SUPPLEMENTARY METHODS

Cell Culture and Reagents

The ATCC was the source for BxPC-3, MIA PaCa-2, Capan-1, PANC-1, SU.86.86, IMR-90, and HPNE-hTERT KRAS G12D cells. HPNE-hTERT KRAS WT was a generous gift from Dr. Laufey Amundadottir, NIH. PK-1 was sourced from RIKEN. Cell lines were maintained either in (MIA PaCa-2, IMR-90, PANC-1) DMEM or in (BxPC-3, Capan-1, SU.86.86, PK-1) RPMI supplemented with 10% FBS. We have used either DMEM (D6429-Sigma) or RPMI (R8758-Sigma) supplemented with 10% FBS (F4135-Sigma) for culturing the cell lines. HPNE-hTERT cells were cultured in base medium following the instructions from ATCC. In short, the base medium for this cell line is: 75% DMEM without glucose (Sigma Cat #D-5030 with additional 2 mM L-glutamine and 1.5 g/L sodium bicarbonate) and 25% Medium M3 Base (INCELL Corp. Cat #M300F-500). To make the complete growth medium, we added the following components to the base medium: fetal bovine serum 5% (final conc.), 10 ηg/ml human recombinant EGF (#PHG0311L, Gibco), 5.5 mM D-glucose (1g/L), and 750 ηg/ml puromycin (Gibco).

NRF2 overexpressing stable cell lines were generated by using Capan-1 and BxPC-3 cell lines. Before generating stable cell lines, cells were authenticated via short tandem repeat analysis with resulting allelic profiles matching those found in online databases. Cells were then seeded into six-well plates and incubated overnight at a density that yielded 60-70% confluency at the time of transduction. Media was removed from the cultures and replaced with one milliliter of media containing 8 µg/mL hexadimethrine bromide (Sigma-Aldrich, St. Louis, MO). One

well from each plate was harvested and counted to calculate the amount of virus to add for each condition. Lentivirus volume corresponding to multiplicities of infection (MOI) of 50 for Capan-1 and 10 for BxPC-3 were mixed into the hexadimethrine bromide-containing media. After a 24-hour incubation at 37º C, 5% CO_2, the virus-containing media was removed and replaced with pre-warmed complete media. Cells were incubated for an additional 24 hours before the media was replaced with pre-warmed selection media containing Blasticidin (8 μg/mL).

For glutamine starvation experiments, cells were rinsed with Hank's Balanced Salt Solution (Sigma H9394) twice the day after seeding and then (depending on cell line culturing media) placed in glutamine-free Dulbecco's modified Eagle medium (DMEM) (Sigma D5546) or Glutamine-free RPMI (Sigma R0883) supplemented with 10% dialyzed FBS (DFBS) (Sigma F0392). Control wells (labeled CM) contain 4 mM Gln (Sigma G7513) and 10% DFBS for the whole experiment treatment time.

For stress granule formation experiments, 15d-PGJ2 treatment was performed when cells had reached ~70-80% confluency. Beforehand, treatment cells were serum starved for 18 hours. Then they were treated with 50μM 15d-PGJ2 (ab141717, Abcam) for 1 hr.

Western Blot Analysis

Antibodies were obtained from the following sources: NRF2 (ab62352), Vinculin (ab129002), Hsp60 (ab45134), NQO1 (ab34173), GCLC (ab190685), AKR1C1 (ab192785), GLS1 (ab156876), GCLM (ab124827), GDH1 (ab168352), GOT1 (ab170950), and GPX4 (ab125066) were obtained from Abcam. Antibodies

against HMOX1 (#5853), IDH1 (#8137), xCT (#12691), Chk1 (#2360), p-Chk1 Ser345 (#2348), ATM (#2873), p-ATM Ser1981 (#4526), and actin (#4970) were obtained from Cell Signaling Technology.

For tissue harvesting purposes, we used a homogenization buffer (Thermo Fisher Scientific T-PER Cat. No. 78510) plus HALT protease inhibitor (Thermo Scientific Cat. No. 78440). The samples were homogenized using a bead mill homogenizer (Fisher Scientific) with steel beads (Fisher Scientific 15-340-151). Samples were homogenized for 3.5 mins at maximum power and then processed for protein concentration measurement (NanoDrop/Thermo Fisher Scientific), followed by the standard procedure for western blot analysis.

