Supplemental Methods

Generation of FLT3-expressing Ba/F3 cells

Murine stem cell virus containing FLT3-ITD and green fluorescent protein genes (pMSCV-FLT3-ITD-IRES-GFP) was a generous gift from Dr. Scott Lowe (Memorial Sloan-Kettering). Point mutations were introduced by site-directed mutagenesis as previously described and were confirmed by DNA sequencing (Hartwell Center for Bioinformatics and Biotechnology, St. Jude Children's Research Hospital).[1] Subsequently, Eco-HEK293 cells were transfected with FLT3 vectors (one preparation for each FLT3 variant) using FuGENE HD Transfection Reagent (Promega, Madison, WI) according the manufacturer's instructions and viral supernatant was collected over a 36-h period. Viral supernatant was added to retronectin-coated plates and centrifuged at 2100 x g for 2 h. Ba/F3 cells (1×10^6) were then transduced with virus for 48 h followed by GFP FACS analysis. GFP-positive cells were selected for IL3-independent cell growth over a period of 7 days. Cells were maintained in RPMI 1640 media (Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) (Atlanta Biologicals, Lawrenceville, GA) (complete RPMI) at 37°C in a humidified atmosphere containing 5% CO_2.

Generation of luciferase-expressing cell lines

MV4-11, MOLM-13, and Ba/F3 FLT3-ITD/D835H cells were transduced with MSCV containing firefly luciferase and yellow fluorescent protein genes (pMSCV-luc-IRES-YFP), which was obtained from the Vector Core Laboratory, St. Jude Children's Research Hospital. Viral supernatant was prepared in the Vector Core Laboratory, and after transduction, YFP-positive cells were single-cell sorted by flow cytometry and expanded in culture. For the Ba/F3 FLT3-

ITD/D835H cells, dual GFP/YFP-positive cell sorting was performed followed by cell expansion in culture.

Generation of drug-resistant MOLM-13 cells

Luciferase-expressing FLT3-ITD-positive MOLM-13 (MOLM-13-luc) cells were continuously cultured in a gradually increasing concentration of the FLT3 inhibitor tandutinib (LC Laboratories, Woburn, MA). After 6 months, MOLM-13-luc cells grew in the presence of 5 μM drug, at which time DNA sequence analysis revealed the acquisition of a FLT3 D835Y mutation. Tandutinib-resistant MOLM-13-luc (MOLM-13-RES-luc) cells were maintained in 5 μM tandutinib-containing complete RPMI and were re-suspended in drug-free media three days before experimentation.

Measurement of cell viability

The effects of a 72-h drug treatment on AML cell line viability were determined by the WST-1 cell proliferation reagent (Roche Applied Science; Indianapolis, IN); cell viability was determined in Ba/F3 cells using the MTT cell proliferation reagent (Roche Applied Science) according to the manufacturer's instructions.

For combination studies of crenolanib and sorafenib, AML cell lines were exposed to drug at a fixed concentration ratio for 72 h (MOLM-13 and MOLM-13-luc: crenolanib 0.29-75 nM combined with sorafenib 0.39-100 nM; MV4-11: crenolanib 0.08-20 nM combined with sorafenib 0.08-20 nM) and cell viability was assessed using the WST-1 cell proliferation reagent. For crenolanib/AraC combination studies, cells were plated on 96-well filter plates (Millipore, Billerica, MA) and treated with drug at a fixed concentration ratio. For non-continuous drug

treatment, cells were washed three times with complete RPMI at the indicated time and replaced with media containing drug or DMSO. Cell viability after 72 h was assessed by WST-1 reagent. The following schedules and sequences were evaluated: (A) simultaneous 72 h continuous exposure (MOLM-13: AraC 0.04-10 μM combined with crenolanib 0.31-80 nM; MV4-11: AraC 0.23-60 μM combined with crenolanib 0.08-20 nM) (B) simultaneous 4 h pulse exposure followed by 68 h crenolanib exposure (MOLM-13: AraC 0.24-63 μM combined with crenolanib 0.29-75 nM; MV4-11: AraC 3.9-1000 μM combined with crenolanib 0.08-20 nM); (C) (pre-AraC) 4 h AraC exposure followed by 68 h crenolanib exposure (MOLM-13: AraC 0.24-63 μM combined with crenolanib 0.29-75 nM; MV4-11: AraC 3.9-1000 μM combined with crenolanib 0.08-20 nM); and (D) (pre-crenolanib) 24 h crenolanib exposure followed by 48 h AraC exposure (MOLM-13: AraC 0.01-2.5 μM combined with crenolanib 0.04-100 nM; MV4-11: AraC 0.08-25 μM combined with crenolanib 0.04-100 nM).

Pediatric AML bone marrow samples were obtained at tyrosine kinase inhibitor (TKI) resistance and processed as previously described.[1] Frozen primary blasts were thawed (viability (mean ± SEM): 82.8 ± 2.3%) and resuspended in complete RPMI supplemented with 1% penicillin/streptomycin (Life Technologies) followed by drug treatment for 72 h. Cell viability was assessed using the MTT cell proliferation reagent. The viability of untreated cells after 72 h in culture was 78.8 ± 4.5%.

Crenolanib tolerability and pharmacokinetic studies in mice

Initial crenolanib pharmacokinetic studies were performed in male and female non-leukemia-bearing FLT3-ITD transgenic C57Bl/6 mice (Stock number: 011112; Jackson Laboratories, Bar Harbor, ME). Crenolanib besylate (AROG Pharmaceuticals, LLC.) for intraperitoneal (i.p.) injection was formulated in 5% glycerol formal (Sigma-Aldrich, St. Louis, MO). Crenolanib besylate for oral administration was formulated in 5% methylcellulose (Sigma-

Aldrich). Serial blood samples were obtained from individual mice at 0.5, 1, 2, 4, and 6 h after administration of crenolanib 15 mg/kg via i.p. injection or 0.5, 1, 1.5, 2.5, and 4 h after oral gavage (3 mice per group).

Eight female *NOD.Cg*-Prkdc^{scid} Il2rg^{tm1Wjl}/*SzJ* (NSG; Stock number: 005557; Jackson Laboratories, Bar Harbor, ME) mice were engrafted with MV4-11-luc cells and forty days later were given crenolanib 15 mg/kg i.p. and serial blood samples were obtained at 0.083, 2, 5, 8, 12 and 24 h. Blood samples were collected in heparinized capillary tubes, transferred and centrifuged to isolate plasma, and samples were stored at -80°C until analysis. Aliquots of plasma were prepared for analysis using liquid-liquid extraction with *tert*-butyl methyl ether, and crenolanib concentrations were assayed by a validated high-performance liquid chromatography method with tandem mass spectrometer (LC-MS/MS).[2]

The concentration-time data were fit by a two-compartment model as implemented in NONMEM.[3] Estimated parameters included central compartment volume (V_c), elimination rate constant (k_e), and intercompartmental rate constants (k_{12} and k_{21}). The area under the concentration-time curve (AUC) was calculated as the dose divided by the post hoc estimate of the clearance, $D/(V_c \times K_{el})$. Concentration-time curves for a QD and BID regimen were simulated by a Monte Carlo differential equation solver algorithm (n = 1000) using the lognormal distributions of parameter values obtained during model fitting.

