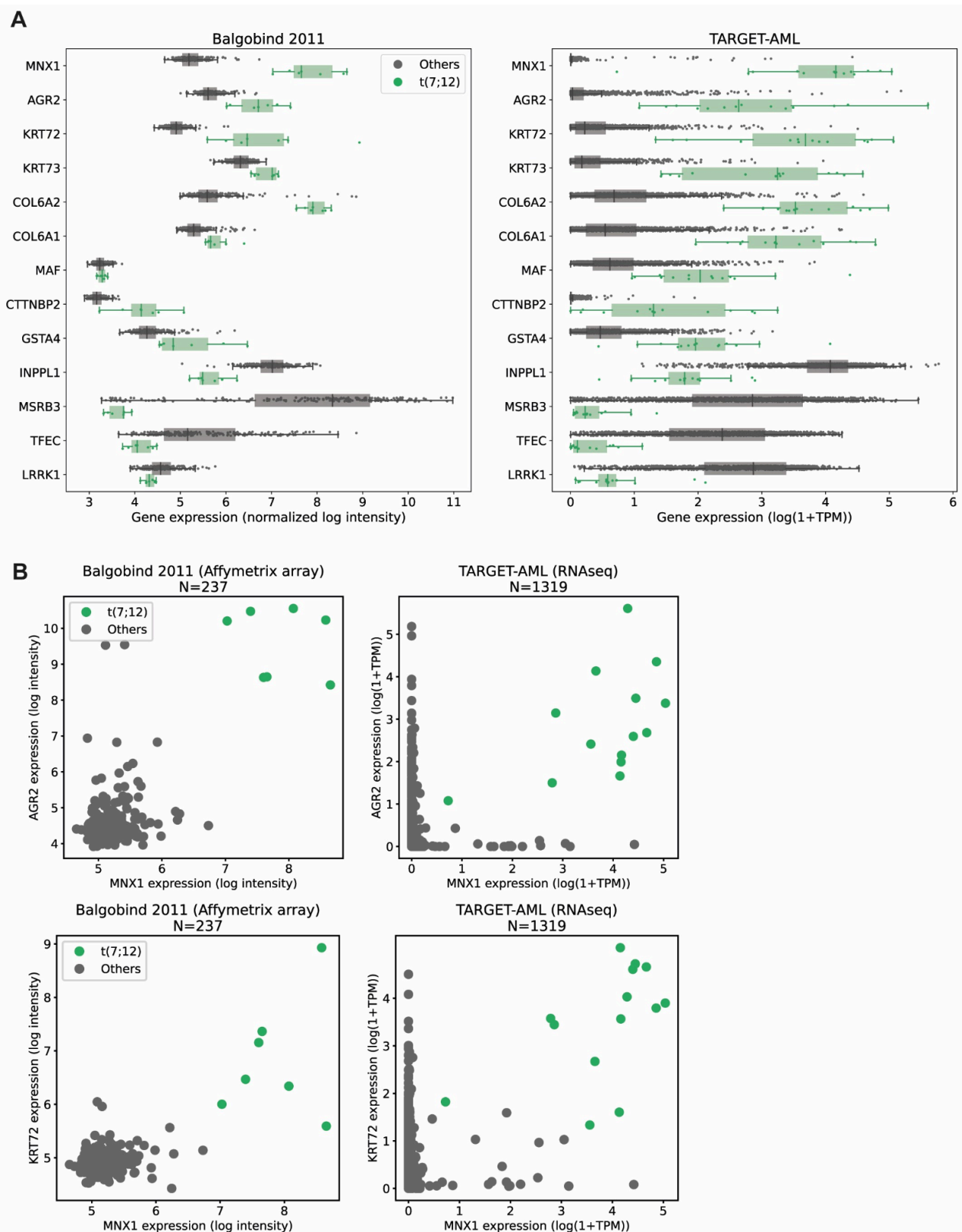


Supplemental Information to

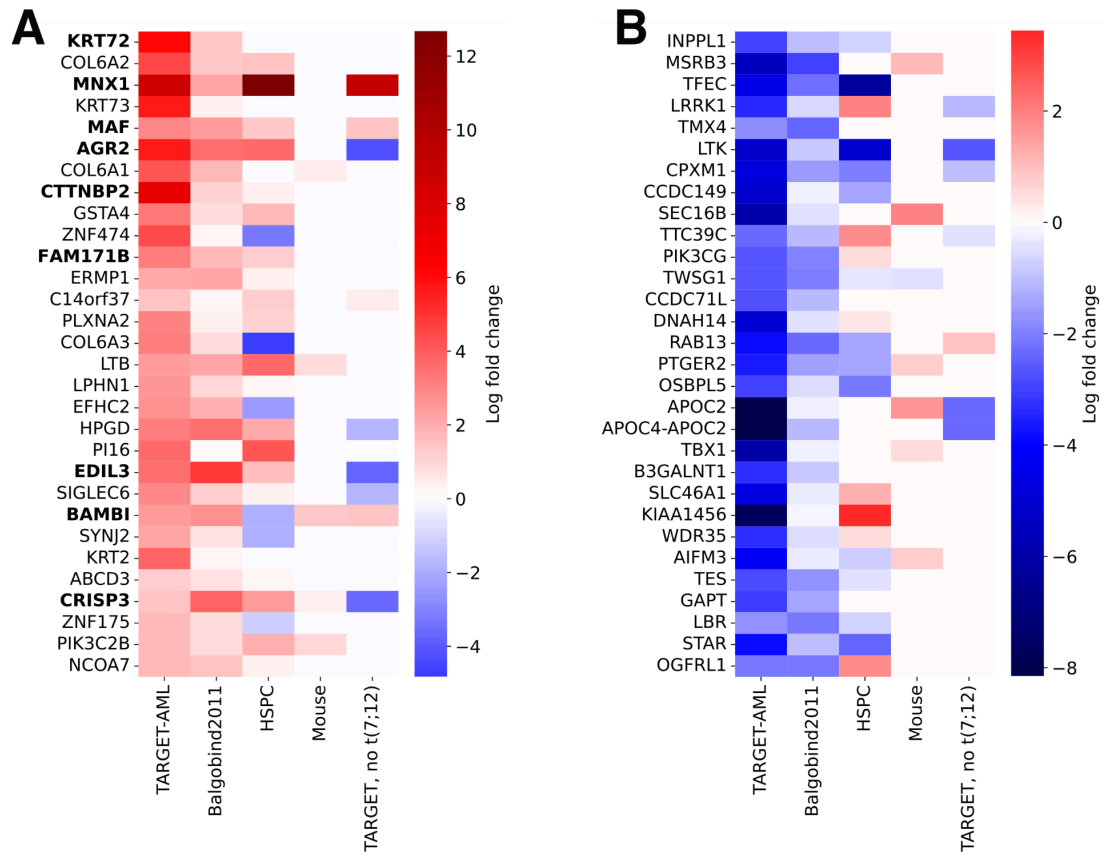
**Altered enhancer-promoter interaction leads to *MNX1* expression
in pediatric acute myeloid leukemia with t(7;12)(q36;p13)**

