

Supplemental materials

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Table S1. Leukemic organ infiltration.

Table S2. Primer sequences.

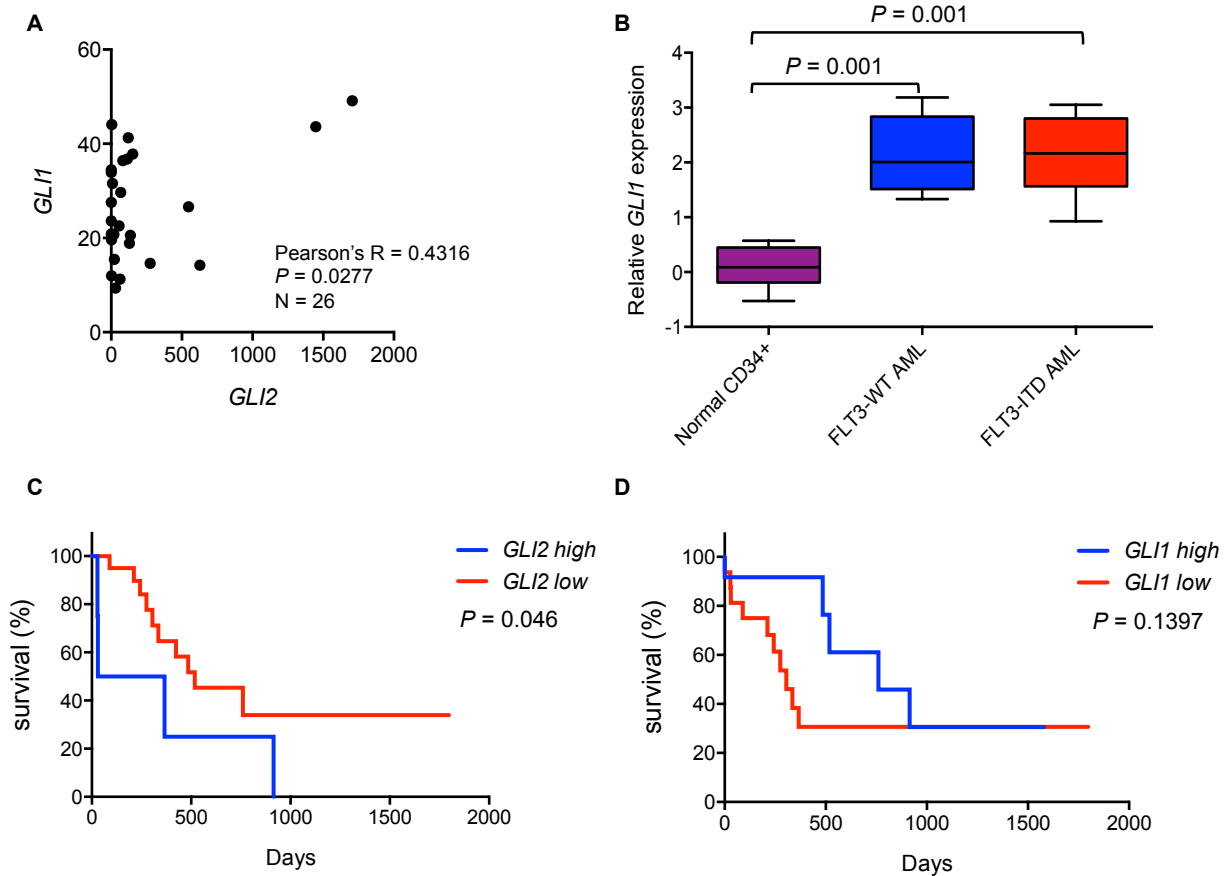


Figure S1. GLI2 is expressed in FLT3-ITD AML clinical specimens. (A) Correlation of *GLI1* and *GLI2* expression in *FLT3*-ITD AML patients from TCGA AML dataset. (B) *GLI1* gene expression in AML patient samples relative to normal human CD34+ cells. (C) Survival of patients with *FLT3*-ITD AML grouped by *GLI2* expression. Patients with *FLT3*-ITD were stratified on the basis of *GLI2* expression as high (above average) and low (below average) groups [average=204, expression determined by RNAseq (RSEM)]. *GLI2* mRNA expression data and *FLT3* mutation status were obtained from cBioPortal (www.cbioportal.org), and clinical data including survival were obtained from TCGA (cancergenome.nih.gov). *P* value was determined with the Gehan-Breslow-Wilcoxon test. (D) Survival of patients with *FLT3*-ITD AML grouped by *GLI1* expression.

