EXTENDED EXPERIMENTAL PROCEDURES

Generation of Plasmid Construct and Transgenic Mice

To generate the *tetO_Lox-Stop-Lox-Kras*^{G12D} transgene, a fragment containing LoxP-Kozak-ATG-Stop sequence was inserted into the EcoRI/NheI site of pTRE-Tight (Clonetech). A splice acceptor sequence was inserted into the NheI/HindIII site of pTRE-Tight and a Stopper-LoxP sequence was inserted into the HindIII/SalI site to generate pTRE-Tight-LSL. The tetO_LSL fragment was isolated from pTRE-tight-LSL with XhoI/SalI digestion and inserted into the XbaI site of pBS-KS containing the SV40 poly(A) sequence inserted into the BamHI site. A fragment containing mutant murine *Kras*^{G12D} cDNA (Johnson et al., 2001) was inserted into the EcoRV site of pKS-Tight-LSL. *TetO_LKras*^{G12D} mice were produced by injecting the construct into FVB/N blastocysts according to a standard protocol. Transgenic founders were screened by Southern blotting.

p48-Cre, *ROSA26-LSL-rtTA-IRES-GFP* and *p53*^{L} mice have been described previously (Belteki et al., 2005; Kawaguchi et al., 2002; Marino et al., 2000). Mice were interbred and maintained on FVB/C57Bl6 hybrid background in pathogen-free conditions at Dana-Farber Cancer Institute. For doxy treatment, mice were fed with doxy (Dox) water (doxy 2g/l, sucrose 20 g/l). For BrdU injection, mice were i.p. injected with BrdU at 60 mg/kg every 12 hr for 48 hr before necropsy. All manipulations were performed with IACUC approval protocol number 04116.

In Vivo Imaging

MRI was performed using T1-weighted pulse sequences after administration of Gd-DTPA using a 4.7 T Bruker Pharmascan. PET was performed using a Siemens Inveon PET-CT scanner 1 hr after injection of ~500 μCi of ^{18}FDG (Engelman et al., 2008). Respiratory rate was monitored with a BioVet (M2M Imaging), and was used to gate the CT.

Antibodies for Immunohistochemistry and Western Blot Analysis

The primary antibodies used for immunohistochemistry or western blot were: SMA (NB600-531, Novus), BrdU (ab1293, Abcam), cleaved-Caspase3 (9664, Cell Signaling), phospho-Erk (4376, Cell Signaling), phospho-S6 (4858, Cell Signaling), Actin (sc-1615, Santa Cruz), Ras (05-516, Millipore), O-GlcNAc (ab2739, Abcam), Myc (sc-42, Santa Cruz). Gfpt1 antibody was a kind gift from Immuno-Biological Laboratories Co., Japan.

LC-MS/MS

Tumor cells were maintained in basal media in the presence or absence of doxycycline for 24 hr. Fresh media were added 2 hr before the experiment. For metabolite collection, media from biological triplicates (in 10 cm dishes at ~70% confluence) was fully aspirated and 4 ml of 80% (v/v) methanol was added at dry ice temperatures. Cells and the metabolite-containing supernatants were collected into conical tubes. Insoluble material in lysates was centrifuged at 2,000*g* for 15 min, and the resulting supernatant was evaporated using a refrigerated SpeedVac. Samples were re-suspended using 20 μl HPLC grade water for mass spectrometry. Ten microliters were injected and analyzed using a 5500 QTRAP hybrid triple quadrupole mass spectrometer (AB/SCIEX) coupled to a Prominence UFLC HPLC system (Shimadzu) via selected reaction monitoring (SRM) of a total of 254 endogenous water soluble metabolites for steady-state analyses of samples. Some metabolites were targeted in both positive and negative ion mode for a total of 285 SRM transitions using positive/negative switching. ESI voltage was +4900 V in positive ion mode and –4500 V in negative ion mode. The dwell time was 4 ms per SRM transition and the total cycle time was 1.89 s. Approximately 9–12 data points were acquired per detected metabolite. Samples were delivered to the MS via normal phase chromatography using a 4.6 mm i.d x 10 cm Amide Xbridge HILIC column (Waters Corp.) at 300 μl/min. Gradients were run starting from 85% buffer B (HPLC grade acetonitrile) to 42% B from 0-5 min; 42% B to 0% B from 5–16 min; 0% B was held from 16–24 min; 0% B to 85% B from 24–25 min; 85% B was held for 7 min to re-equilibrate the column. Buffer A was comprised of 20 mM ammonium hydroxide/20 mM ammonium acetate (pH = 9.0) in water:acetonitrile (95:5). Peak areas from the total ion current for each metabolite SRM transition were integrated using MultiQuant v2.0 software (AB/SCIEX). For ^{13}C labeled experiments, SRMs were created for expected ^{13}C incorporation in various forms for targeted LC-MS/MS. Data analysis was performed in Cluster3.0 and TreeViewer.