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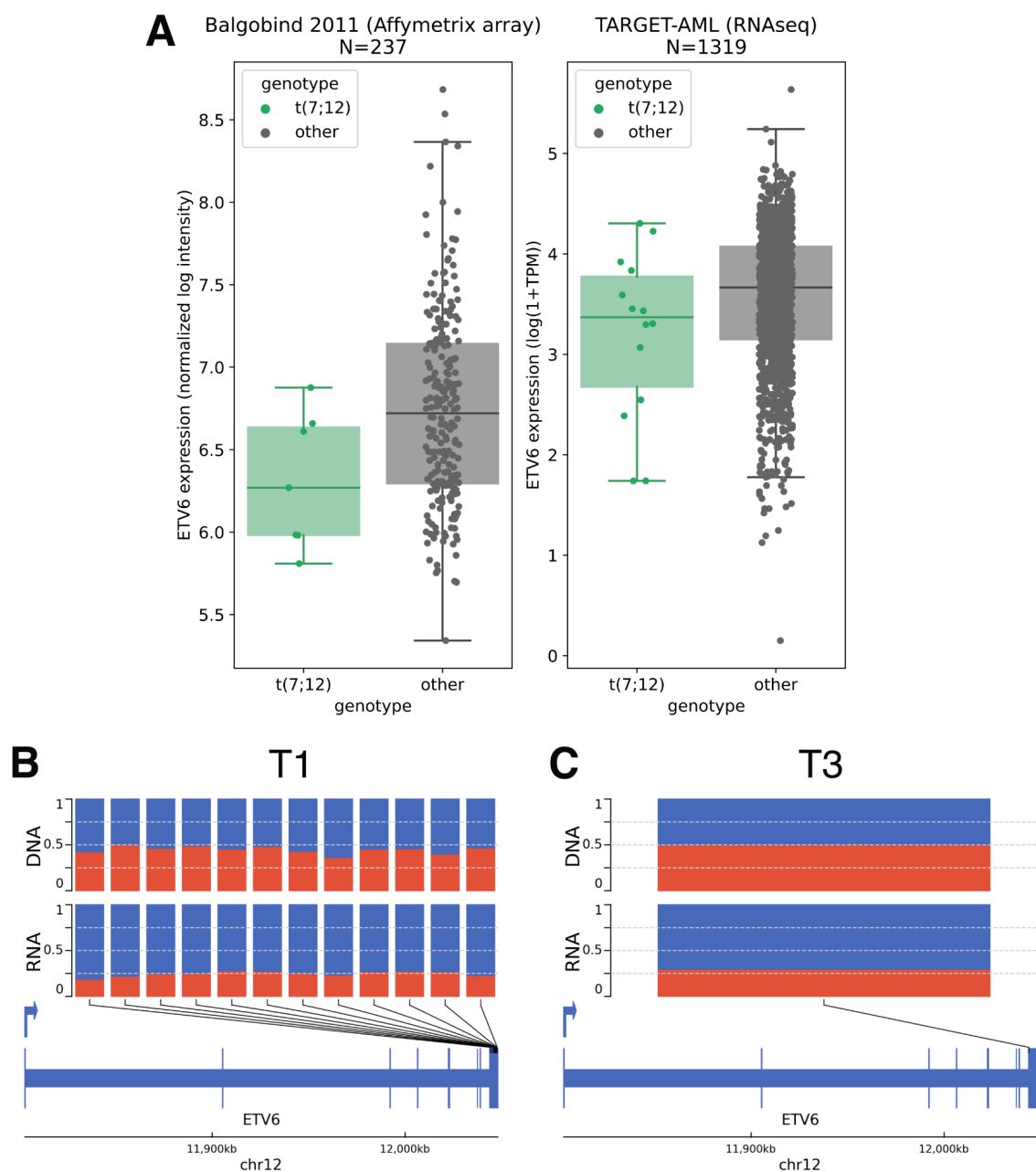
Supplemental Figure 1: Differentially expressed genes in AML with or without t(7;12)(q36;p13)

A, Boxplots for the expression levels (normalized log intensity or log(1+TPM)) of the most differentially expressed genes between t(7;12) AML and all other samples, in the Balgobind and TARGET-AML cohorts. **B**, Scatter plots of the joint expression of *MNX1* and *AGR2*, or of *MNX1* and *KRT72*.



