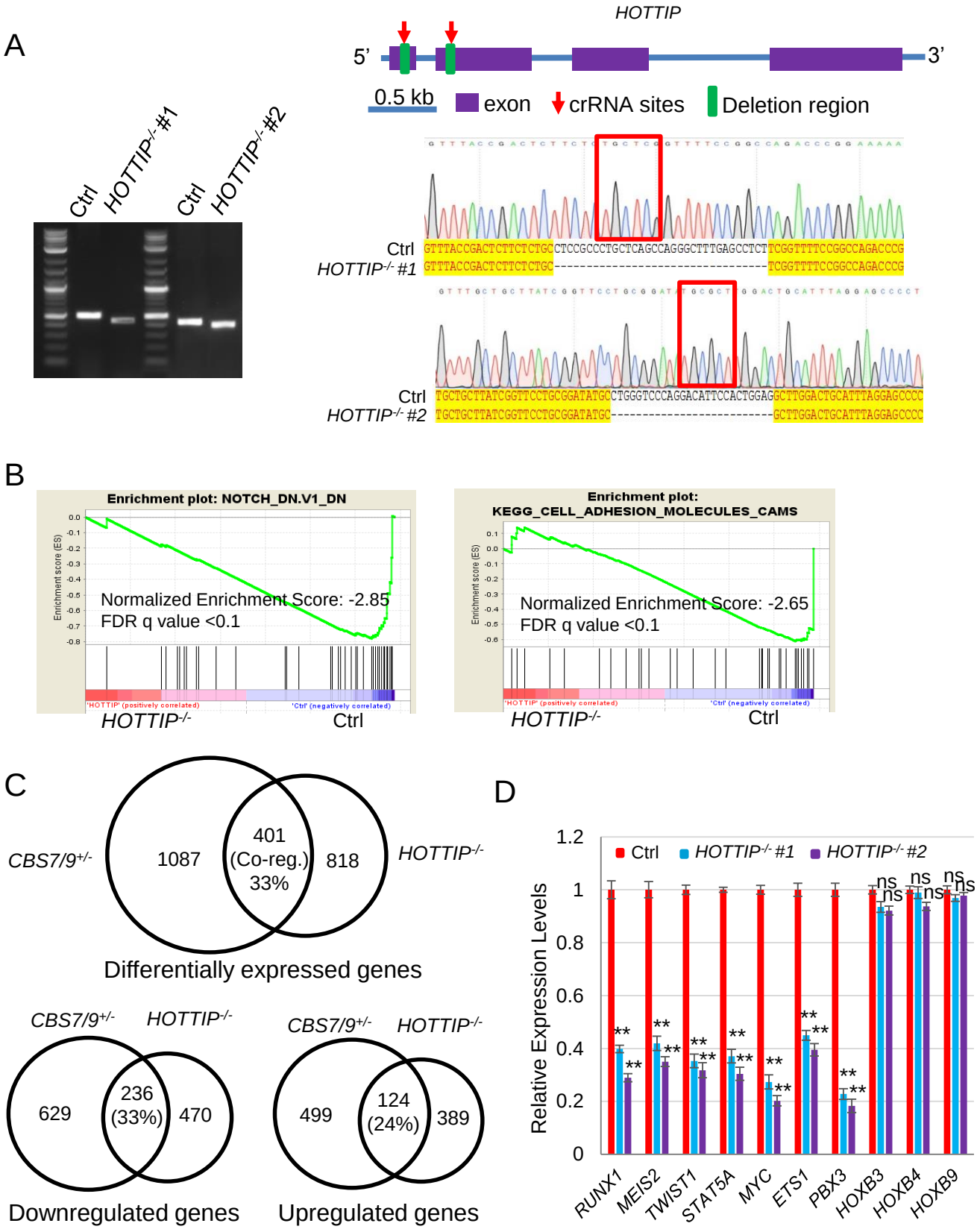
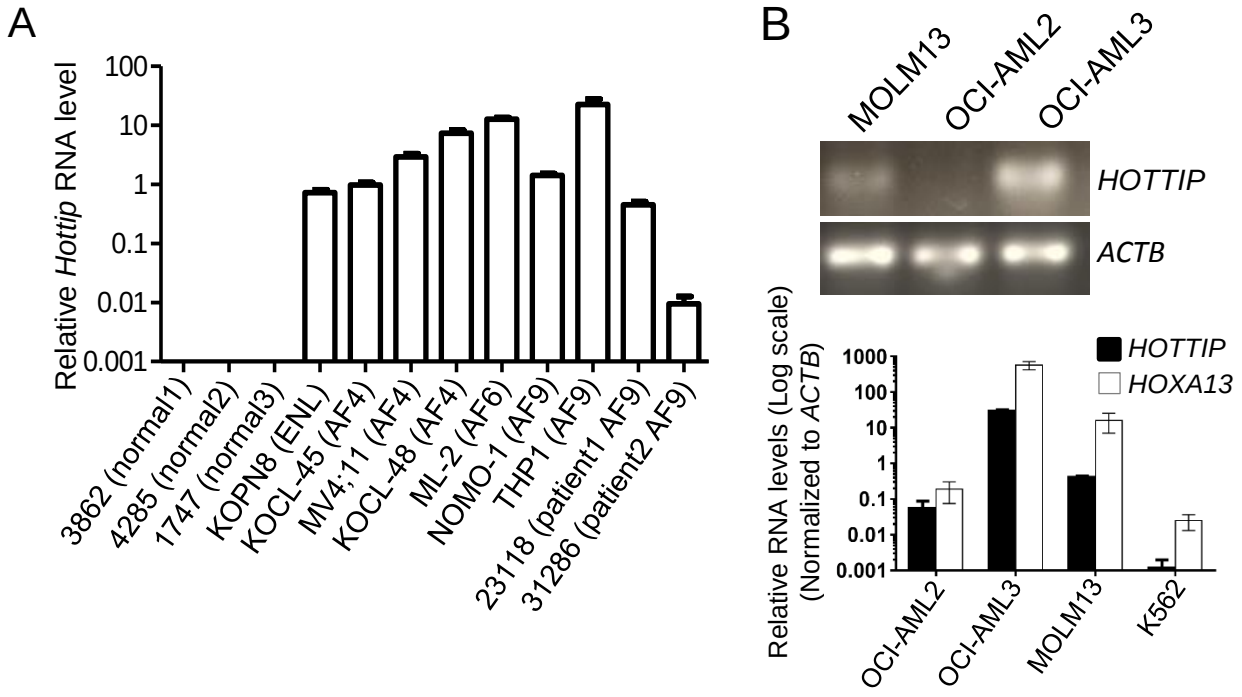
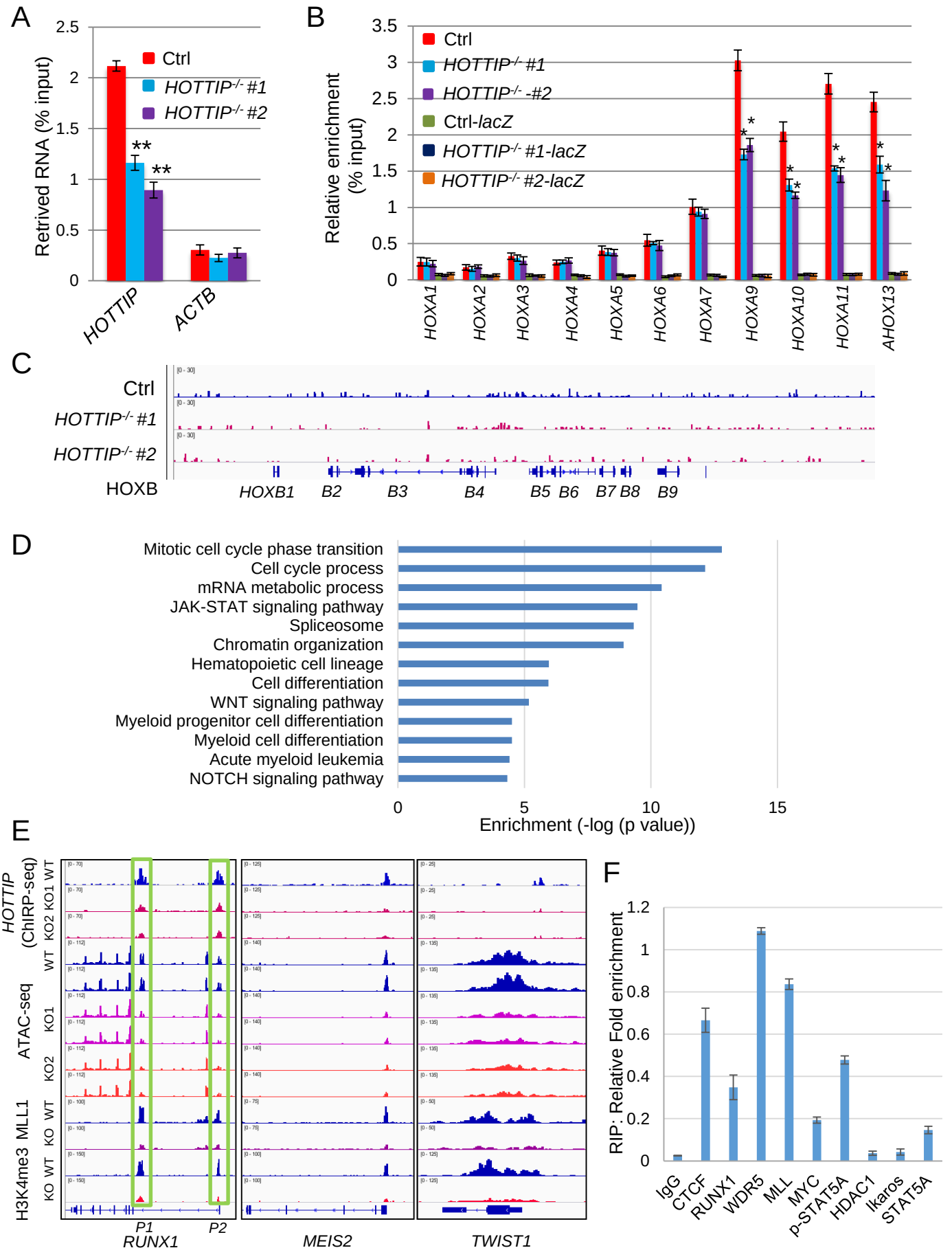
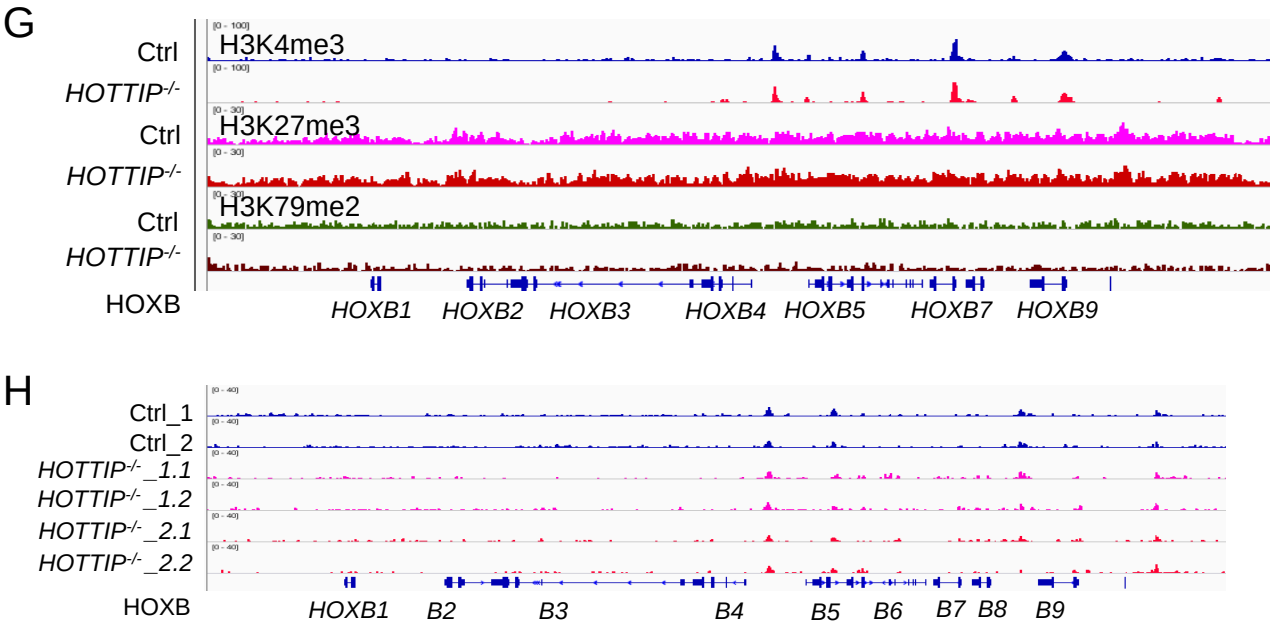

