SUPPLEMENTAL DATA

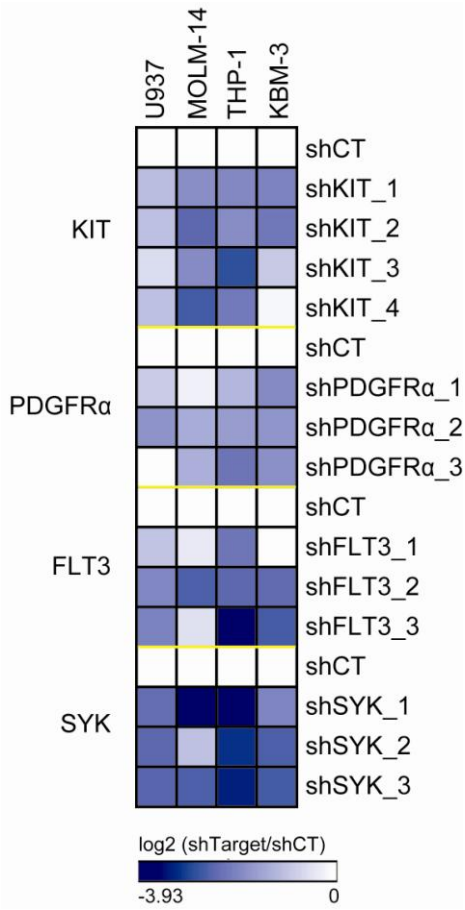


Figure S1, related to Figure 1. Heatmap showing level of KIT, PDGFRα, FLT3 or SYK knockdown, evaluated by flow cytometry, in five AML cell lines transduced with at least three shRNAs per gene. Normalized data are presented as a log2-ratio (shTarget/shCT).

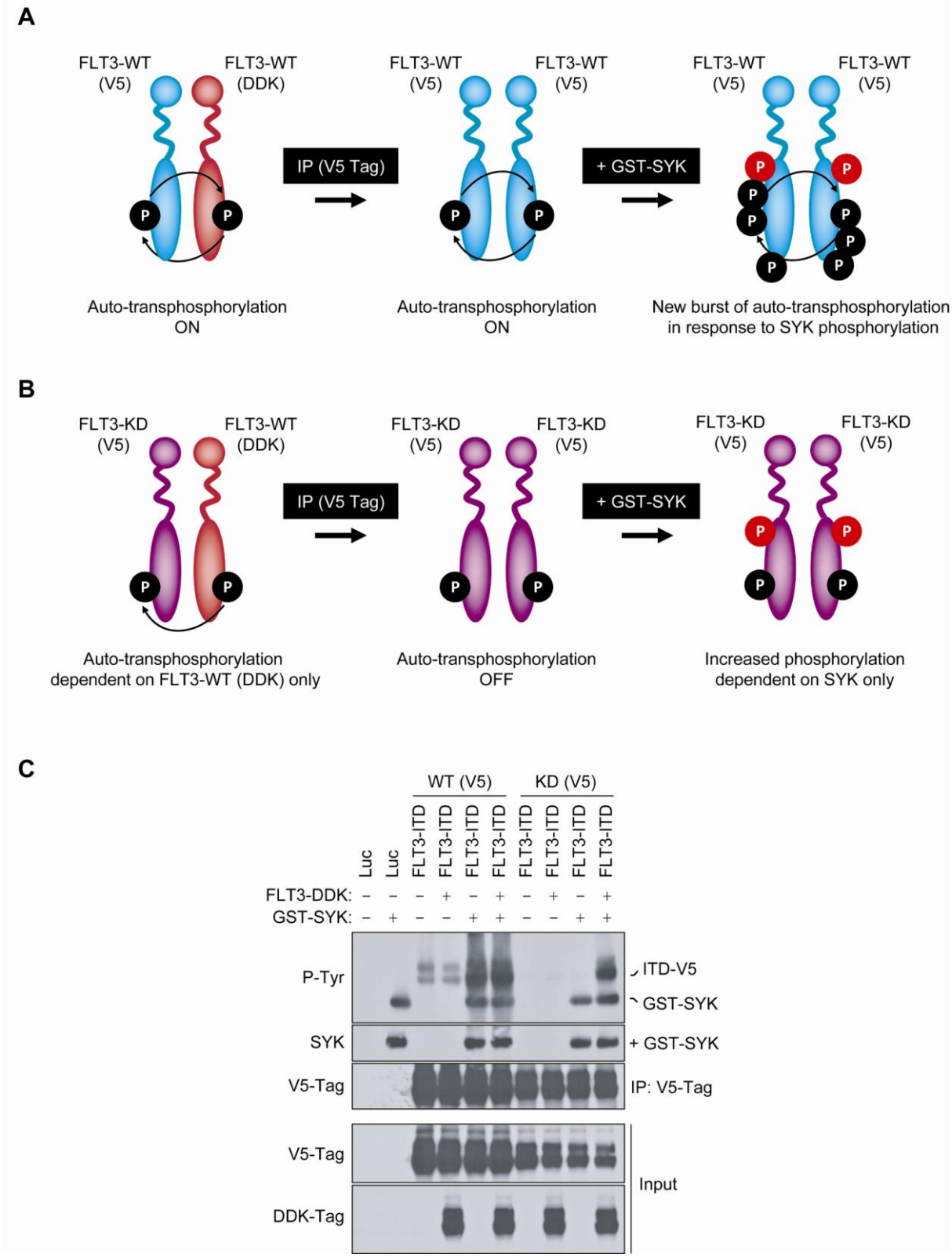


Figure S2, related to Figure 2. (**A and B**) Schema depicting the design of the experiment presented in Figure 2D. Because SYK targets a kinase, the detection of the direct SYK-phosphorylated tyrosine site(s) is rendered difficult by the possibility of

FLT3 auto-transphosphorylation in response to the initial SYK phosphorylation. (**A**) In the control condition, the co-transfection of V5-tagged and DDK-tagged *FLT3* WT vectors induces tyrosine auto-transphosphorylation necessary for receptor activation. By immunoprecipitating the activated V5-tagged FLT3 WT and incubating with GST-SYK, we promote new bursts of auto-transphosphorylation impeding detection of the sites initially phosphorylated by SYK. (**B**) An alternative method is to overexpress a kinase dead (KD) mutant of V5-tagged FLT3 such that its capacity for auto-transphosphorylation is blocked. However, this FLT3-KD is maintained at a basal activated state by DDK-tagged FLT3 WT. DDK-tagged FLT3 WT can then be removed to prevent new bursts of auto-transphosphorylation subsequent to phosphorylation by SYK. We are then able to identify by site-directed mutagenesis the tyrosine(s) phosphorylated directly by SYK. (**C**) Requirement for FLT3-ITD pre-phosphorylation before further activation by SYK. V5-tagged *FLT3-ITD* (*FLT3-ITD* (V5)) or Kinase Dead (FLT3-ITD KD (V5)) were co-transfected into 293E cells along with a DDK-tagged FLT3 WT (FLT3-DDK) vector. V5-tagged constructs were then V5-tag immunoprecipitated to purify out the FLT3-DDK protein before being incubated with GST-SYK for an *in vitro* kinase assay. Global FLT3 phosphorylation level was detected by immunoblot using an anti-phospho-tyrosine (P-Tyr) antibody.

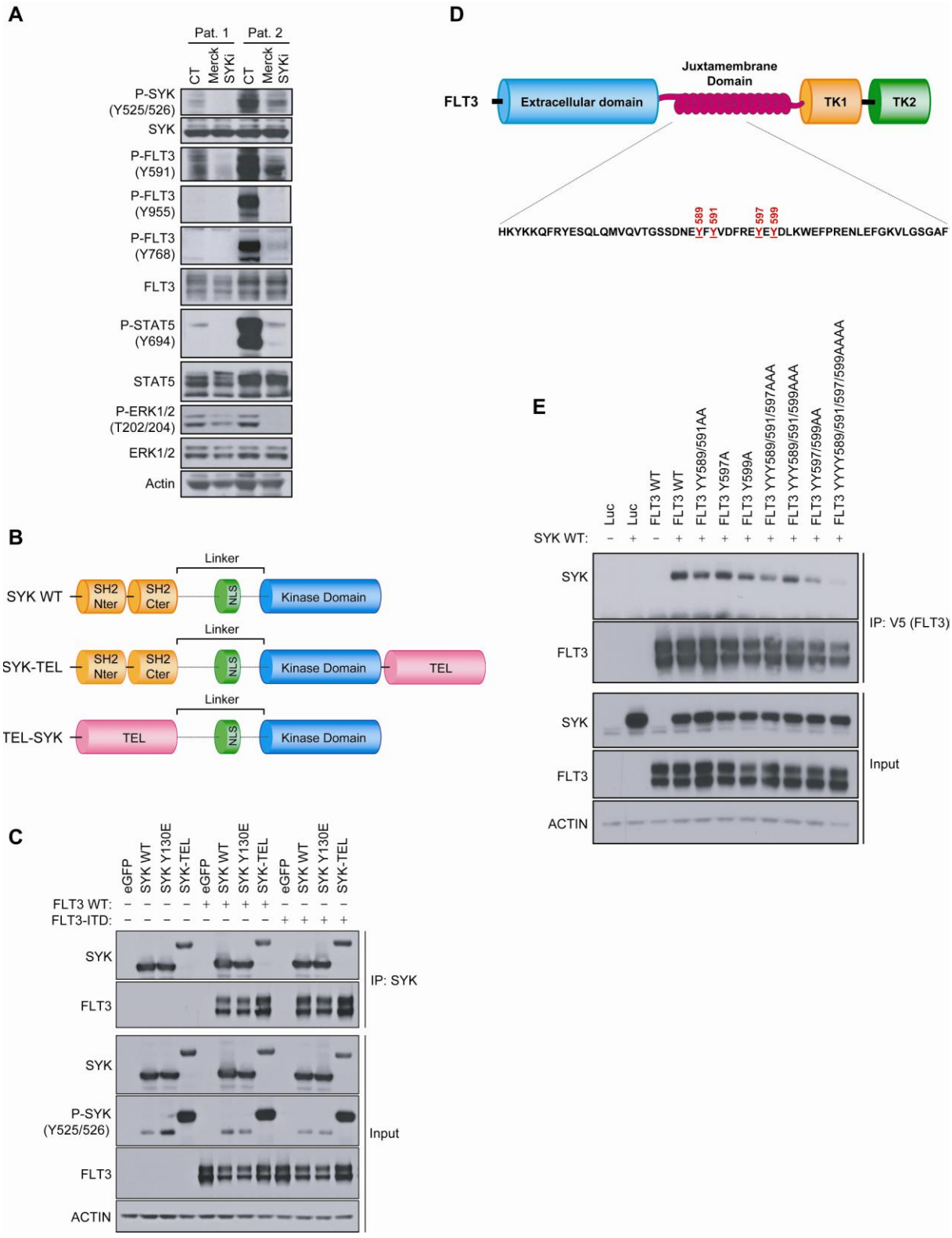


Figure S3, related to Figure 3. (**A**) Effect of the Merck SYKi on FLT3 and its downstream effectors. Western blot for indicated proteins on lysates from two *FLT3-ITD* positive primary patient samples treated for six hours with 5 µM Merck SYKi. (**B**)

Schematic representation of SYK WT and two constitutively activated forms of SYK, SYK-TEL and TEL-SYK. (**C**) Level of interaction of SYK WT, SYK Y130E and SYK-TEL constructs with FLT3 WT and FLT3-ITD. Using anti-SYK antibody, immunoprecipitation of SYK from 293E cells co-expressing indicated combinations of SYK and FLT3 constructs and western blot using anti-P-SYK (Y525/526), anti-SYK, and anti-FLT3 antibodies. (**D**) Schematic representation of the FLT3 kinase composed of an extracellular domain, a juxtamembrane domain and two tyrosine kinase domains (TK1 and TK2). Four tyrosine sites from left to right (Y589, Y591, Y597 and Y599) from the juxtamembrane region are highlighted in red. (**E**) Using anti-V5 antibody, immunoprecipitation of several mutated forms (key tyrosine sites mutated into alanines) of FLT3 from 293E cells co-expressing these constructs and untagged SYK WT. The western blot used anti-SYK and anti-FLT3 antibodies.

