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# Supplementary Materials for

## INTS11 regulates hematopoiesis by promoting PRC2 function

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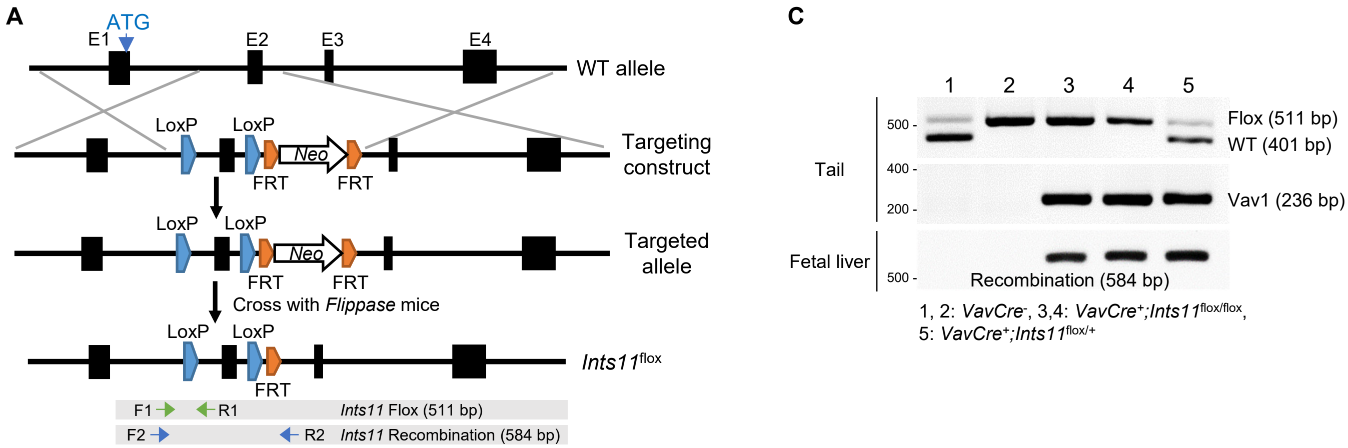



**B**

| | | *VavCre*⁻ | *VavCre*⁺;*Ints11*^flox/+ | *VavCre*⁺;*Ints11*^flox/flox |
|---|---|---|---|---|
| | Expected | 50% | 25% | 25% |
| E13.5 | Observed (n = 44) | 50.0% (n = 22) | 22.7% (n = 10) | 27.3% (n = 12) |
| E14.5 | Observed (n = 40) | 52.5% (n = 21) | 25.0% (n = 10) | 22.5% (n = 9) |
| E15.5 | Observed (n = 34) | 44.1% (n = 15) | 32.4% (n = 11) | 23.5% (n = 8) |
| E16.5 | Observed (n = 35) | 57.1% (n = 20) | 14.3% (n = 5) | 28.6% (n = 10) |
| E17.5 | Observed (n = 37) | 59.4% (n = 22) | 18.9% (n = 7) | 21.6% (n = 8) |
| E18.5 | Observed (n = 22) | 50.0% (n = 11) | 40.9% (n = 9) | 9.09% (n = 2) |

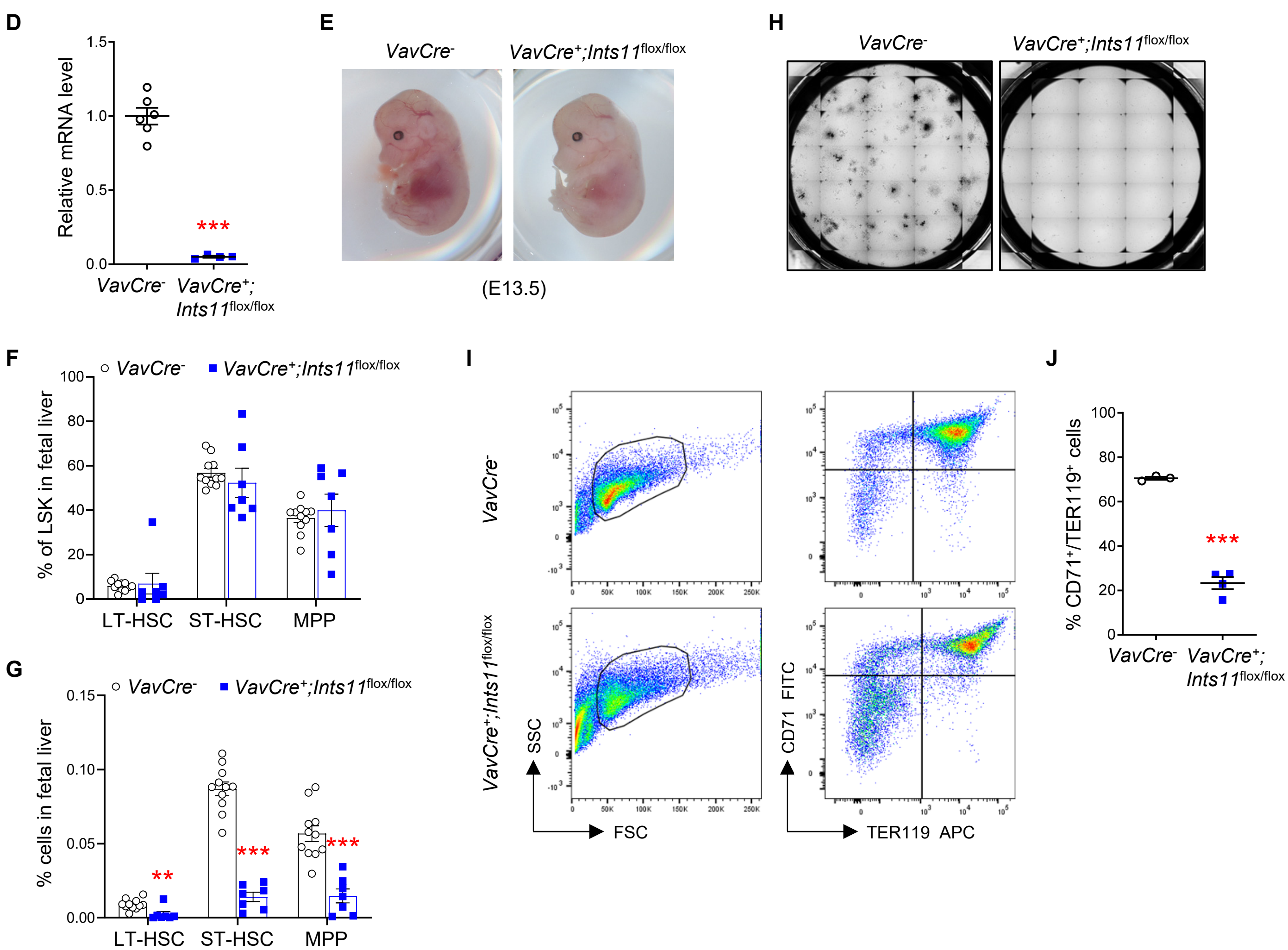


**Fig. S1. INTS11 is required for fetal hematopoiesis.** (A) Schematic of the targeted mouse *Ints11* allele. Targeting vector contained exon 2 flanked by LoxP sites (blue) and a neomycin resistance gene flanked by FRT (orange). The locations of primers are shown. (B) Genotype analysis of embryos after mating of *Vav1Cre*$^{+}$*;Ints11*$^{flox/+}$ (female) x *Vav1Cre*$^{-}$*;Ints11*$^{flox/flox}$ (male) mice. (C) Genotyping of *Vav1Cre*$^{+}$;*Ints11*$^{flox/flox}$ embryos and controls. (D) mRNA levels of *Ints11* in fetal liver CD45$^{+}$ cells from *Vav1Cre*$^{-}$ (*n* = 6) and *Vav1Cre*$^{+}$;*Ints11*$^{flox/flox}$ (*n* = 4) embryos at E13.5. (E) Representative images of E13.5 embryos from *Vav1Cre*$^{+}$*;Ints11*$^{flox/flox}$ and control littermates. Photo Credit: Peng Zhang, University of Texas Health Science Center at San Antonio. (F and G) Quantification of the percentages of LT-HSC, ST-HSC, and MPP cell populations from control (*n* = 11) and *Vav1Cre*$^{+}$*;Ints11*$^{flox/flox}$ (*n* = 7) fetal livers at E13.5. LT-HSC, Lin$^{-}$Sca1$^{+}$cKit$^{+}$CD34$^{-}$CD135$^{-}$; ST-HSC, Lin$^{-}$Sca1$^{+}$cKit$^{+}$CD34$^{+}$CD135$^{-}$; MPP, Lin$^{-}$Sca1$^{+}$cKit$^{+}$CD34$^{+}$CD135$^{+}$. (H) Representative images of colony formation for fetal liver cells from *Vav1Cre*$^{+}$*;Ints11*$^{flox/flox}$ and controls are shown. The images were taken on the 7$^{th}$ day of the assay. (I) Flow cytometric analysis of erythroid cells from control and *Vav1Cre*$^{+}$*;Ints11*$^{flox/flox}$ fetal livers at E13.5. (J) Quantification of the percentages of CD71$^{+}$/TER119$^{+}$ cells from control (*n* = 3) and *Vav1Cre*$^{+}$*;Ints11*$^{flox/flox}$ (*n* = 4) fetal livers at E13.5. Data are shown as the mean ± SEM. Unpaired Student's *t*-test; $^{**}P < 0.01$, $^{***}P < 0.001$.

