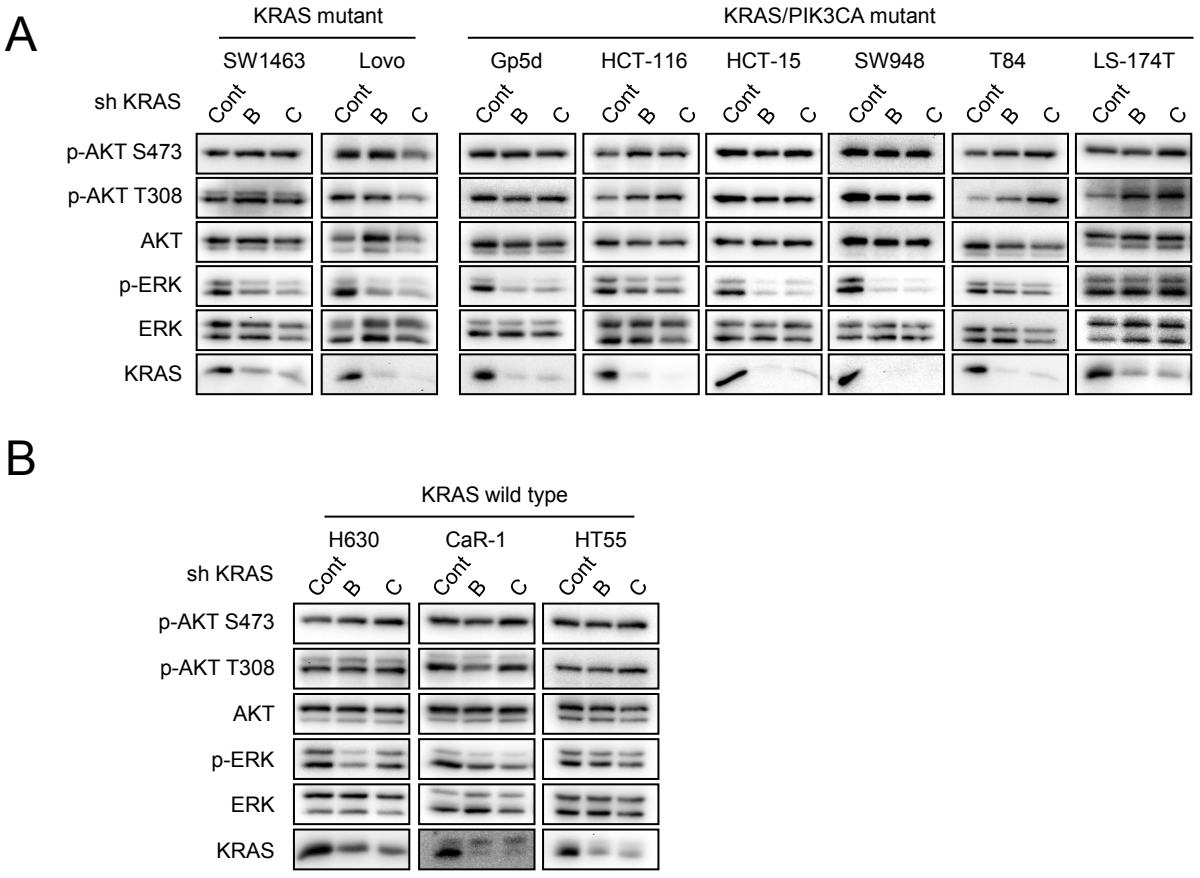
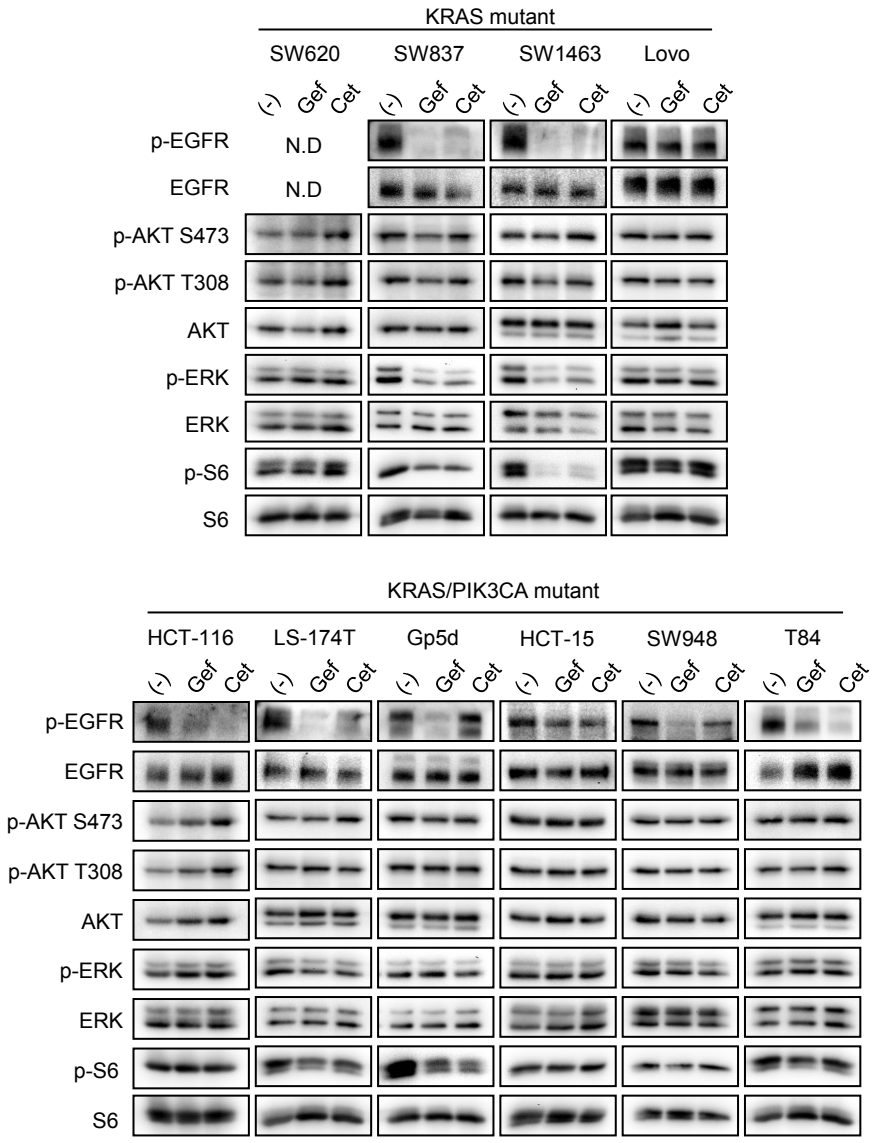