A
HOTTIP
5'
3'
0.5 kb
exon
crRNA sites
Deletion region
Ctrl
HOTTIP-/- #1
Ctrl
HOTTIP-/- #2
Ctrl
HOTTIP-/- #1
Ctrl
HOTTIP-/- #2
B
Enrichment plot: NOTCH_DN.V1_DN
Normalized Enrichment Score: -2.85
FDR q value <0.1
HOTTIP-/-
Ctrl
Enrichment plot:
KEGG_CELL_ADHESION_MOLECULES_CAMS
Normalized Enrichment Score: -2.65
FDR q value <0.1
HOTTIP-/-
Ctrl
C
CBS7/9+/-
1087
401
(Co-reg.)
33%
818
HOTTIP-/-
Differentially expressed genes
CBS7/9+/-
HOTTIP-/-
629
236
(33%)
470
Downregulated genes
CBS7/9+/-
HOTTIP-/-
499
124
(24%)
389
Upregulated genes
D
Ctrl
HOTTIP-/- #1
HOTTIP-/- #2
Relative Expression Levels
RUNX1
MEIS2
TWIST1
STAT5A
MYC
ETS1
PBX3
HOXB3
HOXB4
HOXB9

Supplementary Figure 1. Related to Figure 1; *HOTTIP* loss led to inhibition of posterior HOXA genes and other genes critical for hematopoiesis and leukemogenesis (A) PCR based genotyping and Sanger sequencing confirmation of two CRISPR-Cas9 mediated *HOTTIP*$^{-/-}$ clones in MOLM13 cells. **(B)** Enrichment of downregulated target genes involved in NOTCH signaling and cell adhesion/migration pathways in the *HOTTIP*$^{-/-}$ clones compared to WT control as shown by GSEA. **(C)** Overlap between differentially expressed genes by comparing RNA-seq data obtained from the *HOTTIP*$^{-/-}$ and the CBS7/9 boundary attenuated MOLM13 cells. **(D)** RT-qPCR validation of the key altered hematopoietic/leukemic genes identified by RNA-seq analysis of the WT control and two *HOTTIP*$^{-/-}$ clones. Data is presented as mean ± SD from three or four independent experiments; *p<0.05; **p<0.01 by Student's t-test.


A
Relative Hottip RNA level
100
10
1
0.1
0.01
0.001
3862 (normal1)
4285 (normal2)
1747 (normal3)
KOPN8 (ENL)
KOCL-45 (AF4)
MV4;11 (AF4)
KOCL-48 (AF4)
ML-2 (AF6)
NOMO-1 (AF9)
THP1 (AF9)
23118 (patient1 AF9)
31286 (patient2 AF9)
B
MOLM13
OCI-AML2
OCI-AML3
HOTTIP
ACTB
Relative RNA levels (Log scale)
(Normalized to ACTB)
HOTTIP
HOXA13
1000
100
10
1
0.1
0.01
0.001
OCI-AML2
OCI-AML3
MOLM13
K562

Supplementary Figure 2. Related to Figure 2; *HOTTIP* is aberrantly expressed in AML patients and cell lines. (A) Expression of *HOTTIP* in normal healthy individual, MLL rearranged AML cell lines and patients by RT-qPCR. Data represents mean ± SD from three independent experiments. **(B)** Expression of *HOTTIP* in MOLM13, OCI-AML3 and OCI-AML2 cells determined by RT-PCR or RT-qPCR.


A
Retrived RNA (% input)
Ctrl
HOTTIP-/- #1
HOTTIP-/- #2
HOTTIP
ACTB
B
Relative enrichment (% input)
Ctrl
HOTTIP-/- #1
HOTTIP-/- -#2
Ctrl-lacZ
HOTTIP-/- #1-lacZ
HOTTIP-/- #2-lacZ
HOXA1
HOXA2
HOXA3
HOXA4
HOXA5
HOXA6
HOXA7
HOXA9
HOXA10
HOXA11
AHOX13
C
Ctrl
HOTTIP-/- #1
HOTTIP-/- #2
HOXB
HOXB1
B2
B3
B4
B5
B6
B7
B8
B9
D
Mitotic cell cycle phase transition
Cell cycle process
mRNA metabolic process
JAK-STAT signaling pathway
Spliceosome
Chromatin organization
Hematopoietic cell lineage
Cell differentiation
WNT signaling pathway
Myeloid progenitor cell differentiation
Myeloid cell differentiation
Acute myeloid leukemia
NOTCH signaling pathway
Enrichment (-log (p value))
E
HOTTIP (ChIRP-seq)
ATAC-seq
H3K4me3 MLL1
WT
KO1
KO2
KO
P1
P2
RUNX1
MEIS2
TWIST1
F
RIP: Relative Fold enrichment
IgG
CTCF
RUNX1
WDR5
MLL
MYC
p-STAT5A
HDAC1
Ikaros
STAT5A



Supplementary Figure 3. Related to Figure 3; Deletion of *HOTTIP* in AML cells perturbs its binding to chromatin as well as target gene chromatin structure and accessibility. (A) RT-qPCR analysis of RNA retrieved by the complementary *HOTTIP*-tiling probes and *lacZ* probes compared WT and *HOTTIP*$^{-/-}$ MOLM13 cells. Error bars show mean ± SD from three to four independent experiments; *p<0.05; **p<0.01 by Student's t-test. **(B)** ChIRP-qPCR analysis of the *HOTTIP* RNA enrichment at the *HOXA* locus compared WT and *HOTTIP*$^{-/-}$ MOLM13 cells. Error bars show mean ± SD from three to four independent experiments; *p<0.05; **p<0.01 by Student's t-test. **(C)** ChIRP-seq analysis of *HOTTIP* lncRNA binding in the *HOXB* locus compared WT and *HOTTIP*$^{-/-}$ MOLM13 cells. **(D)** The genes and pathways regulated by the *HOTTIP* bound intergenic regions were analyzed and annotated by the Gene Ontology analysis. **(E)** Alterations in *HOTTIP* binding (ChIRP-seq), chromatin accessibility (ATAC-seq), MLL1 recruitment and H3K4me3 enrichment (ChIP-seq) in the *HOTTIP trans* regulated genes, *RUNX1*, *MEIS2*, and *TWIST1*, compared WT and *HOTTIP* KO MOLM13 cells. P1: promoter 1 of *RUNX1*; P2: promoter 2 of *RUNX1.* **(F)** RT-qPCR analysis of *HOTTIP* RNA retrieved by antibodies against RUNX1, WDR5, MLL1, MYC, and p-STAT5A precipitated from the MOLM13 nuclear extract. **(G)** ChIP-seq analysis of changes in H3K4me3, H3K27me3, and H3K79me2 modification levels in the *HOXB* locus in the MOLM13 cells compared with the WT and the *HOTTIP*$^{-/-}$. **(H)** ATAC-seq analysis of changes in chromatin accessibility upon *HOTTIP*$^{-/-}$ in MOLM13 cells. Shown are altered promoter chromatin accessibility in the *HOXB* locus.

Table S1: Transcription factor bound Motifs analyzed and annotated using ChIRP-seq and ATAC-seq datasets. Related to Figure 3.

ATAC associated			***HOTTIP*-CHIRP**	
Motif	**p value**		**Motif**	**p value**
ETS1	1.00E-465		E-box	1.00E-658
CTCF	1.00E-341		CRE	1.00E-520
E-Box	1.00E-265		RUNX1	1.00E-467
CRE	1.00E-231		HOXA13	1.00E-360
RUNX1	1.00E-215		c-Myc	1.00E-320
c-Myc	1.00E-180		MEIS1	1.00E-280
Max	1.00E-139		USF1	1.00E-259
STAT5	1.00E-122		CTCF	1.00E-220
USF1	1.00E-118		Max	1.00E-215
E2F	1.00E-98		STAT5	1.00E-142
STAT1	1.00E-87		TEAD4	1.00E-120
PU.1	1.00E-81		Smad3	1.00E-101
STAT3	1.00E-70		SOX9	1.00E-91
Nanog	1.00E-49		HLF	1.00E-78
YY1	1.00E-35		MYB	1.00E-69
ATF3	1.00E-22		CDX4	1.00E-60
FOXA1	1.00E-18		ELF4	1.00E-48
SP2	1.00E-14		USF2	1.00E-36
ATF1	1.00E-12		STAT1	1.00E-27
FLI1	1.00E-10		PU.1	1.00E-16
KLF6	1.00E-08		TCF12	1.00E-10
MYB	1.00E-08		Tbx20	1.00E-09
NFAT	1.00E-07		ERG	1.00E-08
ELF4	1.00E-06		TCF4	1.00E-07
TBX5	1.00E-05		AP1	1.00E-07
E2A	1.00E-05		LHX1	1.00E-06
TCF4	1.00E-05		ATF1	1.00E-05
			FRA1	1.00E-05

Table S2: Statistical analysis of ATAC differential peaks and p value. Related to Figures 3, 5 and 8.