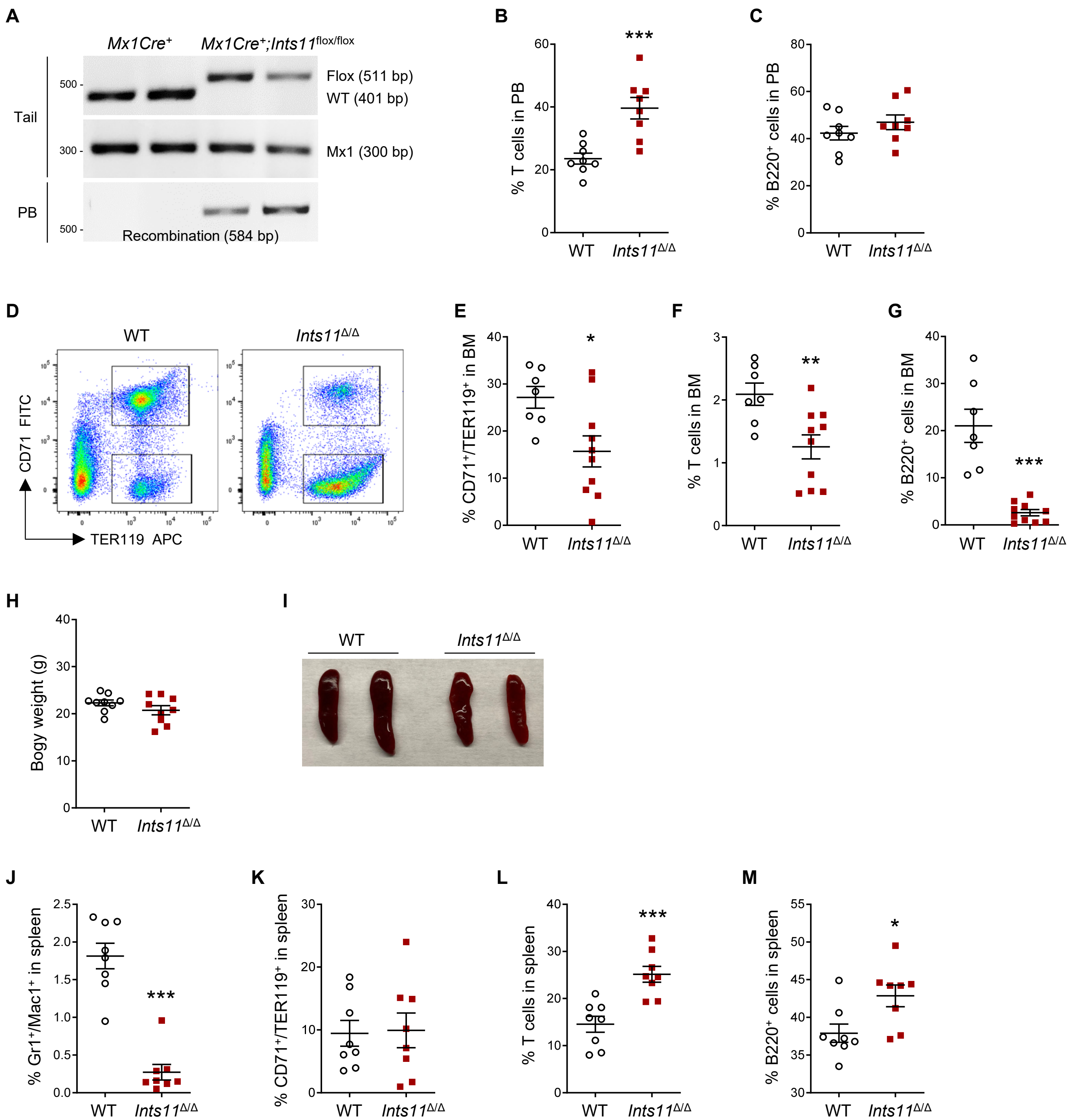

A
Mx1Cre+
Mx1Cre+;Ints11flox/flox
Tail
500
300
Flox (511 bp)
WT (401 bp)
Mx1 (300 bp)
PB
500
Recombination (584 bp)
B
% T cells in PB
***
WT
Ints11Δ/Δ
C
% B220+ cells in PB
WT
Ints11Δ/Δ
D
WT
Ints11Δ/Δ
CD71 FITC
TER119 APC
E
% CD71+/TER119+ in BM
*
F
% T cells in BM
**
G
% B220+ cells in BM
***
H
Bogy weight (g)
I
WT
Ints11Δ/Δ
J
% Gr1+/Mac1+ in spleen
***
K
% CD71+/TER119+ in spleen
L
% T cells in spleen
***
M
% B220+ cells in spleen
*

**Fig. S2. INTS11 deletion leads to hematopoietic failure.** (A) Genotyping of *Mx1Cre*$^{+}$;*Ints11*$^{flox/flox}$ (*Ints11*$^{\Delta/\Delta}$) and *Mx1Cre*$^{+}$ (WT) control mice. (B and C) Frequencies of T (CD4$^{+}$, CD8$^{+}$, and CD4$^{+}$/CD8$^{+}$, B) and B cells (B220$^{+}$, C) in PB from WT and *Ints11*$^{\Delta/\Delta}$ mice ($n$ = 8 per genotype) 12 days after poly(I:C) injection. (D) Flow cytometric analysis of erythroid cells in BM from WT and *Ints11*$^{\Delta/\Delta}$ mice 12 days after poly(I:C) injection. (E-G) Frequencies of erythroid (CD71$^{+}$/TER119$^{+}$, E), T (CD4$^{+}$, CD8$^{+}$, and CD4$^{+}$/CD8$^{+}$, F) and B cells (B220$^{+}$, G) in BM from WT ($n$ = 7) and *Ints11*$^{\Delta/\Delta}$ mice ($n$ = 10) are shown. (H) Body weight of WT and *Ints11*$^{\Delta/\Delta}$ mice ($n$ = 9 per genotype) 12 days after first poly(I:C) injection. (I) The gross appearance of spleen from WT and *Ints11*$^{\Delta/\Delta}$ mice. Photo Credit: Peng Zhang, University of Texas Health Science Center at San Antonio. (J-M) Frequencies of myeloid cells (Gr1$^{+}$/Mac1$^{+}$, J), erythroid cells (CD71$^{+}$/TER119$^{+}$, K), T cells (CD4$^{+}$, CD8$^{+}$, and CD4$^{+}$/CD8$^{+}$, L), and B cells (B220$^{+}$, M) in the spleens from WT and *Ints11*$^{\Delta/\Delta}$ mice ($n$ = 8 per genotype) 12 days after poly(I:C) injection. Data are shown as the mean ± SEM. Unpaired Student's *t*-test; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

