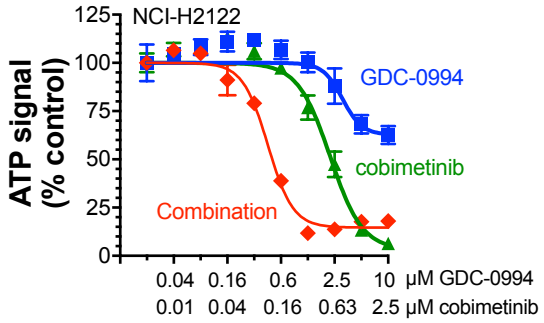
Figure S3

a



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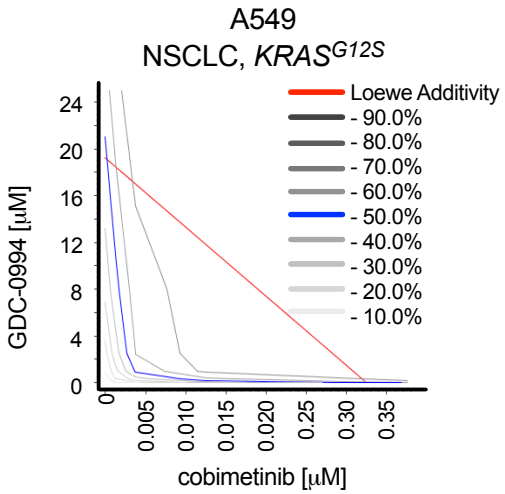
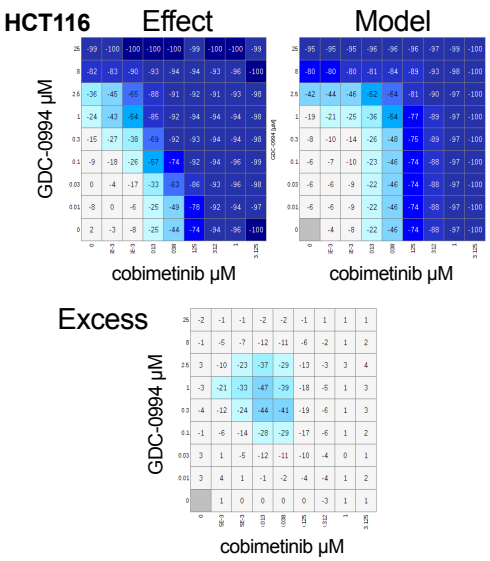
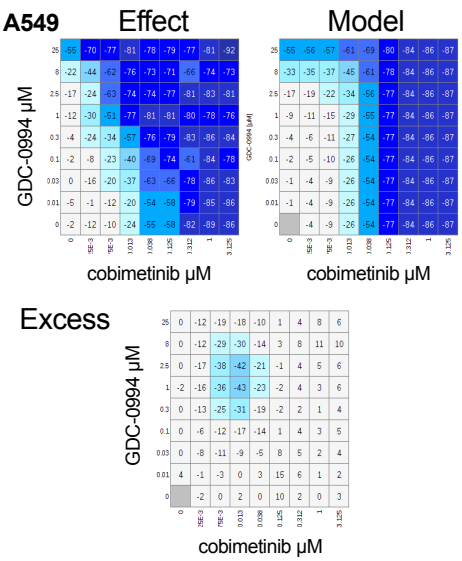


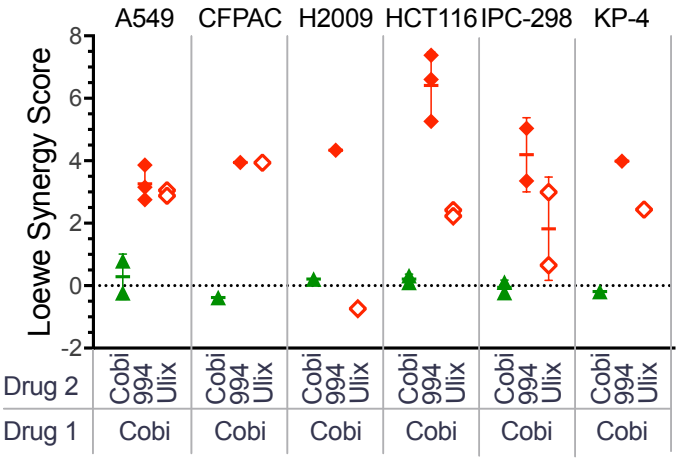
c



d



e



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}).