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Supplementary Information for

KLF7 Promotes Pancreatic Cancer Growth and Metastasis by Upregulating ISG Expression and Maintaining Golgi Complex Integrity

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SI Materials and Methods

shRNA and lentivirus preparation

pLKO.1 lentiviral vector-based shRNAs targeting specific candidate genes and NS control shRNAs were obtained from Horizon Discovery. Details about the shRNAs is provided in Key Resources Table. Lentivirus particles were prepared by transfecting 293T cells with either gene-specific shRNA plasmids or NS shRNA plasmids along with lentiviral packaging plasmids, as described in detail at https://portals.broadinstitute.org/gpp/public/resources/protocols. All lentiviral transfections were performed using Effectene Transfection Reagent (QIAGEN, Hilden, Germany). Stable cell lines were generated by infecting PDAC cells with shRNA lentivirus particles on 12-well plates, followed by selection with appropriate concentrations of puromycin (0.5 to 1.5 μg/ml) to enrich the infected cells. For the pLX304-Blast-V5-based lentivirus, infected PDAC cells were selected with 2 μg/ml blasticidin (Thermo Fisher Scientific, Waltham, MA, USA).

RNA preparation, cDNA preparation, and qRT-PCR analysis

Total RNA was extracted with TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) and purified using the RNeasy Mini Kit (QIAGEN). Then, cDNA was generated using the M-MuLV First Strand cDNA Synthesis Kit (New England Biolabs, Ipswich, MA, USA) according to the manufacturer's instructions. Quantitative RT-PCR was performed with gene-specific primers using the Power SYBR-Green Master Mix (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The *ACTINB* gene

was used as a normalization control. The primer sequences for all the genes analysed in the study are provided in Key Resources Table.

PDAC datasets for KLF7 and ISG expression analysis

Datasets that compared gene expression between pancreatic cancer and normal pancreatic tissues were identified by searching the Oncomine cancer profiling database. The Badea, Pei, Ishikawa, Buchholz, Grutzmann, and Iacobuzio-Donahue datasets were used for data analysis (1-6). The Badea dataset includes paired samples of pancreatic ductal adenocarcinoma (n=39) and normal pancreas (n=39) from 36 patients, three of which were duplicated, analyzed on a Human Genome U133 Plus 2.0 Array. The Pei dataset includes 36 pancreatic carcinoma samples and 16 paired normal samples analyzed on a Human Genome U133 Plus 2.0 Array with metadata including age and sex. The Ishikawa dataset includes 24 pancreatic ductal adenocarcinoma samples and 25 normal pancreatic duct samples analyzed on Affymetrix U133A/B microarrays with metadata including cancer type, patient age and sex, atypical cell proportion, clinical stage, and cytological grade. The Buchholz dataset includes 24 pancreatic intraepithelial neoplasia samples, 8 pancreatic ductal adenocarcinoma samples, and 6 normal pancreatic duct samples analyzed on Human Genome Oligo-Set-Version 2.0 microarrays. The Grutzmann dataset includes 14 microdissected pancreatic ductal adenocarcinoma samples and 11 normal pancreatic duct samples analyzed on Affymetrix U133A/B microarrays with metadata including cancer type, grade, and TNM stage and patient ages and sex. The Iacobuzio-Donahue dataset includes 14 pancreatic carcinoma cell lines, 17 primary pancreatic carcinoma samples of various histologies, and 5 normal pancreas samples analyzed using cDNA microarrays. For

analyzing the expression of KLF7 in TCGA dataset, we used Gene Expression Profiling Interactive Analysis (GEPIA) using the following website; http://gepia.cancer-pku.cn. The expression of KLF7 was analyzed in 179 PDAC tumors and 171 normal samples (Match TCGA + GTEx data).

Chromatin immunoprecipitation

The *KLF7* promoter sequence was downloaded from the University of California, Santa Cruz (UCSC) genome browser and analyzed using PROMO 3.0 (http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3) and rVista 2.0 (https://rvista.dcode.org). ChIP experiments were performed as described previously (7). Cell lysates were incubated with specific antibodies (listed in Key Resources Table) or with the IgG antibody as a control. Normalized Ct (ΔCt) values were calculated by subtracting the Ct value obtained with input DNA from that obtained with immunoprecipitated DNA [ΔCt = Ct(IP) − Ct(input)]. The relative fold enrichment of a given factor at the target site was then calculated using the formula $2^{-[\Delta Ct(T) - \Delta Ct(Actb)]}$, where ΔCt(T) and ΔCt(Actb) are ΔCt values obtained using primers for the target factor and *ACTINB* (negative control), respectively. The primer sequences for the ChIP analysis are listed in Key Resources Table.

Soft-agar assay

Soft-agar assays were performed by seeding 5×10^3 to 2×10^4 PDAC cells stably expressing the indicated shRNA or ORF constructs onto 0.4% low-melting-point agarose (Sigma-Aldrich) layered on top of 0.8% agarose. After 3–4 weeks of incubation, colonies

were stained with a 0.005% crystal violet solution and imaged using a microscope. Colony size was measured using microscopy and ImageJ software (https://imagej.nih.gov/ij/) and plotted as the per cent relative colony size compared with control cells. Statistical analysis was performed using Student's t-tests in the GraphPad Prism 7 software.

Matrigel-invasion assay

Invasion assays were performed in BioCoat Growth Factor Reduced Matrigel Invasion Chambers (Cat#354483, BD Biosciences, Franklin Lakes, NY, USA) using PDAC cells expressing the indicated shRNAs. Cells were serum-starved for 6 h and then seeded in triplicate into the top chamber at a density of 5×10^4 cells/insert in low-serum medium (0.2% FBS). The cells were incubated for 20 h to allow invasion toward the serum-rich medium (10% FBS) in the lower chamber. The number of cells invading the Matrigel was quantified by DAPI staining and imaging; 8–12 fields per membrane were counted, and nuclei were quantified using ImageJ (https://imagej.nih.gov/ij/).

Wound-healing assay

PDAC cells expressing the indicated shRNAs were seeded at a density of 2×10^5 cells per well and grown on 12-well plates until fully confluent. A scratch was then created using a sterile 20 µl pipette tip, and cell migration into the wound was monitored at 0 h, 12 h, 24 h, 48 h, and 72 h using light microscopy. The wound healing was quantified using ImageJ (https://imagej.nih.gov/ij/).

RNA-seq and data analysis

PANC1 cells stably expressing two different shRNAs against *KLF7* were used to prepare total RNA, which was then used for gene-expression analysis on an Illumina HiSeq 2500 system. Total RNA was extracted using Trizol reagent (Invitrogen) according to the manufacturer's instructions and then purified on RNAeasy mini columns (Qiagen) according to the manufacturer's instructions. mRNA was purified from approximately 500 ng total RNA using oligo-dT beads and then sheared by incubation at 94°C. Following first-strand synthesis with random primers, second-strand synthesis was performed with dUTP to generate strand-specific sequencing libraries. The cDNA library was then end-repaired and A-tailed. Adapters were then ligated, and second-strand digestion was performed using Uracil-DNA-Glycosylase. Indexed libraries that met appropriate cut-offs for both were quantified by qRT-PCR using a commercially available kit (KAPA Biosystems). The insert size distribution was determined using LabChip GX or an Agilent Bioanalyzer. Samples with a yield ≥ 0.5 ng/μl were used for sequencing on the Illumina HiSeq 2500 system. Images generated by the sequencers were converted into nucleotide sequences by the base-calling pipeline RTA 1.18.64.0 and stored in FASTQ format. The raw sequencing data in the FASTQ files were subjected to a quality check (FastQC), removal of adapter content, and quality thresholding (removal of reads with Phred score < 30). Reads that passed the quality thresholds were mapped to the latest stable version of the human reference genome hg38 (**GRCh38.p12**, Ensembl) using Bowtie2 and Tophat 2.1.1. The expression of the assembled transcriptomes was estimated using Cufflinks 2.2.1 (8). Briefly, the quality of the assemblies was assessed, and the normalized gene and transcript expression profiles were computed for each sample. The normalization was

performed using the classic fragments per kilobases per million fragments (FPKM) method followed by Log2 transformation. The gene-level differential expression between conditions was estimated using the Log2-transformed FPKM values of transcripts sharing each gene ID. The uncorrected p-value of the test statistic and the FDR-adjusted p-value of the test statistic (q-value) were estimated for differentially expressed genes (DEGs). Any gene with a p-value greater than the false discovery rate, after Benjamini-Hochberg correction for multiple testing, was deemed to be differentially expressed between the test condition and the control condition. The accession number for our RNA-seq data is GSE and is available via GEO link GSE107184.