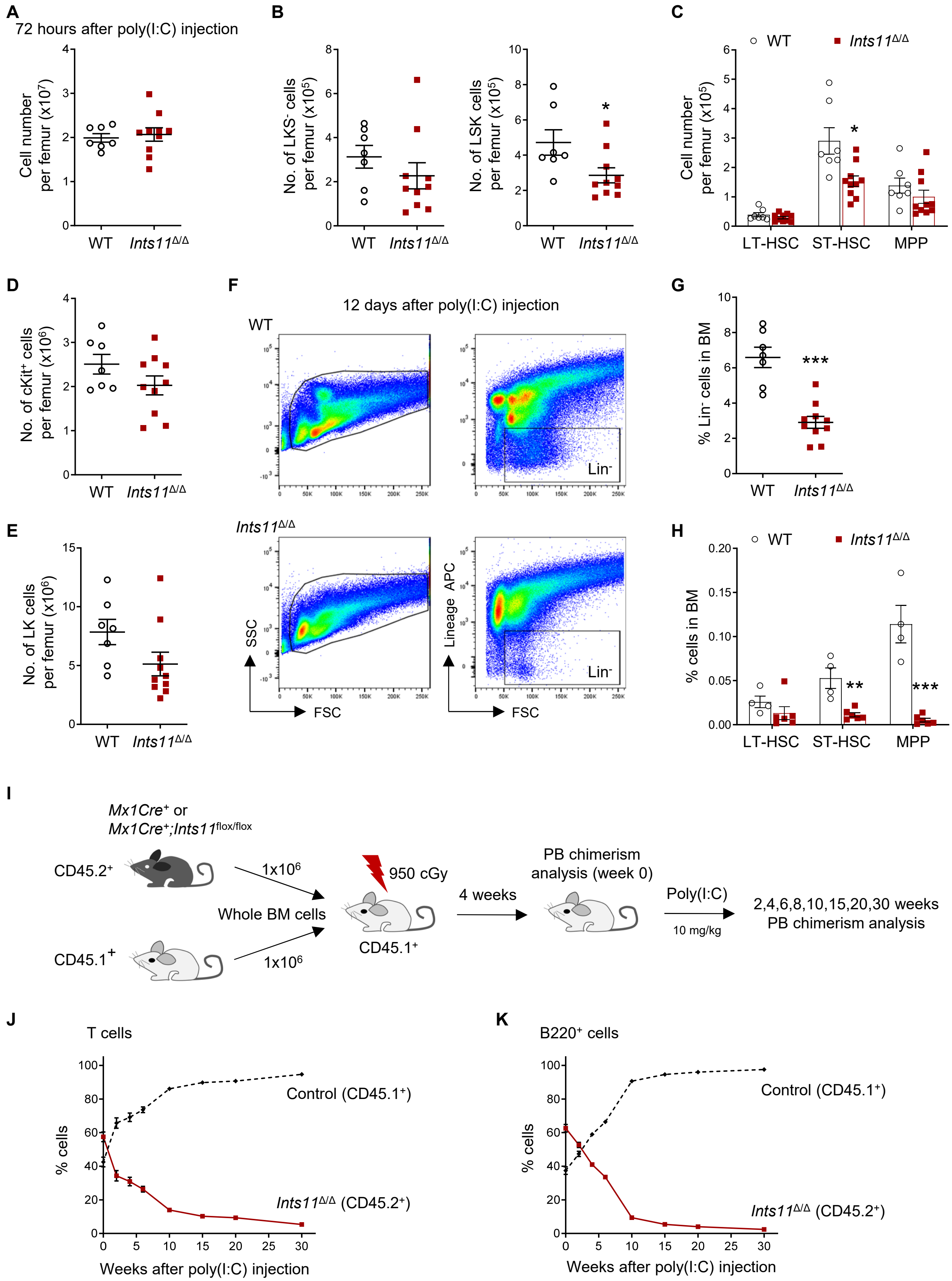

A
72 hours after poly(I:C) injection
Cell number per femur (x10^7)
WT
Ints11^Δ/Δ
B
No. of LKS- cells per femur (x10^5)
No. of LSK cells per femur (x10^5)
C
WT
Ints11^Δ/Δ
Cell number per femur (x10^5)
LT-HSC
ST-HSC
MPP
D
No. of cKit+ cells per femur (x10^6)
E
No. of LK cells per femur (x10^6)
F
12 days after poly(I:C) injection
WT
Ints11^Δ/Δ
SSC
FSC
Lineage APC
Lin-
G
% Lin- cells in BM
H
% cells in BM
I
Mx1Cre+ or
Mx1Cre+;Ints11^flox/flox
CD45.2+
CD45.1+
1x10^6
Whole BM cells
950 cGy
CD45.1+
4 weeks
PB chimerism analysis (week 0)
Poly(I:C)
10 mg/kg
2,4,6,8,10,15,20,30 weeks PB chimerism analysis
J
T cells
% cells
Control (CD45.1+)
Ints11^Δ/Δ (CD45.2+)
Weeks after poly(I:C) injection
K
B220+ cells
Control (CD45.1+)
Ints11^Δ/Δ (CD45.2+)
Weeks after poly(I:C) injection

**Fig. S3. Loss of *Ints11* results in HSPC defects and impairs hematopoietic reconstitution.** (A) BM cellularity of WT ($n$ = 7) and *Ints11*$^{\Delta/\Delta}$ mice ($n$ = 10) 72 hours after first poly(I:C) injection. (B-E) Absolute number of HSPC populations from WT ($n$ = 7) and *Ints11*$^{\Delta/\Delta}$ mice ($n$ = 10) 72 hours after poly(I:C) injection are shown. (F) Flow cytometric analysis of lineage negative (Lin$^-$) cells in BM from WT and *Ints11*$^{\Delta/\Delta}$ mice 12 days after first poly(I:C) injection. (G) Quantification of the percentage of Lin$^-$ cells in total BM cells from WT ($n$ = 7) and *Ints11*$^{\Delta/\Delta}$ mice ($n$ = 10) 12 days after poly(I:C) injection. (H) The percentages of LT-HSC, ST-HSC, and MPP in total BM cells from WT ($n$ = 4) and *Ints11*$^{\Delta/\Delta}$ mice ($n$ = 6) 12 days after poly(I:C) injection are shown. Data are shown as the mean ± SEM. Unpaired Student's $t$-test; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. (I) Schematic for competitive repopulation assay. CD45.2$^+$ BM cells from *Mx1Cre*$^+$ or *Mx1Cre*$^+$;*Ints11*$^{flox/flox}$ mice were mixed with equal numbers of CD45.1$^+$ competitor cells and transplanted into lethally irradiated CD45.1$^+$ recipient mice. *Ints11* deletion was induced upon confirmation of comparable engraftment rates. (J and K) The percentages of *Ints11*$^{\Delta/\Delta}$-derived (CD45.2$^+$) versus CD45.1$^+$ cells in the populations of T cell (J) and B cell (K) from PB of recipient animals at indicated time points ($n$ = 4).

