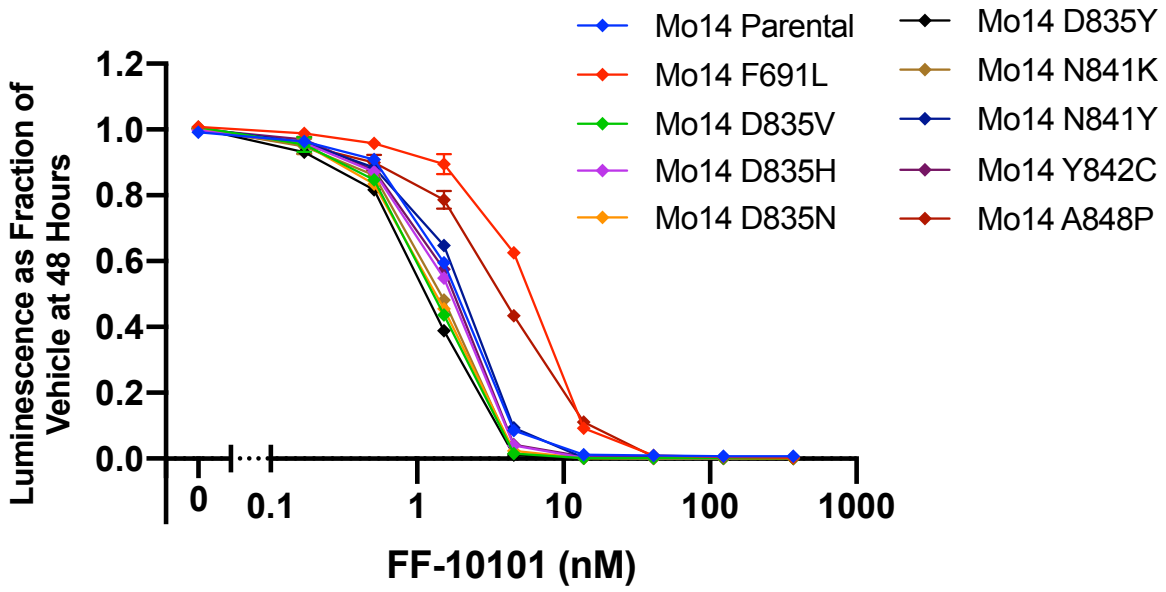
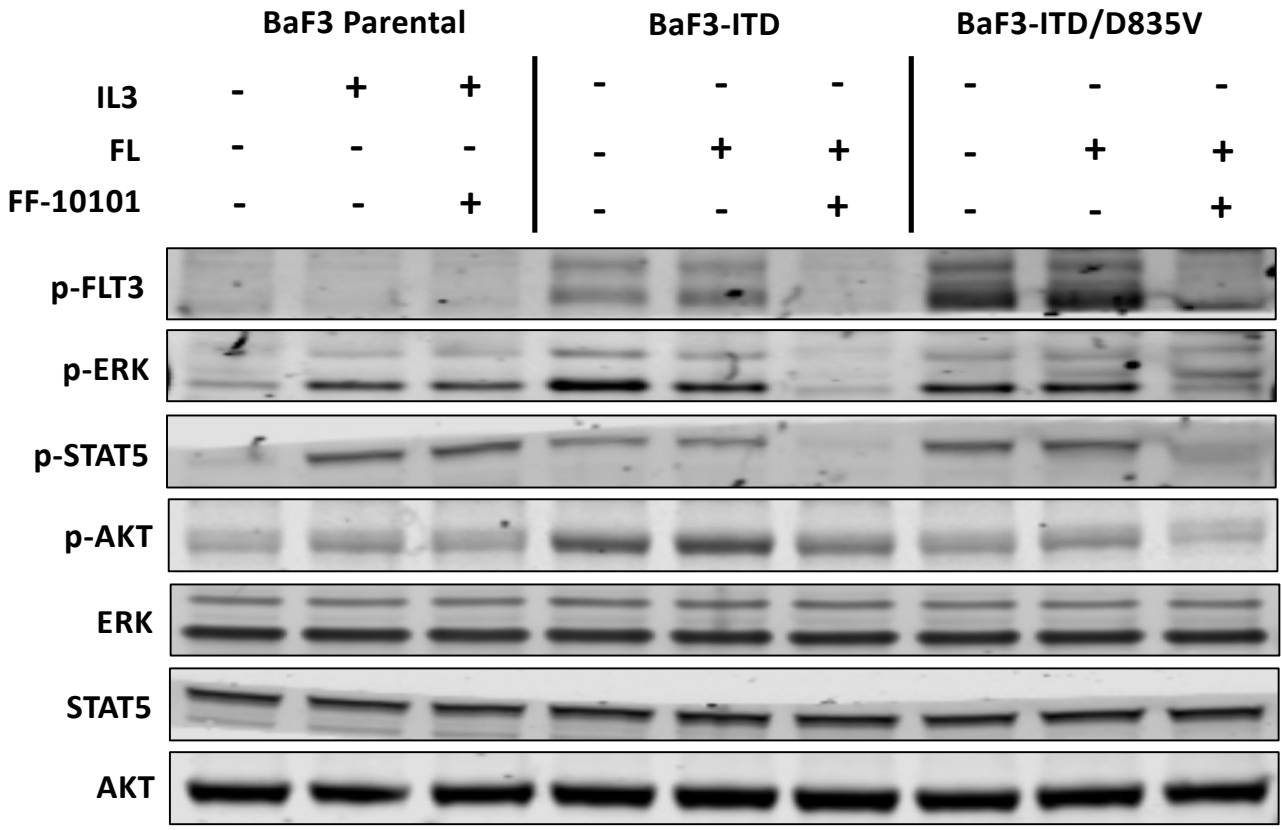
Supplementary Figure 1



