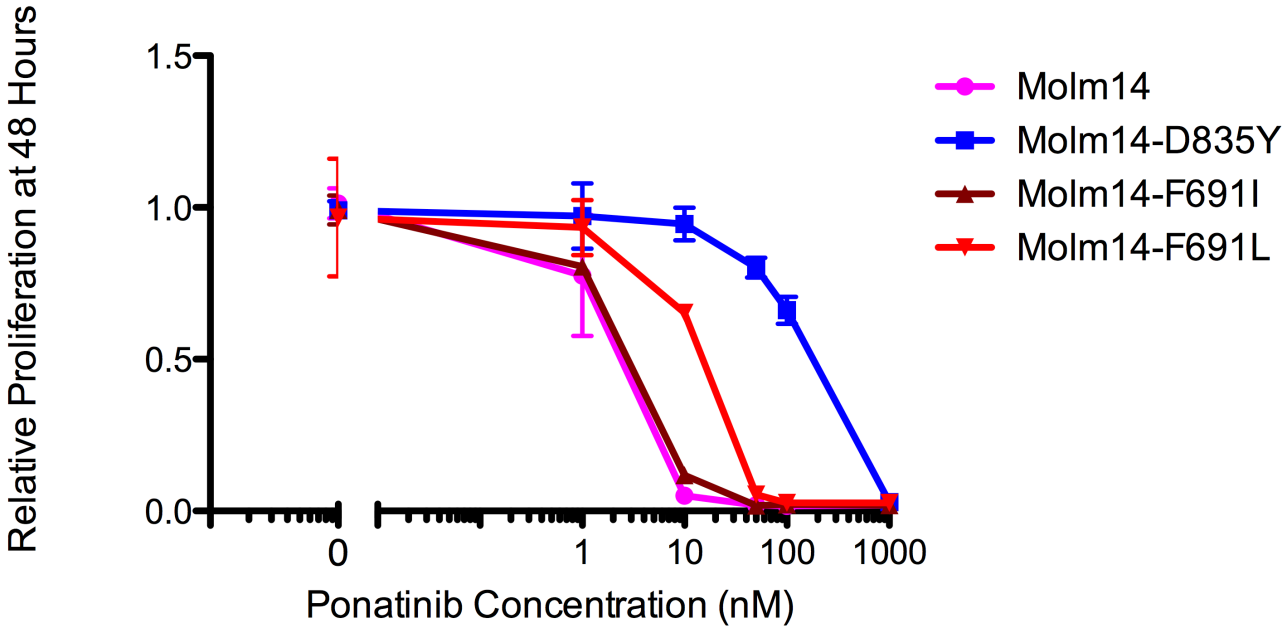
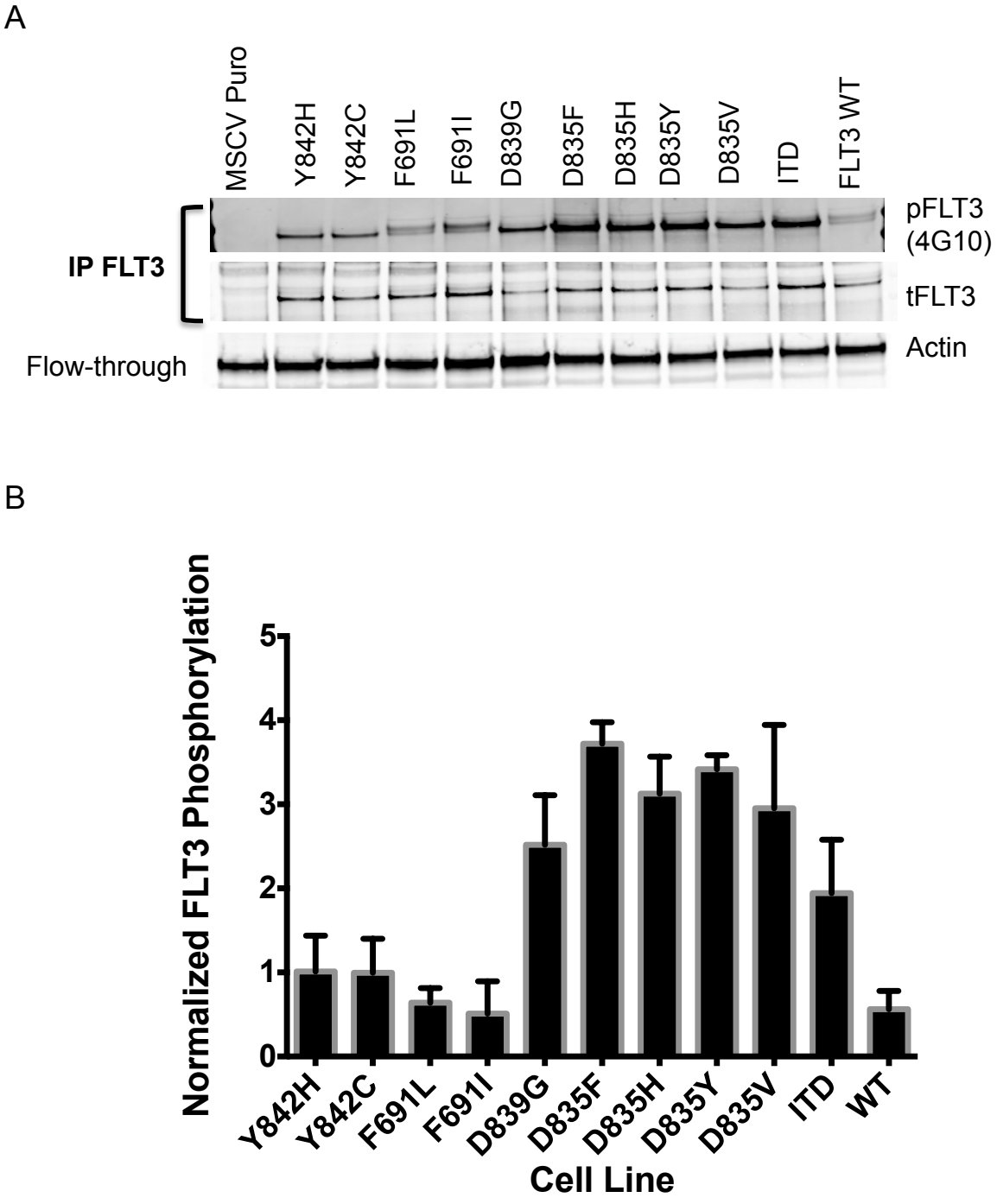
Supplemental Figure 1



