SUPPLEMENTARY METHODS

In vivo studies

All animal studies were approved by the Northwestern University Institutional Animal Care and Use Committee. For the flank tumor xenograft mouse model, 1.5 million WT and SLFN5 KO PANC-1 and MIA-Pa-Ca-2 cells [100 µl cell suspension of 50% PBS and 50% Matrigel (Thermo Fisher)], were implanted into the left flank of athymic NUDE mice, 5-7 weeks of age, by subcutaneous injection (Taconic). Once tumors reached a measurable size, tumor volume was measured every other day until the criterion for euthanasia was reached (2 cm³ tumor size). Tumor length and width were measured using calipers every other day, and volume calculated according to the formula: \( \frac{D \times d^2}{2} \), where \( D \) is the longest diameter and \( d \) is the shorter diameter.

For the orthotopic xenograft mouse model, 2 million WT and SLFN5 KO MIA-Pa-Ca-2–luciferase expressing cells were implanted directly into the pancreas of each mouse using an aseptic surgical technique. A 25 µl cell suspension was prepared in 30% PBS and 70% Matrigel. The cell suspension was loaded into a Hamilton syringe. The spleen was located and an incision was made in the skin to create a pocket. Another incision was made through the peritoneal muscle, to have access into the peritoneal cavity. The spleen was gently removed from the peritoneal cavity with the attached pancreas exposed. Cells were injected into the tail of the pancreas such that the cell suspension formed a clear bubble on the pancreatic surface. The needle was kept in the injection site for ~30 sec and then gently removed to prevent leakage and loss of cells. The spleen and pancreas were then returned into the peritoneal cavity and the incisions were sutured closed. Mice received post-surgical care according to IACUC guidelines.

For in vivo imaging, a Lago/Lago X - Spectral Instruments Imaging system was used. Mice were anesthetized with isoflurane and were imaged 10 minutes after injection.
of luciferin (150 mg/kg). Signal intensities were quantified using Aura software and each image was normalized to its background bioluminescence signal for each reading. Mice were euthanized when BLI signal was ≥ 2.5X10^{10} Radiance within the ROI (Region Of Interest), calculated by the Aura software using the formula: photons/sec/cm^2/sr [number of photons per second that leave a square centimeter of tissue and radiate into a solid angle of one steradian (sr)].

**3D culture of pancreatic cancer cell lines and tumor spheroid assay**

WT and SLFN5 KO PANC-1, MIA-Pa-Ca-2 cells, and SLFN5-KO+SLFN5 PANC-1/MIA-Pa-Ca-2 cells were dissociated with TrypLE and seeded into low-adhesion flasks for suspension culture (6 million cells per 75 cm^2 flask), in cancer stem cell medium: DMEM/F12 (Thermo Fisher), EGF 20ng/ml (Peprotech), FGF 20 ng/ml (Peprotech), heparin 5µg/ml (Sigma Aldrich), B27 2% (Thermo Fisher) and gentamycin 0.1 mg/ml (Sigma Aldrich). The cells were allowed to grow and form spheres for 14 days. Subsequently, pancreas-spheres were dissociated into single cells and seeded (4000 cells/well) into 96 well round bottom ultra-low attachment plates (Fisher/Corning). The spheres were allowed to grow for 14 days and then stained with 0.1 µg/ml of acridine orange (Sigma Aldrich) for 1 hr. The spheres were visualized using a Cytation 3 Cell Imaging Multi-Mode Reader, to determine cross-sectional area.

**Generation of Flag-SLFN5 constructs**

pReciever-M12 containing 3XFlag-SLFN5 (GeneCopoeia) served as a PCR template using Primers with BamHI and KpnI restriction sites for cloning of the SLFN5 ORF into the pENTR4-FLAG vector, (gift from Eric Campeau & Paul Kaufman,
FLAG-SLFN5 was then moved into pLenti-CMV-Hygro-DEST (gift from Eric Campeau & Paul Kaufman, Addgene)\(^1\), using Gateway cloning.