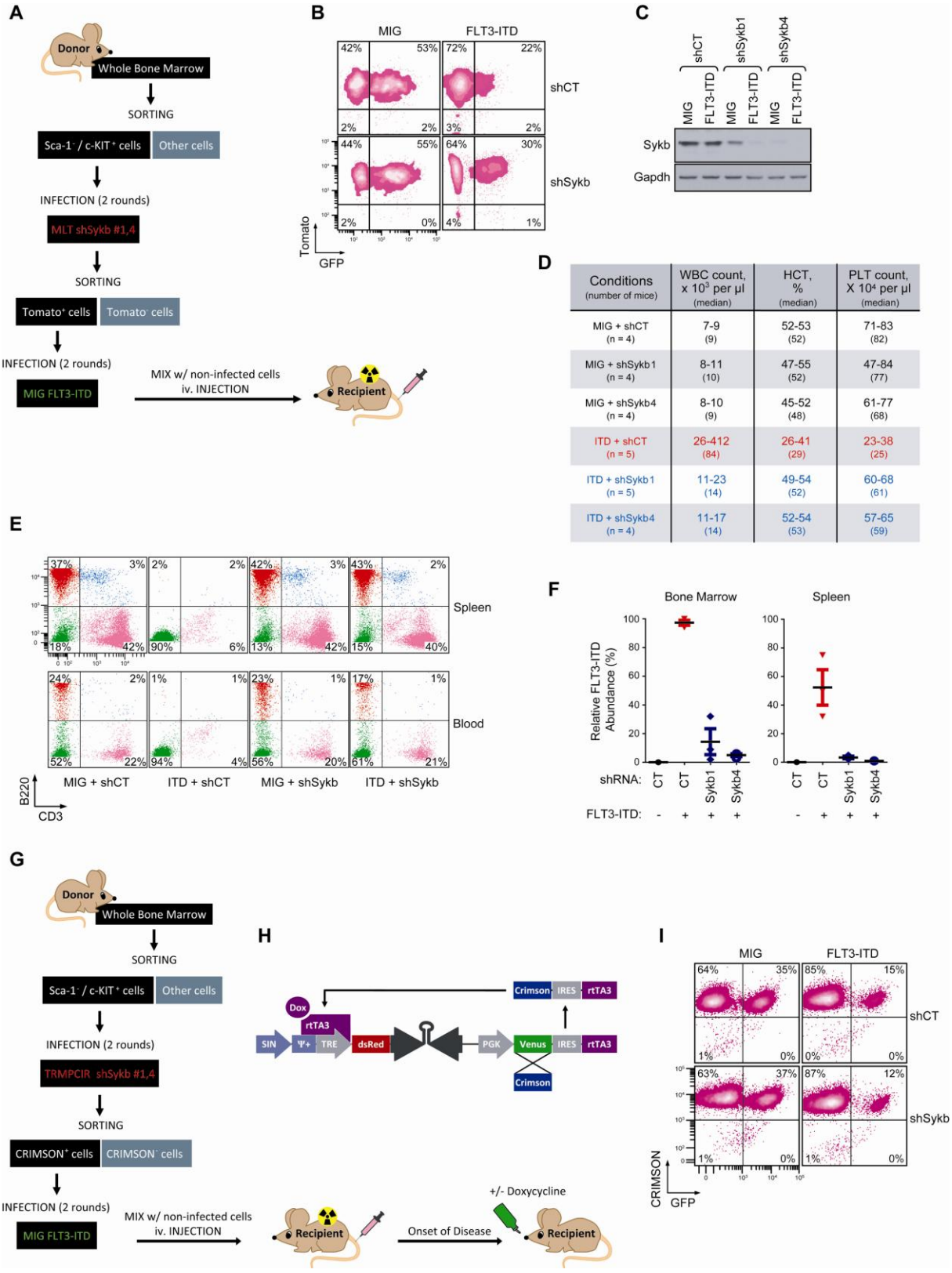


Figure S4, related to Figure 4. (**A**) Model of the bone marrow transplantation assay developed to characterize the effect of Sykb knockdown on leukemia driven by FLT3-ITD. The sca-1$^-$/c-KIT$^+$ myeloid progenitor fraction was sorted from the whole bone

marrow of donor mice and transduced twice with one scrambled mir30-shRNA or two hairpins directed against *Sykb* (mir30-sh Sykb 1 and mir30-sh Sykb 4) coupled with a tomato fluorescent reporter. After a second round of sorting, the tomato-positive myeloid progenitor subpopulation was then transduced twice with the MSCV-EGFP empty (MIG) or *FLT3-ITD* vector, before being mixed with other non-infected bone marrow cells and injected into the lateral tail vein of lethally irradiated syngeneic recipient mice. (**B**) FACS analysis of tomato (Control or *Sykb*-directed mir30-shRNA) and GFP (MIG empty or *FLT3-ITD*) expressing sca-1$^-$/c-KIT$^+$ population injected into recipient mice. A representative FACS plot for each mir30-shRNA CT and Sykb1 is shown. (**C**) Western blot indicating the level of Sykb knock-down in the sca-1$^-$/c-KIT$^+$ cellular fraction seven days after injection into recipient mice. (**D**) Table showing the minimum and the maximum values and the median for WBC count, HCT and PLT count in the blood harvested from the indicated number of mice per group. (**E**) Impairment of lymphoid maturation by *FLT3-ITD*-promoted myeloid expansion. FACS analysis of B220 and CD3 expressing populations in spleen and blood. A representative FACS plot from each group is shown. (**F**) Detection by Fragment Analyzer of *FLT3-ITD* sequence on genomic DNA extracted from bone marrow and spleen cells of mice transplanted with cells expressing different combination of MSCV and mir30-shRNA vectors. Values over groups and organs are shown relative to the highest value measured in the *FLT3-ITD* + mir30-shCT group in the bone marrow, with error bars representing the mean +/- SEM of three mice per group. (**G**) Model of the bone marrow transplantation assay developed to characterize the effect of Sykb knockdown on the progression of FLT3-ITD-induced myeloproliferative disease. The sca-1$^-$/c-KIT$^+$ myeloid progenitor fraction was sorted from the whole bone marrow of donor mice and transduced twice with a doxycycline-inducible TRMPCIR vector

containing either a scrambled mir30-shRNA or a hairpin directed against *Sykb* (mir30-sh Sykb 1 or mir30-sh Sykb 4) and coupled with a crimson fluorescent reporter. After a second round of sorting, the crimson-positive myeloid progenitor subpopulation was then transduced twice with the MSCV-EGFP empty (MIG) or *FLT3-ITD* vector, before being mixed with other non-infected bone marrow cells and injected into the lateral tail vein of lethally irradiated syngeneic recipient mice. After detection of the disease by WBC count, mice were treated with doxycycline to induce shRNA expression. (**H**) Vector schematic of TRMPCIR, which was constructed using the previously described TRMPVIR vector by substitution of the venus for the crimson fluorescent reporter. (**I**) FACS analysis of crimson (Control or *Sykb*-directed mir30-shRNA) and GFP (MIG empty or *FLT3-ITD*) expressing sca-1$^-$/c-KIT$^+$ population injected into recipient mice. A representative FACS plot for each mir30-shRNA CT and Sykb1 is shown.

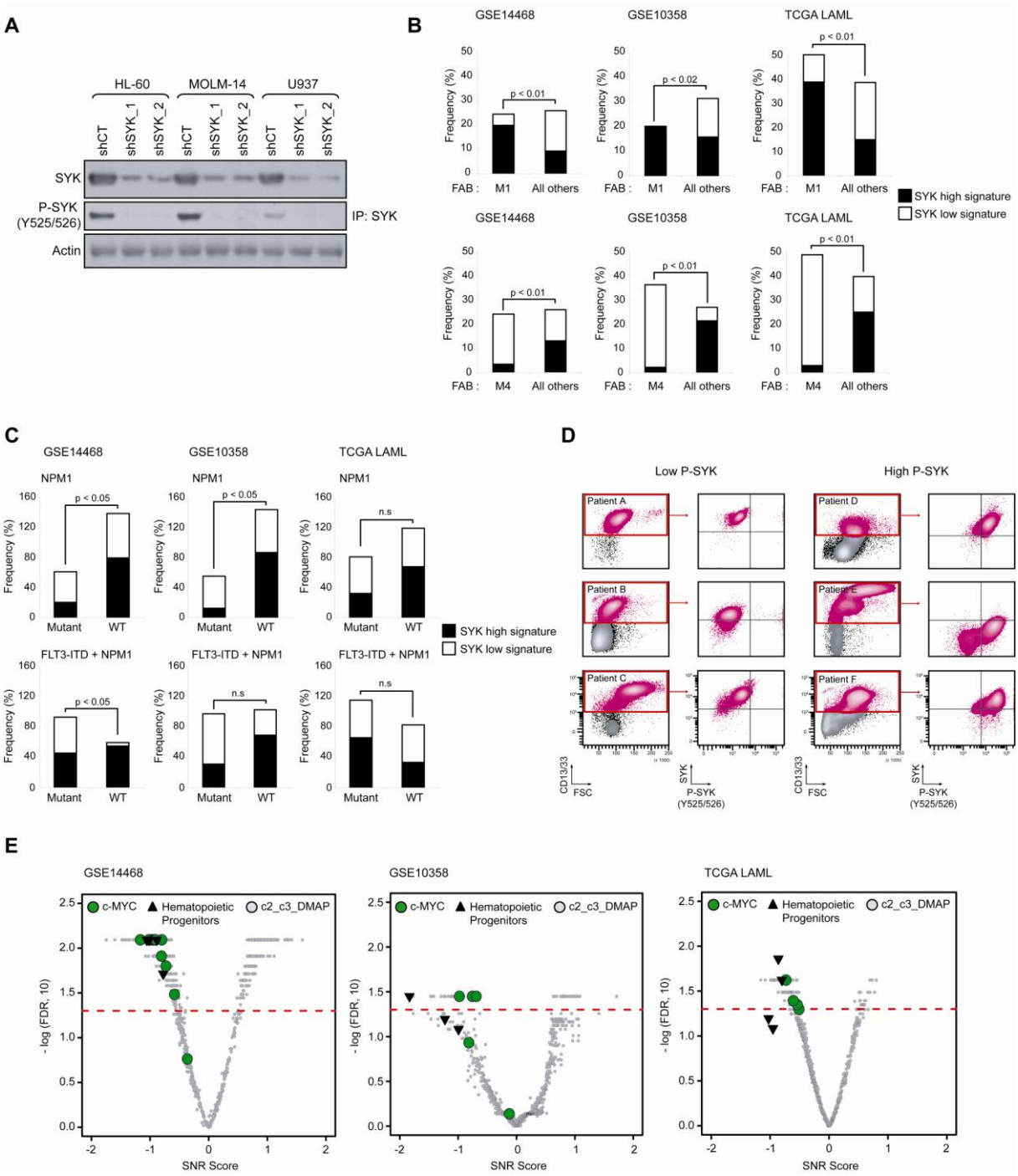


Figure S5, related to Figure 5. (**A**) Western blot related to the genome-wide transcriptional profiling showing SYK knockdown across the three tested AML cell lines: HL-60, MOLM-14 and U937. SYK was immunoprecipitated before blotting with anti-P-SYK (Y525/526) antibody. (**B and C**) Bar graphs showing the frequency of (**B**) FAB M1-AML and M4-AML, or (**C**) NPM1 mutant primary patient samples displaying SYK high versus low signatures in three cohorts GSE14468 (n=526, Wouters et al.,

2009), GSE10358 (n=279, Tomasson et al., 2008), and TCGA LAML (n=179, Ley et al., 2013). p value calculated using Fisher exact test. (**D**) Representative FACS plots of high versus low P-SYK (Y525/526) samples used for patient classification. (**E**) Gene sets depleted in the *FLT3-ITD* patient cohort from three different cohorts (GSE14468, GSE10358, and TCGA LAML) displaying a SYK low versus high signature. Data are presented as a volcano plot of -log (FDR, 10) versus SNR for each evaluated gene set. Green dots indicate sets for *MYC*, black triangles for myeloid progenitors, and gray for all other available gene sets.