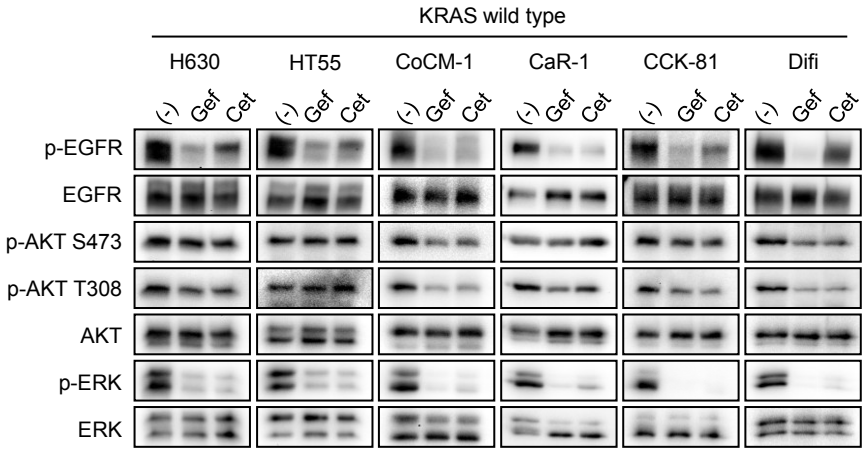
Supplemental Figure 1. KRAS is required for activation of MEK-ERK in *KRAS* mutant colorectal cancers

KRAS mutant and KRAS/PIK3CA double mutant (A) and KRAS wt (B) colorectal cancer cells were infected with two shRNA targeting KRAS (B, C) or control shRNA. 3 days following infection, protein lysates were prepared and probed with the indicated antibodies.



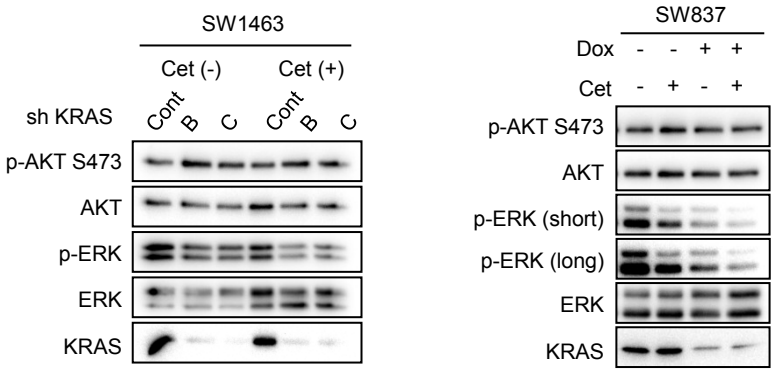
Supplemental Figure 2. EGFR does not regulate PI3K-AKT signaling in *KRAS* mutant colorectal cancers

The indicated cell lines, grouped according to mutational status, were treated with Gefitinib (Gef) 1μM or Cetuximab (Cet) 10 μg/mL for 6 hours. Lysates were probed with the indicated antibodies.

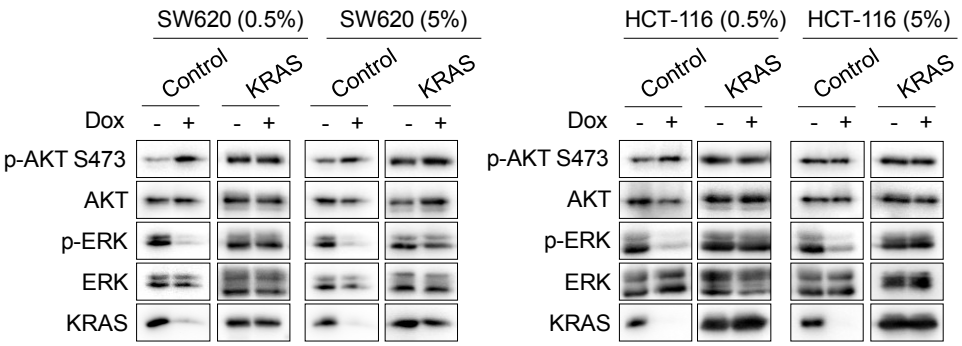


Supplemental Figure 3. EGFR inhibition suppresses MEK-ERK signaling pathway in *KRAS* wildtype cancers

KRAS wildtype cancer cell lines were treated with 1μM of gefitinib (Gef) or 10 μg/mL of cetuximab (Cet) for 6 hours, and lysates were probed with the indicated antibodies.