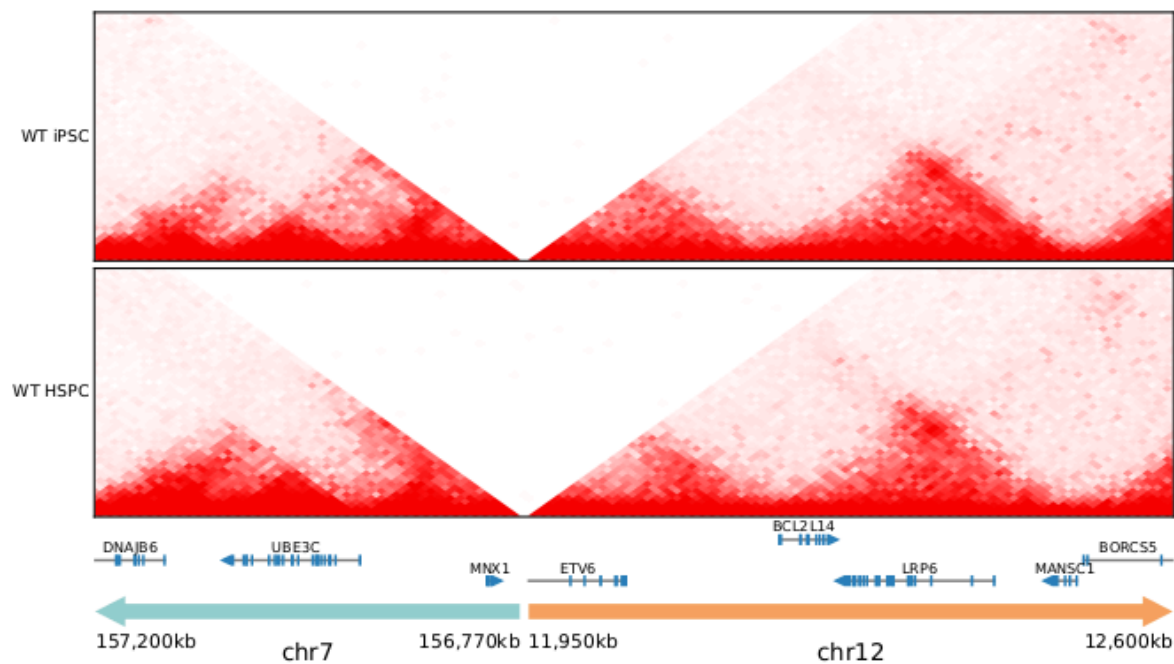
Supplemental Figure 2: Recapitulation of the t(7;12) gene signatures by different experimental models.

Heatmap showing the genes upregulated (**A**) or downregulated (**B**) in t(7;12) AML, where the genes identified by Balgobind et al. 2011 were highlighted in bold. The two first columns show the log-fold change of expression between t(7;12) and wt samples of the TARGET-AML and Balgobind cohorts. The third column shows the log fold change of expression between the t(7;12) HSPCs and the wt HSPCs in the engineered t(7;12) model from Nilsson et al. 2022. The fourth column represents the log fold change of expression between the MNX1-overexpressing and the WT mouse HSPCs from the study of Waraky et al. 2023. The fifth column represents the log fold change between *MNX1*-expressing samples vs other samples, when excluding all samples with a t(7;12) translocation.



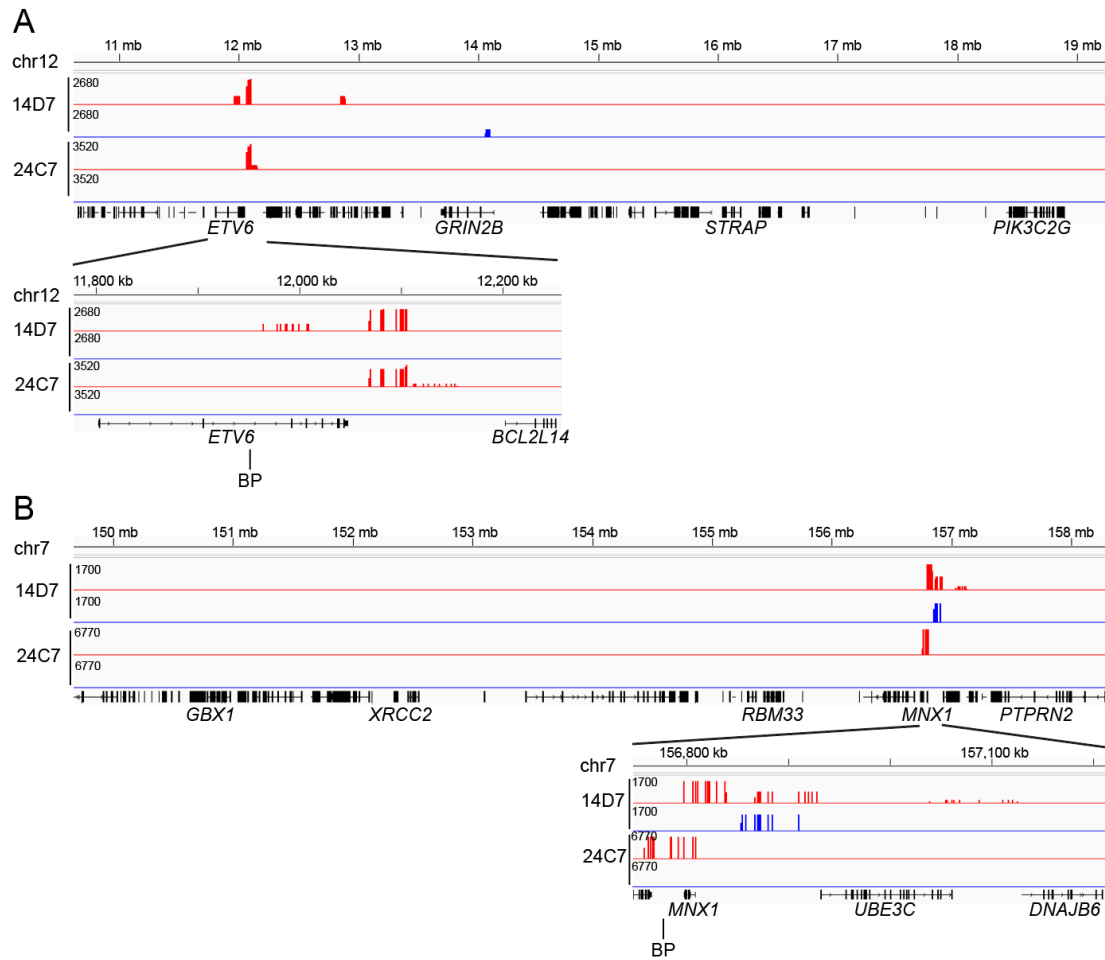
Supplemental Figure 3: *ETV6* haploinsufficiency in t(7;12) samples.