Immunofluorescence and Immunohistochemistry

Cells were grown on six-well glass bottom plate micro wells with Poly D Lysine coating (#P06-20-1.5-N-PDL, Biomedtech Laboratories) and followed by specific treatments as indicated in the text. On sample collection day, cells were washed with DPBS (#14040133, Life Technologies) and permeabilized by using ice-cold 4% formaldehyde solution (#R37814, Invitrogen) for 30 mins. After treatment with 0.1% triton X-100 solution (#HFH10, Invitrogen) for 30 mins, cells were incubated with 2% goat serum (ab138478, Abcam) for 45 mins. Subsequently, cells were incubated with the respective primary antibodies followed by incubation with secondary antibodies for 45 mins. Primary antibodies for G3BP (ab56574) and NRF2 (ab31163) was purchased from Abcam, whereas eIF4G (#2469) was obtained from Cell Signaling Technology. Secondary antibodies for anti-mouse Alexa fluor 546 (A11003), anti-rabbit Alexa fluor 647 (A21245), and anti-rabbit

Alexa fluor 546 (A11035) were purchased from Invitrogen. Afterwards, cells were washed with DPBS and nuclei were stained with DAPI (#D1306, Invitrogen). Images were captured by either a Nikon Spinning Disk microscope (eclipse Ti) or an Olympus (UTBI90) inverted microscope.

For quantifying stress granules, we used the following method: the images of G3BP-, DAPI-, and eIF4G-stained cells were captured with the inverted florescence microscope (Olympus/UTBI90) with the appropriate filters. Stress granules were quantified using ImageJ as described by Bar-Sagi and colleagues, with minor modifications. In short, 200 cell images were captured randomly for each condition. The cell outline was drawn to estimate the cell area and cell number. The “analyze particle” plug-in from ImageJ was used to identify the stress granules above the threshold intensity that remain consistent across all conditions in a given experiment. Then, we calculated the number of stress granules divided by number of cells (#SG/cell), the SG index, by computing the total area captured by stress granules/total cell area. SG area was plotted by averaging the size of the stress granules.

Immunohistochemical (IHC) staining was performed using an NRF2 antibody (#ab62352, Abcam). For the tissue microarray study, we used a pancreas tissue microarray chip (#PA805b, US Biomax, Inc.) that contains 58 cases of duct adenocarcinoma, 1 each of acinic cell carcinoma and squamous cell carcinoma, and 10 each of adjacent normal pancreas tissue and normal pancreas tissue, single core per case. For ethical materials, scanned images of H&E staining, and corresponding clinical information, please refer to https://www.biomax.us/tissue-

arrays/Pancreas/PA805b. The IHC staining was performed at the US Biomax facility by following the regular method. Briefly, the tissues were formalin fixed, paraffin embedded (FFPE). Array sections were mounted on positively charged SuperFrost Plus glass slides. The tissue microarray sections were cut at 5 microns thick. Individual cores were 1.5 mm in diameter. Slides were stained on a Ventana Discovery Ultra automated system using the manufacturer's protocol. Slides were deparaffinized with EZ Prep solution (Ventana, Tucson, AZ). Heat-induced antigen retrieval (HIER) was performed with CC1, pH 8.5 (Ventana) for 72 minutes at 95^{o} C. Peroxidase inhibitor was applied. Protein blocking was applied with Background Sniper (Biocare Medical, Concord, CA). The primary antibody was incubated at 37^{o} C for 40 mins. The secondary antibody used was Discovery anti-Rabbit HQ and anti-HQ HRP, a hapten-based detection system that improves sensitivity (Ventana). Antibody dilution was determined by serial titration and optimization of the antibody on a small test array consisting of normal and tumor tissue. DAB detection was done using Ventana's ChromoMap kit. Slides were then counterstained with hematoxylin. Stained slides were scanned on an Aperio Versa with a 40x objective. The NRF2 immunostaining results were scored according to the accumulated intensity as 0, absent or negative; 1+, weak staining; 2+, mild staining; and 3+, strong staining.

