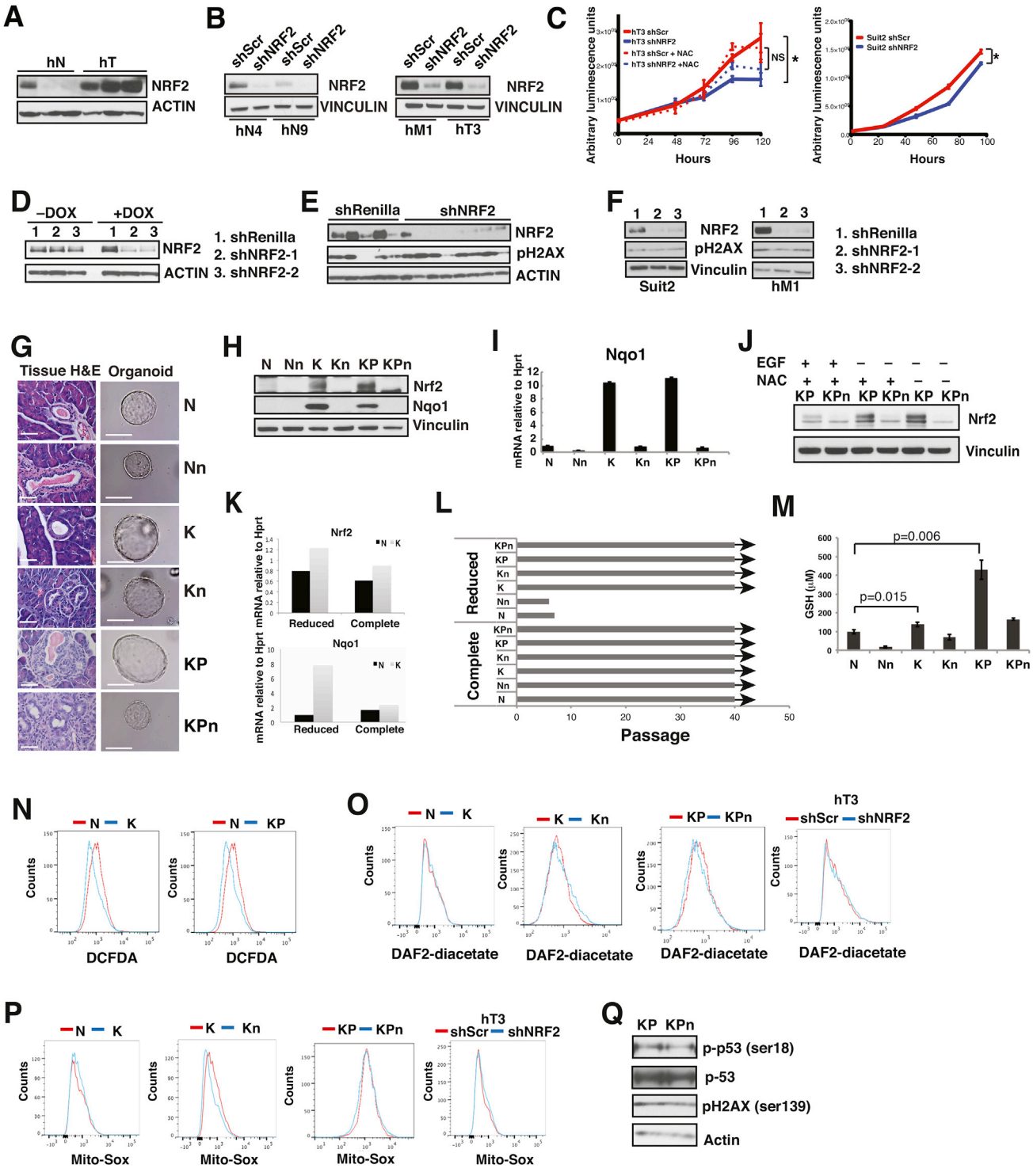


Figure S1. Oncogenic Kras Drives Expression of Nrf2 to Regulate Redox Homeostasis in Pancreatic Ductal Organoids, Related to Figure 1

(A) Immunoblot analysis of NRF2 protein in human normal (hN) and PDA (hT) organoid lines. ACTIN, loading control.

(B) Immunoblot analysis for NRF2 protein in hN and hT lines bearing shScramble or sh*NRF2*. VINCULIN, loading control.

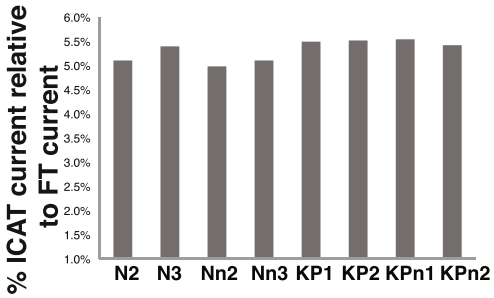
(C) Proliferation of human tumor organoids (hT3) and Suit2 cells bearing shScramble or sh*NRF2*, grown in reduced media measured by CellTiter-Glo. Data are mean ± SEM (n = 5, Student's t test).

(D) Immunoblot analysis of NRF2 protein in Suit2 cells bearing sh*Renilla* or sh*NRF2* in the presence or absence of 1 μg/ml doxycycline for 48 hr.

(legend continued on next page)

(E) Immunoblot analysis of subcutaneous Suit2 tumors bearing sh*Renilla* (n = 5) or sh*NRF2* (n = 8) harvested 8 days post doxycycline administration in drinking water
(F) Immunoblot analysis of Suit2 cells and human PDA organoids bearing sh*Renilla* or sh*NRF2* 48 hr post 1 μg/ml doxycycline treatment.
(G) Early passage murine pancreatic ductal organoids (scale bars, 100 μm) prepared from tissues of 8 week-old mice (scale bars, 50 μm). Representative images from 3 biological replicates.
(H) Immunoblot analysis of Nrf2 and Nqo1 levels in pancreatic organoids. Vinculin, loading control.
(I) qRT-PCR analysis of mRNA expression of a Nrf2 target gene, *Nqo1*, in passage one pancreatic ductal organoids. Data are mean ± s.d. (n = 3)
(J) Immunoblot analysis of Nrf2 levels in KP and KPn organoids under different media conditions.
(K) qRT-PCR of *Nrf2* and *Nqo1* in N and K pancreatic ductal organoids grown in reduced media (no EGF and NAC) compared to complete media. Data are normalized to *Hprt* expression level.
(L) Passaging capacity of murine organoids in reduced media compared to complete media. (average of 2 biological replicates)
(M) Levels of reduced glutathione (GSH) by mass spectrometry of organoids lysed and processed in anoxia. Data are mean ± s.d. (n = 3). Student's t test.
(N) ROS levels in N, K and KP organoids measured by flow cytometry using CM-H2DCFDA. Representative from 3 biological replicates.
(O) Reactive nitrogen species levels in organoids measured by flow cytometry using DAF2-diacetate. Representative from 3 biological replicates.
(P) Mitochondrial superoxide levels in organoids measured by flow cytometry using Mito-SOX. Representative from 3 biological replicates.
(Q) Immunoblot analysis of markers for DNA damage response.

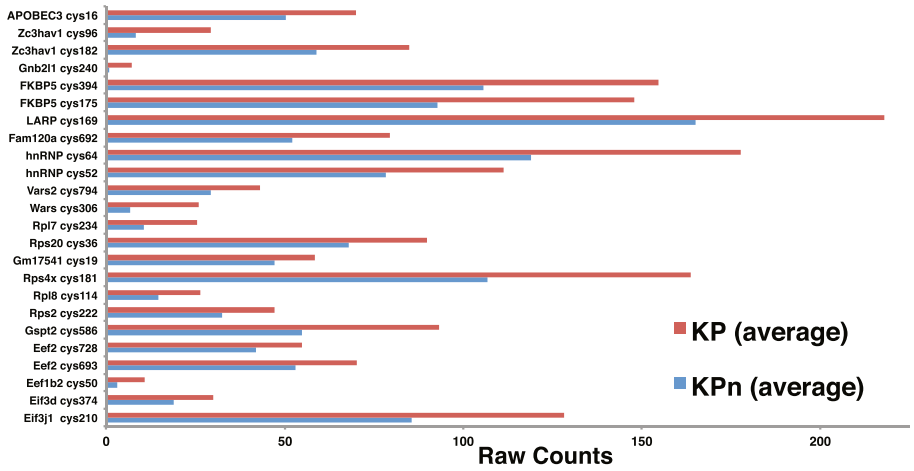
A



B

Protein	Relation to Translation
hnRNPK	mRNA metabolism and translation (stress response) (Ostareck-Lederer et al MCB, 2002, Shen et al, Protoemics 2008, Fukuda et al, Genes Cells 2009)
Fam120A	Binds polyA tail, link to PI3K signaling (Tanaka et al, MCB, 2009)
LARP	Regulates TOP mRNA translation through mTOR (Fonseca et al, JBC, 2014, Tcherkezian et al, G&D, 2014)
FKBP5	Intracellular receptor to Rapamycin. Selection biomarker for Gem and Akt in treatment of PDA (Hou and Wang, Plos One 2012)
Gnb2l1	Implicated in stress response and translation regulation (Nilsson et al, EMBO Rep, 2004)
Zc3hav1	Suppressor of translation. Blocks eIF4A and eIF4G binding (Zhu et al, EMBO J 2012)
Apobec3, KSRP	RNA modification and processing (Wichroski et al, Plos Pathog 2006; Trabucchi et al, Nature 2009)