A, Boxplots showing *ETV6* expression in the Balgobind 2011 and TARGET-AML cohorts, separated between t(7;12) samples and others. **B**, **C**, Allele-specific expression of *ETV6* in T1 (**B**) and T3 (**C**). The minor allele frequency in DNA and RNA is shown for each heterozygous exonic SNP in *ETV6*.

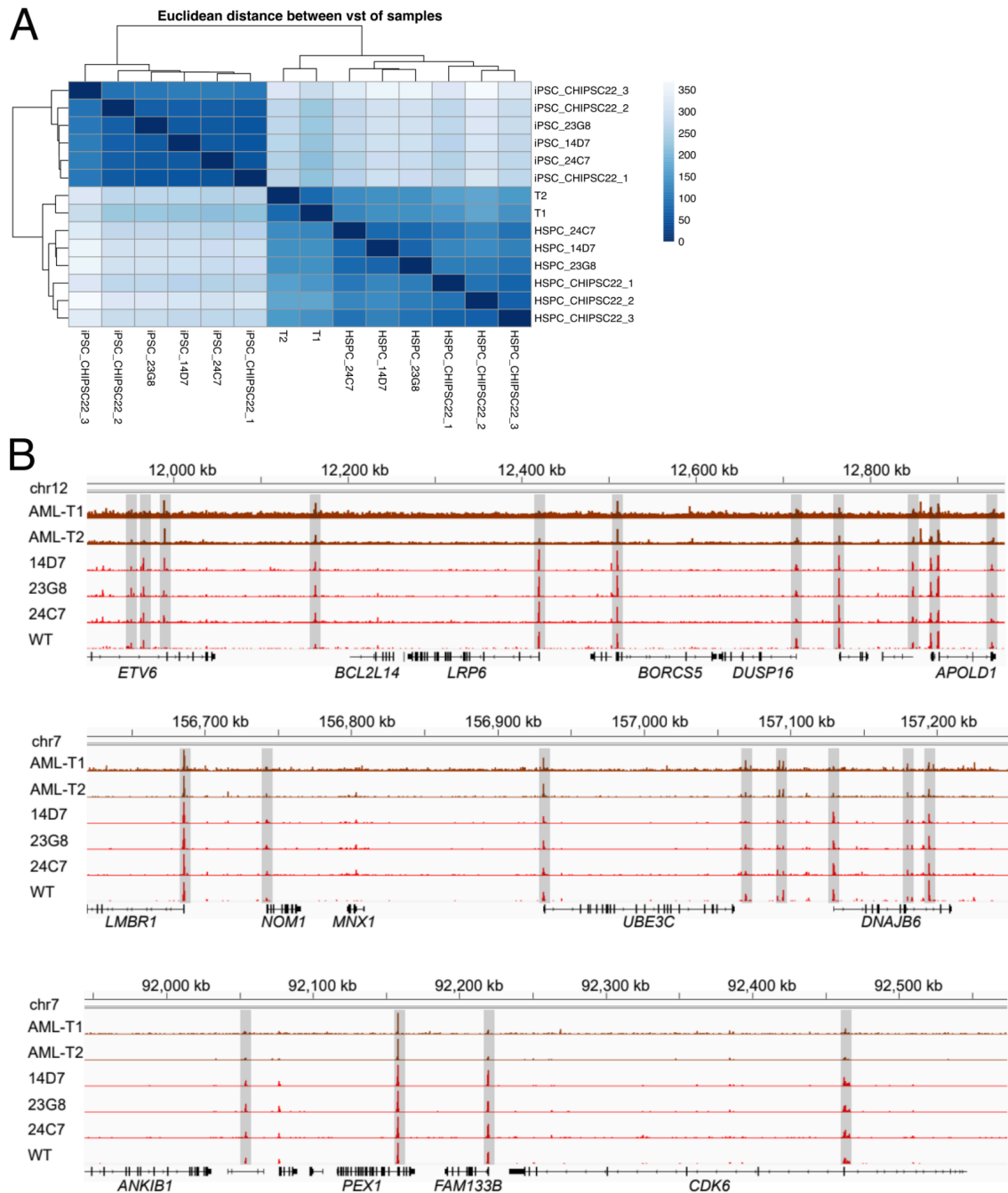


Supplemental Figure 4: Interactions between *ETV6* region and *MNX1* promoter in ChiPSC22^{WT} cells.