KPC Mouse tissue samples were received from the Center for Advanced Preclinical Research (CAPR), Frederick National Laboratory for Cancer Research, National Cancer Institute at Frederick. Tissue slides consisted of 10μm CryoJane frozen sections prepared on SuperFrost slides (Thermo Fisher

Scientific). Tissue samples were freshly frozen in O.C.T. Tissue-Tek compound (VWR) over a bed of isobutanol/dry ice. IHC staining was done by following the above described method. H&E staining of murine tissue samples were performed by the CAPR shared resource at the Frederick National Laboratory. All human tissues were collected under HIPAA-approved protocols, and all animal tissues were collected under IACUC protocol.

Flow Cytometric Analysis

Sample preparation for cell cycle analysis was done by following the methods previously described (1). In brief, cells were washed twice in PBS and harvested. Cell suspensions were resuspended in the following fixing solution: 7ml PBS, 2% BSA, 5mM EDTA, and 0.1% NaN_3. Three milliliters of 100% ethanol were added dropwise. Fixed cells were centrifuged, washed using PBS, and then resuspended in 500 µl sorting buffer and incubated at 37^{o} C for 30 min. The cells were filtered through 70-µm mesh to remove cell aggregates. The DNA content was analyzed by flow cytometry (BD LSRFortessa, BD Biosciences), and percentages of cells within each phase of the cell cycle were determined using BD FACSDiva software (BD Biosciences).

Cell Proliferation and Viability Assays

The cell proliferation assay was performed as previously described (1). The proliferation experiment was carried out for five days post treatments and then collected. Cells were counted either by a Moxi Z automated cell counter (Orflo Technologies) or by hemocytometer after staining with 0.1% crystal violet (#109218, Sigma).

For the cell survival assay, cells were seeded in a 10cm plate and then treated the next day with no-glutamine media or complete media for three days then treated with gemcitabine for 24 hrs. The cells were then trypsinized and plated in six-well plates in complete media. After eight days of proliferation, colonies were fixed with formaldehyde solution (# sc-281692, Santa Cruz Biotechnology) and stained with crystal violet solution. Dye was extracted with 10% acetic acid, and relative proliferation was determined by absorbance measured at 595 ηm (Epoch, BioTek). Relative cell survival was determined as a percentage of the DMSO-treated control.

For the CellTiter-Glo assay, cells were plated in 96-well plates (# 07-000-166, Fisher Scientific) and treated by serial dilutions of gemcitabine the day after plating for 48 hours of incubation. After adding CellTiter-Glo reagent, the plates were read on an Envision plate reader (Perkin-Elmer). The percentage of inhibition or IC50 values were calculated in GraphPad Prism 8 using three-parameter nonlinear regression analysis.

Non-viable cell counting was determined by trypan blue exclusion, as mentioned previously. In short, after various treatments, cells were harvested and treated with trypan blue (# T8154, Sigma-Aldrich). Trypan blue uptake (dead cells) was determined by either Countless II automated cell counter (Invitrogen) or by counting on a hemocytometer.

Metabolomic and lipidomic analysis

For metabolomics study, 20 million cells were collected for each sample. After harvesting, the cell number was counted, and cell pellets were frozen at -80^{o} C

before being sent to the West Coast Metabolomics Center at UC Davis (NIH WCMC) for untargeted primary metabolomic analysis by ALEX-CIS GC-TOF MS. An Agilent 6890 GC equipped with a Gerstel automatic liner exchange system (ALEX) that includes a multipurpose sample (MPS2) dual rail as well as a Gerstel cold injection system (Gerstel, Mülheim, Germany) with temperature program was used in this study. A Leco Pegasus IV time-of-flight mass spectrometer controlled by the Leco ChromaTOF software vs. 2.32 (St. Joseph, MI) was used for mass spectrometer settings in this study. We performed Student's t test on each of the compounds to compare the control vs. treatment. To deal with the multiple comparisons problem, we used the Benjamini-Hochberg procedure for false discovery rate (FDR) correction. We also applied hierarchical clustering analysis (HCA) (distance="euclidean", method="complete") with heatmap visualization using the FDR significant identified compounds. For Metabolite Set Enrichment Analysis (MSEA), over-representation analysis (ORA) was performed on significant compounds with Student's t test, $p < 0.05$. In the MSEA, the fold change is calculated as the average of treatment divided by the average of control. If the fold change is greater than 1, then that treatment is higher than the control. ORA was implemented using the hypergeometric test to evaluate whether a particular metabolite set was represented more than expected by chance within the given compound list. One-tailed p values were provided after adjusting for multiple testing. For pathway networking visualization, we used fold change, raw p-value as input of the metamapp (http://metamapp.fiehnlab.ucdavis.edu/) and used Cytoscape (https://cytoscape.org/) to visualize the compound network. From the