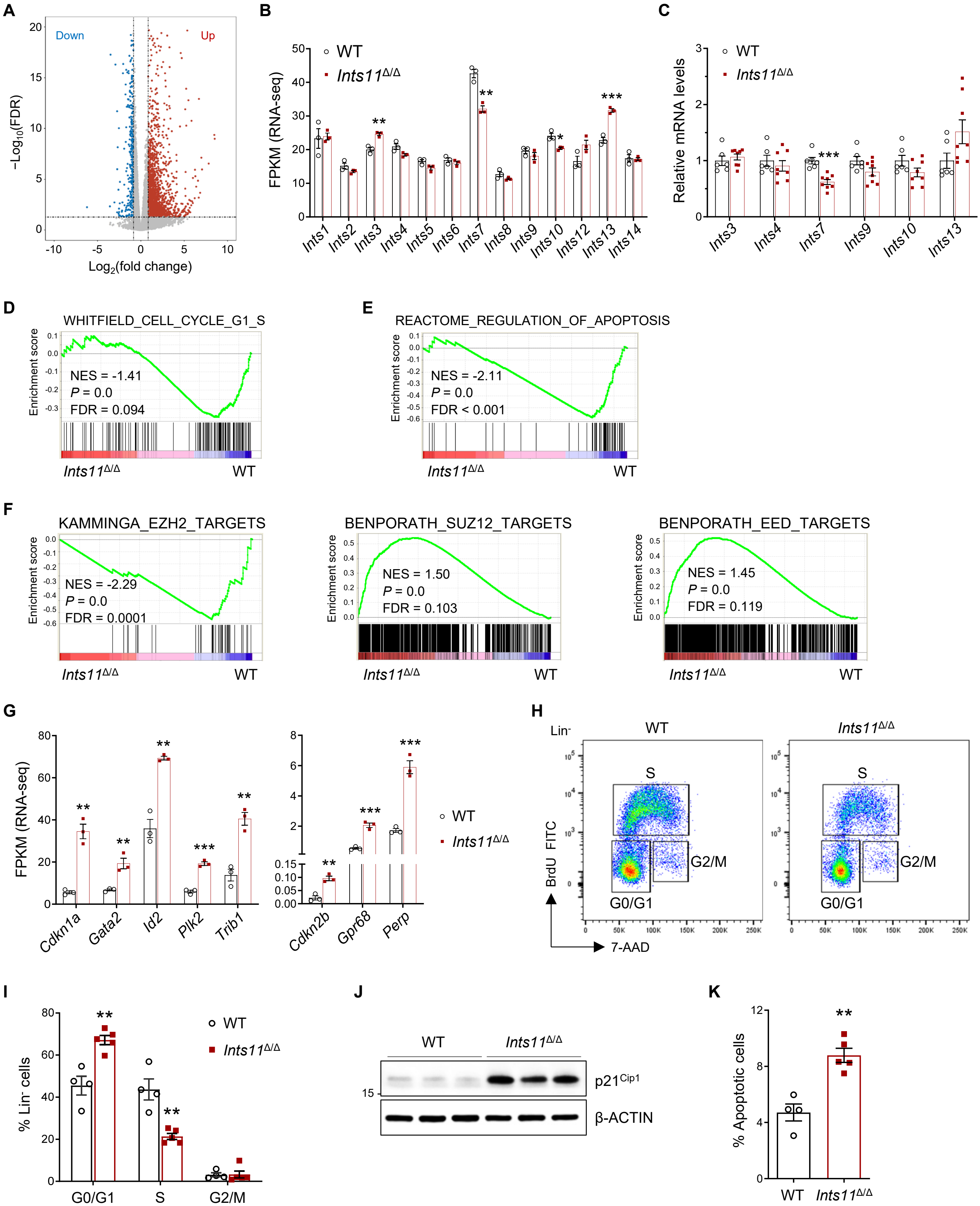

A
Down
Up
−Log10(FDR)
Log2(fold change)
B
WT
Ints11Δ/Δ
FPKM (RNA-seq)
Ints1
Ints2
Ints3
Ints4
Ints5
Ints6
Ints7
Ints8
Ints9
Ints10
Ints12
Ints13
Ints14
C
WT
Ints11Δ/Δ
Relative mRNA levels
Ints3
Ints4
Ints7
Ints9
Ints10
Ints13
D
WHITFIELD_CELL_CYCLE_G1_S
Enrichment score
NES = -1.41
P = 0.0
FDR = 0.094
Ints11Δ/Δ
WT
E
REACTOME_REGULATION_OF_APOPTOSIS
Enrichment score
NES = -2.11
P = 0.0
FDR < 0.001
Ints11Δ/Δ
WT
F
KAMMINGA_EZH2_TARGETS
Enrichment score
NES = -2.29
P = 0.0
FDR = 0.0001
Ints11Δ/Δ
WT
BENPORATH_SUZ12_TARGETS
Enrichment score
NES = 1.50
P = 0.0
FDR = 0.103
Ints11Δ/Δ
WT
BENPORATH_EED_TARGETS
Enrichment score
NES = 1.45
P = 0.0
FDR = 0.119
Ints11Δ/Δ
WT
G
FPKM (RNA-seq)
Cdkn1a
Gata2
Id2
Plk2
Trib1
Cdkn2b
Gpr68
Perp
WT
Ints11Δ/Δ
H
Lin-
WT
Ints11Δ/Δ
S
G2/M
G0/G1
BrdU FITC
7-AAD
I
% Lin- cells
WT
Ints11Δ/Δ
G0/G1
S
G2/M
J
WT
Ints11Δ/Δ
15
p21Cip1
β-ACTIN
K
% Apoptotic cells
WT
Ints11Δ/Δ

**Fig. S4. Loss of *Ints11* derepresses PRC2 target genes in HSPCs.** (A) Volcano plot showing the significantly dysregulated genes in *Ints11*$^{\Delta/\Delta}$ Lin$^{-}$cKit$^{+}$ (LK) cells compared with WT controls (FDR < 0.05 and |fold change| > 1.8). (B) Fragments per kilobase of transcript per million (FPKM) values of indicated members of the Integrator complex in RNA-seq are shown. (C) Relative mRNA levels of indicated members of the Integrator complex in LK cells are shown. (D-F) GSEA plots show that transcriptional signatures associated with cell-cycle transition (D), apoptosis (E), and PRC2 targets (F) are dysregulated in *Ints11*$^{\Delta/\Delta}$ LK cells. The NES, *P*-value and FDR are shown. (G) FPKM values of genes associated with cell-cycle, apoptosis, and PRC2 targets in RNA-seq are shown. (H and I) Cell-cycle analysis of HSPCs from WT ($n$ = 4) and *Ints11*$^{\Delta/\Delta}$ ($n$ = 5) mice 72 hours after poly(I:C) injection. Representative flow cytometry plots (H), and percentages of cells in each stage (I) are shown. (J) Western blot showing the protein level of p21$^{Cip1}$ in LK cells 72 hours after first poly(I:C) injection. (K) Apoptosis analysis of HSPCs from WT ($n$ = 4) and *Ints11*$^{\Delta/\Delta}$ ($n$ = 5) mice 72 hours after poly(I:C) injection by Annexin V/7-AAD staining. Data are shown as the mean ± SEM. Unpaired Student's $t$-test; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

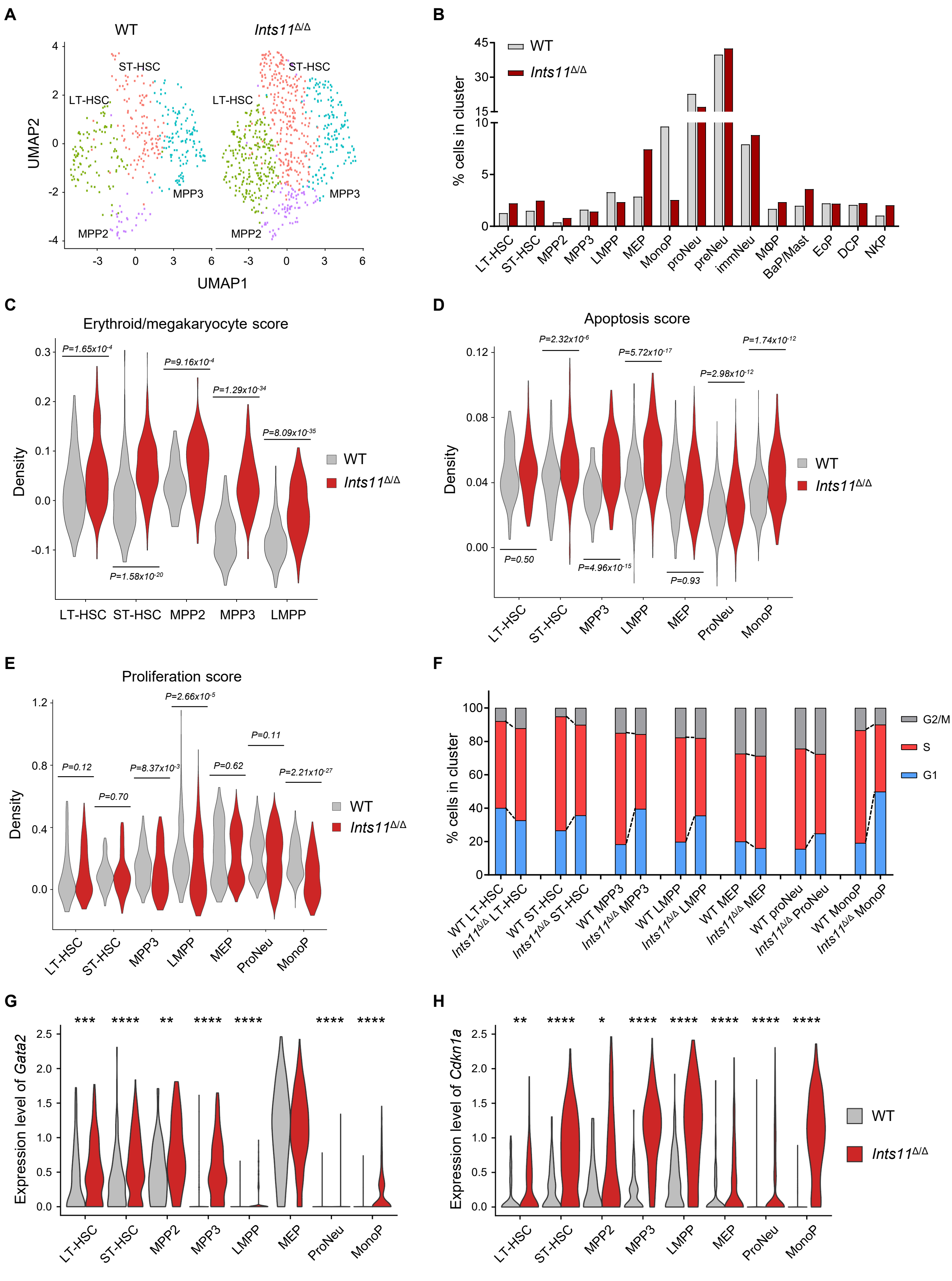

A
WT
Ints11Δ/Δ
ST-HSC
LT-HSC
MPP3
MPP2
UMAP2
UMAP1
B
WT
Ints11Δ/Δ
% cells in cluster
LT-HSC
ST-HSC
MPP2
MPP3
LMPP
MEP
MonoP
proNeu
preNeu
immNeu
MΦP
BaP/Mast
EoP
DCP
NKP
C
Erythroid/megakaryocyte score
Density
WT
Ints11Δ/Δ
D
Apoptosis score
E
Proliferation score
F
G2/M
S
G1
G
Expression level of Gata2
H
Expression level of Cdkn1a

**Fig. S5. Transcriptomic changes of HSPC populations in *Ints11*-deficient cKit$^+$ cells.** (A) UMAP visualization of LT-HSC, ST-HSC, MPP2, and MPP3 cells for WT and *Ints11*$^{\Delta/\Delta}$, respectively. Each dot represents one cell, and cluster identity is color-coded (Seurat). (B) Percentages of each hematopoietic cluster in WT and *Ints11*$^{\Delta/\Delta}$ cKit$^+$ cells are shown. (C-E) Violin plots of erythrocyte/megakaryocyte (C), apoptosis (D), and proliferation (E) transcription signatures in indicated populations are shown. The scores were calculated based on the expression values for genes in given gene sets. (F) Cell cycle phase distributions in different populations of WT and *Ints11*$^{\Delta/\Delta}$ cells. (G and H) Violin plots showing the log-transformed normalized expression levels of *Gata2* (G) and *Cdkn1a* (H) in HSPC populations. Mann-Whitney U test; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

