Supplementary Methods

Chemicals, Peptides, and Recombinant Proteins

Caerulein (Cayman, Cat#24408), Gemcitabine (Teva Parenteral Medicine Inc, Cat#NDC0703-5778-01), Aminooxyacetic acid (AOA; MedChemExpress, Cat#HY-107994), BPTES (Sigma-Aldrich, Cat#SML0601), EGCG (Cayman, Cat#70935), Matrigel Matrix (Corning, Cat#354234), [U-$^{13}C_5$] glutamine (Cambridge Isotope Laboratories, Cat# CLM-1822-H-PK), DAPI (Invitrogen, Cat#P36935), Hematoxylin (Leica, Cat#3801570), Alcoholic Eosin (Leica, Cat#3801615), Turbofect transfection reagent, (Thermo Fisher, Cat#R0532), Dispase II (Sigma-Aldrich, Cat#D4693), TRIzol (Thermo Fisher, Cat#15596026), Pierce IP Lysis Buffer (Thermo Fisher, Cat#87788), Epitope Retrieval Solution (pH 6; Leica, Cat#6064204), PowerUP SYBR Green Mastermix (Applied Biosystems, Cat#A25742), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma Aldrich, Cat#M2128), Nonidet P-40 (Sigma Aldrich, Cat#74385-1L), Tris-HCl (Sigma Aldrich, Cat#T5941-500G), EDTA (Sigma Aldrich, Cat#E6758-500G), SRT2104 (MedChemExpress, Cat#HY-15262), Honokiol (Sigma-Aldrich, Cat#42612), Skim Milk (BD Difco, Cat#232100), B-27™ Supplement (Thermo Fisher, Cat#17504044), PGE2 (Sigma-Aldrich, Cat#A0409-1MG), Nicotinamide (Sigma-Aldrich, Cat#A0636-100G), N-acetylcysteine (Sigma-Aldrich, Cat#A9165-100G), hGastrin I (TOCRIS, Cat#3006), hFGF10 (PeproTech, Cat#100-26), mNoggin (PeproTech, Cat#250-38), hEGF (PeproTech, Cat# AF-100-15), A 83-01 (TOCRIS, Cat#2939), DMEM/F12 (Sigma-Aldrich, Cat#DF-041), HEPES (Sigma-Aldrich, Cat#H4034), Matrigel (Corning, Cat#354234), Y-27632 (Hello Bio, Cat#HB2297), Collagenase XI (Sigma-Aldrich, Cat#C7657-25MG), DNAse I (Sigma-Aldrich, Cat#11284932001).

Commercial Assays

CellTiter-Glo cell Viability Assay Kit (Promega, Cat#G7570), Verso cDNA Synthesis Kit (Thermo Fisher Scientific, Cat#1453A), VetScan Comprehensive Diagnostic Profile (Abaxis, Cat#500-0038-48), Plasmid Maxi kit (Qiagen, Cat#12163), Vectastain ABC Kit (Vector Biolabs, Cat#PK6101), DAB Substrate Kit (Vector Biolabs, Cat#SK-4105), Alcian Blue Stain Kit (IHC World, Cat#IW-3000), GOT Activity Assay Kit (Sigma Aldrich, Cat#MAK055), Glutaminase Activity Assay Kit (Biomedical Research Service, Cat#E-133), GDH Activity Assay Kit (Biomedical Research Service, Cat#E-123), KOD-Plus-Mutagenesis Kit (Toyobo, Cat#SMK101), SIRT1 Activity Assay Kit (Abcam, Cat#ab156065), SIRT3 Activity Assay Kit (Abcam, Cat#ab156067), SIRT5 Activity Assay Kit (Abcam, Cat#ab210074), Mycoplasma PCR Detection Kit (abm, Cat#G238).

