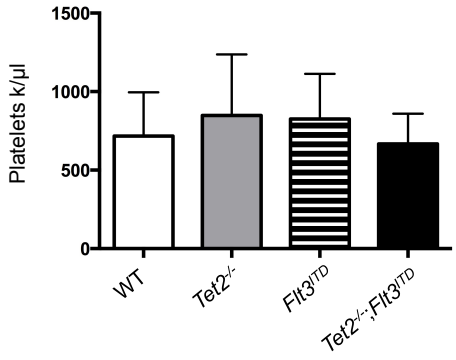
Supplemental Data

A



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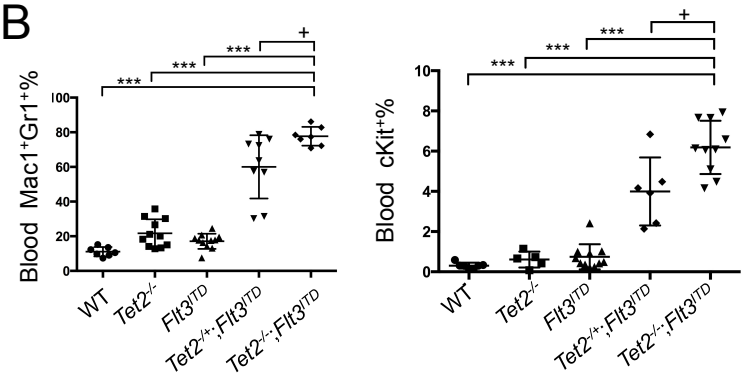

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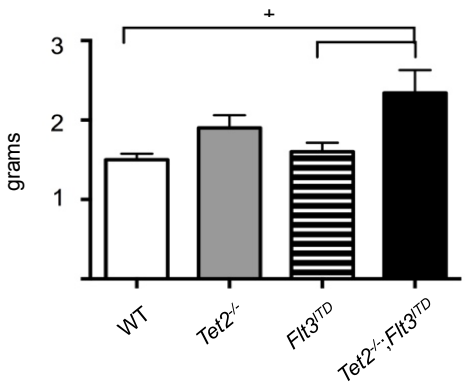

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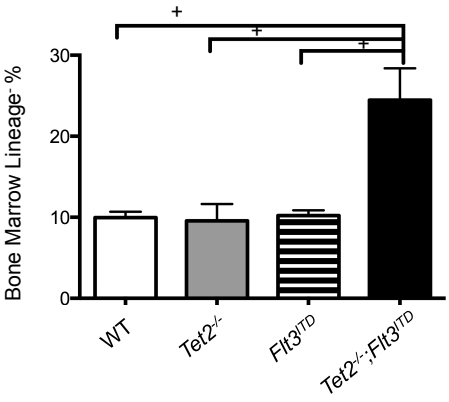

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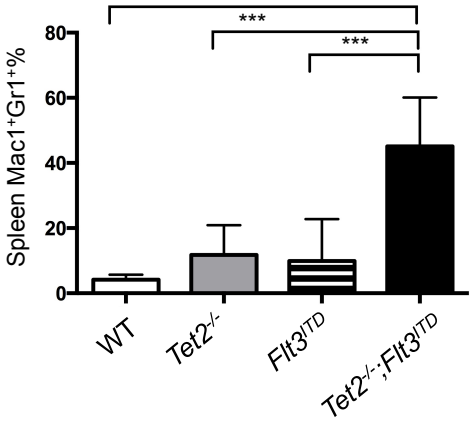

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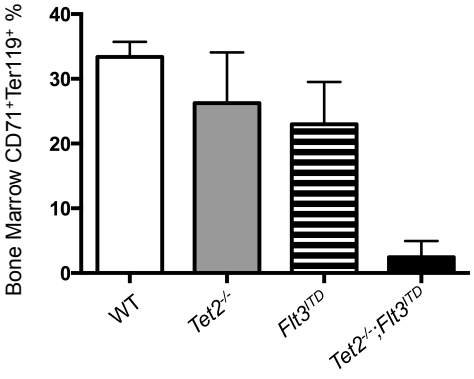

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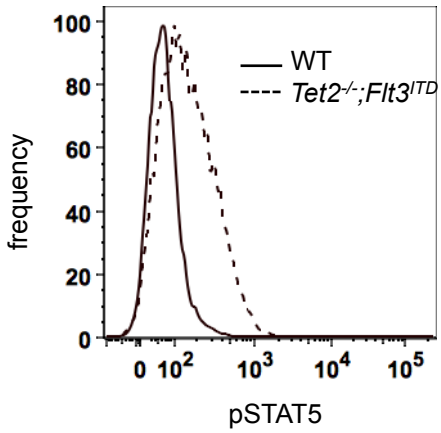
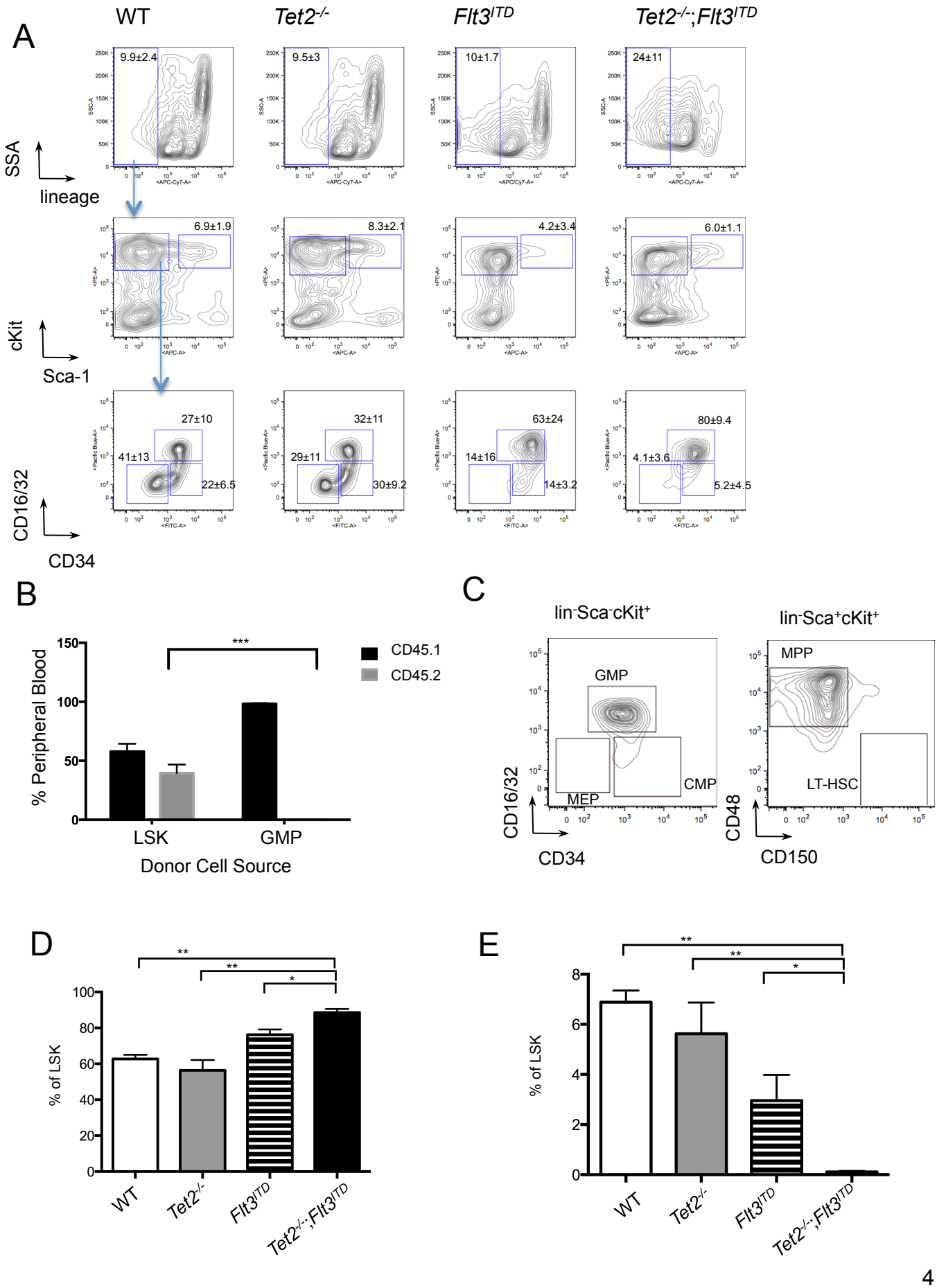