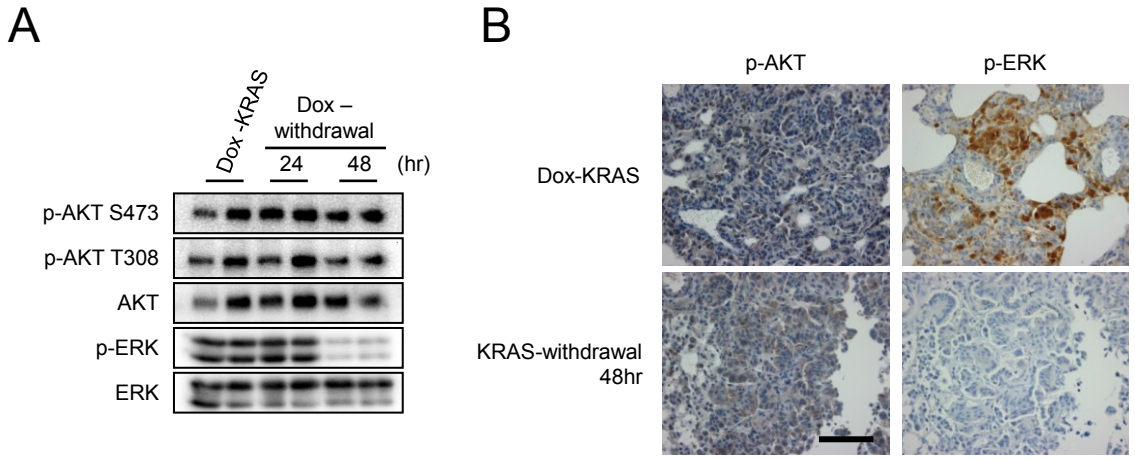


Supplemental Figure 4. KRAS exerts a more dominant regulation on ERK signaling than EGFR in SW1463 and SW837 cell lines. (*Left*) SW1463 cells were infected with one of two independent shRNA targeting KRAS (B, C) or control shRNA. 3 days following infection, cells were treated with vehicle or cetuximab (10 μg/mL) for 6 hours. The cells were lysed and western blots were probed with the indicated antibodies. (*Right*) SW837 cells infected with lentivirus expressing a doxcycyline-inducible KRAS shRNA were cultured in the presence or absence of doxycycline (20 ng/mL) for 72 hours. Then cells were treated with cetuximab for 6 hours. The cells were lysed and western blots were probed with the indicated antibodies.



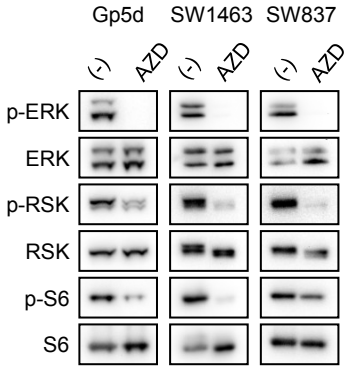
Supplemental Figure 5. Restored expression of KRAS rescues the effects of KRAS knockdown on downstream signaling.

SW620 and HCT-116 cells harboring doxycyline-inducible KRAS or control shRNA (Figure 1F) were infected with GFP-expressing lentivirus encoding KRAS(12V) or control cDNA and sorted for low GFP fluorescence intensity by FACS. Since the KRAS shRNA targets the 3'UTR, the KRAS expression was restored in the cells infected with the KRAS(12V) expressing constructs. Stable polyclonal cell lines were then cultured in the presence or absence of doxycycline (10 ng/mL) in either full serum (5% FBS) or low serum (0.5% FBS) for 72 hours. Lysates were probed with the indicated antibodies.