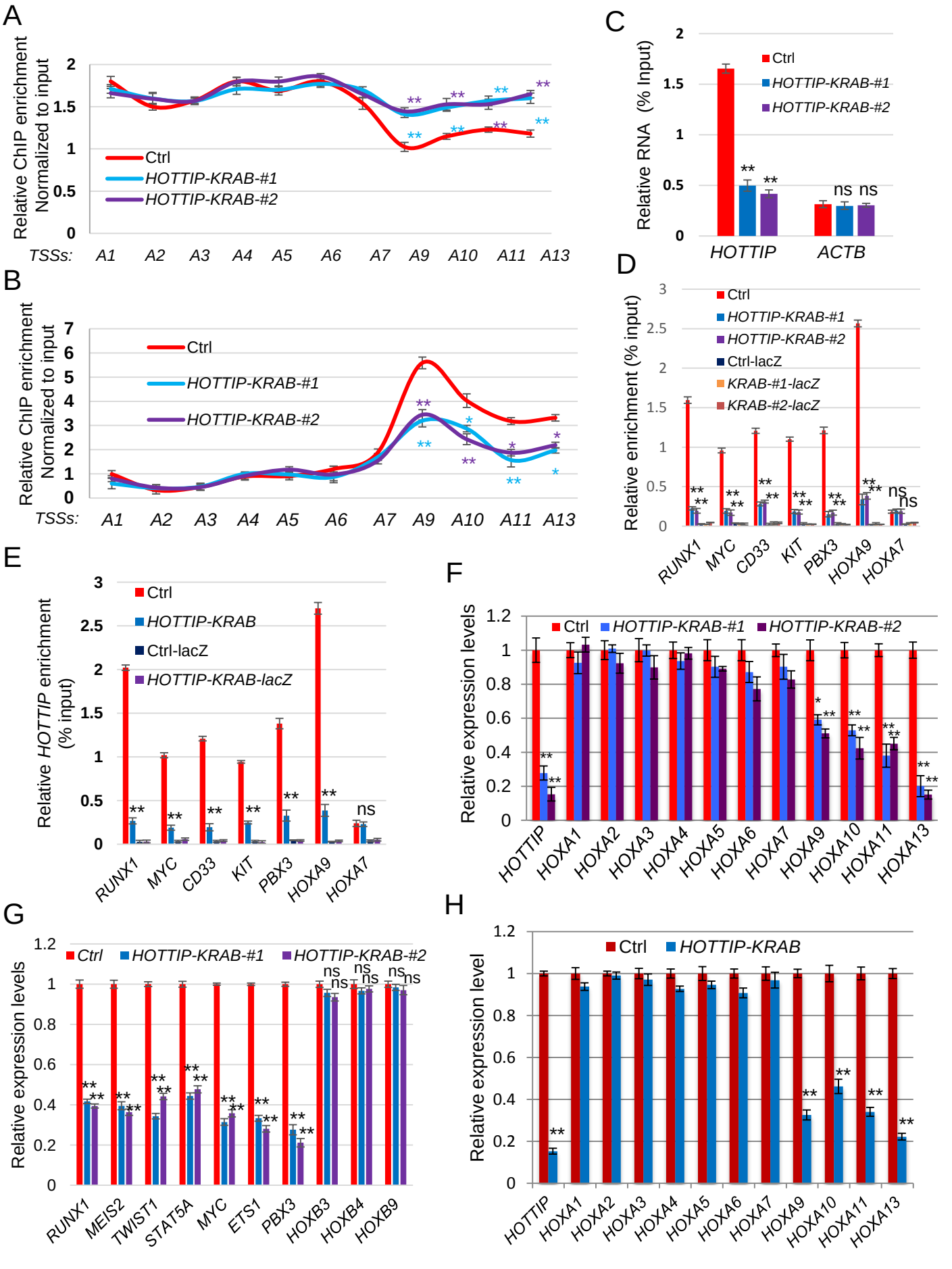
Ctrl vs *HOTTIP*$^{-/-}$					
Chr	**start**	**end**	**log2FoldChange (Ctrl vs KO)**	**Adj p value**	**Genes**
chr7	27239961	27240137	2.653	3.28E-13	*HOTTIP*
chr7	27200117	27201441	2.385	1.45E-08	*HOXA9*
chr7	27239593	27240001	1.917	1.58E-10	*HOXA13*
chr21	36260643	36261680	1.614	5.63E-06	*RUNX1*
chr17	40440299	40440741	1.445	1.72E-08	*STAT5A*
chr8	128747648	128748815	1.411	3.61E-06	*MYC*
chr7	27219354	27220019	1.395	4.17E-05	*HOXA10*
chr7	27224552	27225091	1.378	3.63E-05	*HOXA11*
chr19	51728058	51728855	1.356	4.11E-06	*CD33*
chr7	19156515	19157393	1.346	5.20E-08	*TWIST1*
chr15	37393235	37393784	1.338	5.19E-05	*MEIS2*
chr9	128509129	128509991	1.327	3.45E-06	*PBX3*
chr4	55523944	55524288	1.214	7.64E-07	*KIT*

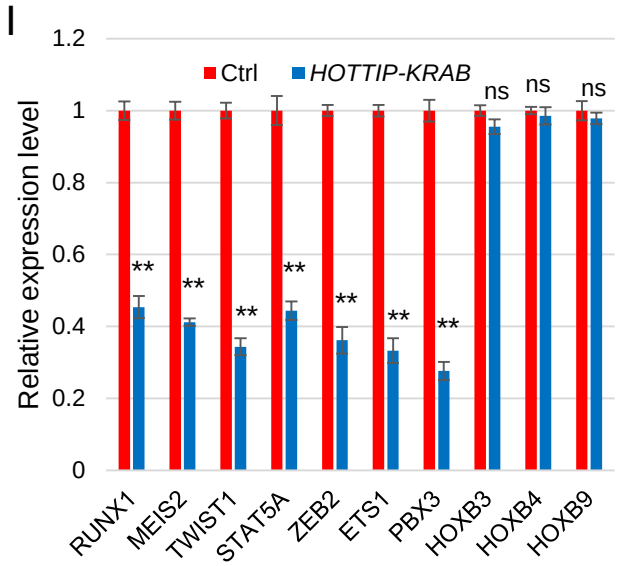
HOTTIP-VP vs *CBS7/9*$^{+/-}$					
Chr	**start**	**end**	**log2FoldChange (VP vs KO)**	**Adj p value**	**Genes**
chr7	27239612	27239999	2.882	5.56E-17	*HOXA13*
chr7	27239945	27240143	2.265	3.51E-16	*HOTTIP*
chr21	36260648	36261668	1.831	8.35E-10	*RUNX1*
chr15	37393239	37393779	1.776	6.44E-09	*MESI2*
chr7	19156523	19157390	1.482	1.08E-07	*TWIST1*
chr2	239756363	239757156	1.387	1.01E-06	*TWIST2*
chr2	66662301	66663195	1.352	1.98E-05	*MEIS1*
chr7	27200112	27201446	1.323	2.04E-09	*HOXA9*
chr17	40440313	40440733	1.302	5.33E-07	*STAT5A*
chr8	128747661	128748802	1.281	6.43E-07	*MYC*
chr2	145277521	145278173	1.256	4.11E-06	*ZEB2*
chr9	128509121	128510008	1.245	8.97E-05	*PBX3*
chr7	27219332	27220028	1.230	5.34E-06	*HOXA10*
chr7	27224556	27225095	1.202	7.95E-05	*HOXA11*
chr11	128391555	128392392	1.198	5.49E-06	*ETS1*

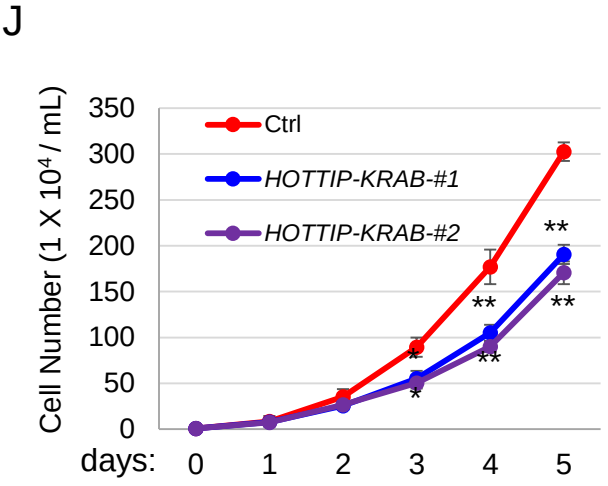
Continued

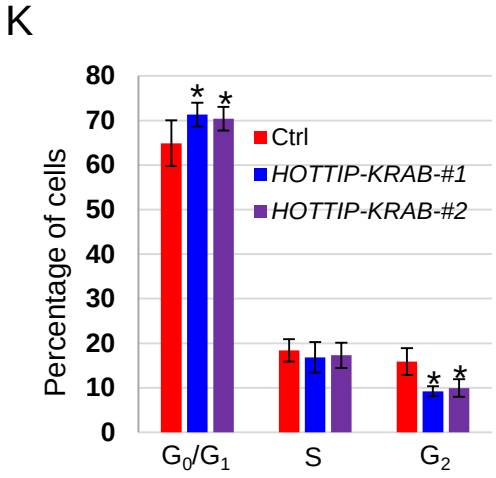
***Hottip*-Tg vs Ctrl LT-HSC**					
Chr	**start**	**end**	**log2FoldChange (HT vs Ctrl)**	**Adj p value**	**Genes**
chr6	52212340	52213115	2.268	6.89E-21	*Hottip*
chr6	52172626	52173303	2.090	1.21E-20	*Hoxa9*
chr6	52209155	52210718	1.971	3.35E-19	*Hoxa13*
chr16	92826082	92826426	1.789	1.21E-16	*Runx1*
chr6	52189793	52191345	1.747	2.26E-18	*Hoxa10*
chr2	34227091	34227906	1.521	6.48E-12	*Pbx3*
chr11	18918453	18919407	1.342	6.48E-10	*Meis1*
chr15	61816553	61817375	1.267	3.26E-13	*Myc*
chr12	34642150	34643195	1.204	3.42E-09	*Twist1*
chr6	52195231	52196121	1.145	4.98E-07	*Hoxa11*

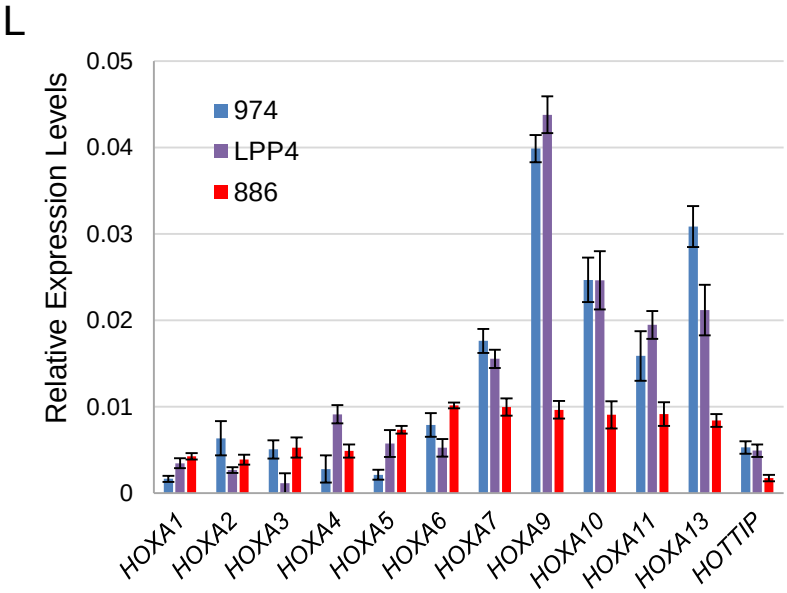
***Hottip*-Tg vs Ctrl ST-HSC**					
Chr	**start**	**end**	**log2FoldChange(HT vs Ctrl)**	**Adj p value**	**Genes**
chr6	52212348	52213124	2.395	1.18E-28	*Hottip*
chr6	52172626	52173303	1.805	4.33E-17	*Hoxa9*
chr6	52209148	52210720	1.744	2.13E-11	*Hoxa13*
chr2	34227085	34227911	1.673	1.06E-15	*Pbx3*
chr16	92826076	92826431	1.512	9.67E-13	*Runx1*
chr6	52189788	52191356	1.486	1.20E-18	*Hoxa10*
chr15	61816547	61817382	1.399	9.91E-12	*Myc*
chr6	52195236	52196129	1.376	9.21E-08	*Hoxa11*
chr11	18918478	18919416	1.246	6.43E-12	*Meis1*
chr12	34642143	34643190	1.213	8.64E-07	*Twist1*