network mapping graph, we can visualize the enrichment of the compound cluster. All statistical analysis in the metabolomics study used statistical programming language R (3.4.0). The HCA used the packages “pheatmap.”

For Lipidomic analysis, cells were collected, resuspended in D-PBS (without Mg and Ca), and frozen at -80^{o}C. The final concentration of resuspended cells was 3,000 cells/μL. Samples were then shipped to Lipotype, GmbH, Germany, for untargeted basic lipidomic analysis. The Lipotype Shotgun Lipidomics technology was used for the lipidomics analysis. The Lipotype Shotgun Lipidomics platform consists of the automated extraction of samples, an automated direct sample infusion, and high-resolution Orbitrap mass spectrometry including lipid class-specific internal standards to assure absolute quantification of lipids. Lipids were extracted using chloroform and methanol. Samples were spiked with lipid class-specific internal standards prior to extraction. After drying and re-suspending in MS acquisition mixture, lipid extracts were subjected to mass spectrometric analysis. Mass spectra were acquired on a hybrid quadrupole/Orbitrap mass spectrometer equipped with an automated nano-flow electrospray ion source in both positive and negative ion mode. Automated processing of acquired mass spectra and identification and quantification of detected molecular lipid species were performed using LipotypeXplorer software. LipotypeXplorer (Lipotype) was used to identify lipids in the mass spectra. Further data processing and analyses was performed using LipotypeZoom (Lipotype) tool. The identified lipid molecules were quantified by normalization to a lipid class-specific internal standard. The amounts in picomoles of individual lipid molecules (species of subspecies) of a given lipid

class were summed to yield the total amount of the lipid class. The amounts of the lipid classes were normalized to the total lipid amount yielding percent per total lipids.

Bioinformatics Analysis

cBioPortal (http://www.cbioportal.org/) was used to analyze PDAC sample (2) data for NRF2/KRAS pathway co-occurrent alteration and OncoPrint information. Considered parameters, including tumor grade, p value, log odds ratio, detailed cancer type, sample source, and reference of the study, were mentioned in the figure.

For assessing NFE2L2 expression in TCGA, we used the RNASeq-derived normalized TCGA gene expression data. Mutation data for PAAD was downloaded from Broad's firehose site (https://gdac.broadinstitute.org). Gene expression for NFE2L2 expression was retrieved for all samples, and samples were then grouped by the mutant KRAS allele. The sample counts were: G12D – 55 samples; G12R – 26 samples; G12V – 40 samples; and KRAS wild type – 45 samples. The p values for each allele compared to wild types using the T-test are: G12D – 0.023; G12R – 0.013; and G12V – 0.008.

For The Cancer Genome Atlas data (TCGA) clinical attributes and GSEA analysis, TCGA clinical files and RNASeqV2 files were downloaded using the TCGA Data Matrix. Customized R scripts were used to examine the clinical patient files from each cancer, in which the patient ID, histologic subtype, and American Joint Committee on Cancer pathologic tumor stage were recorded. Clinical drug files examined for gemcitabine and related drug names and the clinical measure