Clonogenic Assay

Four hundred cells were seeded into each well of 6-well plate in duplicate, and colonies were stained 7–10 days later with 0.2% crystal violet in 80% methanol.

Anchorage-Independent Growth Assay

10,000 cells per well were seeded in medium containing 0.4% low-melting agarose on top of bottom agar containing 1% low-melting agarose in regular medium. After 14–21 days, colonies were stained with iodonitrotetrazoliumchloride (Sigma) and counted with Totallab TL100 software.

Expression Profiling and Bioinformatics Analysis

mRNA expression profiling was performed at the Dana-Farber Microarray Core facility using the Mouse Genome 430 2.0 Array (Affymetrix). To identify differentially expressed genes between doxy treatment and withdrawal, the raw intensities were

log_2-transformed and quantile normalized. The software package LIMMA (Linear Models for Microarray Data) (Smyth, 2004) was applied to detect significantly differentially expressed probes using Benjamini-Hochberg adjusted p values. Complete profiles are available at GEO at GSE23926. For GSEA, gene sets collection from MSigDB 3.0 and Kyoto Encyclopedia of Genes and Genomes (KEGG) were included in the analysis. Promoter analysis was performed as described (Zheng et al., 2008).

Lentiviral-Mediated shRNA Targeting

The clone IDs for the shRNA are: TRCN0000031644 (shGfpt1-1), TRCN0000031645 (shGftp1-2), TRCN0000031648 (shGfpt1-3), TRCN0000181768 (shRpia), TRCN0000186910 (shRpe), TRCN0000042514 (shMyc-1) and TRCN0000042517 (shMyc-2). Lentiviruses were produced in 293T cells with packing mix (ViraPower Lentiviral Expression System, Invitrogen) as per manufacturer's instruction.

Establishment of Primary Pancreatic Adenocarcinoma Cell Lines

Establishment of primary PDAC lines were performed as described (Aguirre et al., 2003). Cells were maintained in RPMI1640 medium containing 10% FBS and 1 μg/ml doxy.

Reagents

Doxycycline (Research Product International), BSO (Sigma), AZD8330 (Selleckchem), BKM120 (Selleckchem), Ras activation assay kit (Millipore), G6PD activity assay kit (BioVision), and GSH/GSSG-Glo assay kit (Promega).

