Supplementary Figure Legends

Supplementary Figure 1. Cellular potency of GDC-0941. GDC-0941 was tested in a panel of cell lines in a 96-hour viability assay as measured by CellTiterGlo. The cell lines are plotted as described in Figure 1.

Supplementary Figure 2. Western blots of PD samples for single agent efficacy studies. Tumor lysates were analyzed by Western blot as described in the Methods for pharmacodynmic markers.

Supplementary Figure 3. Tumor cells are more sensitive than matched normal cells to combined MEK and PI3K inhibition. Three cancer cell lines and normal matched immortalized lines were treated with 1X and 4 X EC_{50} GDC-0973 (MEKi) and GDC-0941 (PI3Ki) as single agents or in combination and assayed for cell death using a Nucleasomal ELISA assay. 1X EC_{50} drug concentrations were 1.4 μM GDC-0973 and 2.5 μM GDC-0941 (H2122 and Hs888.Lu cells), 0.3 μM GDC-0973 and 2.5 μM GDC-0941 (Colo829 and Colo829BL), and 2.5 μM GDC-0973 and 0.15 μM GDC-0941 (Hs578T and Hs578Bst).

Supplementary Figure 4. In vitro combinatorial activity of GDC-0941 and GDC-0973 in melanoma cells. Melanoma cell lines were treated with GDC-0973 and GDC-0941 as single agents or in combination for 96 hours and assayed by Cell Titer Glo assay. The fraction of viable cells at EC_{50} drug concentration is shown. GDC-0973 EC_{50} values are indicated in Supplementary Table 2 and 2.5 μM GDC-0941 was used as 1X EC_{50} value for all cell lines.

Supplementary Figure 5. S6 and Bim are synergistically regulated by GDC-0973 and GDC-0941 in melanoma cell lines and tumor xenografts. A. A375, 624MEL and C32 cell lines were treated with GDC-0973 and GDC-0941 as single agents and in combination. Lysates were prepared after 24 hours of

treatment and analyzed by immunoblot. B. A2058 xenograft tumors were treated with 5 mg/kg GDC-0973 and 30 mg/kg GDC-0941 in combination for 4 days. Tumors were harvested at 2 hours after the final dose. Lysates were prepared and analyzed by immunoblot. C. A375 xenograft tumors were treated an analyzed as in B.

Supplementary Figure 6. Efficiency of RNAi-mediated knockdown of Bim. A. A2058 cells were treated with siRNA against Bim for 72 hours and lysates were analyzed by immunoblot for protein levels of Bim. B. A2058 cells were treated with individual siRNA oligonucleotides to Bim for 72 hours and subsequently treated for 24 hours with the indicated concentrations of GDC-0941 or GDC-0973 and analyzed by Cell Death Detection ELISA assay. Differences in cell death induction by GDC-0973 and GDC-0941 combination in the presence or absence of Bim were statistically significant ($p < 0.05$).

Supplementary Figure 7. Transient treatment of GDC-0973 + GDC-0941 increases cell death and decreases proliferation. A. A2058 cells were treated with a $1XEC_{50}$ concentration of GDC-0973 and GDC-0941 for 24 hours or a $4XEC_{50}$ concentration for 8 hours prior to exchanging media to remove the compounds. Cells were analyzed at the 24 hours time point for apoptosis using the Cell Death Detection ELISA assay. B. A2058 cells were treated via the same protocol and assayed for BrdU incorporation (Cell Proliferation ELISA, Roche). C. A2058 tumor-bearing mice were administered 30 mg/kg GDC-0941 and 5 mg/kg GDC-0973 daily for 4 days and tumors were collected as indicated following the final dose. Tumors were harvest 2 or 24 hrs after the final dose and formalin fixed paraffin-embedded tissue sections were stained with an antibody specific to pS6 using standard techniques. A modified TissueMap solution in Definiens Developer (version 7.0.4) was used to sample all cells present in the tumor tissue and quantify pS6 staining.

Supplementary Figure 8. Intermittent dosing of GDC-0980 in combination with daily dosing of GDC-0973 results in increased anti-tumor efficacy in the A2058 melanoma xenograft tumor model. An in vivo combination efficacy studies was run in the A2058 ($BRAF^{V600E}$, $PTEN^{null}$) melanoma xenograft tumor model. Vehicle (black open circles and lines), GDC-0973 (red triangles and lines), GDC-0980 (blue circles and lines), or a combination of the two drugs (green diamonds and lines) were dosed orally, daily (QD) or weekly (QWk) over a 21 day period, as indicated in the panels. GDC-0980 dosed QD at 1.5 mg/kg (A) or QWk at 10 mg/kg (B) demonstrated combination efficacy in combination with GDC-0973 dosed QD at 5 mg/kg. Group mean tumor volumes and standard error of the mean (SEM) is shown. Percent tumor growth inhibition (%TGI) is listed in Supplementary Table 3.