of response and samples were classified as “Complete Response” (CR) or “Clinical Progressive Disease” (CPD) by designated rules that follow clinical treatment standards and common practice and were applied to both stage II patients and all-stage patients. Normalized RSEM gene result files from the TCGA UNC IlluminaHiSeq RNASeqV2 were further examined for each patient. Only RNASeq files examining the primary tumor were examined. Patients who did not have pancreas-adenocarcinoma ductal type histology or did not have mRNA or RNAseq data for the primary tumor were not utilized for further analysis. All available normal adjacent tissues in the pancreas cohort were averaged. Each patient was compared in an expression fold-wise manner relative to the normal tissue to make a GSEA pre-ranked file in which any genes that were tied due to a 0 value in the RNAseq data in either individual tumor samples or the mean of normal samples were removed to meet the criteria of GSEA pre-ranked analysis or GSEA analysis using a user-supplied ranked list of genes. Each patient's pre-ranked gene list file was submitted to the GenePattern sever to run the GSEA pre-ranked analysis (version 6.0.10) as a batch for databases including: KEGG, REACTOME, GOBP, and BioCarta. Customized R scripts were then used to collate the individual GSEA pre-ranked analysis result files of each patient for pathways or terms from these databases and collect the normalized enrichment score (NES). Normalized enrichment scores were then compared for each of gemcitabine's treatment response categories (CR vs. CPD) and analyzed by customized R scripts for two-sample Student’s t-Test or Wilcoxon rank sum Test (Mann-Whitney U Test).

For GLS and NFE2L2 gene Expression (RSEM in log scale) in the above-mentioned gemcitabine-treated PAAD patients, normalized RSEM values as the expression levels of selected genes (GLS and NFE2L2) were extracted from downloaded UNC Illumina HiSeq RNASeqV2 data files from The Cancer Genome Atlas (TCGA). Box plots were used to compare the RSEM values in log scale (log2) between CR and CPD classes, which was further analyzed by two-sample Student's t-test or Wilcoxon rank sum Test (Mann-Whitney U Test) to assess the statistical significance using customized R scripts.

For analyzing upregulated expression of metabolic genes in TCGA PAAD, RNAseq and clinical data for PAAD was downloaded from the TCGA portal. Normalized RSEM values for the selected genes were used for comparative analysis between stage I vs III or Grade 1 vs Grade 3. t-test was used to assess the statistical significance. Oncomine (https://www.oncomine.org/resource/) was used for various gene copy number analyses for TCGA PAAD, as mentioned in the figure. Parameters including p-value, fold change, reporter information, sample numbers, reference of the study, and grade information of TCGA PADD samples for each Oncomine data analysis were utilized as per Oncomine database instructions.

Xenograft Studies

For the PANC-1 survival study, treatment was discontinued after day 38, and tumors were allowed to re-grow until they reached 1,500 mm^3. Once the tumors reached 1,500 mm^3, the mice were sacrificed. Tumors were measured once every two days with micro-calipers, and tumor volume was calculated as (length x width

x width)/2. All animals were weighed daily to assess possible differences in animal weight among treatment groups as an indication of possible toxicity resulting from the treatments. The dosing solutions were prepared fresh on each day of dosing. CB-839 (BOC Sciences) was supplied as a crystalline powder and dissolved in the vehicle as previously described (3). The vehicle consisted of 25% (w/v) hydroxypropyl-β-cyclodextrin (HPBCD; Roquette) in 10 mM citrate, pH 2. CB-839 was formulated as a solution at 20 mg/mL (w/v) in vehicle. Gemcitabine for injection USP was purchased from Intas Pharmaceuticals and 0.95% NaCl for injection USP was supplied from Hospira Inc. CB-839 was given orally (twice daily) at 200 mg/kg for 15 days and then at 100 mg/kg twice daily until end of the treatment. Gemcitabine was given at 40 mg/kg (PANC-1, SU.86.86) or 20 mg/kg (MIA PaCa-2) by IP injection every three days until Day 15 and then at 20 mg/kg (PANC-1, SU.86.86) or 10 mg/kg (MIA PaCa-2) every three days until end of the treatment. Mice were euthanized when tumor volume exceeded 1,500 mm^3, and survival was followed (PANC-1 survival study) until Day 68. No evidence of treatment-related toxicity was observed in mice studies. There were no animal deaths or euthanasia other than for the reason of tumor volume, and there were no statistically significant differences in weight change. The relative tumor volume for each tumor was calculated by dividing the tumor volume on each day by the tumor volume on day 1 and expressing the result as a percentage. Statistical differences between treatment groups were determined using Mann-Whitney Rank Sum or ANOVA tests with a critical value of 0.05. All xenograft studies were carried

out by Pharma Models, LLC (Marlborough, MA) by following procedures approved by the Institutional Animal Care and Use Committee (IACUC).