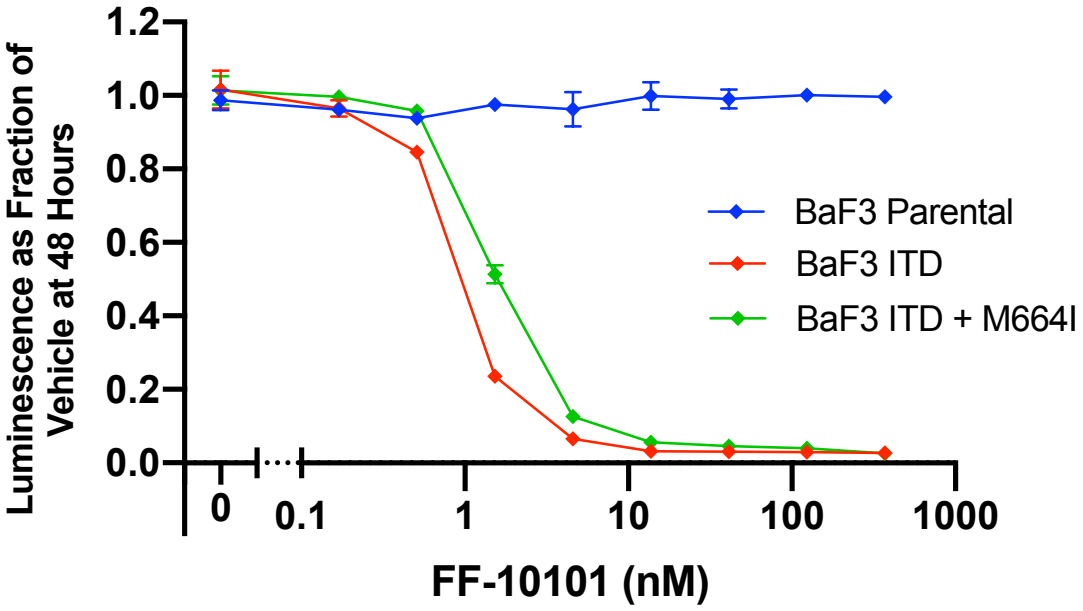
Supplementary Figure 1. FF-10101 exhibits activity against a subset of clinically described FLT3 TKD mutations that confer resistance to type I and II FLT3 inhibitors. 48-hour dose response of FF-10101 against MOLM-14 *FLT3*-ITD+ cells harboring indicated secondary TKD mutations. Error bars represent standard deviation of three technical replicates in a single experiment representative of three independent experiments.