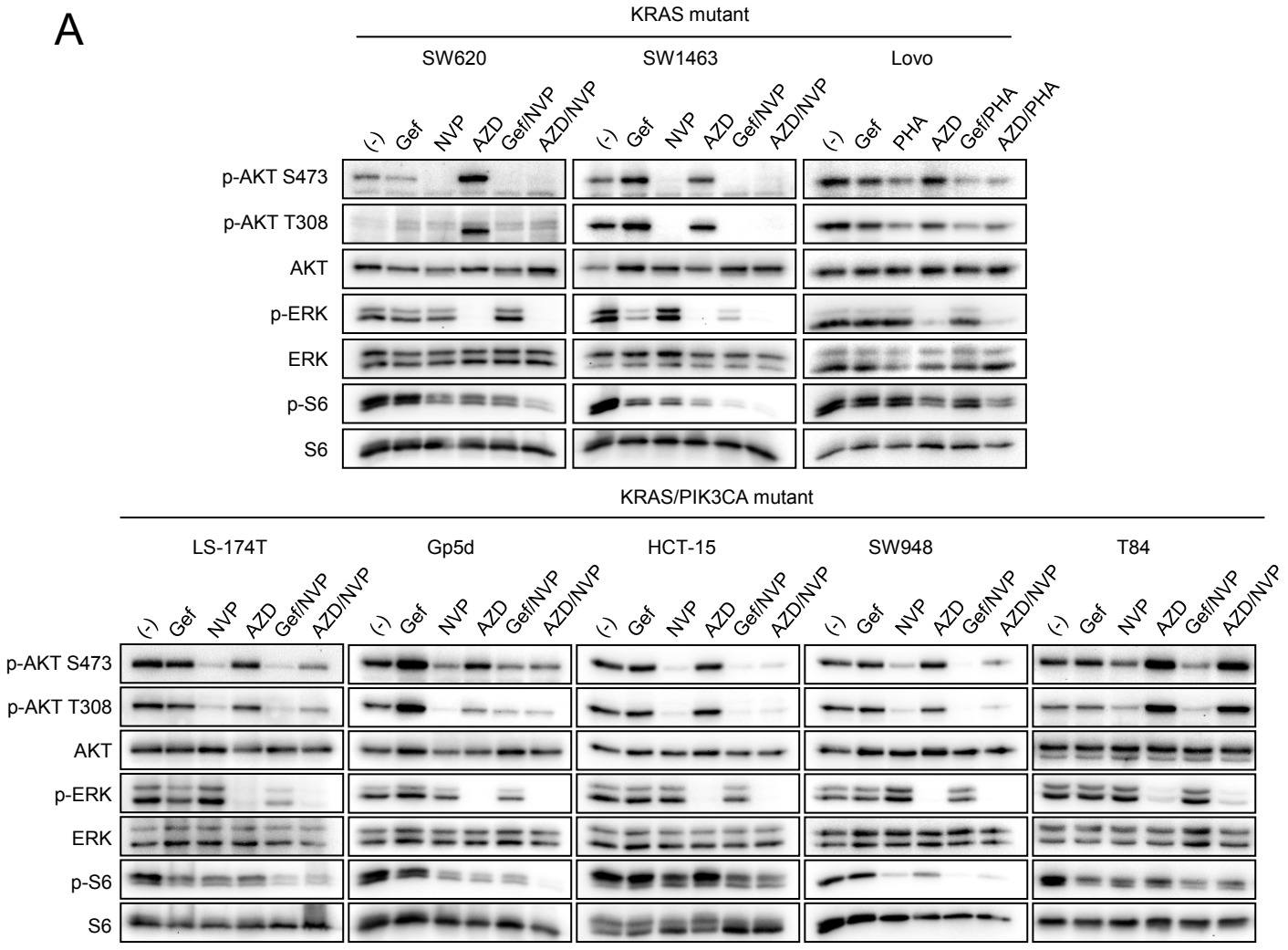


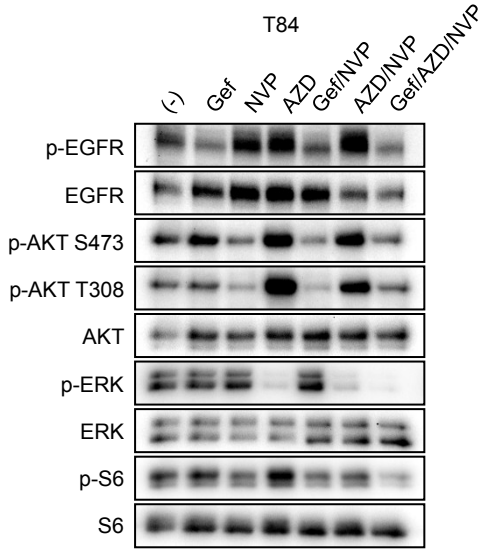
Supplemental Figure 6. KRAS knockdown does not suppress PI3K signaling

(A, B) Tet-op-mutant *Kras* mice were induced to develop lung tumors with doxycycline. The doxycycline was removed and lungs were harvested 24 and 48 hours later. The lungs were assessed by western blot analyses (A) and immunohistochemical analyses (B) with the indicated antibodies. Scale bar, 100μM.



Supplemental Figure 7. MEK-ERK signaling regulates S6 phosphorylation in multiple *KRAS* mutant cancer cell lines. Cells were treated with AZD6244 1μM for 6 hours. Protein lysates were blotted with the indicated antibodies.

