**Supplementary Methods**

**Cell Culture**

PDAC cell lines were maintained in DMEM supplemented with 10% FCS, penicillin/streptomycin, gentamycin/glutamine and sodium pyruvate and cultured in a humidified incubator at 37 ⁰C with 5% CO2. 779E cells were established from a pancreatic cancer xenograft derived from a resected T2N1 moderately-to-poorly differentiated PDAC, following standard procedures [51]. HPNE cells without or with oncogenic KRas expression were maintained in medium composed of 75% DMEM, 25% M3 base (Incell Corp.), 5% FBS, 10 ng/ml EGF and 750 ng/ml puromycin [34].

**DNA constructs and transfections**

Constructs encoding shRNAs that target eIF5A1 and eIF5A2 were purchased from Thermo (V3LHS\_341459, V3LHS\_360415) and Sigma (TRCN0000062548, TRCN0000147855). The siRNA targeting eIF5A1 was purchased from Thermo (M-015739-00-0005), eIF5A2 and PEAK1 siRNA were from Sigma (SASI\_Hs01\_00211442 and SASI\_Hs02\_00357289, respectively). The siRNA targeting K-Ras was from Qiagen (SI02662051). Plasmids encoding human eIF5A1 or eIF5A2 were kindly provided by Dr. Myung-Hee Park (National Institute of Dental and Craniofacial Research). Plasmids for stable overexpression of eIF5A1 and eIF5A2 were generated by inserting the coding sequence of eIF5A isoforms amplified from the above plasmids into pCDH-MCS-IRES-Puro vector (System Biosciences) using the EcoRI-BamHI site. Plasmids encoding GFP (pTriEx-GFP) and GFP-tagged PEAK1 (pTriEx-GFP-PEAK1) was as described previously [30]. Cell lines stably expressing shRNAs or eIF5A isoforms were generated by puromycin selection (1 g/ml) after transfection using PEI (Polyethylenimine) or transduction by lentivirus, as described previously [30]. Transient transfection was performed by PEI as described previously [30]. siRNA transfection was performed using RNAiMAX (Invitrogen) according to the manufacturer’s instructions.

**Immunohistochemistry**

Human pancreatic cancer tissue array was purchased from US Biomax. Sections of spontaneous PanIN tissues from 3-, 6- and 9-month old PDX-1-Cre:LSL-KRASG12D mouse pancreas were isolated and processed for immunohistochemical staining following standard protocol [52]. Samples from patients BK-17 and BK-19 were collected and processed as described previously [30], in accordance with UCSD (La Jolla, CA) Institutional Review Board (IRB) #071136X. Determination of eIF5A1, eIF5A2 and hypusinated eIF5A1 protein expression was performed as described previously using specific antibodies (ab32443 (Abcam) for eIF5A1, H00056648-M01 (Abnova) for eIF5A2 and NIH353 for hypusinated eIF5A1). Images were collected with a Leica DM2500 microscope using a 40x objective lens.

**Western blotting**

Cell extracts were prepared in RIPA (radioimmunoprecipitation assay) buffer (20 mM Tris-HCl pH7.4, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with Complete protease inhibitor cocktail (Roche). Protein concentrations were determined by bicinchoninic acid assay (Pierce). Equal amounts of cell extracts were resolved by SDS-PAGE, transferred to nitrocellulose membranes, and probed with appropriate primary and HRP-conjugated secondary antibodies (Jackson ImmunoResearch). When necessary, membranes were stripped using Restore Western Blot Stripping Buffer (Thermo) and reprobed with a different antibody.

**Soft agar growth assay**

2x104 PANC1 cells expressing shRNA were resuspended in 0.3% agarose and seeded over a cushion of 0.6% agarose in the same medium in 6-well plates. Cells were cultured in the soft agar for 25 days and visible colonies were counted from 10 different fields using an inverted microscope with a 10x objective (Olympus). Bright-field images of the colonies were acquired using Nikon Eclipse Ti inverted microscope (10x objective), and individual colony size was determined using ImageJ software (NIH). Colony size was determined for at least 30 colonies from 5 different fields from each experiment.