A
Relative ChIP enrichment Normalized to input
Ctrl
HOTTIP-KRAB-#1
HOTTIP-KRAB-#2
TSSs: A1 A2 A3 A4 A5 A6 A7 A9 A10 A11 A13
B
Relative ChIP enrichment Normalized to input
Ctrl
HOTTIP-KRAB-#1
HOTTIP-KRAB-#2
TSSs: A1 A2 A3 A4 A5 A6 A7 A9 A10 A11 A13
C
Relative RNA (% Input)
Ctrl
HOTTIP-KRAB-#1
HOTTIP-KRAB-#2
HOTTIP
ACTB
D
Relative enrichment (% input)
Ctrl
HOTTIP-KRAB-#1
HOTTIP-KRAB-#2
Ctrl-lacZ
KRAB-#1-lacZ
KRAB-#2-lacZ
RUNX1 MYC CD33 KIT PBX3 HOXA9 HOXA7
E
Relative HOTTIP enrichment (% input)
Ctrl
HOTTIP-KRAB
Ctrl-lacZ
HOTTIP-KRAB-lacZ
RUNX1 MYC CD33 KIT PBX3 HOXA9 HOXA7
F
Relative expression levels
Ctrl
HOTTIP-KRAB-#1
HOTTIP-KRAB-#2
HOTTIP HOXA1 HOXA2 HOXA3 HOXA4 HOXA5 HOXA6 HOXA7 HOXA9 HOXA10 HOXA11 HOXA13
G
Relative expression levels
Ctrl
HOTTIP-KRAB-#1
HOTTIP-KRAB-#2
RUNX1 MEIS2 TWIST1 STAT5A MYC ETS1 PBX3 HOXB3 HOXB4 HOXB9
H
Relative expression level
Ctrl
HOTTIP-KRAB
HOTTIP HOXA1 HOXA2 HOXA3 HOXA4 HOXA5 HOXA6 HOXA7 HOXA9 HOXA10 HOXA11 HOXA13


I
Ctrl
HOTTIP-KRAB
Relative expression level
1.2
1
0.8
0.6
0.4
0.2
0
ns
**
RUNX1
MEIS2
TWIST1
STAT5A
ZEB2
ETS1
PBX3
HOXB3
HOXB4
HOXB9

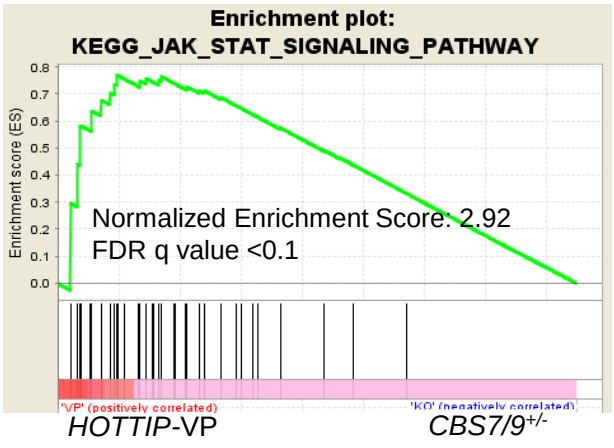

J
Cell Number (1 X 10⁴ / mL)
350
300
250
200
150
100
50
0
Ctrl
HOTTIP-KRAB-#1
HOTTIP-KRAB-#2
days:
0
1
2
3
4
5
*
**


K
Percentage of cells
80
70
60
50
40
30
20
10
0
Ctrl
HOTTIP-KRAB-#1
HOTTIP-KRAB-#2
G0/G1
S
G2
*


L
Relative Expression Levels
0.05
0.04
0.03
0.02
0.01
0
974
LPP4
886
HOXA1
HOXA2
HOXA3
HOXA4
HOXA5
HOXA6
HOXA7
HOXA9
HOXA10
HOXA11
HOXA13
HOTTIP

Supplementary Figure 4. Related to Figures 4; *dCas9-KRAB* mediated inhibition of *HOTTIP* lncRNA resulted in inhibition of active histone modifications and elevated repressive histone modifications in the posterior *HOXA* domain. (A, B) ChIP analysis of H3K9me2 **(A)** and H3K4me3 **(B)** enrichment at the *HOXA* locus compared WT control and *HOTTIP-dCas9-KRAB* MOLM13 clones. **(C)** RT-qPCR analysis of RNA retrieved by the complementary *HOTTIP*-tiling probes compared WT and *HOTTIP-dCas9-KRAB* inhibited MOLM13 cells. **(D)** ChIRP-qPCR analysis of the *HOTTIP* RNA enrichment at the *HOXA9* and other hematopoietic/leukemia specific genes compared WT and *HOTTIP-dCas9-KRAB* MOLM13 cells. **(E)** ChIRP-qPCR analysis of the *HOTTIP* RNA enrichment at the *HOXA9* and other hematopoietic/leukemia specific genes compared WT and *HOTTIP-dCas9-KRAB* OCI-AML3 cells carrying $NPM1^{C+}$ mutation. **(F)** RT-qPCR analysis of *HOXA* gene expression in *MLL*-rearranged MOLM13 cells compared the WT control and the *HOTTIP-dCas9-KRAB* clones. **(G)** RT-qPCR validation of the key altered hematopoietic/leukemic genes identified by RNA-seq analysis compared among the WT control and two *HOTTIP- dCas9-KRAB* clones. **(H)** RT-qPCR analysis of *HOXA* gene expression in $NPM1^{C+}$ mutated OCI-AML3 cells compared the WT control and the *HOTTIP-dCas9-KRAB* clones. **(I)** RT-qPCR validation of the key altered hematopoietic/leukemic genes identified by RNA-seq analysis compared among the WT control and *HOTTIP-KRAB* OCI-AML3 clones. **(J)** Proliferation curves of WT control and the *HOTTIP-dCas9-KRAB* MOLM13 clones were measured by cell viability count. **(K)** FACS analysis of cell cycle was carried out using propidium Iodide staining of the WT control or the *HOTTIP-dCas9-KRAB* MOLM13 clones. **(L)** RT-qPCR analysis of the expression levels of *HOTTIP* and *HOXA* genes in primary AML patient BM cells carrying $MLLr^{+}$ (LPP4), $NPM1^{C+}$/$Flt3\text{-}ITD^{+}$ (974), or $NPM1^{C-}$/$FLT3\text{-}ITD^{+}$ (886). For statistics, data is presented as mean ± SD from three to four independent experiments; *p<0.05; **p<0.01 by Student's t-test.

A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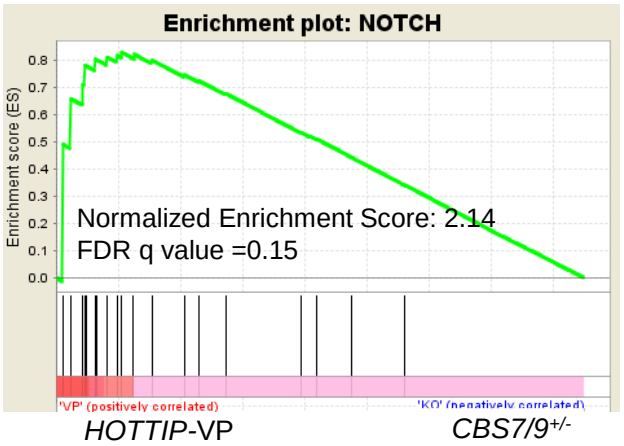
KEGG_JAK_STAT_SIGNALING_PATHWAY
Enrichment score (ES)
Normalized Enrichment Score: 2.92
FDR q value <0.1
'VP' (positively correlated)
'KO' (negatively correlated)
HOTTIP-VP
CBS7/9+/-


Enrichment plot: NOTCH
Enrichment score (ES)
Normalized Enrichment Score: 2.14
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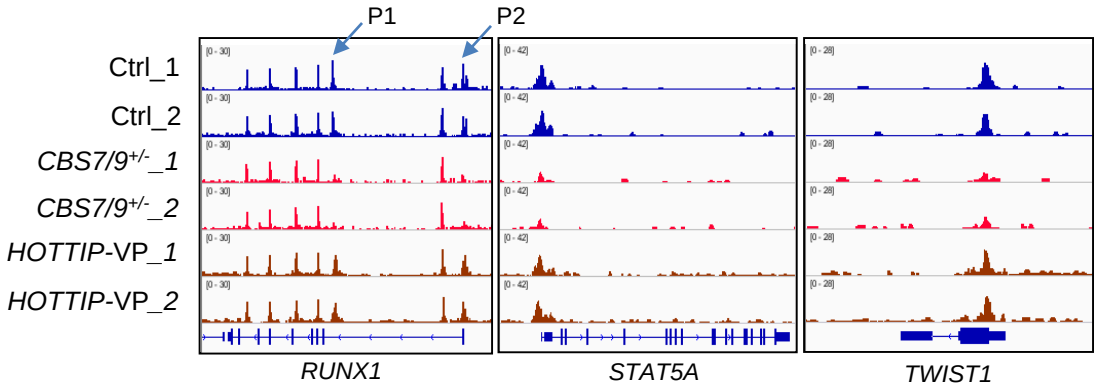
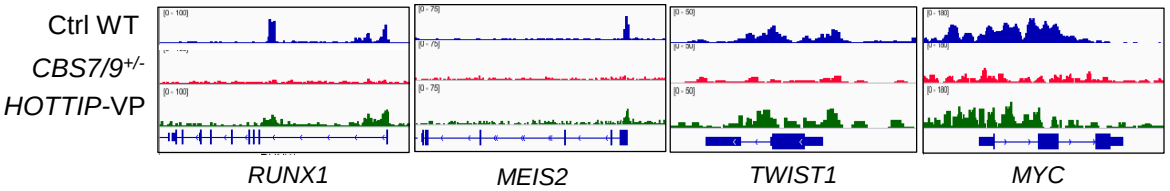
'VP' (positively correlated)
'KO' (negatively correlated)
HOTTIP-VP
CBS7/9+/-