C



D

Category	Term	Count	%	PValue	Fold Enrichm	Bonferroni	Benjamini	FDR
GOTERM_BP_FAT	GO:0006412~translation	57	7.63052209	9.37E-20	4.1291664	1.83E-16	1.83E-16	1.61E-16
GOTERM_BP_FAT	GO:0006396~RNA processing	63	8.43373494	5.10E-17	3.33148088	9.96E-14	4.98E-14	8.74E-14
GOTERM_BP_FAT	GO:0006091~generation of precursor metabolites and energy	41	5.48862115	2.31E-12	3.63012485	4.50E-09	1.50E-09	3.95E-09
GOTERM_BP_FAT	GO:0006397~mRNA processing	40	5.35475234	1.10E-11	3.52806772	2.15E-08	5.39E-09	1.89E-08
GOTERM_BP_FAT	GO:0008380~RNA splicing	34	4.55153949	3.07E-11	3.90895861	6.00E-08	1.20E-08	5.27E-08
GOTERM_BP_FAT	GO:0016071~mRNA metabolic process	42	5.62248996	6.36E-11	3.21381268	1.24E-07	2.07E-08	1.09E-07
GOTERM_BP_FAT	GO:0006457~protein folding	26	3.48058902	1.50E-10	4.73094435	2.92E-07	4.18E-08	2.57E-07
GOTERM_BP_FAT	GO:0034660~ncRNA metabolic process	33	4.41767068	1.60E-10	3.77520711	3.12E-07	3.90E-08	2.74E-07
GOTERM_BP_FAT	GO:0045333~cellular respiration	18	2.40963855	2.58E-10	7.05015566	5.04E-07	5.60E-08	4.43E-07
GOTERM_BP_FAT	GO:0015980~energy derivation by oxidation of organic compounds	22	2.94511379	8.73E-10	5.18769957	1.70E-06	1.70E-07	1.50E-06
GOTERM_BP_FAT	GO:0055114~oxidation reduction	64	8.56760375	4.41E-09	2.20084224	8.61E-06	7.83E-07	7.56E-06
GOTERM_BP_FAT	GO:0043038~amino acid activation	15	2.00803213	4.51E-09	7.53549246	8.81E-06	7.34E-07	7.73E-06
GOTERM_BP_FAT	GO:0043039~tRNA aminoacylation	15	2.00803213	4.51E-09	7.53549246	8.81E-06	7.34E-07	7.73E-06
GOTERM_BP_FAT	GO:0006418~tRNA aminoacylation for protein translation	15	2.00803213	4.51E-09	7.53549246	8.81E-06	7.34E-07	7.73E-06
GOTERM_BP_FAT	GO:0046907~intracellular transport	45	6.02409639	1.00E-07	2.41275629	1.95E-04	1.50E-05	1.72E-04
GOTERM_BP_FAT	GO:0006006~glucose metabolic process	23	3.0789826	1.39E-07	3.79645287	2.71E-04	1.93E-05	2.38E-04
GOTERM_BP_FAT	GO:0019320~hexose catabolic process	14	1.87416332	2.15E-07	6.22161172	4.19E-04	2.80E-05	3.68E-04
GOTERM_BP_FAT	GO:0006007~glucose catabolic process	14	1.87416332	2.15E-07	6.22161172	4.19E-04	2.80E-05	3.68E-04
GOTERM_BP_FAT	GO:0019318~hexose metabolic process	25	3.34672021	2.67E-07	3.41846798	5.21E-04	3.26E-05	4.57E-04
GOTERM_BP_FAT	GO:0051186~cofactor metabolic process	26	3.48058902	2.89E-07	3.30126336	5.64E-04	3.32E-05	4.95E-04

E

Protein Name	
G elongation factor, mitochondrial 1	eukaryotic translation initiation factor 4B
G1 to S phase transition 1	glycyl-tRNA synthetase
alanyl-tRNA synthetase	immature colon carcinoma transcript 1
aminoacyl tRNA synthetase complex-interacting multifunctional protein	isoleucine-tRNA synthetase 2, mitochondrial
asparaginyl-tRNA synthetase	leucyl-tRNA synthetase
aspartyl-tRNA synthetase	mitchondrial ribosomal protein S7
cysteinyl-tRNA synthetase	mitochondrial ribosomal protein L16
eukaryotic translation elongation factor 2	mitochondrial ribosomal protein L39
eukaryotic translation elongation factor 1 beta 2	phenylalanyl-tRNA synthetase, alpha subunit
eukaryotic translation initiation factor 2, subunit 3	ribosomal protein L34
eukaryotic translation initiation factor 2B, subunit 5 epsilon	ribosomal protein L37
eukaryotic translation initiation factor 3, subunit D	ribosomal protein S11
eukaryotic translation initiation factor 3, subunit F	seryl-aminoacyl-tRNA synthetase
eukaryotic translation initiation factor 3, subunit J	threonyl-tRNA synthetase
eukaryotic translation initiation factor 4A1	tyrosyl-tRNA synthetase 2 (mitochondrial)

(legend on next page)

Figure S2. Oxidation of Cysteine Residues on Proteins Involved in Translational Regulation upon Nrf2 Deficiency, Related to Figure 2

(A) Percentage of normalized oxidized peptides (ICAT/iTRAQ) in N, Nn, KP and KPn organoids (data shown from one 8-plex run). FT, iTRAQ labeled flow through.

(B) Proteins implicated in translational regulation are among those exhibiting significant cysteine oxidations in KPn organoids.

(C) Merged cysteine peptide counts of selected proteins involved in translational regulation, normalized to total protein expression level in KP (red) and KPn (blue) organoids, demonstrating a decrease in reduced cysteine peptide counts in KPn organoids. Average of 2 biological replicates

(D) DAVID gene ontology analysis for pathway enrichment of significantly oxidized peptides in T organoids treated with 100 μM BSO compared to untreated controls (data from 2 biological replicates).

(E) List of proteins in the core translation machinery that are oxidized in BSO treated T organoids.

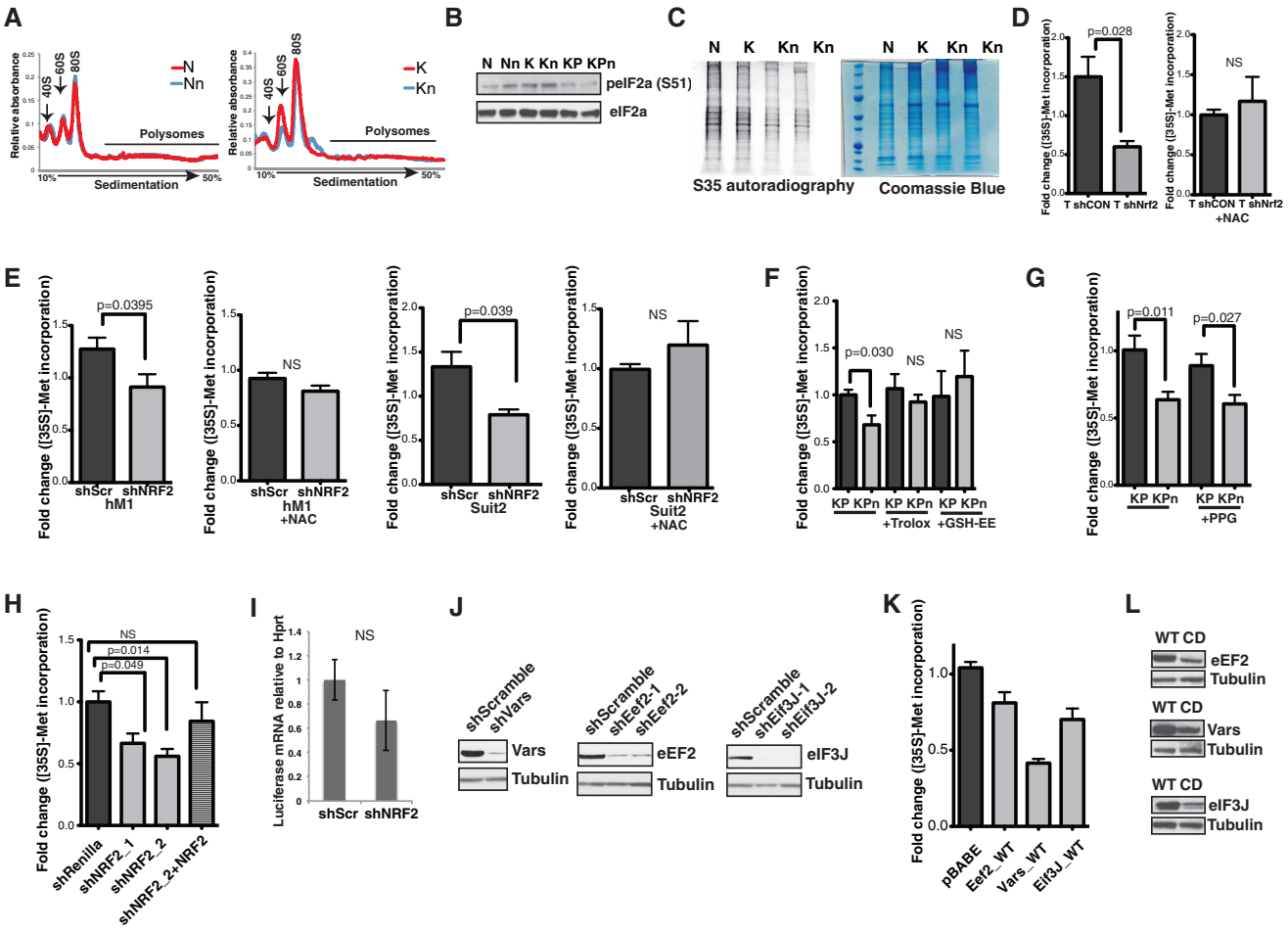


Figure S3. Nrf2 Deficiency Impairs Protein Synthesis through Impairing Redox Homeostasis, Related to Figure 3

(A) Polysome profiles of N, Nn organoids (left panel) and K, Kn organoids (right panel) treated with 100 μg/ml cycloheximide for 10 min. Absorbance light at 254 nm.

(B) Immunoblot analysis of eIF2 activation in murine organoids.