Single-dose tolerability experiments were performed at crenolanib 15, 30, or 60 mg/kg administered i.p. The primary acute toxicities observed at doses >15 mg/kg were lethargy and gait abnormalities, which were reversible within approximately 30 minutes of drug injection. Multiple-dose tolerability studies were performed at crenolanib 15 and 30 mg/kg administered once daily (Monday – Friday) for 4 consecutive weeks. The primary toxicity with multiple dosing was weight loss >15%, which occurred at crenolanib 30 mg/kg. The maximum tolerated

crenolanib dose for single- and multiple-dose administration (up to 4 weeks) was determined to be 15 mg/kg i.p.

In vivo xenograft mouse models

Mice (8-12 weeks old) were housed in a 12-h light/dark cycle with access to food and acidified water *ad libitum*. Male or female *NOD.Cg*-Prkdc^{scid} Il2rg^{tm1Wjl}/*SzJ* (NSG) (Stock number: 005557; Jackson Laboratories, Bar Harbor, ME) animals were given an intravenous injection of 5 x 10^6 luciferase-expressing MV4-11 (MV4-11-luc) cells. Cell engraftment was assessed after injection of D-luciferin (150 mg/kg i.p.; Perkin Elmer, Waltham, MA) on a Xenogen IVIS-200 imaging system (Alameda, CA), and mice were randomized to treatment groups based on signal intensity. Leukemic burden was monitored weekly by noninvasive luciferase imaging, and mice were observed daily and euthanized humanely upon signs of terminal illness (hind limb paralysis, inability to eat/drink, moribund lethargy). MV4-11-luc cells engrafted in the bone marrow by day 7 in males and day 9 in females after cell injection (Supplemental Figure 1). At the time of terminal illness, >90% leukemic cell infiltration in the bone marrow was observed (Supplemental Figure 1). Drug treatments were started on days 10 or 17 after cell injection. Crenolanib besylate was formulated in 5% glycerol formal and administered i.p. Sorafenib was dissolved in 50/50 cremophor EL/ethanol (Sigma-Aldrich) followed by a 4-fold dilution in water and administered by oral gavage.

Female NSG mice were given an intravenous injection of 5 x 10^6 luciferase-expressing MOLM-13-RES (MOLM-13-RES-luc) cells and leukemic burden was monitored twice weekly as described above. Cells engrafted in the bone marrow by day 4 after cell injection (Supplemental Figure 2). At the time of terminal illness, >75% leukemic cell infiltration was observed in the bone marrow (Supplemental Figure 2). Drug treatment was started on day 3 after cell injection.

Male NSG mice were given an intravenous injection of 5 x 10^4 luciferase-expressing Ba/F3 FLT3-ITD/D835H (Ba/F3 FLT3-ITD/D835H-luc) cells. Leukemic burden was monitored twice weekly as described above. Because of the extremely aggressive nature of the Ba/F3 FLT3-ITD/D835H model, we were unable to obtain mouse imaging data before the initiation of drug treatment 1 day after cell injection; however, image analysis on day 3 after cell injection showed similar engraftment of cells between each study arm (Supplemental Figure 3). All animal studies were approved by the Animal Care and Use Committee at St. Jude Children's Research Hospital.

FLT3 mutation analysis

To amplify *FLT3* exons 14 through 20, RNA was isolated from 9 x 10^6 cells using TRIzol REAGENT (Life Technologies) according to the manufacturer's instructions. RNA integrity was assessed using an Agilent 2200 TapeStation System (Agilent Technologies, Santa Clara, CA) then cDNA was synthesized from 1 µg of RNA using SuperScript III First-Strand Synthesis SuperMix (Life Technologies) according the to manufacturer's instructions. FLT3 exons 14 through 20 were amplified using forward primer 5'-CCCTTCCCTTTCATCCAAGA-3' and reverse primer 5'-ATGCCTTCAAACAGGCTTTC-3'. The 25-µL PCR reaction contained 1x 5 PRIME Hotmaster Mix, 200 nM forward and reverse primer, and 2 uL undiluted cDNA. The reaction was run on an Eppendorf Mastercycler ep gradient S (Eppendorf, Hamburg, Germany), using a program consisting of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 68°C for 45 seconds for 35 cycles, with an initial heating at 94°C for 2 minutes and a final extension at 68°C for 3 minutes. The PCR products were then run on a 1% agarose gel stained with ethidium bromide and visualized under ultraviolet light to confirm amplified products were of the correct size. Reactions that produced the appropriate 992 bp PCR product were cloned into pCR2.1-TOPO (Life Technologies) and transformed using One

Shot TOP10 chemically competent E. Coli (Life Technologies) according to the manufacturer's instructions. Colonies were cultured overnight in selective media. Plasmid DNA was isolated using the QuickLyse miniprep kit (Qiagen, Germantown, MD) according to the manufacturer's instructions. Plasmid DNA was then sequenced with the same primers used in amplification as described above. Forward and reverse sequences were aligned and analyzed using the Sequencher software (Gene Codes Corporation, Ann Arbor, MI).

Western blot analysis

Cells were lysed in radioimmunoprecipitation assay buffer supplemented with protease (Roche Applied Science) and phosphatase (Merck KGaA, Darmstadt, Germany) inhibitors. For FLT3 immunoprecipitation, 200 µg total cell lysate was incubated with FLT3 antibodies and protein A/G agarose (Santa Cruz Biotechnology, Santa Cruz, CA) for 2 h at 4°C with constant agitation. Total cell lysate (30-50 µg) or FLT3 immunoprecipitation eluent was separated by SDS-polyacrylamide gel electrophoresis according to the manufacturer's instructions (Life Technologies) and transferred to PVDF membranes followed by Western blot analysis using the indicated antibodies as previously described.[1]

References

1. Baker SD, Zimmerman EI, Wang YD, et al. Emergence of polyclonal FLT3 tyrosine kinase domain mutations during sequential therapy with sorafenib and sunitinib in FLT3-ITD-positive acute myeloid leukemia. *Clin Cancer Res*. 2013 Aug 22 [Epub ahead of print].

2. Roberts MS, Turner DC, Owens TS, et al. Determination of crenolanib in human serum and cerebrospinal fluid by liquid chromatography-electrospray ionization-tandem mass

spectrometry (LC-ESI-MS/MS). *J Chromatogr B Analyt Technol Biomed Life Sci*. 2013;929C:1-5.

3. Beal SL. NONMEM User's Guide. Introduction to version IV: ICON Development Solutions; 2006.

4. Hu S, Niu H, Inaba H, et al. Activity of the multikinase inhibitor sorafenib in combination with cytarabine in acute myeloid leukemia. *J Natl Cancer Inst*. 2011;103(11):893-905.

5. Davis MI, Hunt JP, Herrgard S, et al. Comprehensive analysis of kinase inhibitor selectivity. *Nat Biotechnol*. 2011;29(11):1046-1051.

Table S1. Inhibition of AML cell viability by crenolanib and sorafenib

AML Cell Line	FLT3 Status	Crenolanib IC50 (nM)	Sorafenib IC50 (nM)
MOLM-13	ITD	4.9	17
MV4-11	ITD	1.3	4.9
OCI-AML3	WT	2800	3900**
PL21	ITD*	2900	13000
THP-1	WT	3500	6200**
U937	WT	3100	6900**

* - Contains an intronic ITD mutation.

** - As reported in Hu et al.[4]

Abbreviations: ITD, internal tandem duplication.

Table S2. Pharmacokinetic parameters of crenolanib in leukemia-bearing female NSG mice.