Table S1, related to Figure 5. Provided as an Excel file.

Table S2, related to Figure 5.

GSE 14468	GeneSet	SNR	Pval	FDR
MYC	SCHUHMACHER_MYC_TARGETS_UP	1.354	0.002	0.008
	ORKIN_MYC_Human	1.166	0.002	0.008
	DANG_MYC_TARGETS_UP	1.086	0.002	0.008
	ZELLER_MYC_CORE	1.020	0.002	0.008
	DANG_REGULATED_BY_MYC_UP	0.984	0.002	0.008
	ZELLER_Human-Mouse_CoreMycTarget	0.968	0.002	0.008
	KIM_MYC_AMPLIFICATION_TARGETS_UP	0.951	0.002	0.008
	V$MYCMAX_B	0.800	0.002	0.008
	YU_MYC_TARGETS_UP	0.778	0.008	0.019
	V$MYCMAX_01	0.730	0.006	0.016
	CACGTG_V$MYC_Q2	0.584	0.016	0.033
Hematopoietic Progenitors	DMAP_HSC_eERY_UP	1.597	0.002	0.008
	DMAP_MONO.vs.HSC_DN	1.414	0.002	0.008
	DMAP_HSC_UP	1.352	0.002	0.008
	DMAP_GMP.vs.HSC_UP	1.094	0.002	0.008

GSE 10358	GeneSet	SNR	Pval	FDR
MYC	YU_MYC_TARGETS_UP	0.981	0.002	0.036
	ZELLER_MYC_CORE	0.979	0.002	0.036
	SCHUHMACHER_MYC_TARGETS_UP	0.880	0.002	0.036
	ZELLER_Human-Mouse_CoreMycTarget	0.758	0.002	0.036
	ORKIN_MYC_Human	0.690	0.002	0.036
Hematopoietic Progenitors	DMAP_MONO.vs.HSC_DN	1.716	0.002	0.036
	DMAP_HSC_UP	1.039	0.002	0.036
	DMAP_HSC_eERY_UP	0.911	0.002	0.036

TCGA LAML	GeneSet	SNR	Pval	FDR
MYC	ORKIN_MYC_Human	0.510	0.018	0.050
	ZELLER_Human-Mouse_CoreMycTarget	0.539	0.008	0.045
	ZELLER_MYC_CORE	0.607	0.006	0.040
	CACGTG_V$MYC_Q2	0.732	0.002	0.024
Hematopoietic Progenitors	DMAP_GMP.vs.HSC_UP	0.435	0.036	0.041
	DMAP_HSC_UP	0.465	0.018	0.050
	DMAP_MONO.vs.HSC_DN	0.802	0.002	0.024
	DMAP_HSC_eERY_UP	0.603	0.010	0.051

Table of selective gene sets enriched in primary patient AML samples with *FLT3-ITD* from three different cohorts GSE 14468, GSE 10358, and TCGA LAML displaying a SYK high versus low signature based on single sample GSEA (SNR = signal-to-noise ratio, FDR = False Discovery Rate and FC = Fold Change).

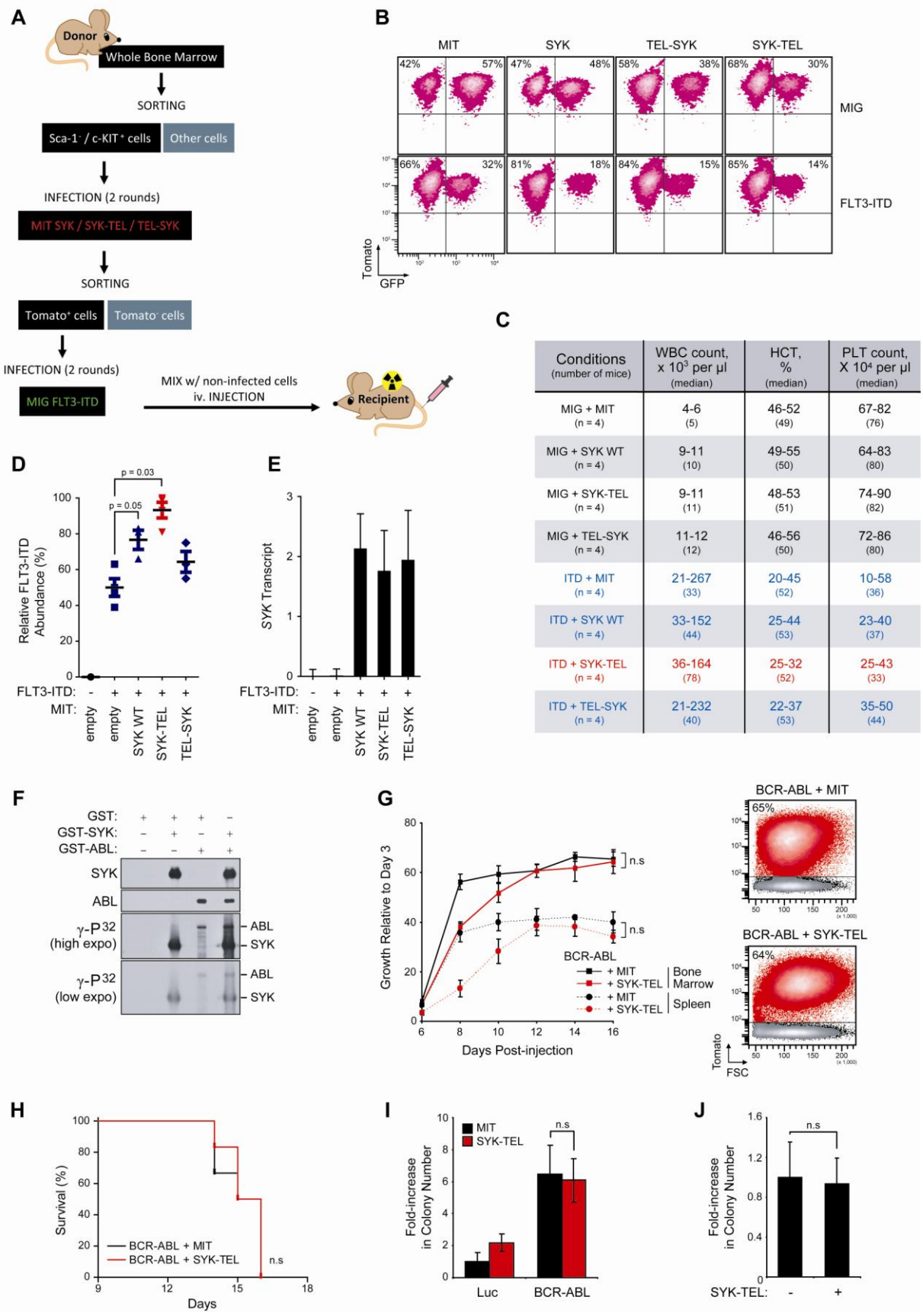
Table S3, related to Figure 5.

GSE 14468	GeneSet	SNR	Pval	FDR
MYC	SCHUHMACHER_MYC_TARGETS_DN	-0.516	0.038	0.066
	ORKIN_MYC_Human	-1.166	0.002	0.008
	DANG_MYC_TARGETS_DN	-0.365	0.118	0.173
	ZELLER_MYC_CORE	-1.020	0.002	0.008
	DANG_REGULATED_BY_MYC_DN	-0.930	0.002	0.008
	ZELLER_Human-Mouse_CoreMycTarget	-0.968	0.002	0.008
	KIM_MYC_AMPLIFICATION_TARGETS_DN	-0.911	0.002	0.008
	V$MYCMAX_B	-0.800	0.002	0.008
	YU_MYC_TARGETS_DN	-0.806	0.004	0.012
	V$MYCMAX_01	-0.730	0.006	0.016
	CACGTG_V$MYC_Q2	-0.584	0.016	0.033
Hematopoietic Progenitors	DMAP_HSC_eERY_DN	-1.057	0.002	0.008
	DMAP_MONO.vs.HSC_UP	-1.012	0.002	0.008
	DMAP_HSC_DN	-0.886	0.002	0.008
	DMAP_GMP.vs.HSC_DN	-0.775	0.008	0.019

GSE 10358	GeneSet	SNR	Pval	FDR
MYC	YU_MYC_TARGETS_DN	-0.817	0.022	0.117
	ZELLER_MYC_CORE	-0.979	0.002	0.036
	SCHUHMACHER_MYC_TARGETS_DN	0.223	0.517	0.728
	ZELLER_Human-Mouse_CoreMycTarget	-0.758	0.002	0.036
	ORKIN_MYC_Human	-0.690	0.002	0.036
Hematopoietic Progenitors	DMAP_MONO.vs.HSC_UP	-1.227	0.006	0.064
	DMAP_HSC_DN	-1.834	0.002	0.036
	DMAP_HSC_eERY_DN	-0.994	0.012	0.083

TCGA LAML	GeneSet	SNR	Pval	FDR
MYC	ORKIN_MYC_Human_DN	-0.510	0.018	0.050
	ZELLER_Human-Mouse_CoreMycTarget_DN	-0.539	0.008	0.045
	ZELLER_MYC_CORE_DN	-0.607	0.006	0.040
	CACGTG_V$MYC_Q2_DN	-0.732	0.002	0.024
Hematopoietic Progenitors	DMAP_GMP.vs.HSC_DN	-0.952	0.002	0.082
	DMAP_HSC_DN	-0.860	0.002	0.014
	DMAP_MONO.vs.HSC_UP	-1.026	0.002	0.064
	DMAP_HSC_eERY_DN	-0.800	0.002	0.024

Table of selective gene sets depleted in primary patient AML samples with *FLT3-ITD* from three different cohorts GSE 14468, GSE 10358, and TCGA LAML displaying a SYK low versus high signature based on single sample GSEA (SNR = signal-to-noise ratio, FDR = False Discovery Rate and FC = Fold Change).



Conditions (number of mice)	WBC count, x 10^3 per µl (median)	HCT, % (median)	PLT count, X 10^4 per µl (median)
MIG + MIT (n = 4)	4-6 (5)	46-52 (49)	67-82 (76)
MIG + SYK WT (n = 4)	9-11 (10)	49-55 (50)	64-83 (80)
MIG + SYK-TEL (n = 4)	9-11 (11)	48-53 (51)	74-90 (82)
MIG + TEL-SYK (n = 4)	11-12 (12)	46-56 (50)	72-86 (80)
ITD + MIT (n = 4)	21-267 (33)	20-45 (52)	10-58 (36)
ITD + SYK WT (n = 4)	33-152 (44)	25-44 (53)	23-40 (37)
ITD + SYK-TEL (n = 4)	36-164 (78)	25-32 (52)	25-43 (33)
ITD + TEL-SYK (n = 4)	21-232 (40)	22-37 (53)	35-50 (44)