**Generation of SLFN5 overexpressing cells**

Lentivirus was generated using Lenti-X Packaging Single Shots (VSV-G) (Clontech) and Lenti-X 293T cells according to the manufacturer’s instruction. The titer was estimated using Lenti-X GoStick (Clontech). The lentiviral particles were concentrated using Lenti-X Concentrator (Clontech), according to the manufacturer’s instructions, and stored at -80°C. For generation of PANC-1-TetON-SLFN5-Myc-Flag stable cell lines, and SLFN5-KO+SLFN5 PANC-1 and SLFN5-KO+SLFN5 MIA-Pa-Ca-2 stable cell lines, the TransDux MAX Lentivirus Transduction enhancer (System Biosciences) was used according to the manufacturer’s instruction. PANC-1-TetON-SLFN5-Myc-Flag cells were expanded under Puromycin (Thermo Fisher) selection. Cells were treated with 10\(\mu\)g/ml doxycycline for 48 hrs to induce expression of SLFN5. Cells left untreated were used as negative controls. SLFN5-KO+SLFN5 PANC-1 and SLFN5-KO+SLFN5 MIA-Pa-Ca-2 cells were expanded under Puromycin and Hygromycin B (Thermo Fisher).

**Generation SLFN5 KO cell lines using CRIPR/Cas9 technology**

Cells were transfected using Turbofect (Thermo Fisher). Specifically, PANC-1 cells were transfected with plasmid carrying Cas9n (D1OA nickase mutant) and single guide RNA (sgRNA) targeting specifically SLFN5 gene, and MIA-Pa-Ca-2 cells were transfected with Cas9, sgRNA targeting SLFN5 gene and HDR (Homology Direct Repair) plasmids. Cells were maintained under Puromycin selection for two weeks and then sorted by flow cytometry for high expression of GFP (Green Florescent Protein) for PANC-1 cells, and RFP (Red florescent protein) for MIA-Pa-Ca2 cells. The sorted cells
were then seeded into 96 well plates, one cell per well, in order to obtain single clones. Different clones were then tested for SLFN5 expression by Western Blot analysis. We proceeded with using clone #6 for PANC-1 cells and clone #21 for MIA-Pa-Ca-2 cells, for all experiments. SLFN5 WT MIA-Pa-Ca-2-LUC and SLFN5 KO MIA-Pa-Ca-2-LUC cells used for the *in vivo* experiments were transduced using lentivirus with pFULT-Luciferase-Tomato, as described above.

**siRNA-mediated knockdown**

Control and *E2F7* targeting siRNAs were from Santa Cruz Biotechnology and used with Lipofectamine RNAiMAX reagent and Opti-MEM medium (Thermo Fisher), as previously described.²

**Hematoxylin and eosin (H&E) and immunohistochemistry (IHC) of thin sections**

Stained slides were from banked patient samples from the Biorepository of the Pathology Core Facility of the Robert H. Lurie Comprehensive Cancer Center. Samples in the Biorepository are collected after obtaining informed consent approved by the Institutional Review Board of Northwestern University. For the orthotopic pancreatic adenocarcinoma mouse model, the pancreas from mice injected with WT MIA-Pa-Ca-2 (n=4) or SLFN5 KO MIA-Pa-Ca-2 (n=5) cells, was harvested 25 days after implantation of tumor cells, and fixed in 10% formalin for 24 hours. H&E staining of thin sections and IHC were performed by the Human Pathology Core of Northwestern University, as previously described.² Sections were analyzed for the presence and phenotype of the tumor by a board-certified pathologist, Dr. Guang-Yu Yang. The presence of an orthotopic tumor was detected for all the mice injected with WT MIA-Pa-Ca-2 cells, but only in 3 of the 5 mice injected with SLFN5 KO MIA-Pa-Ca-2 cells. Slides were scanned using a Hamamatsu NanoZoomer Digital slide scanner and
images were exported using NDP.view2 Viewing software and analyzed using QuPath software.