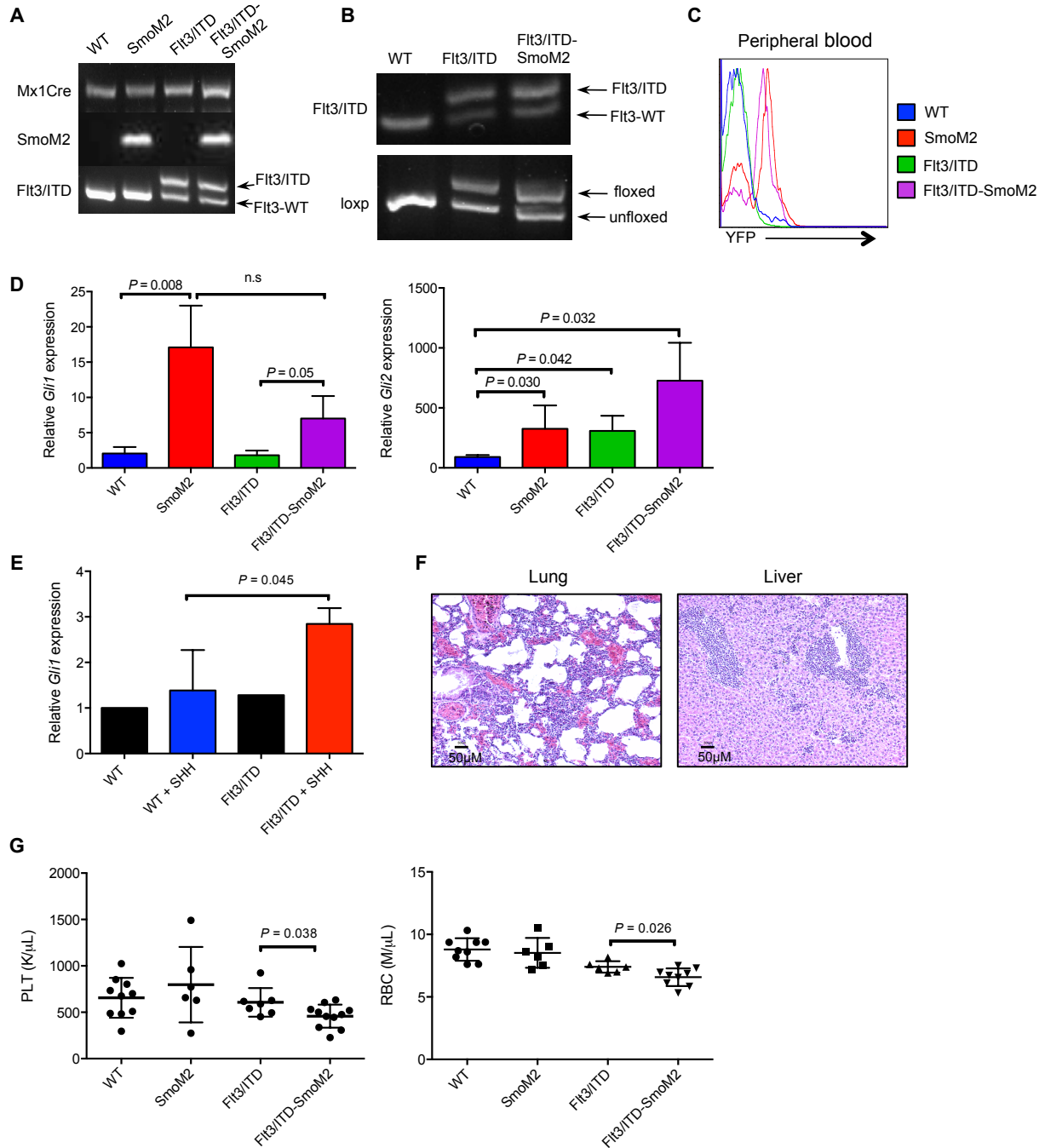


Figure S2. *Flt3/ITD-SmoM2* transgenic mice express Hh pathway genes and develop clinically relevant AML. (A) Genotyping of *Flt3/ITD-SmoM2* transgenic animals. Each lane represents a mouse genotype and each band the detected allele. **(B)** PCR showing excision of *Flt3-ITD* after poly(I:C) administration. **(C)** Peripheral blood flow cytometry of YFP expression after poly(I:C) administration. **(D)** *Gli1* and *Gli2* expression in mouse whole bone marrow cells (n=3). Data represent mean \pm SD. **(E)** *Gli1* expression in mouse bone marrow after SHH treatment (n=3). Data represent mean \pm SD. **(F)** Hematoxylin and eosin stained sections of lung and liver. Bars represent 50 μ M. **(G)** Peripheral red blood cell and platelet counts. Each data point represents one mouse.