Immunoblotting analysis

Whole-cell protein extracts were prepared using IP lysis buffer (Pierce) containing Protease Inhibitor Cocktail (Roche) and Phosphatase Inhibitor Cocktail (Sigma-Aldrich, St. Louis, MO). Lysed samples were centrifuged at 12,000 rpm for 40 min, and clarified supernatants were stored at –80°C. Protein concentrations were determined using Bradford Protein Assay Reagent (Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts of protein samples were electrophoresed on 10% or 12% sodium dodecyl sulfate (SDS)-polyacrylamide gels and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Burlington, MA, USA) using a wet-transfer apparatus from Bio-Rad. The membranes were blocked with 5% skim milk and probed with primary antibodies. After washing, the membranes were incubated with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2,000) (GE Healthcare Life Sciences, Marlborough, MA, USA). The blots were developed using SuperSignal West Pico or

Femto Chemiluminescent Substrate (Thermo Fisher Scientific). All antibodies used for immunoblotting are listed in Table S2.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue microarray slides containing PDAC and matched normal pancreas tissues were obtained from US Biomax, Inc. (Cat. No. HPan-Ade150CS-02; Derwood, MD, USA). Briefly, following deparaffinization of the slides, antigen retrieval was performed in citrate buffer (pH 6.0) at 97°C for 20 min using the Lab Vision PT Module (Thermo Scientific). Endogenous peroxides were blocked with hydrogen peroxide for 30 min and then washed with 1X Tris-buffered saline (TBS). Proteins were then blocked using 0.3% bovine serum albumin (BSA) for 30 min. The slides were incubated with KLF7 antibody (dilution 1:100) followed by secondary anti-rabbit HRP-conjugated antibody (Dako, Jena, Germany). The slides were then stained using the Dako Liquid DAB+ Substrate Chromogen System (Dako) and counterstained with Dako Automation Hematoxylin Histological Staining Reagent (Dako). The KLF7 staining was scored by Dr. Guoping Cai, who was blinded to the identity of each slide. All antibodies used for immunohistochemistry analyses are listed in Table S2.

Mouse tumorigenesis experiments with cells expressing shRNAs

Athymic nude (NU/J) mice (Stock No. 002019, Jackson Laboratory), aged 5–6 weeks, were injected subcutaneously with 5×10^6 PDAC cells expressing different *KLF7* shRNAs or an NS shRNA. Tumor volume was measured every week. Tumor size was calculated using the following formula: length × width^2 × 0.5. All protocols for mouse experiments

were approved by the Institutional Animal Care and Use Committee (IACUC) of Yale University.

Tail vein injection of cells expressing shRNAs for lung metastasis experiments

PANC1 cells stably expressing firefly luciferase under control of the cytomegalovirus (CMV) promoter were generated by co-transfection of the transposon vector piggyBac GFP-Luc and the helper plasmid Act-PBase, as described previously (9). Cells with stable transposon integration were selected using blasticidin S (Thermo Fisher Scientific). PANC1-GFP-F-Luc cells (5×10^4) expressing *KLF7* shRNAs or an NS shRNA were then injected into NSG mice (Stock No. 005557, Jackson Laboratory) via the tail vein. Images were taken of the mice using the IVIS Spectrum In Vivo Imaging System (Perkin Elmer). Total luminescence counts of the tumor-bearing areas were measured using the Living Image *in vivo* imaging software (Perkin Elmer). All protocols for mouse experiments were approved by the IACUC of Yale University.

Caspase-3 activity assay

Caspase-3 activity was measured using the Caspase-3 calorimetric assay kit from Biovision, Inc. (Cat# K106) as per the manufacturer's recommendation. Briefly, 5×10^6 PANC1 cells expressing either NS or *KLF7*shRNAs in combination with *IFIT1* expression vector or empty vector (pLX304) control were lysed in chilled cell lysis buffer provide with the kit and incubated on ice for 10 min. Lysed samples were centrifuged at 10,000 rpm at 4°C for 5 min, and clarified supernatants (cytosolic extracts) were transferred to fresh tubes. Protein concentrations were determined using Bradford Protein Assay

Reagent (Bio-Rad Laboratories, Hercules, CA, USA). Reaction buffer (2X) containing 10 mM DTT was added to the samples containing equal amounts of protein (100 μg). Caspase 3 substrate, DEVD-pNA substrate (200 μM final concentration) was added to the samples, and incubated at 37°C for 2 hr. Thereafter, absorbance of the samples was measured at 405 nm using a microtiter plate reader. The caspase-3 activity was determined in the samples after subtracting the background readings from the blank.

Chemical inhibitors

Brefeldin A and golgicide A were obtained from Sigma-Aldrich. Trametinib was purchased from Cayman chemicals and Wortmannin were purchased from Calbiochem. The information regarding the inhibitors is listed in Key Resources Table. The treatment concentrations and duration of treatment information for each inhibitor is given in the relevant figures and figure legends.

Golgi complex staining and quantitation of Golgi fragmentation

Immunofluorescence staining was performed as described previously (10). In brief, human pancreatic cancer cells (PANC1 and MIAPaCa2) expressing gene-specific shRNAs (KLF7, DLG3, or STX5), NS shRNA, or DLG3 ORF were plated in a multi-well chambered slide. After 24 h, the cells were washed with PBS and fixed with 3.7% paraformaldehyde. The cells were then permeabilized using 0.3% Triton X-100. The slides were then washed in PBS and blocked using 5% BSA in PBS. Then, the cells were probed with GM130 primary antibody (1:200) (Table S2) to visualize the structure of the Golgi membranes. The cells were washed again and incubated with AlexaFluor-488 anti-mouse

antibody (1:1000) (Table S2). The nuclei were then stained with DAPI. Fluorescence images were collected using a LEICA SP5 Confocal Laser Scanning Microscope or an Olympus IX71 inverted fluorescence microscope. The cells were quantified on the basis of three categories of Golgi membrane organization: compact, partially fragmented, and fragmented (11). At least 200 cells were scored per condition.

Glycoprotein carbohydrate content estimation

The glycoprotein content in cells was estimated using the Glycoprotein Carbohydrate Estimate Kit (Thermo Scientific # 23260). Prior to the assay, MIAPaCa2 and PANC1 cells expressing either NS shRNA or *KLF7* shRNA were plated on 100 mm tissue-culture dishes. When the cells were about 95% confluent, protein was extracted using M-PER^{TM} mammalian protein extraction reagent (Thermo Scientific #78501). The protein concentration in the extracts was measured using the Bradford protein assay method. The Glycoprotein Standard Set (Thermo Scientific # 23259) was used as an estimation control and to generate standards by which to calculate glycosylation. Fifty microliters of each standard and each protein sample was placed on 96 well microplates in triplicate. For a background, 50 µl glycoprotein assay buffer was used as a blank. Twenty-five microliters of freshly prepared sodium meta-periodate solution was added to each well, and the plates were then mixed properly and incubated for 10 min at room temperature. Then, 150 µl freshly prepared glycoprotein detection reagent was added, and the plates were mixed gently and incubated for 1 h at room temperature. The plates were then analyzed using a microplate reader at 550 nm. The glycoprotein carbohydrate content of the samples was calculated by comparison to the standards of known glycoprotein carbohydrate content

according to the manufacturer's instructions. For the cell fractionation-based glycoprotein carbohydrate content estimation, cells were fractionated into cytosolic, membrane, nuclear and cytoskeletal fractions using ProteinExtract Subcellular Proteome Extraction Kit (Sigma-Aldrich) as per the manufacturer's instructions. The glycoprotein carbohydrate content was estimated as described above.

Human chemokine array analysis

Human Chemokine Antibody Arrays were purchased from RayBiotech (Cat. No. AAH-CHE-1) and used according to the manufacturer's instructions. Briefly, 2×10^6 MIAPaCa2 cells expressing either NS or *KLF7* shRNAs were plated on 100 mm tissue culture dishes. After 36 h, the cells were washed with 1× PBS. Then, 10 ml reduced-serum OPTI-MEM medium was added to each dish, and the cells were grown for 72 h. Cell culture supernatants (conditioned media) were collected and concentrated using centricon tubes (3 kDa). The array membranes were blocked with blocking buffer at room temperature for 1 h and then incubated with 1 ml primary biotin-conjugated antibody cocktail at 4°C overnight. The membranes were then washed and incubated with 2 ml 1000-fold diluted HRP-conjugated streptavidin at room temperature for 2 h. Then, the array membrane signals were detected using the Super Signal West Pico Chemiluminescent Substrate kit (Thermo Fisher Scientific) and the Super Signal West Femto Chemiluminescent Substrate kit (Thermo Fisher Scientific). The signal intensities were quantified using Image J (https://imagej.nih.gov/ij/). Positive controls on the array membranes were used to normalize the results across different membranes.