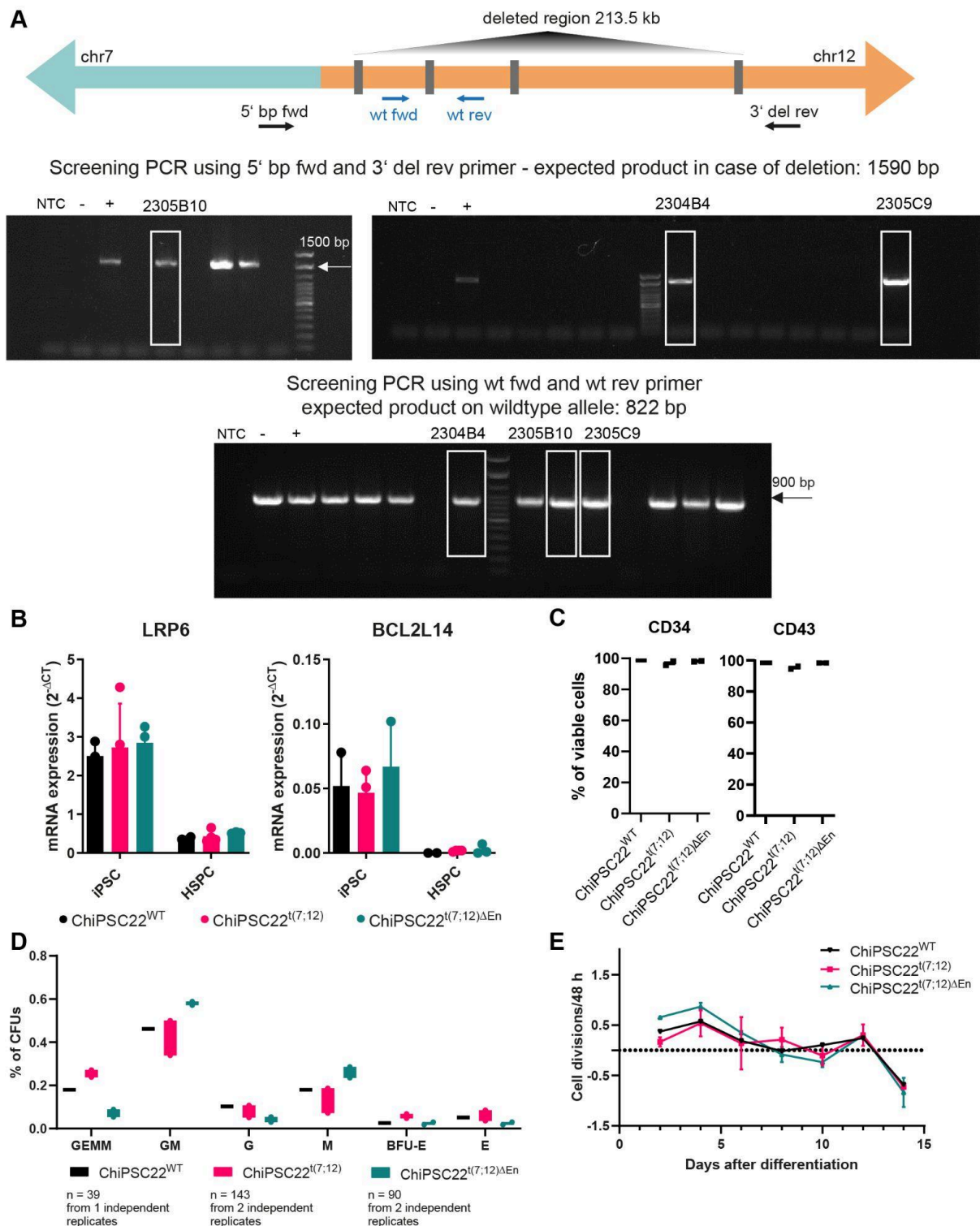
Chromatin interactions analyzed by Hi-C seq in the genomic region flanking the translocation breakpoint in the ChiPSC22^{WT}, either as iPSCs (top) or HSPCs (bottom), visualized with figeno (<https://github.com/CompEpigen/figeno>).



Supplemental Figure 5: Interaction between *MNX1* and the *ETV6* region indicated by reciprocal 4C-data with an *MNX1* viewpoint (A) and an *ETV6* viewpoint (B) of the two ChiPSC22^{t(7;12)} sublines 14D7 and 24C7. Zoom-in views with higher resolution are shown below the respective extended chromosomal regions of about 8 Mb. Red tracks: HSPC, blue tracks: iPSC; numbers in tracks indicate pairwise read numbers in bins; BP: breakpoint.



