SUPPLEMENTAL DATA

Supplemental Methods

Cell Culture. Human colon, pancreas and lung cancer cell lines were maintained as suggested by ATCC. The growth media for all the cell lines was supplemented with 2mM Glutamine, 50 units/ml of penicillin and streptomycin and 10% fetal bovine serum (Gemini Bioproducts, Calabasa, CA), and incubated at 37°C in 5% CO_2, unless otherwise indicated. The HCT116 and DLD-1 isogenic cell lines were cultured in McCoy's 5A medium supplemented with 2 mM glutamine, 50 units/ml penicillin, 50 units/ml streptomycin, and 10% heat inactivated fetal bovine serum.

Cell Proliferation Assay. Cells were plated in 96-well plates at a density of 2-5 $x10^3$ cells per well. The following day cells were treated with DMSO control or PD0325901 at range of concentrations, for 5 days. Each day, the Alamar Blue viability assay (AccuMed International, OH) was performed as previously described (1). Briefly, Alamar Blue (25uL) was added to the culture media, cells incubated for 4 hours and fluorescence measured with a SpextraMax $M2^e$ fluorometer (Molecular Devices, Sunnyvale, CA). The dose required to inhibit growth by 50% compared to control (IC_{50}) was calculated using the SoftMaxPro ver.5 software (Molecular Devices).

Western Blot Analysis. Cells were lysed in Nonidet P-40 buffer (50 mM Tris, pH 7.5, 1% Nonidet P-40, 150 mM NaCl, 2.5 mM Na_3VO_4, 10 mM phenylmethylsulfonyl fluoride, and 10 μM each leupeptin, aprotinin, and soybean trypsin inhibitor) and protein resolved by SDS-PAGE, as previously described (2). Antibodies for ERK, p-ERK (Thr202/Tyr204), AKT, p-AKT (Ser-473), activated (cleaved) Caspase-3 and cleaved PARP were obtained from Cell Signaling Technology (Beverly, MA). KRAS (F234), Cyclin D1 (M-20) and Cyclin D2 (C-

17) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-HA antibody was obtained from Covance (Princeton, NJ).

Animal Studies (additional details). Four- to six-week-old athymic BALB/c female mice (NCI-Frederick Cancer Center) were maintained in pressurized ventilated cages. Tumors were generated by injecting subcutaneously 3-5x10^6 cells, mixed 1:1 with Matrigel (Collaborative Research, Bedford, MA) into the right flank of the animal (200µl/mouse). After 7–10 days, mice bearing tumors 6–7 mm in diameter were randomized to receive the drug or vehicle only as control. The average tumor diameter (average of two perpendicular axes of the tumor) was measured in control and treated groups using vernier calipers.Tumor volumes were calculated with the following formula: $\pi/6 \times$ larger diameter $\times$ (smaller diameter)^2. To prepare lysates, mice were euthanized and tumor tissue removed and homogenized in 2% SDS lysis buffer and processed as described above. For immunohistochemical staining, tumors were excised promptly after euthanasia and immediately placed in 4% paraformaldehyde. The tumors were fixed overnight in paraformaldehyde and then dehydrated and embedded in paraffin. Sections of 8 µm were cut for immunochemical staining with human specific antibody for cleaved PARP (#9541, Cell Signaling Technology, Beverly, MA).

Supplemental References

1. White, M. J., DiCaprio, M. J., and Greenberg, D. A. Assessment of neuronal viability with Alamar blue and granule cell cultures. J Neurosci Methods, *70:* 195-200, 1996.

2. Pratilas, C. A., Hanrahan, A. J., Halilovic, E., et al. Genetic predictors of MEK dependence in non-small cell lung cancer. Cancer Res, *68:* 9375-9383, 2008.

Supplemental Figure Legends

Figure S1: Mutational status, AKT and ERK signaling expression profile in the panel of cell lines.