	Cmax (ng/ml)	Cmax (μM)	$AUC_{(0\text{-}12h)}$ (ng/ml*h)	$AUC_{(0\text{-}24h)}$ (ng/ml*h)	Css, ave (ng/ml)	Css, ave (μM) (once daily)	Css, ave (μM) (twice daily)	$T_{1/2}$ (h)
Crenolanib 15 mg/kg	1370	3.1	2130	2140	89.3	0.2*	0.4*	1.2

* - Simulated from parameters derived from a single 15 mg/kg dosage study.

Abbreviations: AUC, area under the concentration-time curve; Cmax, maximum plasma concentration; Css, ave, average tough plasma concentration at steady-state; $T_{1/2}$, half-life

Table S3. Crenolanib activity against BaF/3 cells expressing various FLT3 mutants.

Cell Line	Crenolanib IC50 (nM)	Sorafenib IC50 (nM)*
GFP + IL3	860	3900
D835H	0.30	81
D835Y	0.06	210
ITD	1.8	1.1
ITD D835H	38	460
ITD D835Y	35	3300
ITD F691L	62	1300

* - As reported in Baker et al.[1]

Abbreviations: ITD, internal tandem duplication.

Table S4. Distribution of FLT3 D835Y mutations on WT and ITD FLT3 alleles.*

Cell Line	ITD-positive clones with D835Y mutation	ITD-negative clones with D835Y mutation
MOLM13	0/10 (0%)	0/8 (0%)
MOLM13-RES	3/7 (43%)	0/7 (0%)

* - Determined from cloning and sequencing of FLT3 exons 14 to 20.

Abbreviations: ITD, internal tandem duplication.

Table S5. Pediatric AML patient characteristics

Patient Number	Age (years)	Sex	Sample Type	FLT3 D835 Status	Blast %
1	11	M	TKI resistant	D835H/Y	88
2	12	M	TKI resistant	D835H/Y	70

Abbreviations: ITD, internal tandem duplication; TKI, tyrosine kinase inhibitor

Supplemental Figure Legends

Figure S1. MV4-11-luc mouse model of FLT3-ITD-positive AML. (A) Male and (B) female NSG mice were engrafted with MV4-11-luc cells and leukemic cell infiltration was monitored by whole body noninvasive luciferase imaging of the ventral and dorsal surfaces. Whole body luciferase images of representative male mice on day 7 and female mice on days 9 and 16. (C) Representative images of bone marrow infiltration of MV4-11-luc cells. Two female mice were euthanized due to signs of terminal disease on days 40-45 and the bone marrow was harvested. Modified Wright-Giemsa staining of bone marrow cytospins was performed (40X).

Figure S2. MOLM-13-RES-luc mouse model of FLT3-ITD D835Y-positive AML. Female NSG mice were engrafted with MOLM-13-RES-luc cells. Leukemic cell infiltration was monitored by whole body noninvasive luciferase imaging of the (A) ventral and (B) dorsal surface. Whole body luciferase images on day 4 are shown. (C) Representative images of bone marrow infiltration of MOLM-13-RES-luc cells. Two female mice were euthanized due to signs of terminal disease on days 19-22 and the bone marrow was harvested. Modified Wright-Giemsa staining of bone marrow cytospins was performed (40X).

Figure S3. Luciferase activity in mice engrafted with Ba/F3 FLT3-ITD/D835H-luc cells. Male NSG mice engrafted with Ba/F3 FLT3-ITD/D835H-luc cells were treated with vehicle, crenolanib 15 mg/kg i.p. twice daily, or sorafenib 15 mg/kg orally once daily beginning on day 1. Whole body noninvasive luciferase imaging was performed on day 3 (images of representative mice are shown).

Figure S4. Crenolanib binds to FLT3 kinase variants with high affinity. (A-B) Representative dose-response graphs for (A) crenolanib and (B) sorafenib binding to recombinant WT FLT3 and FLT3 mutants as determined by the KdELECT assay.

Figure S5. Crenolanib has greater affinity for active Abl kinase, similar to other type 1 kinase inhibitors. (A-B) Dose-response graphs for crenolanib binding to recombinant active and inactive (A) WT Abl and (B) Abl Q252H, as determined by the KdELECT assay. (C-D) The active/inactive K_d ratio for crenolanib and validated type I and type II kinase inhibitors for (C) WT and (D) Q252H Abl. Data for other type I kinase inhibitors taken from Davis et al.[5]

Figure S6. Crenolanib and cytarabine (AraC) combination synergistically inhibit FLT3-ITD-positive cell viability in vitro. Cells were treated with DMSO or increasing concentrations of crenolanib, AraC or both at a fixed concentration ratio under different schedules and sequences: (A) simultaneous 72 h drug exposure; (B) simultaneous 4 h drug exposure followed by 68 h crenolanib exposure; (C) (pre-AraC) 4 h AraC exposure followed by 68 h crenolanib exposure; or (D) (pre-crenolanib) 24 h crenolanib exposure followed by 48 h AraC exposure. Data are from 2-4 independent experiments with 6 replicates each (n = 12-24). Combination index (CI) values were generated using Calcusyn software (CI >1.0: antagonism; CI = 1.0: additivity; CI <01.0: synergism); all R values from the fit of data were > 0.90.

Figure S7. Crenolanib pharmacokinetics in mice. (A-B) Male and female FLT3-ITD transgenic C57Bl/6 mice (3/group) received crenolanib 15 mg/kg administered via (A) i.p. injection or (B) oral gavage and serial blood sampling was performed. Crenolanib plasma concentrations were determined by LC-MS/MS. Values represent the mean ± SEM. (C) Female NSG mice were engrafted with MV4-11-luc cells. Forty days later 8 mice were given crenolanib 15 mg/kg i.p. and serial blood sampling was performed. Mean (solid line) and 95% confidence interval (shaded region) of population crenolanib plasma concentration-time curves for day 1 and steady-state dosing interval derived from a 1,000-subject Monte Carlo simulation on a twice daily dosing regimen (top panel) and once daily dosing regimen (bottom panel). Solid circles represent observed concentration-time data from a single-dose pharmacokinetic study.

Figure S8. Anti-leukemic activity of crenolanib in a MV4-11 xenograft AML mouse model. (A-C) Male NSG mice engrafted with MV4-11-luc cells were treated with crenolanib 15 mg/kg i.p. once daily (Monday - Friday) for 3 consecutive weeks beginning on day 10. (A) Leukemic infiltration in the bone marrow was monitored by noninvasive luciferase imaging. (B) Whole body luciferase images of representative mice. (C) Kaplan-meier analysis of animal survival. Black bar denotes treatment period.

Figure S9. MOLM-13-RES cells express a D835Y mutation. (A) DNA from MOLM-13 and MOLM-13-RES cells was isolated, FLT3 exon 17 was amplified by PCR, and the WT and ITD alleles were separated by gel electrophoresis (top panel). DNA sequencing of codon 835 (lower panel) (GAT: Asp; TAT: Tyr). (B-C) MOLM-13 and MOLM-13-RES cells were lysed and (B) FLT3 was immunoprecipitated and (B-C) Western blot analysis was performed using the indicated antibodies.

Figure S10. Crenolanib delays MOLM-13-RES-luc cell infiltration. Female NSG mice engrafted with MOLM-13-RES-luc cells were treated with vehicle, sorafenib 15 mg/kg orally once daily, or crenolanib 15 mg/kg i.p. once or twice daily (Monday - Friday) for 3 consecutive weeks beginning on day 3. Leukemic infiltration was monitored by noninvasive luciferase imaging. Representative whole body luciferase images from each treatment group are shown.