(C) [^{35}S]-Met incorporation into protein from murine organoids. Total protein extracts were resolved by SDS-PAGE and visualized by Coomassie Blue staining (right panel) or autoradiography (left panel).

(D) [^{35}S]-Met incorporation into proteins from murine tumor organoids (T) bearing shScr or sh*Nrf2* cultured in reduced media or media supplemented with 1.25 mM NAC. Data are mean ± SEM (n = 3, Student's t test).

(E) [^{35}S]-Met incorporation into protein from human tumor organoids or Suit2 cells bearing shScr or sh*NRF2*, cultured in reduced media or media supplemented with 1.25 mM NAC. Data are mean ± SEM (n = 3, Student's t test).

(F) [^{35}S]-Met incorporation into protein from KP and KPn organoids, cultured in reduced media for 48hrs after treatment with 250 μM Trolox or 1 mM GSH ethyl ester (EE). Data are mean ± SEM (n = 3, Student's t test).

(G) [^{35}S]-Met incorporation into proteins from KP and KPn organoids pretreated for 24 hr with 2.5 mM propargylglycine (PPG). Data are mean ± SEM (n = 3, Student's t test).

(H) [^{35}S]-Met incorporation into protein from human tumor organoids bearing sh*Renilla*, sh*NRF2* and *NRF2* cDNA, cultured in reduced media for 48hrs after treatment with 1 μg/ml doxycycline. Data are mean ± SEM (n = 3, Student's t test).

(I) qPCR analysis of firefly luciferase mRNA level in transfected Suit2 cells. Data are mean ± s.d. (n = 3, Student's t test). NS = not significant.

(J) Immunoblot analysis of murine tumor organoids bearing shRNA targeted against the indicated translation regulatory proteins. Tubulin, loading control.

(K) [^{35}S]-Met incorporation into proteins from KP expressing pBABE or ectopically expressing the indicated translation factors. Data are mean ± SEM (n = 3).

(L) Immunoblot analysis of KP organoids expressing wild-type (WT) and cysteine to aspartic acid mutants (CD) of indicated translation factors. Tubulin, loading control.

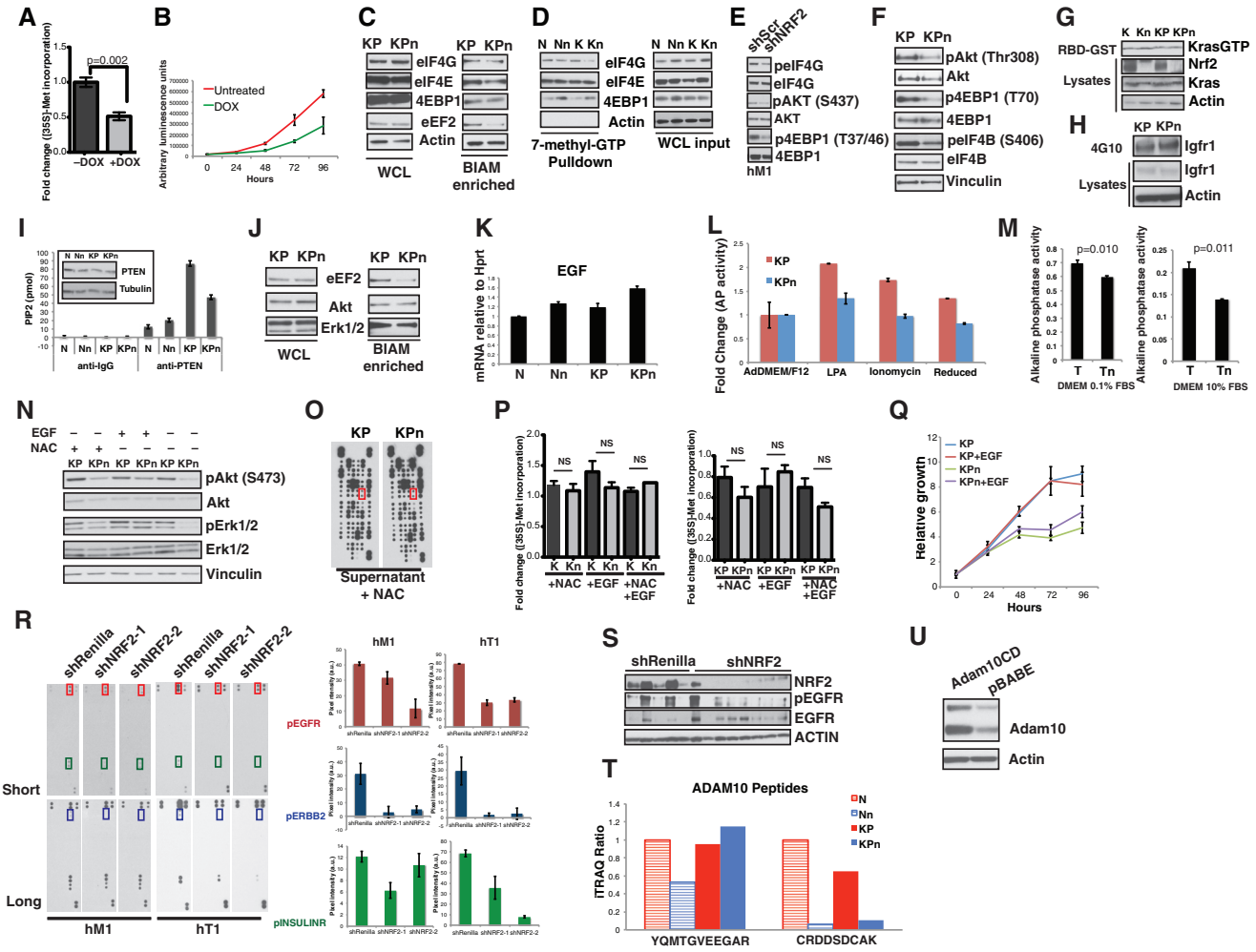


Figure S4. Nrf2 Deficiency Impairs Growth Factor Signaling Cascades Upstream of eIF4F Complex Formation and Cap-Dependent Translation, Related to Figure 4

(A) [^{35}S]-Met incorporation into proteins from KP organoids ectopically expressing 4EBP1-4A mutant (Thoreen et al., 2012), with or without 1 μg/ml doxycycline treatment for 48 hr. Data are mean ± SEM (n = 3, Student's t test).

(B) Proliferation of KP organoids stably expressing 4EBP1-4A mutant in the presence or absence of 1 μg/ml doxycycline. Data are mean ± SEM (n = 5).

(C) Lysates from KP and KPn organoids were subjected to (N-(Biotinoyl)-N'-(Iodoacetyl)Ethylenediamine) (BIAM) enrichment, and analyzed for the indicated proteins. Oxidation of eEF2 serves as positive control.

(D) Lysates from N, Nn, K and Kn organoids were subjected to 7-methyl-GTP pull-downs, and analyzed for indicated proteins.

(E) Immunoblot analysis for growth factor signaling pathway activation in human PDA oragnoids (hM1), bearing shScr or sh*NRF2*. p = phospho.

(F) Immunoblot analysis for growth factor signaling pathway activation in KP and KPn organoids. p = phospho.

(G) Immunoblot analysis for Kras and Kras-GTP by RBD-GST pull-down in K, Kn, KP and KPn organoids.

(H) Immunoblot analysis for IGF1 receptor tyrosine phosphorylation in KP and KPn organoids. 4G10, phospho-tyrosine antibody.

(I) PTEN phosphatase activity measured by quantification of the amount of PI(4,5)P2 (PIP2) converted from PI(3,4,5)P3 by immunoprecipitated PTEN from organoids. Absorbance light at 450 nm. Data are mean ± s.d. (n = 3). Inset, Immunoblot analysis of Pten protein levels in murine organoids.

(J) Lysates from KP and KPn organoids were subjected to (N-(Biotinoyl)-N'-(Iodoacetyl)Ethylenediamine) (BIAM) enrichment, and analyzed for the indicated proteins. eEF2 serves as positive control.

(K) qRT-PCR analysis of *Egf* mRNA expression normalized to *Hprt*. Data are mean ± s.d. (n = 3).

(L) KP and KPn organoids were cultured in reduced media or treated for 1hr with 10 μg/ml lysophosphatidic acid (LPA) or 10 μM ionomycin in Advanced DMEM/F12 to induce ectodomain shedding. Induced EGF shedding was determined by the ratio of alkaline phosphatase activity in the supernatant relative to total alkaline phosphatase activity in the supernatant and the cell lysates in KP and KPn organoids transiently expressing EGF-alkaline phosphatase fusion protein (n = 2). Data are presented as fold increase relative to cells grown in Advanced DMEM/F12.

(M) Constitutive EGF shedding determined by alkaline phosphatase activity in the supernatant from monolayer murine KPC tumor cell lines (T). Data are mean ± s.d. (n = 3, Student's t test).

(N) Immunoblot analysis for growth factor signaling pathway activation in KP and KPn organoids.

(O) Protein array analysis for growth factors secreted into culture medium in KP and KPn organoids supplemented with 1.25 mM NAC. Murine EGF, red box. (n = 2)

(legend continued on next page)

(P) [^{35}S]-Met incorporation into protein from murine organoids grown in reduced media, reduced media supplemented with 1.25 mM NAC, reduced media supplemented with 50ng/ml EGF, or reduced media supplemented with both NAC and EGF, pulsed for 30 min with [^{35}S]-Met. Data are mean +/ s.d. (n = 3, Student's t test).

(Q) Proliferation of KP and KPn organoids grown in reduced media or media supplemented with 50 ng/ml murine EGF, measured by CellTiter-Glo over four days. Data are mean ± SEM (n = 5).

(R) Phospho-receptor tyrosine kinase arrays from human PDA organoids expressing short hairpin against *Renilla* luciferase or *NRF2*, 48hrs after doxycycline treatment. 300 μg cell lysates were used per array. Pixel intensities quantified by ImageJ (right). Data are mean ± s.d. (n = 2).