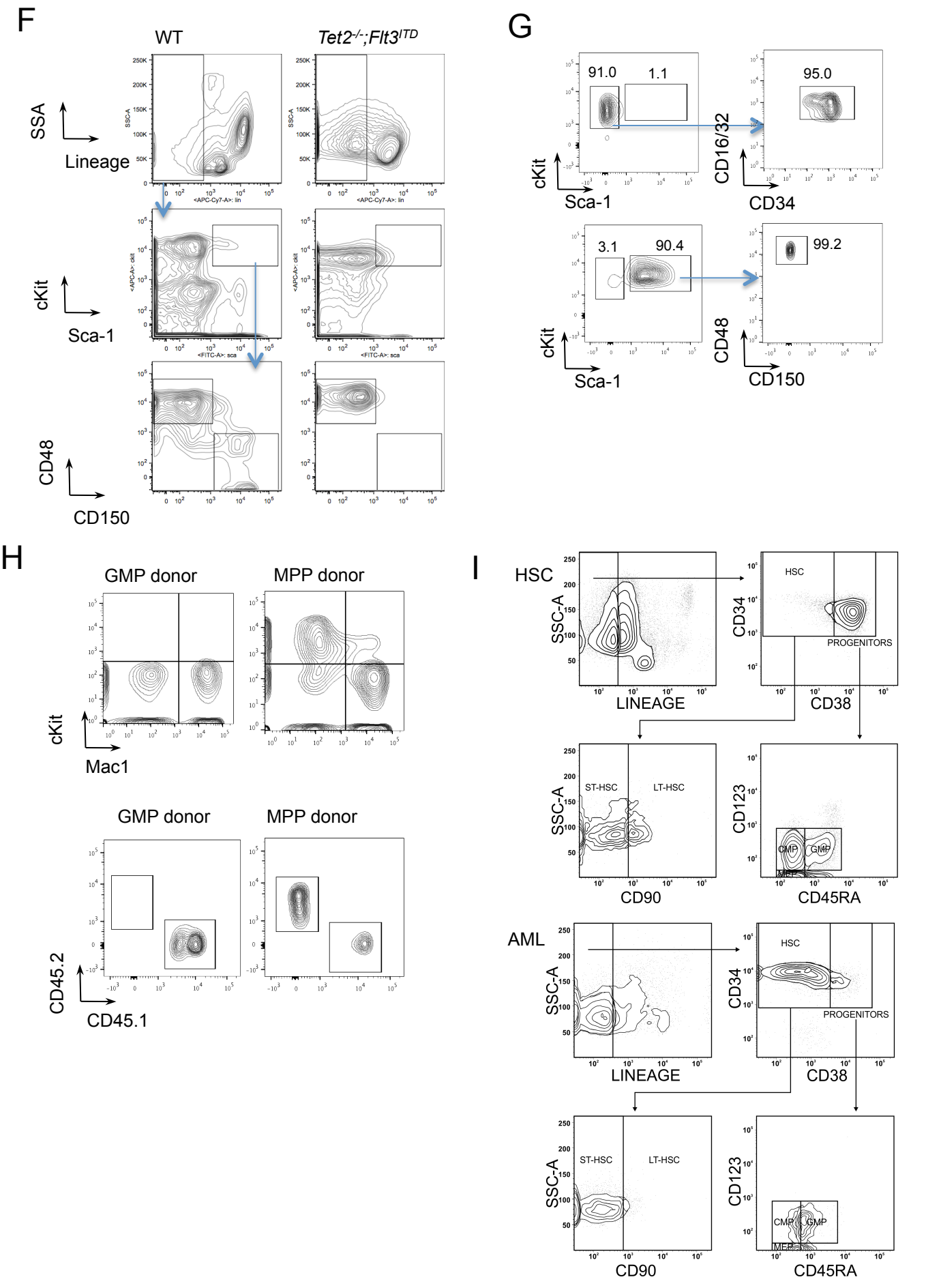
H

CHROM	POS	REF	ALT	GENE NAME	SNPEFF_EFFECT	SNPEFF_AA_CHANGE	T Alt	T Ref	N Alt	N Ref
Tumor1										
5	129022952	G	A	*Ran*	DOWNSTREAM	NA	3	26	0	22
14	21847546	T	G	*Vdac2*	DOWNSTREAM	NA	7	17	1	10
15	77392947	G	A	*Apol7a*	DOWNSTREAM	NA	6	31	2	16
6	86750782	C	G	*Anxa4*	EXON	NA	5	9	0	12
1	136424687	A	G	*Ddx59*	INTRON	NA	3	18	0	22
9	20942478	G	A	*Dnmt1*	INTRON	NA	4	33	1	25
11	80592961	A	C	*Myo1d*	INTRON	NA	5	37	2	33
11	105810422	T	A	*Tanc2*	INTRON	NA	8	97	1	46
14	55829674	A	C	*Nfatc4*	INTRON	NA	6	30	0	10
14	68633351	A	T	*Adam28*	INTRON	NA	4	15	1	14
12	54203685	T	C	*Egln3*	UTR_5_PRIME	NA	7	13	0	8
10	77826817	G	A	*Gm7137*	INTRON	NA	4	74	0	48
10	99113352	C	T	*Galnt4*	DOWNSTREAM	NA	3	7	0	8
19	47668192	A	T	*Col17a1*	DOWNSTREAM	NA	4	11	0	11
2	43589320	T	A	*Kynu*	INTRON	NA	3	27	0	22
2	166048355	T	G	*Ncoa3*	INTRON	NA	4	7	0	10
7	97035338	T	C	*Nars2*	INTRON	NA	4	54	0	46
2	70256394	T	A	*Myo3b*	INTRON	NA	8	26	0	20
Tumor2										
5	124599570	G	T	*Gtf2h3*	DOWNSTREAM	NA	16	26	1	37
16	90726975	A	C	*Mis18a*	EXON	NA	6	7	0	11
1	8678965	C	A	*Sntg1*	INTRON	NA	5	125	0	54
1	78393277	C	G	*Sgpp2*	INTRON	NA	3	18	1	22
2	70256396	A	T	*Myo3b*	INTRON	NA	5	25	1	19
2	130240363	A	T	*Tmc2*	INTRON	NA	3	15	0	11
4	115532819	A	C	*Cyp4a10*	INTRON	NA	8	39	0	15
4	147682444	G	C	*Zfp534*	INTRON	NA	14	116	1	112
5	3972660	T	G	*Akap9*	INTRON	NA	8	67	0	31
5	23839932	A	C	*Tomm7*	INTRON	NA	11	72	0	27
5	45581367	A	G	*Fam184b*	INTRON	NA	7	34	0	25
5	53837766	T	G	*Tbc1d19*	INTRON	NA	8	38	0	20
5	107598397	G	T	*Rpap2*	INTRON	NA	21	139	2	97
6	41374845	T	G	*Prss3*	INTRON	NA	13	82	2	59
6	73037798	G	C	*Dnahc6*	INTRON	NA	4	13	0	12
7	49304707	A	G	*Nav2*	INTRON	NA	3	15	0	21
7	91711646	C	T	*Dlg2*	INTRON	NA	8	32	0	34
8	33257076	A	G	*Wrn*	INTRON	NA	5	40	1	66
8	80989946	T	G	*Usp38*	INTRON	NA	9	76	0	34
8	87582492	C	G	*4933402J07Rik*	INTRON	NA	6	30	0	30
9	99551463	T	G	*Armc8*	INTRON	NA	9	63	0	40
10	94864588	T	G	*Plxnc1*	INTRON	NA	14	135	3	61
15	83703673	G	T	*Scube1*	INTRON	NA	3	41	0	14
16	15714290	A	C	*Prkdc*	INTRON	NA	22	41	0	30
17	57004894	C	T	*Gtf2f1*	INTRON	NA	3	34	0	26
18	43575650	G	T	*Jakmip2*	INTRON	NA	8	81	0	33
19	16742294	T	G	*Vps13a*	INTRON	NA	7	34	0	10
X	26039427	T	G	*Gm5168*	INTRON	NA	15	280	0	125
X	73262316	T	G	*Xlr3c*	INTRON	NA	9	82	1	43
15	66016468	G	T	*Kcnq3*	NON_SYNONYMOUS_CODING	p.Gln416Lys/c.1246G>T	4	8	2	16
X	164944124	A	G	*Mospd2*	INTRON	NA	13	40	0	26
10	100568245	A	T	*4930430F08Rik*	DOWNSTREAM	NA	4	15	0	8
17	57419516	T	C	*Emr1*	INTRON	NA	4	16	0	17
Tumor3										
4	133416542	A	G	*Slc9a1*	DOWNSTREAM	NA	4	63	2	41
15	77424554	C	T	*Apol7b*	DOWNSTREAM	NA	7	112	1	70
17	57306947	A	T	*Vav1*	DOWNSTREAM	NA	6	34	1	19
1	19212542	C	G	*Tfap2b*	INTRON	NA	3	22	1	36
1	93063023	C	T	*Kif1a*	INTRON	NA	3	33	0	18
2	92427096	A	T	*Cry2*	INTRON	NA	7	13	0	8
2	93675178	T	A	*Alx4*	INTRON	NA	4	20	1	20
9	21124799	A	C	*Tyk2*	INTRON	NA	4	25	0	11
17	80352851	A	T	*Arhgef33*	INTRON	NA	3	16	0	11
15	77424576	A	G	*Apol7b*	DOWNSTREAM	NA	9	152	1	104
5	144794497	T	C	*Trrap*	INTRON	NA	8	40	0	21
X	89755060	G	A	*4932429P05Rik*	UTR_3_PRIME	NA	12	111	1	56
4	136052816	G	A	*Rpl11*	EXON	NA	4	62	0	30
19	11307561	A	T	*NA*	INTERGENIC	NA	4	25	0	22
3	152322561	C	T	*Fam73a*	INTRON	NA	10	21	0	17
5	137473436	T	A	*Zan*	INTRON	NA	3	13	0	12
17	57279487	G	A	*Vav1*	INTRON	NA	4	40	0	23
17	57419516	T	C	*Emr1*	INTRON	NA	4	21	0	12
18	33921753	A	T	*Epb4.1l4a*	INTRON	NA	9	29	0	20
10	100341470	C	A	*Gm4302*	NON_SYNONYMOUS_CODING	p.Ser205Arg/c.615C>A	4	24	0	21
8	104524304	C	G	*Nae1*	DOWNSTREAM	NA	5	20	0	21
7	3911858	T	A	*Lilra6*	EXON	NA	5	63	0	31
7	97035348	T	C	*Nars2*	INTRON	NA	6	42	0	24
3	109934836	G	A	*Ntng1*	NON_SYNONYMOUS_CODING	p.Thr207Met/c.620G>A	4	72	0	43

