Supplementary Information

Supplementary Methods

Cell Impedance Assay:

For assessment of relative cell growth by cell electrode impedance response, cells (1E5) were cultured in 96 well gold microelectrode E-plates (Roche Diagnostics GmbH, Mannheim, Germany) for 44 hours prior to drug treatment. Media exchange with MEKi or PI3Ki were added at the indicated doses, at which time impedance measurements were normalized. Real-time measurements were taken at 10 minute intervals using the Xcelligence System (Roche Diagnostics, Mannheim, Germany). Data were generated as a dimensionless parameter called the Cell Index (CI; derived as a relative change in measured electrical impedance). Data are presented as the slope of the resultant impedance calculated for the duration of the treatment. RTCA Software 1.2 was used for both data acquisition and data analysis.

Cell Death Assay:

To determine the induction of cell death, as measured by the determination of cytoplasmic histone-associated-DNA fragments, the Cell Death Detection Elisa-plus assay (Roche Diagnostics) was employed according to manufactures' instructions. Briefly, cells were transfected with non-targeting siRNA or siRNA targeting BIM. Two days later, cells were treated with MEKi (10 nM) and PI3Ki (100 nM) at specified concentrations. After 48 hours, 20 ul of cell lysate was plated in streptavidin-coated microplates, incubated with 80ul of immunoreagent (antihistone-biotin, anti-DNA-

peroxidase), rinsed with incubation buffer, then incubated with ABTS solution. Following the addition of ABTS stop solution fluorometric reading (405 nm) were taken and compared to baseline.

Supplemental Figures

Supplemental Figure 1. Baseline protein expression of phosphorylated MAPK1/2, total MAPK1/2, phosphorylated AKT, total AKT in uveal melanoma cell lines.

Supplemental Figure 2. Uveal melanoma cells (UPMD1 and OMM1) were treated with increasing concentrations of MEKi (0 – 1000 nM) or PI3Ki (0 – 1000 nM) x 4hours. Western blot analysis of phosphorylated MAPK1/2, total MAPK2, phosphorylated AKT, and total AKT following treatment are presented.

Supplemental Figure 3. Uveal melanoma cells were treated with increasing doses of MEKi or PI3Ki and impedance measurements ascertained at 10 min intervals. All impedance values were normalized to the time immediately preceding treatment, and effects of treatment were normalized to the effect of no treatment (horizontal green line). Vertical bars represent the magnitude of impedance measurement slope over the course of treatment (red = negative, blue = positive). Asterisks demarcate experiments in which positive slope values were derived.

Supplemental Figure 4. Cell death assessment following combination MEKi + PI3Ki treatment in BIM siRNA targeted uveal melanoma cells. In the lower panels, western blot analysis of cells transfected with two distinct siRNAs targeting BIM. The effect of BIM siRNA targeting was compared to a non-targeting siRNA control. In the upper panels, BIM siRNA transfection followed by treatment with combination MEKi (10nM) and PI3Ki (100nM). Histone-associated-DNA fragments were measured by ELISA assay.

Supplemental Figure 5. Effect of MEKi and/or PI3Ki treatment on p-cjun protein expression. Cells were treated with MEKi alone, PI3Ki alone or the combination of MEKi + PI3Ki for 4 hours followed by western blot analysis.