Figure S6, related to Figure 6. (**A**) Model of the bone marrow transplantation assay developed to evaluate the impact of SYK activation on the modulation of FLT3-ITD oncogenic activity. The sca-1$^-$/c-KIT$^+$ myeloid progenitor fraction was sorted from the whole bone marrow of donor mice and transduced twice with either MSCV-Tomato empty (MIT), *SYK*, *SYK-TEL* or *TEL-SYK* vectors. After a second round of sorting, the tomato-positive myeloid progenitor subpopulation was then transduced twice with the MSCV-EGFP empty (MIG) or *FLT3-ITD* vector, before being mixed with other non-infected bone marrow cells and injected into the lateral tail vein of lethally irradiated syngeneic recipient mice. (**B**) FACS analysis of tomato (MIT empty, *SYK*, *TEL-SYK* or *SYK-TEL*) and GFP (MIG empty or *FLT3-ITD*) expressing sca-1$^-$/c-KIT$^+$ population injected into recipient mice. A representative FACS plot is shown. (**C**) Table showing the minimum, maximum and median values for WBC count, HCT, and PLT count in the blood harvested from indicated number of mice per group when *FLT3-ITD* + *SYK-TEL* mice became moribund. (**D**) Detection by Fragment Analyzer of *FLT3-ITD* sequence on genomic DNA extracted from spleen cells of mice transplanted with cells expressing different combinations of the indicated vectors. Values across groups are shown relative to the highest value measured in the *FLT3-ITD* + *SYK-TEL* group, with error bars representing the mean +/- SEM of three mice per group. (**E**) qRT-PCR showing relative expression levels of SYK in purified cells infected with different *SYK* constructs. Error bars represent mean +/- SD of four technical replicates. (**F**) Effect of high SYK activation on progression of BCR-ABL-induced leukemia. *In vitro* kinase assay showing incorporation of γ-^{32}P in response to the incubation of active GST-ABL (27-end) with active GST-SYK. (**G**) 1x10^6 BCR-ABL-positive ALL cells expressing either MIT empty or *SYK-TEL* vector were tail vein injected into C57/BL6 recipient mice. At each indicated time point, spleen and bone

marrow were harvested and percent of tomato-positive cells was determined by FACS analysis. Growth is shown relative to the day three (time of significant detection of tomato-positive cells by FACS) values, with error bars representing the mean +/- SD of three mice sacrificed per time point. Two representative FACS plots corresponding to each condition are shown on bone marrow at day 16 post-injection. Statistical significance determined using a Mann-Whitney test. n.s = non significant. (**H**) Kaplan-Meier curves showing overall survival of mice (n=6 per group) transplanted with BCR-ABL-positive ALL cells expressing either MIT empty or *SYK-TEL* vector. Statistical significance determined by Log-Rank (Mantel-Cox) test. n.s = non significant ($p > 0.05$). (**I**) Purified CD34+ human primary cells were infected with *BCR-ABL* in combination with constitutively active *SYK-TEL*. (**J**) Purified CD34+ BCR-ABL positive cells from a patient with CML were infected with *SYK-TEL*. (**I and J**) After 10 to 14 days, colony number was evaluated after MTT staining. n.s = non significant using a Mann-Whitney test ($p > 0.05$). Error bars representing the mean +/- SD of three replicates per condition.

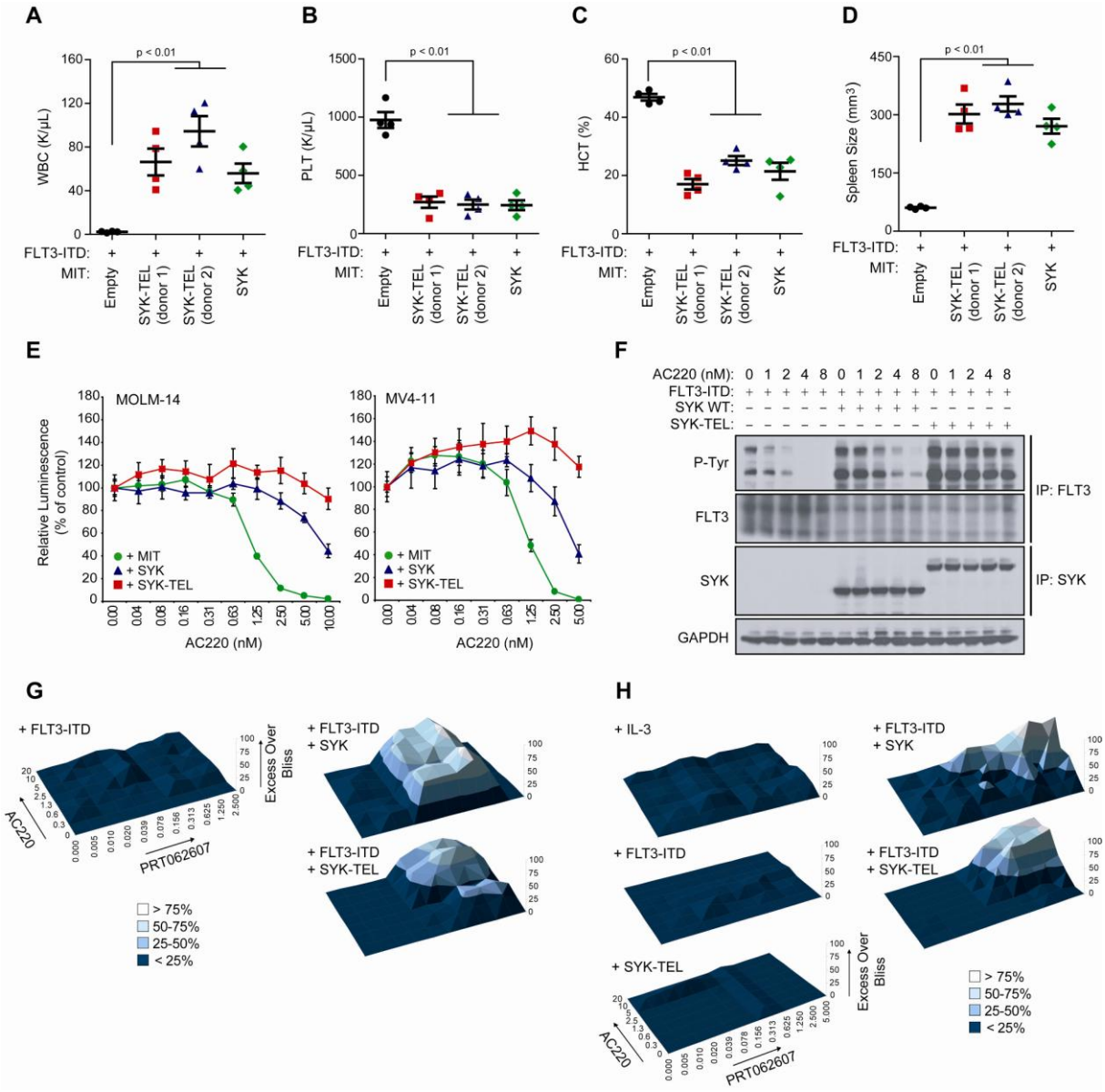


Figure S7, related to Figure 7. (A-D) Secondary transplantation of myeloid cells expressing activated SYK and FLT3-ITD recapitulates features of AML disease. (**A**) WBC count, (**B**) PLT count, and (**C**) HCT in the blood harvested from four mice per group. p value calculated using a Mann-Whitney test. (**D**) Spleen size of four mice per group when *FLT3-ITD* + *SYK-TEL* mice became moribund. (**A-D**) Error bars representing the mean +/- SEM. (**E**) High SYK activation impairs the targeting of FLT3-ITD-driven AML disease with AC220. Growth inhibition of MOLM-14 and MV4-11 AML cells transduced with indicated *SYK* constructs and treated with increasing

doses of AC220. Values are shown relative to day zero (time of seeding), with error bars representing the mean +/- SD of eight replicates per condition. (**F**) Western blot for indicated proteins on lysates from FLT3-ITD-positive primary murine cells expressing either wild-type (WT) or constitutive active (SYK-TEL) SYK and treated for eight hours with indicated doses of AC220. (**G and H**) Effect of the combination of small-molecule inhibitors of SYK (PRT062607) and FLT3 (AC220) on the viability of myeloid murine primary (**G**) or Ba/F3 (**H**) cells co-expressing *FLT3-ITD* and either *SYK* WT or *SYK-TEL*. The data were analyzed using excess above Bliss additive.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell Culture

HL-60, U937, Mono-Mac-6, Kasumi-3, UCSD-AML1, and KG-1 cell lines were purchased from the American Type Culture Collection. KBM-3, NB-4, NOMO-1, and EOL-1 were provided by Dr. Ross Levine; THP-1, MV4-11, and MOLM-14 cell lines by Dr. Scott Armstrong; and MOLM-13 and SKM-1 cell lines by Dr. Benjamin Ebert. All cell lines except MOLM-13, SKM-1, KBM-3, Kasumi-3, and UCSD-AML1 were maintained in RPMI 1640 (Cellgro) supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum (FBS, Sigma-Aldrich) at 37 °C with 5% CO2. MOLM-13 and SKM-1 were maintained in RPMI 1640 supplemented with 1% penicillin-streptomycin and 20% FBS and KBM-3 in IMDM (Gibco) supplemented with 1% penicillin-streptomycin and 10% FBS. Kasumi-3 and UCSD-AML1 were maintained in RPMI 1640 supplemented with 1% penicillin-streptomycin and 10% FBS with 20 ng per ml GM-CSF (Peprotech). The 293 cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal calf serum (FCS) (Invitrogen) and 100 units per ml penicillin/streptomycin (Invitrogen). Ba/F3 cells were maintained in RPMI 1640 (Cellgro) supplemented with 1% penicillin-streptomycin, 5 ml L-glu 100X, 10% WEHI 3CM as a source of IL-3 and 10% FBS. After transduction with either *FLT3-ITD*, *TEL-SYK*, or *SYK-TEL* vector, selection was made by removing WEHI conditioned medium form the culturing medium. Ultimately, cells expressing indicated combination of vectors were selected by flow sorting of double positive GFP/Tomato cells.

Primary patient AML blasts were collected from peripheral blood or bone marrow aspirate after obtaining patient informed consent under a Dana-Farber Cancer Institute Internal Review Board-approved protocol. Mononuclear cells were isolated using Ficoll-Paque Plus (Amersham Biosciences) and red blood cells were lysed before staining for confocal microscopy or flow cytometry analysis.

Chemicals

Retinoic acid (ATRA) was purchased from Sigma-Aldrich. R406 and PRT062607 were purchased from SelleckChem. AC220 and R788 (Fostamatinib, R406 prodrug) were purchased from Haoyuan Chemexpress Co., Ltd. Merck SYKi was kindly provided by Merck & Co., Inc. (Boston, MA).

Plasmids and shRNA Constructs

The pLenti6.2-EGFP and pLenti6.2-SYK vectors were generated by enzyme-mediated site-specific recombination between pDONR221-EGFP and pDONR223-SYK (gifts from Dr. Jesse Boehm, Broad Institute, Boston, MA, USA) and pLenti6.2/V5-DEST™ Gateway® Vector destination vector (Invitrogen). To generate the TEL-SYK construct, RT-PCR was used to isolate the sequences encoding amino acids 1–336 of human ETV6 (TEL) and amino acids 266–635 of human SYK. The assembled cDNA was subcloned in place of mCherry in the lentiviral plasmid FUW-Luc-mCherry-puro (Kimbrel et al., 2009) to give the plasmid FUW-Luc-TEL-SYK-puro. The SYK-TEL construct was generated using the same *TEL* sequence from *ETV6* subcloned down the full-length sequence of *SYK* in a pWZL-Neo Retroviral vector (Cell Biolabs, Inc.). The pCL-Neo empty, FLT3 WT, D835Y, and ITD vectors were obtained from Dr. James D. Griffin. The PLX302-Luc and PLX302-FLT3-V5

vectors were generated by enzyme-mediated site-specific recombination between pDONR223-Luc and pDONR223-FLT3 donor vectors and PLX302-V5 destination vector (gift from Dr. Jesse Boehm, Broad Institute, Boston, MA, USA). The pMSCV-IRES-EGFP (pMIG) and the pMIG-FLT3-ITD (N51) plasmids were generously provided by Dr. Scott Armstrong. The pMSCV-IRES-Tomato (pMIT) and the pMIT-SYK, pMIT-SYK-Tel, and pMIT-Tel-SYK constructs were generated by cloning SYK-TEL and TEL-SYK inserts into the EcoRI sites of the pMIT vector, which was a generous gift from Dr. Scott Armstrong.