Figure S1. Related to Figure 1. Characterization of *VTet2*$^{-/-}$ *Flt3*^{ITD} mice

(A-F) Platelet count (A), Mac1^{+}Gr1^{+} and cKit^{+} peripheral blood frequencies (B), liver weight (C), lineage^{-} bone marrow frequencies (D), spleen Mac1^{+}Gr1^{+} frequencies (E), and CD71^{+}Ter119^{+} bone marrow frequencies (F) of WT, *Tet2*$^{-/-}$, *Flt3*^{ITD}, *Tet2*$^{-/-}$*;Flt3*^{ITD} mice (n=5 to 10 per group). (G) Phospho-STAT5 phosphoflow in WT and *Tet2*$^{-/-}$*;Flt3*^{ITD} mouse bone marrow (representative plot from n=3). (H) Table of somatic nucleotide polymorphisms (SNPs) from 3 *Tet2*$^{-/-}$*;Flt3*^{ITD} leukemias determined through exome sequencing and MuTect analysis. N/A- does not affect amino acid change. CHROM, chromosome; POS, position; REF, reference nucleotide; ALT, alternate nucleotide; SNPEFF_EFFECT, location of SNP in gene structure; SNPEFF_AA_CHANGE, effect of SNP on amino acid change; T, leukemia tumor; N, normal; Alt, number of alternate nucleotide reads; Ref, number of reference nucleotide reads. + p<=.05, * p<=.01 , ** p<=.001, *** p<=.0001. p values using unpaired Student's t-test. Graphs mean±SEM.


A
WT
Tet2-/-
Flt3ITD
Tet2-/-;Flt3ITD
SSA
lineage
cKit
Sca-1
CD16/32
CD34
9.9±2.4
9.5±3
10±1.7
24±11
6.9±1.9
8.3±2.1
4.2±3.4
6.0±1.1
27±10
41±13
22±6.5
32±11
29±11
30±9.2
63±24
14±16
14±3.2
80±9.4
4.1±3.6
5.2±4.5
B
% Peripheral Blood
CD45.1
CD45.2
***
LSK
GMP
Donor Cell Source
C
lin-Sca-cKit+
lin-Sca+cKit+
GMP
CMP
MEP
MPP
LT-HSC
CD16/32
CD34
CD48
CD150
D
% of LSK
**
**
*
WT
Tet2-/-
Flt3ITD
Tet2-/-;Flt3ITD
E
% of LSK
**
**
*
WT
Tet2-/-
Flt3ITD
Tet2-/-;Flt3ITD


F
WT
Tet2-/-;Flt3ITD
SSA
Lineage
cKit
Sca-1
CD48
CD150
G
91.0
1.1
95.0
cKit
Sca-1
CD16/32
CD34
3.1
90.4
99.2
cKit
Sca-1
CD48
CD150
H
GMP donor
MPP donor
cKit
Mac1
GMP donor
MPP donor
CD45.2
CD45.1
I
HSC
SSC-A
LINEAGE
CD34
CD38
HSC
PROGENITORS
SSC-A
CD90
ST-HSC
LT-HSC
CD123
CD45RA
CMP
GMP
MEP
AML
SSC-A
LINEAGE
CD34
CD38
HSC
PROGENITORS
SSC-A
CD90
ST-HSC
LT-HSC
CD123
CD45RA
CMP
GMP
MEP