Proteomic Immunoprecipitation Analysis using LC-MS/MS

Cell lysates were prepared in NP40 lysis buffer and immunoprecipitation performed using FLAG-M2 conjugated sepharose beads (Sigma Aldrich). Samples from cells not exposed to Dox were used as negative controls. Samples were washed 5 times in NP40 lysis buffer and then boiled in 2x Laemmli sample buffer for 10 min. For sample preparation, protein samples were resolved by SDS-PAGE gel and a gel lane (sample) was divided into 10 gel fractions based on molecular weight before performing in-gel digestion. Gel fractions were washed in 100 mM Ammonium Bicarbonate (AmBic)/Acetonitrile (ACN) and reduced with 10 mM dithiothreitol at 50°C for 30 minutes. Cysteines were alkylated with 100 mM iodoacetamide in the dark for 30 minutes at room temperature. Gel fractions were then washed in 100mM AmBic/ACN prior to adding 600 ng trypsin for overnight incubation at 37 °C. Supernatants containing peptides were collected. Gel fractions were washed at room temperature for 10mins with gentle shaking in 50% ACN/5% FA (formic acid), and supernatants were collected. This wash step was repeated for 80% ACN/5% FA, and 100% ACN, and all supernatants were pooled, then subjected to speedvac drying. After lyophilization, peptides were reconstituted with 5% ACN/0.1% FA, in water. Peptides were analyzed by LC-MS/MS using a Dionex UltiMate 3000 Rapid Separation nanoLC coupled to Orbitrap Elite Mass Spectrometer (Thermo Fisher Scientific Inc, San Jose, CA). Samples were loaded onto the trap column, which was 150 μm x 3 cm in-house packed with 3 um ReproSil-Pur® beads. The analytical column was a 75 μm x 10.5 cm PicoChip column packed with 3 um ReproSil-Pur® beads (New Objective, Inc. Woburn, MA). The flow rate was
maintained at 300nL/min. Solvent A was 0.1% FA in water and Solvent B was 0.1% FA in ACN. The peptides were separated on a 60-min analytical gradient from 5% ACN/0.1% FA to 40% ACN/0.1% FA. MS\(^1\) scans were acquired from 400-2000m/z at 60,000 resolving power and automatic gain control (AGC) set to 1x10\(^6\). The 15 most abundant precursor ions in each MS\(^1\) scan were selected for fragmentation by collision-induced dissociation (CID) at 35% normalized collision energy in the ion trap, Previously selected ions were dynamically excluded from re-selection for 60 seconds. Proteins were identified from the MS raw files using the Mascot search engine (Matrix Science, London, UK; version 2.5.1). MS/MS spectra were searched against a Homo Sapiens database (Swiss-Prot, 2019). All searches included carbamidomethyl cysteine as a fixed modification and oxidized Met, deamidated Asn and Gln, acetylated N-term as variable modifications. Three missed tryptic cleavages were allowed. The MS\(^1\) precursor mass tolerance was set to 10 ppm and the MS\(^2\) tolerance was set to 0.6 Da. The search result was visualized by Scaffold (version 4.9.0. Proteome Software, INC., Portland, OR). A 1% false discovery rate cutoff was applied at the peptide level. Only proteins with a minimum of two peptides above the cut-off were considered for further study.

5-Ethynyl-2’-deoxyuridine (EdU) incorporation assay

Cells were plated in triplicate in 96 well plates at 70% confluency, and 24 hrs later cells were serum starved in 0.5% FBS DMEM and maintained for 36 hrs. Cells were allowed to reattach to the bottom of the wells by adding 10% FBS medium for 3 hrs. Then, cells were synchronized in S phase by adding 2mM Hydroxyurea (HU) to the medium for 16 hrs. Cells were then released into 10% FBS medium containing EdU for 6 hrs, or left synchronized in 2mM HU media containing EdU. Subsequently, cells were processed and stained with Click-it™ EdU Alexa Fluor™ 488 HCS Assay kit (Thermo
Fisher/Invitrogen) according to the manufacturer’s instruction. The plate was scanned using a Cytation 3 Cell Imaging Multi-Mode Reader.