(S) Immunoblot analysis of EGFR activation status in Suit2 tumors expressing shRenilla (n = 5) or sh*NRF2* (n = 8), from mice administered with doxycycline water for 8 days. ACTIN, loading control. (NRF2 and ACTIN immunoblot same as in Figure S1E as a reference).

(T) iTRAQ peptide counts of immunoprecipitated Adam10. Representative non-cysteine containing peptide and cysteine-containing peptide in disintegrin domain shown.

(U) Immunoblot analysis of Adam10 in KP organoids. CD, cysteine to aspartic acid mutant expressed in pBABE vector.

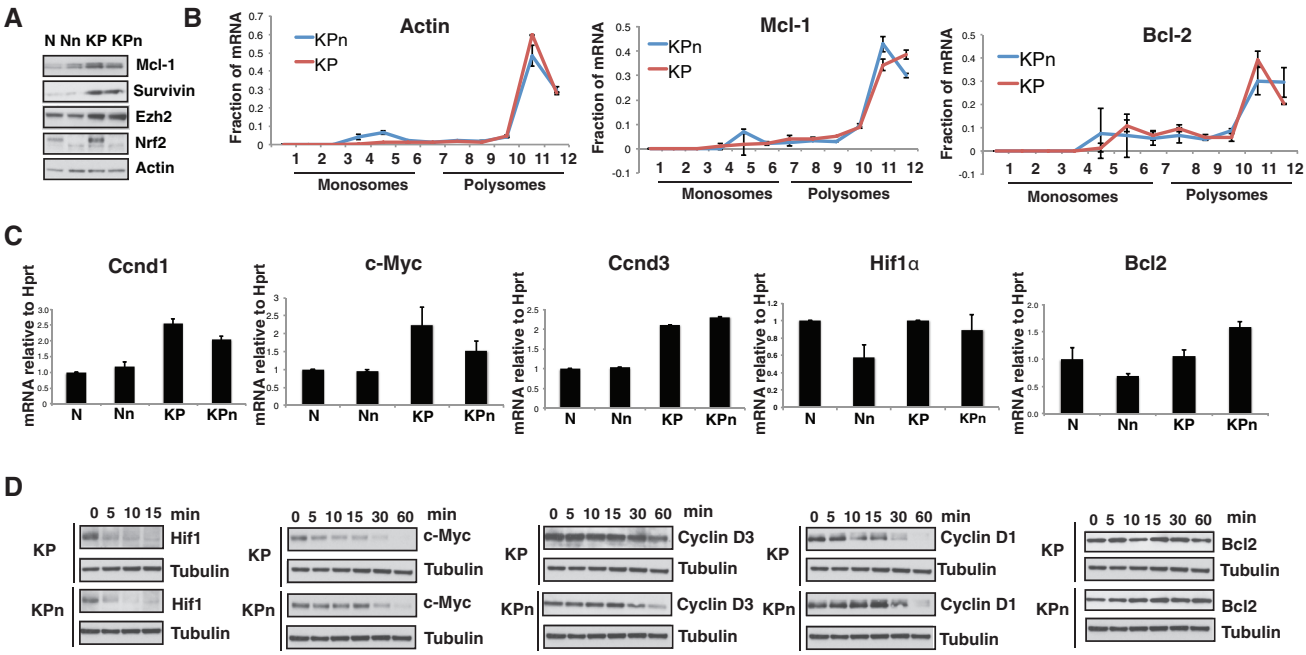


Figure S5. Nrf2 Promotes mRNA-Specific Translation, Related to Figure 5

(A) Immunoblot analysis of oncoproteins in murine organoids. (Nrf2 and actin blots same as Figure 5A for reference).

(B) Polysome qRT-qPCR analysis of *Actin*, *Mcl-1* and *Bcl2* mRNA in KP and KPn organoids. ΔCT calculated against 80S fraction. Data are means normalized against the sum ± s.d. (n = 2 biological replicates). Fractions 1-6: Monosomes. Fractions 7-12: Polysomes.

(C) qRT-qPCR analysis of the expression of indicated mRNA transcripts in organoids normalized to *Hprt*. Data are mean ± s.d. (n = 3).

(D) KP and KPn organoids were exposed to cycloheximide (10 μg/ml) for the indicated times (in minutes). Protein levels were analyzed by immunoblotting for Hif1, c-Myc, CyclinD3, CyclinD1, Bcl2 and Tubulin.

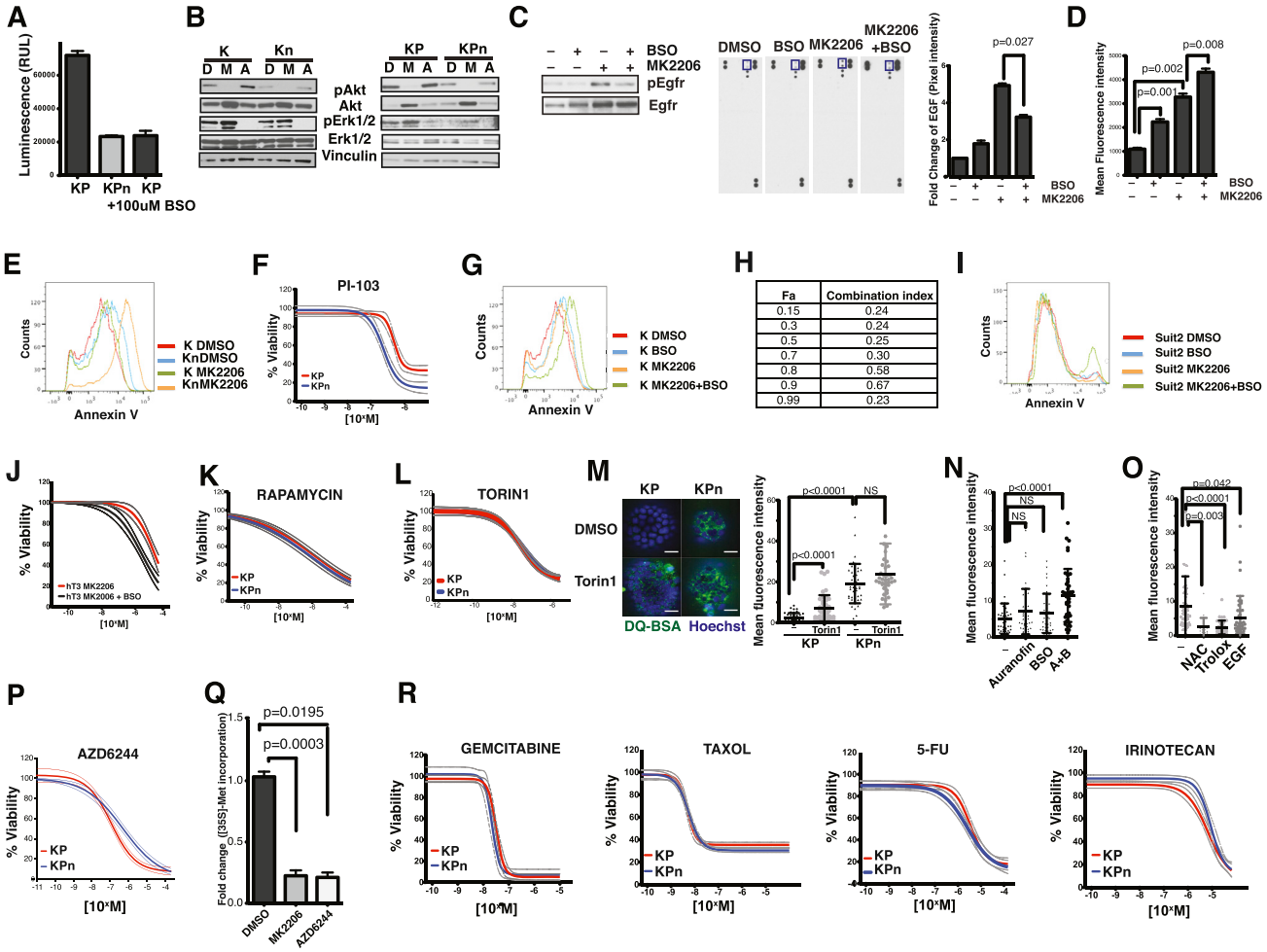


Figure S6. Nrf2 Deficiency Sensitizes Kras Mutant Cells to Inhibitors of Protein Synthesis, Related to Figure 6

(A) Quantification of reduced glutathione by GSH-glo luminescence in KP, KPn and KP organoids treated with 100 μM buthionine sulfoximine (BSO) for 48 hr. Data are mean ± s.d. (n = 3).

(B) Immunoblot analysis of growth factor signaling pathway changes in K, Kn organoids (left) or KP, KPn organoids (right) treated with vehicle DMSO (D), 1 μM pan-AKT inhibitor MK2206 (M) or 1 μM MEK inhibitor AZD6244 (A) for 48 hr.

(C) Immunblot (left) and phospho-receptor tyrosine kinase arrays (middle) from KP organoids under treatment with vehicle only, 1 μM MK2206, 100 μM BSO, or in combination for 48 hr. 100 μg cell lysates were used per array. Blue box, pEGFR. Pixel intensities quantified by ImageJ (right). Data are mean ± s.d. (n = 2, Student's t test).

(D) ROS levels in KP organoids under treatment with vehicle only (DMSO), 1 μM MK2206, 100 μM BSO, or in combination for 48 hr, measured by flow cytometry using the CM-H2DCFDA dye. Data are mean ± SEM (n = 3, Student's t test).

(E) Flow cytometric analysis of apoptosis through Annexin V staining in K and Kn organoids treated with vehicle (DMSO) or 1 μM AKT inhibitor (MK2206) for 48 hr. Representative data from 2 biological replicates.