Figure S1

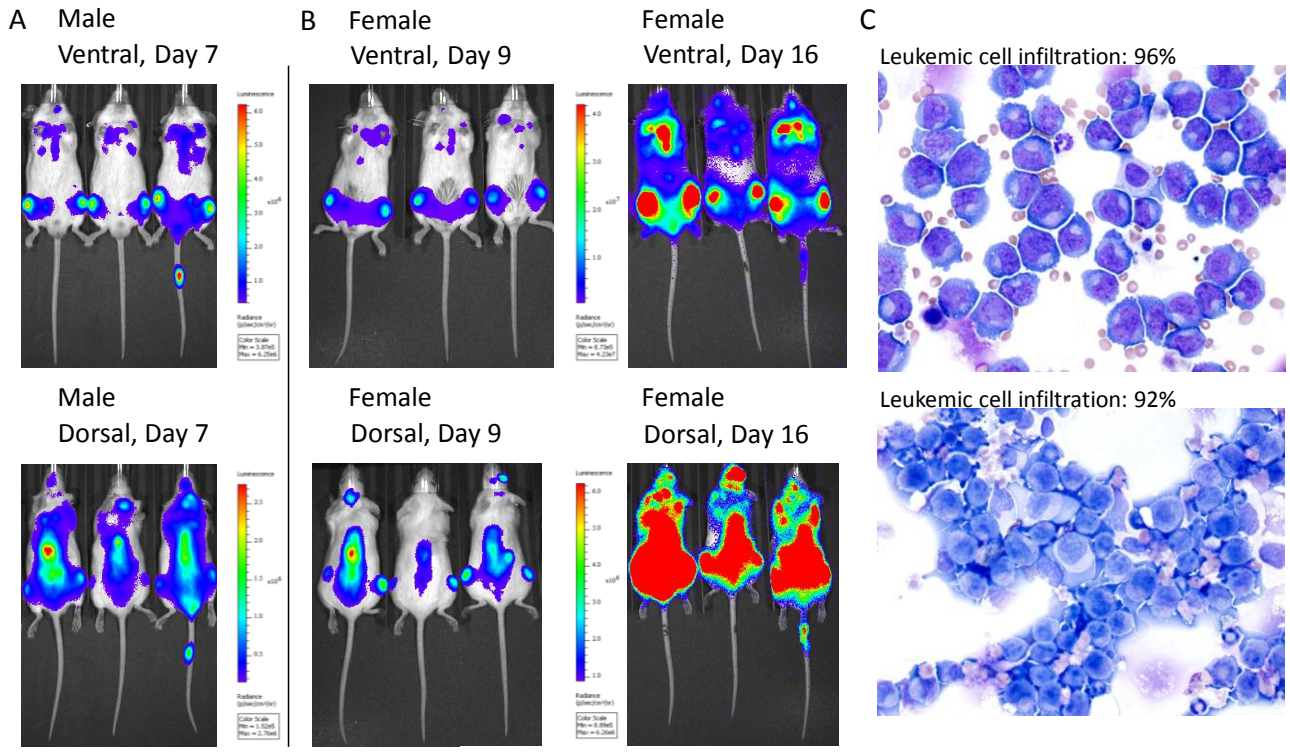

A
Male
Ventral, Day 7
B
Female
Ventral, Day 9
Female
Ventral, Day 16
C
Leukemic cell infiltration: 96%
Male
Dorsal, Day 7
Female
Dorsal, Day 9
Female
Dorsal, Day 16
Leukemic cell infiltration: 92%

Figure S2

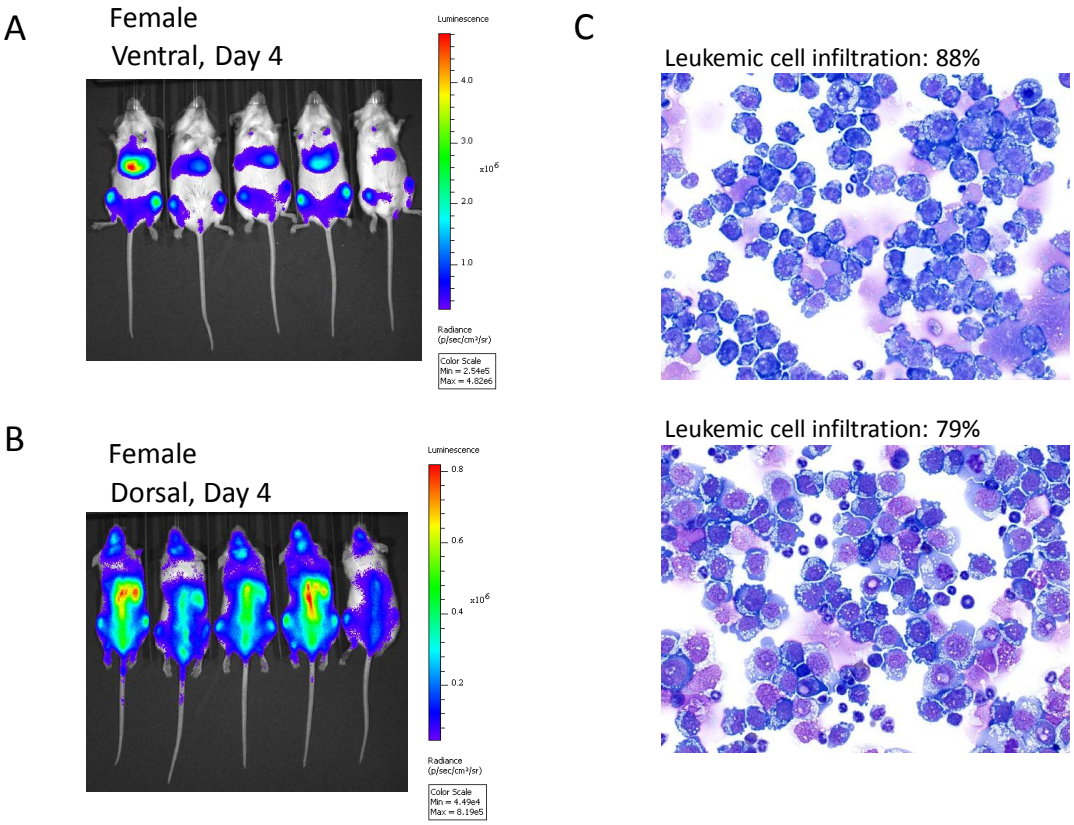

A
Female
Ventral, Day 4
Luminescence
4.0
3.0
x10^6
2.0
1.0
Radiance (p/sec/cm²/sr)
Color Scale
Min = 2.54e5
Max = 4.62e6
B
Female
Dorsal, Day 4
Luminescence
0.8
0.6
x10^6
0.4
0.2
Radiance (p/sec/cm²/sr)
Color Scale
Min = 4.49e4
Max = 8.19e5
C
Leukemic cell infiltration: 88%
Leukemic cell infiltration: 79%