SUPPLEMENTAL REFERENCES

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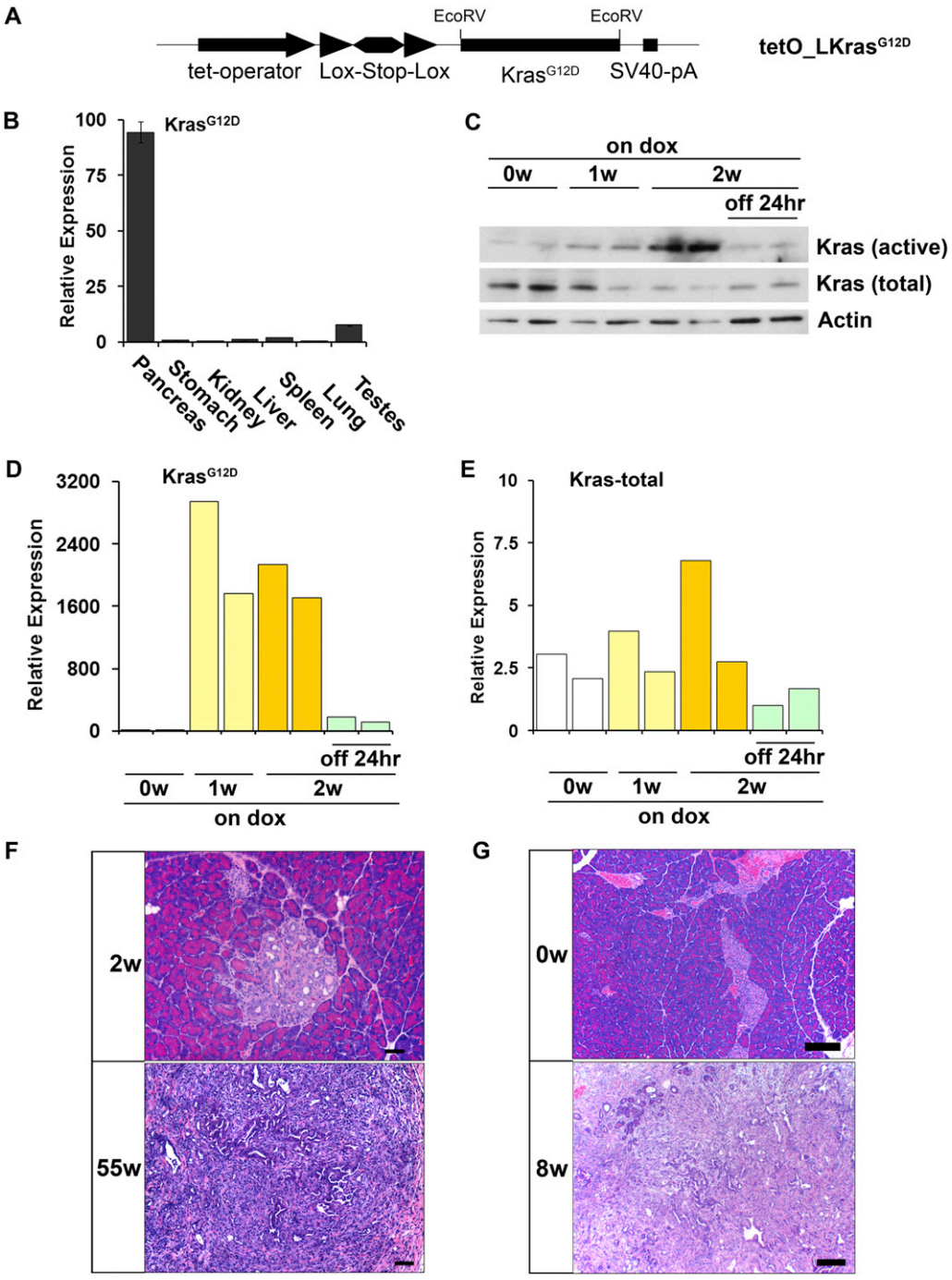

Figure S1. Characterization of the *iKras* Model, Related to Figure 1

(A) Construct for the *tetO_Lox-Stop-Lox-Kras*^{G12D} transgenic allele.

(B) *iKras* mice (n = 3) were fed with doxycycline-containing water for 2 weeks starting at 3 weeks of age. Total RNA was prepared from the indicated tissues and Kras^{G12D} expression was measured by QPCR with transgene-specific primers. Error bars represent SD of the mean.

(C) Pancreatic tissue lysate was prepared from iKras mice fed with doxycycline-containing water for the indicated time period. Pancreatic Ras activity was measured with Raf-RBD pull-down assay.

(D) Total pancreatic RNA was prepared from iKras mice fed with doxycycline-containing water for the indicated time period. *Kras*^{G12D} expression was measured by QPCR with transgene-specific primers.

(E) Expression of total *Kras* (endogenous *Kras* and *Kras*^{G12D}) in samples from (D) was measured with primers common to both the wild-type and transgenic alleles.

(F) H&E staining of pancreatic lesions for iKras mice at indicated ages.

(G) H&E staining of iKras p53$^{L/+}$ pancreas at indicated ages. Scale bar represents 100 μm.