(F) Cell viability of KP and KPn organoids over increasing concentrations of PI-103 for 72 hr. Dotted lines, 95% confidence intervals. (n = 5).

(G) Flow cytometric analysis of apoptosis through Annexin V staining in K organoids treated with vehicle (DMSO), 1 μM MK2206, 100 μM BSO, or in combination, for 48 hr. Representative data from 2 biological replicates.

(H) Combination index analysis to determine synergy between MK2206 and BSO, carried out using the Calcusyn software as described under Extended experimental procedures.

(I) Flow cytometric analysis of apoptosis through Annexin V staining in Suit2 cells treated with vehicle (DMSO), 1 μM MK2206, 100 μM BSO, or in combination, for 48 hr. Representative data from 2 technical replicates.

(J) Cell viability of human organoids (hT3) determined by CellTiter-Glo after treatment with increasing doses of the AKT inhibitor MK2206 in combination with buthionine sulfoximine (BSO) for 72 hr. Dotted lines, 95% confidence intervals. (n = 5).

(K and L) Cell viability of KP and KPn organoids over increasing concentrations of Rapamycin (K) or Torin1 (L) for 72 hr. Dotted lines, 95% confidence intervals. (n = 5).

(M) Lysosomal degradation of DQ-BSA in KP and KPn organoids after 12 hr DQ-BSA uptake in the presence or absence of 250 nM Torin1. Quantification of DQ-BSA fluorescence of cells on right panel. Data are mean ± s.d. (n ≥ 5 fields of view with > 20 cells each, 2 biological replicates, Student's t test).

(N) Quantification of DQ-BSA fluorescence of KP organoids treated with 250 nM Auranofin, 100 μM BSO, or in combination, for 48 hr. Data are mean ± s.d. (n ≥ 5 fields of view with > 20 cells each, 2 biological replicates, Student's t test).

(legend continued on next page)

(O) Quantification of DQ-BSA fluorescence of KPn organoids treated with 1 mM NAC, 250 μM Trolox, or 50 ng/ml EGF, for 48 hr. Data are mean ± s.d. (n ≥ 5 fields of view with > 20 cells each, 2 biological replicates, Student's t test).
(P) Dose response curves of KP and KPn organoids after treatment with increasing doses of AZD6244 for 72 hr. Dotted lines, confidence intervals. (n = 5).
(Q) [^{35}S]-Met incorporation into protein from murine KP organoids treated with 1 μM MK2206 or 1 μM AZD6244 for 72hrs, pulsed for 30 min with [^{35}S]-Met. Data are mean +/ SEM (n = 3, Student's t test).
(R) Dose response curves of KP and KPn organoids after treatment with increasing doses of chemotherapeutics for 72 hr. Dotted lines, confidence intervals. (n = 5).

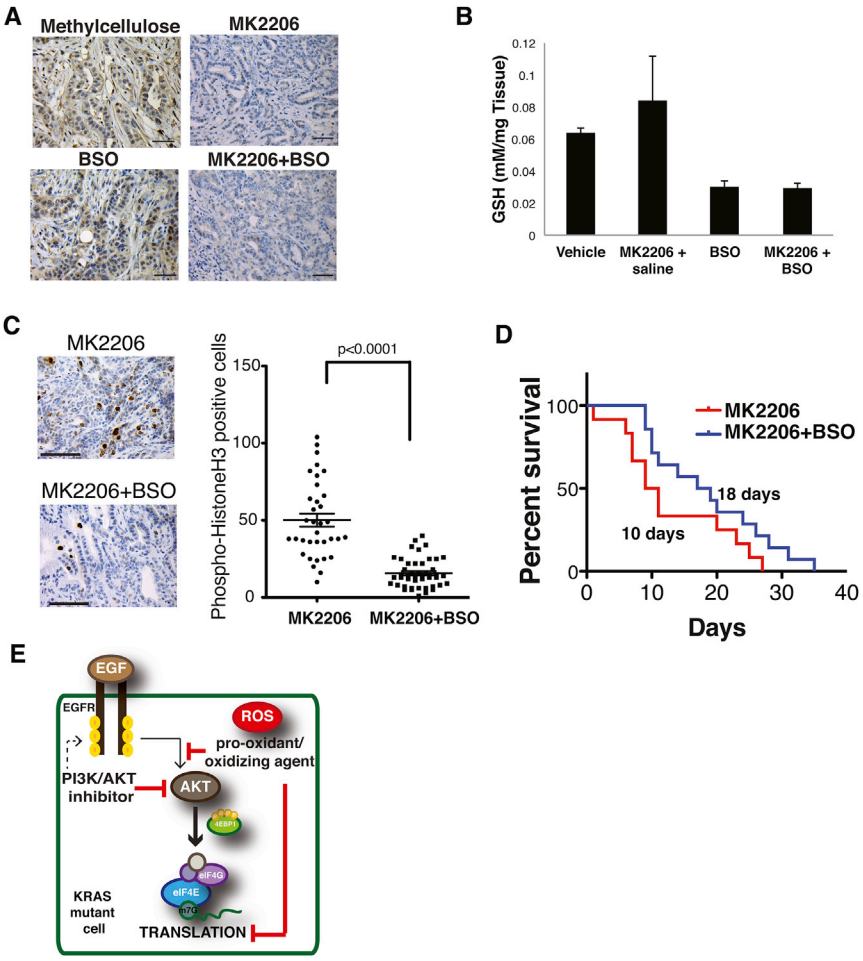


Figure S7. Combined Inhibition of AKT and GSH Synthesis in PDA, Related to Figure 7

(A) Immunohistochemistry of phospho-Akt in tumor tissues from KPC mice treated with vehicle (methylcellulose), BSO only, MK2206 only, or in combination, for 7 days. Scale bar, 50 μm. Representative image from 5 samples (methylcellulose, BSO) and 11 samples (MK2206, MK2206+BSO).

(B) Levels of reduced glutathione per mg of tissue by mass spectrometry. Tumor tissues from KPC mice treated with vehicle (methylcellulose), BSO only, MK2206 only, or in combination, for 7 days were snap frozen and lysed in anoxia. Date are mean ± s.d., n = 3 individual samples per treatment group.

(C) Phospho-histone H3 immunohistochemistry of representative KPC tumors from mice treated with MK2206 or MK2206+BSO at humane endpoint (Left). Quantification of pH 3 positivity (Right). Data are mean ± s.e.m (n ≥ 5 fields of view). Student's t test.

(D) Kaplan-Meier survival analysis for KPC mice treated with MK2206 (n = 12) or MK2206+BSO (n = 14). p = 0.088 by Mantel-Cox (log rank) test.

(E) Model. Solid line, pathway activation; dotted line, pathway adaptive response upon AKT inhibition. m7G, 5′7-methyl guanosine cap of mRNAs.

Cell, Volume *166*

Supplemental Information

NRF2 Promotes Tumor Maintenance by Modulating mRNA Translation in Pancreatic Cancer

Iok In Christine Chio, Seyed Mehdi Jafarnejad, Mariano Ponz-Sarvise, Youngkyu Park, Keith Rivera, Wilhelm Palm, John Wilson, Vineet Sangar, Yuan Hao, Daniel Öhlund, Kevin Wright, Dea Filippini, Eun Jung Lee, Brandon Da Silva, Christina Schoepfer, John Erby Wilkinson, Jonathan M. Buscaglia, Gina M. DeNicola, Herve Tiriac, Molly Hammell, Howard C. Crawford, Edward E. Schmidt, Craig B. Thompson, Darryl J. Pappin, Nahum Sonenberg, and David A. Tuveson

Animals

Trp53$^{+/LSL\text{-}R172H}$, *Kras*$^{+/LSL\text{-}G12D}$, Nrf2$^{-/-}$ (Chan et al., 1996) and *Pdx1-Cre* strains in 129J/C57Bl/6 background were interbred to obtain *Pdx1-Cre; Kras*$^{+/LSL\text{-}G12D}$ (KC), *Pdx1-Cre; Kras*$^{+/LSL\text{-}G12D}$*; Nrf2*$^{-/-}$ (KCn), *Pdx1- Cre; Kras*$^{+/LSL\text{-}G12D}$*; Trp53*$^{+/LSL\text{-}R172H}$ (KPC) (Hingorani et al., 2005), and *Pdx1- Cre; Kras*$^{+/LSL\text{-}G12D}$*; Trp53*$^{+/LSL\text{-}R172H}$*; Nrf2*$^{-/-}$ (KPCn) mice. C57Bl/6 mice were purchased from the Jackson Laboratory. All animal experiments were conducted in accordance with procedures approved by the IACUC at Cold Spring Harbor Laboratory (CSHL).

Xenograft model

1 x 10^5 Human Suit2 cells transduced with lentivirus expressing sh*Renilla* or two independent hairpin sequences against *NRF2* were subcutaneously transplanted into the flanks of athymic nude mice (7 weeks old, males). 21 days after transplantation and tumor establishment, mice were given doxcycyline (2 mg/ml) in drinking water supplemented with sucrose (10 mg/ml) for the first week and then switched to dox chow until the end of the experiment. Tumor volume was monitored by caliper measurements using the following formula: tumor volume [mm^3] = (length [mm]) x (width [mm]) x (height [mm]) x (π/6).

Organoid isolation and culture

Detailed procedures to isolate and propagate mouse and human, normal and neoplastic pancreatic organoids have been described previously (Boj et al., 2015; Huch et al., 2013). In brief, normal pancreatic mouse ducts were manually picked after enzymatic digestion of pancreas with 0.012% (w/v) collagenase XI (Sigma) and 0.012% (w/v) dispase (GIBCO) in DMEM media containing 1% FBS (GIBCO) and were seeded in growth factor-reduced (GFR) Matrigel (BD). For tumors (mT), bulk tissues were minced and digested overnight with collagenase XI and dispase and embedded in GFR Matrigel. In the case of human primary and metastatic pancreatic tumor organoid cultures (hT), tumor tissue was minced and digested with collagenase II (5 mg/ml, GIBCO) in human complete medium at 37°C for a maximum of 16 hours. The material was further digested with TrypLE (GIBCO) for 15 minutes at 37°C, and embedded in GFR Matrigel. Normal samples were processed as above, except that the collagenase digestion was done for a maximum of 2 hours in the presence of soybean trypsin inhibitor (1 mg/ml, Sigma).