(A) Mutational status of KRAS and PIK3CA in the panel of cell lines utilized in this report, grouped by tissue type. **(B)** AKT and ERK signaling expression profile was compared across the panel of KRAS mutant cell lines studied in this report. Tumor histology is indicated: Colon (C), Pancreas (P), NSCLC (L). Two control cell lines, one with deleted PTEN (PC-3, Pancreas) and one with mutant BRAF (Colo205, colon) were used for comparison.

Figure S2: Effects of MEK inhibition on the growth of KRAS mutant tumors *in vivo.*

A and B: Immunoblots of tumor lysates showing the effects of a single 25 mg/kg dose of PD0325901 in SW620 and HCT-15 xenograft tumors. Tumors were excised pretreatment and at the indicated times.

C and D: Mice with established H747 (KRAS mut/PIK3CA wt) and DLD-1 (KRAS mut/PIK3CA mut) xenografts were treated with indicated doses of PD0325901 x 5 days/week x 3 weeks or vehicle only as a control. The results represent the mean percent increase in tumor volume ± SE (n = 5 mice/group).

Figure S3. Elimination of the mutant *PIK3CA* allele renders KRAS mutant tumors MEK/ERK dependent.

A: Isogenic HCT116 KRAS mutant/PIK3CA wild-type (wt PIK3CA, red) and HCT116 KRAS mutant/PIK3CA mutant (mut PIK3CA, blue) cells were grown in the presence of PD0325901 in 10% FBS growth medium and proliferation was measured using Alamar blue

assay. Results are represented as the percentage of respective untreated control on day 3, plotted as a function of the drug concentration.

B: wt PIK3CA HCT116 cells were treated with 50 nM PD0325901, in media supplemented with 1% or 10% FBS. Cells were harvested at indicated times post treatment, lysed and immunoblotted with the indicated antibodies.

C and D: Isogenic DLD-1 KRAS mutant/PIK3CA wild-type (wt PIK3CA, red) and DLD-1 KRAS mutant/PIK3CA mutant (mut PIK3CA, blue) cells were grown in the presence PD0325901 in medium supplemented with either 1% or 10% FBS and proliferation measured using Alamar blue assay. Results are shown as percentage of respective untreated control on day 3 in **(C)** 10% FBS and **(D)** 1% FBS growth media plotted as a function of the drug concentration.

Figure S4: Effects of inhibiting KRAS expression in MEK-dependent and MEK-independent cells

A: Immunoblots showing effects of inhibiting KRAS expression in MEK-dependent (SW403) and MEK-independent (HCT116) cells. Cells were transfected with lipid carrier control (C), non-targeting control (NT) or KRAS siRNA, harvested at 48 and 72 hours post- transfection and cell lysates were immunoblotted with the indicated antibodies.

B: SW403 and HCT116 cells were transfected with either non-targeting (NT) or KRAS siRNA, collected 48hr later and analyzed by flow cytometry. The representative graphs show cell cycle distribution presented as the mean of two independent experiments performed in triplicate.

C: wt PIK3CA and mut PIK3CA isogenic HCT116 cells were transfected with non-targeting control (NT) or KRAS siRNA, harvested 48 hr post transfection and analyzed by flow cytometry. The graph shows the percent of cells with S phase DNA content presented as the mean ± SE from two independent experiments done in triplicate.

Figure S5: Chronic administration of both PD0325901 and AKT-1/2 together was well tolerated in treated animals.