Figure S3

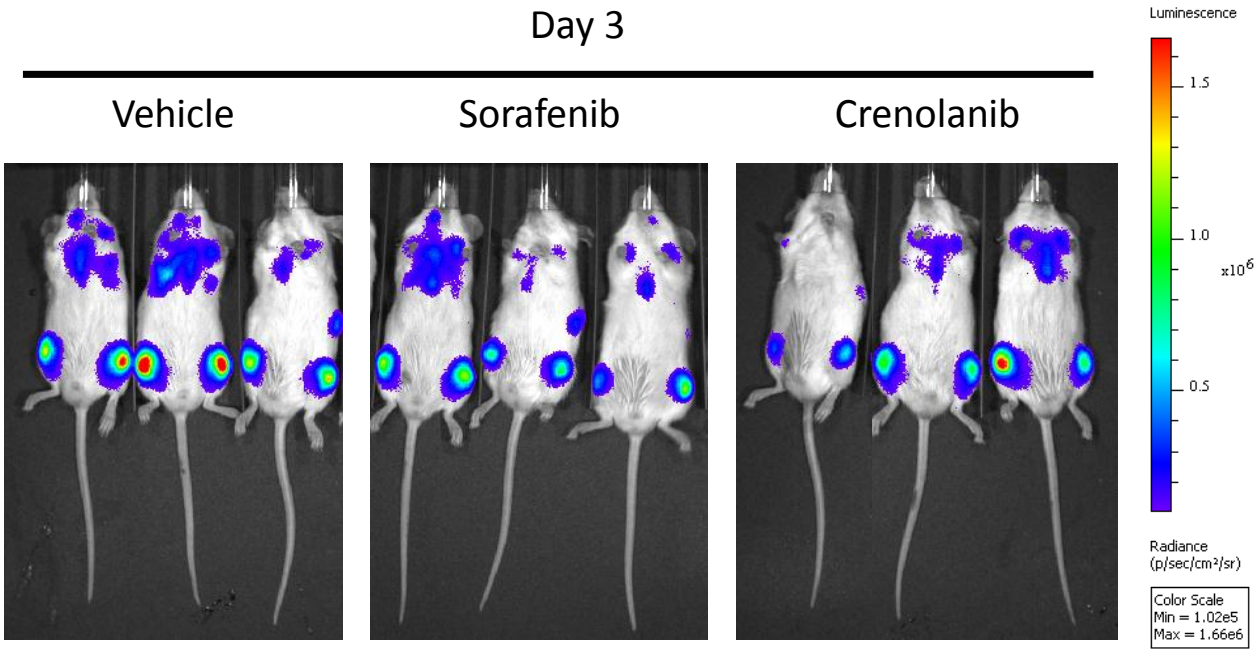

Day 3
Vehicle
Sorafenib
Crenolanib
Luminescence
1.5
1.0
x10^6
0.5
Radiance
(p/sec/cm²/sr)
Color Scale
Min = 1.02e5
Max = 1.66e6

Figure S4

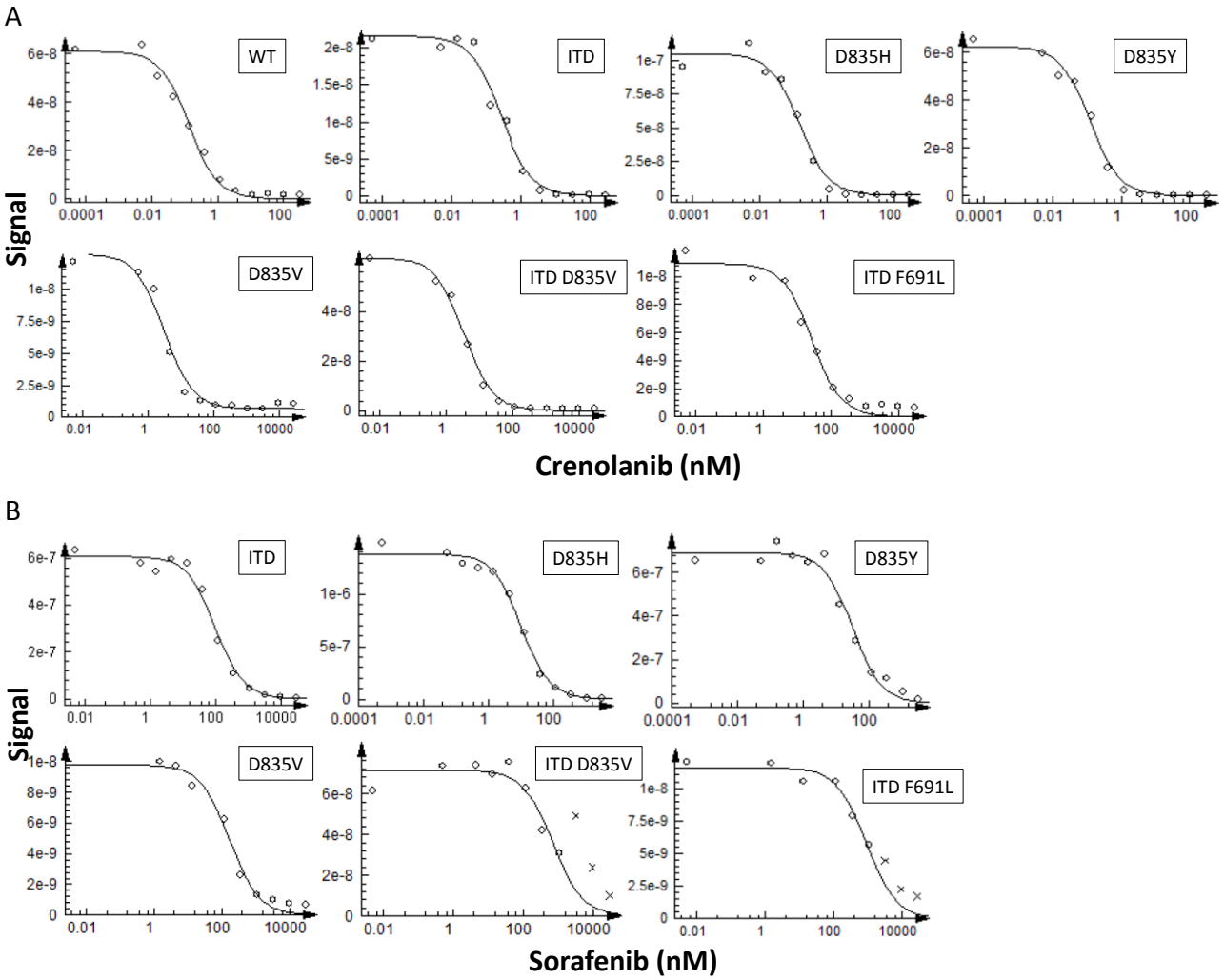

A
WT
ITD
D835H
D835Y
D835V
ITD D835V
ITD F691L
Signal
Crenolanib (nM)
B
ITD
D835H
D835Y
D835V
ITD D835V
ITD F691L
Signal
Sorafenib (nM)

