Figure S3

a) ATP signal (% control) for NCI-H2122, GDC-0994, and cobimetinib, with a combination curve.

b) Loewe Synergy Score for GDC-0994 and cobimetinib in A549 NSCLC, KRAS<sup>G12S</sup> cells.

C) HCT116 Effect and Model for GDC-0994 and cobimetinib.

D) A549 Effect and Model for GDC-0994 and cobimetinib.

Supplemental Figure 3. Dual node targeting is synergistic in KRAS mutant setting.

(a) Cell viability in NCI-H2122 (KRAS$^{G12C}$, NSCLC) cells were treated with cobimetinib and GDC-0994 at the indicated concentrations and cell viability was measured after 72 hr of culture (CellTiter-Glo®). (b) Isobologram analysis of EdU incorporation was utilized to evaluate the combination of cobimetinib and GDC-0994 in A549 (KRAS$^{G12S}$, NSCLC) cells. Predicted Loewe additivity is shown in red, whereas fitting of the 50% effect values is plotted in blue. (c) Response data from the isobologram studies in HCT116 (KRAS$^{G13D}$, colorectal) and (d) A549 with cobimetinib and GDC-0994 were used to plot the “Effect” vs. the assumed “Model” of additivity with the calculated “Excess” plotted below. (e) Synergistic antiproliferative effect of ERK inhibitors with cobimetinib on RAS mutant cell lines. Cells were treated with the indicated drug combinations where cobimetinib (Drug 1) was combined with either itself (Cobi, closed green triangles) or the ERK inhibitors GDC-0994 (994, filled red diamonds) or ulixertinib (BVD-523, Ulix, open red diamonds) (Drug 2) in a dose-matrix format. Cells were grown for 48 hours and the proliferative fraction was determined by EdU labeling using high-content imaging. Synergy was determined using the Loewe additivity model and summarized as a weighted average of excess activity over predicted additivity. Cobimetinib combined with itself in both arms of the matrix serves as sham control resulting in a score of zero. Individual symbols represent independent experiments with mean and SD indicated. Cell lines tested: A549 (NSCLC, KRAS$^{G12C}$), CFPAC (pancreatic adenocarcinoma, KRAS$^{G12V}$), H2009 (lung adenocarcinoma KRAS$^{G12A}$), HCT116 (colon carcinoma, KRAS$^{G13D}$), IPC-298 (melanoma, NRAS$^{Q61L}$), KP-4 (pancreatic carcinoma, KRAS$^{G12D}$).