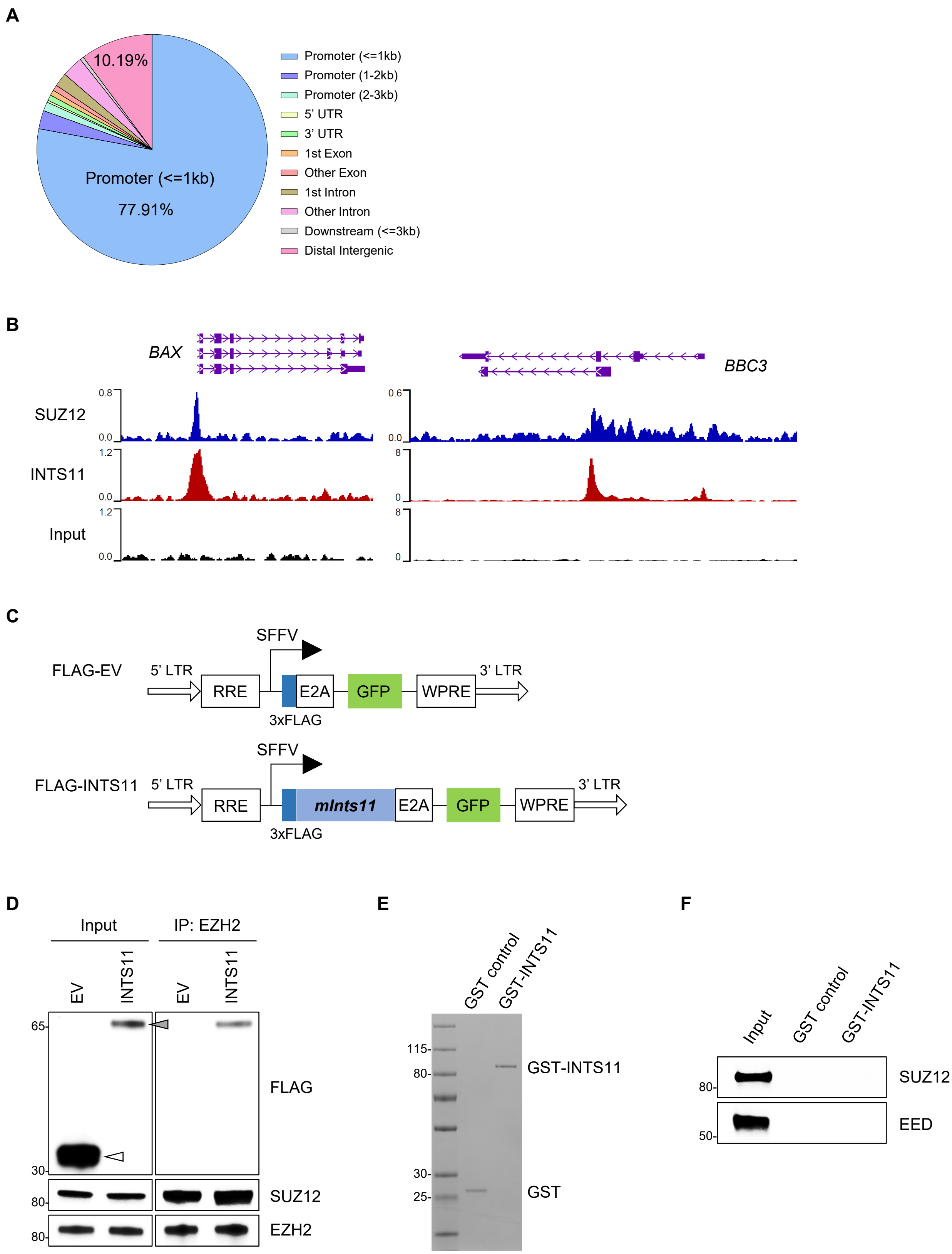
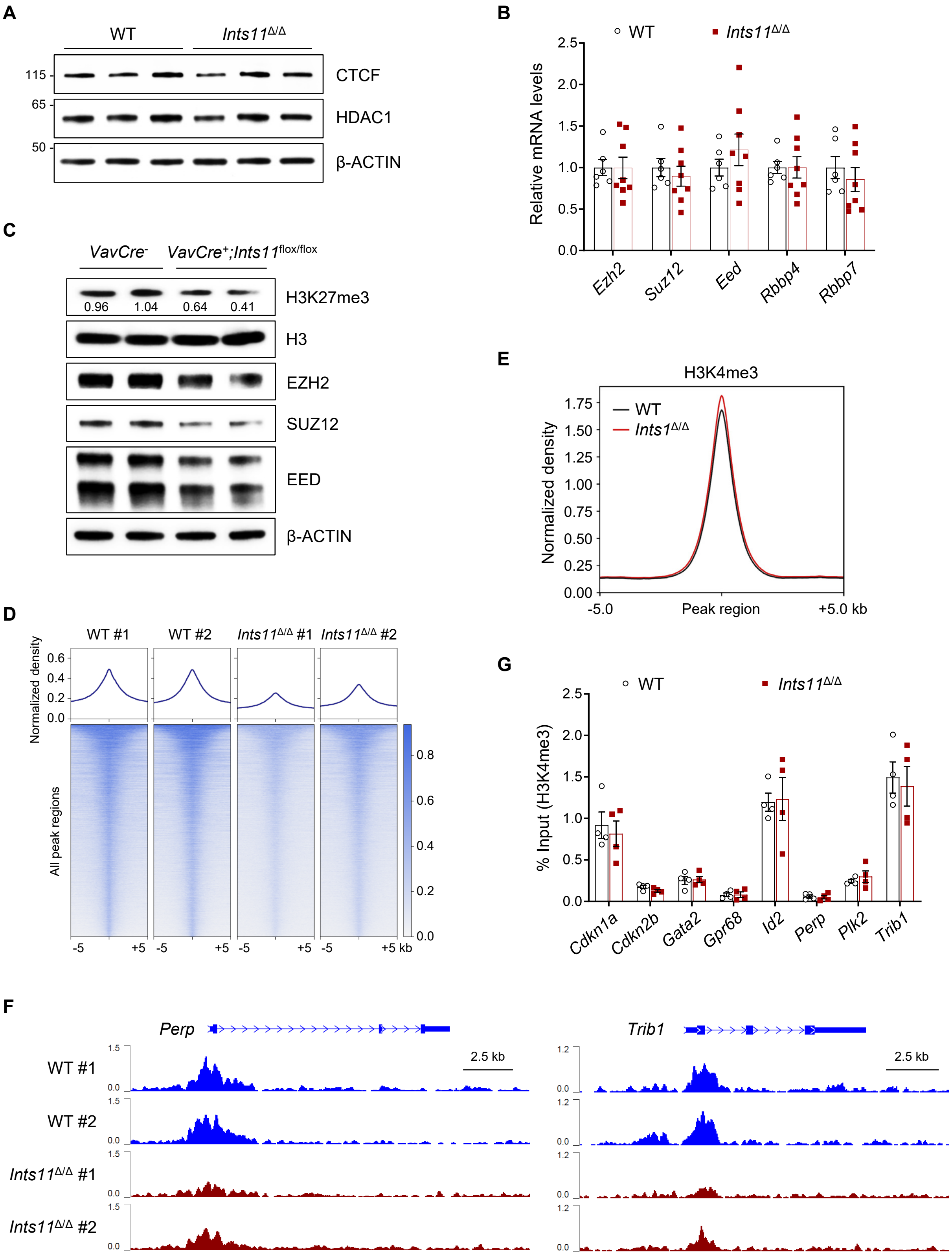
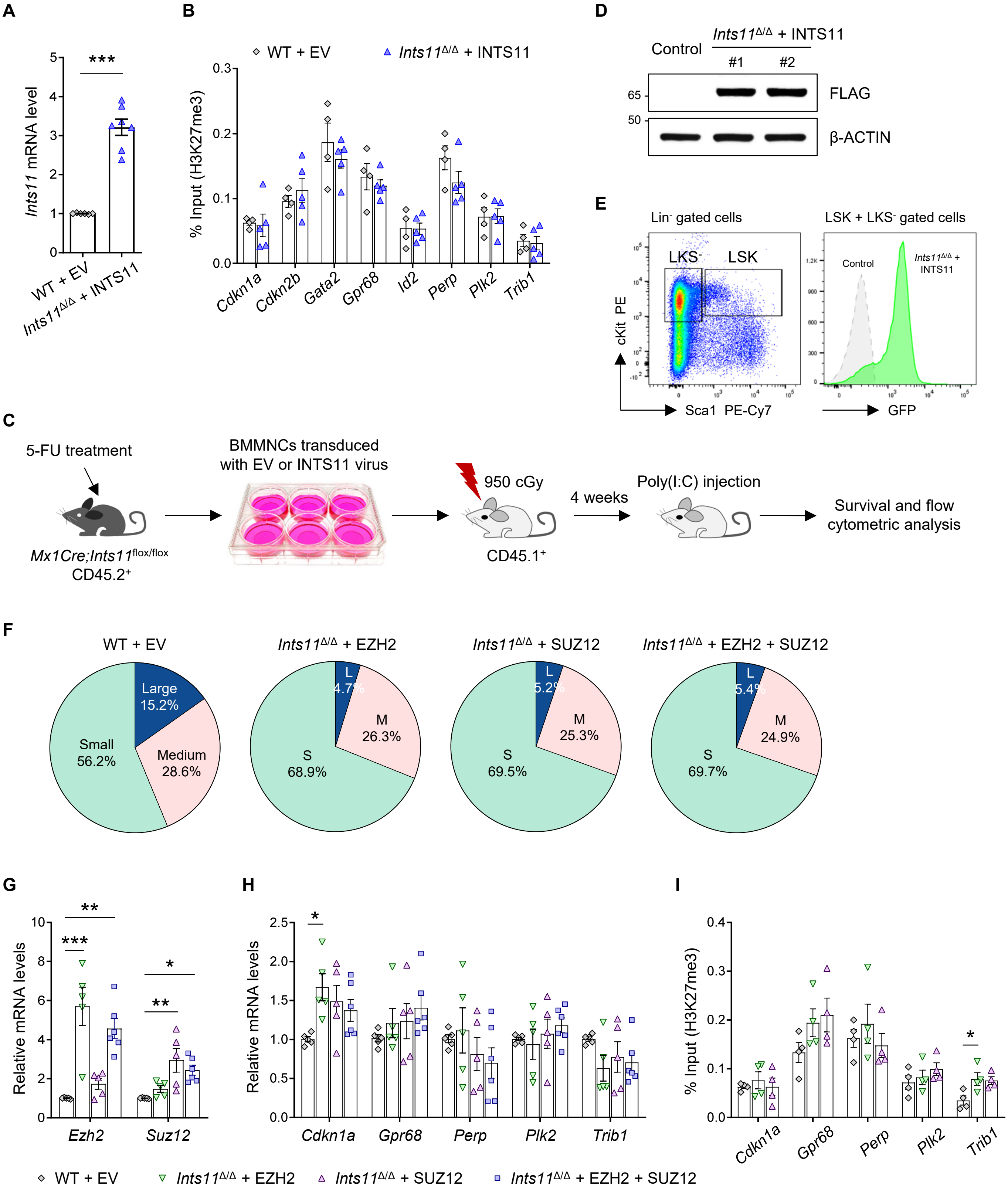