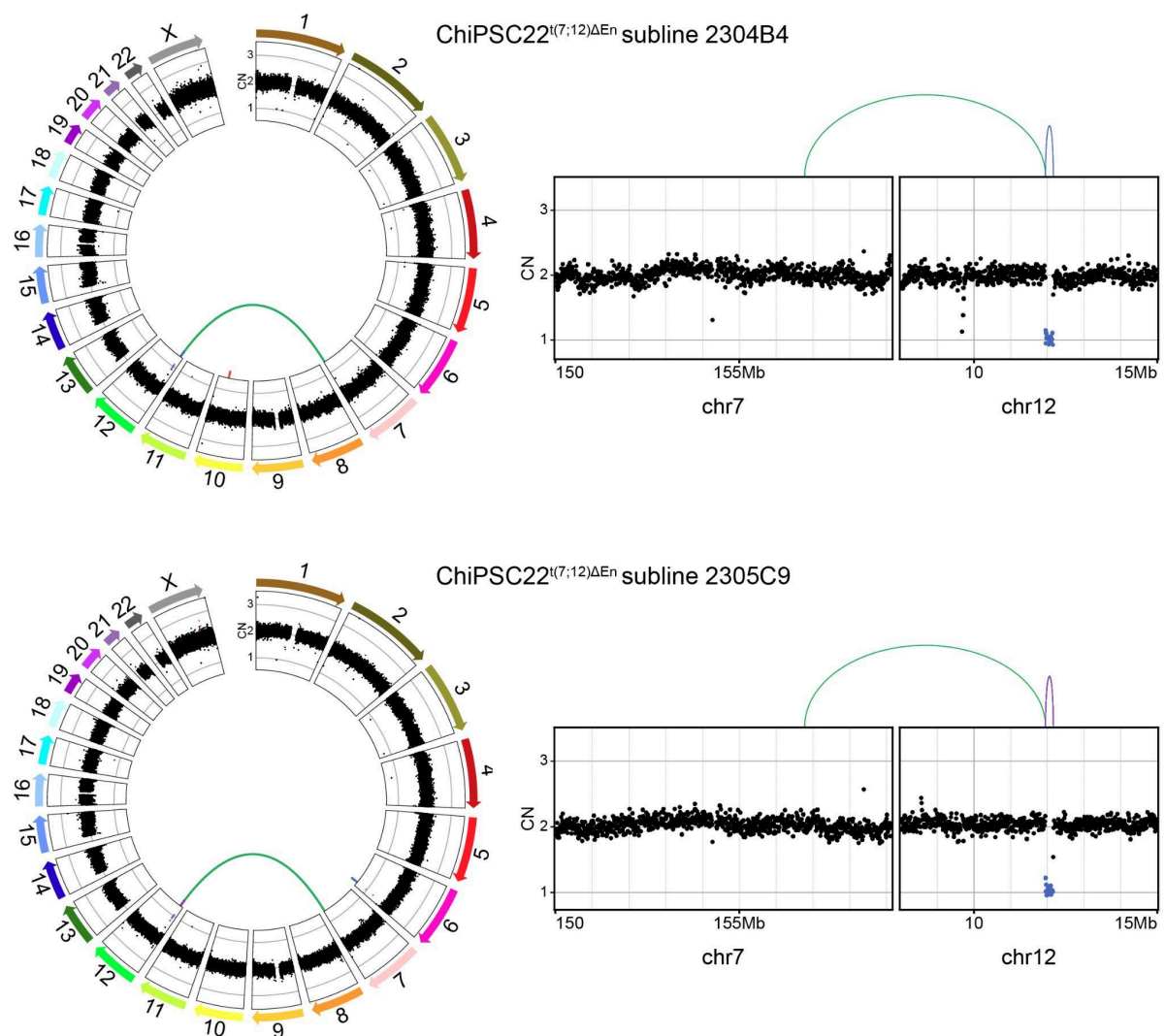
Supplemental Figure 6: Similarity between the open chromatin (ATAC) profiles of AML patients and HSPCs. T1 and T2 are patient samples and 14D7, 23G8 and 24C7 are three ChiPSC22 sublines with t(7;12). **A.** Heatmap of sample distances, based on normalized read counts (vst transformation) of the 10,000 most variable peaks in the ATAC-seq data. **B.** ATAC-seq tracks in the *ETV6*, *MNX1* and *CDK6* regions. Common peak positions are highlighted by a gray shading.



Supplemental Figure 7: Validation of enhancer deletion and clone characteristics

A, Schematic showing the location of the screening primers used to verify the presence of the deletion on the translocated allele and the presence of a wildtype *ETV6* region. Upper PCR results in 1590 bp product if enhancer deletion is present on the translocated allele and lower PCR results in 822 bp product if wildtype *ETV6* locus is present. Cell lines used in the study are highlighted in white box and showed PCR products in both PCRs. **B**, Gene expression in HSPCs derived from ChiPSC22^{WT} (n=2), ChiPSC22^(7;12) (n=2, from 2 independent sublines) and ChiPSC22^{(7;12)ΔEn} (n=3, from 2 independent cell lines) measured via qRT-PCR and shown as $2^{-\Delta Ct}$ vs. *GUSB* as endogenous reference. **C**, Percentage of

CD34⁺ and CD43⁺ cells for ChiPSC22^{WT}, ChiPSC22^{t(7;12)} and ChiPSC22^{t(7;12) Δ En} determined by flow cytometry. All cell lines (ChiPSC22^{WT}, ChiPSC22^{t(7;12)} sublines 14D7 and 24C7 and ChiPSC22^{t(7;12) Δ En} sublines 2304B4 and 2305B10) were analyzed after one round of differentiation. **D**, Colony-forming unit (CFU) assay using 10,000 or 5,000 HSPCs derived from ChiPSC22^{WT}, ChiPSC22^{t(7;12)} sublines 14D7 and 24C7 and ChiPSC22^{t(7;12) Δ En} sublines 2304B4 and 2305B10. The total number of colonies per group was n = 39 for ChiPSC22^{WT}, n = 143 for ChiPSC22^{t(7;12)} and n = 90 for ChiPSC22^{t(7;12) Δ En}. GEMM: granulocyte, erythrocyte, macrophage, megakaryocyte, GM: granulocyte, macrophage, G: granulocyte, M: macrophage, BFU-E: burst-forming unit-erythroid, E: erythroid. **E**, Proliferation capacity of HSPCs derived from ChiPSC22^{WT}, ChiPSC22^{t(7;12)} sublines 14D7 and 24C7 and ChiPSC22^{t(7;12) Δ En} sublines 2304B4 and 2305B10 was analyzed by continuous culture for 14 days..