**Chromatin Immunoprecipitation (ChIP)**

Cells were synchronized in S phase as described above. ChIP was performed using the SimpleChIP® Enzymatic Chromatin IP Kit with Magnetic Beads from Cell Signaling, as per the manufacturer’s instructions. Antibody for E2F7 was purchased from Bethyl. Normal rabbit IgG was used as a negative control. qPCR was performed on purified immunoprecipitated DNA for the E2F1 promoter, CDC6 promoter and ACTIN promoter as a negative control using SsoAdvanced™ Universal SYBR® Green Supermix according to the manufacturer’s instructions with previously published primers listed below: E2F1 FW-AGGGCTCGATCCCGCTCCG; E2F1 REV-TAAAGCCAATAGGAACCGCCG; CDC6 FW-AAAGGCTCTGTGACTACAGCCA; CDC6 REV-GATCCTTCTCAGTCTCACA; ACTIN FW-ATCGTGCGTGACATTAAGGAGAAG; ACTIN REV-CTGGAAGCAGCCGTGGCCGTCTCTTG. All qPCR signals were normalized to the input DNA.

**Cell Cycle analysis by Flow Cytometry**

Cells were seeded in 10cm² dishes to be 50% confluent, then 24 hrs later cells were treated with 2mM thymidine (Sigma-Aldrich) for 14 hrs, and subsequently released into 10% FBS medium for 10 hrs, then 2mM thymidine was added again to the medium.
for 14 hrs. Cells were either left synchronized or released into 10% FBS medium for 6, 8, 10, 12, 14 and 24 hrs. At each time point, cells were harvested, fixed in 100% cold methanol and stored at -80°C. At the time of flow cytometry analysis, cells were washed twice in PBS, resuspended in 1 ml of PBS, stained with 1μl/ml of Propidium Iodide (PI) (stock 10mg/ml) (Sigma-Aldrich) and 4 μl/ml of RNAse A (stock 10 mg/ml) (Thermo Fisher) added, and the suspension incubated for 30 min in the dark. Subsequently, cells were analyzed by flow cytometry. Data were analyzed using the Cell Cycle tool of the FlowJo software.

SUPPLEMENTARY REFERENCES

1 Campeau E, Ruhl VE, Rodier F, Smith CL, Rahmberg BL, Fuss JO et al. A versatile viral system for expression and depletion of proteins in mammalian cells. PLoS One 2009; 4: e6529.

2 Eckerdt F, Bell JB, Beauchamp EM, Clymer J, Blyth GT, Kosciuczuk EM et al. Potent Antineoplastic Effects of Combined PI3Kalpha-MNK Inhibition in Medulloblastoma. Mol Cancer Res 2019; 17: 1305-1315.

3 Bankhead P, Loughrey MB, Fernandez JA, Dombrowski Y, McArt DG, Dunne PD et al. QuPath: Open source software for digital pathology image analysis. Sci Rep 2017; 7: 16878.
### Supplementary Table S3: Key resources table