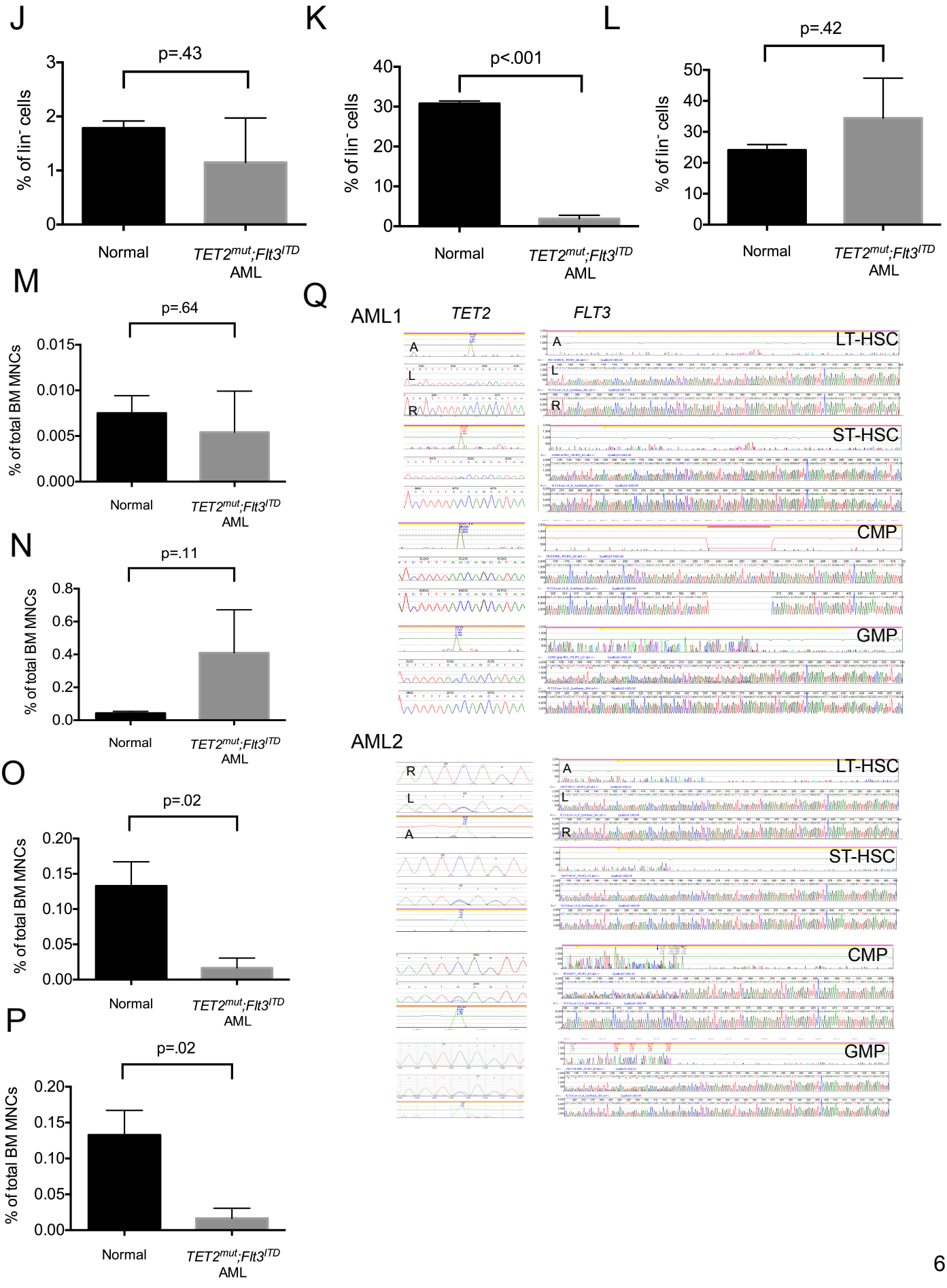

J
p=.43
% of lin- cells
3
2
1
0
Normal
TET2mut;Flt3ITD
AML
K
p<.001
% of lin- cells
40
30
20
10
0
Normal
TET2mut;Flt3ITD
AML
L
p=.42
% of lin- cells
50
40
30
20
10
0
Normal
TET2mut;Flt3ITD
AML
M
p=.64
% of total BM MNCs
0.015
0.010
0.005
0.000
Normal
TET2mut;Flt3ITD
AML
N
p=.11
% of total BM MNCs
0.8
0.6
0.4
0.2
0.0
Normal
TET2mut;Flt3ITD
AML
O
p=.02
% of total BM MNCs
0.20
0.15
0.10
0.05
0.00
Normal
TET2mut;Flt3ITD
AML
P
p=.02
% of total BM MNCs
0.20
0.15
0.10
0.05
0.00
Normal
TET2mut;Flt3ITD
AML
Q
AML1
TET2
FLT3
A
L
R
LT-HSC
ST-HSC
CMP
GMP
AML2
R
L
A
LT-HSC
ST-HSC
CMP
GMP

Figure S2. Related to Figure 2. LSK cells act as leukemic initiating cell in *VTet2*$^{-/-}$*Flt3*^{ITD} leukemia

(A) Analysis of myeloid progenitor and LSK frequencies. gate mean ± SEM. (B) Peripheral blood CD45.1 (normal host marker) and CD45.2 (donor leukemia marker) frequencies in LSK or GMP *Tet2*$^{-/-}$*;Flt3*^{ITD} transplanted mice at 2 weeks. (C) Progenitor and SLAM LSK analysis in LSK *Tet2*$^{-/-}$*;Flt3*^{ITD} transplanted mice. (D-F) Relative frequency of MPP (D) and LT-HSC (E) in WT, *Tet2*$^{-/-}$, *Flt3*^{ITD}, and *Tet2*$^{-/-}$*;Flt3*^{ITD} LSK cells, and gating strategy (F) (n=4 to 6 per group). (G) Post sort purity analysis of sorted GMP and MPP cells from *VTet2*$^{-/-}$*Flt3*^{ITD} bone marrow. % of gate. (H) Peripheral blood Mac1, cKit, CD45.1, CD45.2 analysis from GMP and MPP *VTet2*$^{-/-}$*Flt3*^{ITD} donor transplants. (I) Sorting scheme of normal subjects (HSC) and human AMLs with *TET2* and *FLT3*^{ITD} mutations. (J-P) Frequency of LT-HSC (J), CMP (K), and GMP (L) as % of lin^{-} bone marrow and LT-HSC (M), ST-HSC/MPP (N), CMP (O), and GMP (P) as % of total bone marrow mononuclear cells (BM MNCs) in normal subjects and *TET2*;*FLT3*^{ITD} mutant AMLs (n=9). LT-HSC, long term hematopoietic stem cell; ST-HSC, short term hematopoietic stem cell; MPP, multipotent progenitor; CMP, common myeloid progenitor; GMP, granulocyte-macrophage progenitor. (Q) Examples of *TET2* and *FLT3*^{ITD} mutations found in LT-HSCs, ST-HSCs, CMPs, and GMPs. Order of sequence traces (analysis, leukemia, or reference) are as indicated for LT-HSC sample. A, analysis of sequencing with peaks indicating variation from reference; L, leukemia sequencing; R, corresponding reference sequence. * p<=.01 , ** p<=.001, *** p<=.0001. p values using unpaired Student's t-test. Graphs mean±SEM.

