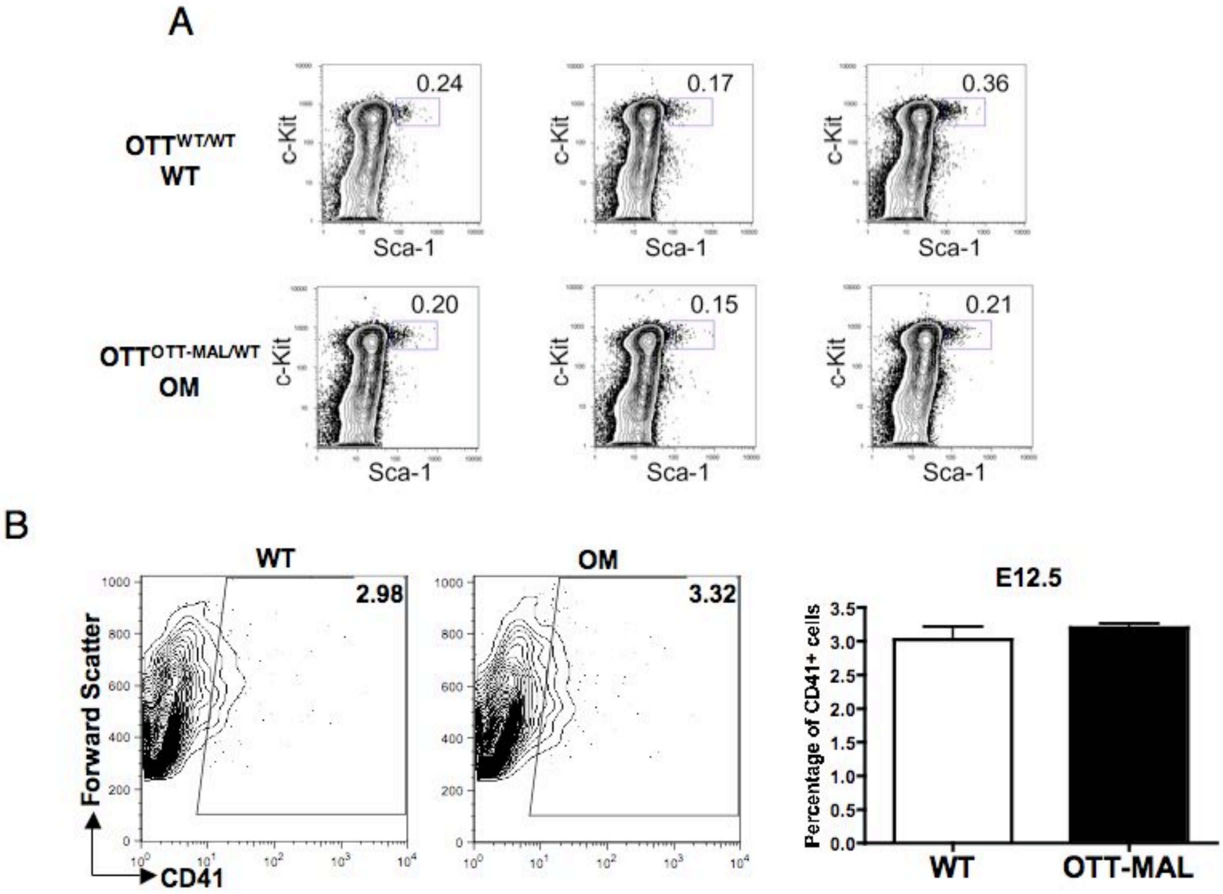
Figure S1


A
OTT$^{WT/WT}$
WT
OTT$^{OTT-MAL/WT}$
OM
c-Kit
Sca-1
0.24
0.17
0.36
0.20
0.15
0.21
B
WT
OM
2.98
3.32
Forward Scatter
CD41
1000
800
600
400
200
0
10^0
10^1
10^2
10^3
10^4
E12.5
Percentage of CD41+ cells
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
WT
OTT-MAL