Supplemental Figure 8: Whole genome sequencing of enhancer deletion cell lines

Circos plot (left) showing the genome-wide rearrangements observed using WGS between ChiPSC22^{t(7;12) Δ En} sublines 2304B4 and 2305C9 and ChiPSC22^{WT} and rearrangements detected by WGS (right), zoomed in around the t(7;12) breakpoint.

Supplemental Table 1: Pediatric AML expressing *MX1*

Sample ID	Cohort	Available cytogenetic information	Age at diagnosis [month]
T1	Germany	47,XY,t(7;12)(q36;p13),+19[17]/47,idem,t(1;15)(p34;q21)[3]/48,idem,+8[2]. ish der(7)t(7;12)(5'TEL+),der(12)t(7;12)(5'TEL-)[19/19]. nuc ish 12p13(TELx2)(5'TEL sep 3'TELx1)[93/100]	4 months
T2	Germany	47,XY,t(7;12)(q36;p13),+19[20]	15 months
T3	Germany	47,XX,t(7;12)(q36;p13),+19[14]/46,XX[1]	7 months
T4*	Sweden	47,XY,t(7;12)(q36;p13),+19[13]/46,XY[2]	6 months
TARGET-20-PARUNX	TARGET-AML	48,XX,+der(6)t(1;6)(q21;q27),t(7;12)(q36;p13),+19[18] [t(7;12) nuc ish ETV6 sep]	77 months
TARGET-20-PASIBG	TARGET-AML	47,XY,t(7;12)(q36;p13),+19[20]	25 months
TARGET-20-PAWUTL	TARGET-AML	49,XX,+6,del(7)(q22),add(12)(p13.3),+19,+22[19]/46,XX[1]	10 months
TARGET-20-PAVXZL	TARGET-AML	47,XY,t(7;12)(q36;p13),+19[17]/47,idem,dup(2)(q13q33)[6]/47,idem,del(11)(q14q25)[2]	11 months
TARGET-20-PAXMPG	TARGET-AML	47,XX,?add(7)(q32), cryp ins(7;12)(q36;p13.2p13.2),+19[20]	11 months
TARGET-20-PAXHGR	TARGET-AML	47,XX,t(7;12)(q36;p13),+19[20]	5 months
TARGET-20-PAUYCA	TARGET-AML	46,XY[20]	176 months
TARGET-20-PAWNHH	TARGET-AML	47,XX,t(7;12)(q36;p13),+19[20]	17 months
TARGET-20-PAVCJB	TARGET-AML	51,XY,+6,t(7;12;14)(q36;p13;q32.1),+8,+15,+19,+21[20]	8 months
TARGET-20-PAXEWS	TARGET-AML	47,XY,t(7;12)(q36;p13),+19[20]	6 months
TARGET-20-PAVXPB	TARGET-AML	49,XY,+6,t(7;12)(q36;p13),+16,+19[20]	24 months
TARGET-20-PAWBTJ	TARGET-AML	46,XX,der(7)del(7)(q22q36)t(7;12)(q36;p13.3),der(12)t(7;12)(q36;p13.3)[20]	9 months
TARGET-20-PAVFDW	TARGET-AML	46,XX[20]	209 months
TARGET-20-PAVAAM	TARGET-AML	46,XX,t(6;11)(q27;q23)[20]	56 months
TARGET-20-PAWNYK	TARGET-AML	46,XX,t(7;12)(q36;p13)[7]/46,XX[13]	20 months
TARGET-20-PAVNGY	TARGET-AML	46,XY[20]	161 months
TARGET-20-PAWYKA	TARGET-AML	46,XY[30]	102 months
TARGET-20-PAVDGM	TARGET-AML	46,XY[29]	199 months
TARGET-20-PAWMLK	TARGET-AML	46,XX[60]	194 months

TARGET-20-PAUYZY	TARGET-AML	46,XY,del(11)(q21q24),der(11)ins(11;11)(p15;q21q23)[11]/46,XX[9]	15 months
TARGET-21-PASVJS	TARGET-AML	46,XX,[20]	63 months
TARGET-20-PAUSBP	TARGET-AML	46,XY,t(6;11)(q27;q23)[18]/46,XY[2]	140 months
TARGET-20-PAXKAL	TARGET-AML	46,XY,t(6;17)(q21;p13),der(7)t(7;7)(q36;p13),add(9)(p13),add(18)(q23)[cp3]/46,sl,del(1)(q32)[2]/46,sl,der(7)t(?4;7)(q21;q?32)[3]/46,sdl2,der(7)del(7)(p13)t(7;7)(q36;p13),add(19)(q13.3)[9]/90,sdl3x2,-X,-21[cp2]	176 months
gsm446011**	Balgobind 2011	t(7;12)	<18 months
gsm445924**	Balgobind 2011	t(7;12)	<18 months
gsm446153**	Balgobind 2011	t(7;12)	<18 months
gsm445951**	Balgobind 2011	t(7;12)	<18 months
gsm445920**	Balgobind 2011	t(7;12)	<18 months
gsm446155**	Balgobind 2011	t(7;12)	<18 months
gsm446154**	Balgobind 2011	t(7;12)	<18 months

*qRT-PCR resulting in 4-fold higher *MNX1* than *ABL1* expression with assays Hs00907365 (*MNX1*, $C_t = 24.4$) and Hs01104728 (*ABL1*, $C_t = 26.4$, reference).

**Microarray expression analysis

All other samples analyzed by RNAseq, and the first six samples analyzed by WGS.