Lentiviral Production and Infection in Organoids

TRC and L3GEPIR shRNA lentiviruses (vector backbone was a gift from Johannes Zuber, Humboldt-University of Berlin, Germany) were produced in 293T cells coexpressing the packaging vectors (pPAX2 and pMD2.G for TRC; VSVG, shPASHA, delta8.2 for L3GEPIR), concentrated with LentiX concentrator (Clontech), and resuspended with organoid culture media supplemented with Y-27632 (10 mM, Sigma). To knock-down expression of Nrf2, short hairpin RNAs against mNrf2 (TRC gene set 12130) and hNRF2 (TRC gene set 7555) were used. Organoid infections were performed as described previously (Koo et al., 2012). In brief, 5x10^4 single cells were resuspended with concentrated retrovirus and spinoculated at 600 RCF for 1 hr at room temperature.

Two days after infection, cells were treated with 1 mg/ml puromycin (GIBCO) for selection. Inducible sh*NRF2* sequences are as follows:

Target location	Label	Antisense Sequence
CDS	NFE2L2_1	TTAAGACACTGTAACTCAGGAA
UTR	NFE2L2_2	TTTATAAAAAAGTACATAGTGG

Orthotopic engraftment in the pancreas

Orthotopic engraftment of mouse organoids was conducted as described (Boj et al., 2015). In brief, mice were anesthetized using isoflurane, and Ketoprofen (5 mg/kg), which was subcutaneously administered. 1 x 10^6 cells prepared from organoid cultures resuspended in 50 µl of Matrigel (Matrigel, BD) and diluted 1:1 with cold PBS were injected per pancreas. The organoid suspension was injected into the tail region of the pancreas using insulin syringes (29 Gauge). The abdominal wall was sutured with absorbable vicryl sutures (Ethicon), and the skin was closed with wound clips (CellPoint Scientific Inc.). Mice were euthanized 4 weeks post transplantation.

Mitochondrial and cellular ROS

For mitochondrial ROS, cells were incubated in phenol red-free Advanced DMEM/F12 with 5 µM MitoSOX (Invitrogen) for 30 min and analysed by flow cytometry. For cellular ROS, cells were labelled in phenol-red-free Advanced DMEM/F12 media containing 5 µM DAF2-diacetate (Cayman Chemical) or 10 µM 2′,7′-dichlorofluorescein diacetate (Molecular probes) for 30 min, and analysed by flow cytometry.

Apoptosis Analysis

Single cell suspensions in BD binding buffer were stained for Annexin V (BD Pharmingen) and 7AAD. Data was collected using a BD FACS Calibur flow cytometer to measure Annexin V and 7AAD iodide levels. At least 10,000 events were recorded for each replicate and analyzed using FlowJo software.

Quantitative RT-PCR

RNA was extracted from cell cultures using TRIzol reagent (Invitrogen). cDNA was synthesized using 1 mg of total RNA and TaqMan Reverse Transcription Reagents (Applied Biosystems). All targets were amplified (40 cycles) using gene-specific Taqman primers and probe sets (Applied Biosystems) on a QuantStudio 6-flex Real time-PCR instrument (Applied Biosystems). Relative gene expression quantification was performed using the DDCt method with the QuantStudio Real-Time PCR software v1.1 (Applied Biosystems). Expression levels were normalized to *Hprt.*

Taqman probes used:
Hprt Mm00446968_m1
Nqo1 Mm01253561_m1
Nfe2l2 Mm00477784_m1
Hif1a Mm00468869_m1
c-Myc Mm00487804_m1
Cyclin D1 Mm00432359_m1
Cyclin D3 Mm01612362_m1
Bcl2 Mm00477631_m1

Actin Mm00607939_m1
SybrGreen qPCR primer sequence for luciferase
Forward: GAGCACGGAAAGACGATGACGG
Reverse: GGCCTTTATGAGGATCTCTCTG

7-Methyl-GTP and Phospho-tyrosine pull-down assays

7-Methyl-GTP (m7 GTP)-Sepharose 4B (Jena Biosciences) was incubated with organoid extracts (300 μg) in buffer containing 20 mM Tris, 100 mM KCl and 1 mM EDTA at 4 °C for overnight and then washed seven times. The proteins attached to the washed Sepharose were then subjected to 4-12% SDS-PAGE followed by Western blotting.
For immunoprecipitation (IP) of tyrosine-phosphorylated proteins, 500 μg of cell lysate was subjected to IP by the addition of 5 μl anti-phosphotyrosine antibody (05321, Millipore), and the immunocomplex formed was captured by incubation with 20 μl protein A Dynabeads for 4 hours with gentle rotation at 4°C.

Western blot analysis

Standard techniques were employed for immunoblotting of organoids. Organoids were quickly harvested using cold PBS on ice. Protein lysates were prepared using 1% SDS lysis buffer and separated on 4–12% Bis-Tris NuPAGE gels (Life technologies), transferred onto a PVDF membrane (Millipore) and incubated with the following antibodies: Tubulin (2148, Cell Signaling); Actin (8465, Cell Signaling), phospho-ERK1/2 (4370, Cell Signaling), pan-ERK1/2 (4695, Cell Signaling), phospho-Akt (4060, 2965, Cell Signaling), pan-Akt (4685, Cell Signaling), phospho-ribosomal S6 (4858, Cell Signaling), and S6 Ribosomal Protein (2317, Cell Signaling). 4EBP1 (9452, Cell signaling), p4EBP1 (2855, 9455, Cell signaling), eIF4G (2498, Cell signaling), peIF4G (2441, Cell signaling), eIF4E (2067, Cell signaling), peIF4E (9741, Cell signaling), pEgfr (3777, Cell signaling), phospho-eIF4B (5399, Cell signaling), phospho-H2AX (9718, Cell signaling), Histone H3 (Ab1791, Abcam), IGF1R (3021, Cell Signaling), CyclinD1 (2978, Cell signaling), CyclinD3 (2936, Cell signaling), Bcl2 (2870, Cell signaling), Egfr (15669, Abcam), Hsp90a (072174, Millipore); Ezh2 (3748, Abcam), Survivin (2808, Cell signaling), Mcl-1 (5453, Cell signaling), Kras (Sc-30, Santa-Cruz), Eef2 (2332, Cell signaling), eIF3J (3261, Cell signaling), Vars (NBP1-55366, Novus Biologicus), Adam10 (Ab1997, Abcam), affinity-purified rabbit-anti-full-length mouse Nrf2 antibody (made by JR Prigge, Montana State University) was a provided by Dr. Ed. Schmidt (Montana State University). All immunoblots are representative of at least three experiments.

Kras-GTP activity assay

The levels of Kras-GTP were determined using the Kras activation assay (Sta-400-k, Cell Biolabs) according to the manufacturer's instructions.

Protein stability and half-life

Organoids were treated with 10 $\mu g\,ml^{-1}$ cycloheximide (Sigma Aldrich) for indicated time points and total cell lysates extracted and separated by SDS–PAGE. Gels were transferred to nitrocellulose (Biorad), and membranes were probed with anti-Hif1a (Cayman Chemicals), Bcl2 (Cell signaling), c-Myc (Cell signalling), CyclinD1 (Cell signalling) and Cyclin D3 (Cell signalling) antibody.

Proliferation Assay

Organoids were dissociated into single cells by first triturating them in media through a fire-polished glass pipette, and then by enzymatic dissociation with 2 mg/ml dispase dissolved in TrypLE (Life Technologies), until the organoids appeared as single cells under the microscope. Cells were counted, and diluted to 10 cells/μl in a mixture of complete media, Rho Kinase inhibitor Y-27632 (10.5 μM final concentration, Sigma), and Growth factor-reduced Matrigel (GFR-Matrigel, 10% final concentration). 100 μl of this mixture (1000 cells per well) was plated in 96-well plates (Nunc), whose wells had been previously coated with a bed of GFR-Matrigel to prevent attachment of the cells to the bottom of the plate. Cell viability was measured every 24 hr using the CellTiter-Glo assay (Promega) and SpectraMax I3 microplate reader (Molecular Devices). Five replicate wells per time point were used. Luminescence data were analyzed with GraphPad Prism.

DQ-BSA fluorescence

For live imaging of DQ-BSA fluorescence dequenching, organoids were incubated for 12 hr with 0.2 mg/ml DQ Green BSA and indicated drugs. 2 hr prior to imaging, 50 nM LysoTracker Red and 0.2 μg/ml Hoechst were added. Organoids were imaged using a Zeiss LSM 5 LIVE. DQ-BSA fluorescence was quantified using the particle analyzer function of Fiji (Schindelin et al., 2012). Mean fluorescence intensity was determined by calculating the integrated signal density / area for individual, randomly chosen organoids across the entirety of each sample. Two independent organoid lines were analyzed for each genotype.