KPC Allograft Study

Ten-twelve-week-old syngeneic C57bl/6J mice received a subcutaneous (left flank) inoculum of low passage mouse pancreatic adenocarcinoma KPC cells (10^5 cells/ 100 μl of serum-free DMEM/F12 media). Upon engraftment, animals have been allowed to develop palpable lesions (<4 mm^3 tumor volume) and randomized into four treatment groups, 10 animals in each experimental arm. Mice have been dosed with a combination of vehicles (saline, intraperitoneally at 10ml/kg, q3d; and 10mM citrate buffer (pH= 2)/25% HPBCD (Sigma # H107), orally, 10ml/kg, bid) for control cohort; Gemcitabine (NIH Pharmacy, intraperitoneally, 40mg/kg, q3d); CB-839 (BOC Biosciences, orally, 200mg/kg, bid); and combination of gemcitabine and CB-839 for respective cohorts during the study. Mice have been weighted daily to monitor for the possible toxicity side-effects. Animals registering the body weight decrease in excess of 10% of the enrollment weight have been taken off treatment until recovering their body weights to less than 5% loss of the corresponding enrollment weights. Potential treatment toxicity effects have been monitored in all study animals by daily measurement of body weights (BW) and computing relative BW changes using the weights at enrollment as reference points (relative BW = [BW at indicated time point – BW at enrollment]/BW at enrollment). No overt BW loss has been documented for either of four treatment arms arguing that none of the treatments leads to significant systemic toxicity. Measurements of subcutaneous tumors have been conducted once weekly and

tumor volumes calculated using Volume = (Lenght^2 x Width)/2 estimation. In line with previous observations (data not published) made in subcutaneous KPC allograft models, several animals in each of four treatment groups have been removed from the study prior to concluding the drug treatment procedure due to developing ulcerated lesions, following the ACUC recommendations. Mice have been treated for three weeks with control vehicle (n=6), gemcitabine (n=8), CB-839 (n=8), or gemcitabine in combination with CB-839 (n=8), as described above. Tumor volume analysis in four groups of animals carrying KPC allografts is represented as 'Box and whiskers' plots of tumor volumes and experimental study arms evaluated pairwise by two-way unpaired ANOVA test to compute statistical significance of tumor responses to different treatments.

Animals have been kept on a 12/12 light-dark cycle. No evidence of treatment-related toxicity was observed in KPC mice study. Statistical differences between treatment groups were determined using Two-way unpaired ANOVA tests with a critical value of 0.05. All experimental procedures have been approved by and done in accordance with the National Cancer Institute- Frederick Animal Care and Use Committee recommendations and AALAS guidelines.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary figure S1, Related to Figure 1. NRF2-mediated pathway is upregulated in KRAS mutant PDAC: A, HPNE-hTERT KRAS WT and KRAS G12D cells were plated and harvested as in **1A.** Effectors of the NRF2 pathway (NQO1, GCLC, AKR1C1, and HMOX) protein levels were determined by western

blot analysis. Vinculin serves as the loading control. Representative image from at least two independent experiments. **B,** Levels of NRF2 in Jimeno pancreatic carcinoma with KRAS mutation (n = 28) cohort versus KRAS wild type (n = 6) and fold change of NRF2 over-expression in KRAS mutant Jimeno pancreas with significant p-value. Data were obtained from Oncomine. **C,** cBioPortal analysis for 456 PDA samples suggested that the NFE2L2 and KRAS gene set is altered in 96 samples. The analysis also evaluated the significant co-occurrent tendency for this gene pair in queried samples. **D,** Kaplan-Meier survival curve generated for PDA patients grouped according to high (n = 43) versus low (n = 127) levels of NFE2L2 and KRAS expression in TCGA-PAAD samples. Using PROGgene V2, the cohort was divided at the 75th percentile of NFE2L2-KRAS combined gene expression (top) and p-value, hazard ratio, and median survival (bottom) were generated. **E,** Survival curve for TCGA-PADD patients, similar to Fig S1D**.** The cohort was bifurcated at the 25th percentile based on combined gene expression of NFE2L2, KRAS. **F,** Gene expressions for NFE2L2 from TCGA-PADD were grouped by the mutant KRAS allele. The p-values for each allele were compared to wild type. **G,** KRAS mutation statuses in PDAC cell lines used in this study were obtained from CCLE or the COSMIC database.

