓	✓
	Rps14	✓	✓
	Rps13		
	Rps12	✓	✓
	Rps11		
	Rps10		
	Rps9		
	Rps8	✓	✓
	Rps7		
	Rps6	✓	✓
	Rps5	✓	✓
	Rps4x		
	Rps3a1	✓	✓
	Rps3	✓	✓
	Rps2	✓	✓
	Rpsa		

Figure S5, related to Figure 4.

Direct activation of RPGs by SETBP1 (A) and SETBP1(D/N) (B) in LSK cells. Large (upper panels) and small (lower panels) ribosomal subunit genes are listed. Genes showing expression increase greater than 2-fold after *Setbp1* or *Setbp1(D/N)* expression in mouse LSK cells and binding by SETBP1 or SETBP1(D/N) in the same cells are indicated by “✓”.

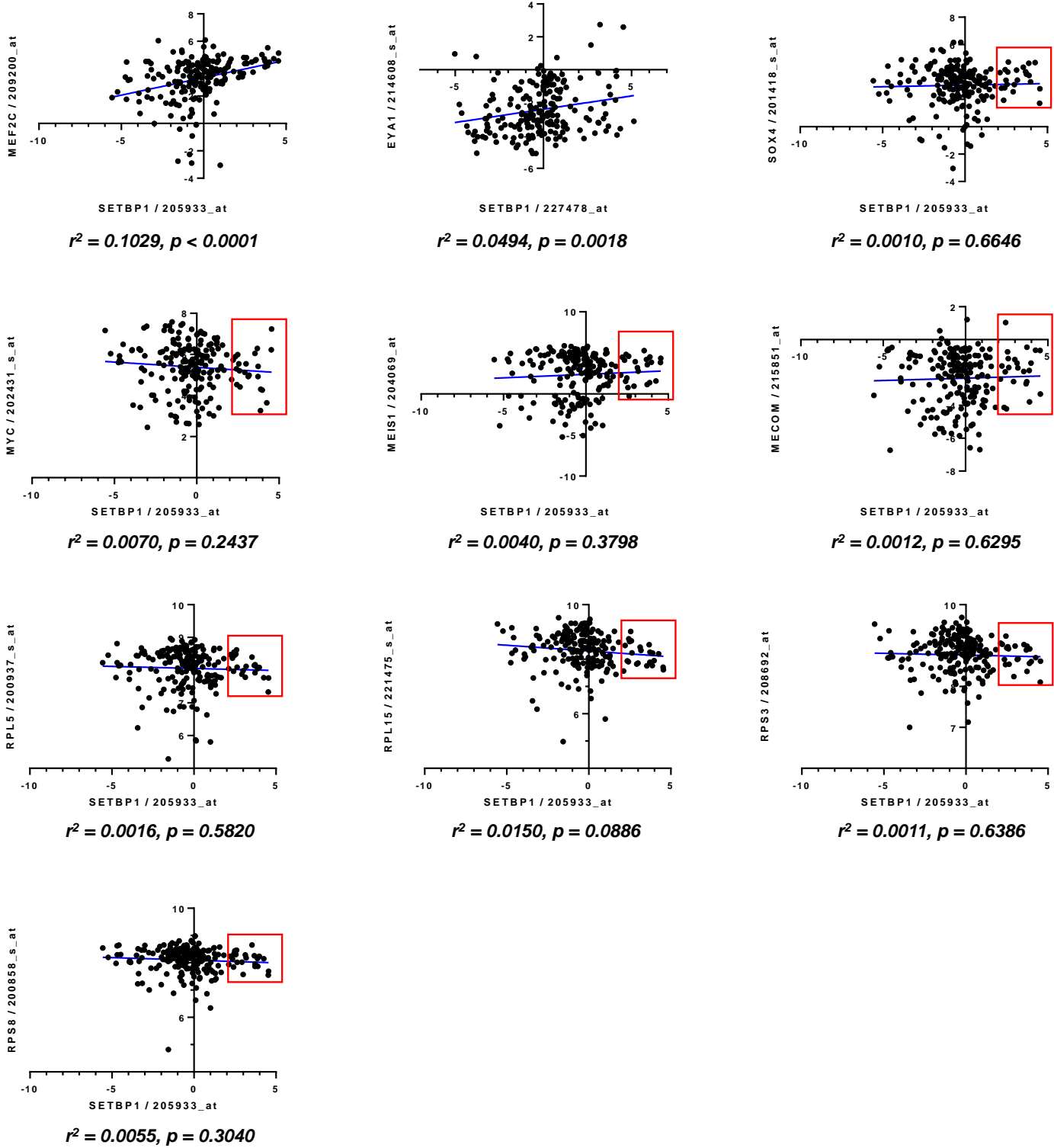


Figure S6, related to Figure 4.