Figure S5

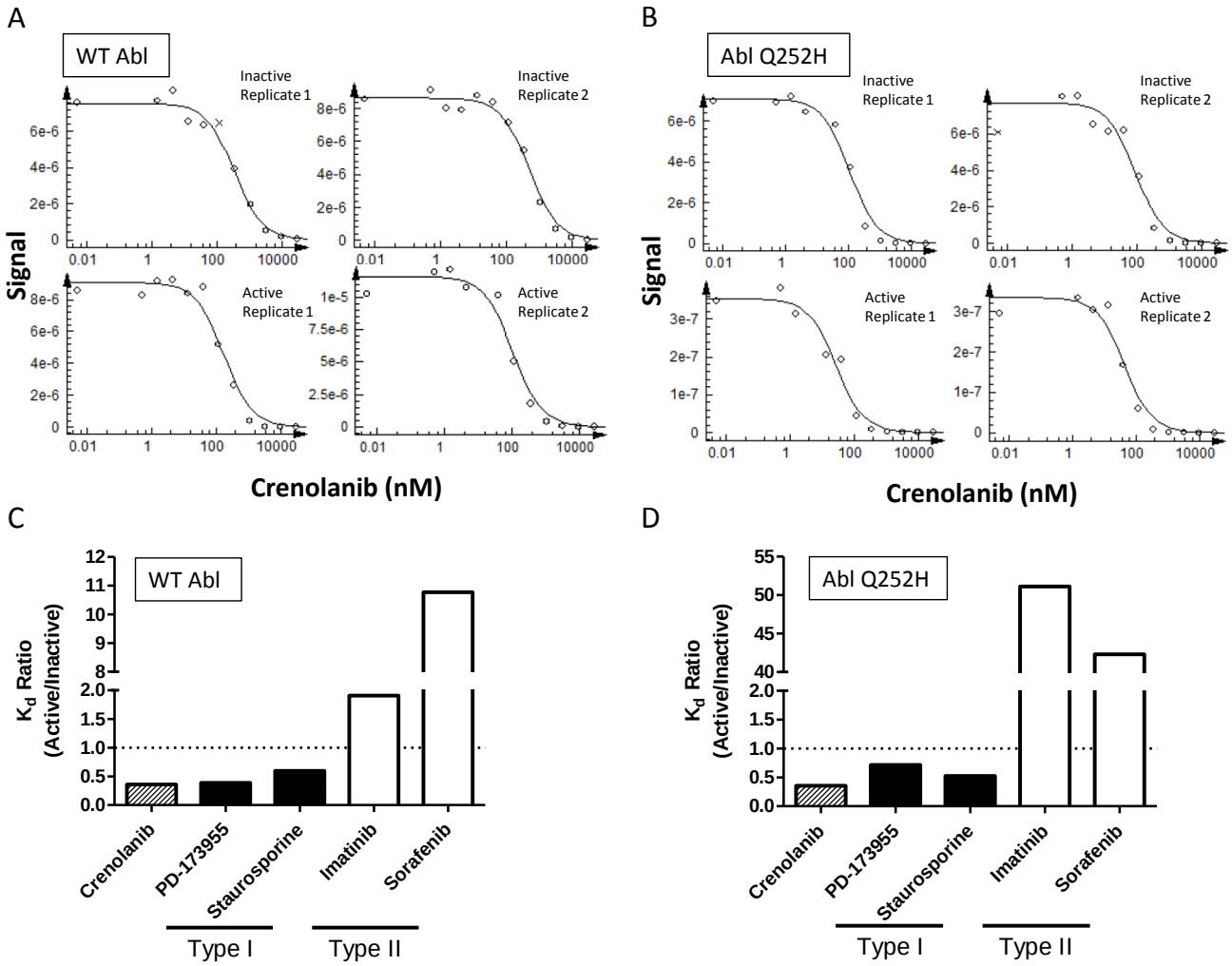

A
WT Abl
Inactive Replicate 1
Inactive Replicate 2
Active Replicate 1
Active Replicate 2
Signal
Crenolanib (nM)
B
Abl Q252H
Inactive Replicate 1
Inactive Replicate 2
Active Replicate 1
Active Replicate 2
Signal
Crenolanib (nM)
C
WT Abl
Kd Ratio (Active/Inactive)
Crenolanib
PD-173955
Staurosporine
Imatinib
Sorafenib
Type I
Type II
D
Abl Q252H
Kd Ratio (Active/Inactive)
Crenolanib
PD-173955
Staurosporine
Imatinib
Sorafenib
Type I
Type II

Figure S6

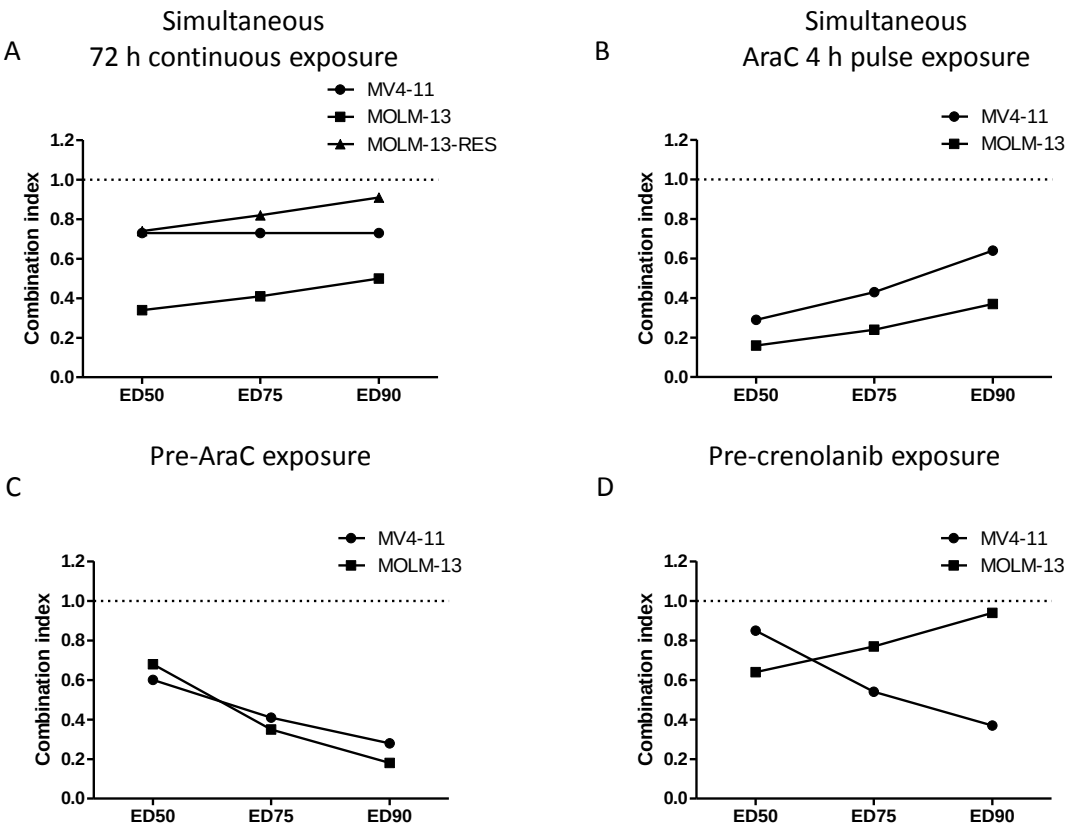

A
Simultaneous
72 h continuous exposure
MV4-11
MOLM-13
MOLM-13-RES
Combination index
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ED50
ED75
ED90
B
Simultaneous
AraC 4 h pulse exposure
MV4-11
MOLM-13
Combination index
C
Pre-AraC exposure
MV4-11
MOLM-13
Combination index
D
Pre-crenolanib exposure
MV4-11
MOLM-13
Combination index