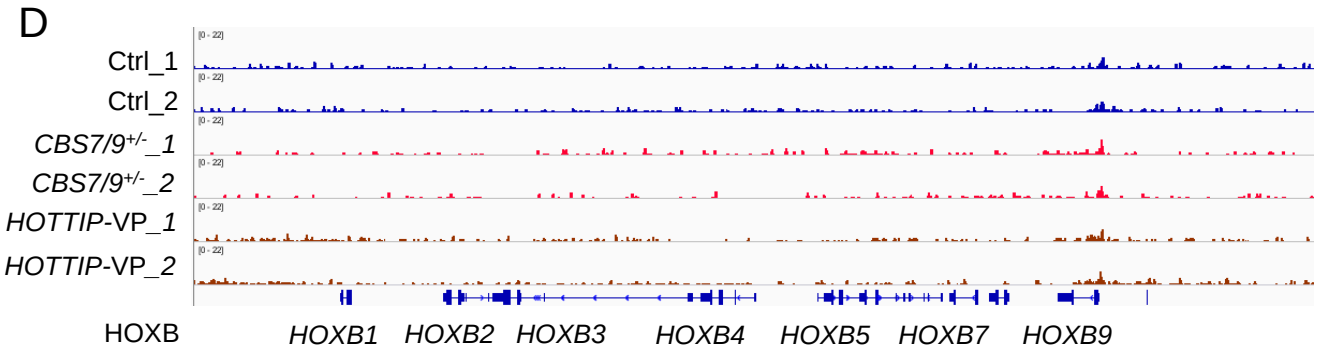
B


P1
P2
Ctrl_1
Ctrl_2
CBS7/9+/-_1
CBS7/9+/-_2
HOTTIP-VP_1
HOTTIP-VP_2
RUNX1
STAT5A
TWIST1

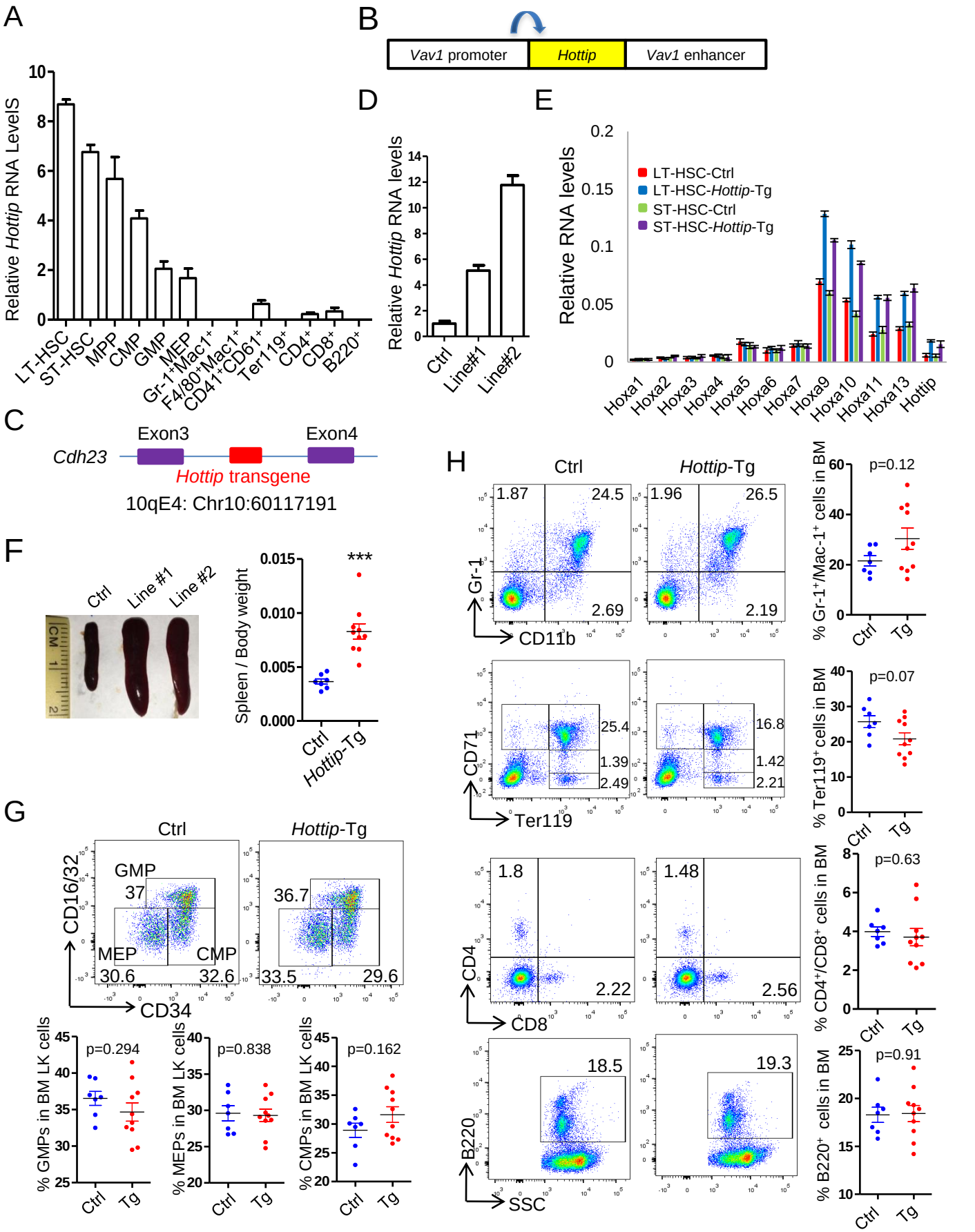
C

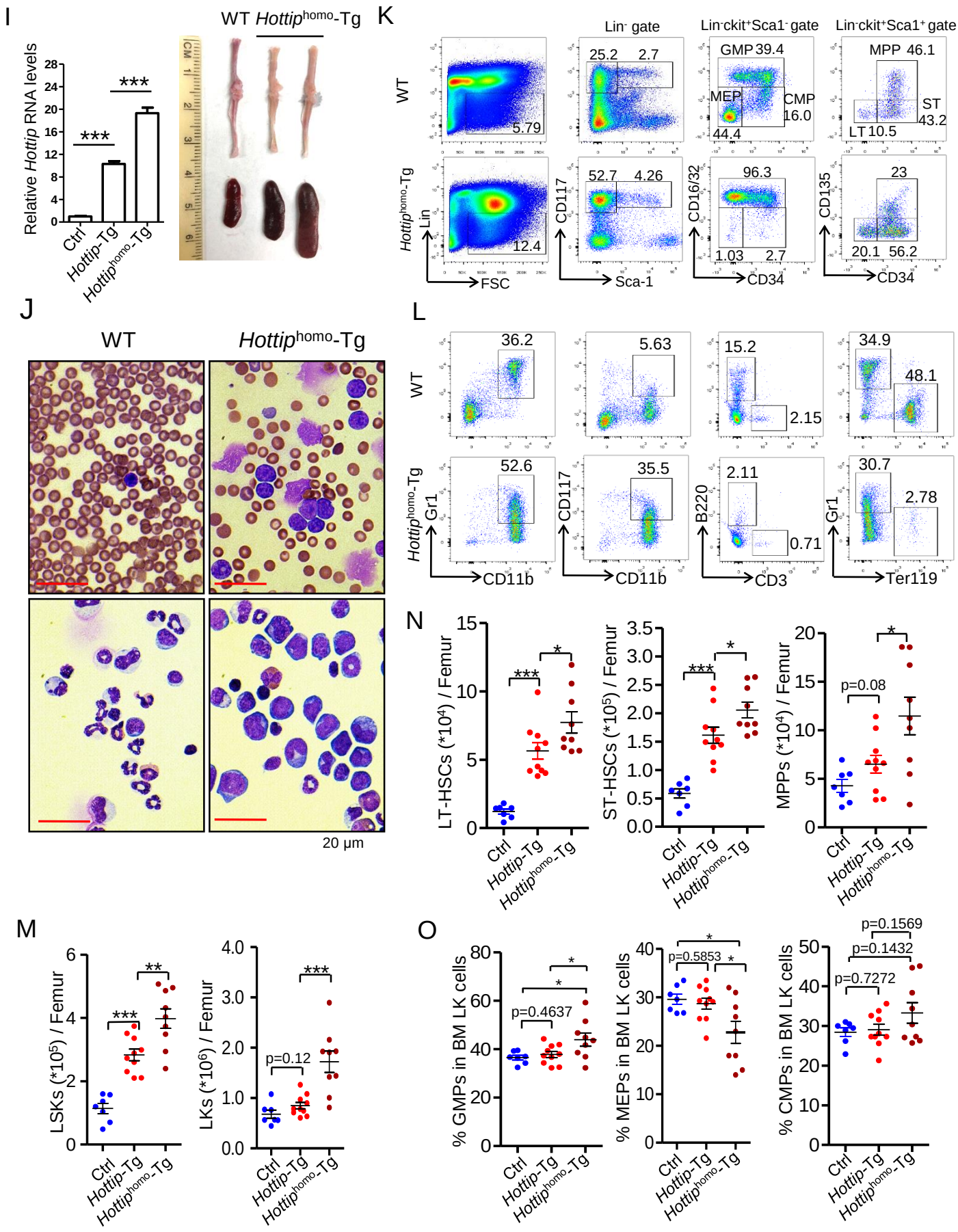

Ctrl WT
CBS7/9+/-
HOTTIP-VP
RUNX1
MEIS2
TWIST1
MYC

D


Ctrl_1
Ctrl_2
CBS7/9+/-_1
CBS7/9+/-_2
HOTTIP-VP_1
HOTTIP-VP_2
HOXB
HOXB1
HOXB2
HOXB3
HOXB4
HOXB5
HOXB7
HOXB9

Supplementary Figure 5. Related to Figure 5; Reactivation of *HOTTIP* lncRNA rescues the posterior *HOXA* chromatin defects and gene expression in the *CBS7/9* boundary-disrupted AML cells. (A) Enrichment of upregulated target genes involved in JAK-STAT signaling and NOTCH signaling pathways in the *dCas9-VP-160* mediated *HOTTIP* activated *CBS7/9*$^{+/-}$ MOLM13 clones compared to the *CBS7/9*$^{+/-}$ MOLM13 cells as shown by GSEA. **(B)** Snap shot of ATAC-seq analysis of *RUNX1*(Left), *STAT5A* (Middle), and *TWIST1* (Right) loci compared among WT MOLM13 control, *CBS7/9*$^{+/-}$ MOLM13 cells, and the *dCas9-VP-160* mediated *HOTTIP* activated *CBS7/9*$^{+/-}$ MOLM13 clones. **(C)** ChIP-seq analysis of MLL1 recruitment in the Non-*HOX* targets compared among WT MOLM13 control, *CBS7/9*$^{+/-}$ MOLM13 cells, and the *dCas9-VP-160* mediated *HOTTIP* activated *CBS7/9*$^{+/-}$ MOLM13 clones. **(D)** ATAC-seq analysis of the alteration of chromatin accessibility in the *HOXB* locus compared among WT MOLM13 control, *CBS7/9*$^{+/-}$ MOLM13 cells, and the *dCas9-VP-160* mediated *HOTTIP* activated *CBS7/9*$^{+/-}$ MOLM13 clones.