**Orthotopic implantation experiments**

Orthotopic implantation experiments were performed essentially as described previously [30]. Briefly, 4-6 weeks old female athymic mice were anesthetized by intramuscular injection of ketamine. Subsequently, a small incision was made in the left lateral flank through the skin and peritoneum. 1x106 PANC1 or 779E cells expressing shRNAs or overexpressing eIF5A isoforms were injected into the tail of the pancreas in a total volume of 10 μL of PBS using a Hamilton syringe. The pancreas was returned to the abdomen, and the peritoneum and skin were closed using Polysorb surgical suture. The mice were sacrificed at the indicated time points, and the primary tumor weight was measured. For shRNA expressing cells, open images of mice bearing tumors were acquired using the OV-100 fluorescence imaging system (Olympus). The number of mice used was 10 per group for PANC1 cells expressing shRNAs (Fig. 5A); 6 for 779E cells containing control shRNA and eIF5A1 shRNA, 7 for eIF5A2 shRNA-expressing cells (Fig. 5B); 5 for PANC1 cells containing control vector, 8 for eIF5A1- or eIF5A2-overexpressing PANC1 cells (Fig. 5C). The presence of knockdown or overexpression was validated by qPCR using RNA extracted from orthotopic tumors (Fig. S3C-E).

**Clonogenic Survival assay**

Clonogenic survival assay was performed essentially as described previously [53]. Briefly, cells treated with the indicated dose of gemcitabine for 24 hrs were replated into 6-well plates (50 cells/well) and allowed to form colonies for 11 days. Subsequently, cells were fixed and stained with crystal violet, and the number of colonies was manually counted.

**Cell proliferation assay**

Analysis of cell growth was conducted using the CyQuant direct cell proliferation assay kit according to the manufacturer's protocol (Invitrogen) and as described previously [30]. Briefly, cells were plated in quadruplicate into 96-well plates at a density of 250 cells (for shRNA expressing cells) or 500 (for drug treated cells) per well in complete media. For drug treatment, cells were exposed to the indicated agents 24 hrs after plating, and relative viable cell number was measured 3 days after based on CyQuant Green fluorescence emission at 525 nm. For shRNA expressing cells, measurement was done 8 days after the cells were plated. For experiments described in Fig. 4B, 6E and F, 1x105 cells were transfected with siRNA in a 6-well plate and seeded into 96-well plates at the density of 250 cells/well the following day. For experiments described in Fig. 7B, cells were plated at a density of 2x105 cells per well into 6-well plates and transfected with pTriEx-GFP or pTriEx-GFP-PEAK1 plasmids (2 g/well) the following day, replated into 96-well plates 24 hrs after, and cultured for another 120 hrs before measurement. For dose response of PANC1 cells to cisplatin (Fig. 7C, lower left panel), PANC-1 cells plated in 6-well plates (2x105 cells/well) were treated with 0, 5 and 10 M cisplatin for 72 hrs. Subsequently, cells were trypsinized and the number of viable cell was determined by trypan blue exclusion assay. For time course analyses of the growth of PANC1 cells or 779E cells with shRNA or eIF5A1/2 overexpression, 1 x 105 cells were seeded per well in 6-well plates and the number of cells as well as the percentage of dead cells was determined 2, 4 and 6 days after plating by trypan blue exclusion method. These analyses were repeated three times for each cell line.

**Combination index calculation**

Combination index (CI) was calculated using Compusyn (Combosyn, Inc.; http://www.combosyn.com/), a freely accessible software that allows calculation of combination index based on the Chou-Talalay method [54]. CI provides quantitative definition for additive effect (CI = 1), synergism (CI < 1), and antagonism (CI > 1) in drug combinations.

**Supplementary Figure Legend**

**Supplementary Figure 1.** A, Validation of isoform specific antibodies to eIF5A. PANC1 cells were transiently transfected with siRNAs directed at the indicated target or both eIF5A1 and eIF5A2 (A1+A2) (left panel), or plasmids encoding eIF5A1 or eIF5A2 (right panel), and then lysed 72 hrs later and subjected to western blotting with anti-eIF5A1, eIF5A2 and hypusinated eIF5A (Hyp-eIF5A) antibodies. GAPDH was used as a loading control. B and C, Immunohistochemical staining of DHPS and DOHH expression in human PDAC or normal pancreatic tissue sections. The TMA representing normal pancreatic tissues and PDAC tissues was stained for DHPS (B) or DOHH (C). Representative figures from normal, well- and poorly differentiated tumors are presented alongside with quantitative immunohistochemical scoring. Boxed regions show normal ducts with corresponding high magnification images. Bar= 100 m. \* represents P values of < 0.05 as determined by Student’s t-test.