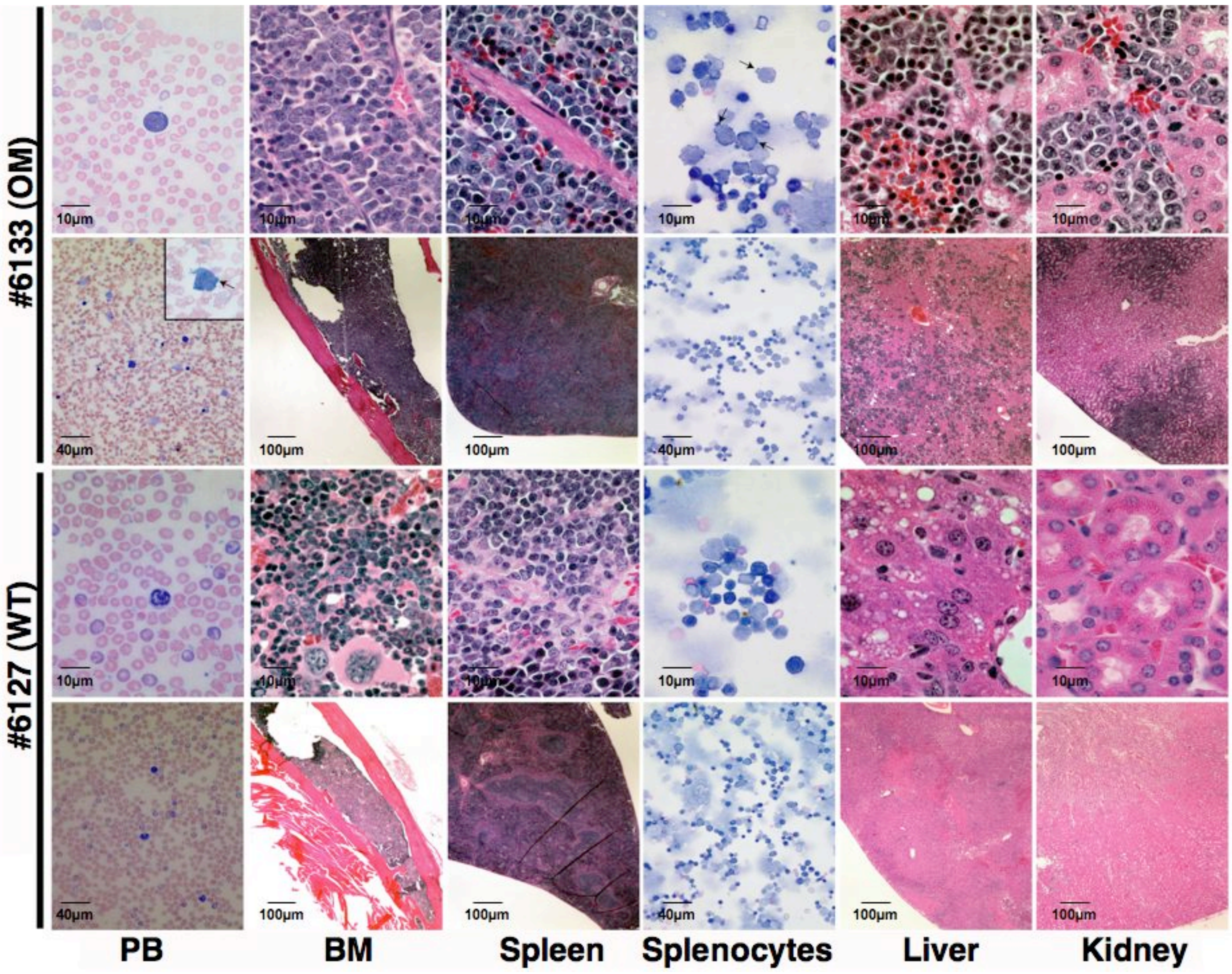


Figure S2

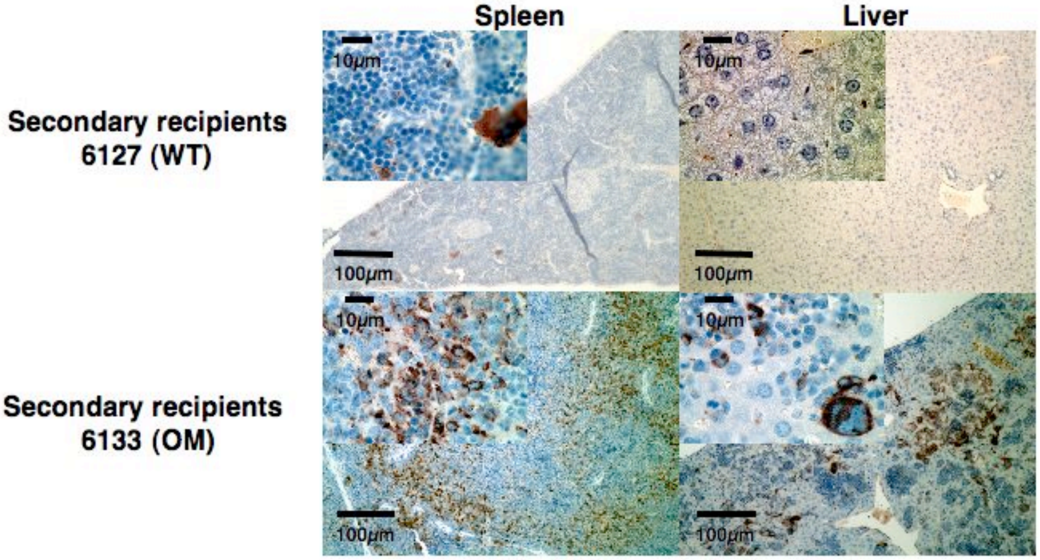
Mercher et al.

Figure S3

A

	Spleen (mg)	Liver (mg)	WBC (K/µl)	HCT (%)	Platelets (K/µl)
Secondary RBM15-MKL1 (n=8)	232 (96-451)	1611 (694-2995)	35 (3.7-118)	46 (34-56)	366 (222-527)
Secondary WT (n=5)	43 (16-101)	858 (512-1207)	2 (1.5-3.22)	50 (36-58)	397 (112-499)
Tertiary RBM15-MKL1 (n=8)	780 (484-912)	3491 (2501-4106)	347 (149-539)	57 (52-64)	1042 (770-1480)
Tertiary WT (n=2)	44 (39-49)	1180 (1131-1229)	2.54 (1.56-3.52)	43 (41-45)	804 (762-845)

B



Von Willebrand Factor staining

Figure S4

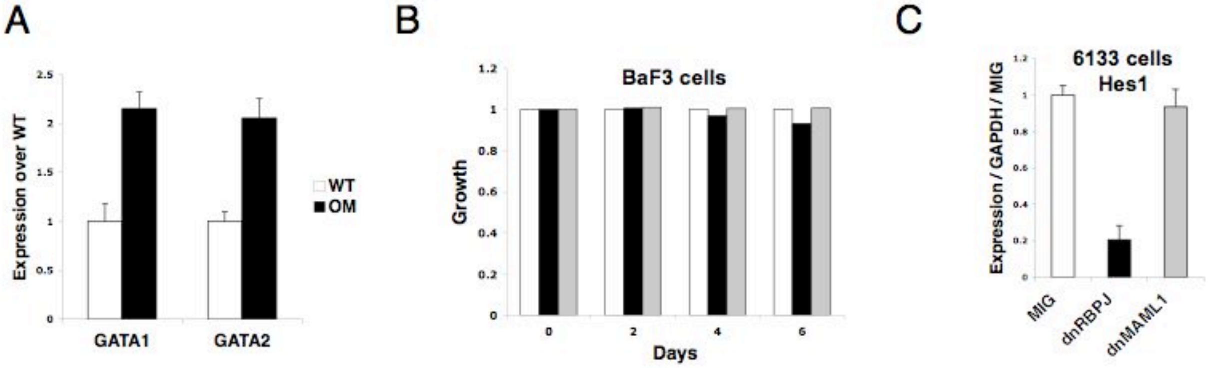

A
Expression over WT
2.5
2
1.5
1
0.5
0
GATA1
GATA2
WT
OM
B
BaF3 cells
Growth
1.2
1
0.8
0.6
0.4
0.2
0
0
2
4
6
Days
C
6133 cells
Hes1
Expression / GAPDH / MIG
1.2
1
0.8
0.6
0.4
0.2
0
MIG
dnRBPJ
dnMAML1

Figure S5

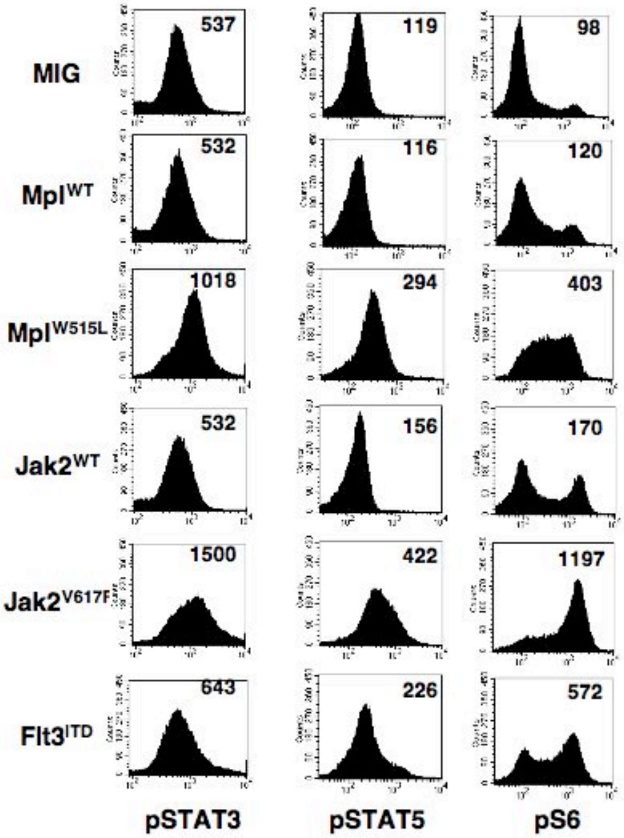

MIG
537
119
98
Mpl^{WT}
532
116
120
Mpl^{W515L}
1018
294
403
Jak2^{WT}
532
156
170
Jak2^{V617F}
1500
422
1197
Flt3^{ITD}
643
226
572
pSTAT3
pSTAT5
pS6