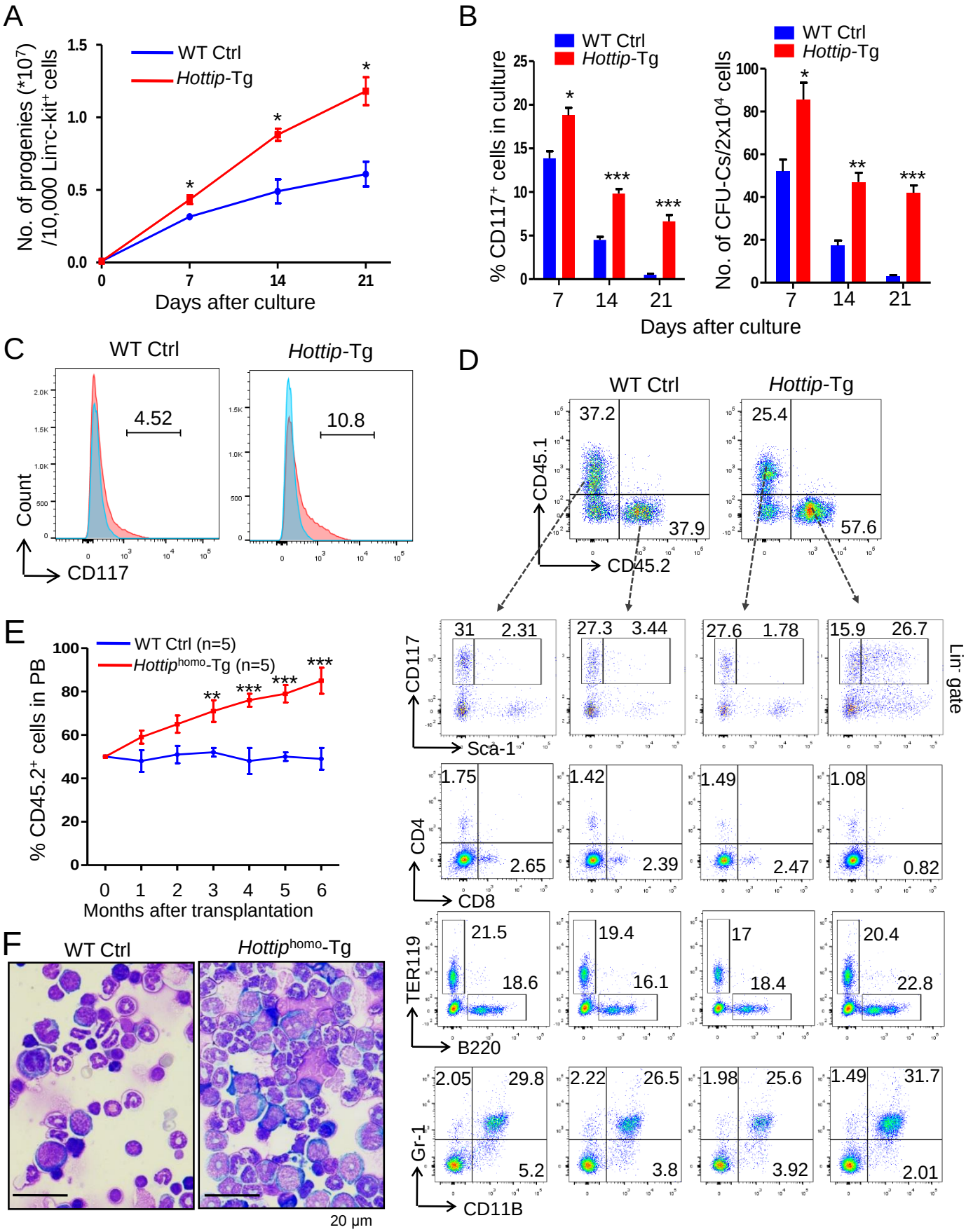

A
Relative Hottip RNA LevelS
LT-HSC
ST-HSC
MPP
CMP
GMP
MEP
Gr-1+Mac1+
F4/80+Mac1+
CD41+CD61+
Ter119+
CD4+
CD8+
B220+
B
Vav1 promoter
Hottip
Vav1 enhancer
D
Relative Hottip RNA levels
Ctrl
Line#1
Line#2
E
Relative RNA levels
LT-HSC-Ctrl
LT-HSC-Hottip-Tg
ST-HSC-Ctrl
ST-HSC-Hottip-Tg
Hoxa1
Hoxa2
Hoxa3
Hoxa4
Hoxa5
Hoxa6
Hoxa7
Hoxa9
Hoxa10
Hoxa11
Hoxa13
Hottip
C
Exon3
Exon4
Cdh23
Hottip transgene
10qE4: Chr10:60117191
F
Ctrl
Line #1
Line #2
Spleen / Body weight
***
Ctrl
Hottip-Tg
G
Ctrl
Hottip-Tg
CD16/32
CD34
GMP
37
MEP
30.6
CMP
32.6
36.7
33.5
29.6
% GMPs in BM LK cells
p=0.294
% MEPs in BM LK cells
p=0.838
% CMPs in BM LK cells
p=0.162
H
Ctrl
Hottip-Tg
Gr-1
CD11b
1.87
24.5
2.69
1.96
26.5
2.19
% Gr-1+/Mac-1+ cells in BM
p=0.12
CD71
Ter119
25.4
1.39
2.49
16.8
1.42
2.21
% Ter119+ cells in BM
p=0.07
CD4
CD8
1.8
2.22
1.48
2.56
% CD4+/CD8+ cells in BM
p=0.63
B220
SSC
18.5
19.3
% B220+ cells in BM
p=0.91
Ctrl
Tg

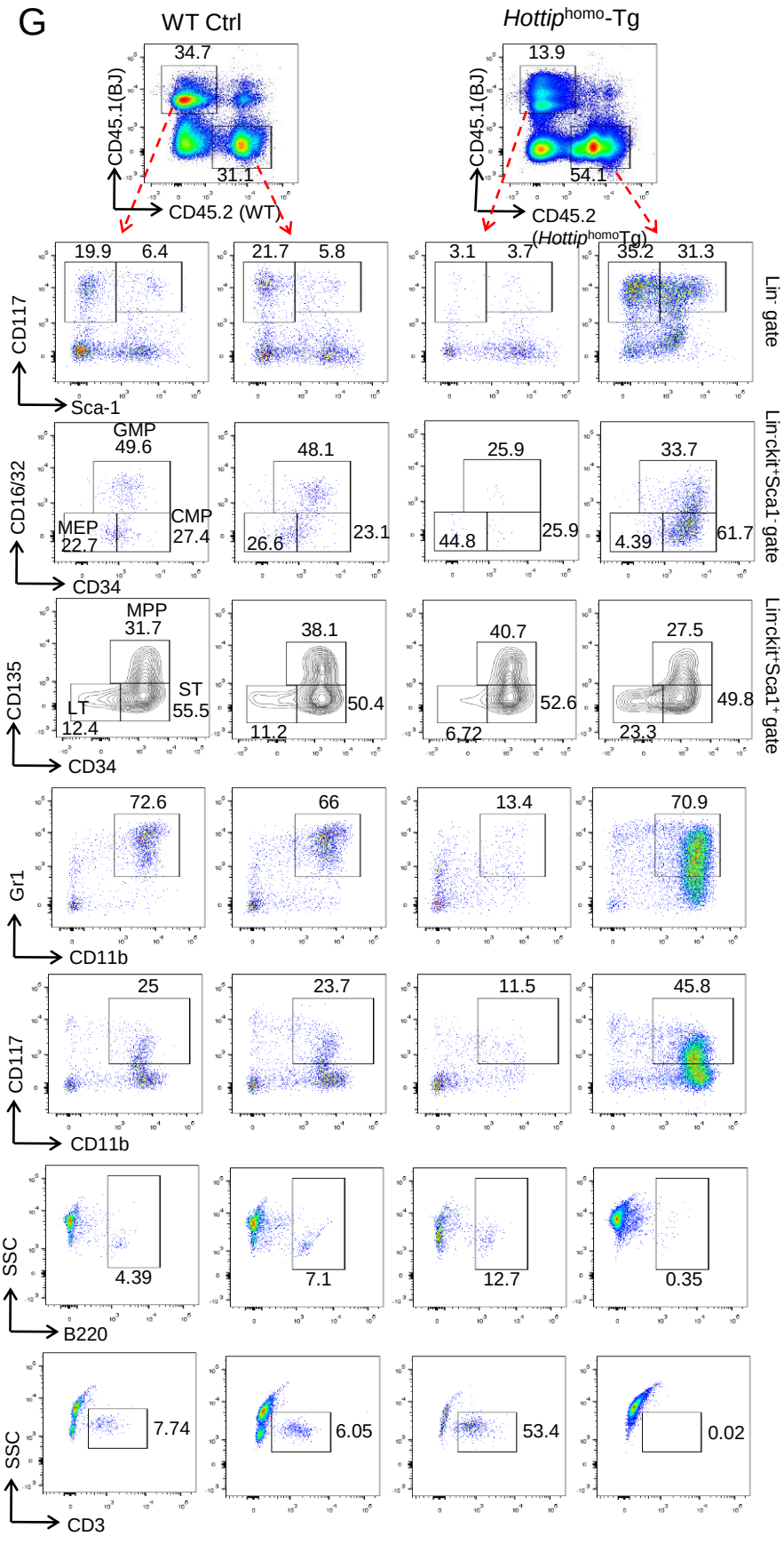

I
WT Hottip^homo-Tg
Relative Hottip RNA levels
28
24
20
16
12
8
4
0
***
***
Ctrl
Hottip-Tg
Hottip^homo-Tg
CM
1
2
3
4
5
6
K
Lin- gate
Lin-ckit+Sca1- gate
Lin-ckit+Sca1+ gate
WT
Hottip^homo-Tg
5.79
25.2
2.7
GMP 39.4
MEP
CMP
16.0
44.4
MPP 46.1
ST
43.2
LT 10.5
12.4
52.7
4.26
96.3
1.03
2.7
23
20.1
56.2
Lin
FSC
CD117
Sca-1
CD16/32
CD34
CD135
CD34
J
WT
Hottip^homo-Tg
20 μm
L
WT
Hottip^homo-Tg
36.2
5.63
15.2
2.15
34.9
48.1
52.6
35.5
2.11
0.71
30.7
2.78
Gr1
CD11b
CD117
CD11b
B220
CD3
Gr1
Ter119
N
LT-HSCs (*10^4) / Femur
ST-HSCs (*10^5) / Femur
MPPs (*10^4) / Femur
***
*
p=0.08
Ctrl
Hottip-Tg
Hottip^homo-Tg
M
LSKs (*10^5) / Femur
LKs (*10^6) / Femur
***
**
p=0.12
O
% GMPs in BM LK cells
% MEPs in BM LK cells
% CMPs in BM LK cells
p=0.4637
p=0.5853
p=0.7272
p=0.1432
p=0.1569

Supplementary Figure 6. Related to Figure 6; *Hottip* lncRNA transgenic expression in hematopoietic compartment perturbs HSC function and results in AML-like disease. (**A**) RT-qPCR analyses of *Hottip* RNA expression pattern of FACS-sorted different cell populations of WT mice. **(B)** Diagram of the *Vav1* promoter driven *Hottip* transgene strategy. **(C)** PCR based transgenic integrating location identification (TAIL) assay maps the *Vav1-Hottip* transgene integration site in the mouse chromosome 10. **(D)** RT-qPCR analysis of *Hottip* RNA expression in BM cells of WT and 2 lines of the *Hottip*-Tg mice. **(E)** RT-qPCR analysis of *Hottip* and *Hoxa* gene expression from purified LT-HSC and ST-HSC populations from WT control and *Hottip*-Tg mice. **(F)** Gross appearance of spleens of representative WT and two lines of *Hottip*-Tg mice (left). Showing is spleen/body weight ratio (right) for age matched WT (n = 7) and the *Hottip*-Tg (n = 10) mice. **(G)** FACS analysis of GMP, MEP and CMP populations within BM LK cells of representative young WT and *Hottip*-Tg mice (Top). Quantitation of the percentage of GMP, MEP and CMP cell populations in the $Lin^{-}ckit^{+}Sca1^{-}$ cells of each genotype of mice are shown (bottom). n=7-10 mice/genotype. **(H)** FACS analysis and quantification of myeloid (Gr-1/Mac-1), erythroid (Ter119/CD71), B (B220/SSC) and T (CD4/CD8) cell populations in the BM of representative young WT and *Hottip*-Tg mice (8-week old). **(I)** The expression levels of *Hottip* in WT, *Hottip*-Tg, and $Hottip^{homo}$-Tg BM mononuclear cells (Left) as well as gross appearance of spleens and femurs of representative WT and moribund $Hottip^{homo}$-Tg mice (Right). **(J)** May-Giemsa stained PB smears and BM cytospins prepared from representative WT and moribund $Hottip^{Homo}$-Tg mice. **(K)** FACS analysis of Lin^{-}, LSK/LK, GMP/CMP, MEP, as well as LT-HSC/ST-HSC/MPP cell populations in the BM of representative young WT and $Hottip^{Homo}$-Tg mice. **(L)** FACS analysis and quantification of myeloid (Gr-1/Mac-1), erythroid (Ter119), B (B220/SSC) and T (CD4/CD8) cell populations in the BM of representative young WT and *Hottip*-Tg mice (8-week old). **(M)** Quantitation of the total LSK and LK cell populations per femur of young WT (n=7), *Hottip*-Tg (n=10), and $Hottip^{Homo}$-Tg (n=9) mice are shown. **(N)** Quantitation of the total LT-HSC, ST-HSC and MPP cell numbers per femur of young WT (n=7), *Hottip*-Tg (n=10), and $Hottip^{Homo}$-Tg (n=9) mice are shown. **(O)** Quantitation of the total GMP. MEP, and CMP cell populations per femur of young WT (n=7), *Hottip*-Tg (n=10), and $Hottip^{Homo}$-Tg (n=9) mice are shown. For statistics, data (Panels F-I, M-O) is presented as mean ± SD.