**Supplementary Figure 2.** A, Time course analyses of growth and death of PANC1 cells containing control, eIF5A1 or eIF5A2 shRNA (shCtrl, sh5A1 and sh5A2, respectively). 1 x 105 cells were plated per well and the number of viable cells (left panel) as well as percentage of dead cells (right panel) was determined by trypan blue dye exclusion assay after 2, 4 and 6 days. B, Time course analyses of growth and death of 779E cells containing control, eIF5A1 or eIF5A2 shRNA (shCtrl, sh5A1 and sh5A2, respectively). The number of viable cells as well as percentage of dead cells is shown as above. C, Time course analyses of growth and death of PANC1 cells overexpressing eIF5A1 or eIF5A2, or containing empty vector (5A1, sh5A2 and Ctrl, respectively). The number of viable as well as percentage of dead cells was determined as above. \* represents P values of < 0.05 as determined by Student’s t-test.

**Supplementary Figure 3.** A, eIF5A knockdown using an independent set of shRNAs. PANC1 cells were transduced with eIF5A1 (5A1) or eIF5A2 (5A2) shRNAs targeting different sequence from the ones used in Fig. 4. Relative cell proliferation and the western blot analysis of eIF5A1, eIF5A2, PEAK1 and tubulin are shown. B, Open images of a representative mouse used in Fig. 5A and B. Mice implanted with PANC1 cells (top) or 779E cells (bottom) bearing the indicated shRNA are shown. Arrows indicate the GFP-positive primary tumors, and bars represent 10 mm. C and D, Validation of eIF5A1 or eIF5A2 knockdown in orthotopic tumor tissues. RNA was extracted from PANC1 or 779E cells before injection (cells) or from tumor tissues (tumors), and subjected to qPCR using primers specific to human eIF5A1 or eIF5A2 to assess the expression levels of human eIF5A1/2. eIF5A1 or eIF5A2 mRNA levels relative to control cells or tumors are shown. E, Validation of eIF5A1 or eIF5A2 overexpression in orthotopic tumor tissues. eIF5A1 or eIF5A2 mRNA levels relative to control cells or tissues were determined and shown as above. \* represents P values of < 0.05 as determined by Student’s t-test.

**Supplementary Figure 4.** A, Western blot analysis of PEAK1 protein levels in 779E cells expressing shRNAs targeting eIF5A1 (5A1) or eIF5A2 (5A2). GAPDH was used as loading control. B, Western blot analysis of PEAK1 protein levels in 779E cells containing both eIF5A1 shRNA and eIF5A2 siRNA (sh5A1/si5A2) compared with cells containing control shRNA and siRNA (shCtrl/siCtrl). C, Combination index analysis showing synergy between CPX and gemcitabine (Gem). Combination index (CI) was calculated based on Chou-Talalay method, for experiments where PANC1 or FG cells were treated with varying dose of Gem together with 5 M of CPX. CI at the indicated dose (100 nM or 1 M) of Gem was calculated for FG and PANC1 cells. D, Analysis of viable cell number for PANC1 cells containing control shRNA or PEAK1 shRNA after treatment with 50 or 100 nM Gem for 7 days. Relative number of viable PANC1 cells with PEAK1 shRNA is presented as % of control cells, as assessed by CyQuant assay. E, Clonogenic survival assay of PANC1 cells containing control (shCtrl) or eIF5A2 (sh5A2) shRNA after Gem treatment (100 nM, 24 hrs). Bar graphs represent the number of colonies in Gem-treated cell relative to vehicle-treated cells. F, Clonogenic survival assay of FG cells containing control (shCtrl), eIF5A1 (sh5A1) and eIF5A2 (sh5A2) shRNA after Gem treatment (20 nM, 24 hrs). Western blots show stable isoform-specific knockdown of eIF5A1 or eIF5A2 in FG cells. Bar graphs represent the number of colonies in Gem-treated cells relative to vehicle-treated cells as above. \* represents P values of < 0.05 as determined by Student’s t-test.

**Supplementary Figure 5.** Heatmap of fold change in the mRNA expression levels of eIF5A isoforms and PEAK1 in tumor samples as derived from Oncomine. Tumor samples where co-upregulation of eIF5A1 and PEAK1 (A), eIF5A2 and PEAK1 (B) or eIF5A1, eIF5A2 and PEAK1 (C) was observed are presented. Data are publicly available on Oncomine.

**Supplementary References**

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