**SUPPLEMENTARY MATERIALS and METHODS**

**β-galactosidase Staining**

Cells were seeded in 35 mm dishes and treated 24 hours later with ribociclib, binimetinib, or with both agents. After 6 days of treatment, cells were fixed and stained for β-galactosidase activity according to the manufacturer’s protocol (Cell Signaling, #9608). Imaging was performed under a 10x/N.A.0.40 objective of a Leica DM400B microscope using a SPOT RT3 slider camera (SPOT Imaging). At least two representative images were acquired per treatment, and although brightness levels were uniformly enhanced using ImageJ (NIH), the images were otherwise unaltered. All experiments were repeated at least twice.

**Real-Time Cell Imaging**

The NBL-S neuroblastoma cell line was seeded into a 6-well plate and treated with 1/2x IC50 ribociclib, binimetinib, or the combination of ribociclib and binimetinib, or with 50 nM SN-38 (Santa Cruz), the active metabolite of irinotecan. Cells were incubated for up to 6 days (Zeiss Axio Observer.Z1 microscope outfitted with a Zeiss Axiocam MRm camera) during which time identical fields of cells were imaged at 10x magnification for each treatment group at three-hour intervals. A selection of still images were compiled into **Figure 4D**.

**SUPPLEMENTARY ONLINE TABLES**

**Table S1.** Specific mutations of genes listed in Table 1.

**Table S2.** Foundation Medicine sequencing calls of cancer-specific gene panel.

**Table S3.** Gene lists corresponding to microarray analysis described in Figures 1A and 1B: **(A)** binimetinib and **(B)** ribociclib. Significant genes (in bold type) are defined as having an adjusted p-value of 0.25 or less.

**SUPPLEMENTARY FIGURES**

**Supplementary Figure S1. Neuroblastoma cell lines are sensitive to binimetinib. (A)** growth inhibition curves based on RT-CES analysis of neuroblastoma cell lines (N=22) treated with a 4-log dose range of single agent binimetinib (\*cell line extremely resistant, curve not shown); **(B)** ERK phosphorylation is rapidly reduced in sensitive lines treated with binimetinib (500 nM); **(C)** immunoblots for p21 induction following increasing doses of binimetinib (1-10,000 nM) for 24h (treatment with etoposide was used a positive control for p21 induction); and **(D)** the binimetinib-sensitive cell line NBL-S demonstrates a G1 arrest cell cycle profile following treatment (24h) with binimetinib, in contrast to the resistant cell line IMR-05.

**Supplementary Figure S2. Gene expression profiling of human neuroblastoma cell lines with regards to relative sensitivity to MEK1/2 and CDK4/6 inhibition. (A and B)** k-means clustering of cell line IC50 values to identify associations between the presence of relevant genomic alterations and sensitivity to binimetinib and ribociclib: **(A)** *ALK* mutations, and **(B)** *TP53* mutations.

**Supplementary Figure S3. Combined MEK and CDK4/6 inhibition does not induce significant levels of apoptosis or senescence. (A)** immunoblot analysis performed for cleaved PARP (SN-38 treatment serves as a positive control); **(B)** cell cycle analysis was performed (48 hour treatment) to identify sub-G1 content (Q-VD-OPh peptide blocks caspase activity and identifies caspase-dependent sub-G1 populations); and **(C)** β-galactosidase staining was performed to identify senescent cells (6 day treatment).

**Supplementary Figure S4. G1 arrest induced by MEK1/2 and CDK4/6 inhibition is reversible.** Cells were treated (1x IC50 for 6 days), washed to remove drug and incubated for 3 days prior to cell cycle analysis.

**Supplementary Figure S5. Gene expression profiling of human neuroblastoma cell lines with regards to the synergy observed from combined binimetinib-ribociclib treatment. (A-D)** Clustering of synergyvalues (synergy > 1, non-synergy < 1) to identify associations between the presence of relevant genomic alterations and sensitivity to the binimetinib-ribociclib combination: **(A)** *RAS-MAPK* alterations, **(B)** *MYCN* amplification, **(C)** *TP53* mutations, and **(D)** *ALK* mutations.

**Figure S6. Neuroblastoma xenografts are sensitive to combined binimetinib-ribociclib treatment.** Xenografts were treated with vehicle, single agent binimetinib (3 mg/kg BID), single agent ribociclib (75 mg/kg QD), or the combination. Tumor volume measurements of individual mice and Kaplan-Meier survival curves from the following four neuroblastoma models are shown: **(A)** NB-EBc1, **(B)** NBL-S, **(C)** SK-N-BE(2)C, and **(D)** NGP.