SI Figures and Tables

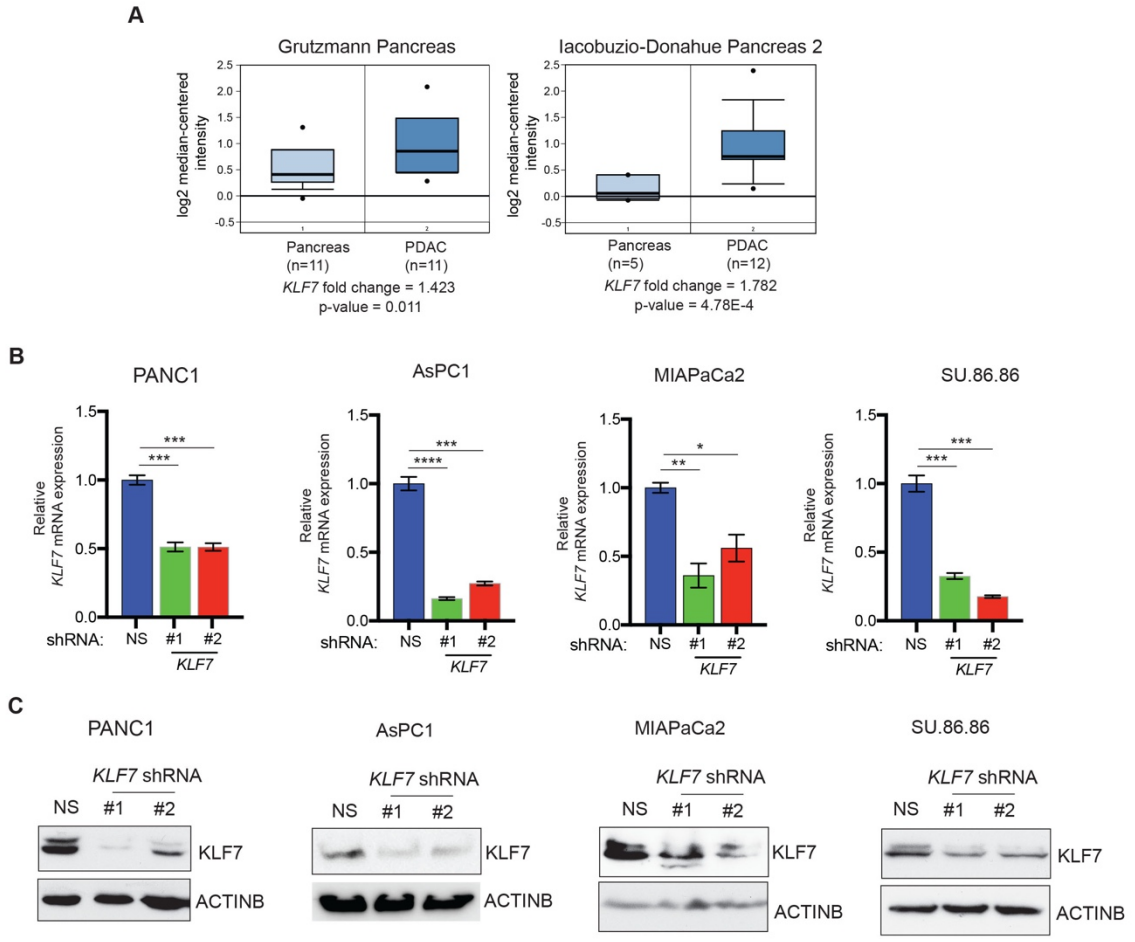

Fig. S1. KLF7 overexpression and knockdown in PDAC cell lines. (A) The indicated PDAC patient datasets were analyzed for *KLF7* mRNA expression. The upregulation of *KLF7* mRNA in PDAC compared with normal pancreas is shown. (B) The indicated PDAC cell lines expressing either non-specific (NS) or *KLF7* shRNAs were analyzed for *KLF7* mRNA expression. The *KLF7* mRNA expression is plotted relative to that in the NS shRNA-expressing cells. *ACTINB* was used for normalization. (C) The indicated PDAC cell lines expressing either non-specific (NS) or *KLF7* shRNAs were analyzed for *KLF7* via immunoblotting. ACTINB was used as loading control. Data are presented as the mean ± SEM, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

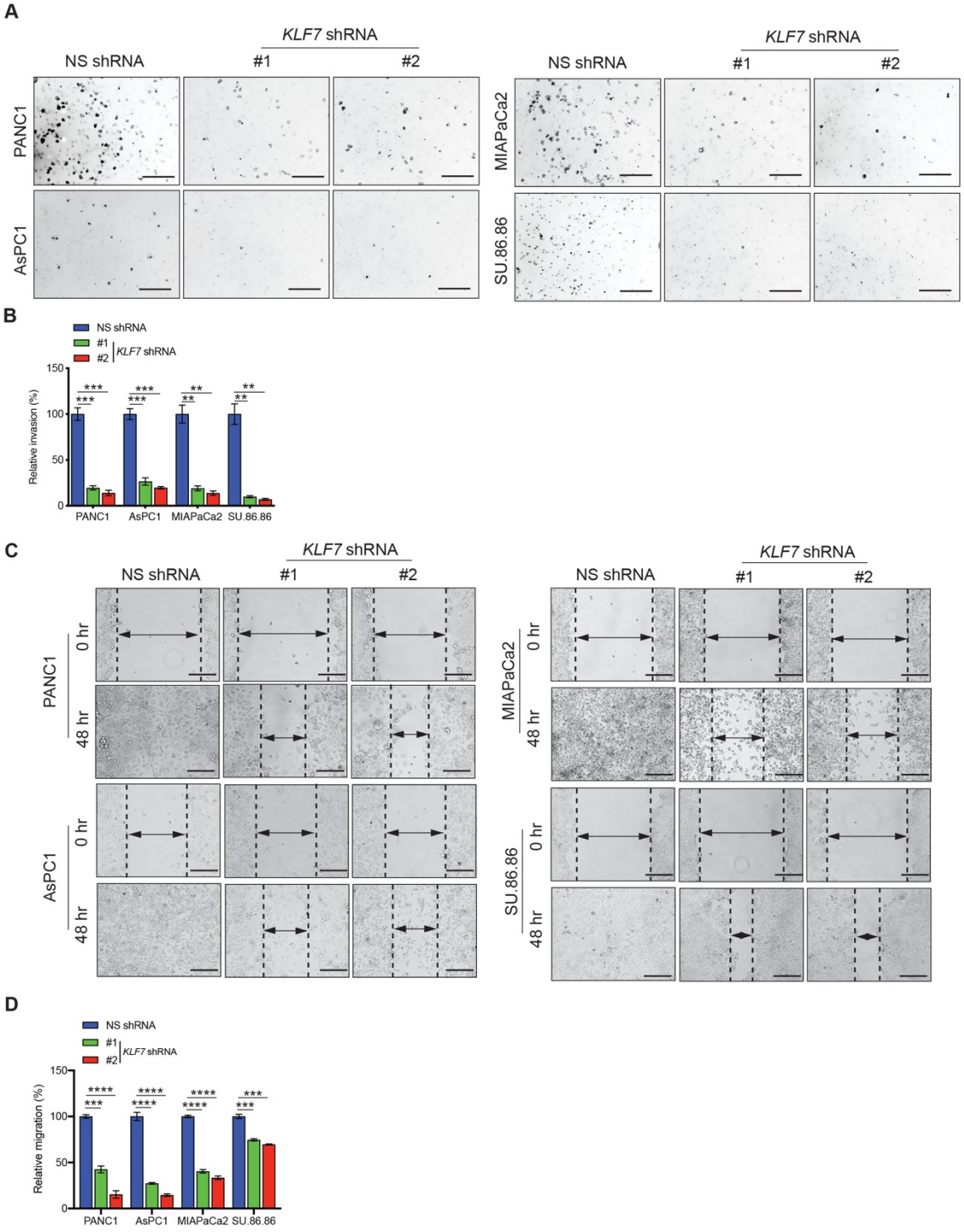

Fig. S2. KLF7 knockdown inhibits metastatic attributes of PDAC cells. (A) The indicated PDAC cell lines expressing either non-specific (NS) or *KLF7* shRNAs were analyzed by Matrigel-invasion assay. Representative images are shown. Scale bar, 200 μm. (B) Quantitation of the results presented in panel A. (C) Migration of PDAC cell lines

expressing either NS or *KLF7* shRNAs was analyzed in a wound-healing assay. Representative images are shown, Scale bar, 200 μm. (D) Quantitation of the data presented in panel C. Data are presented as the mean ± SEM. ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