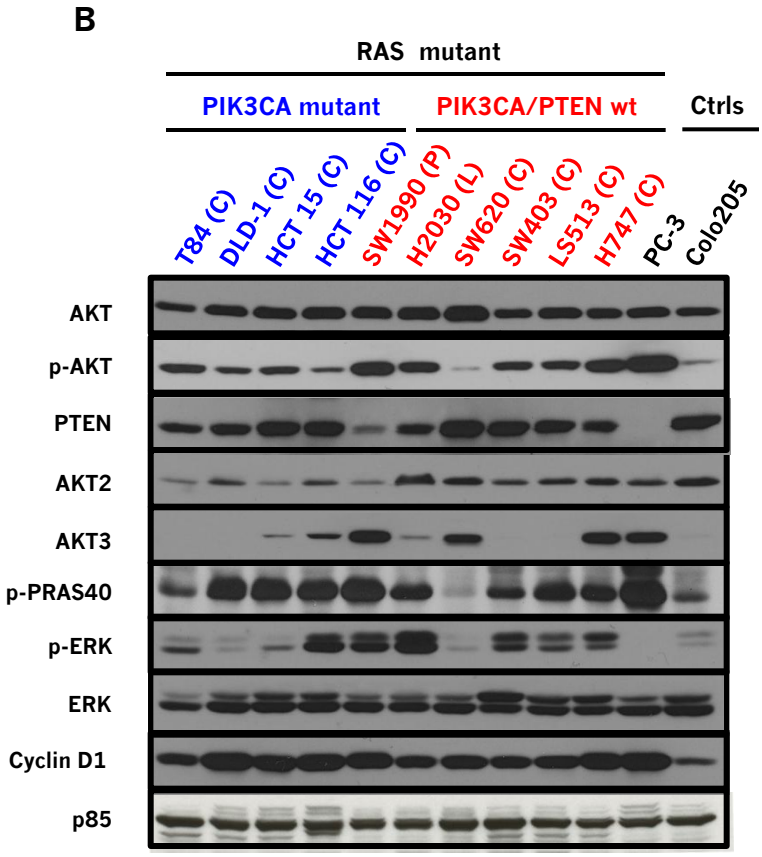
A: Average weight of the mice treated with PD0325901 and AKT-1/2. Mice with established HCT15 (KRAS mutant/PIK3CA mutant) xenografts were dosed with 5 mg/kg of PD0325901 and 100mg/kg of AKTi-1/2 alone or in combination. The mice were treated with inhibitors or vehicle only as a control 5 days/week for indicated number of days. The results represent the average mouse weight ± SE (n = 5 mice/group).

Halilovic et al., Supplemental Figure S1

A

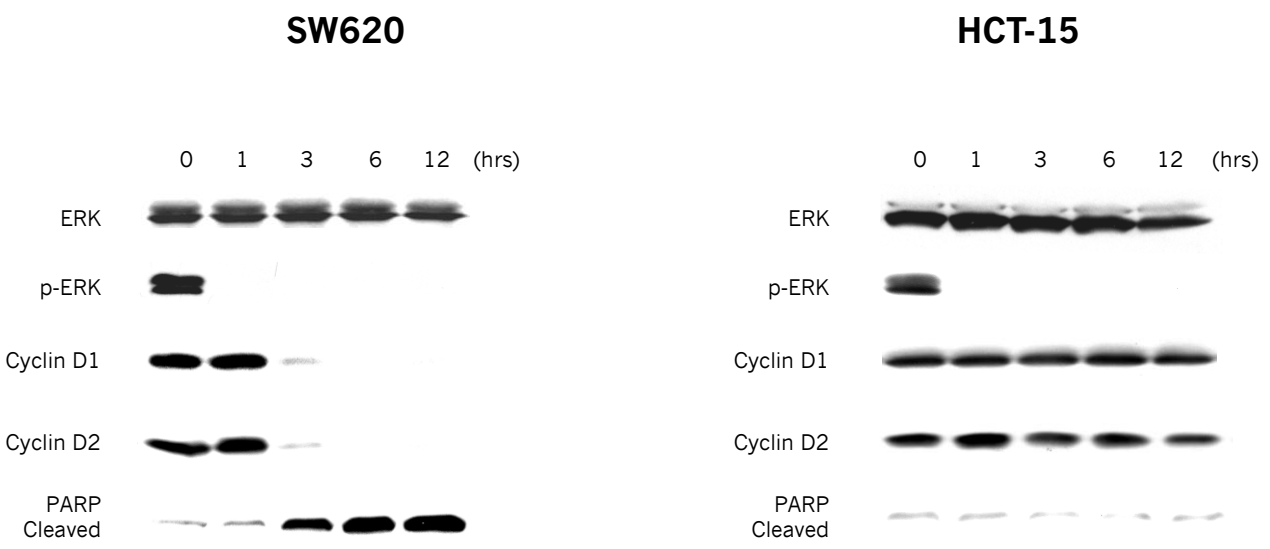
Cell Line		
	Colon	
T84	KRAS (G13D)	PIK3CA (E542K)
DLD-1	KRAS (G13D)	PIK3CA (E545K)
HCT-15	KRAS (G13D)	PIK3CA (E545K)
HCT 116	KRAS (G13D)	PIK3CA (H1047R)
SW1116	KRAS (G12A)	
SW 620	KRAS (G12V)	
SW403	KRAS (G12V)	
H747	KRAS (G13D)	
LS513	KRAS (G12D)	
	Pancreas	
SW 1990	KRAS (G12D)	
HPAF-II	KRAS (G12D)	
Mia PaCa-2	KRAS (G12C)	
	NSCLC	
H460	KRAS (Q61K)	PIK3CA (E545K)
H2030	KRAS (G12C)	
H1734	KRAS (G13C)	
H358	KRAS (G12C)	