Therapeutic experiments with organoids

MK2206 and AZD6244 were dissolved in DMSO. Irinotecan, Gemcitabine, 5-Fluorouracil and Taxol were dissolved in saline. The final concentration of DMSO was no higher than 0.2%. Nine different doses and a vehicle were used for the cytotoxicity assays for MK2206: 1 nM, 5 nM, 10 nM, 50 nM, 100 nM, 500 nM, 1 μM, 5 μM, 10 μM and 100 μM. A similar range was used for other drugs based on the solubility of each drug. When MK2206 was used in combination with BSO the dose of BSO was fixed to 100 or 200 μM as indicated in figure legends. For EC50 analysis, organoids were dissociated to single cells by triturating organoids in media through a fire-polished glass pipette, and then by enzymatic dissociation with 2 mg/mL dispase dissolved in TrypLE (Life Technologies) at 37°C, until the organoids appeared as single cells under the microscope (15-45 min). Cells were counted, and diluted to 10-30 cells/μL in a mixture of complete media, Rho Kinase inhibitor Y-27632 (10.5 μM final concentration, Sigma), and GFR-Matrigel (10% final concentration). 100 μL of this mixture (1000-3000 cells per well) was plated in a 96 well plate (Nunc) previously coated with a bed of GFR-Matrigel. Once organoids reformed (between 36-48 hr post-plating, confirmed by microscopy), drugs were added in 100μL of media. As described above, nine different doses plus a vehicle control were used for each drug, and five replicate wells were treated with each dose. 72 hr after the addition of the drug, cell viability was measured using a luminescence ATP-based assay (CellTiter-Glo, Promega) and a plate reader (I3, Molecular Devices). Synergistic effects were determined by using the Chou–Talay

method to calculate the combination index (CI) CIs of <1, 1, and >1 indicate synergism, additive effect, and antagonism, respectively.

Bicistronic CAP/IRES Luciferase Reporter Assays

SUIT2 cells and organoids were transiently transfected with bicistronic transgenic luciferase reporter for cap- and IRES-mediated translation (SV40-HCV-IRES or SV40-CPV-IRES). 24hrs after transfection, cells were analyzed directly for Firefly and Renilla luciferase expression using a Dual Luciferase Assay kit (Promega) measured on a luminometer (Junior LB 9509).

Transfection and Ectodomain-Shedding Assay

Full length murine EGF was cloned into AP-tag5 (GenHunter) by Gibson assembly. 2D cancer cell lines and organoids were transfected with X-tremeGENE 9 DNA Transfection Reagent (Roche) and Amaxa P3 Primary Cell 4D nucleofector, respectively. Cells were washed with PBS and replaced with serum-free DMEM or Advanced DMEM-F12 for 4 hrs to monitor constitutive shedding. Alkaline phosphatase (AP) activity in the supernatant and cell lysates was measured at an absorbance of 405nm after incubation with the AP substrate 4-nitrophenyl phosphate. No AP activity was detected in conditioned media of non-transfected cells. Three identical wells were prepared, and the ratio between the AP activity in the supernatant and the cell lysate plus supernatant was calculated.

Adam10 activity assay

Sensolyte 520 ADAM10 Activity Assay kit (fluorometric) from AnaSpec was used to detect ADAM10 activity, which was monitored at excitation/emission =490/520 nm. Samples were prepared based on the manufacturer's instructions.

cDNA cloning of retroviral constructs

Human NRF2 cDNA (Addgene, #21555) was used as a PCR template to generate V5-tagged NRF2. This PCR product was subcloned into the MSCV-neo vector (Clontech). Plasmids carrying murine cDNAs of eEF2, eIF3J, Vars (Origene) and Adam10 (Sino Biologicals Inc) were used as PCR templates to generate their corresponding V5-tagged fragments. The mutant-V5 cDNA (Cysteine to Aspartic acid) of eEF2, eIF3J, Vars and Adam10 were further generated by PCR from their wild type counterparts. Cysteine residues mutated in each gene are as follows: $eEF2^{C693D}$, $eIF3J^{C210D}$, $Vars^{C681D}$, and $Adam10^{C525D:C531D}$. Wild type-V5 as well as mutant-V5 cDNA fragments were subcloned into pBABE-puro retroviral vector. For Vars and eIF3J CD mutants, their V5-tagged wild type cDNAs were digested with BSU36i and PfoI, respectively, along with SalI. These digested fragments (containing V5 tag) and PCR products containing CD mutations were ligated into pBabe to generate their corresponding CD mutants. All final cloning products were verified by sequencing and by western blotting. Primers used for cloning are listed in Table S5.

Adam10 immunoprecipitation and Mass Spectrometry

Adam10 was immunoprecipitated from organoids using ab84595 from Abcam.

For immunoprecipitation (IP) Adam10, 500 µg of cell lysate was alkylated with 20 mM iodoacetic acid (lysis buffer containing 50 mM Hepes) followed by IP with 5 µl anti-Adam10 antibody (ab84595, Abcam). The immunocomplex formed was captured by incubation with 20 µl protein A Dynabeads for 4 hours with gentle rotation at 4°C.
The beads for ADAM10 IP samples were reconstituted with 20uL of 50mM triethylammonium bicarbonate buffer (TEAB). RapiGest SF Surfactant was added to a final concentration of 0.1% and *tris*(2-carboxyethyl)phosphine (TCEP) was added to final concentration of 5mM. Samples were then heated to 55°C for 20min, allowed to cool to room temperature and methyl methanethiosulfonate (MMTS) added to a final concentration of 10mM. Samples were incubated at room temperature for 20 min to complete blocking of free sulfhydryl groups. 2ug of sequencing grade trypsin (Promega) was then added to the samples and they were digested overnight at 37°C. After digestion the supernatant was removed from the beads and was dried in vacuo. Peptides were fractionated using a high-low pH reverse phase separation strategy adapted from Gillar et al. (2005) as described in the sections below. Capillary LCMS, mass spectrometry and database searching were also as described below, with the difference that peaklist files were generated using Proteome Discoverer (Thermo).

Lysis, ICAT labeling, Tryptic Digestion, iTRAQ Labeling

Organoids grown in reduced media were washed in cold PBS. Pelleted cells were lysed and processed in an anoxic chamber with 100ul of lysis buffer (50mM HEPES, 0.5mM EDTA, 1% NP-40, 0.1% SDS at pH 8). Protease inhibitor cocktail 1 (Sigma-Aldrich), phosphatase inhibitor cocktail 2 (Sigma-Aldrich), and phosphatase inhibitor cocktail 3 (Sigma-Aldrich) (1 □l each) were added to each sample. The lysate was sonicated for 30 sec and triturated through a 25-gauge needle five times. The lysate was then centrifuged at 14,000 rpm for 5 min at 4°C to pellet any insoluble material. The supernatant was then removed into a fresh 1.5 mL centrifuge tube and a BCA assay (Pierce) was performed on 10 µl of lysate to determine protein concentration. The remaining lysate was snap frozen in liquid nitrogen. For each sample, 1 vial of cleavable ICAT Reagent (AB Sciex) was brought to room temperature. Acetonitrile (10 µl) and 100 µg of lysate was added to each reagent vial and it was incubated for 2 hr at 37°C. This labeling step, performed under oxidizing conditions, reacts out free (reduced) cysteine thiols. The ICAT-labeled proteins were then precipitated by the addition of 4x sample volume of methanol, followed by 2x sample volume of chloroform and 3x volume of water. The samples were then incubated at -20°C for 2 hrs and centrifuged at 14,000 rpm for 10min at 4°C. The upper liquid layer was removed and discarded without disturbing the pellet. Methanol was added to 3x the original sample volume, the sample vortexed and then centrifuged at 14,000 rpm for 10min at 4°C. The entire supernatant was removed and discarded without disturbing the pellet, and the pellet was allowed to dry. The precipitated proteins were reconstituted with 40 µL of 100 mM triethylammonium bicarbonate buffer (TEAB) pH 7.8. *Tris*(2-carboxyethyl)phosphine (TCEP) was added to a final concentration of 5mM, the samples heated to 55°C for 20 mins, then allowed to cool to room temperature. Methyl methanethiosulfonate (MMTS) was added to a final concentration of 10 mM and the samples incubated at room temperature for a further 20 min to complete blocking of free sulfhydryl groups. This step completes reduction and capping of remaining thiols that were not reacted previously with ICAT. Sequencing

grade trypsin (2 μg, Promega) was then added to the samples and they were digested overnight at 37°C and dried in vacuo. Peptides were reconstituted in 50 μl of 0.5 M TEAB/70% isopropanol and labeled with 8-plex iTRAQ reagent for 2 hr at room temperature essentially according to Ross et al (2004). Labeled samples were then acidified to pH 4 using formic acid, combined and concentrated in vacuo until ~10 μl remained. It is important to note that the ICAT reagents were used solely as a specifically cleavable capture reagent to enrich the free-thiol peptides. Quantitation of both flow-through (total protein) and ICAT-selected cysteine peptides was achieved using iTRAQ.

C18 clean-up, ICAT enrichment and cleavage – iTRAQ-labelled peptides were then cleaned up with a Sep-Pak C18 Cartridge (Waters) according to the following protocol. Acetonitrile (2 ml) was aspirated through the cartridge and discarded. Then 2 ml of 0.1% trifluoracetic acid (TFA) was aspirated through and discarded. Samples received 1 ml of 0.1% TFA and the entire sample was aspirated through the cartridge. The flow through was discarded. The cartridges were washed with 2 mL of 0.1% TFA. Finally, peptides were eluted by aspirating with 1 mL of 70% acetonitrile. These peptides were dried in vacuo. Enrichment of the ICAT cysteine peptides was performed using a kit provided by AB Sciex. The avidin cartridge was activated by injecting 2 mL of Affinity Buffer – Elute and diverting to waste. Next, 2 ml of Affinity Buffer – Load was injected and also diverted to waste. Affinity Buffer – Load (0.5 ml) was added to the peptides and the pH was adjusted to 7 (if necessary). The peptide sample was injected onto the cartridge and the flow through was collected. This fraction will later be analyzed as the "flow through" part of the experiment, allowing measurement of global protein changes which could then be used to normalize cysteine peptide changes to their respective protein levels. The cartridge was washed with 3 mL of Affinity Buffer – Wash and 1 ml of water. ICAT peptides were eluted with 800 μl of Affinity Buffer – Elute. The ICAT peptides were dried in vacuo until completion and then dissolved in 90 μl of cleaving reagents A and B in a 95:5 ratio. Samples were incubated for 2 hrs at 37°C and again dried in vacuo until completion.

