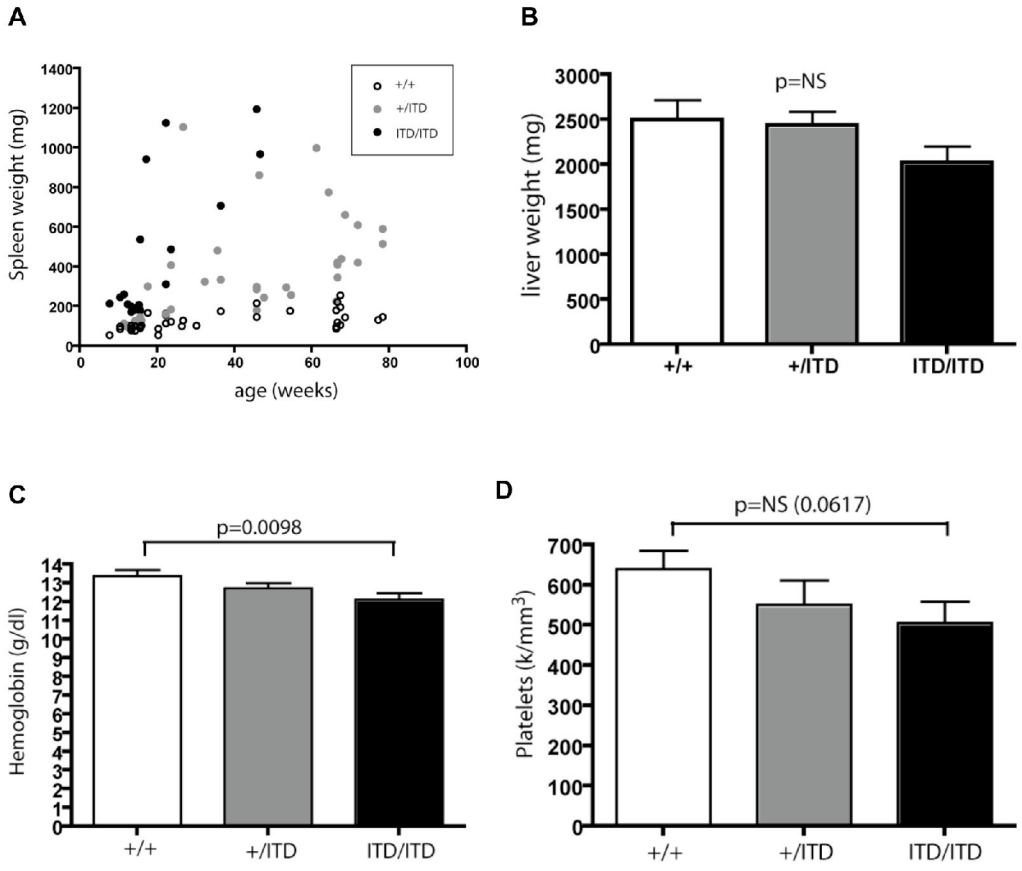
Supplemental Dataset Lee et al.

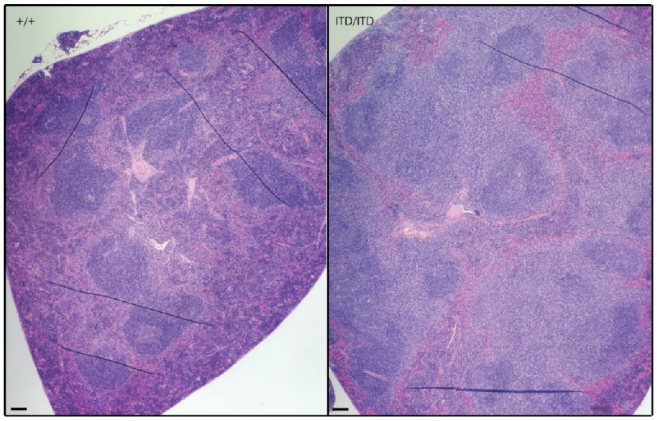


Supplemental Figure 1; Lee et al.