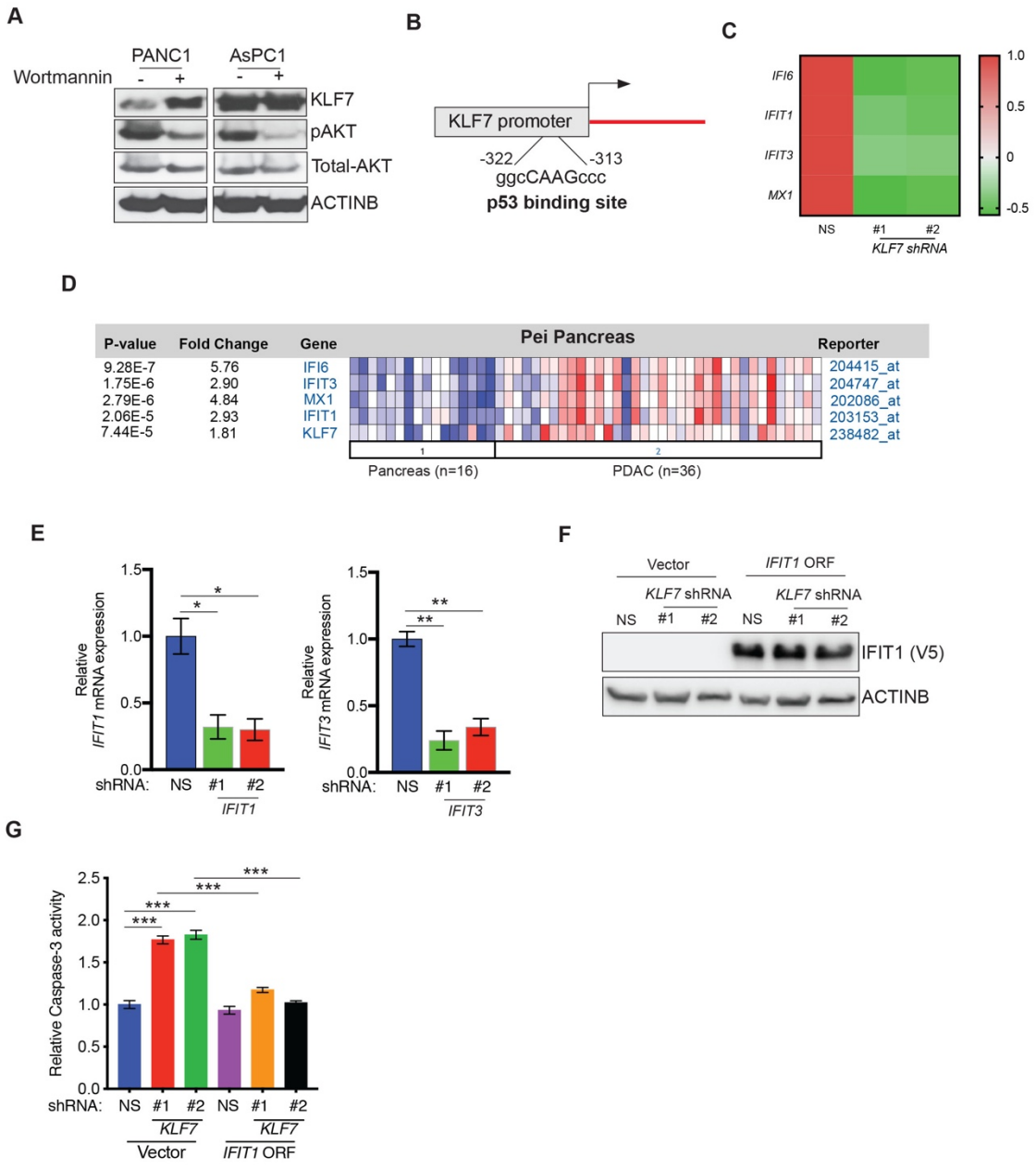

Fig. S3. p53 regulates KLF7, which in turn regulates interferon-stimulated genes (ISGs). (A) Indicated PDAC cell lines were treated with DMSO or wortmannin (1 μM) for 24 h. the indicated proteins were detected by immunoblot. (B) The coordinates of p53-binding sites on the *KLF7* promoter sequence are shown. (C) RNA sequencing was performed using PANC1 cells expressing non-specific (NS) or *KLF7* shRNAs. The heatmap shows the ISGs (IFI6, IFIT1, IFIT3, MX1) expression in *KLF7*-knockdown cells compared with that in NS shRNA-expressing cells. (D) The Pei dataset was analyzed for mRNA expression of *KLF7* and the indicated ISGs using the Oncomine database. Fold changes in expression and p-values for the indicated genes are shown. (E) PANC1 cells expressing either non-specific (NS) or *IFIT1 or IFIT3* shRNAs were analyzed for *IFIT1*

and IFIT3 mRNA expression by RT-qPCR. The *IFIT1 or IFIT3* mRNA expression is plotted relative to NS shRNA-expressing cells. *ACTINB* was used for normalization. (F) PANC1 cells expressing NS or *KLF7* shRNAs were ectopically expressed empty vector or V5-tagged IFIT1 ORF were analyzed for the IFIT1 expression using V5-tagged. The expression of IFIT1 was analyzed using antibody against V5. ACTINB was used for as a loading control. (G) PANC1 cells expressing either *KLF7* shRNAs along with vector, or with V5-tagged IFIT1 ORF were analyzed for apoptosis induction by measuring caspase 3 activity assay. Relative fold changes under indicated conditions is shown. Data are presented as the mean ± SEM, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

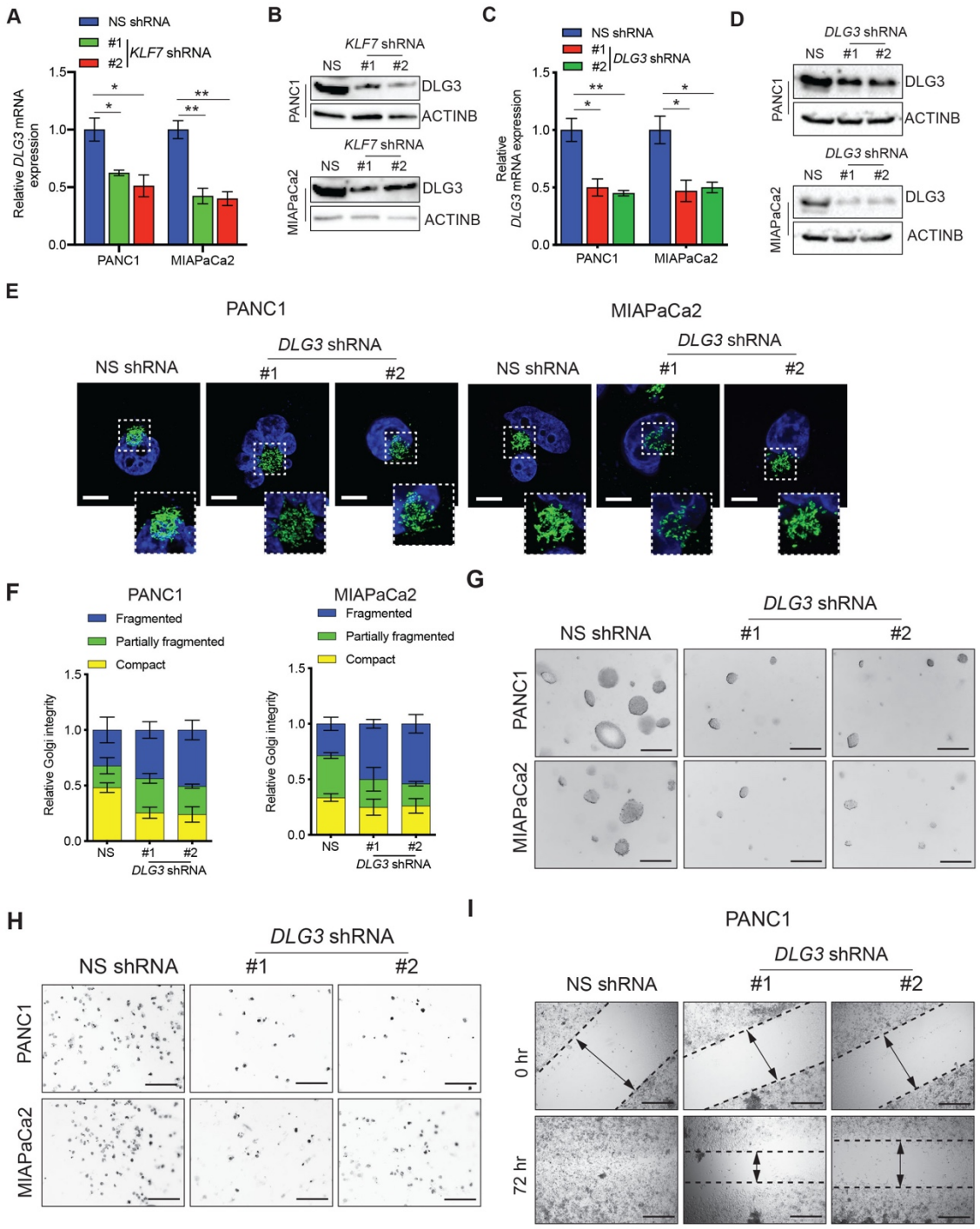

Fig. S4. The role of DLG3 in KLF7-mediated PDAC tumor growth and metastasis. (A) PDAC cell lines expressing either non-specific (NS) or *KLF7* shRNAs were analyzed for *DLG3* mRNA expression. *DLG3* mRNA expression is plotted relative to that in NS shRNA-expressing cells. *ACTINB* was used for normalization. (B) PDAC cell lines expressing either NS or *KLF7* shRNAs were analyzed for expression of the indicated proteins by immunoblotting. ACTINB was used as a loading control. (C) PDAC cell lines

expressing either non-specific (NS) or *DLG3* shRNAs were analyzed for *DLG3* mRNA expression. *ACTINB* was used as internal control. (D) PDAC cell lines expressing either non-specific (NS) or *DLG3* shRNAs were analyzed for DLG3 protein expression by immunoblotting. ACTINB was used as a loading control for immunoblotting. (E) PDAC cell lines expressing either NS or *DLG3* shRNAs were analyzed for Golgi fragmentation using GM130-based immunofluorescence staining. Representative confocal images are shown. Scale bar, 5 μm. (F) Quantitation of cells with compact, partly fragmented, or fragmented Golgi based on the data shown in panel E. (G) PDAC cell lines expressing NS or *DLG3* shRNAs were analyzed for anchorage-independent growth in a soft-agar assay. Representative images taken under the indicated conditions are shown. Scale bar, 500 μm. (H) PDAC cell lines expressing NS or *DLG3* shRNAs were analyzed in a Matrigel-invasion assay. Representative images taken under the indicated conditions are shown. Scale bar, 200 μm. (I) Migration of PDAC cell lines expressing NS or *DLG3* shRNAs was analyzed in a wound-healing assay. Representative images taken under the indicated conditions are shown. Scale bar, 200 μm. Data are presented as the mean ± SEM, * $p<0.05$, ** $p<0.01$.