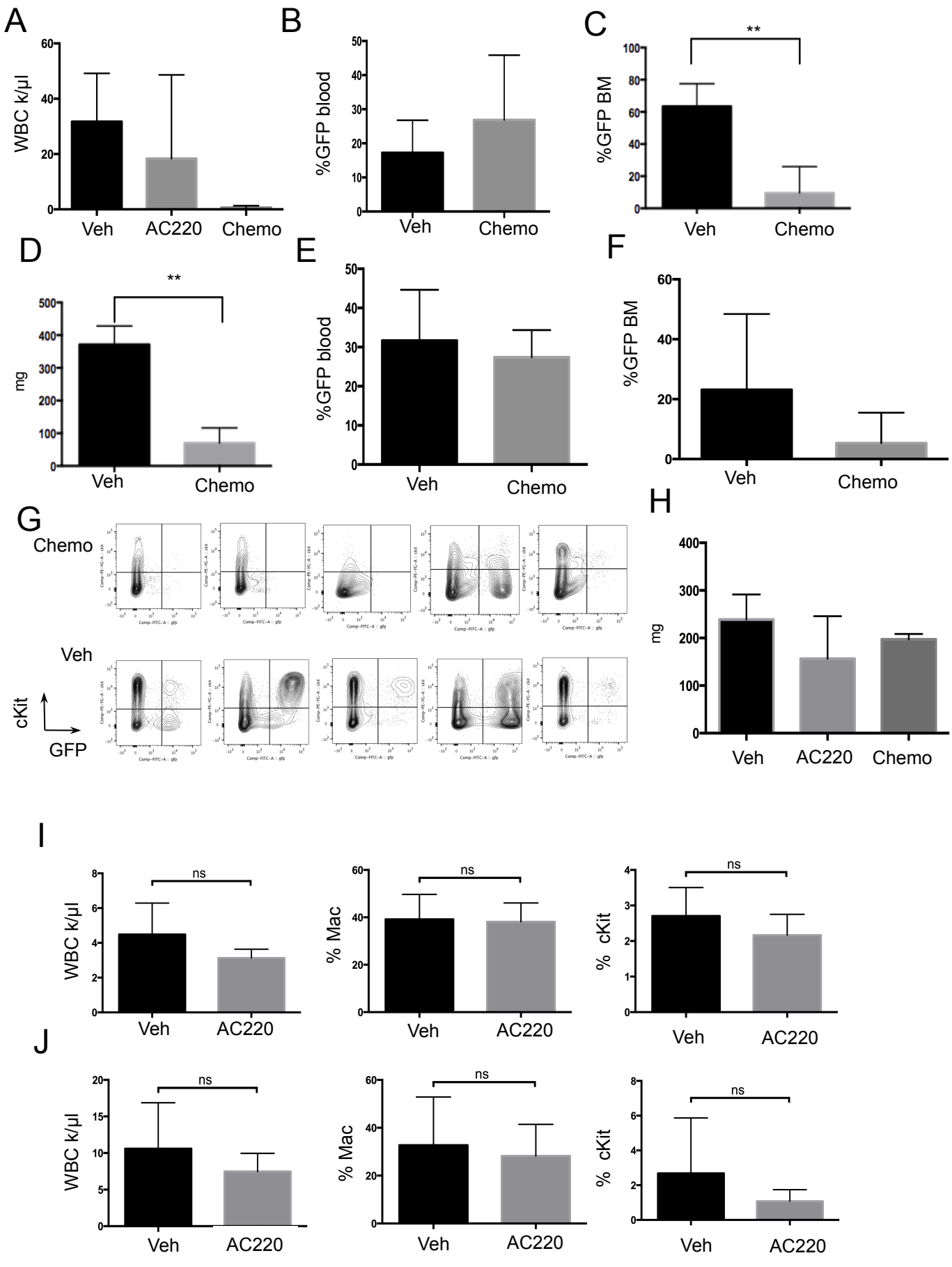

A
WBC k/µl
Veh
AC220
Chemo
B
%GFP blood
Veh
Chemo
C
**
%GFP BM
Veh
Chemo
D
**
mg
Veh
Chemo
E
%GFP blood
Veh
Chemo
F
%GFP BM
Veh
Chemo
G
Chemo
Veh
cKit
GFP
H
mg
Veh
AC220
Chemo
I
ns
WBC k/µl
Veh
AC220
ns
% Mac
Veh
AC220
ns
% cKit
Veh
AC220
J
ns
WBC k/µl
Veh
AC220
ns
% Mac
Veh
AC220
ns
% cKit
Veh
AC220

Figure S3. Related to Figure 3. *VTet2$^{-/-}$Flt3^{ITD}* therapy response

(A) WBC count at 2 weeks of *Tet2$^{-/-}$;Flt3^{ITD}* transplanted mice treated with vehicle, AC220, or chemotherapy. (B) Pre-treatment peripheral blood GFP% of *AML1-ETO-9a* leukemia (n=5 per group). (C,D) Post-treatment bone marrow %GFP (C) and spleen weight (D) from vehicle or chemotherapy treated *AML1-ETO-9a* transplanted leukemia. (E) Pre-treatment peripheral blood GFP% of *AML1-ETO NRAS^{G12D}* leukemia (n=5 per group). (F,G) Post-treatment bone marrow %GFP (F) from vehicle or chemotherapy treated *AML1-ETO NRAS^{G12D}* transplanted leukemia and flow plot of individual mice for GFP^{+}cKit^{+} leukemia cells (G). (H) Spleen weight of mice transplanted with *VTet2$^{-/-}$Flt3^{ITD}* leukemia following treatment for 4 weeks (n=5 per group). (I,J) Peripheral blood analysis of mice transplanted with cells from Vehicle or AC220 treated mice with *Tet2$^{-/-}$;Flt3^{ITD}* leukemia at 28 days (I) and 62 days (J) for WBC, %Mac, and %cKit (n=7 per recipient group). ns, not statistically significant. p values using unpaired Student's t-test. Graphs mean±SEM.

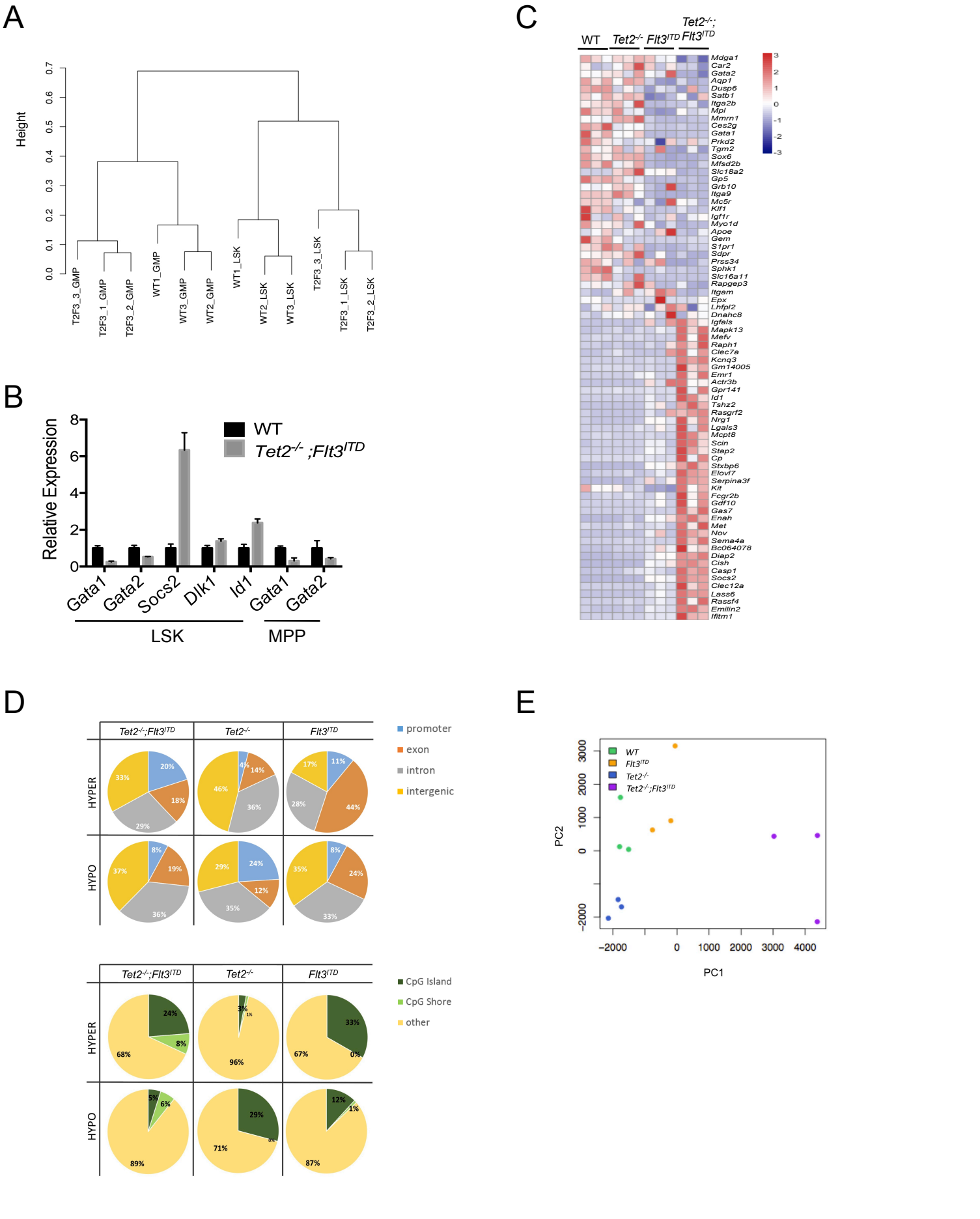

A
Height
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
T2F3_3_GMP
T2F3_1_GMP
T2F3_2_GMP
WT1_GMP
WT3_GMP
WT2_GMP
WT1_LSK
WT2_LSK
WT3_LSK
T2F3_3_LSK
T2F3_1_LSK
T2F3_2_LSK
B
Relative Expression
WT
Tet2-/- ;Flt3ITD
Gata1
Gata2
Socs2
Dlk1
Id1
Gata1
Gata2
LSK
MPP
C
WT
Tet2-/-
Flt3ITD
Tet2-/-;
Flt3ITD
Mdga1
Car2
Gata2
Aqp1
Dusp6
Satb1
Itga2b
Mpl
Mmrn1
Ces2g
Gata1
Prkd2
Tgm2
Sox6
Mfsd2b
Slc18a2
Gp5
Grb10
Itga9
Mc5r
Klf1
Igf1r
Myo1d
Apoe
Gem
S1pr1
Sdpr
Prss34
Sphk1
Slc16a11
Rapgep3
Itgam
Epx
Lhfpl2
Dnahc8
Igfals
Mapk13
Mefv
Raph1
Clec7a
Kcnq3
Gm14005
Emr1
Actr3b
Gpr141
Id1
Tshz2
Rasgrf2
Nrg1
Lgals3
Mcpt8
Scin
Stap2
Cp
Stxbp6
Elovl7
Serpina3f
Kit
Fcgr2b
Gdf10
Gas7
Enah
Met
Nov
Sema4a
Bc064078
Diap2
Cish
Casp1
Socs2
Clec12a
Lass6
Rassf4
Emilin2
Ifitm1
D
Tet2-/-;Flt3ITD
Tet2-/-
Flt3ITD
HYPER
HYPO
promoter
exon
intron
intergenic
CpG Island
CpG Shore
other
E
WT
Flt3ITD
Tet2-/-
Tet2-/-;Flt3ITD
PC1
PC2