Supplementary Figure 2



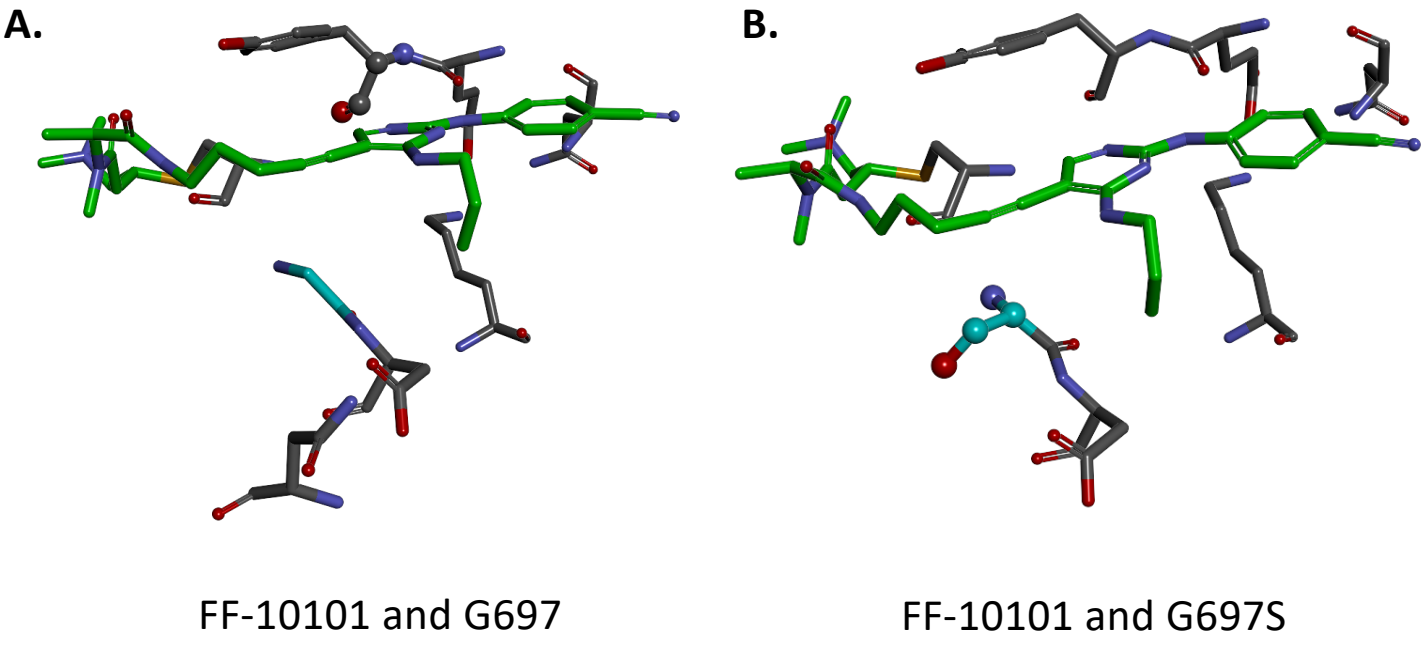
Supplementary Figure 2. FF-10101 inhibition of ERK signaling occurs predominantly through inhibition of FLT3. Assessment of FLT3, ERK, STAT5, and AKT phosphorylation by immunoprecipitation and western blot in Ba/F3 parental, Ba/F3-ITD, and Ba/F3-ITD/D835V cells. Cells were suspended in complete RPMI media supplemented with either IL3 (100 ng/mL) or FL (FLT3 Ligand, 10 ng/mL) where indicated and treated with either vehicle or FF-10101 (100 nM) for 1 hour.