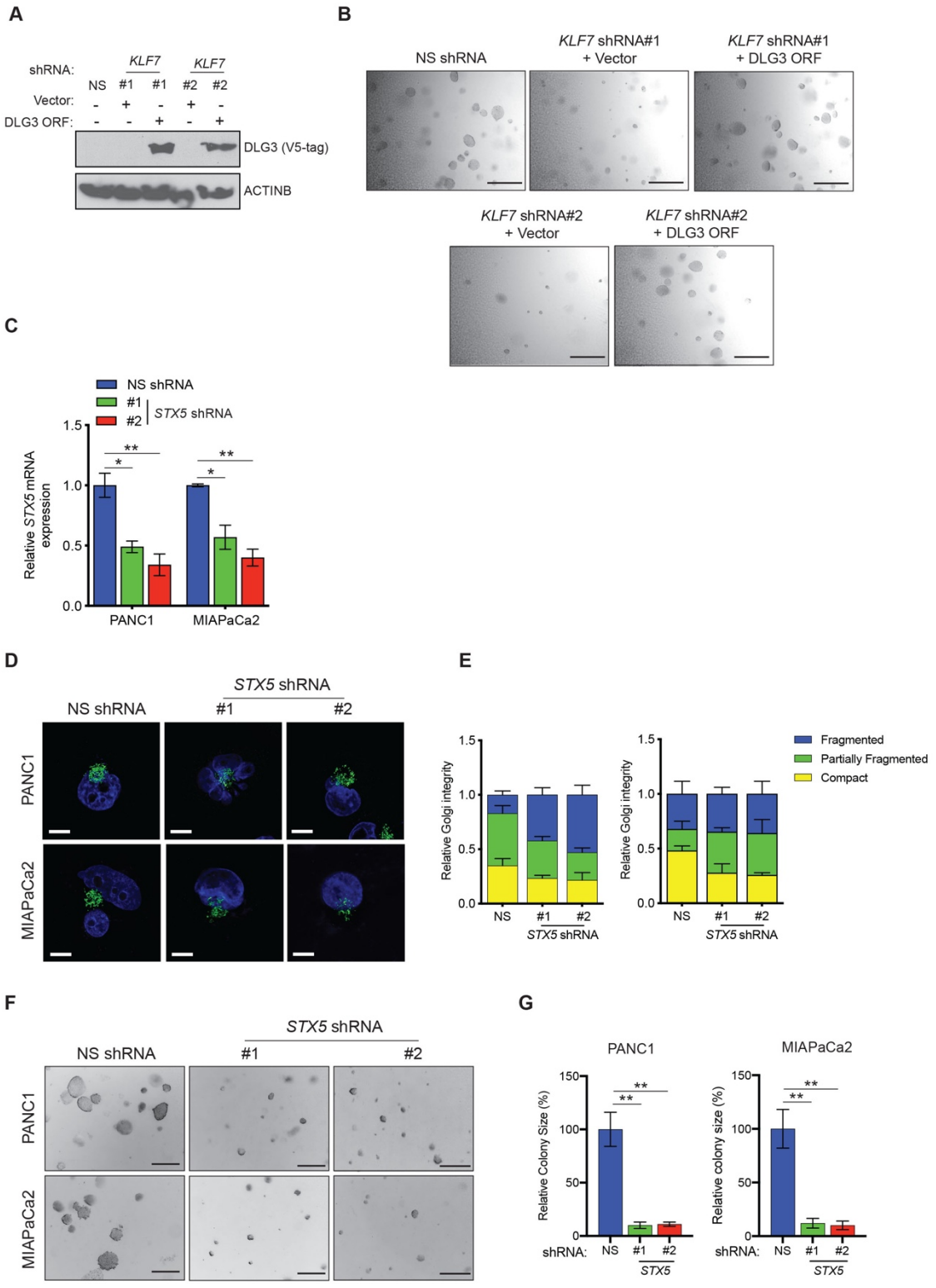

Fig. S5. DLG overexpression rescues Golgi integrity and PDAC tumor growth and metastatic attributes in KLF7 knockdown PDAC cells and STX5 knockdown inhibits

PDAC anchorage-independent growth and metastatic attributes of PDAC cells. (A) PANC1 cells expressing non-specific (NS) or *KLF7* shRNAs along with empty vector or V5-tagged DLG3 ORF were analyzed for DLG3 expression by immunoblotting using V-antibody. ACTINB was used as a loading control. (B) PANC1 cells expressing NS or *KLF7* shRNAs along with empty vector or V5-tagged DLG3 ORF were analyzed for anchorage-independent growth in a soft-agar assay. Representative images are shown. Scale bar, 500 μm. (C) PANC1 cells expressing NS or *STX5* shRNAs were analyzed for *STX5* mRNA expression. STX5 mRNA expression is plotted relative to that in NS shRNA-expressing cells. *ACTINB* was used for normalization. (D) PANC1 and MIAPaCa2 cells expressing either non-specific (NS) or *STX5* shRNAs were analyzed for Golgi fragmentation using immunofluorescence staining with the Golgi marker GM130. Representative confocal images are shown. Scale bar, 5 μm. (E) Quantitation of cells containing compact, partly fragmented, or fragmented Golgi based on the data shown in panel D. F) PANC1 and MIAPaCa2 cells expressing NS or *STX5* shRNAs were analyzed for anchorage-independent growth in a soft-agar assay. Representative images are shown. Scale bar, 500 μm. (G) Relative colony sizes of PANC1 and MIAPaCa2 cells expressing the indicated shRNAs as shown in panel F. Data are presented as the mean ± SEM, * $p<0.05$, ** $p<0.01$.

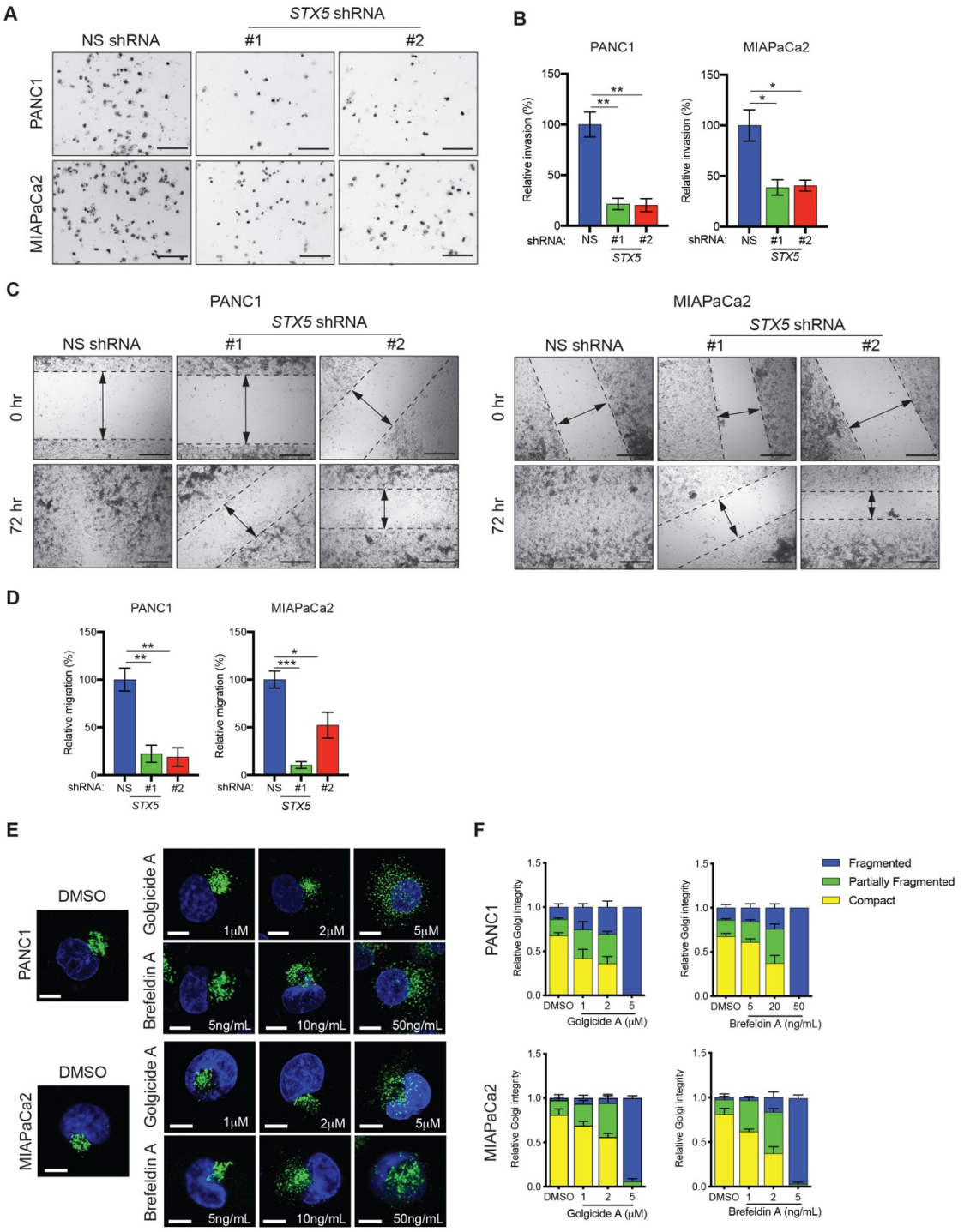

Fig. S6. Induction of Golgi fragmentation by inhibition of STX6 or by pharmacological means blocks PDAC tumor growth and metastasis. ((A) Invasion of PANC1 and MIAPaCa2 cells expressing NS or *STX5* shRNAs was analyzed in a Matrigel-invasion assay. Representative images are shown. Scale bar, 200 μm. (B) Relative invasion of PANC1 and MIAPaCa2 cells expressing the indicated shRNAs as shown in panel A.