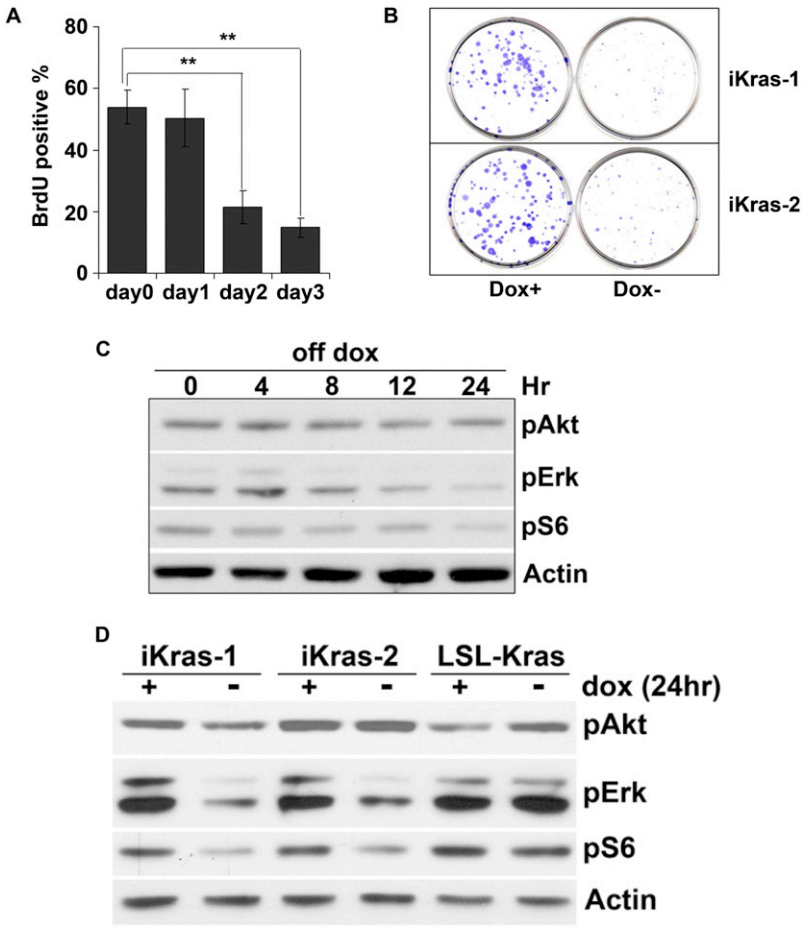

Figure S2. Growth and Downstream Signaling Changes upon Loss of Oncogenic Kras Expression, Related to Figure 2

(A) Quantitation of BrdU-positive tumor cells as shown in Figure 2A. Three mice per time point were analyzed with five random 200x fields counted per mouse. Error bars represent SD of the mean. **$p < 0.01$.

(B) Clonogenic assay *for iKras* $p53^{L/+}$ PDAC cells kept in the presence or absence of doxycycline.

(C) *iKras* $p53^{L/+}$ PDAC cells were pulled off doxycycline for indicated hours, and cell lysates were blotted for phospho-Akt, phospho-Erk, and phospho-S6.

(D) *iKras* $p53^{L/+}$ or *LSL-Kras*^{G12D} $p53^{L/+}$ PDAC cell lines were cultured in the presence or absence of doxycycline for 24 hr, and cell lysates were blotted for phospho-Akt, phospho-Erk, and phospho-S6.

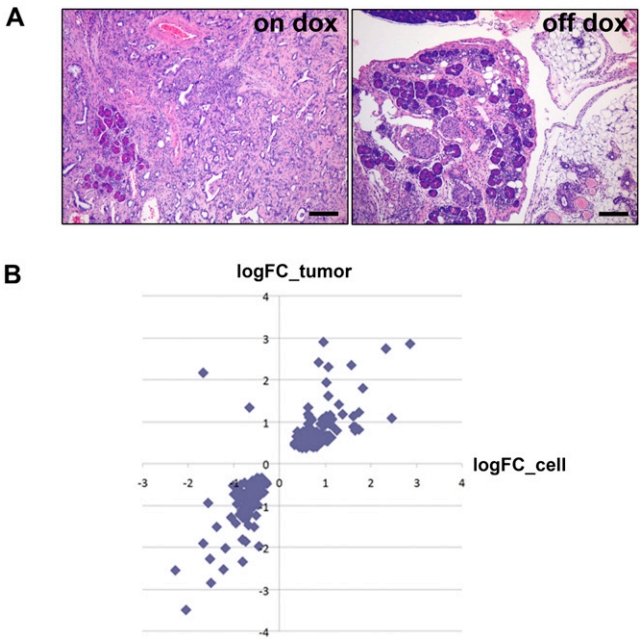

Figure S3. *iKras* Cells Grown Orthotopically in Mice Recapitulate Features of the Primary Tumors, Related to Figure 3

(A) H&E staining of orthotopic xenograft tumors generated with *iKras* $p53^{L/+}$ PDAC cells. For off dox sample, the tumor-bearing animals were pulled off doxycycline for 10 days.