Supplementary Figure 3



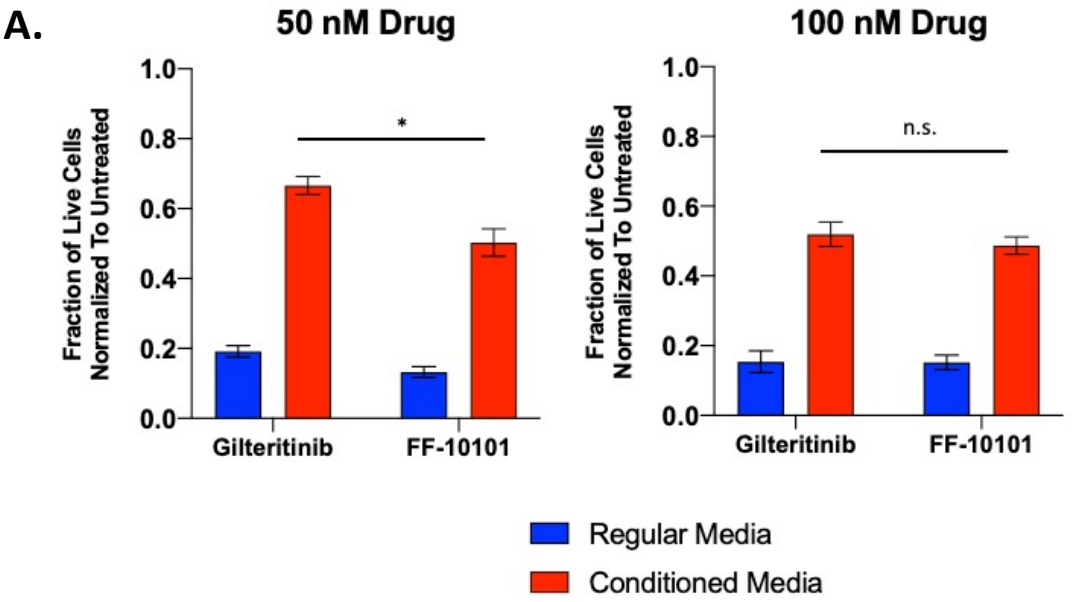
Supplementary Figure 3. FLT3 M664I does not induce resistance to FF-10101. 48-hour dose-response of FF-10101 against Ba/F3-ITD cells expressing *FLT3* M664I. Error bars represent standard deviation of three technical replicates in a single experiment representative of three independent experiments.

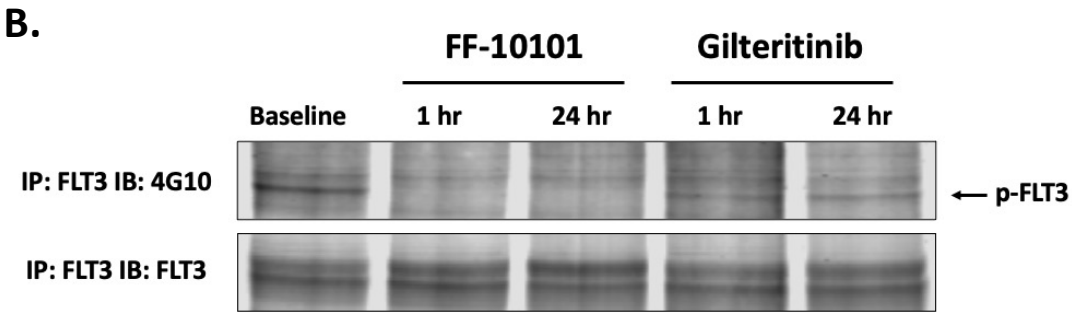
Supplementary Figure 4



Supplementary Figure 4. Structural modeling and chemical structures (A) Structural model of FF-10101 against FLT3 G697 and (B) FLT3 G697S. (C) Chemical structures of FF-10101 and FLA-9430

Supplementary Figure 5





Supplementary Figure 5. Comparing FF-10101 and gilteritinib in MV4-11 cells cultured in regular or HS5 conditioned media. (A) Apoptosis measured in MV4-11 cells following 72-hour treatment with 50 nM (left) and 100 nM (right) gilteritinib or FF-10101 in regular complete RPMI media or HS5 conditioned media. Live cells negative for caspase-3 were normalized to untreated control cells. Results represent aggregate data from two independent experiments each with two technical replicates. Error bars represent SD and statistical analysis performed using paired t-test. *, P=0.0189. (B) Assessment of FLT3 phosphorylation by immunoprecipitation and western blot in MOLM-14 cells treated with FF-10101 (50 nM) or gilteritinib (50 nM) for 1 and 24-hours in HS5 conditioned media.

Supplementary Table 1

Patient/Sample	Age/Gender	Mutations	Cytogenetics
AML #1	61/Male	1. FLT3-ITD (AR not reported) 2. NPM1 W288fs (VAF 38.6%) 3. DNMT3A A380E (VUS, VAF 45.8%)	Normal Male Karyotype
AML #2	69/Female	1. FLT3 D835V (VAF 91.2%) 2. NPM1 W288fs (VAF 39.4%) 3. WT1 S381fs (45.1%)	Normal Female Karyotype
AML #3	80/Female	1. FLT3-ITD (AR 0.79) 2. DNMT3A F414Lfs*7 (VAF 44.9%) 3. IDH1 R132C (VAF 44.6%) 4. RUNX1 R320* (VAF 43.1%) 5. DNMT3A R882H (VAF 43.9%)	Normal Female Karyotype