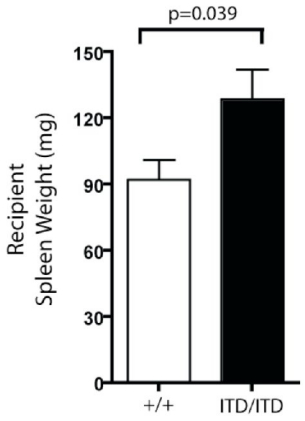
Supplemental Dataset Lee et al.

Supplemental Figure 1. Supplementary hematologic and pathologic data of *Flt3*$^{+/+}$, *Flt3*$^{+/ITD}$, and *Flt3*$^{ITD/ITD}$ mice (A) Splenomegaly in *Flt3*$^{+/ITD}$and *Flt3*$^{ITD/ITD}$ animals is variable and progresses over time. Mild decreased trends in (B) liver weight (C) hemoglobin levels and (D) platelet counts between *Flt3*$^{+/+}$, *Flt3*$^{+/ITD}$, and *Flt3*$^{ITD/ITD}$ mice are observed (plotted for B-D are mean values +/- S.E.M.).

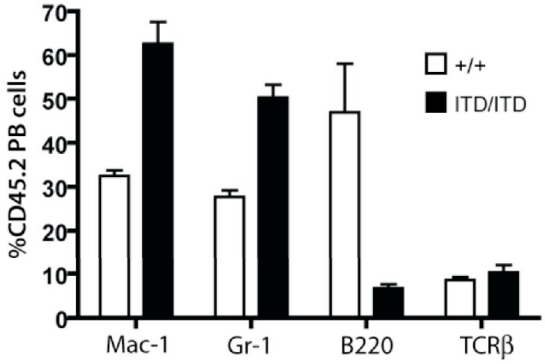
A



B

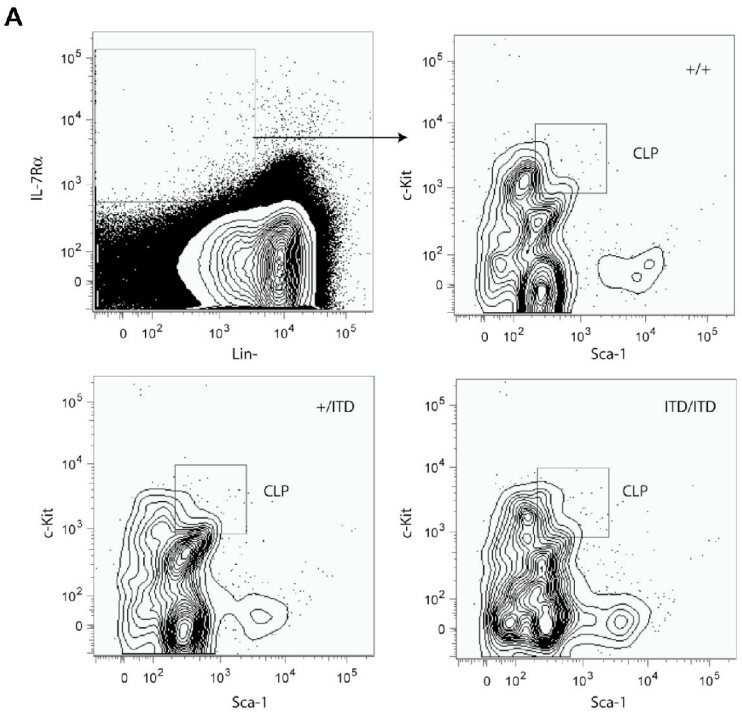


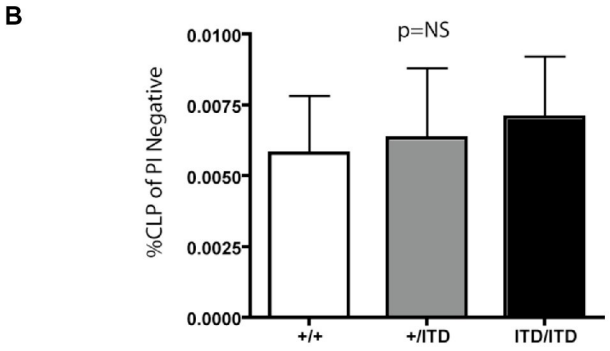
C



Supplemental Figure 2; Lee et al.