A
10.19%
Promoter (<=1kb)
77.91%
Promoter (<=1kb)
Promoter (1-2kb)
Promoter (2-3kb)
5' UTR
3' UTR
1st Exon
Other Exon
1st Intron
Other Intron
Downstream (<=3kb)
Distal Intergenic
B
BAX
BBC3
SUZ12
INTS11
Input
0.8
0.0
1.2
0.0
1.2
0.0
0.6
0.0
8
0
8
0
C
FLAG-EV
5' LTR
RRE
SFFV
3xFLAG
E2A
GFP
WPRE
3' LTR
FLAG-INTS11
5' LTR
RRE
SFFV
3xFLAG
mInts11
E2A
GFP
WPRE
3' LTR
D
Input
IP: EZH2
EV
INTS11
EV
INTS11
65-
30-
80-
80-
FLAG
SUZ12
EZH2
E
GST control
GST-INTS11
115-
80-
30-
25-
GST-INTS11
GST
F
Input
GST control
GST-INTS11
80-
50-
SUZ12
EED

**Fig. S6. INTS11 interacts with the PRC2 complex.** (A) Genome-wide distribution of INTS11-enriched regions in hematopoietic cells (GSE106359). Regions that overlap in the two biological replicates are preserved. (B) Representative ChIP-Seq tracks show INTS11 and SUZ12 co-occupy the gene promoters in hematopoietic cells. (C) Schematic representation of the lentiviral vectors expressing mouse *Ints11* gene (FLAG-INTS11) and the empty vector (EV) control. RRE, Rev Response Element. SFFV, spleen focus-forming virus. WPRE, woodchuck hepatitis virus posttranscriptional regulatory element. (D) Reciprocal EZH2 IP from nuclear extracts of 293T cells transfected with FLAG-tagged INTS11 or EV, and representative immunoblot analysis is shown. Arrowhead indicates the FLAG-fusion protein. (E) Coomassie blue-stained gel shows the GST-INTS11 fusion protein and GST control. (F) The GST-INTS11 fusion protein was immobilized on glutathione-agarose beads and incubated with SUZ12 or EED, respectively. Immobilized GST protein was used as controls.



A
WT
Ints11^Δ/Δ
115
65
50
CTCF
HDAC1
β-ACTIN
B
WT
Ints11^Δ/Δ
Relative mRNA levels
2.5
2.0
1.5
1.0
0.5
0.0
Ezh2
Suz12
Eed
Rbbp4
Rbbp7
C
VavCre-
VavCre+;Ints11flox/flox
0.96
1.04
0.64
0.41
H3K27me3
H3
EZH2
SUZ12
EED
β-ACTIN
E
H3K4me3
WT
Ints1Δ/Δ
Normalized density
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-5.0
Peak region
+5.0 kb
D
WT #1
WT #2
Ints11^Δ/Δ #1
Ints11^Δ/Δ #2
Normalized density
0.6
0.4
0.2
0.0
All peak regions
0.8
0.6
0.4
0.2
0.0
-5
+5
-5
+5
-5
+5
-5
+5 kb
G
WT
Ints11^Δ/Δ
% Input (H3K4me3)
2.5
2.0
1.5
1.0
0.5
0.0
Cdkn1a
Cdkn2b
Gata2
Gpr68
Id2
Perp
Plk2
Trib1
F
Perp
Trib1
2.5 kb
2.5 kb
WT #1
WT #2
Ints11^Δ/Δ #1
Ints11^Δ/Δ #2
1.5
0.0
1.2
0.0

**Fig. S7. INTS11 deletion reduces the H3K27me3 enrichment by destabilizing the PRC2 complex**. (A) The protein levels of CTCF and HDAC1 in LK cells from WT and *Ints11*$^{\Delta/\Delta}$ mice 72 hours after first poly(I:C) injection are shown. (B) qPCR showing the mRNA expression levels of indicated members of PRC2 complex in LK cells. (C) Western blot analysis of H3K27me3 and indicated subunits of the PRC2 complex in fetal liver cells from *Vav1Cre*$^{-}$ and *Vav1Cre*$^{+}$;*Ints11*$^{flox/flox}$ embryos at E13.5. H3 and β-ACTIN were used as loading controls. (D) Heatmaps of normalized H3K27me3 ChIP-seq read densities centered on the midpoints of all H3K27me3 regions. Each row represents a single region. (E) Global levels of H3K4me3 at peaks and 5-kb regions surrounding the peak midpoint. The coverages were normalized by the sequencing depth and averaged in 2 biological replicates. (F) Normalized H3K27me3 signals on *Perp* and *Trib1* gene loci are shown. (G) ChIP-qPCR showing the enrichment of H3K4me3 at the promoters of the genes in Fig. 4F ($n$ = 4 per genotype). Data are shown as the mean ± SEM.



A
B
C
D
E
F
G
H
I
***
Ints11 mRNA level
WT + EV
Ints11Δ/Δ + INTS11
WT + EV
Ints11Δ/Δ + INTS11
% Input (H3K27me3)
Cdkn1a
Cdkn2b
Gata2
Gpr68
Id2
Perp
Plk2
Trib1
Control
Ints11Δ/Δ + INTS11
#1
#2
65
50
FLAG
β-ACTIN
Lin- gated cells
LSK + LKS- gated cells
LKS-
LSK
cKit PE
Sca1 PE-Cy7
Control
Ints11Δ/Δ + INTS11
GFP
5-FU treatment
Mx1Cre;Ints11flox/flox
CD45.2+
BMMNCs transduced with EV or INTS11 virus
950 cGy
CD45.1+
4 weeks
Poly(I:C) injection
Survival and flow cytometric analysis
WT + EV
Large 15.2%
Medium 28.6%
Small 56.2%
Ints11Δ/Δ + EZH2
L 4.7%
M 26.3%
S 68.9%
Ints11Δ/Δ + SUZ12
L 5.2%
M 25.3%
S 69.5%
Ints11Δ/Δ + EZH2 + SUZ12
L 5.4%
M 24.9%
S 69.7%
Relative mRNA levels
Ezh2
Suz12
**
***
**
*
Relative mRNA levels
*
Cdkn1a
Gpr68
Perp
Plk2
Trib1
% Input (H3K27me3)
*
Cdkn1a
Gpr68
Perp
Plk2
Trib1
WT + EV
Ints11Δ/Δ + EZH2
Ints11Δ/Δ + SUZ12
Ints11Δ/Δ + EZH2 + SUZ12