(C) Migration of PANC1 and MIAPaCa2 cells expressing NS or *STX5* shRNAs was analyzed in a wound-healing assay. Representative images are shown. Scale bar, 200 μm. (D) Relative migration of PANC1 and MIAPaCa2 cells expressing the indicated shRNAs as shown in panel C. (E) PANC1 and MIAPaCa2 cells were treated with DMSO or the indicated concentrations of golgicide A or brefeldin A for 24 h and analyzed for Golgi fragmentation using GM130-based immunofluorescence staining. Representative confocal images of cells expressing the indicated shRNAs are shown. Scale bar, 5 μm. (F) Quantitation of cells containing compact, partly fragmented, or fragmented Golgi based on the data shown in panel E. Data are presented as the mean ± SEM, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

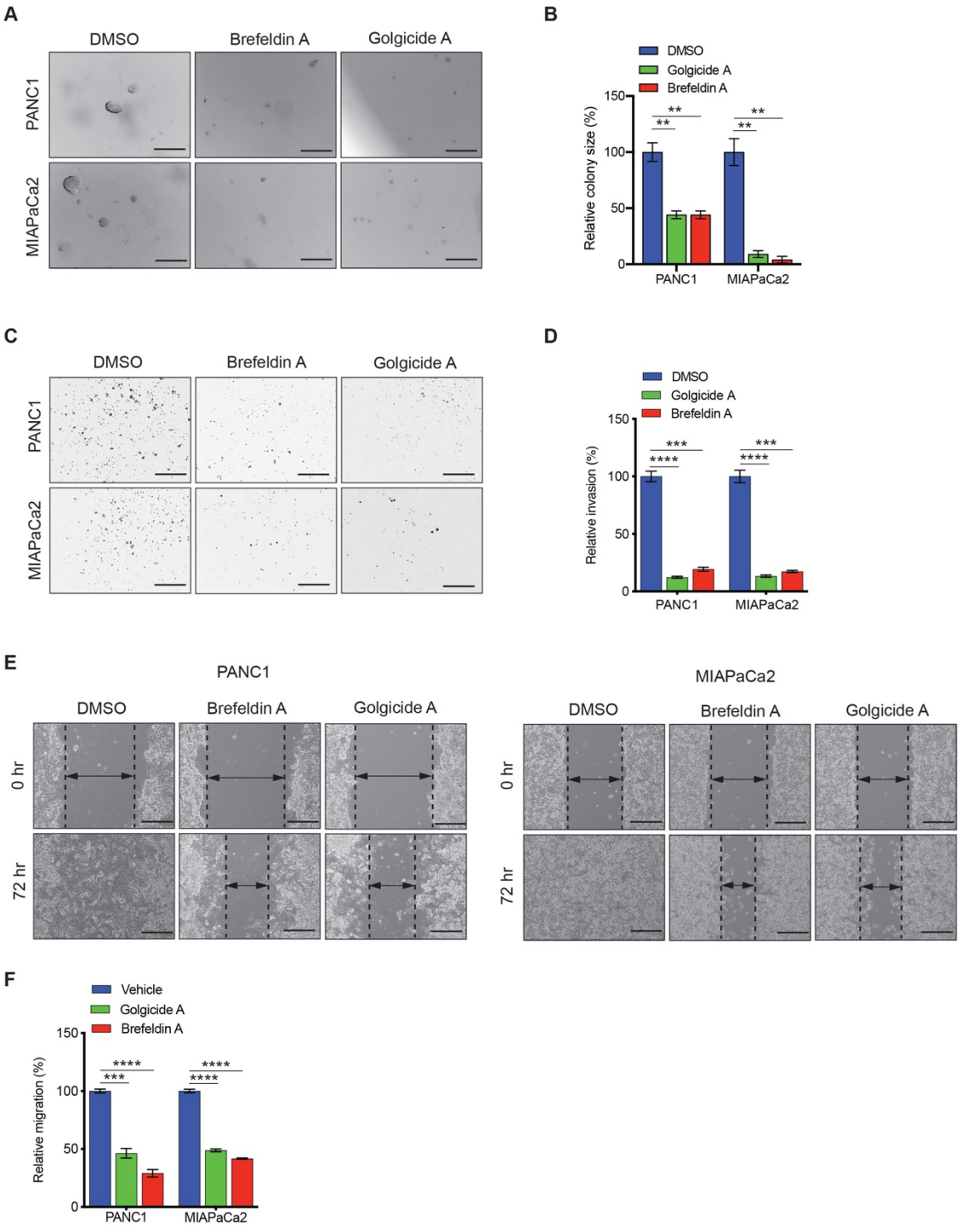

Fig. S7. Pharmacological activators of Golgi fragmentation block PDAC tumor growth. (A) PDAC cell lines were treated with brefeldin A (10 ng/ml) or golgicide A (2 μM) and analyzed for anchorage-independent growth in a soft-agar assay. Representative images taken under the indicated conditions are shown. Scale bar, 500 μm. (B) Relative colony sizes under the indicated conditions as shown in panel A. (C) PDAC cell lines were

treated with brefeldin A (10 ng/ml) or golgicide A (2 μM) and analyzed by Matrigel-invasion assay. Representative images are shown. Scale bar, 200 μm. (D) Relative invasion under the indicated conditions as shown in panel C. (E) PDAC cell lines were treated with brefeldin A (10 ng/ml) or golgicide A (2 μM) and analyzed in a wound-healing assay. Representative images are shown. Scale bar, 200 μm. (F) Relative migration under the indicated conditions as shown in panel E. Data are presented as the mean ± SEM, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

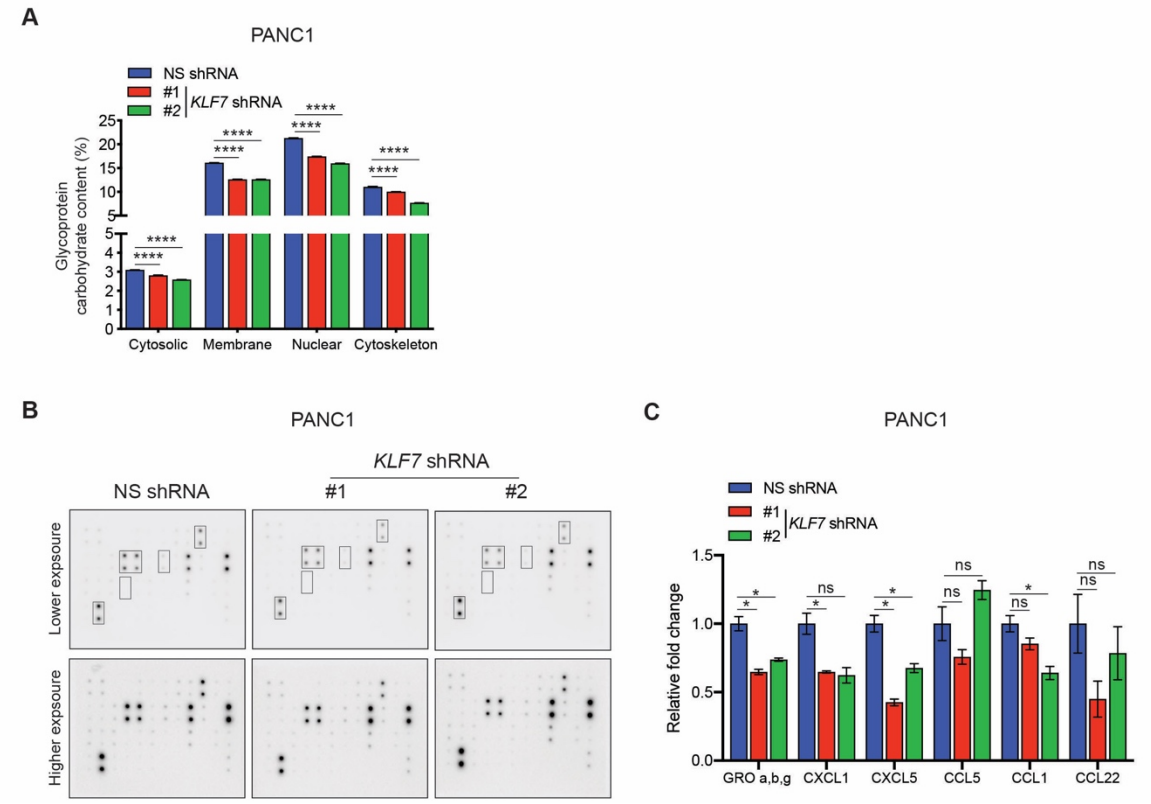

Fig. S8. Golgi fragmentation in *KLF7* knockdown PDAC cells resulted in reduced global protein glycosylation and reduced secretion of growth-promoting chemokines. (A) PANC1 cells expressing non-specific (NS) or *KLF7* shRNAs were analyzed for total glycosylated protein in various cellular fraction. The amounts of glycosylated proteins in cytosolic, membrane, nuclear and cytoskeleton in *KLF7* shRNAs expressing cells relative to those in NS shRNA-expressing cells is shown. (B) Expression of 38 human chemokines was measured in PANC1 cells expressing non-specific (NS) or *KLF7* shRNAs using human chemokine arrays. Representative images of the array membranes are shown. (C) Quantitation of the membranes shown on the left panel B. Data are presented as the mean ± SEM, ns = not significant, * $p<0.05$, **** $p<0.0001$.