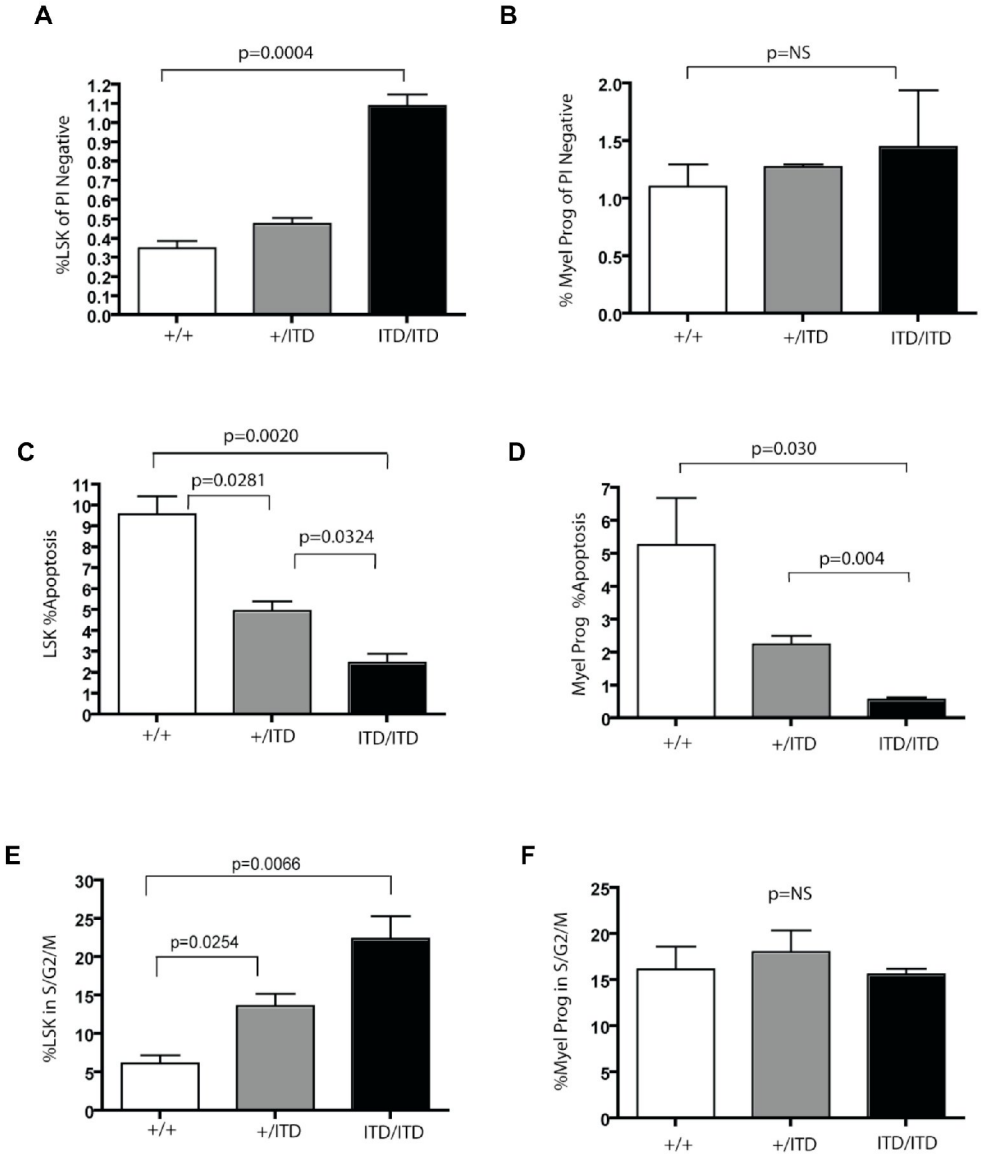
Supplemental Figure 2. Transplantability of Flt3-ITD induced myeloproliferative disease. (A) Image (left; H&E) demonstrates normal preserved splenic architecture from lethally irradiated wild-type recipients (B6 SJL.1) receiving 1x10^6 BM cells from *Flt3*$^{+/+}$ animals. Section (right; H&E) displays a representative spleen from a lethally irradiated wild-type recipient (B6 SJL.1) receiving 1x10^6 BM cells from a *Flt3*$^{ITD/ITD}$ donor animal. Scale bars, 250 μm. The image demonstrates a prominent white pulp expansion by an atypical population of pale mononuclear cells with monocytoid features similar to that observed in the primary mutant *Flt3*$^{ITD/ITD}$ donor animal. (B) Transplanted wild-type (B6 SJL.1) animals receiving either *Flt3*$^{+/+}$ (n=5) or *Flt3*$^{ITD/ITD}$ (n=5) BM (1x10^6 cells) were sacrificed at experimental endpoint 4 months after transplantation. Spleen weights demonstrated a statistically significant degree of splenomegaly in animals receiving *Flt3*$^{ITD/ITD}$ cells versus *Flt3*$^{+/+}$ (mean +/- S.E.M.). (C) Flow cytometric analysis of peripheral blood from lethally irradiated wild-type secondary recipients (B6 SJL.1-which express CD45.1) was performed 4 months after transplantation with either *Flt3*$^{+/+}$ (n=5) or *Flt3*$^{ITD/ITD}$ (n=5) BM (1x10^6 cells). Lineage analysis of peripheral blood donor cells (expressing CD45.2) was measured at time of sacrifice at 4 months, which demonstrated an increased population of myeloid and monocytic populations (Mac-1$^+$, Gr-1$^+$) and decreased B cells (B220 positive) in those animals receiving *Flt3*$^{+/+}$ versus *Flt3*$^{ITD/ITD}$ BM cells, reflecting the phenotype observed in the primary mutant animals. No differences in donor derived peripheral T cells (TCRβ-positive) were noted (values plotted are mean +/- S.E.M.).