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| SLFN5               | Sigma-Aldrich | Cat#: HPA017760, RRID:AB_2189993 |
| GAPDH               | Millipore | Cat#: MAB374, RRID:AB_2107445 |
| FLAG-M2- HRP        | Sigma-Aldrich | Cat#: A8592-0.2mg, RRID:AB_439702 |
| Alpha-tubulin (B-7) | Santa Cruz Biotechnology | Cat#: sc-5286, RRID:AB_628411 |
| Beta-tubulin        | Cell Signaling Technology | Cat#: 2146, RRID:AB_2210545 |
| RPB1 CTD (4H8)      | Cell Signaling Technology | Cat#: 2629, RRID:AB_2167468 |
| E2F7                | Abcam | Cat#: ab56022, RRID:AB_880024 |
| E2F7                | Bethyl | Cat#: A303-037A, RRID:AB_2615234 |
| E2F1 (KH95)         | Santa Cruz Biotechnology | Cat#: sc-251, RRID:AB_627476 |
| CDC6 (C42F7)        | Cell Signaling Technology | Cat#: 3387, RRID:AB_2078525 |
| Cyclin E1 (HE12)    | Cell Signaling Technology | Cat#: 4129, RRID:AB_2071200 |
| Cyclin A2 (BF683)   | Cell Signaling Technology | Cat#: 4656, RRID:AB_2071958 |
| Cyclin B1 (GNS1)    | Santa Cruz Biotechnology | Cat#: sc-245, RRID:AB_627338 |
| Phospho-Aurora A (Thr288) (C39D8) | Cell Signaling Technology | Cat#: 3079, RRID:AB_2061481 |
| Aurora A (D3E4Q)    | Cell Signaling Technology | Cat#: 14475, RRID:AB_2565504 |
| SLFN11 (D-2)        | Santa Cruz Biotechnology | Cat#: sc-515071, RRID:N/A |
| SLFN12 (G13)        | Santa Cruz Biotechnology | Cat#: sc-138619, RRID:AB_11150325 |
| SLFN13               | Abcam | Cat#: ab121737, RRID:AB_11127631 |
| SLFN14               | Abcam | Cat#: ab106406, RRID:AB_10890814 |
| Histone H3 (1B1B2)  | Cell Signaling Technology | Cat#: 14269, RRID:AB_12756816 |
| Ki67                | Abcam | Cat#: ab15580, RRID:AB_443209 |
| **Mouse Anti-rabbit IgG (Conformation Specific) (L27A9) mAb (HRP Conjugate)** | **Cell Signaling Technology** | **Cat#: 5127**  
**RRID:AB_10892860** |
|---|---|---|
| **Rabbit IgG HRP Linked Whole Ab** | **GE Healthcare** | **NA934-1ML**  
**RRID:AB_772206** |
| **Goat Anti-Mouse IgG (H + L)-HRP Conjugate** | **Biorad** | **Cat#:1706516**  
**RRID:AB_11125547** |

**Chemicals, Peptides, and Recombinant Proteins**

| **Doxycycline hyclate** | **Sigma Aldrich** | **Cat#: D9891-5G** |
| **Acridine orange solution** | **Sigma Aldrich** | **Cat#: A9231** |
| **Hydroxyurea** | **Sigma Aldrich** | **Cat#: H8627-1G** |
| **Thymidine** | **Sigma Aldrich** | **Cat#: T1895-1G** |
| **IFNα** | **Infergen** | **Cat#: N/A** |
| **Anti-FLAG M2 affinity gel** | **Sigma Aldrich** | **Cat# A2220-5ML**  
**RRID:AB_10063035** |
| **D-Luciferin 1 g/ Potassium Salt** | **GoldBio** | **Cat#: LUCK-1G** |
| **Propidium iodide solution** | **Sigma Aldrich** | **Cat#: P4864-10ML** |
| **RNase A, DNase and Protease free** | **Thermo Fisher** | **Cat#: EN0531** |
| **Pancreas Whole Human Normal** | **G-Biosciences** | **Cat#: NLH-09** |
| **Pancreas Whole Human Tumor** | **G-Biosciences** | **Cat#: TLH-09** |
| **GE Healthcare Amersham™ ECL™** | **GE Healthcare** | **Cat#: 45-000-875** |
| **Lenti-X Packaging Single Shots (VSV-G)** | **Clontech** | **Cat#: 631275** |
| **Lenti-X GoStick** | **Clontech** | **Cat#: 631280** |
| **Lenti-X Concentrator** | **Clontech** | **Cat#: 631231** |
| **TransDux MAX Lentivirus Transduction enhancer** | **System Biosciences** | **Cat#: LV860A-1** |
| **Turbofect** | **Thermo Fisher** | **Cat#: R0531** |
| **Lipofectamine™ RNAiMAX Transfection Reagent** | **Thermo Fisher** | **Cat#: 13778-150** |
| **E2F7 siRNA (h)** | **Santa Cruz** | **Cat#: sc-44590** |
| **Control siRNA-B** | **Santa Cruz** | **Cat#: sc-44230** |