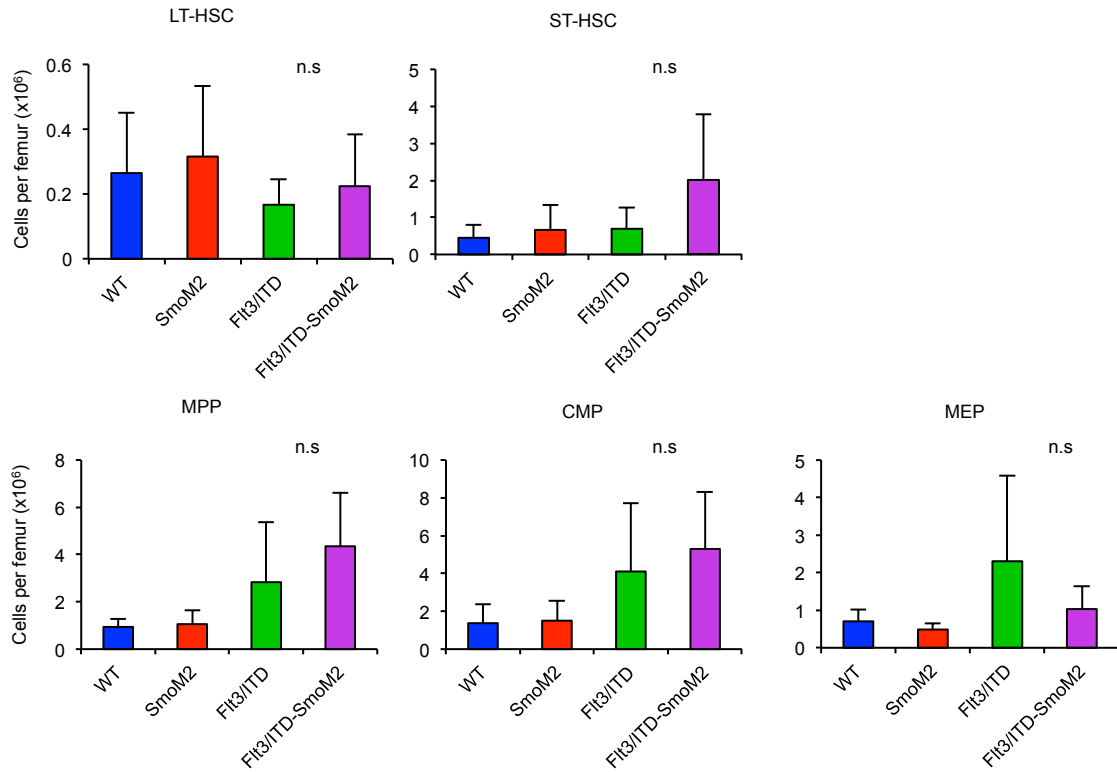
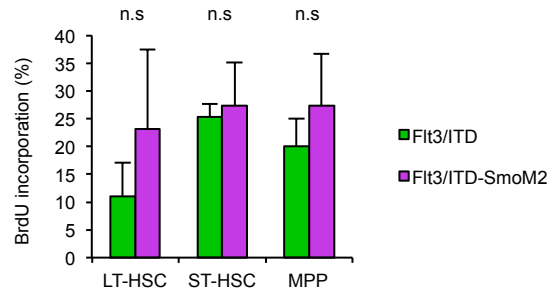
A**B**

Figure S3. Specific bone marrow hematopoietic stem cell and myeloid progenitor compartments are maintained in Flt3/ITD-SmoM2 mice. (A) Total number of immature HSPCs (n=3). Data represent mean \pm SD. n.s.=non-significant. (B) BrdU incorporation by primitive HSPC populations (n=3). Data represent mean \pm SD. n.s.=non-significant.