B

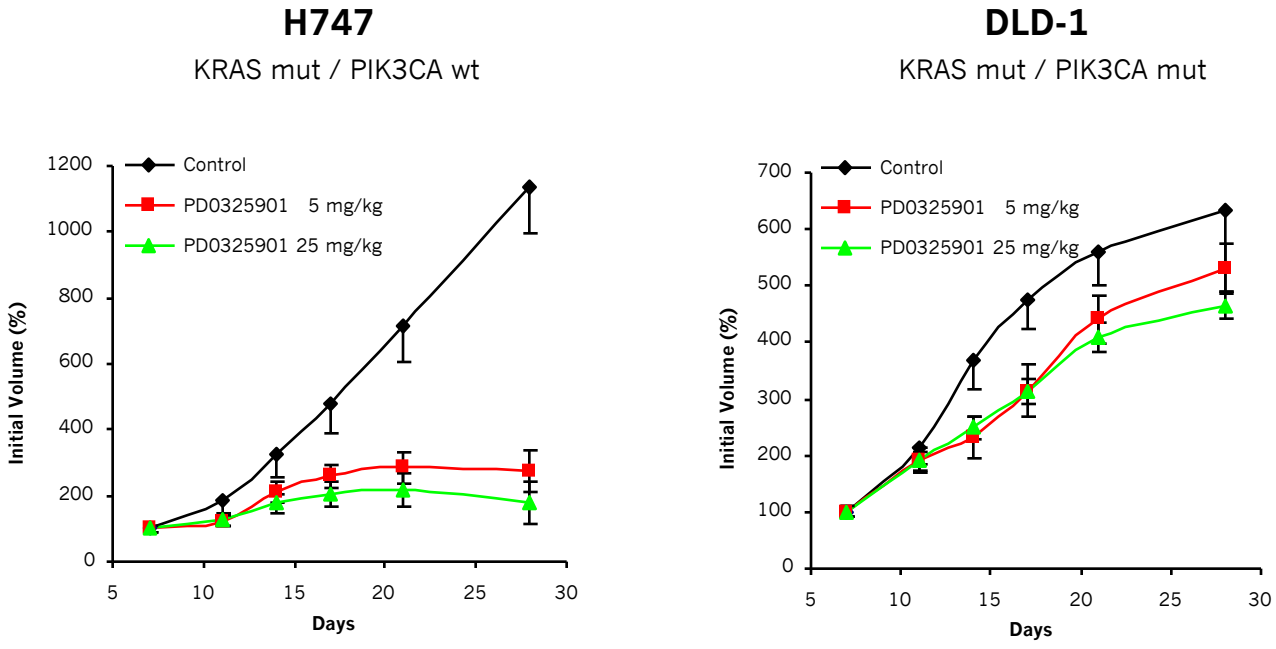


Halilovic et al., Supplemental Figure S2

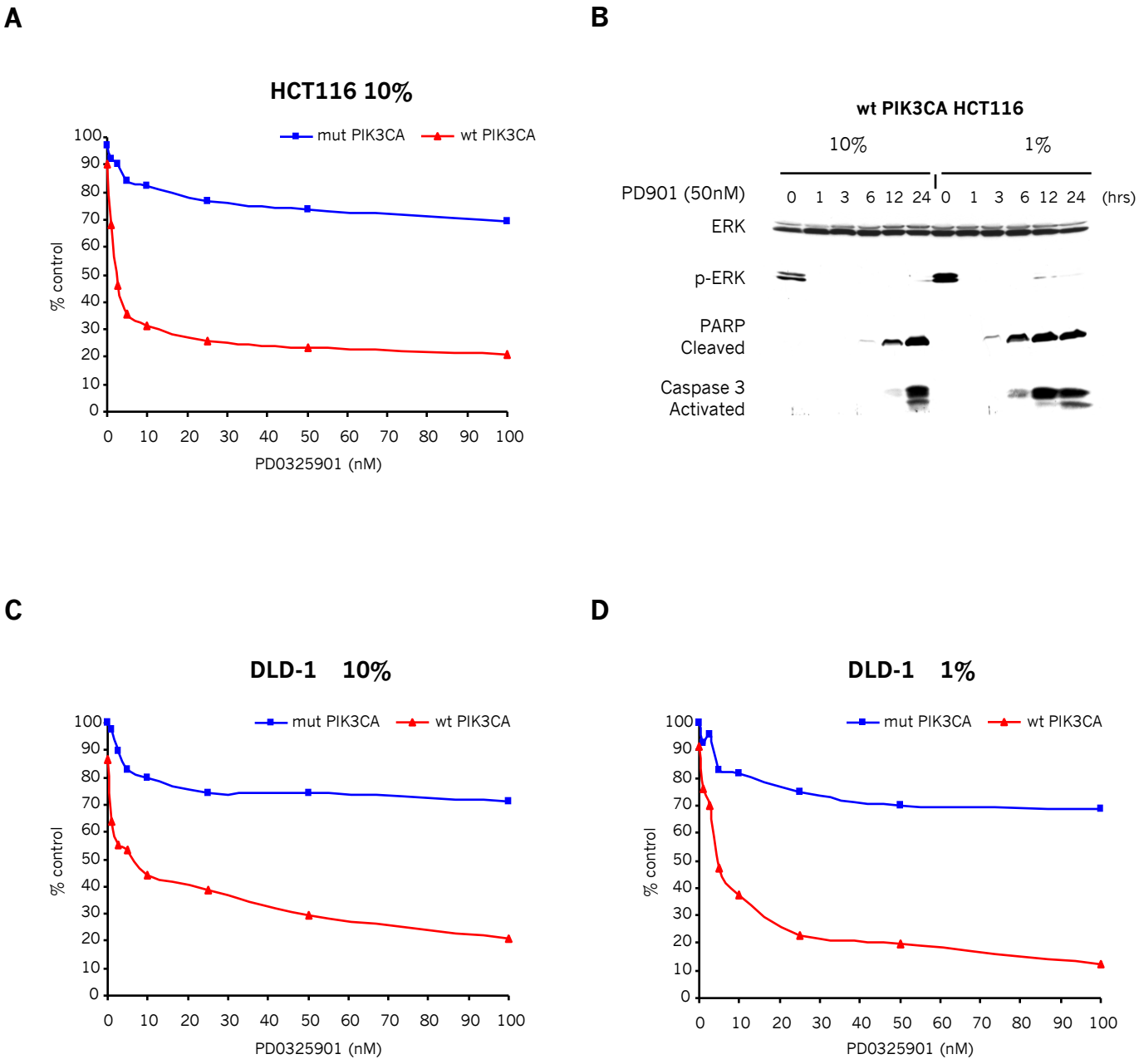
A



