**Supplementary Figures**

**Figure S1. Quizartinib, sorafenib, crenolanib and gilteritinib cytotoxicity in cell lines with FLT3-ITD or FLT3-WT.** Exponentially growing cells were treated with 0-1 M quizartinib, sorafenib, crenolanib or gilteritinib for 96 hours. Cytotoxicity assays were performed as detailed in Materials and Methods. Means + S.E.M. of triplicate experiments are shown. IC50 concentrations are shown in the inserted table. Of note, Ba/F3-WT and 32D/WT cells were plated in medium with 10 ng/ml interleukin-3.

**Figure S2.** **Enhanced apoptosis of Ba/F3-ITD and 32D/ITD cells treated with FLT3 inhibitors at serial concentrations in the presence of AZD1208.** Ba/F3-ITD and 32D/ITD cells were treated in triplicate with quizartinib, sorafenib, crenolanib or gilteritinib at increasing concentrations in the absence and presence of 1 M AZD1208. Percentages of annexin V-positive cells were measured at 48 hours and compared by 2-way ANOVA.

**Figure S3. Pim kinase and FLT3 inhibition does not enhance induction of apoptosis of cells with FLT3-WT. A.** **Combined treatment with Pim and FLT3 inhibitors does not abrogate growth of Ba/F3-WT cells.** Ba/F3-WT cells were cultured at 1x105/ml with AZD1208 at 1 µM and/or quizartinib at 1 nM (left) or 1 M (right), or DMSO control. Cell counts measured at 24, 48 and 72 hours were normalized to 0-hour control. Line graphs represent means + S.E.M. of triplicate values. **B.** **Pim and FLT3 inhibitor co-treatment does not increase percentage of Ba/F3-WT cells in sub-G1 phase.** Ba/F3-WT cells were cultured as above. Cells were collected at serial time points and fixed overnight, and cell cycle was analyzed by flow cytometry. Representative 72-hour data from triplicate experiments are shown. **C.** **AZD1208 and quizartinib co-treatment does not increase annexin V labeling of cells with FLT3-WT.** Ba/F3-WT and 32D/WT cells were cultured with AZD1208 and/or 1 nM or 1 M quizartinib. Cells were stained with annexin V/PI and analyzed by flow cytometry, and percentages of annexin V-positive cells were compared by 2-way ANOVA. Means + S.E.M. of triplicate values are shown. **D.** **AZD1208 and quizartinib co-treatment does not decrease mitochondrial membrane potential in Ba/F3-WT cells.** Ba/F3-WT cells were cultured as above. Mitochondrial membrane potential measured by flow cytometry as increased red fluorescence of JC-1 dye was compared by 2-way ANOVA. Means + S.E.M. of triplicate values are represented in bar graphs.

**Figure S4. Combined AZD1208 and quizartinib treatment does not abrogate growth of KG-1a cells, expressing FLT3-WT, in a xenograft model.** Mice injected subcutaneously with KG-1a cells were treated with 30 mg/kg AZD1208 and/or 1 mg/kg quizartinib, or with vehicle control, and tumor volumes at serial time points were measured and graphed. Means + S.E.M. values are shown. Minimal, and similar, decrease in growth was seen with AZD1208 alone and combined with quizartinib.

**Figure S5. AZD1208 and quizartinib co-treatment was well tolerated in the orthotopic model.** Mice were weighed three days per week. Minimal weight loss was observed.

**Figure S6. AZD1208 and quizartinib co-treatment does not increase cellular ROS generation, but increases mitochondrial ROS generation.** Ba/F3-ITD cells were treated with 1 M AZD1208 and/or 1 nM quizartinib, DMSO control, or H2O2 as a positive control. **A. Cellular ROS.** Labeling with the redox-sensitive dye CM-H2DCFDA was measured. Increased cellular ROS generation was not seen with AZD1208 and quizartinib co-treatment. **B. Mitochondrial ROS.** Percentages of cells labeled with MitoPY1 were measured. Increased mitochondrial ROS was seen with AZD1208 and quizartinib co-treatment.

**Figure S7.** **Post-transcriptional regulation of Mcl-1 expression.** **A.** **AZD1208 and quizartinib co-treatment does not alter expression of miR29b, relative to treatment with quizartinib alone.** Expression of miR-29b in Ba/F3-ITD cells treated with AZD1208 and/or quizartinib, or DMSO control, was measured by RT-qPCR.Means + S.E.M. of triplicate values are shown. **B. AZD1208 and quizartinib co-treatment does not selectively decrease polysomal association of Mcl-1 mRNA.** Ba/F3-ITD cells were collected after 24-hour treatment with quizartinib and/or AZD1208, or DMSO control, and a polysome profile was created. A lower polysomal RNA (P) to uninitiated/untranslated (U) RNA ratio was observed with the combination treatment (top panel), and RT-qPCR revealed decreased association of GAPDH mRNA (middle panel), but not Mcl-1 mRNA (lower panel), with polysomes.

**Figure S8. The USP9X inhibitor WP1130 enhances induction of apoptosis of Ba/F3-ITD and MV4-11 cells by quizartinib in a concentration-dependent manner.** Cells were cultured for 48 hours with and without 1 nM quizartinib with 0, 2, 3, 3.5 and 4 M WP1130, then stained with annexin V/PI and analyzed by flow cytometry. Means + S.E.M. of triplicate values are shown. Percentages of annexin V-positive cells were compared by 2-way ANOVA.