Figure S6

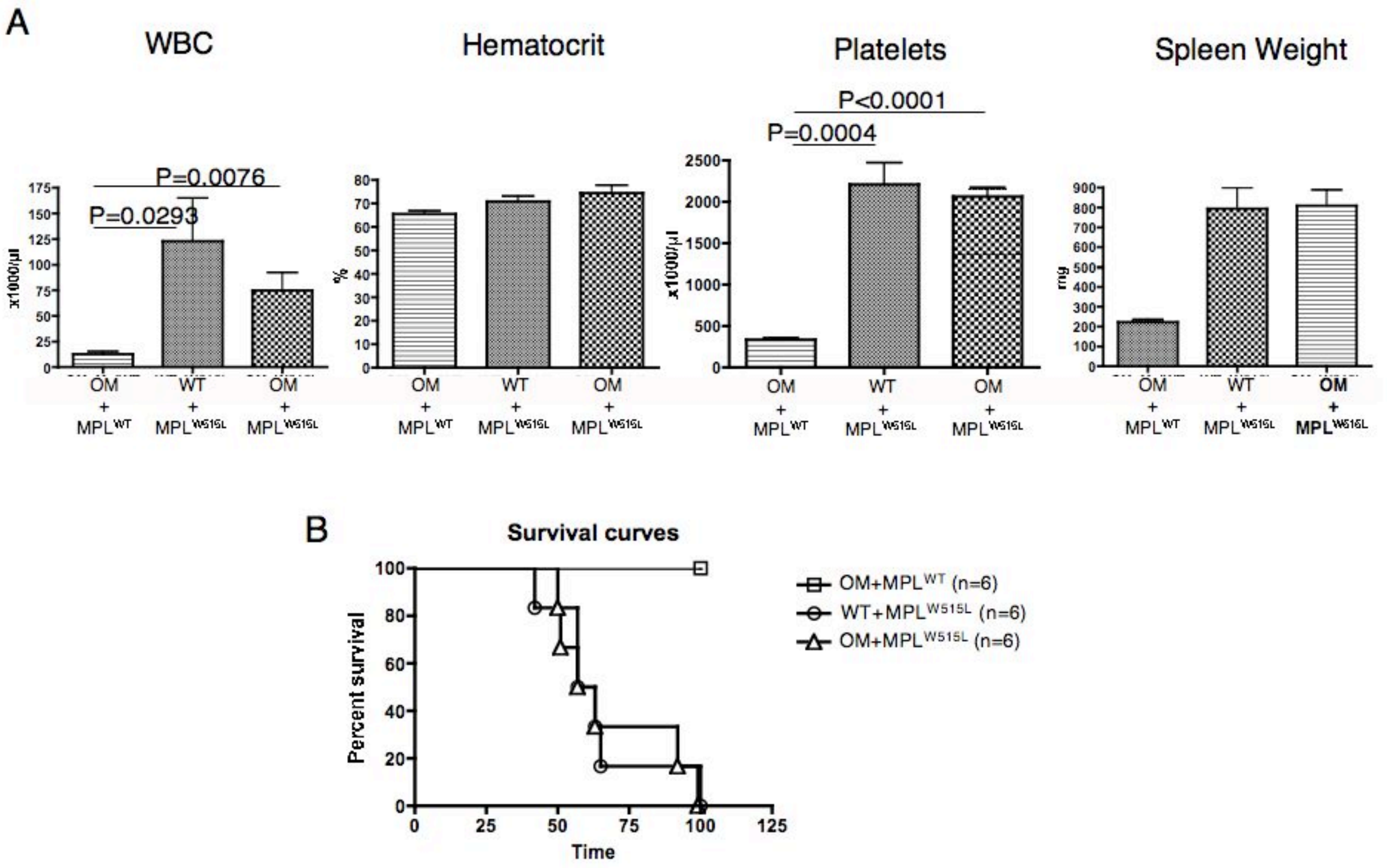

A
WBC
P=0.0076
P=0.0293
x1000/µl
175
150
125
100
75
50
25
0
OM + MPL^WT
WT + MPL^W515L
OM + MPL^W515L
Hematocrit
%
80
70
60
50
40
30
20
10
0
OM + MPL^WT
WT + MPL^W515L
OM + MPL^W515L
Platelets
P<0.0001
P=0.0004
x1000/µl
2500
2000
1500
1000
500
0
OM + MPL^WT
WT + MPL^W515L
OM + MPL^W515L
Spleen Weight
mg
900
800
700
600
500
400
300
200
100
0
OM + MPL^WT
WT + MPL^W515L
OM + MPL^W515L
B
Survival curves
Percent survival
100
80
60
40
20
0
Time
0
25
50
75
100
125
OM+MPL^WT (n=6)
WT+MPL^W515L (n=6)
OM+MPL^W515L (n=6)