**Critical Commercial Assays**

| **AlamarBlue™ Cell Viability Reagent** | **Thermo Fisher** | **Cat#: DAL1025** |
| **SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads)** | **Cell Signaling Technology** | **Cat#: 9003** |
| **NE-PER™ Nuclear and Cytoplasmic Extraction Reagent Kit** | **Thermo Fisher** | **Cat#: 78833** |
| **Click-it™ EdU Alexa Fluor™ 488 HCS Assay** | **Thermo Fisher/Invitrogen** | **Cat#: C10350** |
| **SsoAdvanced™ Universal SYBR® Green Supermix** | **Biorad** | **Cat#: 172-5271** |

**Experimental Models: Cell Lines and reagents**

| **PANC-1** | **ATCC** | **CRL-1469**  
**RRID:CVCL_0480** |
| **Experimental Models: Organisms/Strains** | **Recombinant DNA** |
|------------------------------------------|---------------------|
| NCRNU-F sp/sp (Ncr-Foxn1<sup>nub</sup>) nude mouse | Slfn5 Double Nickase Plasmid (h) |
|                                          | SLFN5 CRISPR/Cas9 KO Plasmid (h) |
|                                          | SLFN5 HDR Plasmid (h) |
|                                          | pLVX-tetON-SLFN5-MYC-FLAG |

| **MIA-Pa-Ca-2** | **ATCC** | **CRL-1420** |
|-----------------|----------|--------------|
| **RRID:**CVCL_0428 |

| **293T** | **Clontech** | **Cat#: 632180** |
|----------|--------------|------------------|
| **RRID:**CVCL_0063 |

| **TrypLE Express** | **Thermo Fisher** | **Cat#: 12604-013** |
|---------------------|-------------------|---------------------|
| **Recombinant Human FGF-basic (154 a.a.)** | **Peprotech** | **Cat#: 100-18B** |
| **Epidermal Growth Factor, Urogastrone, URG** | **Peprotech** | **Cat#: AF-100-15** |
| **Heparin sodium salt** | **Sigma-Aldrich** | **Cat#: H3149-10KU** |
| **B27<sup>®</sup> Serum-Free Supplement** | **Thermo Fisher** | **Cat#: 17504-044** |
| **Gentamycin** | **Sigma-Aldrich** | **Cat#: G1397-10ml** |
| **BD Matrigel, Growth factor–reduced (GFR), Phenol Red and LDEV-Free** | **Thermo Fisher** | **Cat#: 356231** |
| **Puromycin** | **Thermo Fisher** | **Cat#: A11138-03** |
| **Hygromycin B (50mg/ml)** | **Thermo Fisher** | **Cat#: 10687010** |
| **Opti-MEM** | **Thermo Fisher** | **Cat#: 31985-070** |
| **96 well round bottom ultra-low attachment plate** | **Fisher-Corning** | **Cat#: 07-20-680** |
| **25 µL Microliter Syringe Model 702 N, Cemented Needle, 22s gauge, 2 in, point style 2** | **Hamilton** | **Cat#: 80400** |

| **Oligonucleotides** |
|-----------------------|
| ChIP E2F1 FW AGGGCTCTCGATCCCGCTCCG |
| ChIP E2F1 REV TAAAGCCATAGGAACCGCCG |
| ChIP CDC6 FW AAAGGCTCTGTGACTACAGCCA |
| ChIP CDC6 REV GATCCTTCTCAGTCTTCTCAC |
| ChIP Actin FW ATCGTGCGTGACATTAAGGAGAAG |
| ChIP Actin REV CTGGAAGCAGCCGTGGCCGTCTC |