SI References

1. Grutzmann R*, et al.* (2004) Gene expression profiling of microdissected pancreatic ductal carcinomas using high-density DNA microarrays. *Neoplasia* 6(5):611-622.
2. Ishikawa M*, et al.* (2005) Experimental trial for diagnosis of pancreatic ductal carcinoma based on gene expression profiles of pancreatic ductal cells. *Cancer Sci* 96(7):387-393.
3. Pei H*, et al.* (2009) FKBP51 affects cancer cell response to chemotherapy by negatively regulating Akt. *Cancer Cell* 16(3):259-266.
4. Badea L, Herlea V, Dima SO, Dumitrascu T, & Popescu I (2008) Combined gene expression analysis of whole-tissue and microdissected pancreatic ductal adenocarcinoma identifies genes specifically overexpressed in tumor epithelia. *Hepatogastroenterology* 55(88):2016-2027.
5. Buchholz M*, et al.* (2005) Transcriptome analysis of microdissected pancreatic intraepithelial neoplastic lesions. *Oncogene* 24(44):6626-6636.
6. Iacobuzio-Donahue CA*, et al.* (2003) Exploration of global gene expression patterns in pancreatic adenocarcinoma using cDNA microarrays. *Am J Pathol* 162(4):1151-1162.
7. Gazin C, Wajapeyee N, Gobeil S, Virbasius CM, & Green MR (2007) An elaborate pathway required for Ras-mediated epigenetic silencing. *Nature* 449(7165):1073-1077.
8. Trapnell C*, et al.* (2012) Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat Protoc* 7(3):562-578.
9. Ding S*, et al.* (2005) Efficient transposition of the piggyBac (PB) transposon in mammalian cells and mice. *Cell* 122(3):473-483.
10. Malvi P, Janostiak R, Nagarajan A, Cai G, & Wajapeyee N (2019) Loss of thymidine kinase 1 inhibits lung cancer growth and metastatic attributes by reducing GDF15 expression. *PLoS Genet* 15(10):e1008439.
11. Cervigni RI*, et al.* (2015) JNK2 controls fragmentation of the Golgi complex and the G2/M transition through phosphorylation of GRASP65. *J Cell Sci* 128(12):2249-2260.

Table S1. Summary of immunohistochemistry staining for KLF7 in human PDAC patient samples and matched normal adjacent pancreatic ductal tissues.

Staining	**Nuclear**			
Density	0	1+	2+	3+
Percentile	0 = Less than 10%	1+ = 10-25%	2+ = 26-50%	3+ = More than 50%

Tissue	**PDAC**									**Matched adjacent normal tissue**					
No.	**Gender**	**Age**	**Organ**	**Pathology Type**	**TNM**	**Stage**	**Grade**	**Density**	**Percentile**	**Pathology Type**	**TNM**	**Stage**	**Grade**	**Density**	**Percentile**
1	F	63	Pancreas	Ductal adenocarcinoma	T1N0M0	1A	Ⅱ	2+	2+	NAT	-	-	-	0	0
2	M	65	Pancreas	Ductal adenocarcinoma	T1N0M0	1A	Ⅱ	2+	2+	NAT	-	-	-	0	0
3	M	70	Pancreas	Ductal adenocarcinoma	T1N0M0	1A	Ⅱ	1+	0	NAT	-	-	-	0	0
4	M	52	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ	2+	3+	NAT	-	-	-	0	0
5	F	70	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ	2+	2+	NAT	-	-	-	1+	2+
6	M	54	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ	0	0	NAT	-	-	-	0	0
7	M	63	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ	2+	2+	NAT	-	-	-	0	0
8	M	65	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ	2+	2+	NAT	-	-	-	0	0
9	F	57	Pancreas	Adenosquamous carcinoma	T2N0M0	1B	Ⅱ	3+	2+	NAT	-	-	-	0	0
10	M	52	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ	2+	2+	NAT	-	-	-	0	0
11	M	51	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ	1+	2+	NAT	-	-	-	0	0
12	F	62	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ	2+	2+	NAT	-	-	-	1	0
13	F	46	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ	1+	1+	NAT	-	-	-	0	0
14	M	69	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ-Ⅲ	2+	3+	NAT	-	-	-	0	0
15	M	44	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅰ-Ⅱ	1+	1+	NAT	-	-	-	0	0
16	M	46	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅰ-Ⅱ	2+	3+	NAT	-	-	-	2+	1+
17	F	52	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ	1+	1+	NAT	-	-	-	1+	0
18	M	72	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ-Ⅲ	2+	2+	NAT	-	-	-	0	0
19	M	71	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ	1+	1+	NAT	-	-	-	0	0
20	F	77	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ-Ⅲ	1+	1+	NAT	-	-	-	0	0
21	M	61	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ-Ⅲ	2+	2+	NAT	-	-	-	2+	2+

22	M	80	Pancreas	Adenosquamous carcinoma	T2N0M0	1B	Ⅱ-Ⅲ	2+	2+	NAT	-	-	-	1+	1+
23	M	48	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ	3+	3+	NAT	-	-	-	0	0
24	M	64	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ	2+	1+	NAT	-	-	-	0	0
25	M	55	Pancreas	Adenosquamous carcinoma	T2N0M0	1B	Ⅱ-Ⅲ	3+	3+	NAT	-	-	-	1+	0
26	F	63	Pancreas	Ductal adenocarcinoma	T2N0M0	1B	Ⅱ	1+	2+	NAT	-	-	-	0	0
27	F	55	Pancreas	Ductal adenocarcinoma	T2M0	1/2/2016	II	2+	2+	NAT	-	-	-	0	0
28	F	62	Pancreas	Ductal adenocarcinoma	T2M0	1—2	Ⅱ-Ⅲ	2+	3+	NAT	-	-	-	0	0
29	F	78	Pancreas	Ductal adenocarcinoma	T3N0M0	2A	Ⅱ	1+	1+	NAT	-	-	-	1+	0
30	M	63	Pancreas	Ductal adenocarcinoma	T3N0M0	2A	Ⅱ	1+	1+	NAT	-	-	-	0	0
31	M	61	Pancreas	Adenosquamous carcinoma	T3N0M0	2A	Ⅱ-Ⅲ	1+	1+	NAT	-	-	-	0	0
32	M	55	Pancreas	Ductal adenocarcinoma	T3N0M0	2A	Ⅱ	1+	1+	NAT	-	-	-	0	0
33	M	60	Pancreas	Ductal adenocarcinoma	T3N0M0	2A	Ⅱ-Ⅲ	1+	2+	NAT	-	-	-	0	0
34	F	52	Pancreas	Ductal adenocarcinoma	T1N1M0	2B	Ⅱ	1+	1+	NAT	-	-	-	0	0
35	M	61	Pancreas	Ductal adenocarcinoma	T2N1M0	2B	Ⅲ	1+	1+	NAT	-	-	-	1+	0
36	F	57	Pancreas	Ductal adenocarcinoma	T2N1M0	2B	Ⅱ	3+	2+	NAT	-	-	-	0	0
37	F	60	Pancreas	Ductal adenocarcinoma	T2N1M0	2B	Ⅱ-Ⅲ	2+	2+	NAT	-	-	-	1+	1+
38	M	65	Pancreas	Ductal adenocarcinoma	T2N1M0	2B	Ⅲ	2+	3+	NAT	-	-	-	0	0
39	M	41	Pancreas	Ductal adenocarcinoma	T2N1M0	2B	Ⅱ-Ⅲ	2+	1+	NAT	-	-	-	0	0
40	F	55	Pancreas	Ductal adenocarcinoma	T2N1M0	2B	Ⅱ	2+	1+	NAT	-	-	-	1+	1+
41	M	71	Pancreas	Ductal adenocarcinoma	T2N1M0	2B	Ⅱ	1+	1+	NAT	-	-	-	1+	1+
42	M	62	Pancreas	Ductal adenocarcinoma	T2N1M0	2B	Ⅰ-Ⅱ	1+	1+	NAT	-	-	-	2+	1+
43	M	73	Pancreas	Ductal adenocarcinoma	T2N1M0	2B	Ⅱ	2+	2+	NAT	-	-	-	0	0
44	M	71	Pancreas	Ductal adenocarcinoma	T2N1M0	2B	Ⅱ	1+	1+	NAT	-	-	-	0	0
45	M	65	Pancreas	Ductal adenocarcinoma	T2N1M0	2B	Ⅱ	2+	1+	NAT	-	-	-	0	0
46	F	44	Pancreas	Ductal adenocarcinoma	T2— —M0	1—2	III	1+	2+	NAT	-	-	-	0	0
47	F	NA-	Pancreas	Ductal adenocarcinoma	T2N1M0	2B	Ⅰ-Ⅱ	1+	1+	NAT	-	-	-	0	0
48	M	62	Pancreas	Ductal adenocarcinoma	T2N0M1	4	Ⅱ	1+	1+	NAT	-	-	-	0	0
49	F	60	Pancreas	Ductal adenocarcinoma	T2— —M1	4	III	2+	3+	NAT	-	-	-	1+	1+
50	F	60	Pancreas	Ductal adenocarcinoma	T3N1M1	4	II	1+	2+	NAT	-	-	-	0	0