Figure S7

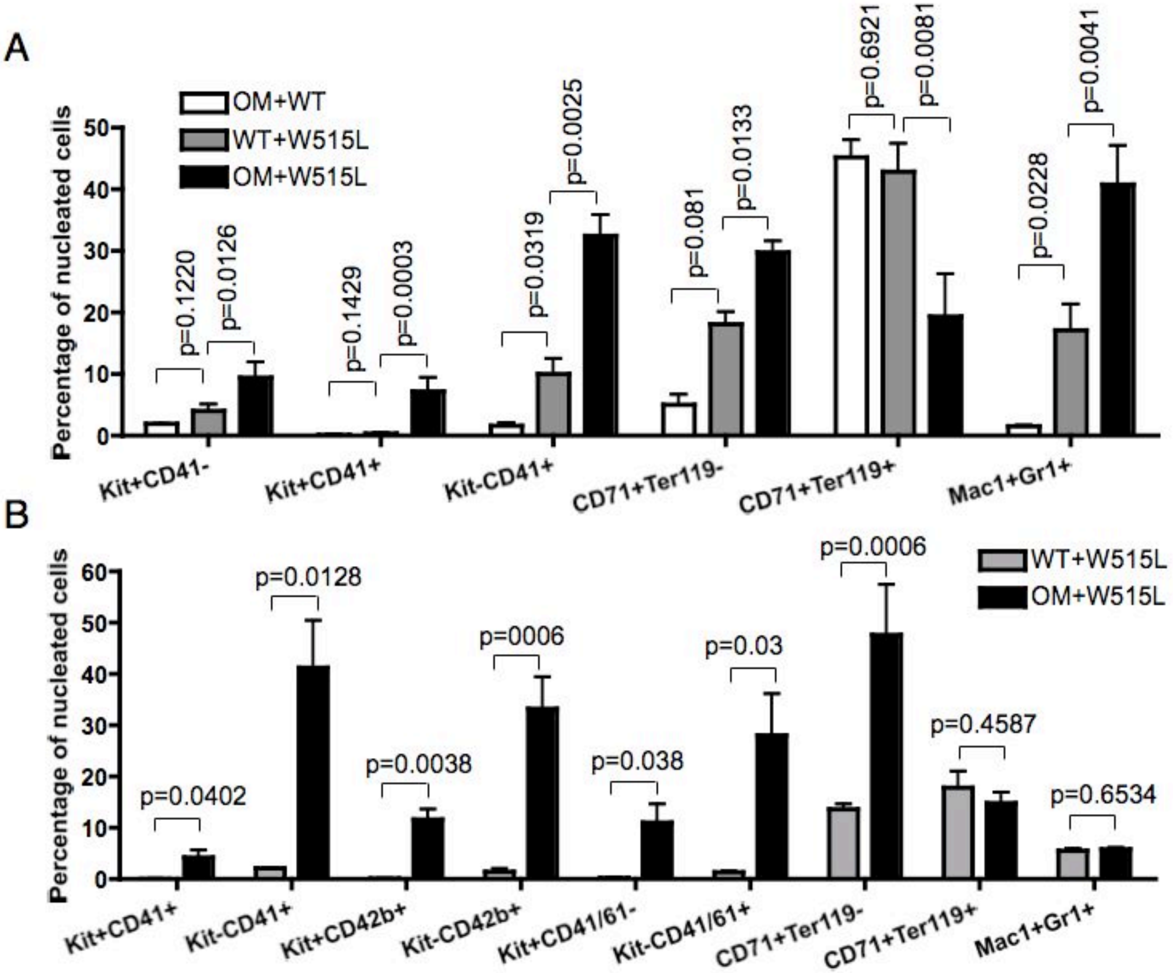

A
OM+WT
WT+W515L
OM+W515L
Percentage of nucleated cells
50
40
30
20
10
0
p=0.1220
p=0.0126
p=0.1429
p=0.0003
p=0.0319
p=0.0025
p=0.081
p=0.0133
p=0.6921
p=0.0081
p=0.0228
p=0.0041
Kit+CD41-
Kit+CD41+
Kit-CD41+
CD71+Ter119-
CD71+Ter119+
Mac1+Gr1+
B
WT+W515L
OM+W515L
Percentage of nucleated cells
60
50
40
30
20
10
0
p=0.0402
p=0.0128
p=0.0038
p=0006
p=0.038
p=0.03
p=0.0006
p=0.4587
p=0.6534
Kit+CD41+
Kit-CD41+
Kit+CD42b+
Kit-CD42b+
Kit+CD41/61-
Kit-CD41/61+
CD71+Ter119-
CD71+Ter119+
Mac1+Gr1+

Figure S8

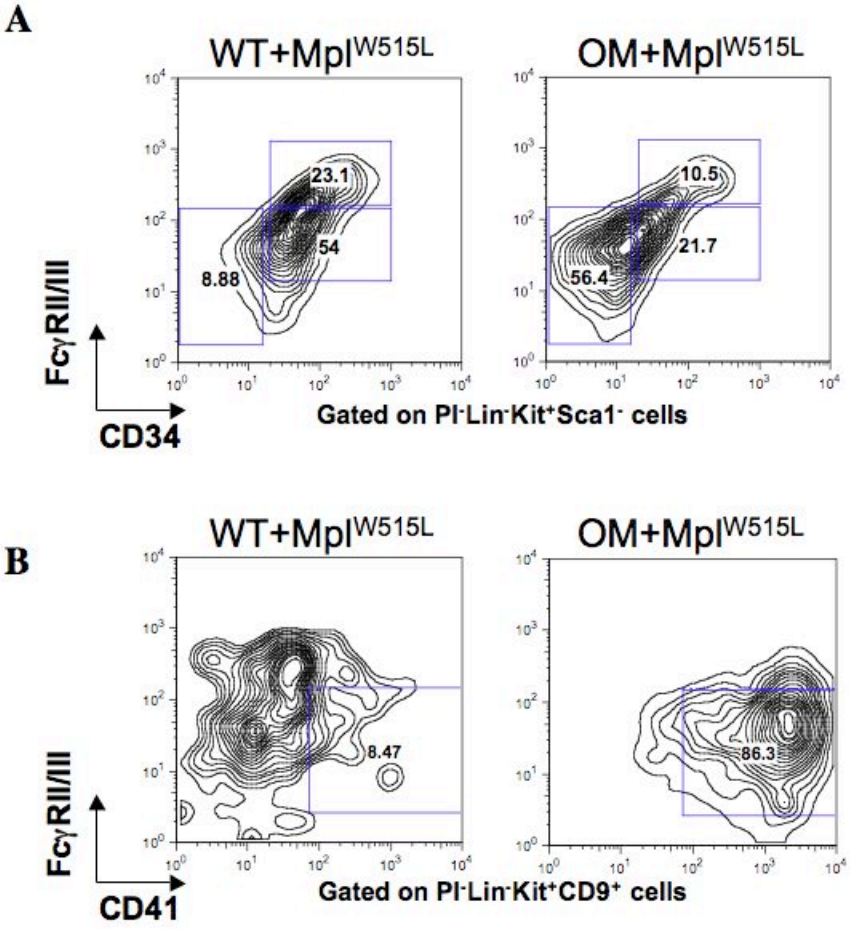

A
WT+Mpl^{W515L}
OM+Mpl^{W515L}
23.1
54
8.88
10.5
21.7
56.4
Fcγ RII/III
CD34
Gated on PI$^-$Lin$^-$Kit$^+$Sca1$^-$ cells
B
WT+Mpl^{W515L}
OM+Mpl^{W515L}
8.47
86.3
Fcγ RII/III
CD41
Gated on PI$^-$Lin$^-$Kit$^+$CD9$^+$ cells
10^0
10^1
10^2
10^3
10^4