2-Dimensional Fractionation

Peptides were fractionated using a high-low pH reverse phase separation strategy adapted from (Gilar et al., 2005). For the first (high pH) dimension, peptides were fractionated on a 10cm x 1.0 mm column packed with Gemini 3u C18 resin (Phenomenex, Ventura, CA) at a flow rate of 100 μl/min. Mobile phase A consisted of 20mM ammonium formate, pH 10, and mobile phase B consisted of 90% acetonitrile / 20 mM ammonium formate pH 10. Total peptide (100 μg) was reconstituted with 50 μL of mobile phase A and the entire sample injected onto the column. Peptides were separated using a 35 min linear gradient from 5% B to 70% B and then increasing mobile phase to 95% B for 10 min. Fractions were collected every min for 80 min and were then combined into 22 fractions using the concatenation strategy described by (Wang et al., 2011). An estimated 1 μg of peptide from each of the 22 fractions was then separately injected into the mass spectrometer using capillary reverse phase LC at low pH, described below. The ICAT enriched cysteine peptide pools and flow-through total peptide pools were analyzed separately in two 22-fraction LCMS experiments, with expression-level changes calculated from iTRAQ reporter ions for each set.

Capillary LC Mass Spectrometry

An Orbitrap Velos Pro mass spectrometer (Thermo Scientific), equipped with a nano-ion spray source was coupled to an EASY-nLC system (Thermo Scientific). The nano-flow LC system was configured with a 180 µm id fused silica capillary trap column containing 3 cm of Aqua 5 µm C18 material (Phenomenex), and a self-pack PicoFrit™ 100 µm analytical column with an 8 µm emitter (New Objective, Woburn, MA) packed to 15 cm with Aqua 3 µm C18 material (Phenomenex). Mobile phase A consisted of 2% acetonitrile/0.1% formic acid and mobile phase B consisted of 90% acetonitrile/ 0.1% formic Acid. Each sample (3 µl), dissolved in mobile phase A, was injected through the autosampler onto the trap column. Peptides were then separated using the following linear gradient steps at a flow rate of 400 nl/min: 5% B for 1 min, 5% B to 35% B over 70 min, 35% B to 75% B over 15 min, held at 75% B for 8 min, 75% B to 8% B over 1 min and the final 5 min held at 8% B.

Eluted peptides were directly electrosprayed into the Orbitrap Velos Pro mass spectrometer with the application of a distal 2.3 kV spray voltage and a capillary temperature of 275°C. Each full-scan mass spectrum (Res=60,000; 380-1700 *m/z*) was followed by MS/MS spectra for the top 12 masses. High-energy collisional dissociation (HCD) was used with the normalized collision energy set to 35 for fragmentation, the isolation width set to 1.2 and activation time of 0.1. Duration of 70 seconds was set for the dynamic exclusion with an exclusion list size of 500, repeat count of 1 and exclusion mass width of 10 ppm. We used monoisotopic precursor selection for charge states 2+ and greater, and all data were acquired in profile mode.

Database Searching

Peaklist files were generated by Mascot Distiller (Matrix Science). Protein identification and quantification was carried using Mascot 2.4 (Perkins et al., 1999) against the Uniprot Human sequence database (88,698 sequences; 35,138,129 residues). Methylthiolation of cysteine and N-terminal and lysine iTRAQ modifications were set as fixed modifications, methionine oxidation and deamidation (NQ) as variable. Trypsin was used as cleavage enzyme with one missed cleavage allowed. Mass tolerance was set at 30 ppm for intact peptide mass and 0.3 Da for fragment ions. Search results were rescored to give a final 1% FDR using a randomized version of the same Uniprot Human database. Protein-level iTRAQ ratios were calculated as intensity weighted, using only peptides with expectation values < 0.05. Global ratio normalization (summed) was applied across all iTRAQ channels. Protein enrichment was then calculating by dividing sample protein ratios by the corresponding control sample channel.

Merging of iTRAQ datasets and normalization of cysteine peptides to their cognate proteins.

iTRAQ data merging across separate experiments was accomplished exactly as described in (Boj et al., 2015) by systematically holding two or more samples constant between experiments ("shared channels"). Only proteins identified and quantified in all experiments were merged. The process of ICAT peptide merging was identical to the iTRAQ merging with two differences: 1) all iCAT observations were averaged prior to merging; and 2) merging was done on a per-peptide rather than per-protein basis. Thus,

only peptides identified and quantified in all experiments were merged. Due to sparser sampling and lower overall numbers of observations, the average CV of normalized channels for ICAT peptides was 10%-15%. Subsequent to iTRAQ and ICAT merging, peptide ICAT values were corrected for protein levels from corresponding iTRAQ protein values by dividing ICAT values by iTRAQ quantifications normalized to a maximum of 1. Thus, ICAT values corresponding to the maximum channel remain unchanged (divided by 1) and, by example, iTRAQ channels with half of the maximum intensity are multiplied by two (specifically they are divided by 0.5). These values represent the final, protein-level corrected ICAT quantifications. This approach means that changes in cysteine peptide levels are normalized to any changes in their repective parent protein. The output thus highlights 'sensitive' cysteine peptides and residues that change differentially to changes in expression levels of the parent protein, an approach that has not been used before in global cysteine proteomics.

GSH/GSSH quantification

Extraction and analysis was adapted from (Zhu et al., 2008). Pelleted cells were lysed with 300 µl of derivatizing solution (1.25 mM ABD-F, 0.25 mM EDTA, 25 mM sodium borate) and sonicated on ice. Lysate was centrifuged at 15,000 x g for 10 min at 4°C. Lysate (20 µg) was added to 120 µL of 5% sulfosalicylic acid and 20 µL of internal standard (1 mM $^{13}C_2{}^{15}N_1$ GSH : 0.5 mM $^{13}C_4{}^{15}N_2$-GSSG in 1 mM EDTA : 0.1 M Sodium Borate). Samples (5 µl) were then injected into the mass spectrometer.

Mass Spectrometry

A Thermo Vantage triple quadrupole mass spectrometer (Thermo Scientific) equipped with an ESI spray source was coupled to an Accela HPLC system (Thermo Scientific). The HPLC system was configured with an XBridge C_{18} HPLC column (5 µm; 2.1 mm x 150 mm, Waters Corp). Mobile phase A consisted of water with 0.1% formic acid and mobile phase B of 80% acetonitrile/ 0.1% formic acid. Each sample (5 µl) was injected through the autosampler onto the column. Targets were then separated using the following gradient: 2% B at 0 min, 2% B at 2.5 min, 8% B at 2.51 min, 8% B at 4.5 min, and 70% B at 4.51 min, 100% B at 11 min, 100% B at 15 min, 2% B at 15.5 min, followed by an 8.5 min equilibration period. The flow rate was 400 µl/min for the duration of the method. Single reaction monitoring (SRM) was used to monitor for 4 transitions in total (see table). Peaks were integrated using Xcalibur software (Thermo Scientific).

	Parent	Product	CE
Light derivatized GSH	505.3	376.2	24
Heavy derivatized GSH	508.3	379.2	24
Light GSSG	613.2	355	24
Heavy GSSG	619.2	361.2	24

References

Boj, S.F., Hwang, C.I., Baker, L.A., Chio, II, Engle, D.D., Corbo, V., Jager, M., Ponz-Sarvise, M., Tiriac, H., Spector, M.S., *et al.* (2015). Organoid models of human and mouse ductal pancreatic cancer. Cell *160*, 324-338.

Chan, K., Lu, R., Chang, J.C., and Kan, Y.W. (1996). NRF2, a member of the NFE2 family of transcription factors, is not essential for murine erythropoiesis, growth, and development. Proc Natl Acad Sci U S A *93*, 13943-13948.

Gilar, M., Olivova, P., Daly, A.E., and Gebler, J.C. (2005). Two-dimensional separation of peptides using RP-RP-HPLC system with different pH in first and second separation dimensions. Journal of separation science *28*, 1694-1703.

Hingorani, S.R., Wang, L., Multani, A.S., Combs, C., Deramaudt, T.B., Hruban, R.H., Rustgi, A.K., Chang, S., and Tuveson, D.A. (2005). Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. Cancer Cell *7*, 469-483.

Huch, M., Bonfanti, P., Boj, S.F., Sato, T., Loomans, C.J., van de Wetering, M., Sojoodi, M., Li, V.S., Schuijers, J., Gracanin, A., *et al.* (2013). Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. The EMBO journal *32*, 2708-2721.

Koo, B.K., Stange, D.E., Sato, T., Karthaus, W., Farin, H.F., Huch, M., van Es, J.H., and Clevers, H. (2012). Controlled gene expression in primary Lgr5 organoid cultures. Nature methods *9*, 81-83.

Perkins, D.N., Pappin, D.J., Creasy, D.M., and Cottrell, J.S. (1999). Probability-based protein identification by searching sequence databases using mass spectrometry data. Electrophoresis *20*, 3551-3567.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., *et al.* (2012). Fiji: an open-source platform for biological-image analysis. Nature methods *9*, 676-682.

Wang, Y., Yang, F., Gritsenko, M.A., Wang, Y., Clauss, T., Liu, T., Shen, Y., Monroe, M.E., Lopez-Ferrer, D., Reno, T., *et al.* (2011). Reversed-phase chromatography with multiple fraction concatenation strategy for proteome profiling of human MCF10A cells. Proteomics *11*, 2019-2026.

Zhu, P., Oe, T., and Blair, I.A. (2008). Determination of cellular redox status by stable isotope dilution liquid chromatography/mass spectrometry analysis of glutathione and glutathione disulfide. Rapid communications in mass spectrometry : RCM *22*, 432-440.