Supplementary figure S2, Related to Figure 1: Isotype control for NRF2 in IHC and survival measure for NRF2: A, Rabbit isotype controls for NRF2 immunohistochemical staining were performed as in Fig.1 E. Images are in 20x view, scale bars 100μm (left), 2mm (right). **B,** PROGgene V2 survival analysis for TCGA-PAAD samples divided at 25th percentile, grouped by high versus low levels

of NFE2L2 expression (top). p-value, hazard ratio, median survival, and upper and lower confidence intervals were evaluated (bottom).

Supplementary figure S3, Related to Figure 2: Knockdown and over-expression of NRF2 in PDAC cells: A, Transient transfection of two different shRNA sequences targeting NRF2 and scrambled shRNA (pLKO.1) in PDAC cells (PANC-1: left and PK-1: right), showing downregulation of NRF2 by western blot analysis. **B,** Western blot analysis of whole cell lysates from PANC-1 cells after DMSO (control) and AI-1 (10μM, 48 hours) treatment, exhibiting NRF2 activation. **C,** Whole cell lysates from Capan-1 and BxPC-3 cells (first two panels) stably expressing empty vector (pLOC- control) or NRF2 overexpression were subjected to western blot analysis with the indicated antibodies. Immunoblot analysis of MIA PaCa-2 cell (last panel) with transiently transfected control plasmid (pLenti6.3) or NRF2 overexpressing plasmid. **D,** NRF2 western blot analysis of whole cell lysates from BxPC-3 (left) and MIA PaCa-2 (right) cells after DMSO (control) and AI-1 treatment. **E,** Transient transfection of two different shRNA sequences targeting NRF2 and scrambled shRNA in MIA PaCa-2 cells, depicting downregulation of NRF2 by western blot analysis. Vinculin serves as the loading control. Representative data (A-E) are shown from at least two independent experiments.

Supplementary figure S4, Related to Figure 3: Effects of NRF2 activation on metabolomics: BxPC-3 cells were treated with DMSO (control) or AI-1 (10μM) for six hours, followed by metabolomic analysis as mentioned in the methods section.

After data processing, the Benjamini-Hochberg false discovery rate (FDR) has been used for correction. Hierarchical clustering analysis (HCA) has been utilized (distance="Euclidean", method="complete") with heatmap visualization using the FDR significant identified compounds. A clear distinction between control (DMSO) and AI-1 treated compounds is detected in **A,** heatmap. Four independent replicates were performed for each group. Student's t test was performed on each of the compounds to compare the control vs. treatment. **B,** The compound network has been visualized using MetaMapp and Cytoscape pathway networking. Red: increase; Blue: decrease.

Supplementary figure S5, Related to Figure 3: Cell cycle analysis of PDAC cells upon NRF2 activation: A, BxPC-3 and **B,** MIA PaCa-2 cells were plated at 30% confluence in 10cm plates in complete growth medium containing 10% serum. After 24 hours, the cells were treated with DMSO (control) or AI-1 (10μM) for 48 hours. Then, cells were harvested and analyzed for cell cycle distribution by measuring DNA content/cell as described in the methods section. Representative flow histograms (right side of A-B) show no significant change in cell cycle population upon AI-1 treatment in BxPC-3 and MIA PaCa-2 cells. The Y-axis of the histogram depicts cell number, while the X-axis indicates DNA content. The error bars represent standard error of mean for experiments repeated three times.