Figure S9

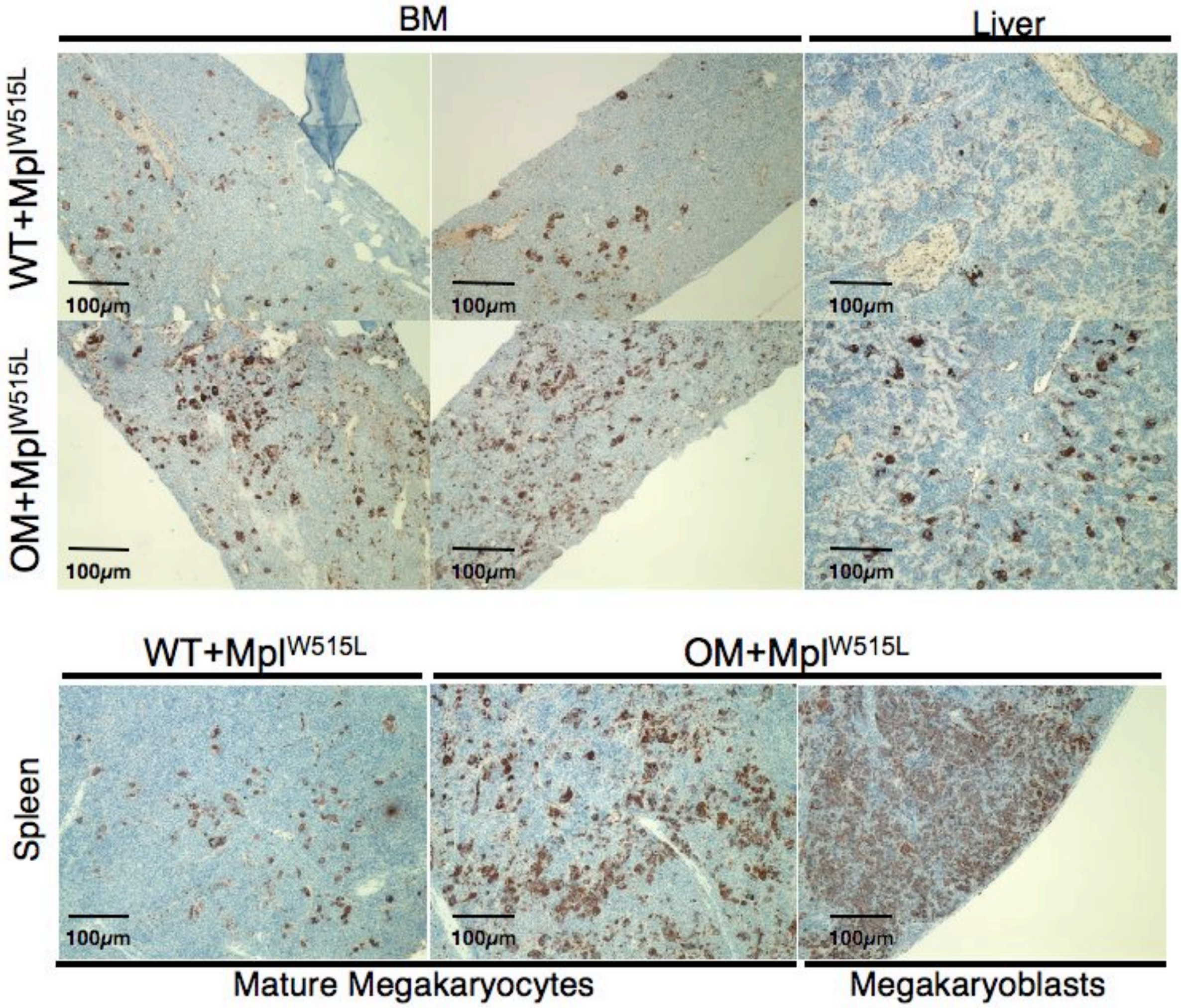

BM
Liver
WT+Mpl^W515L
OM+Mpl^W515L
100μm
WT+Mpl^W515L
OM+Mpl^W515L
Spleen
Mature Megakaryocytes
Megakaryoblasts