Oligonucleotides encoding human shRNAs provided by the RNAi Consortium shRNA library (http://www.broad.mit.edu/rnai/trc/lib) were cloned into pLKO.1 vectors as described previously (Moffat et al., 2006). Sequences targeted by each of these hairpins are listed below:

Designation	Clone ID	Clone Name	Target Sequence
shFLT3_1	TRCN0000000775	NM_004119.x-2461s1c1	CTGGAATTTAAGTCGTGTGTT
shFLT3_2	TRCN0000039706	NM_004119.1-545s1c1	CCCTGCTTTACACATTAAGAA
shFLT3_3	TRCN0000009886	NM_004119.x-139s1c1	CAAGATCTGCCTGTGATCAAG
shKIT_1	TRCN0000195226	NM_000222.1-4825s1c1	CCTTTGTGTTTCTATTGACTT
shKIT_2	TRCN0000195108	NM_000222.1-2018s1c1	CCCTGGTCATTACAGAATATT
shKIT_3	TRCN0000194707	NM_000222.1-1838s1c1	CAACTGCTTATGGCTTAATTA
shKIT_4	TRCN0000196865	NM_000222.1-439s1c1	GACCCAGAAGTGACCAATTAT
shPDGFRα_1	TRCN0000196272	NM_006206.3-5354s1c1	GCTTAATTGCTGATACCATAT
shPDGFRα_2	TRCN0000196928	NM_006206.3-4998s1c1	GCTAGCAATTGCGACCTTAAT
shPDGFRα_3	TRCN0000195132	NM_006206.3-4080s1c1	CTACTACTGTTATCAGTAATG
shSYK_1	TRCN0000197257	NM_003177.3-1527s1c1	GCAGCAGAACAGACATGTCAA
shSYK_2	TRCN0000003163	NM_003177.x-579s1c1	GCAGGCCATCATCAGTCAGAA
shSYK_3	TRCN0000195465	NM_003177.3-2506s1c1	CAGAGGAATTTGGCTGCTTCT
shControl			CCTAAGGTTAAGTCGCCCTCG

shRNA constructs targeting human *SYK* (shSYK_4 and shSYK_5) or murine *Sykb* (shSykb_1 and shSykb_4), (sequences listed below), were designed by adapting BIOPREDsi small interfering RNA predictions. shRNAs were cloned into the MLS

(Dickins et al., 2005), TRMPVIR (Zuber et al., 2010), and TRMPCIR retroviral vectors, containing a miR30 expression cassette, as 116-nt XhoI–EcoRI fragments, which were generated by amplifying 97-mer oligonucleotides (Invitrogen) using 5′miR30-XhoI (TACAATACTCGAGAAGGTATATTGCTGTTGACAGTGAGCG) and 3′miR30-EcoRI (ACTTAGAAGAATTCCGAGGCAGTAGGCA) primers and the Vent polymerase kit (Invitrogen) with the following conditions: 50 µl reaction containing 0.05 ng oligonucleotide template, 1× Vent buffer, 0.3 mM of each dNTP, 0.8 µM of each primer, and 1.25 U Vent polymerase; cycling: 94 °C for 3 min; 35 cycles of 94 °C for 30 s, 54 °C for 30 s, and 75 °C for 20 s; 75 °C for 5 min. shRNAs constructs were then validated by sequencing and their knockdown efficiency verified by western blot.

Designation	Species	Accession Number	Target Sequence
shSYK_4	Homo Sapiens	NM_003177.5, NM_001135052.2, NM_001174167.1, NM_001174168.1	CACAGCGAGACTCTGTCAAAA
shSYK_5	Homo Sapiens	NM_003177.5, NM_001135052.2, NM_001174167.1, NM_001174168.1	CCCGCTCTTAAAGATGAGTTA
shSykb_1	Mus Musculus	NM_001198977.1, NM_011518.2	CCGGAGTGAGAGCTAAGCTAA
shSykb_4	Mus Musculus	NM_001198977.1, NM_011518.2	CTCCTTGTTGCTGCCACTAAA
shControl			AGGAATTATAATGCTTATCTA

The TRMPCIR vector used for *in vivo* experiments was designed by substitution, in the TRMPVIR vector, of the yellow-green fluorescent protein Venus reporter (Zuber et al., 2010) for the sequence encoding the far red fluorescent protein Crimson isolated from the pCMV-E2-Crimson Vector (Clontech).

For virus production, 12 µg of the above plasmids and 6 µg ψ-eco (for retroviral infection of murine cells), 6 µg pCMV-GAG/POL and pCMV-VSVG (for retroviral infection of human cells) or 6 µg pCMV8.9 and pCMV-VSVG (for lentiviral infection of human cells) packaging vectors were transfected into the 293T packaging cell line using X-tremeGENE 9 (Roche), and the resulting viral supernatants were

harvested as previously described (Banerji et al., 2012). The lentiviral viruses were then concentrated using PEG-it™ Virus Precipitation Solution (SBI System Biosciences).

Site-directed Mutagenesis

The different point mutations and deletions in the *SYK* and *FLT3* sequences were obtained using a QuikChange XL Site-directed mutagenesis kit (Stratagene). The sequence of primers used for these mutagenesis reactions are listed below:

Backbone vector	Mutant	Primer	Sequence
Full length SYK	SH2.1^{mut} (42RQS>GGI)	Forward	GGG CTT TAT TTG CTG GGC GGG ATC CGC AAC TAC CTG GG
		Reverse	CCC AGG TAG TTG CGG ATC CCG CCC AGC AAA TAA AGC CC
	SH2.2^{mut} (195RAR>GAL)	Forward	GGA AAG TTC CTG ATC GGA GCC CTA GAC AAC AAC GGC TCC
		Reverse	GGA GCC GTT GTT GTC TAG GGC TCC GAT CAG GAA CTT TCC
	ΔSH2.1	Forward	GGC TGA CAG CGC CAA CCA CCT GCC CTT GCC CTT CAA CCG GCC CCA AGG GGT GC
		Reverse	GCA CCC CTT GGG GCC GGT TGA AGG GCA AGG GCA GGT GGT TGG CGC TGT CAG CC
	ΔSH2.2	Forward	GGC CTG GTC TGC CTC CTC AAG AAG CCC GAC ACG CTC TGG CAG CTA GTC GAG C
		Reverse	GCT CGA CTA GCT GCC AGA GCG TGT CGG GCT TCT TGA GGA GGC AGA CCA GGC C
	Δ(SH1 + SH2)	Forward	GGC TGA CAG CGC CAA CCA CCT GCC CTT CCC CGA GGG AAA GAA GTT CGA CAC GC
		Reverse	GCG TGT CGA ACT TCT TTC CCT CGG GGA AGG GCA GGT GGT TGG CGC TGT CAG CC
	ΔNLS	Forward	GCC AGA ACT TGC ACC CTG GGC TGC ACC CAA GGA GGT TTA CCT GGA CCG
		Reverse	CGG TCC AGG TAA ACC TCC TTG GGT GCA GCC CAG GGT GCA AGT TCT GGC
	Kinase Dead (K402R)	Forward	GTG AAA ACC GTG GCT GTG CGA ATA CTG AAA AAC GAG GC
		Reverse	GCC TCG TTT TTC AGT ATT CGC ACA GCC ACG GTT TTC AC
Full length FLT3	YY589/591AA	Forward	GGT GAC CGG CTC CTC AGA TAA TGA GGC CTT CGC CGT TGA TTT CAG AGA ATA TG
		Reverse	CAT ATT CTC TGA AAT CAA CGG CGA AGG CCT CAT TAT CTG AGG AGC CGG TCA CC
	Y597A	Forward	GAG TAC TTC TAC GTT GAT TTC AGA GAA GCT GAA TAT GAT CTC AAA TGG GAG TTT CC
		Reverse	GGA AAC TCC CAT TTG AGA TCA TAT TCA GCT TCT CTG AAA TCA ACG TAG AAG TAC TC
	Y599A	Forward	CGT TGA TTT CAG AGA ATA TGA AGC TGA TCT CAA ATG GGA GTT TCC
		Reverse	GGA AAC TCC CAT TTG AGA TCA GCT TCA TAT TCT CTG AAA TCA ACG
	Y726A	Forward	GGA ACA CAA TTT CAG TTT TGC CCC CAC TTT CCA ATC ACA TC
		Reverse	GAT GTG ATT GGA AAG TGG GGG CAA AAC TGA AAT TGT GTT CC
	Y768A	Forward	CAC TCT GAA GAT GAA ATT GAA GCT GAA AAC CAA AAA AGG CTG GAA GAA GAG G
		Reverse	CCT CTT CTT CCA GCC TTT TTT GGT TTT CAG CTT CAA TTT CAT CTT CAG AGT G
	Y842A	Forward	GAT ATC ATG AGT GAT TCC AAC GCT GTT GTC AGG GGC AAT GCC CG
		Reverse	CGG GCA TTG CCC CTG ACA ACA GCG TTG GAA TCA CTC ATG ATA TC
	Y899A	Forward	CCG GTT GAT GCT AAC TTC GCC AAA CTG ATT CAA AAT GG
		Reverse	CCA TTT TGA ATC AGT TTG GCG AAG TTA GCA TCA ACC GG
	Y955A	Forward	GAT GCA GAA GAA GCG ATG GCT CAG AAT GTG GAT GGC C
		Reverse	GGC CAT CCA CAT TCT GAG CCA TCG CTT CTT CTG CAT C
	Y969A	Forward	GTT TCG GAA TGT CCT CAC ACC GCC CAA AAC AGG CGA CCT TTC AG
		Reverse	CTG AAA GGT CGC CTG TTT TGG GCG GTG TGA GGA CAT TCC GAA AC
	Kinase Dead (K644R)	Forward	CTC AAT CCA GGT TGC CGT CAG AAT GCT GAA AGA AAA AGC AG
		Reverse	CTG CTT TTT CTT TCA GCA TTC TGA CGG CAA CCT GGA TTG AG
FLT3-YY589/591AA	YYY589/591/597AAA	Forward	GAG GCC TTC GCC GTT GAT TTC AGA GAA GCT GAA TAT GAT CTC AAA TGG GAG TTT CC
		Reverse	GGA AAC TCC CAT TTG AGA TCA TAT TCA GCT TCT CTG AAA TCA ACG GCG AAG GCC TC
FLT3-YY589/591AA	YYY589/591/599AAA	Forward	CGT TGA TTT CAG AGA ATA TGA AGC TGA TCT CAA ATG GGA GTT TCC
		Reverse	GGA AAC TCC CAT TTG AGA TCA GCT TCA TAT TCT CTG AAA TCA ACG
FLT3-Y599A	YY597/599AA	Forward	GAG TAC TTC TAC GTT GAT TTC AGA GAA GCT GAA GCT GAT CTC AAA TGG GAG TTT CC
		Reverse	GGA AAC TCC CAT TTG AGA TCA GCT TCA GCT TCT CTG AAA TCA ACG TAG AAG TAC TC
FLT3-YYY589/591/599AAA	YYYY589/591/597/599AAAA	Forward	GAG GCC TTC GCC GTT GAT TTC AGA GAA GCT GAA GCT GAT CTC AAA TGG GAG TTT CC
		Reverse	GGA AAC TCC CAT TTG AGA TCA GCT TCA GCT TCT CTG AAA TCA ACG GCG AAG GCC TC

High-throughput Kinase Activity Profiling

A Luminex immunosandwich assay was performed on four AML cell lines (MV4-11, MOLM-14, NOMO-1 and THP-1) stably transduced with a pWZL empty or *SYK-TEL* vector encoding for a constitutively activated form of SYK. As previously described by Du *et al.*, 100 µg whole-cell lysates from each cell line and positive control lysates were quantified, and equal concentrations of protein were incubated in quadruplicate overnight at 4°C with a mixture of antibody-coupled Luminex beads directed against 105 protein candidates (Du et al., 2009). The mixture was then washed twice with PBS-1% BSA pH 7.4 and incubated with a secondary anti-phosphotyrosine biotin-labeled 4G10 antibody (Millipore) for 30 minutes at room temperature. The microspheres were washed twice more with PBS-1% BSA pH 7.4 and then incubated with 0.2 µg/mL R-phycoerythrin streptavidin conjugates (SAPE, Molecular Probes) for 10 min at room temperature. The conjugates were washed two additional times and analyzed on a FlexMAP 3D with xPONENT (version 4.0.370.0) software (Luminex) per the manufacturer's specifications. Each microsphere was analyzed with two lasers, one to detect the bead color representative of the tyrosine kinase identity, and the second to detect the R-phycoerythrin signal reflecting the tyrosine phosphorylation level of the tyrosine kinase. The background readings for each capture antibody were obtained using microspheres incubated with 1x cell lysis buffer (Cell Signaling Technology). Values were considered positive if they were 3-fold over the background and represented by log2-transformation of the folds over background. Negative values were threshold to -10. The preprocessed data were converted into .gct files and analyzed with GenePattern 3.0 (The Broad Institute). For a given kinase, the result was expressed as a log2-transformed ratio of the

normalized value corresponding to the SYK-TEL versus the control condition. Heatmap projections were created based on the GENE-E software (http://www.broadinstitute.org/cancer/software/GENE-E/).