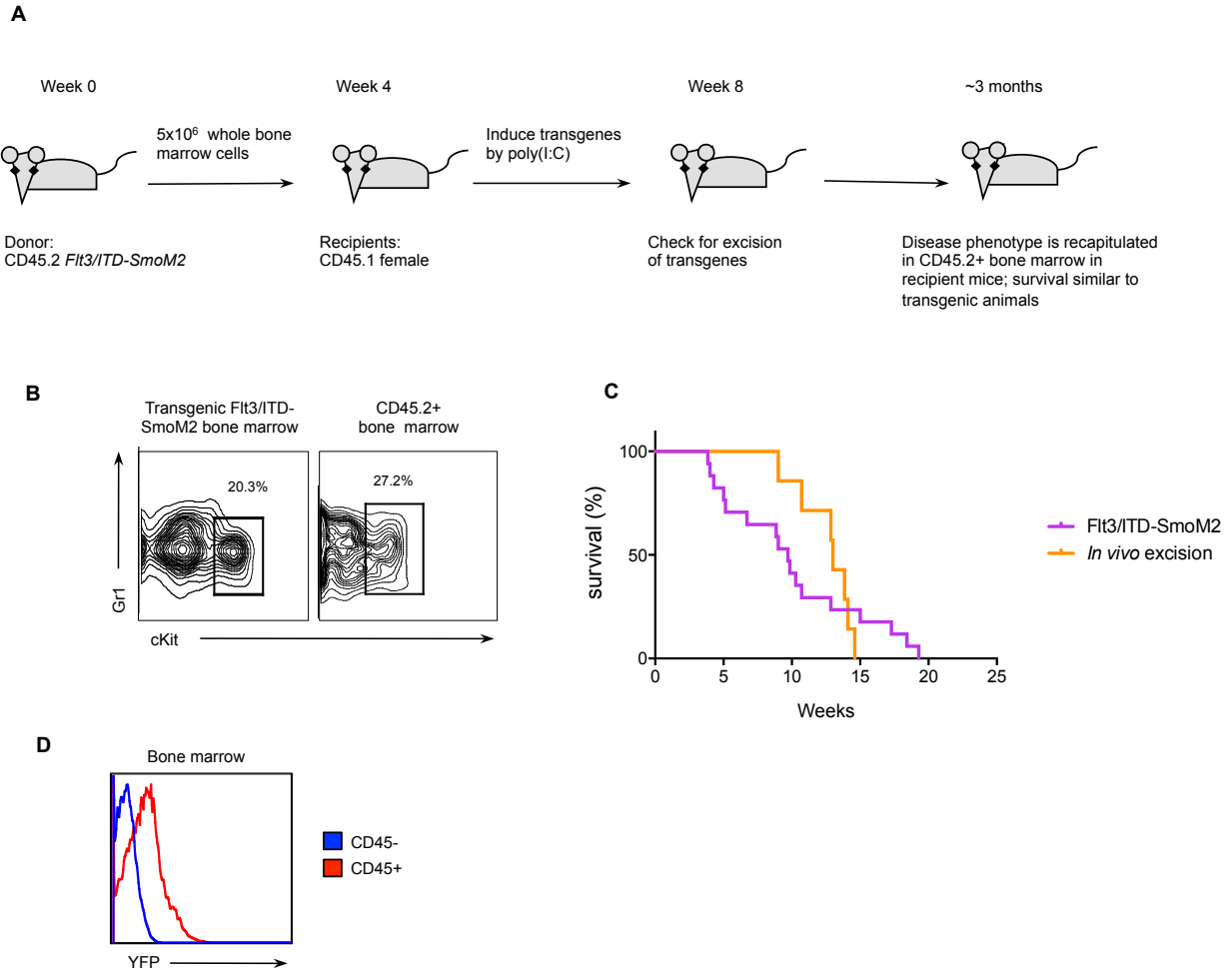


Figure S4. Leukemia formation in *FIt3/ITD-SmoM2* mice is cell-intrinsic. (A) Experimental schema. (B) Recapitulation of disease phenotype in bone marrow of original transgenic animals (left) and transplanted CD45.1 recipients (right). (C) Kaplan Meier survival curve of recipient mice after poly(I:C) administration compared to transgenic *FIt3/ITD-SmoM2* mice. (D) YFP expression in bone marrow of transplanted mice.