(B) Correlation of expression levels of differentially expressed genes obtained from xenograft transcriptome or transcriptome of cultured parental lines upon doxycycline withdrawal.

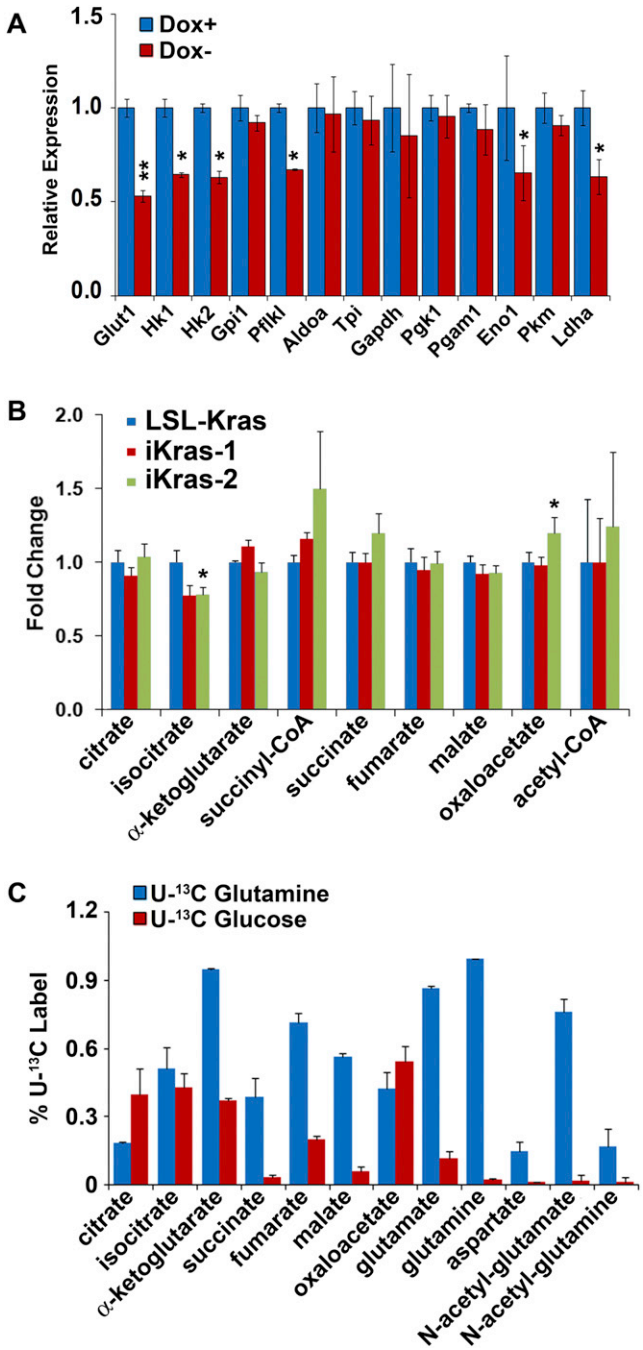

Figure S4. Metabolic Changes upon Loss of Oncogenic Kras Expression, Related to Figure 4

(A) Relative mRNA levels of glycolysis genes in the presence or absence of doxycycline for 24 hr.

(B) Fold changes of metabolites in TCA cycle upon doxycycline withdrawal for 24 hr.

(C) *iKras p53*$^{L/+}$ PDAC cells were cultured in the presence of either U-^{13}C glucose or U-^{13}C glutamine with doxycycline for 24 hr. The bar graph illustrates the percentage of uniformly isotope-labeled TCA cycle intermediates derived from either glutamine (blue) or glucose (red).

Error bars represent SD of the mean. *$p < 0.05$; **$p < 0.01$.