Table S2: Primer sequences for RT-qPCR analysis; clone ID and catalog numbers for shRNAs (Open Biosystems); antibodies used; source and concentration of chemical inhibitors used.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
KLF7 (Immunoblotting)	Abcam	Cat# ab197690
KLF7 (IHC)	Sigma-Aldrich	Cat# HPA030490; RRID: AB_10601922
p53	Santa Cruz	Cat# sc-126 (DO-1); RRID: AB_628082
p21	Santa Cruz	Cat# sc-271532 (B-2) RRID: AB_10650266
ACTINB	Cell signaling	Cat#4970; RRID: AB_2223172
pAKT Ser473	Cell Signaling	Cat#4060; RRID: AB_2315049
Total-AKT	Cell Signaling	Cat#9272; RRID: AB_329827
pERK1/2	Cell Signaling	Cat#4376; RRID: AB_331772
Total-ERK1/2	Cell Signaling	Cat#4695; RRID: AB_390779
V5	Cell Signaling	Cat#13202; RRID: AB_2687461
DLG3	Abcam	Cat# ab3438; RRID: AB_303802
IFIT1	Cell Signaling	Cat# 14769; RRID: AB_2783869
IFITM1	Cell Signaling	Cat# 13126, RRID: AB_2798126
IFITM3	Cell Signaling	Cat# 59212, RRID: AB_2799561
IFI6	Sigma-Aldrich	Cat# SAB1306792
MX1	Cell Signaling	Cat# 37849, RRID: AB_2799122
Biological Samples		
Tissue microarray slide (PDAC and matched normal pancreas tissue)	US Biomax, Inc.	Cat# HPan-Ade150CS-02
Chemicals, Peptides, and Recombinant Proteins		
DMEM	GIBCO	Cat# 11965-092
RPMI	GIBCO	Cat# 11875-093
Fetal Bovine Serum	GIBCO	Cat# 10437-028
Trypsin-EDTA	GIBCO	Cat# 25200-056
Penicillin-Streptomycin	GIBCO	Cat# 15140-122
Effectene Transfection Reagent	QIAGEN	Cat# 301427
Trametinib	Cayman	Cat# 16292

Wortmannin	Calbiochem	Cat# 10010591
Brefeldin A	Sigma-Aldrich	Cat# B6542
Golgicide A	Sigma-Aldrich	Cat# G0923
Agarose, Low gelling	Sigma-Aldrich	Cat# A9045
Matrigel Invasion Chambers	BD Biosciences	Cat# 354483
VECTASHIELD Hardset Antifade Mounting Medium with DAPI	Vector Laboratories	Cat# H-1500
XenoLight D-Luciferin - K+ Salt Bioluminescent Substrate	Perkin Elmer	Cat# 122799
Deposited Data		
RNA-Seq performed with KLF7 KD PANC1 cells	This paper	GEO: GSE107184
Experimental Models: Cell Lines		
293T	ATCC	ATCC CRL-3216
PANC1	ATCC	ATCC CRL1469
AsPC1	ATCC	ATCC CRL-1682
MIAPaCa2	ATCC	ATCC CRL-1420
SU.86.86	ATCC	ATCC CRL-1837
HPNE	ATCC	ATCC CRL-4023
Experimental Models: Organisms/Strains		
Mouse: NU/J homozygous	Jackson Laboratory	Stock No. 002019
Mouse: NSG	Jackson Laboratory	Stock No. 005557
Oligonucleotides		
ACTINB qPCR Forward primer	gtcttcccctccatcgtggg	
ACTINB qPCR Reverse primer	cctctcttgctctgggcctc	
KLF7 qPCR Forward primer	ggcagtccgaggggcttatt	
KLF7 qPCR Reverse primer	cgttgggatcgacgcgaaag	
p53 qPCR Forward primer	tatgagccgcctgaggttgg	
p53 qPCR Reverse primer	ggcacaaacacgcacctcaa	
p21 qPCR Forward primer	gggatgagttgggaggaggc	
p21 qPCR Reverse primer	gaagatcagccggcgtttgg	
DLG3 qPCR Forward primer	tgggcaaagcagtgggaaga	
DLG3 qPCR Reverse primer	gctgtaccgatccccaggac	
IFI6 qPCR Forward primer	tcacttgcagtggggtggag	
IFI6 qPCR Reverse primer	caggatcgcagaccagctca	

IFIT1 qPCR Forward primer	ggtaggctctgcttcccagg	
IFIT1 qPCR Reverse primer	cattgccaaggctgctctcg	
IFIT3 qPCR Forward primer	agtgaggaaatgggccaggg	
IFIT3 qPCR Reverse primer	aaccaccactgcaggcttct	
IFITM1 qPCR Forward primer	ccccagcaccatccttccaa	
IFITM1qPCR Reverse primer	aagcccagacagcaccagtt	
IFITM3 qPCR Forward primer	ctggtcttcgctggacacca	
IFITM3 qPCR Reverse primer	cggatgtggatcacggtgga	
MX1 qPCR Forward primer	tggagcaggacaggttgcaa	
MX1 qPCR Reverse primer	ctcgctatgccgtctgggaa	
ChIP		
KLF7 Promoter p53 binding Forward primer	atagctcgcctcgcaccgc	
KLF7 Promoter p53 binding Reverse primer	ccgcgtgtgaccatgtaagg	
ACTINB promoter p53 binding Forward primer	gaggggagaggggtaaaaa	
ACTINB promoter p53 binding Reverse primer	aaaggcgaggctctgtgct	
shRNAs		
KLF7 shRNA#1	TRCN0000013258	RHS3979-9580947
KLF7 shRNA#2	TRCN0000013261	RHS3979-9580950
TP53 shRNA#1	V3LHS_333919	RHS4430-101168779
TP53 shRNA	V3LHS_333918	RHS4430-101164691
IFIT1 shRNA#1	TRCN0000158439	RHS3979-99219747
IFIT1 shRNA#1	TRCN0000159834	RHS3979-99219755
IFITM1 shRNA#1	TRCN0000057499	RHS3979-9624683
IFITM1 shRNA#2	TRCN0000057502	RHS3979-9624686
IFITM3 shRNA#1	TRCN0000118023	RHS3979-98060061
IFITM3 shRNA#2	TRCN0000118024	RHS3979-98060068
Recombinant DNA		
Plasmid: pLX304-V5-BLAST	Addgene	Cat# 25890
Plasmid: pBabe Puro	Addgene	Cat# 1764
Plasmid: pLX304-IFIT1	Horizon Discovery	Cat# OHS6085-213578947
Plasmid: pLX304-IFITM1	Horizon Discovery	Cat# OHS6085-213574587
Plasmid: pLX304-IFITM3	Horizon Discovery	Cat# OHS6085-213575055
Plasmid: pLX304-MX1	Horizon Discovery	Cat# OHS6269-213579120
Plasmid: pLX304-DLG3	Horizon Discovery	Cat# OHS6058-213573135

Plasmid: piggyBac GFP-Luc	Ding et al., 2005	N/A
Plasmid: Act-PBase	Ding et al., 2005	N/A
Commercial assays and kits		
Glycoprotein Carbohydrate Estimation Kit	Thermo Scientific	Cat# 23260
ProteoExtract Subcellular Proteome Extraction Kit	Millipore Sigma	Cat# 539790
Human Chemokine Array C1	RayBiotech, Inc.	Cat# AAH-CHE-1-4
Caspase-3 calorimetric assay kit	Biovision, Inc.	Cat# K106
Software and Algorithms		
Prism 8.0	GraphPad	www.graphpad.com/scientific software/prism
ImageJ	https://imagej.nih.gov/ij	N/A
Other		
KLF7 mRNA expression in normal and PDAC samples were analyzed and represented as box plot.	Oncomine Research Premium Edition	https://www.oncomine.org/
KLF7 mRNA expression in normal and PDAC samples were analyzed and represented as box plot.	Gene Expression Profiling Interactive Analysis (GEPIA)	http://gepia.cancer-pku.cn