**Fig. S8. Re-expression of INTS11 increases the PRC2 activity and rescues the *Ints11*-deficient phenotypes.** (A) qPCR showing the mRNA levels of *Ints11* in *Ints11*$^{\Delta/\Delta}$ cells transduced with FLAG-INTS11. Each dot represents an individual mouse (*n* = 6 to 7 per group). (B) The enrichment of H3K27me3 at the promoters of the indicated genes in *Ints11*$^{\Delta/\Delta}$ cells transduced with FLAG-INTS11 (*n* = 4 to 5 per group). (C) Schematic representation of *in vivo* rescue assay. BM mononuclear cells from 5-FU treated *Mx1Cre*$^{+}$;*Ints11*$^{\text{flox/flox}}$ mice (CD45.2$^{+}$) were transduced with a lentiviral vector (FLAG-INTS11 or EV control) and then transplanted into lethally irradiated CD45.1$^{+}$ recipient mice. *Ints11* deletion was induced after 4 weeks of transplantation. (D) Western blotting showing the expression of FLAG-INTS11 in cKit$^{+}$ cells from recipient receiving *Ints11*$^{\Delta/\Delta}$ cells transduced with FLAG-INTS11. (E) Flow cytometric analysis of HSPC populations from recipients receiving *Ints11*$^{\Delta/\Delta}$ cells transduced with FLAG-INTS11 after 8 weeks of poly(I:C) injection. The population of HSPCs (LSK and LKS$^{-}$) was restored and confirmed the expression of GFP in the recipient mice. (F) Pie-charts showing the proportion of colony size in *Ints11*-deficient cKit$^{+}$ cells transduced with PRC2 subunits (EZH2 and/or SUZ12). (G and H) qPCR showing the mRNA levels of *Ezh2* and *Suz12* (G) and PRC2 targets (H) in *Ints11*$^{\Delta/\Delta}$ cells transduced with EZH2 and/or SUZ12. Each dot represents an individual mouse (*n* = 5 to 6 per group). (I) The levels of H3K27me3 at the promoters of indicated PRC2 target genes are shown (*n* = 4 per group). Data are shown as the mean ± SEM. Unpaired Student's *t*-test, or one-way ANOVA with Tukey's multiple comparisons test; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

**Table S1.** **Primers used in this study.**

| Name | Forward | Reverse |
|---|---|---|
| Genotyping PCR | | |
| *Ints11-flox* | ATGCCTACGTAAGGAAAGCAGACT | TGAGGTCTAGTGTTGAATGAAGCC |
| *Ints11-Rec* | GATGGCTCCTCAGCGTCATT | CTAGGCCACTGCCGGTTTAT |
| qPCR | | |
| *Ints11* | TCATGTTGGACTGTGGGATG | TGGGTGATGTAAGAAAAGTCAGG |
| *Ints3* | GAGTTACAGAAGGGAAAGGGGA | ACTGGTTGAAAGTAGCAGCCT |
| *Ints4* | CACCTGAAAAAGCGGGTGTAT | AATCGGAGCTTCTTGGTAGCA |
| *Ints7* | TCCGCTCTTATGGAACTGGAC | AGGGAACGGGTACTTCTGGAA |
| *Ints9* | CCCCAGGCTATCTAATCTTCCC | AATCCACAAATACATGACCGGAG |
| *Ints10* | ACACCATCGAACGGAATGCC | GCTGGTCCGGGAAATTCACAA |
| *Ints13* | GTTGTGGATCACTGCCCATAC | GGGAGCCAAAGGGATGATGC |
| *Ezh2* | ACTTCTGTGAGCTCATTGCG | CGACTGCATTCAGGGTCTTT |
| *Suz12* | CCAACGATAAGTCTACAGCTCC | GTAGTCAGCGTCTCCTTAACAG |
| *Eed* | TCCACTTTCCTGACTTTTCTACC | CCATTTTGCCAGGTTTCCAG |
| *Rbbp4* | GACGACGCAGTGGAAGAAC | CTGGGCAGTTAAGCTGGGC |
| *Rbbp7* | GAGCGTGTCATCAACGAAGAG | CCACTGAACGGTAAGACTGGG |
| *Cdkn1a* | CCTGGTGATGTCCGACCTG | CCATGAGCGCATCGCAATC |
| *Cdkn2b* | CCCTGCCACCCTTACCAGA | CAGATACCTCGCAATGTCACG |
| *Gata2* | ATACCCACCTATCCCTCCTATG | AGCCTTGCTTCTCTGCTTAG |
| *Gpr68* | TATCTTGCCCCATCGACCACA | AGTACCCGAAGTAGAGGGACA |
| *Id2* | ATGAAAGCCTTCAGTCCGGTG | AGCAGACTCATCGGGTCGT |
| *Perp* | ATGGAGTACGCATGGGGAC | GGCGAAGAACGAGAGAATGAAG |
| *Plk2* | AGCCAGAAGTCCGATACTACC | TCCCTAGCTTGAGATCCCTGT |
| *Trib1* | AGAACCCAGCTTAGACTGGAA | AAAAGCGTATAGAGCATCACCC |
| ChIP-qPCR | | |
| *Cdkn1a* | GCCCAAAGCGTGAGAATGAA | AAACAGGGCACTTGGTTCAC |
| *Cdkn2b* | GAAACATCCCTCGGCACTAG | GAGACATTGCTCCCAGATAGC |
| *Gata2* | GTCCACAATCCCTAGACTCATG | AGCCCAAATCCAACTGACTC |
| *Gpr68* | TCTCTACCTTCCTCCCAGCT | TCCTGACCTAGAGACTGGCT |
| *Id2* | GCCTTTTATCCTCTTTCTCCCC | CACAAGCACATTACCGAAACG |
| *Perp* | ACCAAAGCTACCCGGATTAAG | CGGATCTACCTTTGGACCTTG |
| *Plk2* | CTTGGGCTCTCACTATCTGATC | TCCAGTTTTCTCGTTTCCCC |
| *Trib1* | TGGGTTTGGCAGAGCAGATA | CCACAGGAAAGCACTCAACC |

**Table S2. Detailed list of reagents used in this study.**

| Reagent or Resource | Source | Identifier |
|---|---|---|
| Antibodies | | |
| Mouse lineage antibody cocktail APC | BD Pharmingen | Cat# 558074; RRID: AB_1645213 |
| Rat monoclonal anti-mouse CD117 (cKit) PerCP-Cy5.5 | BD Pharmingen | Cat# 560557; RRID: AB_1645258 |
| Rat monoclonal anti-mouse CD117 (cKit) PE | BD Pharmingen | Cat# 553869; RRID: AB_395103 |
| Rat monoclonal anti-mouse CD117 (cKit) APC | BD Pharmingen | Cat# 553356; RRID: AB_398536 |
| Rat monoclonal anti-mouse Ly-6A/E (Sca1) PE-Cy7 | BD Pharmingen | Cat# 558162; RRID: AB_647253 |
| Rat monoclonal anti-mouse CD34 FITC | BD Pharmingen | Cat# 553733; RRID: AB_395017 |
| Rat monoclonal anti-mouse CD34 Alexa Fluor 700 | BD Pharmingen | Cat# 560518; RRID: AB_1727471 |
| Rat monoclonal anti-mouse CD135 BV421 | BD Pharmingen | Cat# 562898; RRID: AB_2737876 |
| Rat monoclonal anti-mouse CD16/32 APC-Cy7 | BioLegend | Cat# 101327; RRID: AB_1967102 |
| Hamster monoclonal anti-mouse CD48 FITC | BD Pharmingen | Cat# 557484; RRID: AB_396724 |
| Monoclonal anti-mouse CD150 PE | Thermo Fisher Scientific | Cat# 12-1502-82; RRID: AB_1548765 |
| Rat monoclonal anti-mouse CD71 FITC | BD Pharmingen | Cat# 553266; RRID: AB_394743 |
| Rat monoclonal anti-mouse CD71 PE | BD Pharmingen | Cat# 553267; RRID: AB_394744 |
| Rat anti-mouse TER-119 APC | BD Pharmingen | Cat# 557909; RRID: AB_398635 |
| Rat monoclonal anti-mouse Ly-6G and Ly-6C (Gr1) PerCP-Cy5.5 | BD Pharmingen | Cat# 552093; RRID: AB_394334 |
| Rat monoclonal anti-mouse Ly-6G and Ly-6C (Gr1) PE-Cy7 | BD Pharmingen | Cat# 565033; RRID: AB_2739049 |
| Rat monoclonal anti-mouse CD11b (Mac1) PE | BD Pharmingen | Cat# 553311; RRID: AB_394775 |
| Rat monoclonal anti-mouse CD4 PerCP-Cy5.5 | BD Pharmingen | Cat# 550954; RRID: AB_393977 |
| Rat monoclonal anti-mouse CD4 PE-Cy7 | BD Pharmingen | Cat# 552775; RRID: AB_394461 |
| Rat monoclonal anti-mouse CD8a PE | BD Pharmingen | Cat# 553033; RRID: AB_394571 |
| Rat monoclonal anti-mouse CD45R/B220 APC | BD Pharmingen | Cat# 553092; RRID: AB_398531 |
| Mouse monoclonal anti-mouse CD45.2 PerCP-Cy5.5 | BD Pharmingen | Cat# 552950; RRID: AB_394528 |
| Mouse monoclonal anti-mouse CD45.1 FITC | BD Pharmingen | Cat# 553775; RRID: AB_395043 |
| Mouse monoclonal anti-mouse CD45 PE-Cy7 | BD Pharmingen | Cat# 552848; RRID: AB_394489 |