Correlation between SETBP1 expression and the expression of its targets in human AMLs. Normalized TCGA microarray data was obtained from the OncoPrint database (www.oncoPrint.org) and analyzed. Samples with high SETBP1 expression are circled with red boxes. Note that most samples with high SETBP1 expression express relatively high levels of the identified target genes. The correlation between expression levels was calculated from linear regression.

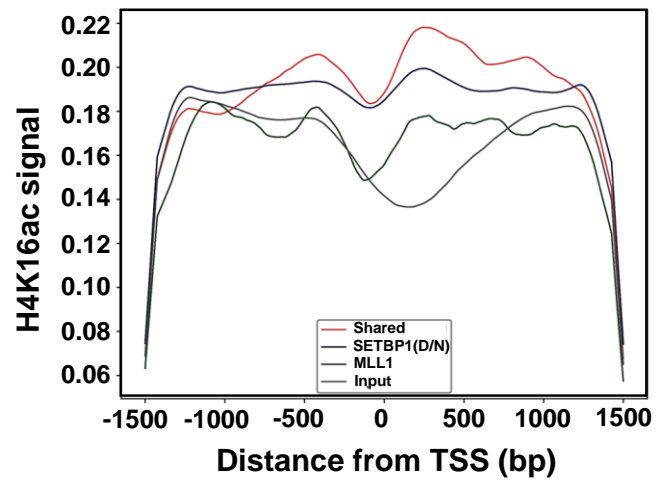
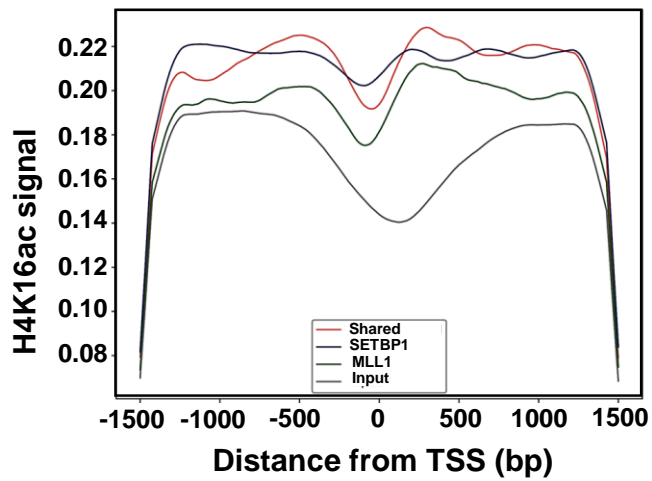


Figure S7, related to Figure 5.

Average binding profiles of H4K16ac at promoter regions of SETBP1-bound, MLL1-bound, and SETBP1/MLL1 co-bound targets in *Setbp1*-expressing LSK cells (left) and SETBP1(D/N)-bound, MLL1-bound, and SETBP1(D/N)/MLL1 co-bound targets in *Setbp1(D/N)*-expressing cells (right).