Supplemental Figure 1. Activity of Ponatinib Against Molm14 cells Expressing FLT3-ITD Kinase Domain Mutations *in vitro*. Relative proliferation of Molm14 cells expressing FLT3-ITD mutant isoforms after 48 hours in various concentrations of ponatinib (error bars represent s.d. of triplicates from the same experiment).

Supplemental Figure 2



Supplemental Figure 2. Autophosphorylation of FLT3 Mutant Isoforms in the Absence of ITD. (A) FLT3 mutant isoforms are immunoprecipitated after transient expression in 293T cells and subjected to Western Blot with anti-phosphotyrosine (4G10) and total anti-FLT3 antibodies. Results shown are representative of triplicate experiments. (B) Quantitation of FLT3 autophosphorylation from FLT3 mutant isoforms after immunoprecipation from transfected 293T cells. Error bars represent standard deviation of triplicate experiments.

Supplemental Methods

Generation of AC220 Resistant Molm14 Cell Lines. Molm14 cells were cultured in increasing concentrations of AC220 in RPMI 1640 + 10% FCS. Exponentially growing cells were plated in 6-well dishes in 0.5nM AC220 at a density of $2x10^5$ cells/mL. AC220 dose was escalated when cells reached a confluency of ~$1x10^6$ cells/mL. Cells were considered resistant when they were capable of proliferating in 20nM AC220. RNA was isolated from resistant cell lines by Trizol extraction and cDNA was generated by SuperScript II reverse transcription (Invitrogen) according to manufacturer's recommendations. The *FLT3* KD was amplified and sequenced as described above.

Immunoprecipitation. 293T cells were plated in 6 cm plates and transfected with MSCVpuroFLT3-ITD plasmid containing FLT3 mutations of interest using Lipofectamine 2000 (Invitrogen) per manufacturer's protocol. After 48 hours, cells were washed with PBS and re-plated in DMEM + 0.1% FCS for 2 hours. Following this, cells were again washed in PMS, collected, lysed and quantitated using BCA assay. FLT3 was immunoprecipitated from 400ug of total protein using anti-FLT3 S18 antibody (Santa Cruz) and subjected to Western immunoblot with 4G10 antibody (Millipore). Visualized bands were quantitated by densitometry and FLT3 phosphorylation was normalized to the total FLT3 signal.