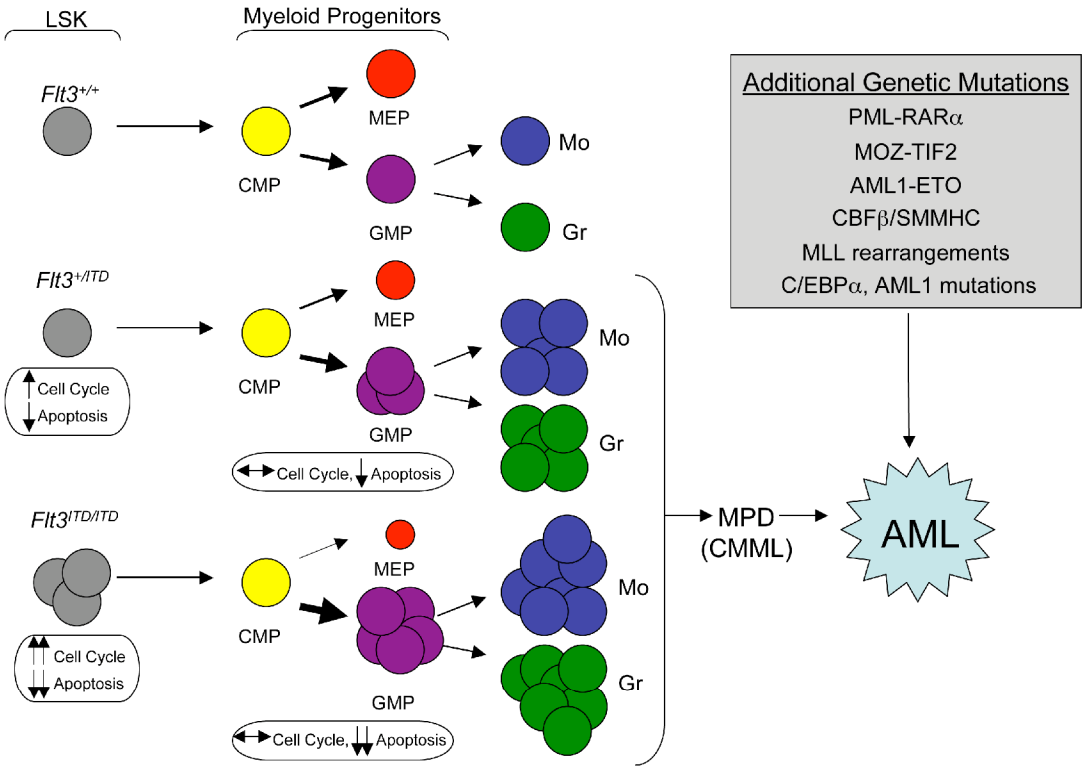
Supplemental Figure 3; Lee et al.