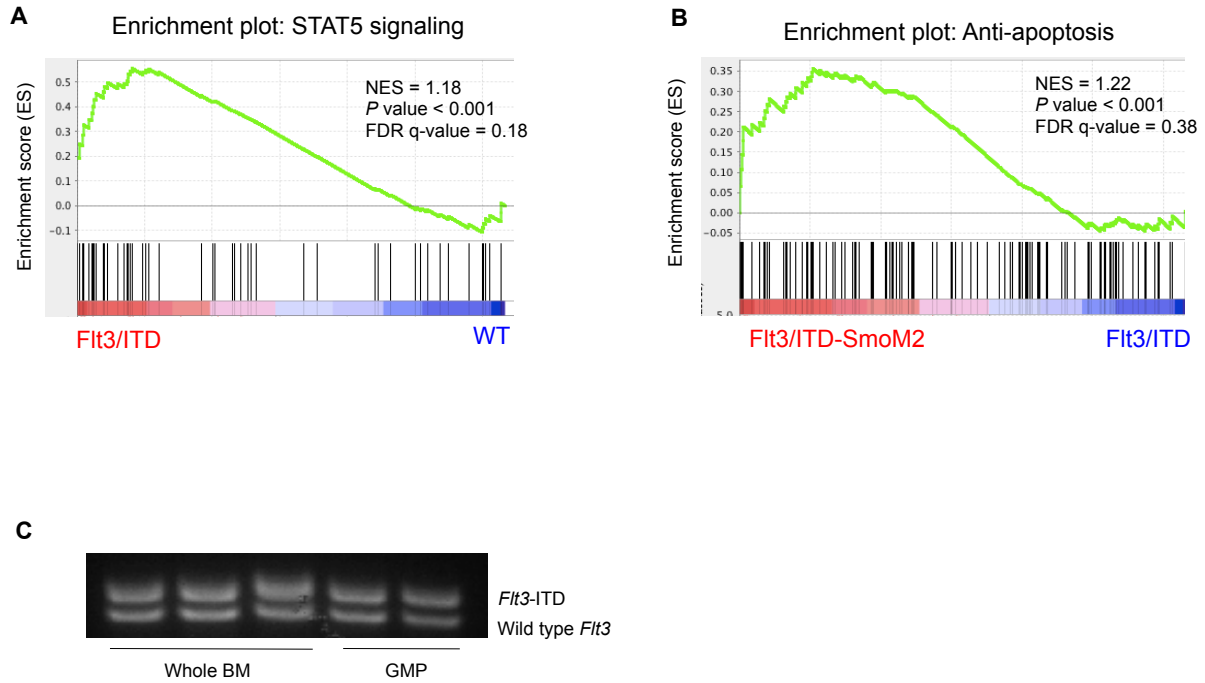


Figure S5. GSEA reveals increased STAT5 signaling in *Flt3/ITD* and anti-apoptotic features in *Flt3/ITD-SmoM2* mice. (A) GSEA analysis comparing *Flt3/ITD* and wild type mice using gene sets representing STAT5 signaling. (B) GSEA analysis comparing *Flt3/ITD-SmoM2* and *Flt3/ITD* mice with gene sets representing anti-apoptosis. (C) PCR analysis of *Flt3/ITD* status in whole bone marrow and GMP cells of *Flt3/ITD-SmoM2* diseased mice. Each lane represents an individual *Flt3/ITD-SmoM2* mouse.