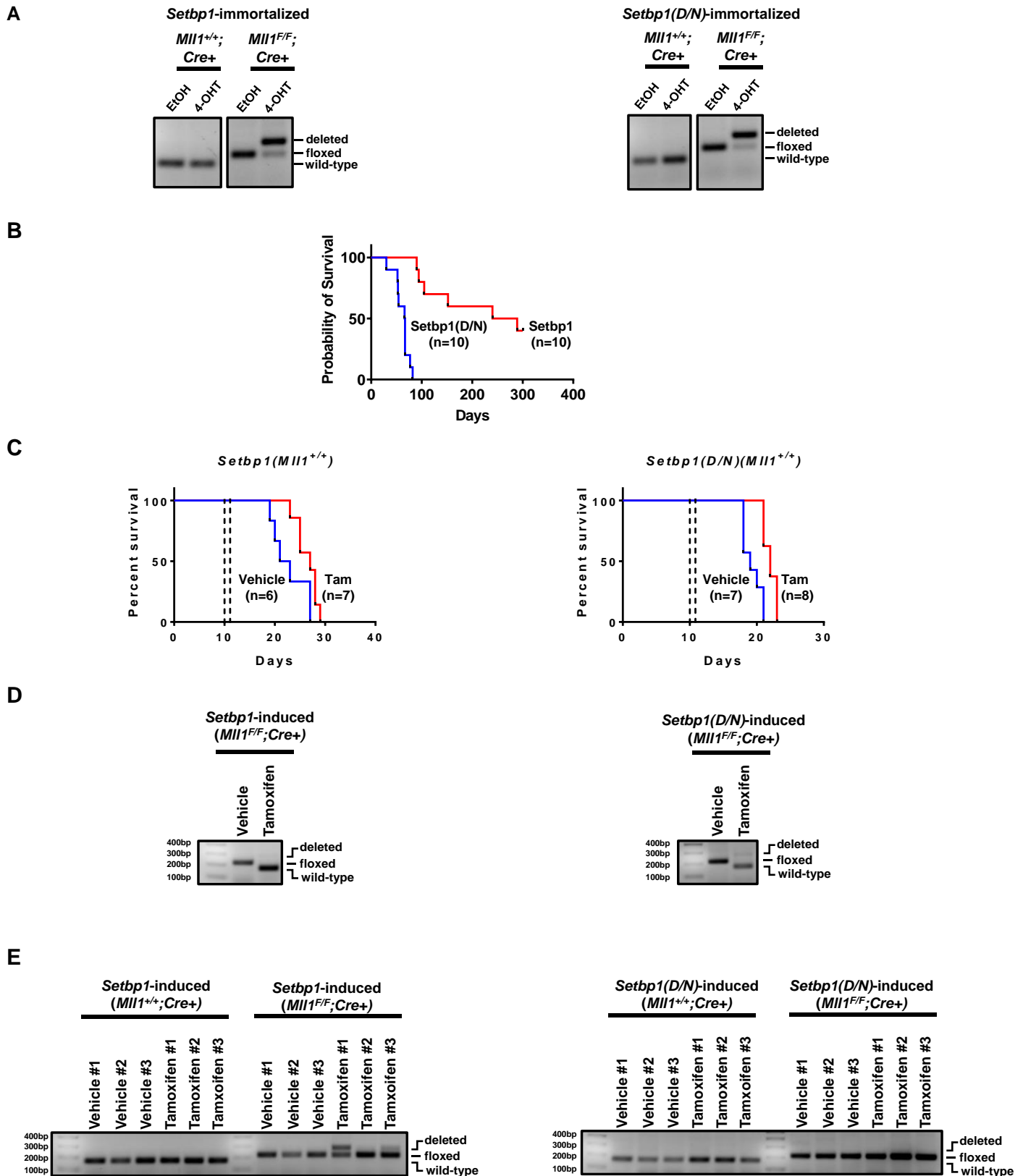


Figure S8, related to Figure 6.

(A) Efficient *Mll1* deletion induced by 4-OHT treatment in *Setbp1*- and *Setbp1(D/N)*-immortalized cells. Genotyping results are shown for *Setbp1*-immortalized (A) and *Setbp1(D/N)*-immortalized

Mll1^{+/+};*Cre*⁺ and *Mll1*^{F/F};*Cre*⁺ cells at 24 hours after treatment with 100 nM 4-OHT or control ethanol (EtOH). (B) Survival curves of mice transplanted with *Mll1*^{F/F} or *Mll1*^{+/+} cells transduced with either *Setbp1* or *Setbp1(D/N)* virus. Note that the latency and penetrance of leukemia development are in line with our previous studies using these viruses (Vishwakarma et al., 2016 and Nguyen et al., 2016). (C) Survival curves of mice receiving 5 x10⁵ *Setbp1*-induced (left panel) or *Setbp1(D/N)*-induced (right panel) *Mll1*^{+/+};*Cre*⁺ AML cells treated with Tamoxifen or control corn oil. Tamoxifen or corn oil treatments are indicated by dotted lines. (D) Genotyping results of bone marrow cells from mice receiving *Setbp1*-induced (left) or *Setbp1(D/N)*-induced (right) *Mll1*^{F/F};*Cre*⁺ AML cells at 10 days after injections of Tamoxifen or corn oil (Vehicle). The loss of floxed allele and the presence of weak deleted allele in mice treated with Tamoxifen suggest efficient *Mll1* deletion in AML cells and the subsequent significant reduction in their number. (E) Genotyping results of bone marrow cells from moribund mice receiving *Setbp1*-induced (left) or *Setbp1(D/N)*-induced (right) *Mll1*^{F/F};*Cre*⁺ AML cells and injections of Tamoxifen or corn oil.