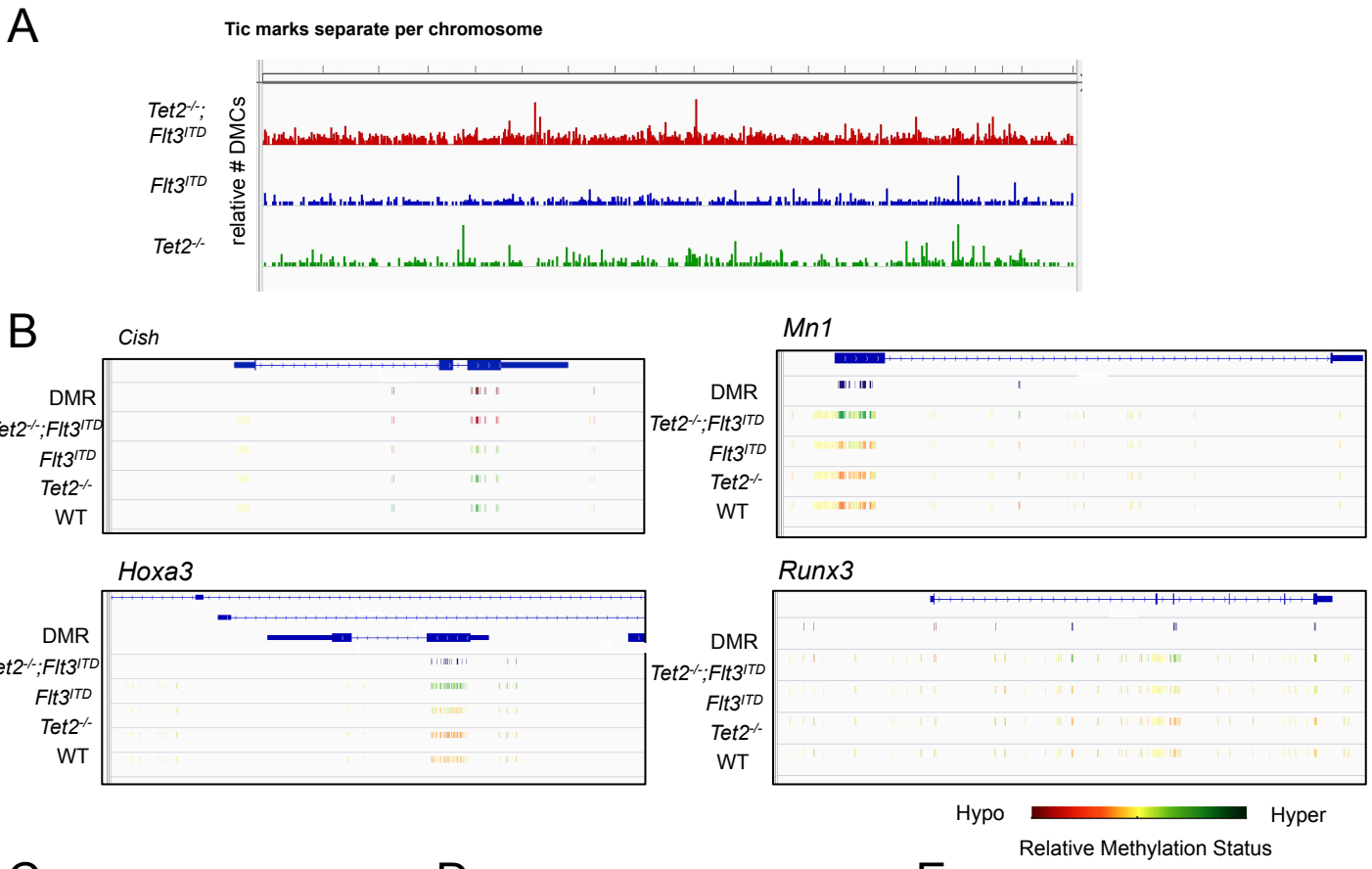
Figure S4. Related to Figure 4. Expression and methylation analysis of *VTet2*$^{-/-}$ *Flt3*^{ITD} LSK cells

(A) Dendrogram clustering of RNA-seq expression from WT and *Tet2*$^{-/-}$;*Flt3*^{ITD} (T2F3) LSK and GMP cells. (B) qRT PCR validation of select *Tet2*$^{-/-}$;*Flt3*^{ITD} differentially expressed genes from LSK and MPP cells. Mean±SEM. (C) Heat map of differentially expressed genes in *Tet2*$^{-/-}$;*Flt3*^{ITD} LSKs (in Figure 4B) compared to WT, *Flt3*^{ITD}, and *Tet2*$^{-/-}$ LSK cells. (D) DMR genomic localization and characterization. (E) Principal component analysis of eRRBS methylation profiles compared to wild-type.

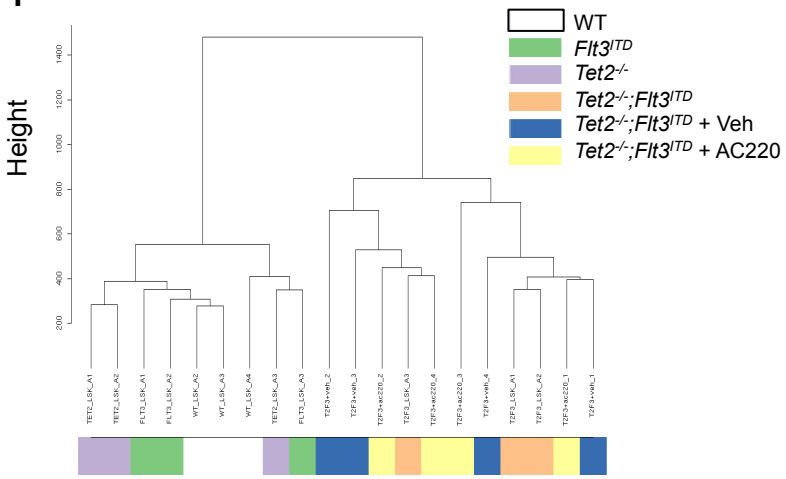
Table S1, related to Figure 4. Provided as an Excel File.

Table S2, related to Figure 4. Provided as an Excel File.

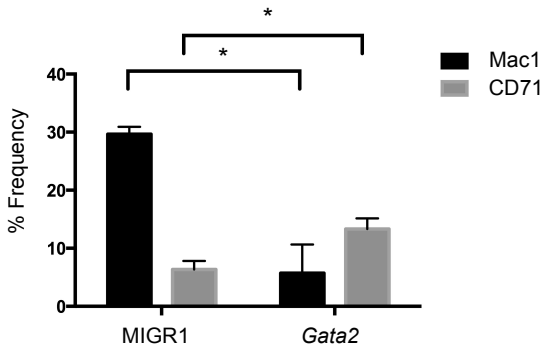
Table S3, related to Figure 4. Provided as an Excel File.