| | | |
|---|---|---|
| 7-AAD | BD Pharmingen | Cat# 559925; RRID: AB_2869266 |
| Rabbit polyclonal anti-INTS11 | Bethyl Laboratories | Cat# A301-274A; RRID: AB_937779 |
| Rabbit polyclonal anti-INTS1 | Bethyl Laboratories | Cat# A300-361A; RRID: AB_2127258 |
| Rabbit polyclonal anti-INTS3 | Bethyl Laboratories | Cat# A302-050A; RRID: AB_1604272 |
| Rabbit polyclonal anti-INTS4 | Bethyl Laboratories | Cat# A301-296A; RRID: AB_937909 |
| Mouse monoclonal anti-β-Actin | MilliporeSigma | Cat# A2228; RRID: AB_476697 |
| Rabbit monoclonal anti-mouse p21 | Abcam | Cat# ab188224; RRID: AB_2734729 |
| Rabbit polyclonal anti-H3K27me2 | Cell Signaling Technology | Cat# 9728; RRID: AB_1281338 |
| Rabbit polyclonal anti-H3K27me3 | MilliporeSigma | Cat# 07-449; RRID: AB_310624 |
| Rabbit polyclonal anti-H3K4me3 | Diagenode | Cat# C15410003; RRID: AB_2616052 |
| Rabbit polyclonal anti-H3 | Abcam | Cat# ab1791; RRID: AB_302613 |
| Rabbit monoclonal anti-EZH2 | Cell Signaling Technology | Cat# 5246; RRID: AB_10694683 |
| Mouse monoclonal anti-EZH2 | MilliporeSigma | Cat# 17-662; RRID: AB_1977568 |
| Rabbit monoclonal anti-SUZ12 | Cell Signaling Technology | Cat# 3737; RRID: AB_2196850 |
| Rabbit monoclonal anti-EED | Cell Signaling Technology | Cat# 85322 |
| Rabbit polyclonal anti-RBBP4/7 | Cell Signaling Technology | Cat# 4633; RRID: AB_1904116 |
| Rabbit polyclonal anti-CTCF | MilliporeSigma | Cat# 07-729; RRID: AB_441965 |
| Rabbit polyclonal anti-HDAC1 | Thermo Fisher Scientific | Cat# PA1-860; RRID: AB_2118091 |
| Mouse monoclonal anti-FLAG M2 | MilliporeSigma | Cat# F3165; RRID: AB_259529 |
| Mouse monoclonal anti-HA | MilliporeSigma | Cat# H3663; RRID: AB_262051 |
| Mouse IgG, HRP-linked whole Ab (from sheep) | GE Healthcare | Cat# NA931; RRID: AB_772210 |
| Rabbit IgG, HRP-linked $F(ab')_2$ fragment (from donkey) | GE Healthcare | Cat# NA9340; RRID: AB_772191 |
| Bacterial and Virus Strains | | |
| NEB 5-alpha Competent E. coli | NEB | Cat# C2987H |
| BL21 (DE3) Competent E. coli | NEB | Cat# C2527H |
| One Shot Stbl3 Chemically Competent E. coli | Thermo Fisher Scientific | Cat# C737303 |
| Chemicals, Peptides, and Recombinant Proteins | | |
| Poly(I:C) | InvivoGen | Cat# tlrl-pic-5 |
| 5-fluorouracil | Selleckchem | Cat# S1209 |
| Methylcellulose medium MethoCult M3134 | STEMCELL Technologies | Cat# 03134 |

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|---|---|---|
| Recombinant Murine Stem Cell Factor | Peprotech | Cat# 250-03 |
| Recombinant Murine Interleukin 3 | Peprotech | Cat# 213-13 |
| Recombinant Murine Thrombopoietin | Peprotech | Cat# 315-14 |
| Recombinant Murine Granulocyte-Macrophage Colony-Stimulating Factor | Peprotech | Cat# 315-03 |
| Recombinant Human Erythropoietin | Peprotech | Cat# 100-64 |
| Recombinant Human Interleukin-6 | Peprotech | Cat# 200-06 |
| Mouse CD45 MicroBeads | Miltenyi Biotec | Cat# 130-052-301 |
| Mouse CD117 MicroBeads | Miltenyi Biotec | Cat# 130-091-224 |
| Mouse Direct Lineage Cell Depletion Kit | Miltenyi Biotec | Cat# 130-110-470 |
| Lipofectamine 3000 Transfection Reagent | Thermo Fisher Scientific | Cat# L3000075 |
| TRIzol Reagent | Thermo Fisher Scientific | Cat# 15596026 |
| Benzonase Nuclease | MilliporeSigma | Cat# E1014 |
| Protease Inhibitor Cocktail Tablets | MilliporeSigma | Cat# S8830 |
| Mouse monoclonal anti-FLAG M2 Affinity Gel | MilliporeSigma | Cat# A2220; RRID: AB_10063035 |
| Mouse monoclonal anti-HA Magnetic Beads | Thermo Fisher Scientific | Cat# 88837; RRID: AB_2861399 |
| Protein A/G PLUS-Agarose | Santa Cruz Biotechnology | Cat# sc-2003; RRID: AB_10201400 |
| Dynabeads Protein G | Thermo Fisher Scientific | Cat# 10004D |
| Pierce Glutathione Agarose | Thermo Fisher Scientific | Cat# 16100 |
| Recombinant EZH2 Protein Complex (human) | Active Motif | Cat# 31337 |
| Recombinant human EZH2 protein | OriGene Technologies | Cat# TP302054 |
| Recombinant human SUZ12 protein | OriGene Technologies | Cat# TP302362 |
| Recombinant human EED protein | OriGene Technologies | Cat# TP319261 |
| Prometheus ProSignal Pico ECL Reagent | Genesee Scientific | Cat# 20-300 |
| Prometheus ProSignal Femto ECL Reagent | Genesee Scientific | Cat# 20-302 |
| Critical Commercial Assays | | |
| FITC BrdU Flow Kit | BD Pharmingen | Cat# 559619; RRID: AB_2617060 |
| PE Annexin V Apoptosis Detection Kit I | BD Pharmingen | Cat# 559763; RRID: AB_2869265 |
| QIAfilter Plasmid Maxi Kit | Qiagen | Cat# 12263 |
| QuantiTect Reverse Transcription Kit | Qiagen | Cat# 205313 |
| Fast SYBR Green Master Mix | Thermo Fisher Scientific | Cat# 4385617 |
| Subcellular Protein Fractionation Kit | Thermo Fisher Scientific | Cat# 78840 |
| TruSeq Stranded mRNA Library Prep | Illumina | Cat# 20020594 |
| Chromium Single Cell 3′ Reagent Kit (v3) | 10x Genomics | Cat# PN-1000075 |
| Chromium Single Cell B Chip Kit (v3) | 10x Genomics | Cat# PN-1000154 |
| MicroPlex Library Preparation Kit (v2) | Diagenode | Cat# C05010013 |
| Deposited Data | | |
| Bulk RNA-Seq data | This paper | GEO: GSE159403 |
| Single-cell RNA-Seq data | This Paper | GEO: GSE159404 |
| ChIP-Seq data | This Paper | GEO: GSE159402 |
| INTS11 ChIP-Seq data | (*16*) | GEO: GSE106359 |
| SUZ12 ChIP-Seq data | (*29*) | GEO: GSE59090 |
| Experimental Models: Cell Lines | | |
| Human: HEK 293TN cell | System Biosciences | Cat# LV900A-1 |

| | | |
|---|---|---|
| Human: K562 cell | ATCC | Cat# CCL-243 |
| Experimental Models: Organisms/Strains | | |
| Mouse: B6.Cg-Commd10Tg(Vav1-icre)A2Kio/J (Vav1-Cre) | The Jackson Laboratory | JAX: 008610; RRID: IMSR_JAX:008610 |
| Mouse: B6.Cg-Tg(Mx1-cre)1Cgn/J (Mx1-Cre) | The Jackson Laboratory | JAX: 003556; RRID: IMSR_JAX:003556 |
| Mouse: B6.SJL-Ptprca Pepcb/BoyJ (BoyJ) | The Jackson Laboratory | JAX: 002014; RRID: IMSR_JAX:002014 |
| Mouse: Ints11 flox | This paper | |
| Recombinant DNA | | |
| pSFFV-FLAG-E2A-GFP | This Paper | N/A |
| pSFFV-FLAG-INTS11-E2A-GFP (mouse) | This Paper | N/A |
| pLV-CMV-HA-EZH2-CMV-mCherry (mouse) | This Paper | N/A |
| pLV-CMV-HA-SUZ12-CMV-GFP (mouse) | This Paper | N/A |
| pET-GST-INTS11 (human) | This Paper | N/A |
| Other | | |
| Superose 6 Increase 10/300 GL | Cytiva | Cat# 29091596 |