| **Recombinant DNA** |
|---------------------|
| Slfn5 Double Nickase Plasmid (h) | Santa Cruz Biotechnology | **Cat#: sc-408333-NIC** |
| SLFN5 CRISPR/Cas9 KO Plasmid (h) | Santa Cruz Biotechnology | **Cat#: sc-435875** |
| SLFN5 HDR Plasmid (h) | Santa Cruz Biotechnology | **Cat#: sc-435875-HDR** |
| pLVX-tetON-SLFN5-MYC-FLAG | BioInnovatise, Inc | **N/A** |
| **ORF expression clone for** SLFN5(NM_144975.3) (Purified plasmid) | Genecopoeia | Cat#: EX-H0940-M12 |
|---|---|---|
| pLenti-CMV Hygro Dest (W117-1) | Addgene | Cat#: 17454 RRID:Addgene_17454 |
| pENTR4-FLAG (w210-2) | Addgene | Cat#: 17423 RRID:Addgene_17423 |
| pFULT-Luciferase-Tomato | Northwestern University Skin Disease Research Core Center | N/A |

### Software and Algorithms

| Software | Company | RRID |
|---|---|---|
| Prism | Graphpad | RRID:SCR_002798 |
| Excel | Microsoft | N/A |
| Scaffold 4 | Proteome Software | RRID:SCR_014345 |
| Metascape | Metascape.org | http://metascape.org |
| FlowJo | Becton, Dickinson & Company | RRID:SCR_008520 |
| Adobe Photoshop | Adobe | N/A |
| QuPath | Bankhead et al., 2017 | N/A |
| Fiji-ImageJ | Schindelin et al., 2012 | N/A |
| NDP.view2 | Hamamatsu | U12388-01 |
Supplementary figure legends

Supplementary Fig. S1: SLFN5 gene expression is associated with poor survival of pancreatic cancer patients (I). Survival analysis of pancreatic cancer patients expressing high (red) versus low (green) levels of SLFN5 (a), SLFN1 (b), SLFN11 (c), SLFN12 (d), SLFN12L (e), SLFN13 (f) and SLFN14 (g) genes. Plots and statistical analyses were generated using PROGeneV2 software using a median score cut-off method and data extracted from GSE50827 dataset.

Supplementary Fig. S2: SLFN5 gene expression is associated with poor survival of pancreatic cancer patients (II). Survival analysis of pancreatic cancer patients expressing high (red) versus low (green) levels of SLFN5 (a), SLFN1 (b), SLFN11 (c), SLFN12 (d), SLFN12L (e), SLFN13 (f) and SLFN14 (g) genes. Plots and statistical analyses were generated using PROGeneV2 software using a median score cut-off method and data extracted from TCGA-PAAD dataset.

Supplementary Fig. S3: Effects of targeted deletion of SLFN5 in PDAC cells on the expression of other SLFN proteins. Western blot analysis of SLFN5 (a), SLFN11 (b), SLFN12 (c), SLFN13 (d) and SLFN14 (e) in lysates from WT and SLFN5 KO PANC-1 and MIA-Pa-Ca-2 cells, as indicated. Levels of Histone H3 were used as loading control.

Supplementary Fig. S4: SLFN5 regulates E2F1 and CDC6 expression. (a) Immunoblots quantification using ImageJ software. Quantified data are means ± SEM pooled from 3 independent experiments, including the one shown in Fig.5a. Fold change of CDC6 or E2F1 protein levels 6 hours after release from Hu is shown. (*, p < 0.05; **, p < 0.01; ****, p < 0.0001 using a one-way ANOVA with Tukey’s multiple comparison test). (b) Negative control data for ChIP experiments shown in Fig.5c using primers for the actin
promoter. Data were normalized to the corresponding IgG control, and are expressed as fold enrichment over WT cells. Shown are means ± SEM of 3 independent experiments.