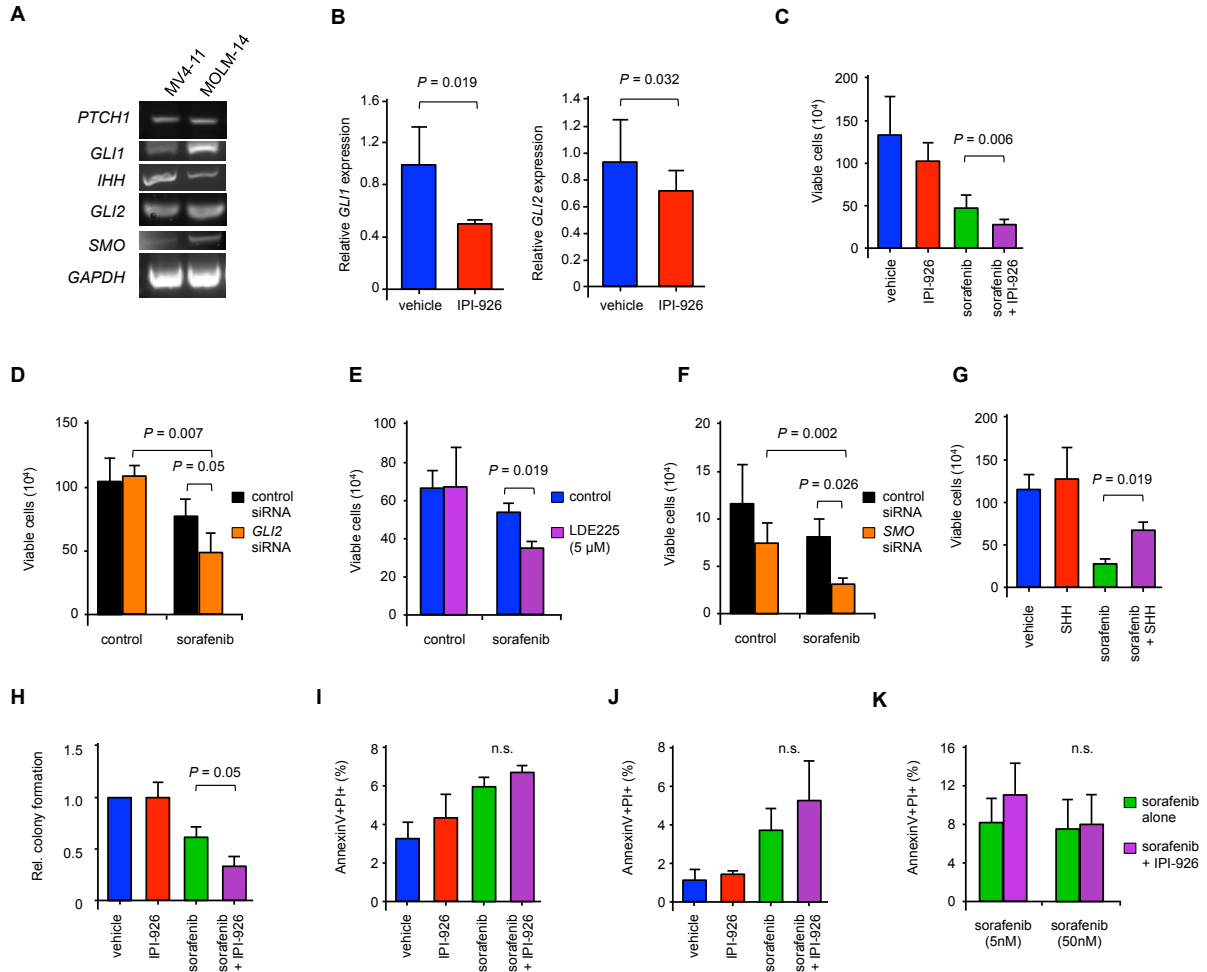


Figure S6. The combination of sorafenib and IPI-926 limit the growth of *FLT3*-ITD AML. (A) Expression of Hh components in AML cell lines. (B) Relative *GLI1* and *GLI2* expression in MV4-11 cells treated with IPI-926 (n=3). Data represent mean \pm SD. (C) Viable cell counts in drug-treated Molm-14 cells. (D) Viable cell counts after transfection of MV4-11 cells with *GLI2* or control siRNA and treated with sorafenib 24 hours later (n=3). Data represent mean \pm SD. (E) Viable cell counts of MV4-11 cells treated with LDE225 and/or sorafenib (n=3). Data represent mean \pm SD. (F) Viable cell counts of MV4-11 cells after transfection of *SMO* or control siRNA followed 24 hours later with sorafenib (n=3). Data represent mean \pm SD. (G) Viable cell counts of MOLM-14 cells treated with SHH and sorafenib (n=3). Data represent mean \pm SD. (H) Relative colony formation of Molm-14 cells after drug treatment (n=3). Data represent mean \pm SD. Apoptosis of drug-treated (I) MV4-11, (J) Molm-14 cells, and (K) HL60 cells (n=3). Data represent mean \pm SD. n.s.=non-significant.