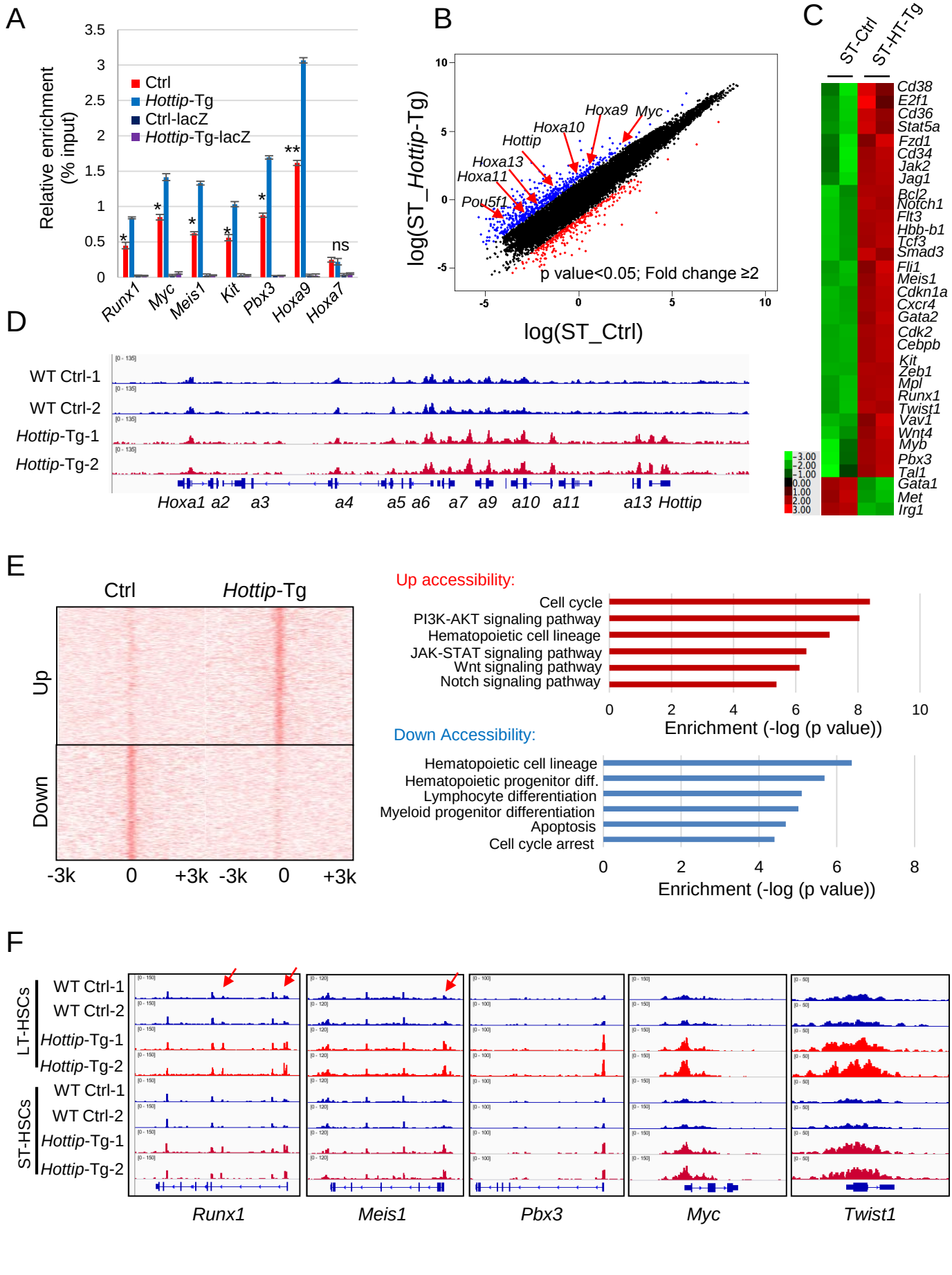
Table S3: Indel and snp variants analysis of whole exome sequencing of the *Hottip*-Tg AML mice. Related to Figure 6.

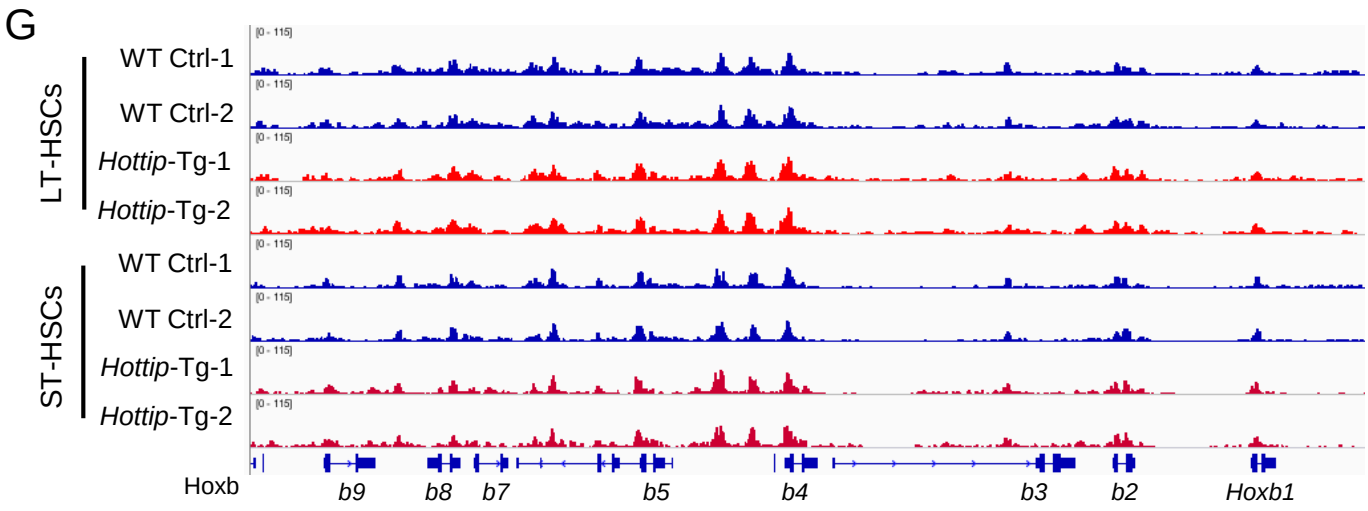
strelka method for exome-seq snp and indel analysis					
sample name #		**indel**	**snp**	**indel_AA_change**	**snp_AA_change**
Hottip-Tg mice	22	39	35	1	15
	39	81	50	3	14
	48	47	63	4	14
	51	49	76	2	27
	55	37	44	3	16
WT mice	69	54	52	4	14
	130	42	42	1	15
Recurrent mutation gene					
Mroh2a					

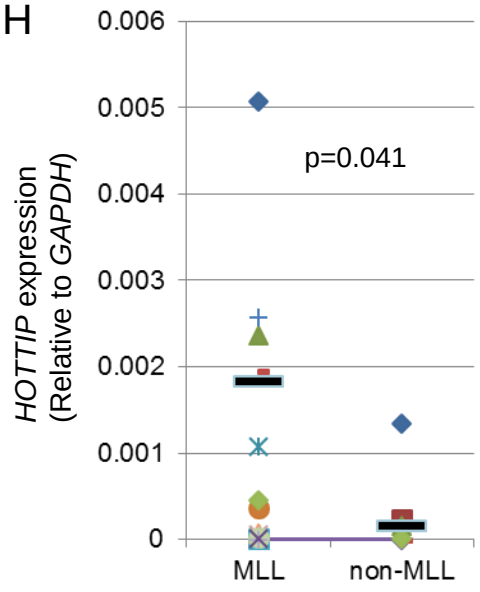

A
No. of progenies (*10^7) /10,000 Lin-c-kit+ cells
WT Ctrl
Hottip-Tg
Days after culture
B
% CD117+ cells in culture
No. of CFU-Cs/2x10^4 cells
WT Ctrl
Hottip-Tg
Days after culture
C
WT Ctrl
Hottip-Tg
4.52
10.8
Count
CD117
D
WT Ctrl
Hottip-Tg
37.2
37.9
25.4
57.6
CD45.1
CD45.2
31
2.31
27.3
3.44
27.6
1.78
15.9
26.7
CD117
Sca-1
Lin- gate
1.75
2.65
1.42
2.39
1.49
2.47
1.08
0.82
CD4
CD8
21.5
18.6
19.4
16.1
17
18.4
20.4
22.8
TER119
B220
2.05
5.2
29.8
2.22
3.8
26.5
1.98
3.92
25.6
1.49
2.01
31.7
Gr-1
CD11B
E
WT Ctrl (n=5)
Hottip homo-Tg (n=5)
% CD45.2+ cells in PB
Months after transplantation
F
WT Ctrl
Hottip homo-Tg
20 μm

