Supporting Information

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Fig. S1. Activated K-Ras with a phosphomimetic amino acid at position 181 does not induce apoptosis. (*A*) Representative phase contrast images of Bax/Bak WT or double knockout (DKO) murine embryonic fibroblasts (MEFs) transfected with indicated constructs showing clonogenic survival. (Scale bar, 100 μm.) (*B*) COS-1 cells transfected with the indicated construct or treated with 1 μM staurosporine, harvested 16 h posttransfection, permeabilized with 0.025% digitonin and separated into pellet [P] and soluble [S] fractions. Fractions were analyzed by SDS/PAGE as cell equivalents and immunoblotted with an anti-cytochrome c antibody. (C) COS-1 cells transfected or treated as in *B* were immunoblotted for poly-ADP ribose polymerase (PARP) (*Upper*) or anticleaved caspase-3 (*Lower*). Numbered lanes correspond to conditions in *B*. (*D*) NIH 3T3 cells transfected with vector or K-Ras12V181E, lysed at the indicated times, and analyzed by SDS/PAGE and immunoblots with anticleaved caspase-3 antibody. Immunoblot of RhoGDI demonstrates equal loading. Immunoblots shown are representative of at least three independent experiments.



Fig. S2. Activated phospho–K-Ras inhibits cell survival in a Bcl-xL–dependent fashion. (*A*) Representative phase contrast images of Bcl-xL^{+/+}, $-^{/-}$, and rescued $-^{/-}$ MEFs transfected with the indicated constructs representing clonogenic survival as the presence or absence of a confluent monolayer. (Scale bar, 100 μ m.) (*B*) HCT116 K-Ras WT and 13D cells as well as Panc-1 cells were treated with the indicated compounds. Cell proliferation was measured on days 1, 3, and 5 by a modified MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Each point represents the average absorbance from five wells. Plots shown are representative of three experiments.



Fig. S3. K-Ras binds the C terminus of inositol trisphosphate receptor (IP3R) in an amino acid 181 phosphorylation-dependent fashion. Affinity purification (AP) of recombinant Ras (5 nM) (A, K-Ras12V181A; E, K-Ras12V181E; H, H-Ras61L; S, K-Ras12V181S) with GST or GST–IP3R1-C in Sf9 insect cell lysates containing baculovirus-expressed human Bcl-xL. Samples were immunoblotted with anti-PanRas and anti–Bcl-xL antibodies. This figure is the same as Fig. 3*B Right* but shows, in addition to the conventional exposure of the Ras affinity purification (*Top*), an overexposed image of the same membrane (second panel) that reveals that, in addition to K-Ras12V181E (E), GST–IP3R1-C also affinity captures phosphorylatable K-Ras12V181S (arrow) but not nonphosphorylatable K-Ras12V181A or H-Ras61L.



Fig. 54. K-Ras12V181E inhibits Bcl-xL-mediated sensitization of IP3Rs in Sf9 cells. Representative current traces of endogenous insect IP3R channel activity measured by patch clamp electrophysiology of nuclei isolated from Sf9 cells. Downward deflections indicate channel openings. Arrows indicate zero-current level. Specified molecules were present in the pipette solution. All pipette solutions contained 10 nM inositol trisphosphate (InsP₃) and 2 μ M free Ca²⁺. Results are representative of three independent experiments.



Fig. 55. Activated phospho–K-Ras induces autophagy in a Bcl-xL–dependent fashion and Atg5 deficiency partially protects against phospho–K-Ras–associated toxicity. (A) Bcl-xL^{+/+}, ^{-/-}, and rescued MEFs transfected with the indicated constructs and mCherry-LC3 were treated with or without 10 mM 3-methyladenine (3-MA) and scored 24 h posttransfection for mCherry-LC3 translocation to cytoplasmic puncta. Bars represent fold change in the percentage of autophagic cells (mean \pm SEM, n = 3). (B) HCT116 K-Ras WT and 13D cells transfected with mCherry-LC3, treated with the indicated compounds and scored 24 h posttransfection for Cherry-LC3 translocation as in A. Bars represent fold difference in the percentage of autophagic cells in 13D cells versus WT cells (mean \pm SEM, n = 3). (C) Representative fluorescent images of mCherry-LC3 in HCT116 K-Ras WT and 13D cells with or without Aplog-1 treatment. (Scale bar, 20 μ m.) (D) Representative electron micrographs of autophagosmes in HCT116 K-Ras 13D cells with Aplog-1 treatment. (Scale bar, 0.5 μ m.) (E) Representative phase contrast images of Atg5^{+/+} and ^{+/-} MEFs transfected with the indicated constructs showing clonogenic survival. (Scale bar, 100 μ m.)