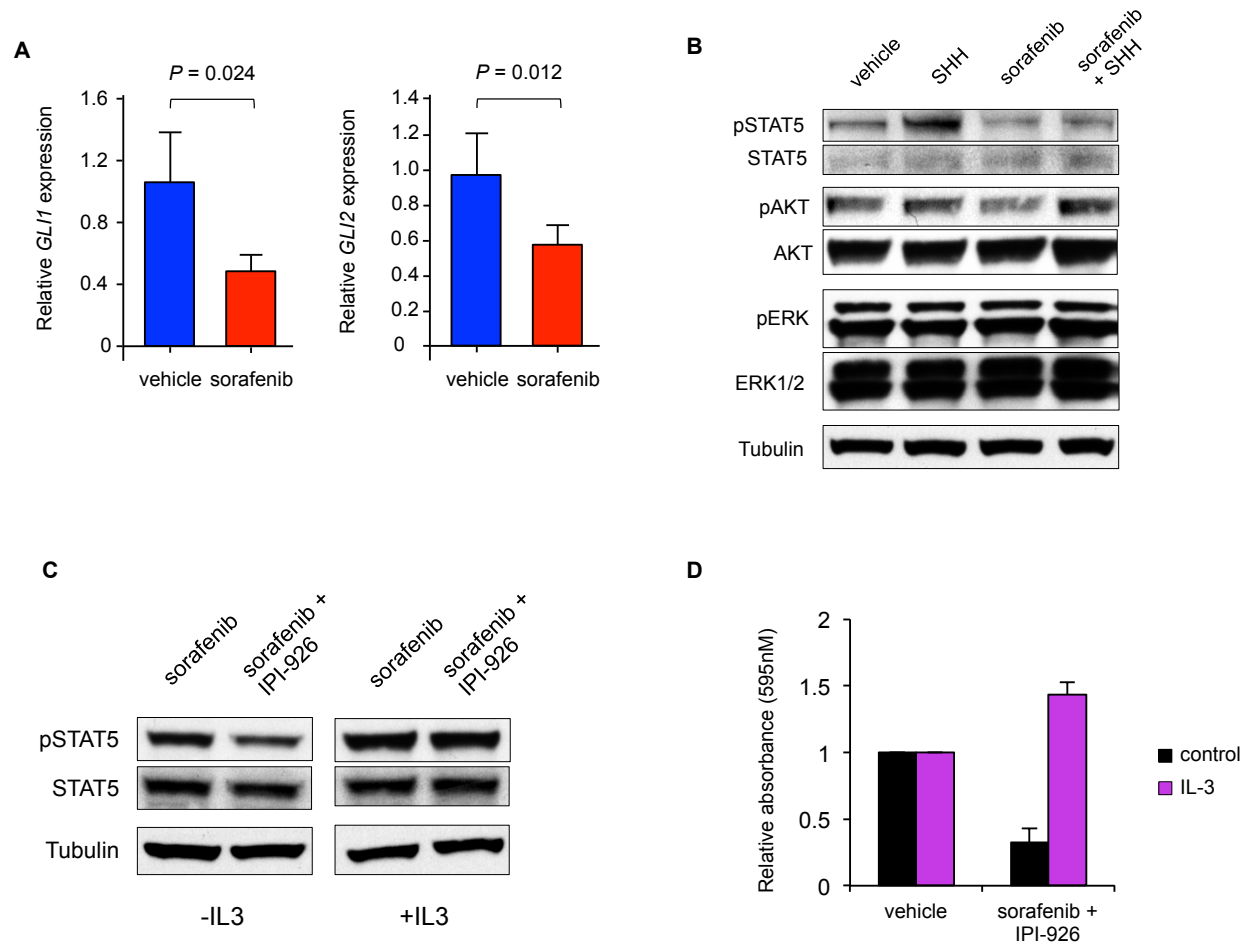


Figure S7. Hh signaling impacts FLT3-ITD at the level of STAT5. (A) Relative *GLI1* and *GLI2* expression in MV4-11 cells after sorafenib treatment (n=3). Data represent mean \pm SD. (B) Western blot showing protein expression in MV4-11 cells after SHH and/or sorafenib treatment. (C) Western blot of pSTAT5 and STAT5 expression in Baf3/ITD cells after drug and IL-3 treatment. (D) MTT assay of Baf3/ITD cells after treatment with sorafenib + IPI-926 with or without IL-3 (n=3). Data represent mean \pm SD.