Supplemental Figure 3. Common lymphoid progenitors (CLP). (A) Multiparameter flow cytometric analysis of BM from representative *Flt3*$^{+/+}$, *Flt3*$^{+/ITD}$, and *Flt3*$^{ITD/ITD}$ mice. (B) Bar graph represents numbers of CLP as a percentage of live cells in mutant and wt littermates (mean +/- S.E.M.) [*Flt3*$^{+/+}$, n=3; *Flt3*$^{+/ITD}$, n=3; *Flt3*$^{ITD/ITD}$, n=3].



Supplemental Figure 4; Lee et al.

Supplemental Figure 4. Analysis of hematopoietic stem cell and progenitor populations of young mutant Flt3 animals. (A-B) Multiparameter flow cytomeric analysis of a cohort of one month old (31 days post natal) mutant Flt3 animals (mean +/- S.E.M.; *Flt3*$^{+/+}$, n=3; *Flt3*$^{+/ITD}$, n=2; *Flt3*$^{ITD/ITD}$, n=3) demonstrates significantly increased numbers of HSC (LSK; Lin$^-$Sca1$^+$ckit$^+$) cells and a trend towards increased myeloid progenitors (Myel Prog) in *Flt3*$^{ITD/ITD}$ mice over heterozygous and wt animals. (C-D) Survival analysis of HSC (Lin$^-$ Sca1$^+$ckit$^+$) and myeloid progenitor (Lin$^-$Sca1$^-$ckit$^+$) cells show a dose-dependent decrease in the percentage of apoptotic cells (7-AAD$^-$/Annexin-V$^+$) in both compartments. (E-F) Analysis of primitive stem and progenitor (Lin$^-$Sca1$^+$ckit$^+$) and myeloid progenitor (Lin$^-$Sca1$^-$ckit$^+$) populations in this same cohort of young mutant FLT3 mice demonstrate an LSK-specific increase of cells in S/G2/M and proportional decrease in G0/G1 in an ITD dose-dependent manner. For (C-F) plotted are mean values +/- S.E.M.; *Flt3*$^{+/+}$, n=3; *Flt3*$^{+/ITD}$, n=2; *Flt3*$^{ITD/ITD}$, n=3).



Supplemental Figure 5; Lee et al.

Supplemental Figure 5. Schematic model illustrating the effects of FLT3-ITD in leukemic hematopoiesis. The ITD allele causes increased cell cycling in a dose-dependent manner (*Flt3*$^{+/ITD}$ and *Flt3*$^{ITD/ITD}$ versus *Flt3*$^{+/+}$) that is restricted within the primitive LSK (Lin^{-}Sca1^{+}ckit^{+}) population and not observed in the myeloid progenitor compartment. Increased survival (decreased apoptosis) is seen in both the LSK and myeloid progenitor populations in an ITD dose-dependent fashion. Constitutive FLT3-ITD signaling promotes expansion of the GMP and a concomitant decrease in MEP ultimately leading to expansion of increased mature monocyte (Mo) and granulocyte (Gr) populations and the myeloproliferative disease (MPD; CMML) observed within these animals.