Western Blot and Immunoprecipitation

Western blot was performed as described previously (Hahn et al., 2009) using cell lysates normalized for total protein content. For coimmunoprecipitations, cells were suspended in lysis buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 20 mM EDTA, 50 mM NaF, 0.5% NP-40, 1 mM dithiothreitol) supplemented with EDTA-free protease inhibitors and PhosSTOP phosphatase inhibitors (Roche). Lysates (500 µg) were then incubated with 2 µg antibody and 35 µL protein G–Sepharose (Invitrogen) at 4 °C overnight. Beads were washed five times with 1 mL lysis buffer before boiling in Laemmli sample buffer and performing SDS–polyacrylamide gel electrophoresis (PAGE) with transfer to nitrocellulose membranes, and western blotting. A list of antibodies used for western blotting and immunoprecipitation experiments is available in the table below the Flow-based Myeloid Differentiation Screening section of the supplemental experimental procedures.

***In Vitro* Kinase Assay**

The assay was performed using a kinase buffer (Cell Signaling Technology) supplemented with 10 mM *p*-nitrophenyl phosphate, 10 mM $MnCl_2$. 2 µg of recombinant active full length GST/SYK (SignalChem) and 2 µg of GST/FLT3 (571-993, SignalChem) fusion proteins were incubated in kinase buffer for 15 min at 30°C and with 50 µM [γ-^{32}P] ATP (Perkin Elmer). The same amount of recombinant protein was used for the assay between GST-SYK and GST-ABL (27-end, SignalChem). The

poly Glu-Tyr universal substrate peptide (P61-58, SignalChem) was used as a positive control for the kinase reaction. The reaction was stopped by addition of Laemmli sample buffer and resolved on SDS–PAGE.

Phosphorylation Site Mapping

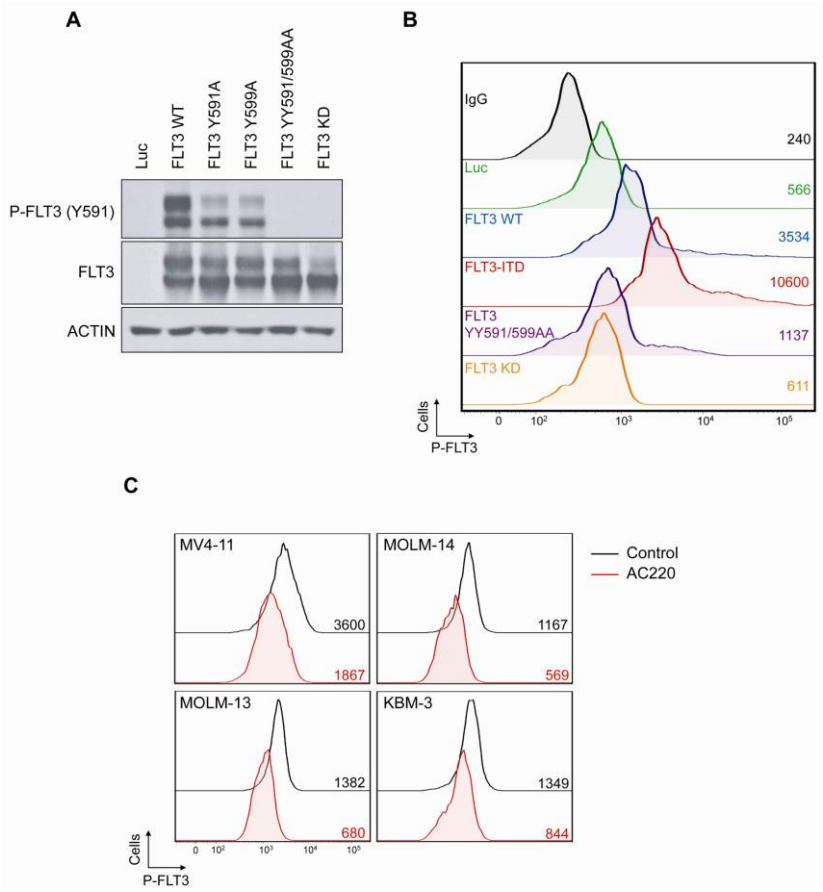
The *in vitro* kinase reaction using both GST-SYK and GST-FLT3 was performed as detailed above, except that the incubation was made in the presence of 500 μM cold ATP. For experiments performed in cells, both proteins were transfected into 293E cells and FLT3 was pulled down using the V5-tag. Either GST-tagged or V5-tagged proteins were separated by SDS-PAGE, the gel was stained with Coomassie blue, and the FLT3 band was excised. Gel slices were subjected to reduction with dithiothreitol, alkylation with iodoacetamide, and in-gel digestion with trypsin overnight at pH 8.3, followed by reversed-phase microcapillary/tandem mass spectrometry (LC-MS/MS). LC-MS/MS was performed using an Easy-nLC nanoflow HPLC (Thermo Fisher Scientific) with a self-packed 75 mm id x 15 cm C18 column connected to a hybrid linear ion trap LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific) in the data-dependent acquisition and positive ion mode at 300 nL/min. MS/MS spectra collected via collision-induced dissociation in the ion trap were searched against the concatenated target and decoy (reversed) Swiss-Prot and FLT3 single entry protein databases using Sequest with differential modifications for Ser/Thr/Tyr phosphorylation (+79.97) and the sample processing artifacts Met oxidation (+15.99), deamidation of Asn and Gln (+0.984) and Cys alkylation (+57.029) as affixed modification. Phosphorylated and unphosphorylated peptide sequences were identified if they initially passed the following Sequest scoring thresholds against the target database: 1+ ions, Xcorr ³ 2.0 Sf ³ 0.4, P ³ 5; 2+ ions,

Xcorr ³ 2.0, Sf ³ 0.4, P ³ 5; 3+ ions, Xcorr ³ 2.60, Sf ³ 0.4, P ³ 5 against the target protein database. Passing MS/MS spectra were manually inspected to be sure that all b- and y- fragment ions aligned with the assigned sequence and modification sites. Determination of the exact sites of phosphorylation was aided using FuzzyIons and GraphMod software (Proteomics Browser Software suite, Harvard University). False discovery rates (FDR) of peptide hits were estimated below 1.2% based on reversed database hits. The ratio of the number of phosphorylated versus non-phosphorylated sequences over each FLT3-derived peptide was used to evaluate the phosphorylation level of a given tyrosine site. To visualize SYK-induced changes in tyrosine phosphorylation level, the Phospho/Nonphospho value measured for each tyrosine site was converted into an exponential. The ratio of the exponential values corresponding to the panel of tyrosine phosphorylated in condition with FLT3 + SYK and those corresponding to the same panel of tyrosine phosphorylated in condition with FLT3 only, was ultimately log2-transformed and projected into a heatmap based on the GENE-E software (http://www.broadinstitute.org/cancer/software/GENE-E/).

Flow Cytometry

Primary patient AML cells were washed in PBS-0.1% BSA-2mM EDTA before a 45-minute incubation with a combination of anti-human PE-Cy7 CD13 and CD33 (used at 1/50) or PE-Cy7 isotype control antibodies. For FLT3 total staining, cells were incubated with an anti-human APC-conjugated FLT3 (used at 1/10) or APC isotype control antibody along with the cocktail of anti-CD13 and -CD33 antibodies. Cells were then washed twice with PBS-0.1%BSA-2mM EDTA before a 20-minute fixation with BD Cytofix/CytoPerm Fixation and Permeabilization Solution (BD Biosciences). Cells were then washed three times with BD Perm/Wash Buffer and incubated for 45

minutes at 4°C with the combination of anti-human PE-conjugated P-SYK (Y525/526) (used at 1/25) and APC-conjugated SYK (used at 1/100), DyLight488-conjugated MYC (used at 1/50), non-conjugated P-FLT3 (Y591) (used at 1/50), or the corresponding combination of isotype control antibodies. Cells were washed three times with Perm/Wash Buffer and non-conjugated P-FLT3 (Y591) condition was incubated for 35 minutes with an Alexa Fluor 488 goat anti-rabbit IgG antibody used at 1/500 (Invitrogen). Cells were washed three times in Perm/Wash Buffer and analyzed using a BD FACSCanto II analyzer. The P-FLT3 (Y591) antibody used in this study was not absolutely specific for the site Y591, as we identified by mutagenesis that it recognized the promiscuous autophosphorylation site Y599 as well (see below, **panel A**). This antibody was nevertheless perfectly efficient to recognize activated form of FLT3 by flow cytometry and western-blot since the overexpression of a constitutive active (ITD) or a kinase dead (KD) form of FLT3 enhanced or diminished the signal detected respectively (see below, **panel A and B**). Finally, the treatment of four FLT3-ITD AML cell lines with the specific FLT3 inhibitor, AC220, reduced the fluorescence emitted by this antibody (see below, **panel C**). A list of antibodies used for flow analysis of human samples is available in the table below the Flow-based Myeloid Differentiation Screening section of the supplemental experimental procedures. To account for any background fluorescence, P-SYK, SYK, P-FLT3 and FLT3 mean fluorescence levels were normalized to the mean fluorescence level of their corresponding isotype controls. P-SYK and P-FLT3 mean fluorescence reflecting level of activation were then normalized to SYK and FLT3 mean fluorescence, showing the cellular expression of both proteins. Each normalized value corresponding to (P-SYK/SYK) and (P-FLT3/FLT3) was then scaled for each patient using the Z-Score calculation across all patient samples.



Validation of P-FLT3 (Y591) antibody for flow staining. (**A**) Western blot and (**B**) FACS analysis for P-FLT3 (Y591) on 293E cells expressing either wild-type (WT), mutant (Y591A, Y599A and YY591/599AA) or inactivated (KD) forms of FLT3. (**C**) FACS analysis for P-FLT3 (Y591) on indicated FLT3-ITD positive AML cell lines treated with 10 nM AC220. For each FACS plot, numbers indicate mean of fluorescence.

Murine bone marrow, spleen and blood cells were collected from each organ, washed with PBS-0.1% BSA-2mM EDTA and lysed for 10 minutes with red blood cell lysis buffer (Sigma). After two washing steps with PBS-0.1% BSA-2mM EDTA, bone marrow cells were stained for 30 minutes at 4°C with a combination of anti-lineage cocktail, sca-1, c-KIT, CD16/32 and CD34 antibodies and spleen and blood cells

were incubated for 5 minutes at 4°C with mouse BD Fc Block™ (BD Biosciences) before staining with the combination of Mac-1/Gr-1 and B220/CD3 antibodies. Stained cell subpopulations were analyzed with BD LSR-II analyzer (BD Biosciences).

RNA Extraction and qRT-PCR Analysis

RNA was extracted from cells with an RNeasy Kit (Qiagen). Primers and probes for human *SYK* (Hs00895377_m1) and murine *Gapdh* (Mm99999915_g1) and *Sykb* (Mm01333032_m1), as well as human and murine control gene *RPL13A* (Hs01926559_g1 and Mm01612986_gH) were obtained from Applied Biosystems. A list of human and murine probes used for designing the MYC transcriptional target mini-library is available below. Data were collected in technical quadruplicate, analyzed using the ΔΔCT method, and plotted as percentage of transcript compared to the negative control condition.

Designation	Manufacturer	Human Probes	Murine Probes
MYC	Invitrogen	Hs00905030_m1	Mm00487804_m1
ABCE1		Hs00759267_s1	Mm01253089_g1
CCT3		Hs00195623_m1	Mm00657873_m1
DHX9		Hs00430767_g1	Mm00456030_m1
DTL		Hs00978565_m1	Mm00712787_m1
G3BP1		Hs01045296_g1	Mm00785370_s1
ILF3		Hs01128096_g1	Mm01175953_m1
NOLC1		Hs01102319_g1	Mm00661731_g1
PAICS		Hs00967251_g1	Mm04208670_g1

Confocal Microscopy

AML patient cells were stained as described in the flow cytometry section using anti-human FITC-conjugated SYK (1/50) and APC-conjugated FLT3 (1/10) antibodies. Cells were incubated with 1 mg/mL of 4′,6-diamidino-2-phenylindole, mounted on

glass slides in Fluoromount-G (Southern Biotechnology Associates), and photographed with a Nikon Ti w/ Spinning Disk Confocal.