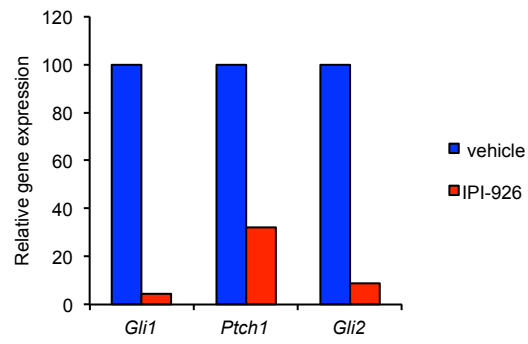


Figure S8. IPI-926 inhibits the expression of Hh target genes in Flt3/ITD-SmoM2 bone marrow cells.

Genotype	Spleen	Bone Marrow	Liver	Lung	Kidney	Thymus
<i>Flt3/ITD-SmoM2</i>	3+	3+	normal	normal	1+	depleted
<i>Flt3/ITD-SmoM2</i>	3+	3+	3+	3+	1+	depleted
<i>Flt3/ITD-SmoM2</i>	3+	3+	1+	2+	normal	depleted
<i>Flt3/ITD-SmoM2</i>	3+	3+	2+	normal	normal	depleted
<i>Flt3/ITD</i>	1+	1+	1+	normal	normal	normal
<i>SmoM2</i>	normal	normal	normal	normal	normal	normal
<i>SmoM2</i>	normal	normal	normal	normal	normal	normal
WT	normal	normal	normal	normal	normal	normal

Table S1. Leukemic organ infiltration.

Normal = similar to wild type

Depleted = decreased tissue cellularity compared to wild type

1+ = low degree of infiltration of tissue with undifferentiated myeloid cells

2+ = intermediate degree of infiltration of tissue with undifferentiated myeloid cells

3+ = high degree of infiltration of tissue with undifferentiated myeloid cells

Table S2. PCR primers

	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
Mouse genotyping		
<i>SmoM2</i>	AAGTTCATCTGCACCACCG	TCCTTGAAGAAGAT GTGCG
<i>Flt3-ITD</i>	CTCTC GGAACTCCCCTTA	TGCAGATGATCCAGGTGACT
<i>Mx1Cre</i>	GCGGTCTGGCAGTAAAACTA TC	GTGAAACAGCATTGCTGTCACTT
<i>loxP</i>	CTTCGTATAATGTATGCTATACG	TCGTATAGCATACATTATACG
Mouse qRT-PCR		
<i>Socs3</i>	CCAAGAACCTACGCATCCAGTG	CGTGGGTGGCAAAGAAAAGG
<i>Fos</i>	CGAAGGGAACGGAATAAGATGG	AGACCTCCAGTCAAATCCAGGG
<i>JunB</i>	CAGCTACTTTTCGGGTCAGGG	GGCTAGCTTCAGAGATGCGC
<i>p57</i>	CAGCGGACGATGGAAGAACT	CTCCGGTTCCTGCTACATGAA
<i>CyclinF</i>	GCCTCTCGCTTCTTCAGCAT	GGCGGATGAAGAGCCAGAT
<i>Pim1</i>	GCCCTCCTTTGAAGAAATCC	GGACCTGGAGTCTGGAATGA
<i>RPS16</i>	CTTGAGGCTTCATCCACAT	ATATTCGGGTCCGTGTGAAG
<i>Mkp1</i>	GTGCCTATCACGCTTCTCGG	TGGTTGTCCTCCACAGGGAT
Human qRT-PCR		
<i>PTCH1</i>	AATAAGGCTGAGGTTGGTCATGGTTAC	AGGGTCGTGGTGGTGAAGGAAAG
<i>IHH</i>	GGCAGCTGTCTCTACACACG	GGGCCTAAGATGGATGGAAT
<i>GLI1</i>	CCACGGGGAGCGGAAGGAG	ACTGGCATTGCTGAAGGCTTTACTG
<i>SMO</i>	GGTGTGGTTTGGTTTGTGGTCCTC	CCTGGTTGAAGAAGTCGTAGAAGTGG
<i>BETA</i>		
<i>ACTIN</i>	ATCCACGAAACTACCTTCAACTCCAT	CATACTCCTGCTTGCTGATCCACATC
Taqman probes (ABI)		
Species	Gene	Probe I.D.
Human	<i>GLI1</i>	Hs01110766_m1
Human	<i>PTCH1</i>	Hs00181117_m1
Human	<i>GLI2</i>	Hs01119974_m1
Mouse	<i>Gli1</i>	Mm00494645_m1
Mouse	<i>Rps18</i>	Mm02601777_g1