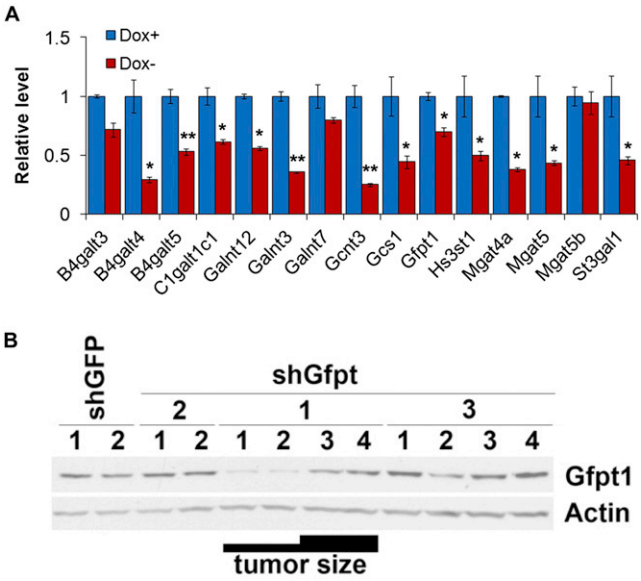

Figure S5. Oncogenic Kras Regulates Expression of Genes Involved in Glycosylation to Promote Tumor Growth, Related to Figure 5

(A) Relative mRNA levels of glycosylation genes in the presence or absence of doxycycline for 24 hr. Error bars represent SD of the mean. *$p < 0.05$; **$p < 0.01$.

(B) Xenograft tumors generated from *iKras p53*$^{L/+}$ PDAC cell lines infected with shRNA against *GFP* or *Gfpt1* were collected, and tissue lysates were blotted for Gfpt1.