Supplementary figure S6, Related to Figure 4 and Table 2: Role of metabolic pathways imparting gemcitabine resistance in pancreatic cancer: **A,**

Oncomine analysis of Garnett Cell Lines suggested that the expression of GLS was elevated in the gemcitabine-resistant group compared to other groups based on gemcitabine sensitivity. **B-E**, Pathway enrichment score comparisons for respective pathways in gemcitabine-treated stage II (B-C) and all-stage (D-E) PDAC patients with complete response or clinical progressive disease. Detailed data points for normalized enrichment scores of GSEA pre-ranked analysis were shown as dots along with a box-and-whisker plot. By using TCGA-PADD data in pathway databases (KEGG, GOBP, BioCarta), metabolic pathway enrichment scores were calculated. Significant p value was then evaluated (as described in the methods section). An enrichment score was generated with comparisons between gemcitabine-treated all-stage or stage 2 PDAC patients with complete response and with clinical progressive disease. **F,** Box plot of normalized RSEM values (in log2 scale) of selected genes (GLS and NFE2L2) to compare their expression level between Complete Response and Clinical Progressive Disease classes. The p-values were reported on the legend at top right corner of each plot.

Supplementary figure S7, Related to Figure 4: Glutamine starvation regulates cell growth and assembly of stress granules: A, SU.86.86 and PK-1 cells were plated in complete media. After 24 hours, cells were shifted to CM or (-Q), harvested, and quantified at indicated times as in Fig. 4C. Error bars represent the SEM for three independent experiments. **B,** Proliferation of PANC-1 cells shown. Cells were treated with different drugs in complete media (CM). Drug treatment conditions were followed as in Fig. 4D. Data is presented as relative percentage

to vehicle-treated (DMSO) control CM. Error bars represent standard error of mean (SEM) for experiments repeated four times. **C,** Number of SGs per cell and stress granule area (SG area) were calculated for respective samples as in Fig. 4G. Error bars indicate SEM for two independent experiments. **D,** PANC-1 cells were treated as in Fig. 4H. NRF2 protein level was assessed by western blot analysis. Vinculin is the loading control. A representative image is shown from at least two independent experiments. **E,** Stress granule area (SG area) was calculated for control (DMSO-treated) and 15d-PGJ2-treated PANC-1 cells in CM and glutamine-starved conditions (-Q) as in Fig. 4I. Error bars indicate SEM for three independent experiments. The SG area based on G3BP immunofluorescence is depicted in green, whereas the eIF4G immunofluorescence-based SG area is grouped in red.

Supplementary figure S8, Related to Figure 5: Effects of glutamine deprivation on cell viability and NRF2 expression: A, PANC-1 cells were plated and shifted to CM or media lacking Q. Cells were additionally treated with (5mM) DMKG and/or (1X) NEAA mixture for 72 hours where indicated. Gemcitabine (2μM, 36 hours) was added for respective samples and the percent of non-viable cells was calculated using the trypan blue dye exclusion assay. **B,** IMR-90 cells were plated at 30% confluence in six-well plates in complete media (CM) containing 10% serum for 24 hours, after which the cells were shifted to CM or medium lacking Q for 48 hours. Then, cells were subsequently treated with or without (2μM) gemcitabine for an additional 24 hours in the presence of pretreatment as indicated. The percent of non-viable cells was determined. **A-B,** Error bars represent SEM for three independent experiments. **C-D,** Tissue lysates of three

representative mouse tumors of indicated groups from MIA PaCa-2 (C) and PANC-1 (D) xenograft studies were analyzed for the NRF2 protein level expression by western blot analysis. Vinculin was used as the loading control.

Supplementary Table S1, Related to Figure 1: Clinical characteristics of NRF2 expression in pancreatic cancer patients: Clinicopathologic parameters used in the pancreatic cancer tissue micro array (TMA) with normal tissue as control, including TNM classification of malignant tumors, clinical stage, and pathological grade, 80 cases/80 cores.

Abbreviations:

DAG: Diacylglycerol, TAG: Triacylglycerol, PA: Phosphatidate, PC: Phosphatidylcholine, PE: Phosphatidylethanolamine, PG: Phosphatidylglycerol, PI: Phosphatidylinositol, PS: Phosphatidylserine, SM: Sphingomyelin, CE: Cholesterol esters, MS: mass spectrometry, DMKG: dimethyl α-ketoglutarate, NEAA: non-essential amino acid.

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