Growth Measurement

To assess growth, cells were plated in 384-well plates. ATP content was measured using CellTiter Glo (Promega) per the manufacturer's instructions. Combination treatment with AC220 and PRT062607 in Ba/F3 was performed across a range of concentrations in 384-well format and synergy assessed using excess over Bliss additive synergy analysis (Berenbaum, 1981). The Bliss model calculates an expected effect of the combined response, C, of two individual agents with the effects A and B using the formula $C=A+B-A \times B$.

Flow-based Myeloid Differentiation Screening

U937, MOLM-14, THP-1, and KBM-3 cells were arrayed in two series of three replicates per shRNA in round bottom 96-well tissue culture plates in 50 µl of medium at 7,500 cells/well and incubated overnight at 37 °C with 5% CO2. The next day, 8 µg/ml polybrene (Sigma) and 10 µl PEG-it™ concentrated virus containing lentiviral pLKO.1 vectors were added to each well, with the exception of 18 uninfected wells/cell lines reserved for chemical controls. Plates were spun for 35 minutes at 1100 rcf and incubated at 37°C with 5% CO2 for 48 hours. Selection was performed with 1 µg/ml puromycin (Sigma) and medium was changed on the other replicate to serve as an unselected control. At this time point, the uninfected wells were treated with DMSO (3 wells/cell line) or 1 µM ATRA (3 wells/cell line). The plates were incubated for 5 days at 37°C with 5% CO2 before staining with FITC CD11b and PE CD14 myeloid differentiation markers. To do so, at day 5 post-puromycin selection,

each well was washed with 100 µl PBS-0.1% BSA-2mM EDTA twice and incubated for 45 minutes at 4°C with the combination of anti-human CD11b/CD14 or isotype control antibodies (used at 1/10). After two other washing steps, plates were then analyzed on FACSCanto II using the BD™ High Throughput Sampler (HTS) system. For each hairpin, the mean fluorescence level for each dye (FITC or PE) was expressed as an average of the three replicates. To account for any background fluorescence, the CD11b/CD14 mean fluorescence level for each triplicate was then normalized to the mean fluorescence level of the corresponding isotype controls. Results were then expressed as a log2-transformed ratio of the normalized mean fluorescence level corresponding to the hairpin of interest and the normalized mean fluorescence level of the control hairpin. Heatmap projections on differentially expressed CD11b and CD14 across each hairpin-transduced cell line were created based on the GENE-E software (http://www.broadinstitute.org/cancer/software/GENE-E/).

The knockdown efficiency of each target was evaluated on separate plates using the same extracellular staining protocol used for CD11b/CD14 except that FLT3, KIT, and PDGFRα expression level was measured using anti-human APC-conjugated FLT3 (1/10), APC-conjugated KIT (1/50), and APC-conjugated PDGFRα (1/50) antibodies (see table below) whose mean fluorescence level was normalized to the corresponding APC-conjugated isotype control. For SYK staining, we used an intracellular staining protocol consisting of washing the cells with 100 µl PBS-0.1% BSA-2mM EDTA twice and then fixing for 20 minutes with 100 µl BD Cytofix/CytoPerm Fixation and Permeabilization Solution (BD Biosciences). After two other wash steps with 100 µl BD Perm/Wash Buffer, cells were incubated for 35

minutes at 4°C with anti-human APC-conjugated SYK (1/100) or with the corresponding isotype control antibody.

Target	Conjugate	Catalog Number	Manufacturer
ABL	Non	sc-23	Santa-Cruz Biotechnology
ACTIN	Non	MS-1295-P	Neomarkers
AKT	Non	9272	Cell Signaling Technology
P-AKT (S473)	Non	4060	Cell Signaling Technology
B220	PE-Cy7	25-0452-81	eBioscience
CALNEXIN	Non	2679	Cell Signaling Technology
CD3	APC	17-0031-81	eBioscience
CD5	APC	17-0051	eBioscience
CD11b	FITC	IM0530U	Beckman Coulter
CD13	PE-Cy7	25-0138-42	eBioscience
CD14	PE	IM0650U	Beckman Coulter
CD16/32	PerCP-Cy5.5	45-0161-82	eBioscience
CD33	PE-Cy7	25-0338-42	eBioscience
CD34	eFluor® 450	48-0341-82	eBioscience
CD45R	APC	17-0452	eBioscience
CD127	APC	17-1271	eBioscience
c-KIT	PE	12-1172-82	eBioscience
DDK-Tag	Non	TA50011	Origene
ERK1/2	Non	9102	Cell Signaling Technology
P-ERK1/2 (T202/Y204)	Non	9101	Cell Signaling Technology
FLT3	Non	sc-20733	Santa-Cruz Biotechnology
FLT3	Non	sc-479	Santa-Cruz Biotechnology
FLT3	APC	MHCD13505	Invitrogen
P-FLT3 (Y591)	Non	3461	Cell Signaling Technology
P-FLT3 (Y591)	Non	3474	Cell Signaling Technology
P-FLT3 (Y768)	Non	Collaboration with Pr. Lars Rönnstrand (Lund University, Malmo, Sweden) (Razumovskaya et al., 2009)	
P-FLT3 (Y955)	Non	AT-7085	MBL International Corporation
P-FLT3 (Y969)	Non	3463	Cell Signaling Technology
GAPDH	Non	sc-25778	Santa-Cruz Biotechnology
GIANTIN	Non	ab24586	Abcam
GR-1	APC	17-5931-81	eBioscience
KIT	Non	3392	Cell Signaling Technology
KIT	APC	313206	BioLegend
Lineage	APC	BDB558074	BD Biosciences
Mac-1	PE-Cy7	101216	BioLegend
MYC	DyLight 488	ab139907	Abcam
PDGFRα	APC	MA1-10097	ThermoScientific
Sca-1	PE-Cy7	25-5981-82	eBioscience
STAT5	Non	9363	Cell Signaling Technology
P-STAT5 (Y694)	Non	9351	Cell Signaling Technology
P-STAT5 (Y694/699)	Non	sc-11761	Santa-Cruz Biotechnology
SYK	Non	sc-1240	Santa-Cruz Biotechnology
SYK	APC	17-6696-42	eBioscience
SYK	FITC	552476	BD Biosciences
SYK	Non	2712	Cell Signaling Technology
P-SYK (Y525/526)	Non	2710	Cell Signaling Technology
P-SYK (Y525/526)	PE	6485	Cell Signaling Technology
TEL	Non	8546	Santa-Cruz Biotechnology
TER-119	APC	17-5921	eBioscience
Phospho-Tyrosine Total	Non	9411	Cell Signaling Technology
V5-Tag	Non	R960-25	Invitrogen
V5-Tag	Non	sc-83849-R	Santa-Cruz Biotechnology

In Vivo Transplantation

The Massachusetts Institute of Technology Committee on Animal Care reviewed and approved all mouse experiments described in this study. BALB/c mice were purchased from Taconic. Each recipient mouse was transplanted with tranduced sca-1^-/c-KIT^+ myeloid progenitors sorted from the total bone marrow of five donor mice. To do so, 4-week-old male donor mice were primed with intraperitoneal injection of 5′-fluorouracil (150 mg/kg) and subsequently sacrificed after 6 days by CO_2 asphyxiation. Bone marrow was harvested from femur, tibia and humerus, and red blood cells were lysed (Red Blood Cell Lysis, RBCL buffer, Sigma). Cells were cultured overnight in transplant medium made with 20 ng/ml IL-3 (#213-13, Peprotech), 20 ng/ml IL-6 (#216-16, Peprotech), 100 ng/ml FLT3-Ligand (#250-31L, Peprotech) and 100 ng/ml SCF (#250-03, Peprotech) in StemSpan SFEM (#09650, StemCell Technologies Inc.). The sca-1^-/c-KIT^+ myeloid progenitor fraction was sorted from the total bone marrow following exclusion of $CD5^+$, $CD127^+$, $CD45R^+$ and TER-119^+ cell populations using FACS Aria (BD Biosciences). The next day, these sorted cells were transduced with either tomato-coupled miR30-shRNAs, TRMPCIR miR30-shRNAs, or pMSCV-IRES vectors by two rounds of spin-infection, at 24 hour intervals. All remaining bone marrow cells were maintained in culture during these steps of spin-infection and were then mixed with the infected myeloid fraction before tail vein injection into recipient mice. For each spin-infection, plates were coated with 50 µg/mL retronectin (Takara) per the manufacturer's instructions and fresh viral supernatant was added onto each retronectin-coated well before spinning for two hours at 2000g at 32 °C to allow virus binding to the retronectin. 10×10^6 cells in 3 mL transplant media containing 5 µg/mL polybrene (Sigma) and 7.5 mM HEPES buffer (Sigma) were then seeded into each of these wells and centrifuged for 6 hours at

1800g to promote cell transduction. After two days, cells were sorted based on expression of the tomato fluorescent protein and the next day, this tomato-positive fraction only was spin-infected twice, at 24-hour intervals, with pMSCV-IRES-EGFP (pMIG) empty or FLT3-ITD vectors using the same procedure. Transduced cells were washed in PBS, resuspended in Hanks balanced salt solution (Life Technologies) and then mixed with the remaining non-infected bone marrow cells before injection into the lateral tail vein of lethally irradiated (2×450cGy) male recipient mice. Mice were housed in microisolator cages with autoclaved chow and acidified water. Two days post-injection in recipient mice, a small fraction of sorted myeloid cells maintained in culture was analyzed by flow cytometry to evaluate the purity of the tomato or the crimson reporter-based sorting and the proportion of cells expressing pMIG empty or FLT3-ITD vectors.

For the experiment using the doxycycline inducible SYK shRNA-based system, the WBC count was evaluated every week. When the WBC count reached approximately 30 K/µl in the FLT3-ITD + miR30-shCT group, 1 mg/ml doxycycline + 3% sucrose was administered every two days in drinking water. At day 75 post-transplanation, doxycycline was removed from drinking water to measure possible relapse of the disease in the FLT3-ITD + miR30-Sykb groups.

To confirm the stable insertion of human FLT3-ITD (W51) into the murine myeloid cells' genomes, when mice became moribund, genomic DNA was extracted from bone marrow and spleen cells using DNeasy Blood and Tissue Kit (Qiagen). The juxtamembrane region of FLT3 was PCR-amplified and the presence of the 21 base pair tandem duplication ITD (W51) was determined by Fragment Analysis, and confirmed by sequencing. To evaluate the relative abundance of FLT3-ITD-expressing cells in one given organ, the sample with the highest signal was used as

a reference to calculate the signal strength of the others. For *in vivo* knockdown experiments, level of Sykb extinction was evaluated by western blot, while *in vivo* overexpression of human *SYK*, *TEL-SYK,* and *SYK-TEL* constructs was quantified by qRT-PCR analysis using primers matching the C-terminal region of human SYK.

The spleen size was evaluated by microtomography (microCT) on anesthetized animals injected with contrast agent. WBC and PLT as well as HCT were measured using Hemavet Automated Veterinary Analyzer on whole blood collected by retro-orbital bleeding.

Infection and Transplantation of BCR-ABL-transformed ALL cells

Murine *p19*$^{Arf\ -/-}$ pre-B cells expressing *Bcr-Abl* fusion protein were cultured in RPMI supplemented with 10% FBS, 4 mM L-glutamine and 5 µM β-mercaptoethanol (Williams et al., 2006). 1.5x10^6 cells were infected in a 6-well plate using 8 µg/ml polybrene and were tomato-based sorted 72 hours post-infection using FACS Aria (BD Biosciences). 1x10^6 cells were injected into 6 week-old immune-competent syngeneic C57/BL6 female mice, and disease progression was evaluated every two days by analysis of the percentage of tomato-positive cells in spleen and bone marrow with a BD LSR-II analyzer.