Figure S10

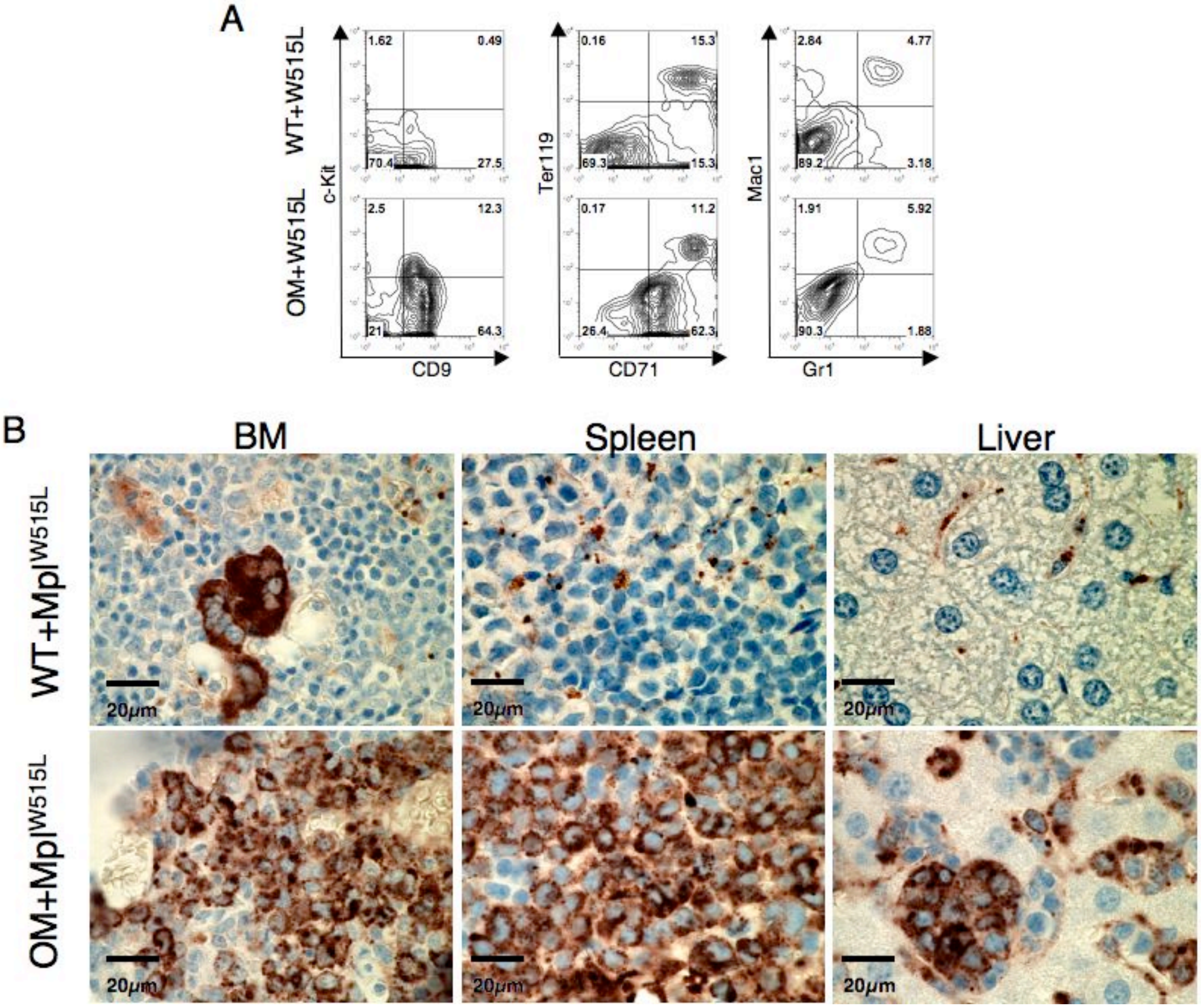

A
WT+W515L
OM+W515L
c-Kit
CD9
1.62
0.49
70.4
27.5
2.5
12.3
21
64.3
Ter119
CD71
0.16
15.3
69.3
15.3
0.17
11.2
26.4
62.3
Mac1
Gr1
2.84
4.77
89.2
3.18
1.91
5.92
90.3
1.88
B
BM
Spleen
Liver
WT+Mpl^{W515L}
OM+Mpl^{W515L}
20μm
20μm
20μm
20μm
20μm
20μm

Figure S11

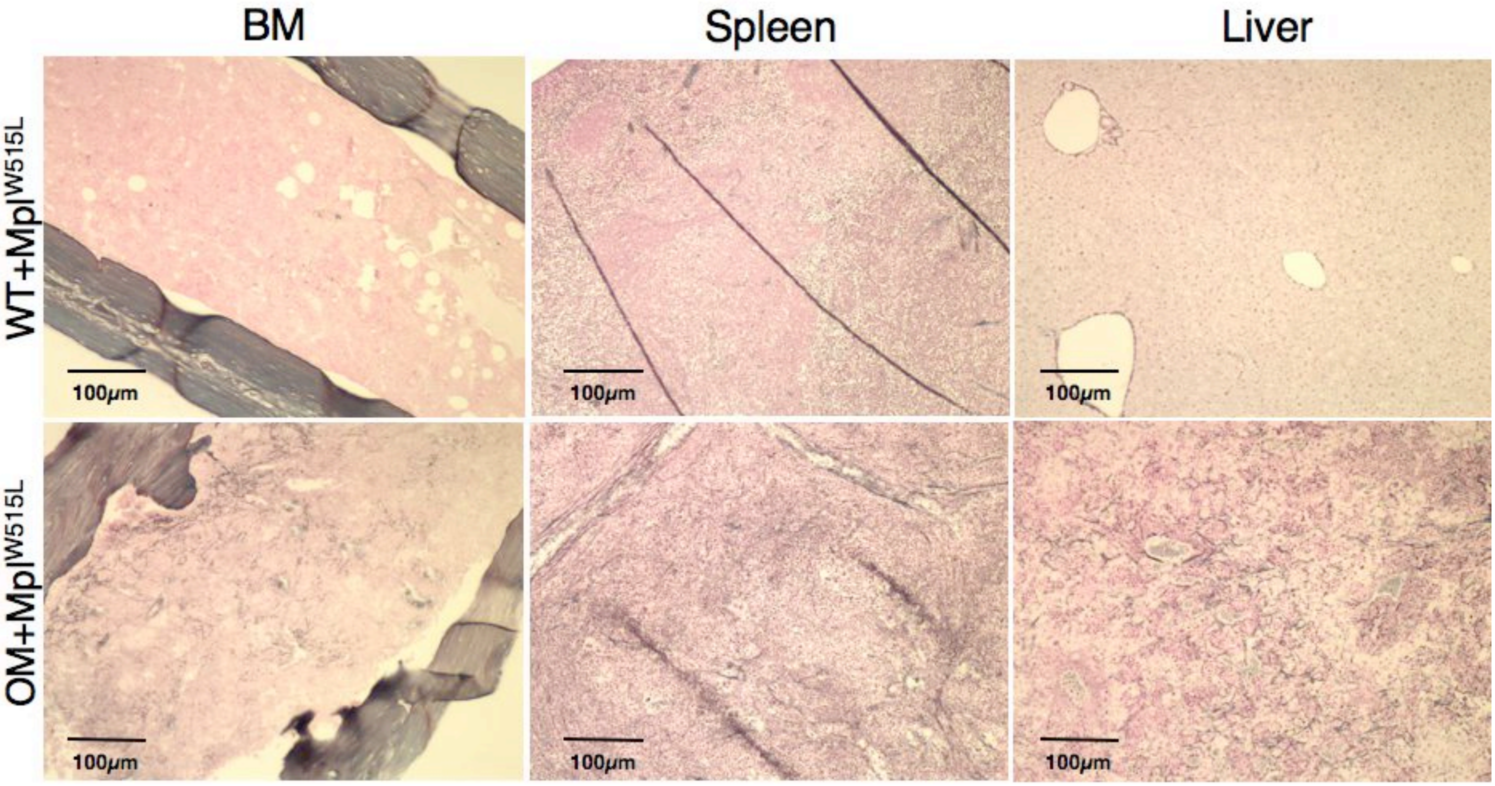

BM
Spleen
Liver
WT+Mpl^{W515L}
OM+Mpl^{W515L}
100μm
100μm
100μm
100μm
100μm
100μm