A
Tic marks separate per chromosome
Tet2-/-; Flt3ITD
Flt3ITD
Tet2-/-
relative # DMCs
B
Cish
Mn1
Hoxa3
Runx3
DMR
Tet2-/-;Flt3ITD
Flt3ITD
Tet2-/-
WT
Hypo
Hyper
Relative Methylation Status
C
Relative Gata2 Expression
1.5
1.0
0.5
0.0
*
WT
Tet2-/-; Flt3ITD AC220
Tet2-/-; Flt3ITD
D
k/µl
100
80
60
40
20
0
Veh
AC220
E
mg
1000
800
600
400
200
0
Veh
AC220
F
mg
8000
6000
4000
2000
0
Veh
AC220
G
PC2
PC1
600
300
0
-300
-600
-200
0
200
400
WT
Tet2-/-
Flt3ITD
Tet2-/-;Flt3ITD
Tet2-/-;Flt3ITD + Veh
Tet2-/-;Flt3ITD + AC220

H


Height
WT
Flt3ITD
Tet2-/-
Tet2-/-;Flt3ITD
Tet2-/-;Flt3ITD + Veh
Tet2-/-;Flt3ITD + AC220

I


% Frequency
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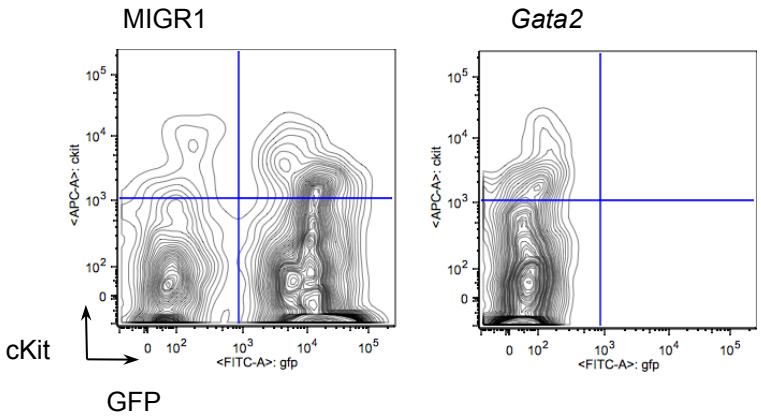
20
10
0
*
*
MIGR1
Gata2
Mac1
CD71

J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Gata2
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cKit
GFP

Figure S5. Related to Figure 5. Cooperativity of *Tet2*$^{-/-}$ and *Flt3*^{ITD} on methylation and gene expression

(A) Whole genome differentially methylated cytosine (DMC) profile in *Tet2*$^{-/-}$*;Flt3*^{ITD}, *Flt3*^{ITD}, and *Tet2*$^{-/-}$ LSK cells vs WT. (B) Example gene loci with differential methylation in *Tet2*$^{-/-}$*;Flt3*^{ITD} LSK cells compared to *Flt3*^{ITD}, *Tet2*$^{-/-}$, and WT LSK. DMR, differentially methylated region. (C) *Gata2* expression with qRT-PCR after treatment with AC220 in LSK bone marrow cells. (D-F) Primary *VTet2*$^{-/-}$ *Flt3*^{ITD} mice response to vehicle or AC220 treatment for WBC (D), spleen weight (E), and liver weight (F). (G,H) Principle component analysis (G) and clustering (H) of eRRBS methylation profiles from WT, *Tet2*$^{-/-}$, *Flt3*^{ITD}, *Tet2*$^{-/-}$*;Flt3*^{ITD}, *Tet2*$^{-/-}$*;Flt3*^{ITD} vehicle treated, and *Tet2*$^{-/-}$*;Flt3*^{ITD} AC220 treated LSK cells. Line in (G) separating clustered groups (WT, *Tet2*$^{-/-}$, and *Flt3*^{ITD} from *Tet2*$^{-/-}$*;Flt3*^{ITD} untreated, vehicle treated, and AC220 treated). (I) Quantitation of CD71 and Mac1 expression in CFU assay cells from *Tet2*$^{-/-}$;*Flt3*^{ITD} bone marrow cells expressing MIGR1 control or *Gata2* (gated on GFP+) (n=3 per group). (J) Bone marrow GFP and cKit expression flow plot from mice transplanted with *VTet2*$^{-/-}$*Flt3*^{ITD} bone marrow cells expressing MIGR1 control or *Gata2*. + p<=.05, * p<=.01. p values using unpaired Student's t-test. Graphs mean±SEM.

Table S4, Related to Figure 5. Genes hypermethylated and decreased expression in *Tet2*$^{-/-}$;*Flt3*^{ITD} LSK cells

List of genes that have at least 3 differentially hypermethylated cytosines within the promoter or gene body and downregulated in expression by RNA-seq. Hyper DMC #, number of hypermethylated cytosines; T2F3, *Tet2*$^{-/-}$;*Flt3*^{ITD} ;log2FC, log 2 fold change.

Gene Symbol	Hyper DMC #	Expression
	T2F3 vs WT	log2FC
MN1	113	-2.24
LTBP3	57	-1.68
GATA2	51	-2.99
ZFPM1	39	-2.85
HOXA3	37	-2.19
HLF	30	-2.74
RUNX3	24	-1.05
RAPGEF3	24	-2.88
TRIM47	21	-3.54
FAM110A	18	-1.26
GSE1	16	-1.42
ADCY9	10	-1.64
CD247	9	-2.55
KIT	8	-1.73
DUSP4	8	-3.02
ARAP3	8	-1.56
SMARCA2	7	-1.12
ZBTB16	7	-2.42
RAI1	7	-1.12
TGFBR2	6	-1.05
PRKG1	6	-5.25
ANK1	6	-3.45
GCNT2	6	-2.61
MICAL3	6	-1.91
DUSP6	5	-2.89
NFIC	5	-1.60
KCTD1	5	-1.05
MDGA1	5	-3.13
GDF3	5	-4.66
RPS4Y2	5	-3.48
PLCG1	5	-1.89
CUEDC1	5	-1.86
SIK1	5	-1.48
DEF8	4	-1.23
HNF4A	4	-1.99
EPOR	4	-5.76
OSGIN1	4	-5.15

TNK2	4	-1.75
PANK1	4	-1.49
PLSCR3	4	-1.43
LHFPL2	3	-1.99
DNTT	3	-2.37
SCUBE3	3	-1.65
IKZF2	3	-1.33
NRIP1	3	-1.19
GATA1	3	-5.75
ITGA9	3	-5.11
PHF21B	3	-3.82
LTBP1	3	-2.98
4930515G01RIK	3	-2.25
SDC4	3	-2.11
HOXB3	3	-2.11
RHD	3	-2.01
VWF	3	-1.92
TCF7	3	-1.78
RUSC2	3	-1.54
SAMD14	3	-1.54
EGFL7	3	-1.36

Table S5, related to Figure 5. Provided as an Excel File

Table S6, Related to Figure 5. Genes with promoter DMCs and differential RNA expression in *Tet2*$^{-/-}$ *Flt3*^{ITD} LSK

List of genes with >=3 promoter Differentially Methylated Cytosines (DMCs) in *Tet2*$^{-/-}$; *Flt3*^{ITD} LSK from pairwise comparison to *Tet2*$^{-/-}$ or *Flt3*^{ITD} LSK profiles and with differential gene expression. Each column represents number of DMCs in the comparison vs WT. log2FC, log2 fold change in gene expression. X indicates presence of DMCs in corresponding gene in *TET2* mutant (*TET2*^{mut}) human AML.

Gene Symbol	*Tet2*$^{-/-}$;*Flt3*^{ITD} vs WT	*Flt3*^{ITD} vs WT	*Tet2*$^{-/-}$ vs WT	log2FC	*TET2*^{mut} AML
GATA2	20	1	0	-2.99	X
ARAP3	15	1	0	-1.56	X
TMEM215	14	0	0	2.35	X
EGFL7	11	0	0	-1.36	X
ANK1	10	0	0	-3.45	X
GSE1	5	0	0	-1.42	X
RPS4Y2	5	0	0	-3.48	
FAM110A	4	0	0	-1.26	X
PLSCR3	4	0	0	-1.43	X
EPOR	4	0	0	-5.76	
FLT1	3	0	0	2.31	X
SULF2	3	0	0	2.00	X
TMEM51	3	0	0	3.61	X
SCUBE3	3	0	0	-1.65	X
TCF7	3	0	0	-1.78	X
GATA1	3	0	0	-5.75	
6030419C18RIK	3	1	0	4.61	
4930515G01RIK	3	0	0	-2.25	
IGFALS	-3	0	0	7.15	X
E330020D12RIK	-3	-1	0	2.95	
ALDH1B1	-4	0	0	1.58	X
SLC17A8	-5	0	0	4.55	X
S1PR3	-5	-2	0	1.38	X
GIMAP4	-5	-5	0	4.61	
TIFAB	-8	-2	0	1.29	
IFI30	-9	-3	0	3.18	X
STAP2	-12	0	0	6.04	X

Table S7. Related to Figure 5. List of Genes and DMC number in the gene promoter region by eRRBS analysis of human ST-HSCs isolated from *TET2;FLT3*^{ITD} mutant AML (n=2) compared to CD34^{+} normal bone marrow cells (n=13). Genes list (n=53) derived from overlap with set of 83 murine genes which had >=5 DMCs in the promoter region of *Tet2*$^{-/-}$*;Flt3*^{ITD} LSKs compared to WT LSKs and with a human ortholog.