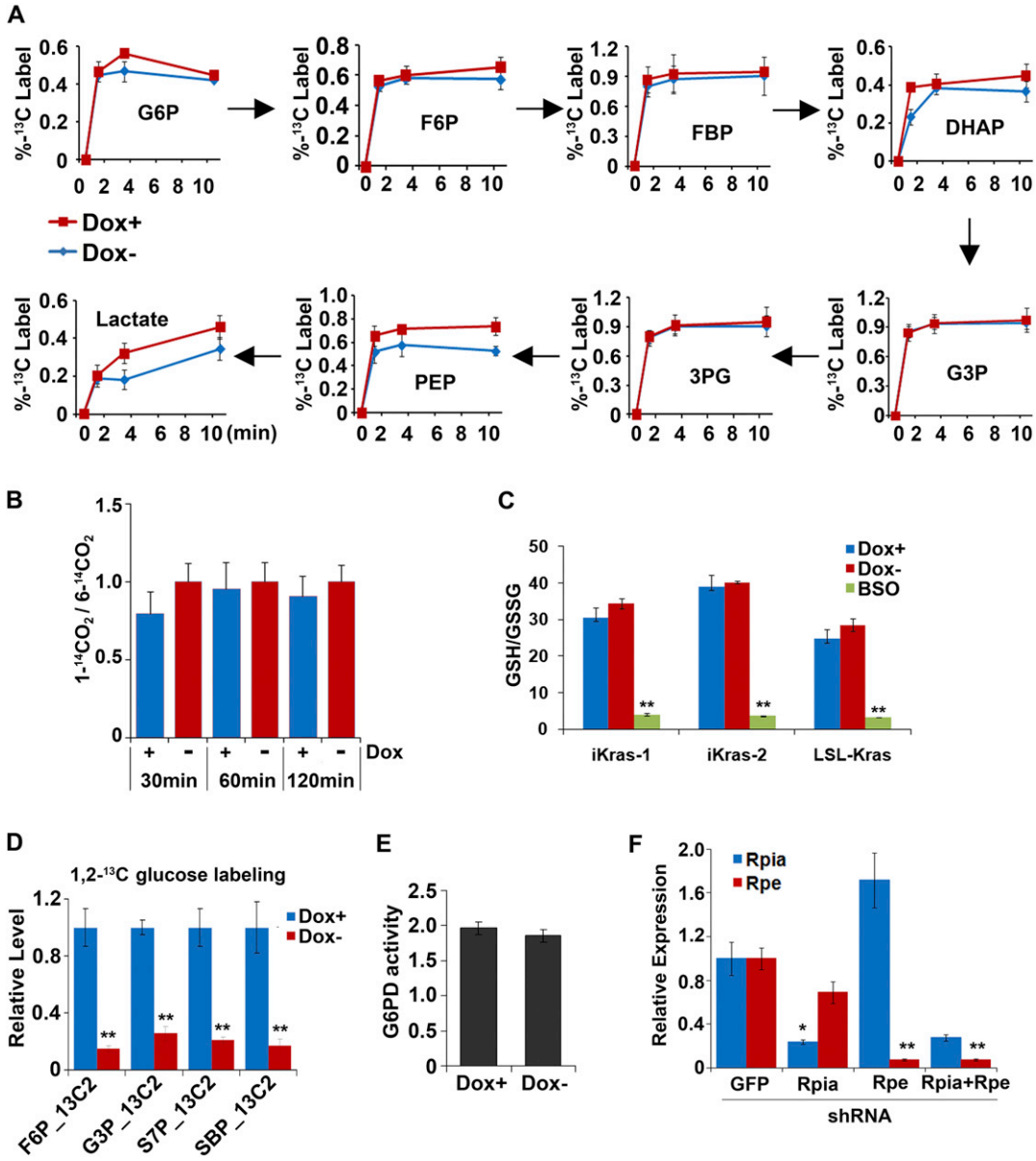

Figure S6. Oncogenic Kras Promotes Glucose Flux through the Nonoxidative PPP to Enhance Ribose Biogenesis without Impacting Cellular Redox State, Related to Figure 6

(A) *iKras p53*$^{L/+}$ cells were maintained in the presence or absence of doxycycline for 24 hr, at which point U-^{13}C glucose labeling kinetics were determined for the indicated metabolites at 1, 3, and 10 min. Data are presented as the percentage of uniformly ^{13}C-labeled metabolite per total metabolite pool at the indicated time points.

(B) *iKras p53*$^{L/+}$ cells were maintained in the presence or absence of doxycycline for 24 hr, at which point 1-^{14}C or 6-^{14}C glucose was introduced into the media. The amount of ^{14}C-labeled CO_2 released from cultured cells was measured at the indicated time points. CO_2 derived from 1-^{14}C glucose can be generated from either oxidative PPP or TCA flux, whereas CO_2 derived from 6-^{14}C glucose can only be generated from TCA flux. Data are normalized to 6-^{14}C glucose to account for potential *iKras*-mediated changes in glucose-derived TCA flux.

(C) *iKras p53*$^{L/+}$ cells were maintained in the presence or absence of doxycycline or treated with 0.5 mM BSO for 24 hr. GSH and GSSG levels were measured and normalized to cell number.

(D) *iKras p53*$^{L/+}$ cells were maintained in the presence or absence of doxycycline for 24 hr, at which point normal growth media was changed to that containing 1,2-^{13}C glucose for another 12 hr. 1,2-^{13}C glucose-incorporation into 13C2-labled G3P, F6P, S7P, and SBP were measured by targeted LC-MS/MS. Data are presented as relative metabolite abundance.

(E) *iKras p53*$^{L/+}$ cells were maintained in the presence or absence of doxycycline for 24 hr, at which point G6PD enzymatic activity was measured and normalized to cell number.

(F) Relative mRNA levels of *Rpia* or *Rpe* in *iKras p53*$^{L/+}$ PDAC cells infected with shRNA against *GFP*, *Rpia*, or *Rpe*.

Error bars represent SD of the mean. *p < 0.05; **p < 0.01.