Supplemental Table 2: Known AML driver genes

ASXL1
ASXL2
BCOR
CEBPA
CEBPG
CREBBP
DNMT3A
DNMT3B
ETV6
EZH2
FLT3
GATA2
IDH1
IDH2
JAK2
JARID2
KAT6A
KDM3B
KDM6A
KIT
KMT2A
KMT2C
KMT2D
KMT2E
KRAS
MED12
NCOR1
NCOR2
NF1
NOTCH1
NOTCH2
NPM1
NRAS
NSD1
PHF6
PTPN11
RB1
RUNX1
SF3B1
SMARCA2
SMARCA4
SMC1A
SMC3
SRSF2
STAG2
SUZ12
TET1
TET2
TP53
U2AF1
WT1
ZRSR2

Supplemental Table 3: TaqMan (Applied Biosystems) gene expression assays

Gene name	Assay ID
<i>MNX1</i>	Hs00907365_m1
<i>AGR2</i>	Hs00356521_m1
<i>MMP9</i>	Hs00957562_m1
<i>CRISP3</i>	Hs00195988_m1
<i>MAF</i>	Hs04185012_s1
<i>GUSB</i>	Hs99999908_m1
<i>ETV6</i>	Hs01045741_m1
<i>LRP6</i>	Hs00233945_m1
<i>BCL2L14</i>	Hs1030396_m1

Supplemental Table 4: Antibodies

Antigen	Source	Purpose	Immunogen	Manufacturer and product number	RRID	Amount or dilution used
β -actin	Mouse monoclonal	Western	β -actin	Santa Cruz sc-47778 HRP	AB_2714189	1:10000
MNX1	Rabbit polyclonal	Western	aa 6-43	Thermo Fisher PA-67195	AB_2662922	1:500
Rabbit immunoglobulins	Goat	Western	HRP-linked anti rabbit IgG	Cell Signaling 7074	AB_2099233	1:5000
Mouse immunoglobulins	Goat	Western	HRP-linked anti mouse IgG	Cell Signaling 7076	AB_330924	1:5000
H3K4me1	Rabbit polyclonal	ACT-seq	aa 1-100 (mono methyl K4)	Abcam ab8895	AB_306847	0.8 μ g
H3K27ac	Rabbit polyclonal	ACT-seq	aa 1-100 (acetyl K27)	Abcam ab4729	AB_2118291	0.8 μ g
H2B	Rabbit monoclonal	ACT-seq	Synthesized peptide from yeast H2B	Boster Biological Technology M30930	AB_2924769	0.8 μ g
IgG	Rabbit serum	ACT-seq	not applicable	Millipore PP64B	AB_97852	0.8 μ g
PE-Cy7 CD43	mouse	Immuno-phenotyping	CD43	BD Pharmingen 563522		
APC CD34	mouse	Immuno-phenotyping	CD34	BD 345804		
PE-Cy7 IgG1k	mouse	Isotype control	IgG1k	BD Pharmingen 557872		
APC IgG1k	mouse	Isotype control	IgG1k	BD Pharmingen 550854		

Supplemental Table 5: Primers used for 4C

Viewpoint region	Viewpoint primer ¹	Sequence ²	hg19 primer coordinate
<i>MNX1</i> 3 kb upstream	HindIII_4cMNX1_R1	GTCTCGTGGGCTCGGAGATG	chr7:156805780-156805801
		TGTATAAGAGACAG <u>GGCTAGG</u>	
	DpnII_4cMNX1_F1	<u>TGTACCTGAAAGCTT</u>	chr7:156806553-156806574
		TCGTCGGCAGCGTCAGATGT	
<i>ETV6</i> intron 2, 4 kb proximal to chr12-breakpoint	HindIII_4cETV6_F1	GTATAAGAGACAG <u>CCCTATGG</u>	chr12:11953871-11953894
		<u>CTGGAATGTAAAAGCTT</u>	
	DpnII_4cETV6_R1	TCGTCGGCAGCGTCAGATGT	chr12:11954291-11954315
		GTATAAGAGACAG <u>AAATGTTT</u>	
		<u>GCTGCAAACCTATTCTTG</u>	

¹Touchdown PCR with ten cycles from 63°C-58°C and 25 cycles at 58°C.

²Genome-specific sequences corresponding to hg19 coordinates are underlined; break point locations are chr7:156778230 and chr12:11950302.

Supplemental Table 6: Two-color FISH oligonucleotides

See separate file for Supplemental Table 6

Supplemental Table 7: PCR primers to confirm engineered deletion on chr12

Name	Sequence 5' to 3'	hg19 position
5' bp fwd	CTTCACTCTATGCCTCCCTACCTGG	chr7: 156778089-156778113
3' del rev	GCAAGTGCCTATGCAAACCA	chr12: 12164774-12164793
wt fwd	CCAGGTGCAAGGCTCTATCC	chr12: 11989035-11989054
wt rev	TTGAACAAGAATGGCGCTGC	chr12: 11989909-11989928
5' del crRNA	TCAGGCACAATCCTTTGTACTGG	chr12: 11951006-11951028
3' del crRNA	AGCACATCCCAACTGGCACTGGG	chr12: 12164572-12164594
ssODN	AAGGATAAGATTTTGAGATTATCTTTCCAAGGAAA ACTTATCAACTATCTATTATGTATCAGGCACAATC CTTTG GCCAGTTGGGATGTGCTGTGCACACCAA GTGCCCCTAGCTCCTCACCTACCTCTGGGATCA CTGACAGTCCTGCT	black: chr12: 11950948-11951022; red: chr12: 12164578-12164652

Supplemental Table 8: Differentially expressed genes in t(7;12)(q36;p13) AML cases from the Balgobind ⁴⁰ and the TARGET ³⁷ cohorts

See separate file for Supplemental Table 8