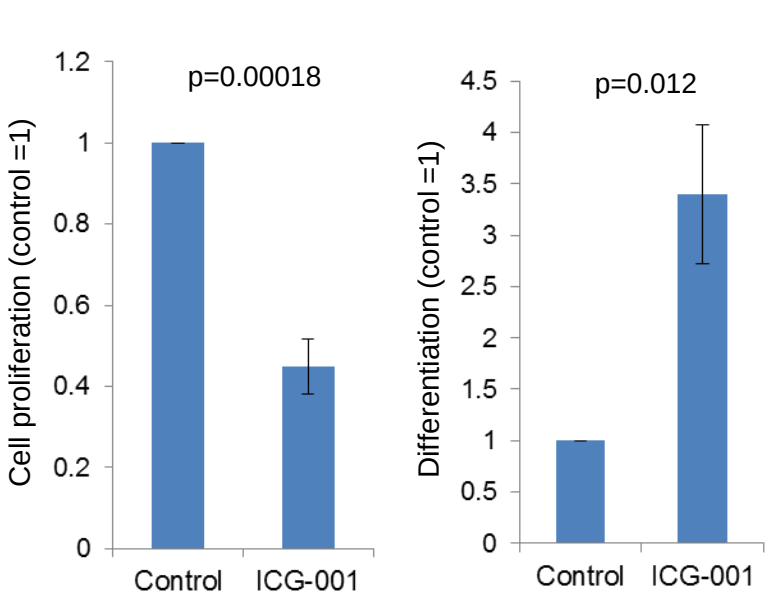

G
WT Ctrl
Hottip homo-Tg
34.7
31.1
CD45.1(BJ)
CD45.2 (WT)
13.9
54.1
CD45.2
(Hottip homoTg)
19.9
6.4
21.7
5.8
3.1
3.7
35.2
31.3
CD117
Sca-1
Lin- gate
GMP
49.6
MEP
22.7
CMP
27.4
48.1
26.6
23.1
25.9
44.8
25.9
33.7
4.39
61.7
CD16/32
CD34
Lin-ckit+Sca1- gate
MPP
31.7
LT
12.4
ST
55.5
38.1
11.2
50.4
40.7
6.72
52.6
27.5
23.3
49.8
CD135
CD34
Lin-ckit+Sca1+ gate
72.6
66
13.4
70.9
Gr1
CD11b
25
23.7
11.5
45.8
CD117
CD11b
4.39
7.1
12.7
0.35
SSC
B220
7.74
6.05
53.4
0.02
SSC
CD3

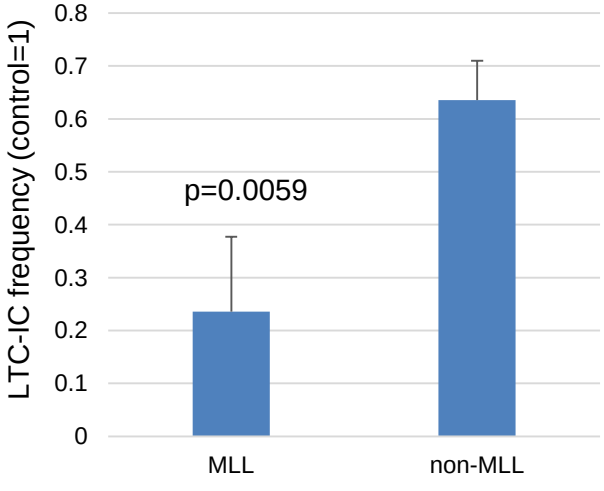
Supplementary Figure 7. Related to Figures 7; *Hottip* regulates self-renewal and proliferation of HSCs. (**A-C**) The expansion and proliferation potential of WT and *Hottip*-Tg HSC/HPCs were examined by culturing BM $Lin^{-}c\text{-}Kit^{+}$ cells in the presence of SCF, TPO, IL-3, G-CSF and EPO. No. of total cells (**A**), percent $c\text{-}Kit^{+}$ cells and No. of CFU-Cs **(B)** in the progenies of $Lin^{-}c\text{-}Kit^{+}$ cells cultured for 7, 14 and 21 days were shown; representative histogram showed the c-Kit (CD117) expression in the cultures of WT and *Hottip*-Tg $Lin^{-}c\text{-}Kit^{+}$ cells for 14 days (**C**). **(D)** FACS analysis showing CD45.2 v.s. CD45.1 chimerism as well as their respective distribution of LSK/LK cell (within Lin^{-} cells) and lineage populations in the BM of representative mice receiving WT or *Hottip*-Tg BM cells. **(E)** Kinetic FACS analyses of CD45.2 (Donor) chimerisms in the PB of recipients (CD45.1) receiving WT or $Hottip^{Homo}$-Tg BM cells. **(F)** Images of May–Grunwald–Giemsa stained cytospin preparations of BM cells from representative mice receiving WT or $Hottip^{Homo}$-Tg BM cells. Scale bar, 20 μm. **(G)** FACS analysis showing CD45.2 vs. CD45.1 chimerism and their respective lineage distribution of LSK/LK, GMP/CMP/MEP, LT-HSC/ST-HSC/MPP cell populations, as well as lineage distribution of myeloid, B and T (CD3/B220), immature myeloid in the BM of representative mice receiving WT or $Hottip^{Homo}$-Tg BM cells. For statistics, data (Panels A, B, and E) is presented as mean ± SD.

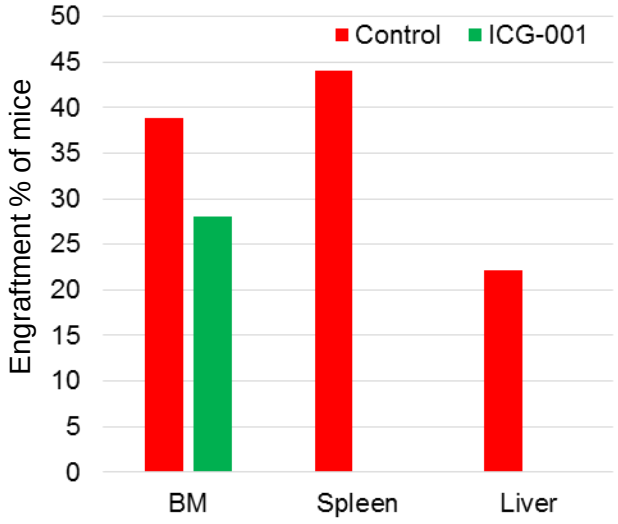

A
Relative enrichment (% input)
Ctrl
Hottip-Tg
Ctrl-lacZ
Hottip-Tg-lacZ
Runx1
Myc
Meis1
Kit
Pbx3
Hoxa9
Hoxa7
ns
B
log(ST_Hottip-Tg)
log(ST_Ctrl)
Hoxa9
Myc
Hoxa10
Hottip
Hoxa13
Hoxa11
Pou5f1
p value<0.05; Fold change ≥2
C
ST-Ctrl
ST-HT-Tg
Cd38
E2f1
Cd36
Stat5a
Fzd1
Cd34
Jak2
Jag1
Bcl2
Notch1
Flt3
Hbb-b1
Tcf3
Smad3
Fli1
Meis1
Cdkn1a
Cxcr4
Gata2
Cdk2
Cebpb
Kit
Zeb1
Mpl
Runx1
Twist1
Vav1
Wnt4
Myb
Pbx3
Tal1
Gata1
Met
Irg1
D
WT Ctrl-1
WT Ctrl-2
Hottip-Tg-1
Hottip-Tg-2
Hoxa1 a2 a3 a4 a5 a6 a7 a9 a10 a11 a13 Hottip
E
Ctrl
Hottip-Tg
Up
Down
-3k 0 +3k -3k 0 +3k
Up accessibility:
Cell cycle
PI3K-AKT signaling pathway
Hematopoietic cell lineage
JAK-STAT signaling pathway
Wnt signaling pathway
Notch signaling pathway
Enrichment (-log (p value))
Down Accessibility:
Hematopoietic cell lineage
Hematopoietic progenitor diff.
Lymphocyte differentiation
Myeloid progenitor differentiation
Apoptosis
Cell cycle arrest
Enrichment (-log (p value))
F
LT-HSCs
ST-HSCs
WT Ctrl-1
WT Ctrl-2
Hottip-Tg-1
Hottip-Tg-2
Runx1
Meis1
Pbx3
Myc
Twist1


G
LT-HSCs
WT Ctrl-1
WT Ctrl-2
Hottip-Tg-1
Hottip-Tg-2
ST-HSCs
WT Ctrl-1
WT Ctrl-2
Hottip-Tg-1
Hottip-Tg-2
Hoxb
b9
b8
b7
b5
b4
b3
b2
Hoxb1


H
HOTTIP expression (Relative to GAPDH)
0.006
0.005
0.004
0.003
0.002
0.001
0
p=0.041
MLL
non-MLL


I
Cell proliferation (control =1)
1.2
1
0.8
0.6
0.4
0.2
0
p=0.00018
Control
ICG-001
Differentiation (control =1)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
p=0.012
Control
ICG-001


J
LTC-IC frequency (control=1)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
p=0.0059
MLL
non-MLL


K
Engraftment % of mice
50
45
40
35
30
25
20
15
10
5
0
Control
ICG-001
BM
Spleen
Liver

Supplementary Figure 8. Related to Figure 8; Transgenic expression of *Hottip* lncRNA remodels chromatin structure and alters hematopoietic transcription programs. (A) ChIRP-RT-qPCR analysis of the *Hottip* RNA enrichment at the *Hoxa7, Hoxa9* and other hematopoietic/leukemia specific genes in the purified BM HSCs (Including LT-HSCs and ST-HSCs) compared WT and *Hottip*-Tg mice. Data is presented as mean ± SD from three to four independent experiments; *p<0.05; **p<0.01 by Student's t-test. **(B)** Scatter blot of RNA-seq analysis of more than two folds of differentially expressed genes upon overexpression of *Hottip* lincRNA in BM ST-HSC populations of the *Hottip*-Tg mice as compared to WT control. **(C)** Heat map analysis for changed expression of representative genes associated with hematopoiesis and leukemogenesis upon *Hottip* overexpression in BM ST-HSC populations of the *Hottip* Tg mice as compared to WT control (Cutoff: Fold change ≥ 2; q value < 0.05). **(D)** ATAC-seq analysis of chromatin accessibility in the *Hoxa* locus compared the WT control and *Hottip* Tg mouse BM ST-HSCs. **(E)** ATAC-seq promoter density map of ST-HSCs sorted from WT and *Hottip* transgenic BM. Significant upregulated ATAC-seq promoter peaks correlate with GO terms of enriched pathways annotated by GREAT analysis compared between WT control and *Hottip* transgenic ST-HSCs (Top). Significant downregulated ATAC-seq promoter peaks correlate with GO terms of enriched pathways annotated by GREAT analysis compared between WT control and *Hottip* transgenic ST-HSCs (Bottom). **(F)** ATAC-seq analysis of *Hottip trans*-regulated genes important for hematopoiesis and leukemogenesis compared between WT control and the *Hottip*-Tg BM LT-HSC (Top) and ST-HSC (Bottom) populations. **(G)** ATAC-seq analysis of *Hoxb* locus compared between WT control and the *Hottip*-Tg BM LT-HSC (Top) and ST-HSC (Bottom) populations. **(H)** Expression of *HOTTIP* by RT-qPCR. 22 MLL-AML cell lines and primary samples and 7 non-MLL-AML primary samples were subjected to RT-qPCR, the *HOTTIP* expression of each sample were normalized to house-keeping gene GAPDH. The black bar represents the mean expression of each group. p value is determined by Students' t-test. **(I)** MLL-AML samples with high expression levels of *HOTTIP* were either treated with DMSO or ICG-001 (500 nM) and the cells were counted 5 days after treatment. The cell number of each sample was normalized to DMSO control (Left) or the cells were stained with 0.1% NBT and the differentiated cells were NBT-positive. The percentage of NBT-positive cells of each sample was normalized to DMSO control (Right). p value is determined by Students' t-test. **(J)** Primary MLL-AML samples that exhibit high levels of *HOTTIP* expression and Non-MLL samples with very low or silent HOTTIP expression levels were either treated with DMSO or ICG-001 (500 nM) and the long-term culture initiating cells (LTC-IC) frequency of each group were determined. The data was normalized with DMSO control which is set to 1. p value is determined by Students' t-test. **(K)** The human engraftment of bone marrow, spleen and liver of leukemic mice were determined by positive on human CD45 and CD33 comparing vehicle (Control; red; n=4) and ICG-001 (50 mg/Kg; green; n=5).