		Promoter Methylation		
Gene Symbol	**Gene Name**	**Hyper**	**Hypo**	**Total**
EGFL7	EGF-like-domain, multiple 7	63	0	63
FGF8	fibroblast growth factor 8 (androgen-induced)	40	0	40
TSSK3	testis-specific serine kinase 3	39	0	39
ZNF503	zinc finger protein 503	34	0	34
CDX1	caudal type homeobox 1	33	0	33
GP1BB	glycoprotein Ib (platelet), beta polypeptide	31	0	31
TMEM215	transmembrane protein 215	31	0	31
S1PR3	sphingosine-1-phosphate receptor 3	29	0	29
B3GNT9	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 9	28	0	28
HOXA5	homeobox A5	28	0	28
OTX1	orthodenticle homeobox 1	27	0	27
GATA2	GATA binding protein 2	18	0	18
TBX1	T-box 1	18	0	18
ANK1	ankyrin 1, erythrocytic	15	2	17
CACNG6	calcium channel, voltage-dependent, gamma subunit 6	16	1	17
HIF3A	hypoxia inducible factor 3, alpha subunit	17	0	17
CYB5D1	cytochrome b5 domain containing 1	16	0	16
MIR196A1	microRNA 196a-1	16	0	16
SF3B2	splicing factor 3b, subunit 2, 145kDa	15	1	16
EEF1D	eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein)	12	1	13
TAGLN	Transgelin	13	0	13
CD164L2	CD164 sialomucin-like 2	11	0	11
HAND1	heart and neural crest derivatives expressed 1	11	0	11
KCND3	potassium voltage-gated channel, Shal-related subfamily, member 3	11	0	11
CEND1	cell cycle exit and neuronal differentiation 1	9	1	10
IFI30	interferon, gamma-inducible protein 30	2	8	10
GPR173	G protein-coupled receptor 173	9	0	9
IRF7	interferon regulatory factor 7	1	5	6
PLEKHB1	pleckstrin homology domain containing, family B (evectins) member 1	6	0	6
ESR1	estrogen receptor 1	5	0	5
KCNC3	potassium voltage-gated channel, Shaw-related subfamily, member 3	5	0	5
MIR147B	microRNA 147b	5	0	5
RARA	retinoic acid receptor, alpha	5	0	5
FAM155B	family with sequence similarity 155, member B	2	2	4
NRSN2	neurensin 2	4	0	4
SLC17A8	solute carrier family 17 (vesicular glutamate transporter), member 8	3	1	4
MMRN2	multimerin 2	3	0	3
RPL38	ribosomal protein L38	3	0	3
ARAP3	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 3	2	0	2
FGFR1	fibroblast growth factor receptor 1	0	2	2
FOXP1	forkhead box P1	2	0	2

LPIN1	lipin 1	2	0	2
MIR219-2		0	2	2
AIM1L	absent in melanoma 1-like	1	0	1
HSF4	heat shock transcription factor 4	1	0	1
KLF9	Kruppel-like factor 9	1	0	1
MCOLN3	mucolipin 3	1	0	1
MYO7A	myosin VIIA	1	0	1
PALM3	paralemmin 3	1	0	1
PWWP2B	PWWP domain containing 2B	1	0	1
SGK223	homolog of rat pragma of Rnd2	1	0	1
STX16-NPEPL1	STX16-NPEPL1 readthrough (NMD candidate)	1	0	1
TRADD	TNFRSF1A-associated via death domain	1	0	1

Supplemental Experimental Procedures

Antibodies

Antibodies used for flow cytometry were as follows: (anti-mouse) Gr1 (Ly6G), B220 (RA3-682), CD34 (RAM34), FCγR (93) Sca-1 (D7), phospho-Stat5 from eBioscience; cKit (2B8), Mac-1 (CD11b) (M1/70), NK1.1 (PK136), Ter119 (Ter119,553673), CD3 (145-2C11), CD45.1 (A20), CD45.2 (104) all from BD Biosciences; and CD150 (TC-15-12F2.2) and CD48 (HM48-1); both from Biolegend. The 'lineage cocktail' included CD3, Gr-1, Mac-1 (CD11b), NK1.1, B220, and Terr-119. Anti-human antibodies include CD34-Pacific Blue (581) and CD90-allophycocyanin (5E10, both from Biolegend) and CD123-Alexa fluor 700 (32703, R&D).

Quantitative real-time PCR

RNA was isolated with the RNeasy-Plus mini kit (Qiagen). The RNA was then used as a template for cDNA synthesis using the Verso cDNA kit (Thermoscientific). Quantitative real-time PCR reactions were carried out using SYBR Green MasterMix on an Applied Biosciences qPCR cycler. Relative expression was determined by the Δ/ΔCT method and normalized to the internal control, GAPDH or Actin.

AML1-ETO transplants and treatment

Mouse bone marrow or hematopoietic fetal liver derived cells transformed with *AML1-ETO-9a* or *AML1-ETO* and $NRAS^{G12D}$ with an IRES GFP element were provided by Stephen Nimer and Scott Lowe from previously published studies (Zuber et al., 2009) (Wang et al., 2011). Mice were treated with a doxorubicin and cytarabine regimen in same fashion as $Tet2^{-/-};Flt3^{ITD}$ mice. Analysis performed a 4 weeks or time of sacrifice for morbidity.

RNA-sequencing analysis

The raw output BAM files from the sequencer were first converted to FASTQ format using PICARD SamToFastq. Adapters were removed using cutadapt and any read shorter than 35bp was discarded. The trimmed reads were then mapped to the Mouse genome (MM9) using TopHat (ver 2) with a transcriptome index using the Ensembl GTF (ver 37.67) and specifying a strand specific library. Reads that were unmapped by TopHat were then re-mapped using BWA bwasw (ver 0.5.9) again to the mouse genome. The mapped reads from the two passes were merged and then quantitated with HTSeq-count with stranded and strict-intersection options. GSEA was performed per (Subramanian et al., 2005).

Exome-sequencing analysis

DNA was prepared from *VTet2*$^{-/-}$*Flt3*^{ITD} derived leukemia cells from the peripheral blood and somatic tail sample. Exome libraries were prepared by the Agilent SureSelectXT V4 51mb Exome kit. Leukemia mouse exomes were sequenced to a targeted average depth of 200x coverage and somatic samples to 100x coverage on an Illumina HiSeq 2500. We aligned the data through BWA and pre-processed it using GATK following standard recommendations. We used Mutect v 1.1.4 to infer single point variants and only kept the variants that pass the standard high confidence filters. We used Somatic Indel Detector v 2.3-9 to infer insertions and deletions (indels) and only considered indels that were supported by more than five reads. Both variants and indels were annotated using SnpEff v4.0e.

Differential Methylation Analysis

Gene annotations were carried out by overlapping the CpG sites and regions with the exons, introns, UTR5, UTR3 and 5kb upstream regions of refseq genes for genome

assembly mm9. In addition to the pairwise comparisons, the cooperative effect between *Tet2* and *Flt3* mutations to the overall methylation was calculated using a logistic regression model with binomial distribution and a logit link function. CpG sites that showed significant p-values ($p<0.01$ after Benjamini-Hochberg correction) and at least 25% methylation contribution for the *Tet2:Flt3* interaction term were taken as synergistic. Methylation genes correlated with expression based on differential RNA-seq expression values and $p<0.1$.

Supplemental References

Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., Paulovich, A., Pomeroy, S. L., Golub, T. R., Lander, E. S., and Mesirov, J. P. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A *102*, 15545-15550.

Wang, L., Gural, A., Sun, X. J., Zhao, X., Perna, F., Huang, G., Hatlen, M. A., Vu, L., Liu, F., Xu, H.*, et al.* (2011). The leukemogenicity of AML1-ETO is dependent on site-specific lysine acetylation. Science *333*, 765-769.