B

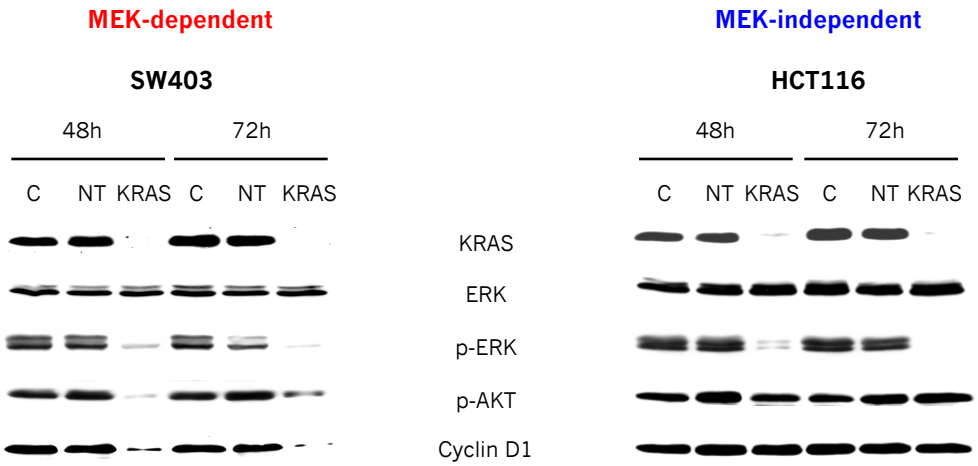


Halilovic et al., Supplemental Figure S3

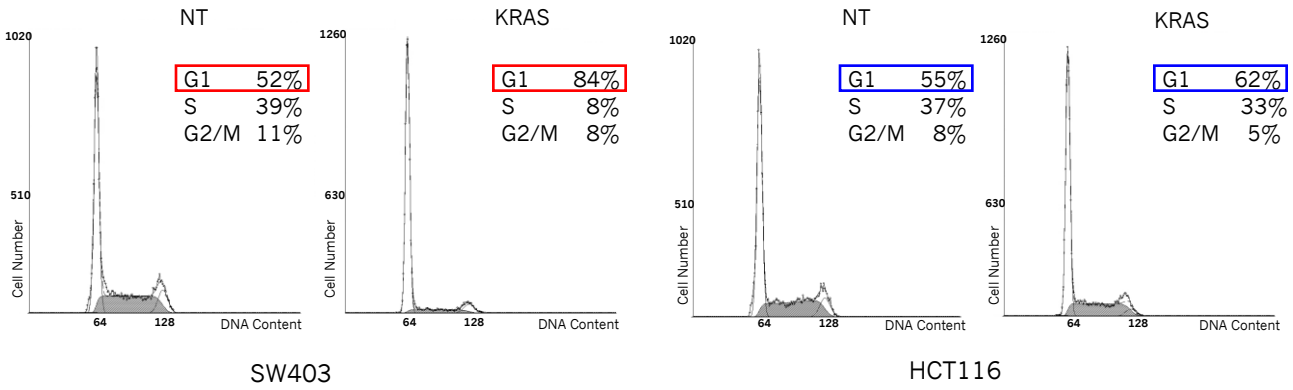


Halilovic et al., Supplemental Figure S4

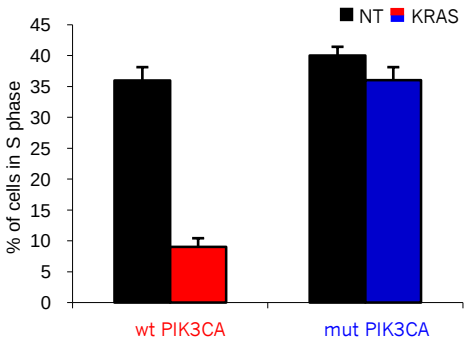
A



B



C



Halilovic et al., Supplemental Figure S5

A