Supplemental Dataset Lee et al.

Development of an acute myeloid leukemia (AML) requires cooperation with additional genetic mutations, examples of which have been shown to be important in previously reported epidemiological studies as well as murine models.

Supplemental Table 1.
Clinical and laboratory characteristics of patients with CMML by FLT3 status

Variable	*FLT3-ITD Positive n=6*		*FLT3 Wild-type n=162*	
	Median	*Range*	*Median*	*Range*
Age, y	66	44-75	68	31-89
Male, no. (%)	4 (67%)	--	113 (70%)	--
Female, no. (%)	2 (33%)	--	49 (30%)	--
Hemoglobin, g/L	10.2	8.0-11.6	10.5	5.9-16.4
Platelet count, x 10^9/L	93	10-146	94.5	2-820
White blood cell count, x 10^9/L	18.0	6.8-37.2	15.5	2.6-173
Neutrophils, %	43	30-61	48	3-89
Neutrophils, 10^9/L	7.9	2.5-13.3	7.2	0.31-74.4
Monocytes, %	22	18-38	23	2-75
Monocytes, 10^9/L	3.9	1.6-14.1	3.3	0.2-50.2
Lymphocytes, %	13	7-29	14	1-50
Lymphocytes, 10^9/L	1.8	1.2-5.2	2.2	0.3-34.5
Eosinophils, %	2	0-7	1	0-29
Basophils, %	1	0-2	0	0-10
Peripheral blood IMCs, %*	11	1-25	4	0-35
Bone marrow blasts, %	7	2-20	5.5	0-27
Bone marrow monocytes, %	8	5-18	11	1-40
Bone marrow lymphocytes, %	5	2-10	6	0-26
Bone marrow erythroid cells, %	19	5-40	15	0-65
Myeloid-erythroid cell ratio	3.3	0.8-13.4	3.7	0.0-88.0
LDH, U/L	718	598-1627	555	191-4759
B^2-microglobulin, mg/L	8.5	5.6-12.4	3.7	0-19.2

*IMCs (immature myeloid cells)

Supplemental Table 2.
Bone marrow (I) and hematological profiles (II) of FLT3-ITD CMML patients

I.	*UPIN*	*Cellularity %*	*Blasts %*	*PBIMC %*	*MC+MMC %*	*Segs %*	*Monos %*	*Lymphs %*	*Normoblasts %*
	5886	30	20	4	33	26	5	2	5
	4536	90	6	1	30	33	5	2	21
	1883	40	7	2	15	15	10	10	40
	7054	95	8	5	35	13	13	7	10
	1339	90	4	4	34	19	18	2	17
	0729	35	2	7	19	15	6	8	33

II.	*UPIN*	*Hb* g/dl*	*Platelets x10^9/L*	*WBC x10^9/L*	*Monos x10^9/L*	*Segs %*	*Lymph %*	*Monos %*	*IMC %*	*LDH iU/L*	*β_2M mg/L*
	5886	8.0(T)	10	22.3	4.0	49	7	13	11	779	5.1
	4536	9.5(T)	135	13.6	3.8	48	9	28	6	1089	9.4
	1883	8.8(T)	48	8.5	1.7	61	14	20	5	656	5.6
	7054	10.8(T)	146	30.8	5.5	30	17	18	9	1627	12.4
	1339	11.6(T)	120	37.2	9.8	36	9	38	13	598	7.6
	0729	11.6	66	6.8	1.6	37	29	24	7	604	2.0

*T=red blood cell transfusion dependent; UPIN (unique patient identifier number); PBIMC (peripheral blood immature myeloid cells); MC+MMC (myelocytes + metamyelocytes); Hb (hemoglobin); WBC (white blood cells); IMC (immature myeloid cells); LDH (lactate dehydrogenase); β_2M (beta 2-microglobulin)