Figure S12

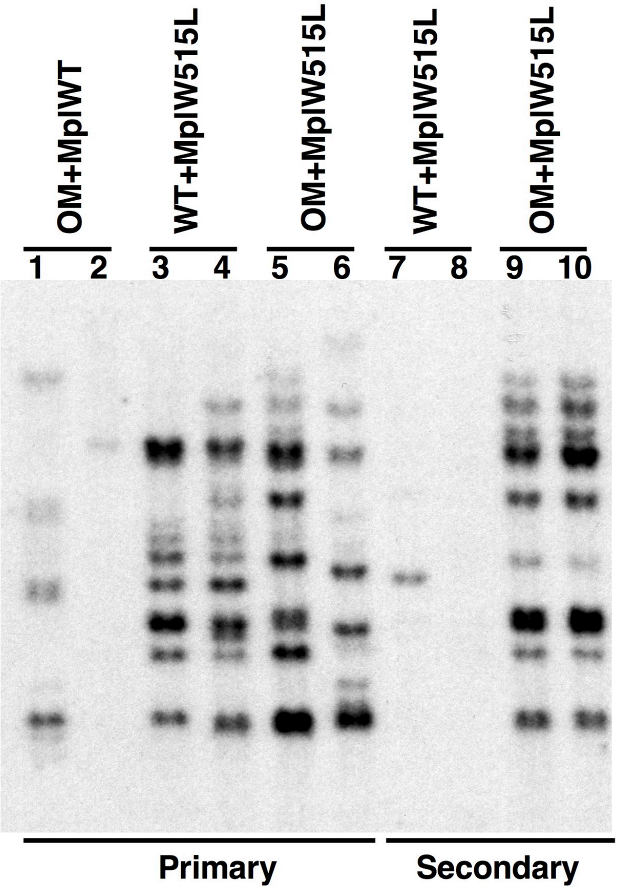

OM+MplWT
WT+MplW515L
OM+MplW515L
WT+MplW515L
OM+MplW515L
1
2
3
4
5
6
7
8
9
10
Primary
Secondary

Table S1:
Notch pathway genes for Gene Set Enrichment Analysis

Probe Set ID	Gene	Gene Title
203395_s_at	Hes1	hairy and enhancer of split 1 (Drosophila)
203394_s_at	Hes1	hairy and enhancer of split 1 (Drosophila)
216674_at	Hes2	hairy and enhancer of split 2 (Drosophila)
214521_at	Hes2	hairy and enhancer of split 2 (Drosophila)
239230_at	Hes5	hairy and enhancer of split 5 (Drosophila)
204849_at	Tcfl5	transcription factor-like 5 (basic helix-loop-helix)
218839_at	HEY1	hairy/enhancer of split related with YRPW motif 1
44783_s_at	HEY1	hairy/enhancer of split related with YRPW motif 1
219743_at	HEY2	hairy/enhancer of split related with YRPW motif 2
201170_s_at	BHLHB2	basic helix-loop-helix domain containing, class B,2
201169_s_at	BHLHB2	basic helix-loop-helix domain containing, class B,2
221530_s_at	BHLHB3	basic helix-loop-helix domain containing, class B,3
202284_s_at	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21,Cip1)
218996_at	TFPT	TCF3 (E2A) fusion partner (in childhood leukemia)
211837_s_at	PTCRA	pre-TCRalpha (Ptcra)
215492_x_at	PTCRA	pre-TCRalpha (Ptcra)
211252_x_at	PTCRA	pre-TCRalpha (Ptcra)
209604_s_at	GATA3	GATA binding protein 3
209602_s_at	GATA3	GATA binding protein 3
209603_at	GATA3	GATA binding protein 3
218751_s_at	FBXW7	F-box and WD-40 domain protein 7 (archipelago)
218902_at	Notch1	notch gene homolog 1 (Drosophila)
212377_s_at	Notch2	notch gene homolog 2 (Drosophila)
210756_s_at	Notch2	notch gene homolog 2 (Drosophila)
202443_x_at	Notch2	notch gene homolog 2 (Drosophila)
202445_s_at	Notch2	notch gene homolog 2 (Drosophila)
203238_s_at	Notch3	notch gene homolog 3 (Drosophila)
203237_s_at	Notch3	notch gene homolog 3 (Drosophila)
205247_at	Notch4	notch gene homolog 4 (Drosophila)
219537_x_at	DLL3	delta3 (Delta-like3/DLL3)
209560_s_at	DLK1	delta-like1 homolog
216268_s_at	JAG1	jagged1 (Alagille syndrome)
209097_s_at	JAG1	jagged1 (Alagille syndrome)
209098_s_at	JAG1	jagged1 (Alagille syndrome)
209099_x_at	JAG1	jagged1 (Alagille syndrome)
32137_at	JAG2	jagged2
209784_s_at	JAG2	jagged2
32137_at	JAG2	jagged2
215732_s_at	DTX2	deltex2 homolog 2 (Drosophila)
49051_g_at	DTX3	deltex3 homolog 3 (Drosophila)
221835_at	DTX3	deltex3 homolog 3 (Drosophila)
49049_at	DTX3	deltex3 homolog 3 (Drosophila)
202604_x_at	Adam10	a disintegrin and metallopeptidase domain 10
214895_s_at	Adam10	a disintegrin and metallopeptidase domain 10
202603_at	Adam10	a disintegrin and metallopeptidase domain 10

213532_at	Adam17	a disintegrin and metallopeptidase domain 17
205746_s_at	Adam17	a disintegrin and metallopeptidase domain 17
205745_x_at	Adam17	a disintegrin and metallopeptidase domain 17
218389_s_at	Aph1a	anterior pharynx defective 1a homolog (C. elegans)
221036_s_at	Aph1b	anterior pharynx defective 1b homolog (C. elegans)
217094_s_at	Itch	itchy homolog E3 ubiquitin protein ligase (mouse)
209743_s_at	Itch	itchy homolog E3 ubiquitin protein ligase (mouse)
209744_x_at	Itch	itchy homolog E3 ubiquitin protein ligase (mouse)
207782_s_at	Psen1	presenilin 1 (Alzheimer disease 3)
203460_s_at	Psen1	presenilin 1 (Alzheimer disease 3)
204262_s_at	Psen2	presenilin 2 (Alzheimer disease 4)
204261_s_at	Psen2	presenilin 2 (Alzheimer disease 4)
211373_s_at	Psen2	presenilin 2 (Alzheimer disease 4)
218302_at	Psen2	presenilin 2 (Alzheimer disease 4)
202360_at	Maml1	mastermind like 1 (Drosophila)
206480_at	Maml1	mastermind like 1 (Drosophila)
207946_at	MAML3	mastermind like 3 (Drosophila)
207785_s_at	RBPSUH	recombining binding protein suppressor of hairless
211974_x_at	RBPSUH	recombining binding protein suppressor of hairless
215270_at	LFNG	lunatic fringe homolog (Drosophila)
212968_at	RFNG	radical fringe homolog (Drosophila)
204153_s_at	MFNG	manic fringe homolog (Drosophila)
204152_s_at	MFNG	manic fringe homolog (Drosophila)
209073_s_at	Numb	numb gene homolog (Drosophila)
207545_s_at	Numb	numb gene homolog (Drosophila)
219286_s_at	Rbm15	RNA binding motif protein 15
202689_at	Rbm15b	RNA binding motif protein 15B
201996_s_at	SPEN	spen homolog, transcriptional regulator (SHARP)
201997_s_at	SPEN	spen homolog, transcriptional regulator (SHARP)
208759_at	NCSTN	nicastrin
204889_s_at	NEURL	neuralized1 (Rfn67, Neurl1)
204888_s_at	NEURL	neuralized1 (Rfn67, Neurl1)
205435_s_at	AAK1	AP2-associated kinase
214998_at	AAK1	AP2-associated kinase
214956_at	AAK1	AP2-associated kinase
211186_s_at	AAK1	AP2-associated kinase
205434_s_at	AAK1	AP2-associated kinase
209988_s_at	ASCL1	Mash1/Hash1 (Ascl1)
209985_s_at	ASCL1	Mash1/Hash1 (Ascl1)
213768_s_at	ASCL1	Mash1/Hash1 (Ascl1)
209987_s_at	ASCL1	Mash1/Hash1 (Ascl1)
201575_at	SNW1	SNW domain containing 1
215424_s_at	SNW1	SNW domain containing 1
222183_x_at	SNW1	SNW domain containing 1
216424_at	Cd4	CD4 molecule
203547_at	Cd4	CD4 molecule