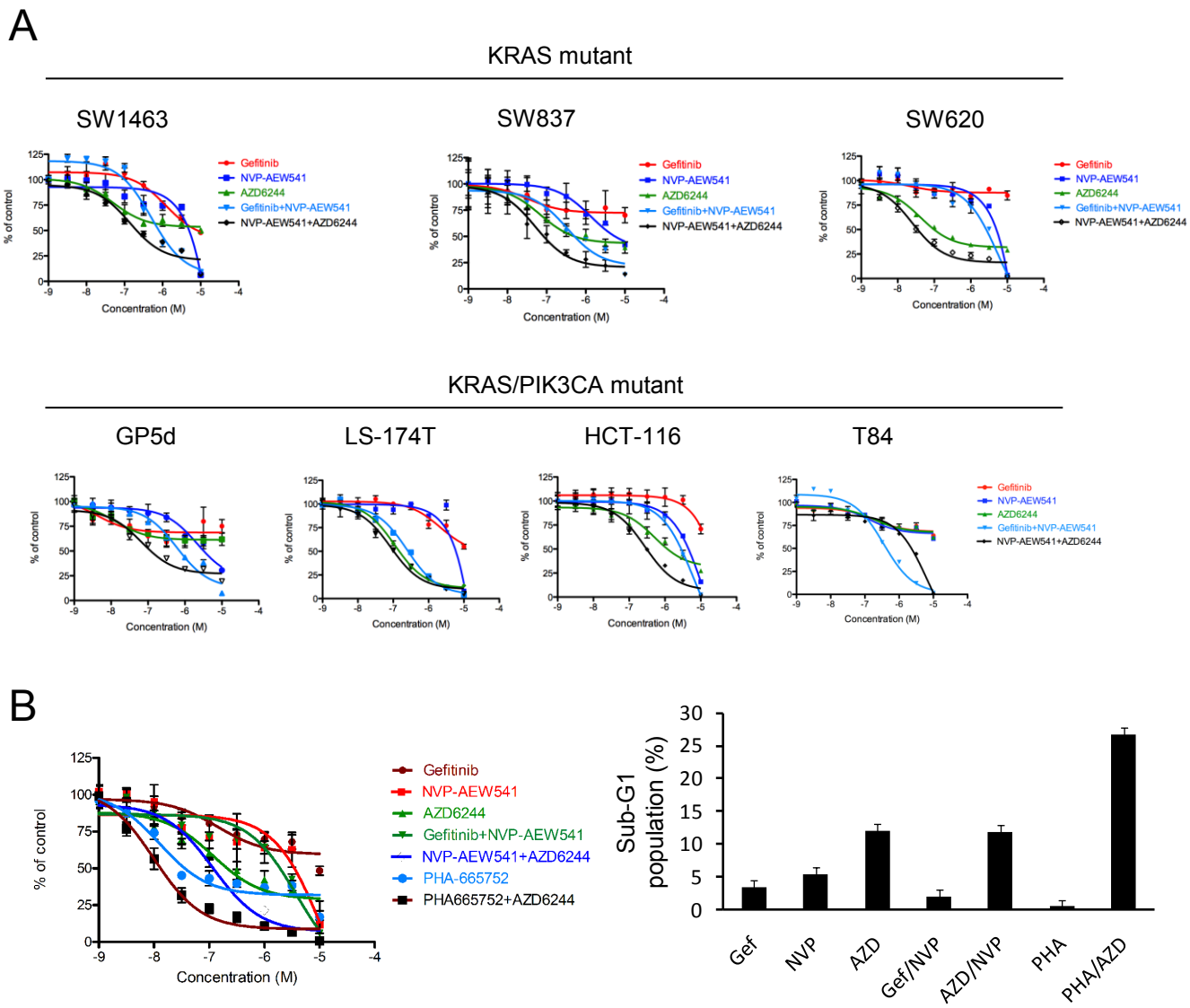




Supplemental Figure 8. Mechanisms of PI3K activation in *KRAS* mutant colorectal cancers

(A) Cells were treated with either DMSO (-) or the indicated drug(s) for 6 hours. Drug concentrations are the same as in Figure 4A. Protein lysates were immunoblotted and probed with the indicated antibodies.

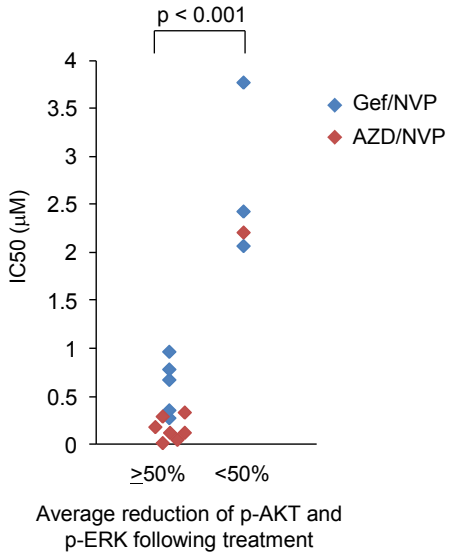
(B) PI3K suppression requires IGF-IR and EGFR inhibition in the presence of MEK inhibitors in T84 cells. Cells were treated with either DMSO (-) or the indicated drug combinations for 6 hours. Drugs were used at 1μM. Protein lysates were probed with indicated antibodies.