**Supplementary Fig. S5:** Targeted deletion of *SLFN5* in PDAC cells suppresses tumor growth *in vivo* and enhances survival. (a) WT (n=5) and *SLFN5* KO (n=5) PANC-1 cells (*left panel*) and WT (n=5) and *SLFN5* KO (n=5) MIA-Pa-Ca-2 cells (*right panel*) were injected subcutaneously into the left flank of athymic NUDE mice. WT and *SLFN5* KO PDAC tumor volumes are shown. Data are expressed as means ± SEM. (**, *p* < 0.01; ***, *p* < 0.001; ****, *p* < 0.0001 using a two-way ANOVA with Sidak’s multiple comparison test). (b) Kaplan-Meier survival curves of mice bearing WT and *SLFN5* KO PDAC tumors. Statistical analysis was performed using Kaplan Meier with a Mantel-Cox (log rank) test.

**Supplementary Fig. S6:** Loss of *SLFN5* leads to complete tumor remission in 5 of 13 mice bearing *SLFN5* KO MIA-Pa-Ca-2 tumors. BLI time course for 5 mice bearing *SLFN5* KO MIA-Pa-Ca-2 tumor cells is shown. The accompanying heat map range: blue represents low BLI and red high BLI.
Supplementary Fig. S2

a) SLFN5

Overall Survival

Days

p = 0.000614

n = 51

3 years

5 years

b) SLFNL1

Overall Survival

Days

p = 0.0066701

n = 42

3 years

5 years

c) SLFN11

Overall Survival

Days

p = 0.1162387

n = 44

3 years

5 years

d) SLFN12

Overall Survival

Days

p = 0.0179332

n = 44

3 years

5 years

e) SLFN12L

Overall Survival

Days

p = 0.158391

n = 43

3 years

5 years

f) SLFN13

Overall Survival

Days

p = 0.0019395

n = 41

3 years

5 years

g) SLFN14

Overall Survival

Days

p = 0.6353071

n = 39

3 years

5 years
Supplementary Fig. S3

**a**

|        | PANC-1 | MIA-Pa-Ca-2 |
|--------|--------|-------------|
| **SLFN5:** | WT | KO | WT | KO |

Blot: anti-SLFN5

Blot: anti-HISTONE H3

**b**

|        | PANC-1 | MIA-Pa-Ca-2 |
|--------|--------|-------------|
| **SLFN5:** | WT | KO | WT | KO |

Blot: anti-SLFN11

Blot: anti-HISTONE H3

**c**

|        | PANC-1 | MIA-Pa-Ca-2 |
|--------|--------|-------------|
| **SLFN5:** | WT | KO | WT | KO |

Blot: anti-SLFN12

Blot: anti-HISTONE H3

**d**

|        | PANC-1 | MIA-Pa-Ca-2 |
|--------|--------|-------------|
| **SLFN5:** | WT | KO | WT | KO |

Blot: anti-SLFN13

Blot: anti-HISTONE H3

**e**

|        | PANC-1 | MIA-Pa-Ca-2 |
|--------|--------|-------------|
| **SLFN5:** | WT | KO | WT | KO |

Blot: anti-SLFN14

Blot: anti-HISTONE H3
Supplementary Fig. S4

(a) Fold change in CDC6 and E2F1 expression in PANC-1 and MIA-Pa-Ca-2 cells with SLFN5 WT and KO conditions.

(b) Actin fold enrichment over WT conditions in PANC-1 and MIA-Pa-Ca-2 cells with SLFN5 WT and KO conditions.
Supplementary Fig. S5

(a) Tumor volume (mm³)

- PANC-1
  - SLFN5 WT
  - SLFN5 KO

- MIA-Pa-Ca-2
  - SLFN5 WT
  - SLFN5 KO

Days after implantation of tumor cells

(b) Survival (%)

- PANC-1
  - SLFN5 WT
  - SLFN5 KO
  - \( p = 0.0330 \)

- MIA-Pa-Ca-2
  - SLFN5 WT
  - SLFN5 KO
  - \( p = 0.0494 \)

Days after implantation of tumor cells
Supplementary Fig. S6

**SLFN5 KO MIA-Pa-Ca-2 tumor cells**

11 days after implantation of tumor cells

18 days after implantation of tumor cells

25 days after implantation of tumor cells

32 days after implantation of tumor cells

43 days after implantation of tumor cells

50 days after implantation of tumor cells

57 days after implantation of tumor cells