Figure S7

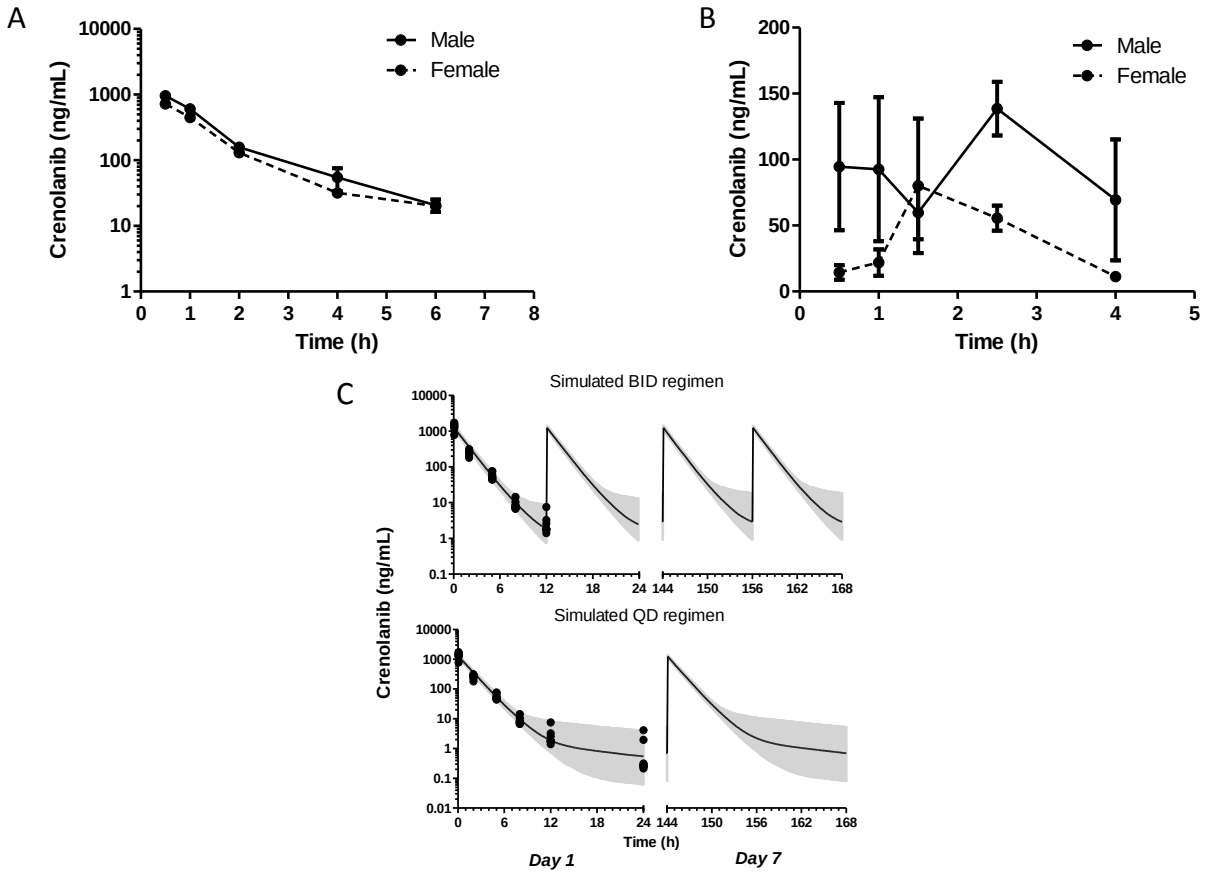

A
Crenolanib (ng/mL)
Time (h)
Male
Female
B
Crenolanib (ng/mL)
Time (h)
Male
Female
C
Simulated BID regimen
Simulated QD regimen
Crenolanib (ng/mL)
Time (h)
Day 1
Day 7

Figure S8

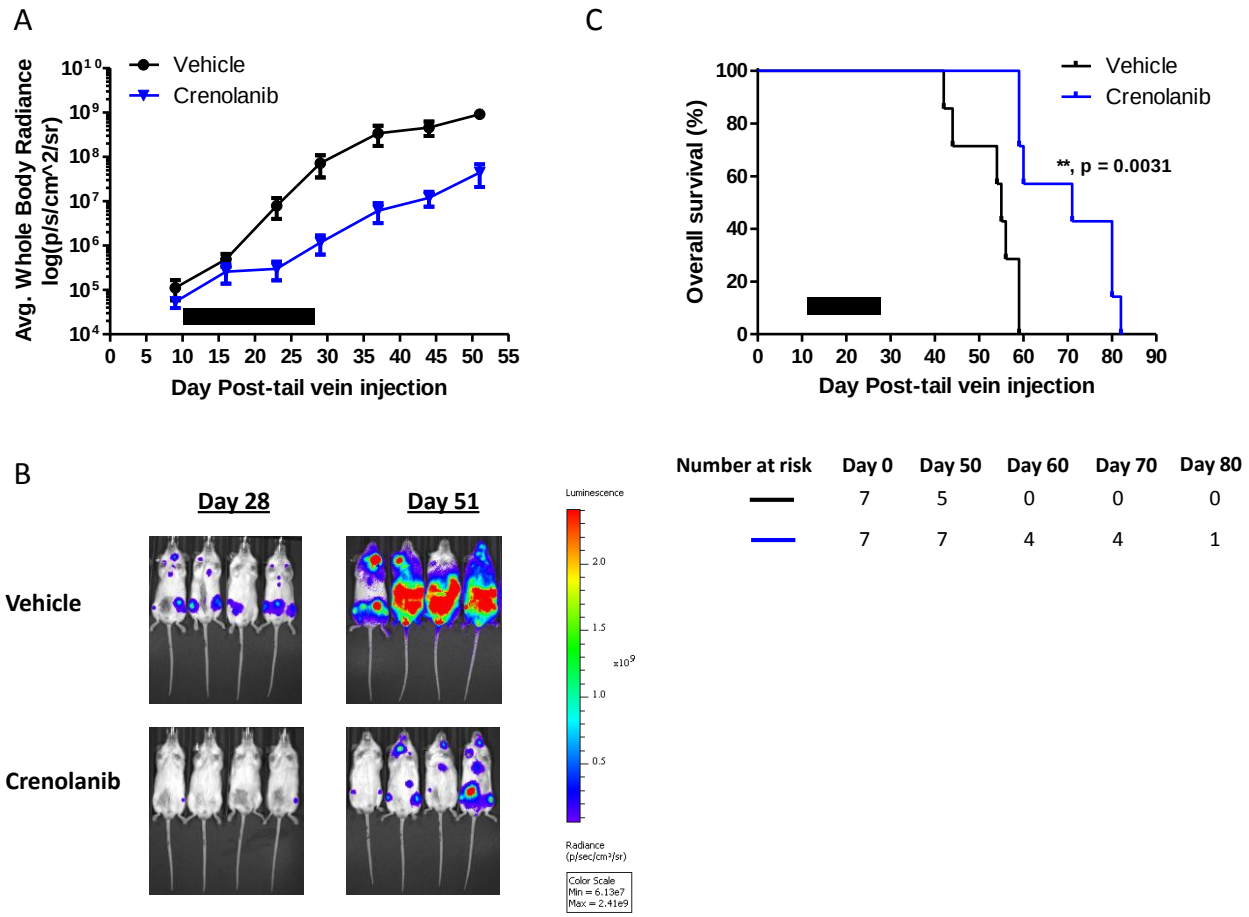

A
Vehicle
Crenolanib
Avg. Whole Body Radiance log(p/s/cm^2/sr)
Day Post-tail vein injection
C
Vehicle
Crenolanib
Overall survival (%)
**, p = 0.0031
Day Post-tail vein injection
Number at risk
Day 0
Day 50
Day 60
Day 70
Day 80
7
5
0
0
0
7
7
4
4
1
B
Day 28
Day 51
Vehicle
Crenolanib
Luminescence
Radiance (p/sec/cm²/sr)
Color Scale
Min = 6.13e7
Max = 2.41e9