The OTT-MAL fusion oncogene activates RBPJ-mediated transcription and induces acute megakaryoblastic leukemia in a knock-in mouse model

Thomas Mercher, Glen D. Raffel, Sandra A. Moore, Melanie G. Cornejo, Dominique Baudry-Bluteau, Nicolas Cagnard, Jonathan L. Jesneck, Yana Pikman, Dana Cullen, Ifor R. Williams, Koichi Akashi, Hirokazu Shigematsu, Jean-Pierre Bourquin, Marco Giovannini, William Vainchenker, Ross L. Levine, Benjamin H. Lee, Olivier A. Bernard, D. Gary Gilliland

Supplemental data legends

Figure S1: Analysis of E12.5 fetal liver cells.

A: HSC (LSK cells) in WT vs. *OTT*$^{OTT\text{-}MAL}$ (OM)

B: E12.5 total fetal liver cells analyzed by flow cytometry for the CD41 marker.

Figure S2: Histopathologic analysis of OTT-MAL-induced AMKL.

Peripheral blood (PB) smears and cytospun splenocytes (Splenocytes) were stained with Wright-Giemsa solutions. Bone marrow (BM), spleen, liver and kidney sections from #6127 (WT control) and #6133 (OM) animals were stained with Hematoxylin&Eosin (H&E) solutions. Arrows indicate megakaryoblasts with cytoplasmic blebs, characteristic of human AMKL, in peripheral blood and splenocytes of #6133.

Figure S3: AMKL developed in *OTT*$^{OTT\text{-}MAL}$ (OM) animal is transplantable to secondary recipients.

A: Average (lowest value, highest value) spleen weight, liver weight, white blood counts (WBC), hematocrit percentage (HCT) and platelet counts in secondary SCID recipients are indicated.

B: Von Willebrand factor immunohistochemistry on spleen and liver sections from secondary recipients is shown.

Figure S4: dnRBPJ inhibits proliferation of 6133 cells but not Ba/F3 cells.

A: RNA was extracted from lineage-negative cells of OM and WT littermate control animals and analyzed by quantitative RT-PCR for Notch target genes compared to Gapdh.

B: Ba/F3 cells were infected with dnRBPJ, dnMAML1 and empty vector, sorted for GFP+ cell after 24 hours and viable cells were counted. Mean number of cells is shown.

C: RNA were extracted from 6133 cells infected with dnRBPJ, dnMAML1 and empty vector, and analyzed for expression of Hes1 transcript by quantitative RT-PCR compared to Gapdh. Mean+/-SD of triplicate experiments is shown.

Figure S5: Phospho-flow cytometry analysis of 6133 cells.

Phosphoflow cytometry analysis of STAT3, STAT5 and S6 phosphorylation status in 6133 cells transduced with different mutants. Median fluorescence intensity is indicated on the histograms.

Figure S6: Characteristic of primary animals transplanted with $OTT^{OTT\text{-}MAL}$ +MPL^{W515L} (OM+MPL^{W515L}) and control bone marrow cells.

A: Counts from WT+*MPL*^{W515L} and OM+*MPL*^{W515L} primary recipients. White blood cell, hematocrit, platelet counts and spleen weights of animals transplanted with WT+*MPL*^{W515L} or OM+*MPL*^{W515L} cells are shown.

B: Kaplan-Meier survival curves of primary recipients.

Figure S7: Histogram representation of flow cytometry results in primary (**A**) and secondary (**B**) transplant animals. Mean+/-SD of three independent animals are shown.

Figure S8: MEP and MKP expansion in the spleens of OM+*MPL*^{W515L} animals.

A: Multi-parameter flow analysis of bone marrow CMP, GMP and MEP populations. Experiment was performed in duplicate and a representative analysis gated on PI$^-$Lin$^-$Sca$^-$Kit$^+$ cells is shown.

B: Multi-parameter flow analysis of the MKP population. MKP are defined as Lin$^-$Kit$^+$CD9$^+$FcγRII/III^{Low}CD41$^+$. Percentage of gated population is indicated.

Figure S9: Increased number of infiltrating megakaryocytes in primary OM+*MPL*^{W515L} animals compared to WT+*MPL*^{W515L}. Von Willebrand factor immunohistochemistry was performed on bone marrow, spleen and liver sections. Megakaryoblasts indicates areas showing poorly differentiated immature hypolobated megakaryocytes almost exclusively.

Figure S10: Secondary recipients have a AMKL phenotype.

A: Flow cytometry analysis of secondary recipient splenocytes.

B: Von Willebrand staining of secondary OM+*MPL*^{W515L} tissue sections reveals infiltration of bone marrow, spleen and liver with poorly differentiated immature megakaryoblasts.

Figure S11: AMKL is associated with increased fibrosis.

Bone marrow, spleen and liver sections were stained for reticulin fibers (black).

Figure S12: Clonality analysis.

DNA from primary (Lane #1-6) and secondary (Lane #7-10) recipients were extracted from bone marrow cells, digested with BamHI and analyzed by Southern blot using a probe in the GFP coding region. Numerous insertions of the MSCV MPL^{W515L} iresGFP are visible in primary and secondary (>10 insertion sites) *OTT*$^{OTT\text{-}MAL}$+MPL^{W515L} recipients (Lanes #5-6 and lanes #9-10, respectively). Secondary recipients (Lanes #9-10) presented here were derived from the primary animal in lane #5. Of note, the variation in the relative intensity between the bands, comparing either the primary animal (Lane #5) with secondary animals (Lanes #9-10) or the two secondary animals, supports the presence of several independent leukemic clones in primary and secondary recipients.

Table S1: Notch signaling pathway genes for gene set enrichment analysis. Genes were included in the list based on the report in the literature of their implication in the Notch signaling pathway. Probe set identification numbers; gene symbols and gene names are shown.