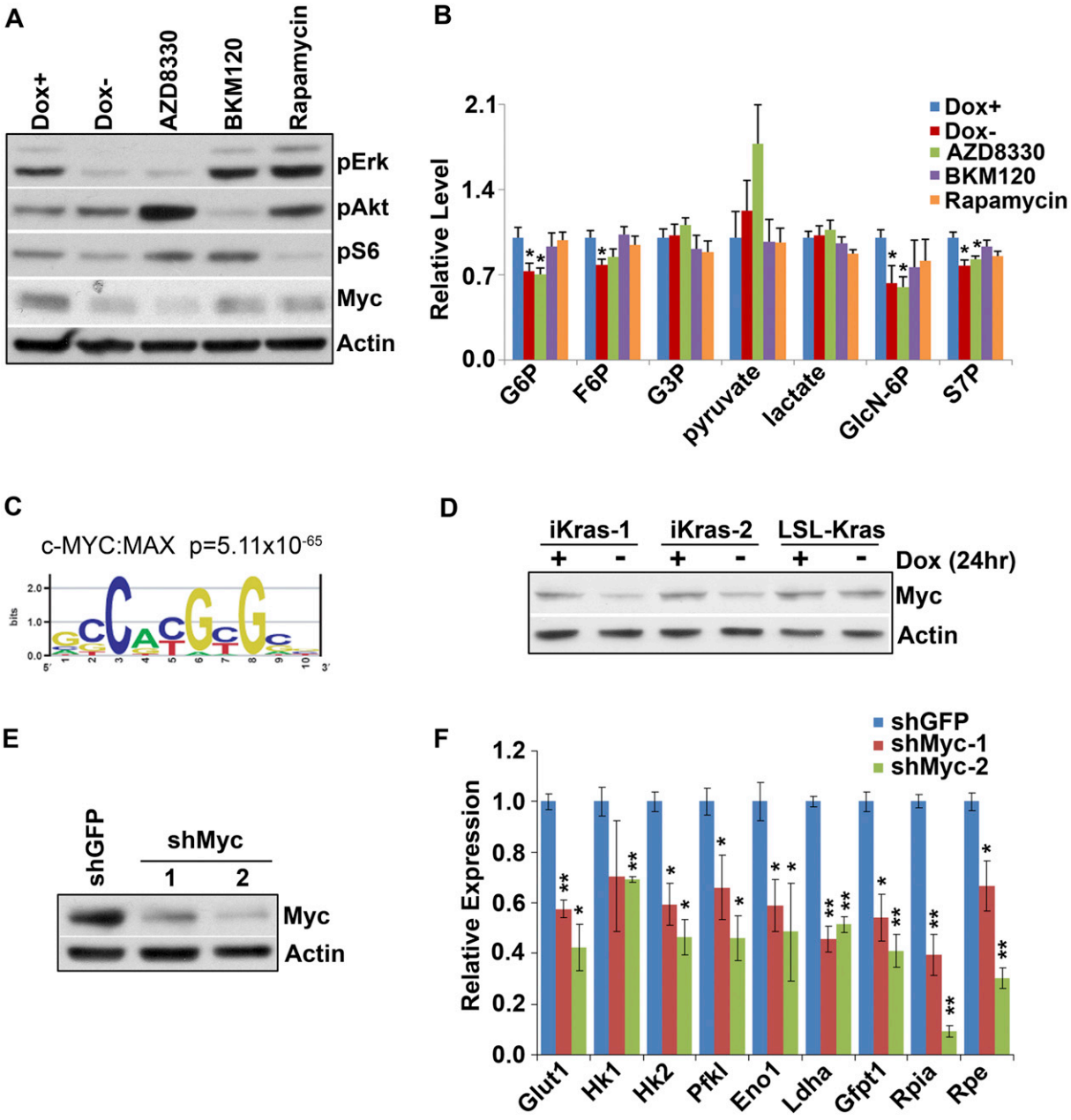

Figure S7. Kras-Induced Metabolic Reprogramming Is Dependent on MAPK and Myc, Related to Figure 7

(A and B) *iKras p53*$^{L/+}$ cells were treated with AZD8330 (50 nM), BKM120 (150 nM), or Rapamycin (20 nM) for 18 hr. As control, cells were cultured in the presence or absence of doxycycline for 24 hr, and (A) cell lysates were blotted for phospho-Akt, phospho-Erk, phospho-S6, and Myc. (B) Relative steady-state levels of indicated metabolites were measured by targeted LC-MS/MS.

(C) In silico promoter analysis of *Kras*^{G12D} inactivation transcriptome showing significant enrichment of Myc binding element.

(D) *iKras p53*$^{L/+}$ or *LSL-Kras*^{G12D} *p53*$^{L/+}$ PDAC cell lines were cultured in the presence or absence of doxycycline for 24 hr and cell lysates were blotted for Myc.

(E and F) Protein levels of Myc (E) and relative mRNA levels of metabolism genes (F) in *iKras p53*$^{L/+}$ cells infected with shRNA against *GFP* or *Myc*.

Error bars represent SD of the mean. *p < 0.05; **p < 0.01.