Figure S9

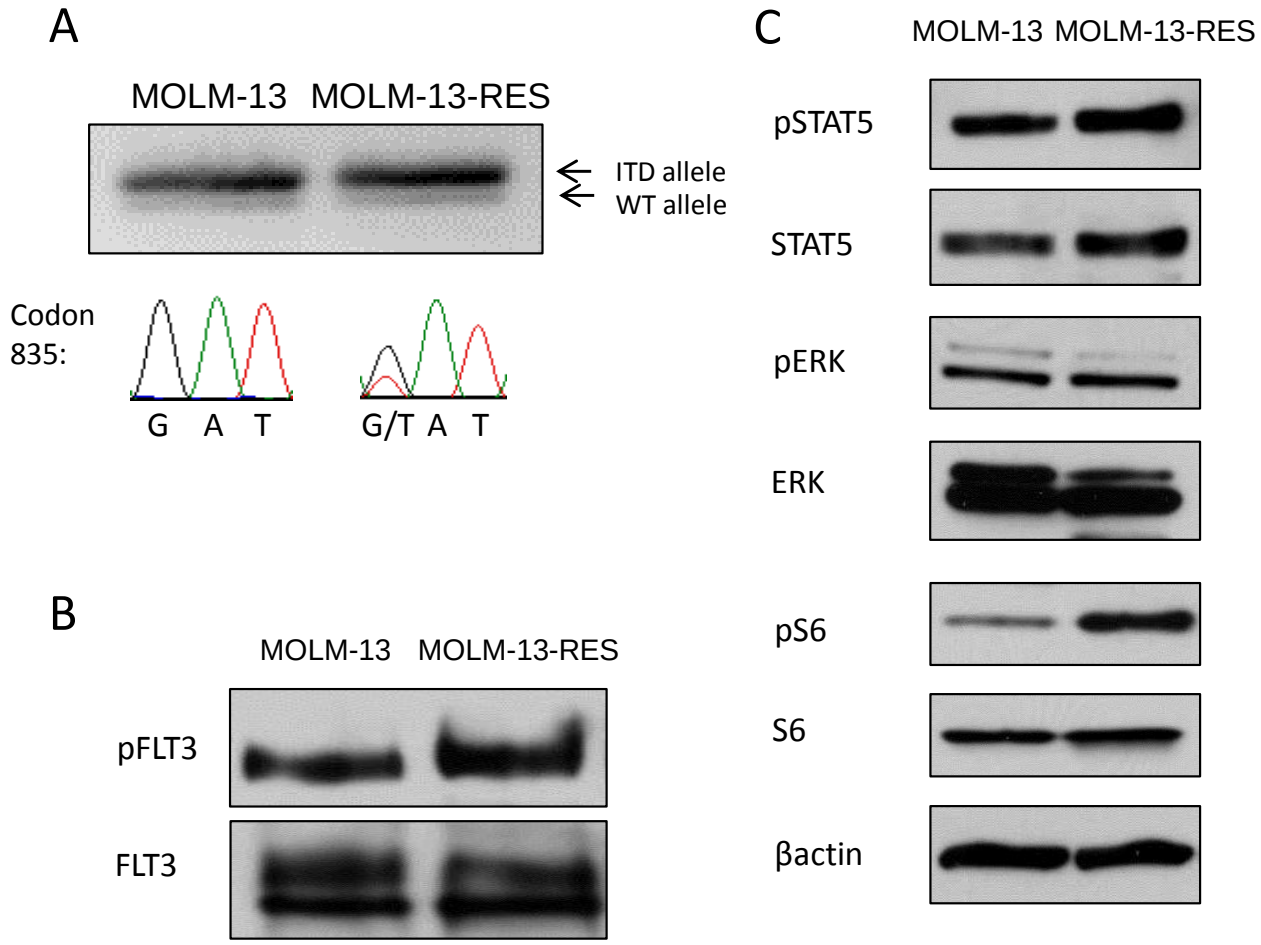
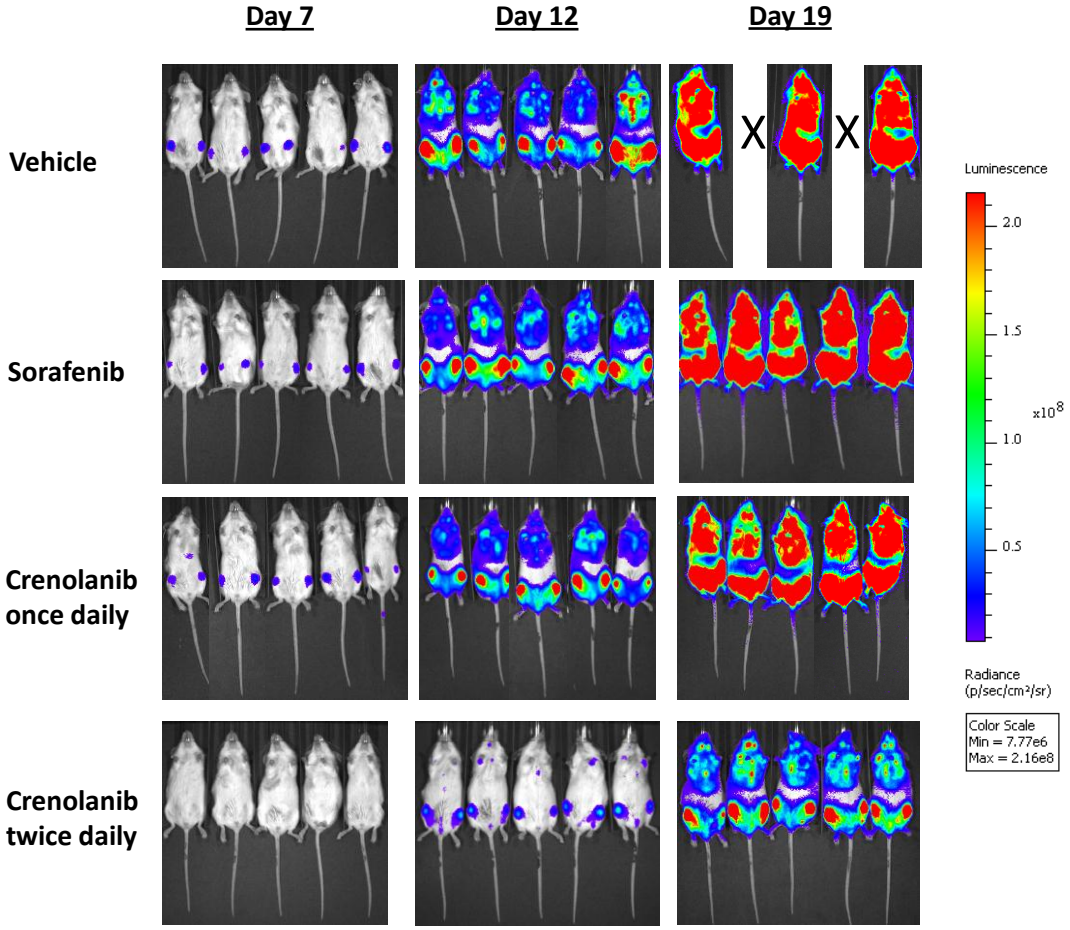

A
MOLM-13
MOLM-13-RES
ITD allele
WT allele
Codon 835:
G A T
G/T A T
B
MOLM-13
MOLM-13-RES
pFLT3
FLT3
C
MOLM-13
MOLM-13-RES
pSTAT5
STAT5
pERK
ERK
pS6
S6
βactin

Figure S10


Day 7
Day 12
Day 19
Vehicle
Sorafenib
Crenolanib once daily
Crenolanib twice daily
X
X
Luminescence
2.0
1.5
x10^8
1.0
0.5
Radiance (p/sec/cm²/sr)
Color Scale
Min = 7.77e6
Max = 2.16e8