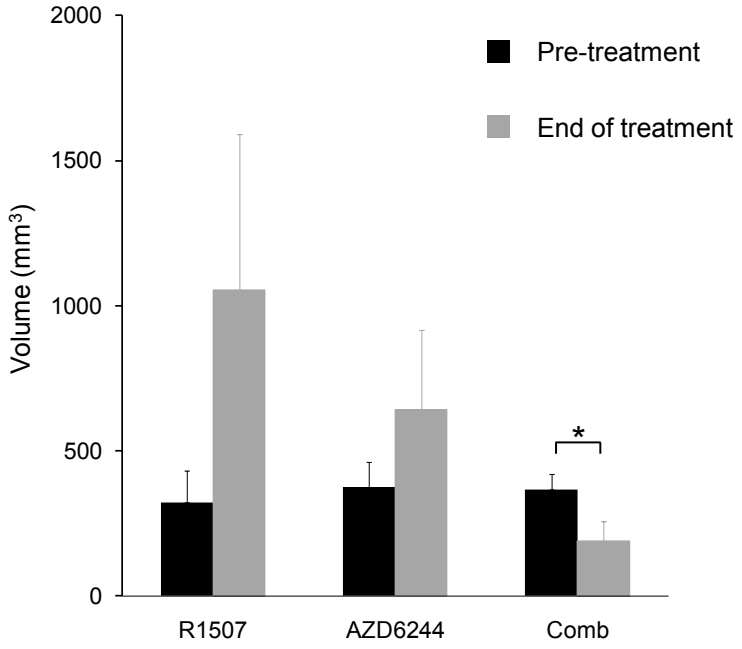
Supplemental Figure 9. Decreased cell viability is induced by combined inhibition of IGF-IR (or MET) and MEK in *KRAS* mt colorectal cancers

(A) Cell viability data for the individual *KRAS* mutant and KRAS/PIK3CA mutant cell lines using the indicated drugs and combinations. Each concentration was assessed in sixplicate, and the averages and standard deviations are shown. All cell lines except LS-174T showed synergy with IGF-IR and MEK inhibitors.

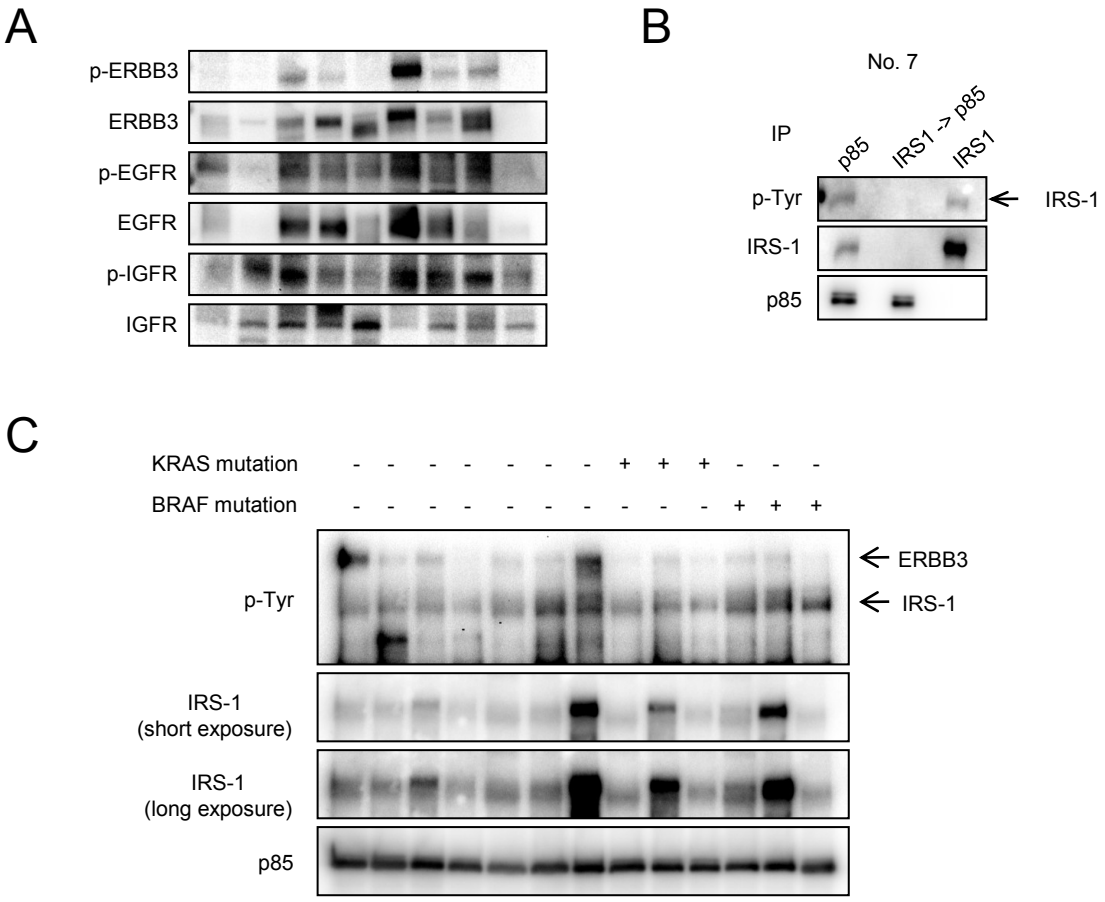
(B) A combination of MET and MEK inhibitors is the most effective in Lovo cells. (*left*) Lovo cells were assessed by a 72-hour survival assay in increasing concentrations of the indicated drugs and combinations. Combined inhibition of MET and MEK showed synergy comparing to single treatments. (*right*) Lovo cells were incubated in the presence of the indicated drug(s), and the percentage of cells with sub-G_0/G_1 DNA content was determined by propidium iodine staining and fluorescence-activated cell sorting analyses.