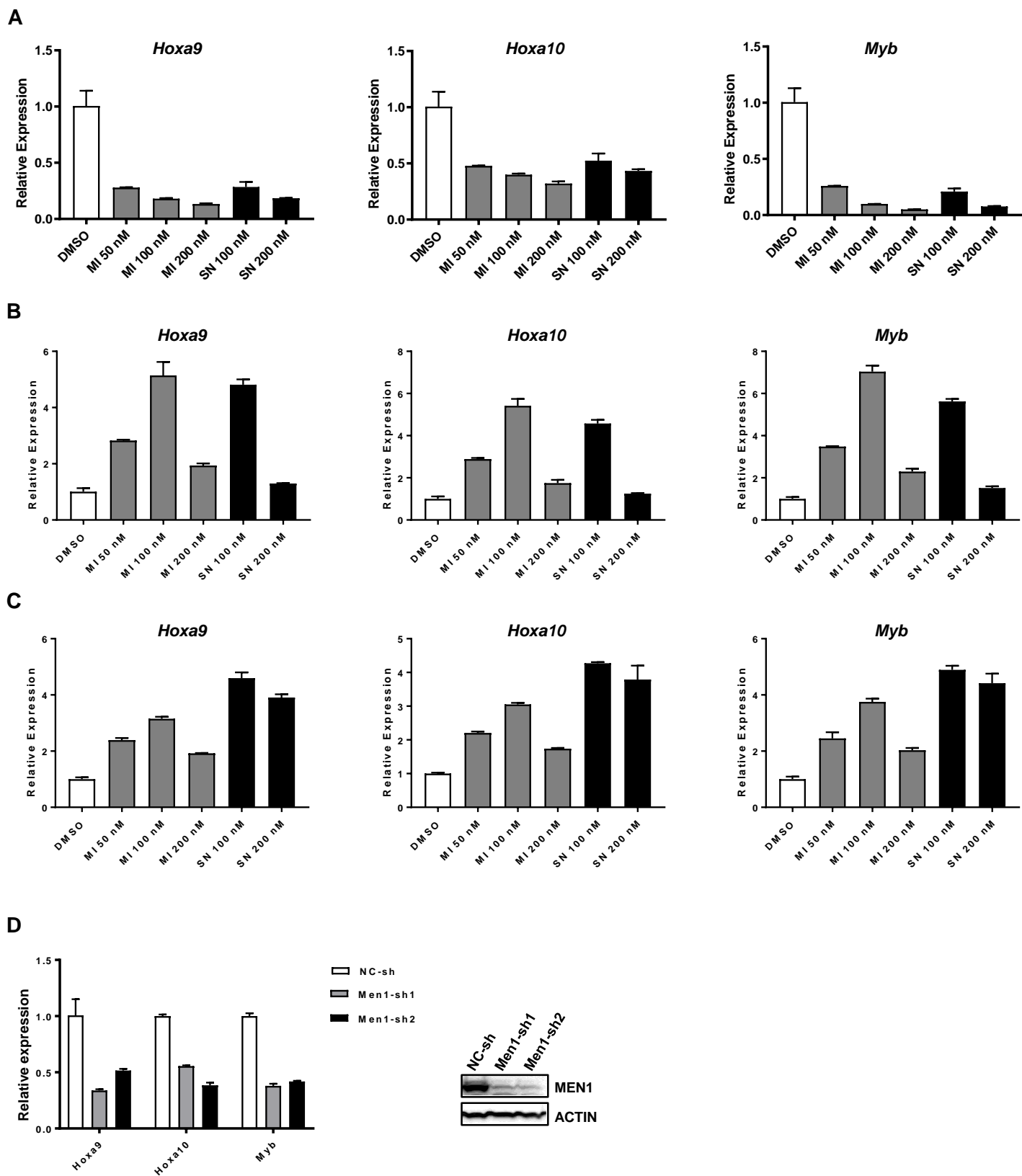


Figure S9, related to Figure 7.

Transcriptional activation induced by Setbp1/Setbp1(D/N) is resistant to MENIN inhibitors. (A) Real-time RT-PCR analysis of indicated *MLL/AF9* targets in *MLL/AF9*-induced mouse AML cells at 3 days

after treatment with MI-3454 (MI) or SNDX-5613 (SN) at indicated concentration or control DMSO. Data are represented as mean \pm SD (n=3). (B) Real-time RT-PCR analysis of indicated SETBP1/MLL1 co-bound targets in *Setbp1*-induced AML cells at 6 days after treatment with MI-3454 (MI) or SNDX-5613 (SN) at indicated concentration or control DMSO. Fresh drug was added after 3 days. Data are represented as mean \pm SD (n=3). (C) Real-time RT-PCR analysis of same genes in *Setbp1(D/N)*-induced AML cells at 6 days after same treatments as in (B). Data are represented as mean \pm SD (n=3). (D) Left panel, real-time RT-PCR analysis of relative *Hoxa9/Hoxa10/Myb* mRNA levels in *Setbp1(D/N)*-induced AML cells after transduction with *Men1*-specific shRNAs (*Men1-sh1* and *-sh2*) or a non-targeting control shRNA (NC-sh). Data are represented as mean \pm SD (n=3). Right panel, Western blotting analysis of the same transduced cells using indicated antibodies.