Combination of small-molecule inhibitors *in vivo*

Approximately 0.75 × 10^6 or 2 × 10^6 spleen-derived murine primary cells expressing FLT3-ITD + SYK-TEL or FLT3-ITD + SYK respectively were tail vein injected as a secondary transplant into sublethally irradiated (400cGy) 6-week-old male BALB/c mice. When the WBC count reached approximately 30 K/µl, either a daily treatment with 80 mg/kg R788 (Fostamatinib) or vehicle control (35% TPGS, 60% PEG 400,

5% propylene glycol; in 100 µl*;* n=6 per condition), a daily treatment with 5 mg/kg AC220 or vehicle control (22% hydroxypropyl-β-cyclodextrin in sterile water; in 200 µl; n = 6 per condition), a twice-daily treatment with 20 mg/kg PRT062607 or vehicle control (0.5% methylcellulose in sterile water; in 100 µl; n=6 per condition), or a combination of AC220 and PRT062606, were administered by oral gavage. Since no significant differences in overall survival were observed between each vehicle-treated group, only the group of mice treated with the combination of vehicles was represented.

Infection of Primary Cells

Normal purified $CD34^+$ human cells were obtained from Lonza, and use of these materials is considered exempt as Human Subjects by the Dana-Farber Cancer Institute Internal Review Board. These cells were maintained in StemSpan SFEM (#09650, StemCell Technologies Inc.) medium supplemented with 20 ng/ml IL-3 (#200-03, Peprotech), 20 ng/ml IL-6 (#200-06, Peprotech), 20 ng/ml GM-CSF (#300-03, Peprotech), 100 ng/ml FLT3-Ligand (#300-19, Peprotech) and 100 ng/ml SCF (#300-07, Peprotech). They were serially transduced with each vector by two rounds of spin-infection (6 hours at 1800g) each, at 24 hour intervals using plates coated with 50 µg/mL retronectin (Takara) per the manufacturer's instructions. At 24 hours after the last spin-infection, cells were extensively washed in phosphate-buffered saline (PBS) before a 48-hour antibiotic selection. Viable cells (1.5×10^3 cells/ml) were then plated into semisolid methylcellulose medium (MethoCult H4236, StemCell Technologies Inc.) supplemented with selective antibiotic, 20 ng/ml IL-3 (#200-03, Peprotech), 20 ng/ml IL-6 (#200-06, Peprotech), 20 ng/ml GM-CSF (#300-03,

Peprotech), and 100 ng/ml SCF (#300-07, Peprotech). After at least 10 days, the colony number was evaluated after MTT staining of the colonies (R&D Systems).

Histopathology

Murine tissues were fixed for at least 72 hours in 10% neutral buffered formalin (Sigma), dehydrated in alcohol, cleared in xylene, and infiltrated with paraffin on an automated processor (Leica). The tissue sections (4 µm thick) from paraffin-embedded tissue blocks were placed on charged slides and deparaffinized in xylene, rehydrated through graded alcohol solutions, and stained with H&E.

Genome-wide Expression Analysis

HL-60, MOLM-14, and U937 cell lines were transduced in triplicate with a control luciferase-directed shRNA and in duplicate with two SYK-directed shRNAs (shSYK_1 and shSYK_2), and were then selected with 1 µg/ml puromycin 48 hours post-infection. At day 5 post-infection, RNA was extracted and profiled using HT HG-U133A arrays (Affymetrix) at the Broad Institute (Cambridge, MA, USA). The computational analysis of the gene expression data was performed through the Genome Space bioinformatics platform (http://www.genomespace.org).

Data Processing. The CEL data files were subjected to quality control and passed tests based on distance between arrays, array intensity distribution, and variance mean dependence which are implemented in the *ArrayQuality* R package available at Bioconductor (www.bioconductor.org). The raw expression data were processed by applying the RMA algorithm in the ExpressionFileCreator module of GenePattern 3.5.0 (http://www.genomespace.org). All probe sets with the maximum

expression below 16 were considered under-expressed and “filtered”. Of the 22,215 probe sets on the Affymetrix HT HG-U133A array, 13,847 probe sets remained after filtering. The data from the 13,847 probe sets were further collapsed to 8,849 non-redundant genes with distinct HUGO symbols based on the Affymetrix HT_HG-U133A.na33 annotations, by assigning to each gene the probe set with the maximum average expression intensity.

Comparative Marker Analysis. The 21 samples available in the data were separated into two groups: one with all the cell lines transduced with the control shRNAs and one with all the cell lines transduced with the SYK shRNAs. The Comparative Marker Selection module from GenePattern 3.5.0 (http://www.genomespace.org) was employed to identify individual genes that were differentially expressed between the control shRNA and the SYK shRNA subgroups. The analysis was performed by applying a 2-sided Signal-to-Noise Ratio (SNR) test followed by 1000 permutations of phenotype labels. The settings for the SNR parameters were *log-transformed-data*:no, *complete*:no, *balanced*:no, *smooth p-values*: yes.

A “core” SYK knockdown signature consisting of 45 genes up-regulated and of 36 genes down-regulated between the control shRNA and the SYK shRNA samples was defined based on the cut-offs SNR permutation p-value ≤ 0.05, Benjamini-Hochberg false discovery rate (FDR) ≤ 0.05, and absolute fold change (FC) ≥ 1.2 across each of the three cell lines. In addition, a “relaxed” SYK knockdown signature consisting of 115 up-regulated and 95 down-regulated genes between the control shRNA and the SYK shRNA groups was defined based on the cut-offs SNR permutation p-value ≤ 0.05, Benjamini-Hochberg false discovery rate (FDR) ≤ 0.05

and absolute fold change (FC) ≥ 1.2 across at least two of the three cell lines. The "core" and the "relaxed" SYK knock-down signatures are presented in **Table S1**.

In silico Tests for SYK high versus SYK Low Signatures in Primary AML Samples

The *in silico* test used to determine the proportion of FLT3-WT and FLT3-ITD patients exhibiting a high SYK versus low SYK signature was carried out on the Affymetrix U133 Plus2 gene expression data GSE144688 (Wouters et al., 2009) including 526 AML patient samples and RNAseq expression data TCGA LAML (Ley et al.) including 179 patient samples. Data were downloaded from the InSilico DB Genomic Datasets Hub (http://www.genomespace.org). These data and one additional cohort GSE10358 (Tomasson et al., 2008) including 279 patients were also queried to determine the FAB categories of patients displaying a different proportion of samples with high SYK signature versus low SYK signature compared to all the other categories. The same analysis was performed to determine the number of patients with known mutation in AML (i.e. *NPM1*, *DNMT3A*, *IDH1/2*, *RUNX1*, *TET2* etc.) displaying high versus low SYK signature. For each patient high SYK and low SYK signature scores were evaluated based on enrichment analysis with the relaxed SYK knockdown signature. The analysis was performed by applying the single sample GSEA (ssGSEA) method available in the Gene Set Enrichment Analysis (GSEA v2.0.10) software.

The ssGSEA method assigns to each individual sample represented as a ranked list of genes an Enrichment Score (ES) with respect to each gene set in a given collection of pathways. Thus ssGSEA provides a representation of the gene

expression data in the space of gene sets. The gene set representation has more biological interpretability and can be further analyzed with statistical and machine learning methods. ES is calculated as a running sum statistic by walking down across the ranked list of genes, increasing the sum when encountering genes in the gene set and decreasing it when encountering genes not in the gene set. The significance of the ES is estimated based on a permutation p-value and adjusted for multiple hypotheses testing through FDR. A positive ES denotes a significant overlap of the signature gene set with groups of genes at the top of the ranked list, while a negative ES denotes a significant overlap of the signature gene set with groups of genes at the bottom of the ranked list. For each sample the ES was further transformed into a z-score by subtracting the average of the ES's assigned to all other samples and by dividing the result to their standard deviation.

For each patient the high SYK signature versus the low SYK signature was evaluated based on the absolute cut-off 1 for the z-score. In the GSE144688, GSE10358 and TCGA LAML datasets, 59, 31 and 37 patients respectively were assigned with a high SYK signature based on a z-score ≥ 1 and 73, 24 and 37 patients respectively were assigned with a low SYK signature based on a z-score ≤ -1. The significance of the differences between the proportions of SYK high versus SYK low patients was evaluated by applying the two tailed Fisher's Exact Test implemented in the function *fisher.test* (library stats, R 2.14, http://cran.r-project.org/).

Functional Enrichments Associated with the High Versus Low SYK Signatures

The ssGSEA method was further applied to the patients displaying high SYK and low SYK signatures in all dataset, across a broad collection of canonical pathways and

gene sets available in the Molecular Signature Database (the c2 collection of 1452 curated pathways and the c3 collection of 615 transcription factor, MSigDB v3.1, http://www.broadinstitute.org/gsea/msigdb/index.jsp) and in the Differentiation Map Portal at the Broad Institute (DMAP, http://www.broadinstitute.org/dmap/home/.) The Comparative Marker Selection method was applied to the ES data to identify the pathways and the gene sets which are significantly differentially enriched in populations exhibiting a high versus low SYK signature. The significance was assessed based on the cut-offs 2 for the fold change, 0.01 for the p-value, and 0.05 for the FDR. The significantly enriched gene sets were then grouped into functional clusters based on their biological function annotations.

Gene expression data from this study are available from Gene Expression Omnibus Expression (GEO) (accession number: **GSE54065**).

SUPPLEMENTAL REFERENCES

Banerji, V., Frumm, S. M., Ross, K. N., Li, L. S., Schinzel, A. C., Hahn, C. K., Kakoza, R. M., Chow, K. T., Ross, L., Alexe, G.*, et al.* (2012). The intersection of genetic and chemical genomic screens identifies GSK-3alpha as a target in human acute myeloid leukemia. J Clin Invest *122*, 935-947.

Berenbaum, M. C. (1981). Criteria for analyzing interactions between biologically active agents. Adv Cancer Res *35*, 269-335.

Dickins, R. A., Hemann, M. T., Zilfou, J. T., Simpson, D. R., Ibarra, I., Hannon, G. J., and Lowe, S. W. (2005). Probing tumor phenotypes using stable and regulated synthetic microRNA precursors. Nat Genet *37*, 1289-1295.

Du, J., Bernasconi, P., Clauser, K. R., Mani, D. R., Finn, S. P., Beroukhim, R., Burns, M., Julian, B., Peng, X. P., Hieronymus, H.*, et al.* (2009). Bead-based profiling of tyrosine kinase phosphorylation identifies SRC as a potential target for glioblastoma therapy. Nat Biotechnol *27*, 77-83.

Kimbrel, E. A., Lemieux, M. E., Xia, X., Davis, T. N., Rebel, V. I., and Kung, A. L. (2009). Systematic in vivo structure-function analysis of p300 in hematopoiesis. Blood *114*, 4804-4812.

Moffat, J., Grueneberg, D. A., Yang, X., Kim, S. Y., Kloepfer, A. M., Hinkle, G., Piqani, B., Eisenhaure, T. M., Luo, B., Grenier, J. K.*, et al.* (2006). A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen. Cell *124*, 1283-1298.

Razumovskaya, E., Masson, K., Khan, R., Bengtsson, S., and Ronnstrand, L. (2009). Oncogenic Flt3 receptors display different specificity and kinetics of autophosphorylation. Exp Hematol *37*, 979-989.

Tomasson, M. H., Xiang, Z., Walgren, R., Zhao, Y., Kasai, Y., Miner, T., Ries, R. E., Lubman, O., Fremont, D. H., McLellan, M. D.*, et al.* (2008). Somatic mutations and germline sequence variants in the expressed tyrosine kinase genes of patients with de novo acute myeloid leukemia. Blood *111*, 4797-4808.

Wouters, B. J., Lowenberg, B., Erpelinck-Verschueren, C. A., van Putten, W. L., Valk, P. J., and Delwel, R. (2009). Double CEBPA mutations, but not single CEBPA mutations, define a subgroup of acute myeloid leukemia with a distinctive gene expression profile that is uniquely associated with a favorable outcome. Blood *113*, 3088-3091.

Zuber, J., McJunkin, K., Fellmann, C., Dow, L. E., Taylor, M. J., Hannon, G. J., and Lowe, S. W. (2010). Toolkit for evaluating genes required for proliferation and survival using tetracycline-regulated RNAi. Nat Biotechnol *29*, 79-83.