Supplemental Figure 10. The effects of inhibitor combinations on the activation MEK/ERK and PI3K/AKT pathways correlates with their efficacy. The levels of phosphorylated ERK and AKT after treatment with gefitinib and NVP-AEW541 or AZD6244 and NVP-AEW541 were quantified for each cell line examined (raw data shown in Figure 4A and Supplemental Figure 8A). The average of suppression of AKT and ERK phosphorylation was calculated in each cell lines. Using a cutoff of 50% reduction of phosphorylation, cells were plotted versus IC50s.



Supplemental Figure 11. Tumor volume at the end of the treatment. Tumor volumes at the beginning and the end of the experiment shown in Figure 5 are presented as the average + SEM. Combination treatment showed significant reduction in tumor volume (* indicates $p < 0.05$ by paired t-test).

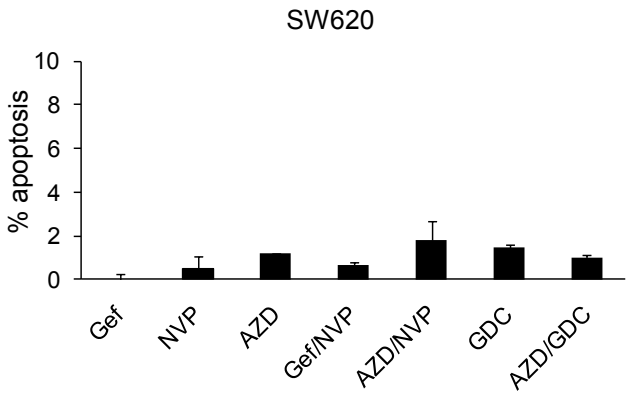


Supplemental Figure 12. IRS-1 associates with PI3K in human colorectal cancers

(A) Lysates from the human colorectal cancer specimens. Whole cell lysates from nine different human colorectal cancer specimens used for the immunoprecipitations in Figure 6 were immunoblotted for the indicated antibodies. Phospho-MET was not detected in any samples.

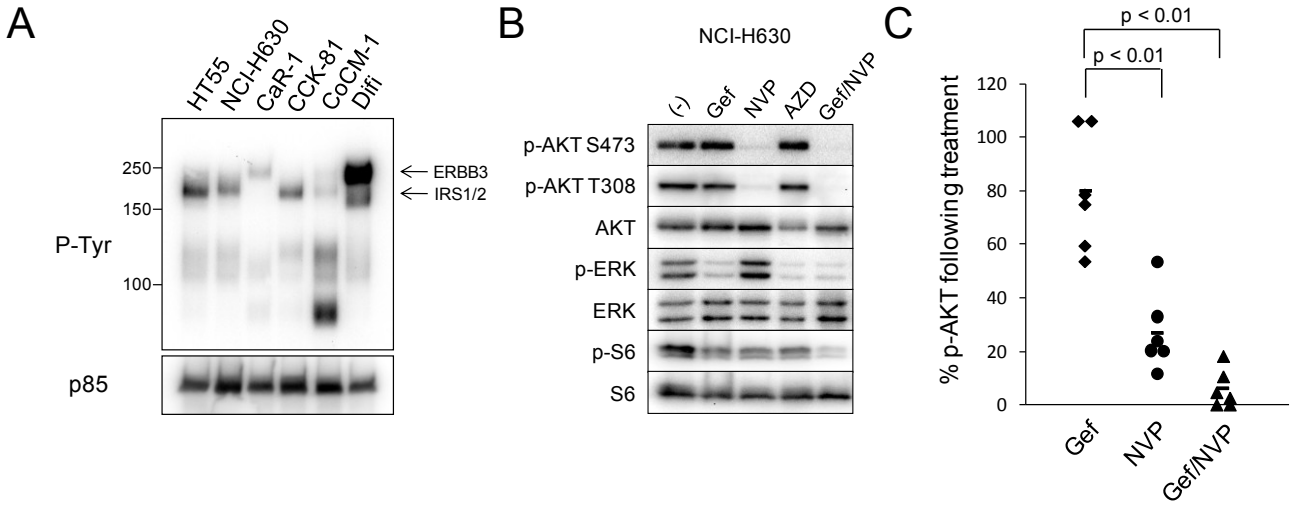
(B) The protein lysate from patient #7 (7^{th} lane of Figure 6A) was immunoprecipitated with p85 (lane 1) and IRS1 (lane 3). Supernatant from the IRS-1 IP was subsequently followed by p85 IP (lane 2). Please note that IP of IRS-1 cleared the pTyr band associated with the p85 IP.

(C) p85 was immunoprecipitated from additional 13 human colorectal cancer specimens followed by western blotting with the indicated antibodies. The KRAS and BRAF mutational status of each cancer specimen is indicated.



Supplemental Figure 13. Concomitant inhibition of PI3K-AKT and MEK-ERK signaling does not lead to apoptosis in SW620 cells

SW620 cells were treated with indicated drugs and combinations for 72 hours. The percent of cells undergoing apoptosis, as measured by annexin V positivity, is shown relative to untreated cells. The average $\pm$ SD of three independent experiments is shown. GDC-0941 (GDC) is a pan-PI3 kinase inhibitor.

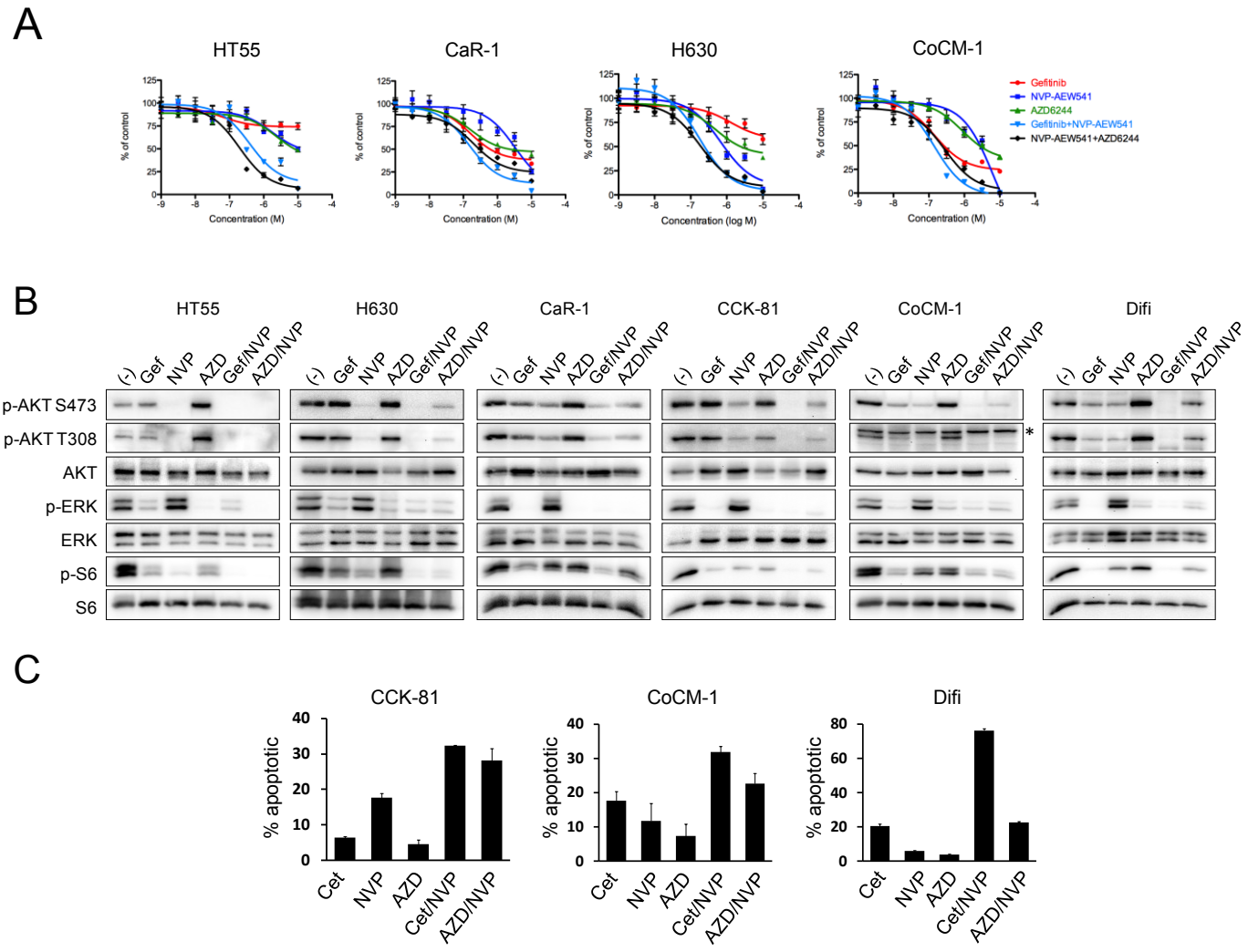


Supplemental Figure 14. Regulation of the PI3K-AKT signaling pathway in *KRAS* wildtype cancers

(A) Cell extracts from the indicated cell lines were immunoprecipitated with an antibody to p85 and blotted with an anti-pTyr antibody.

(B) NCI-H630 cells were treated with control, gefitinib (1 μM), NVP-AEW541 (NVP) (1 μM), a MEK inhibitor AZD6244 (AZD) (1 μM), and gefitinib and NVP-AEW541 (Gef/NVP) for 6 hours. Cell lysates were probed with the indicated antibodies.

(C) The normalized levels of phosphorylated AKT were quantified for each of the KRAS wildtype cell lines 6 hours following the indicated treatments (blots are shown in Supplemental Figure 3 and 15B). Each point represents the results from a single cell line. Bars indicate mean values.



Supplemental Figure 15. Combined EGFR and IGF-IR inhibition downregulate both AKT and ERK phosphorylation leading to apoptosis in *KRAS* wt cancers

(A) Cell viability data for the individual *KRAS* wt cell lines using the indicated drugs and combinations. Each concentration was assessed in sixplicate, and the averages and standard deviations are shown.

(B) Cells were treated with either DMSO (-) or the indicated drug or drug combinations for 6 hours. Drugs were used at the same concentrations as in Supplemental Figure 8A. Protein lysates were probed with the indicated antibodies. *, non-specific bands.

(C) KRAS wt cells were treated with indicated drugs and combinations for 72 hours. The percent of cells undergoing apoptosis, as measured by annexin V positivity, is shown relative to untreated cells. The average $\pm$ SD of three independent experiments is shown.