Organoid Culture and Experiments

The detailed procedure for generating organoids was performed as previously identified [1]. In brief, the tissue from the patient-derived xenografts (PDX) were minced and then incubated in the digestion media (Human Complete Medium containing 10 µg/mL DNAse I, 1 mg/mL Collagenase XI, 10.5 µM Y-27632) at 37°C with gentle rotation for 1 hour. Cells were collected and then seeded with Matrigel (cat#354234, Corning). The organoids were cultured in Human Complete Feeding Medium (advanced DMEM/F12 containing 10 mM HEPES, 1X Glutamax, 500 nM A83-01, 50 ng/mL hEGF, 100 ng/mL mNoggin, 100 ng/mL hFGF10, 0.01 µM hGastrin I, 1.25 mM N-acetylcysteine, 10 mM nicotinamide, 1 µM PGE2, 1X B27 supplement, 10% R-spondin1 conditioned media, and 50% Wnt3A-conditioned media). For the indicated experiments, 2×10^3 organoid cells were suspended in 50 µL Matrigel per well and seeded in 24 well plates. Cytation 3 Cell Imaging Multi-Mode Reader was used to capture the size of the organoids. CellTiter-Glo kit (Promega; Madison, WI, USA) was used to measure the cell viability of organoids 72-hours post-treatment.

***In Vivo* Mouse Studies**

We utilized the following mice strains: Athymic *Foxn1*^{nu}/*Foxn1*^{nu} (Taconic Biosciences), C57BL/6 (The Jackson Laboratory), NOD scid gamma (NSG™; The Jackson Laboratory), *Sirt5*$^{flox/flox}$ (Provided by Dr. Johan Auwerx), *LSL-Kras*$^{G12D/+}$ (NCI), *LSL-Trp53*$^{R172H/+}$ (NCI), *Pdx-1-Cre* (NCI). All animal experiments were conducted under the protocols approved by the institutional animal care and use committee (IACUC) at the University of Nebraska Medical Center (UNMC). The animal facility of UNMC bred and housed the athymic nude mice (*Foxn1*^{nu}/*Foxn1*^{nu}) as well as the NOD *scid* gamma (NSG™; NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) mice used for orthotopic implantations. For the *in vivo* tumor growth experiments, 5×10^5 T3M4-shScramble or T3M4-shSIRT5 cells were injected into the pancreatic tissue of six-week-old female NOD-SCID mice. After 6 days following implantation, tumor diameters were measured by calipers every three days, and tumor volumes were calculated using the following formula: volume = 0.5 × (longer diameter × shorter diameter^2). For the AOA treatment studies, 2×10^6 T3M4-shScramble and T3M4-shSIRT5 were injected into the pancreas of eight-week-old female nude mice. When the tumor size reached approximately 100 mm^3, the mice were randomized into the following two groups: vehicle control group (saline) and AOA group, which was treated daily with 10 mg/kg AOA. Body weight and tumor volume were measured twice a week during the treatment. At the end of treatment, mice were euthanized to collect tumor weight and tumor volume. Mice organs and tumor tissues were excised, frozen in liquid nitrogen or fixed with 4% paraformaldehyde, and embedded in paraffin for further analysis.

Patient-Derived Xenograft (PDX) Studies

Eight to ten weeks old male NOD *scid* gamma (NSG™; NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) mice were used to implant orthotopic PDX tumors. The PDX (PA137) tumors were cut into 3-5 mm pieces and then orthotopically implanted to the mouse pancreas. When the tumor size reached around 100 mm^3, mice were randomized into four groups with similar tumor volumes: control group, gemcitabine (diluted in saline, 25 mg/kg, twice per week), MC3138 [diluted in DMSO: corn oil (1:9) solution, 200 mg/kg, daily] and gemcitabine with MC3138. Gemcitabine and MC3138 were administered by intraperitoneal injections. and gemcitabine with MC3138. Blinding was not possible for the mouse studies. Tumor diameters and body weight were measured every four days. After 20 days of treatment, mice were euthanized to collect tumor weight and tumor volume. Mice organs and tumor tissues were excised, frozen in liquid nitrogen or fixed with 4% paraformaldehyde, and embedded in paraffin for further analysis.

RNA Extraction and qRT-PCR Analysis

Total RNA from cell lines and tissues were extracted using TRIzol reagent. Reverse transcription analyses used the Verso-cDNA synthesis kit, according to the manufacturer's instructions. QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific; Waltham, MA, USA) and SYBR Green PCR Master Mix were used to perform the qPCR according to the manufacturer's protocol. Quantification of target genes was normalized by 18S ribosomal RNA using the 2-ΔΔCt method [2]. The primer sequences used for qPCR analysis are listed in Supplementary Table S4.

Antibodies

Rabbit Anti-SIRT5 (HPA022002; RRID: AB_1856913; Sigma-Aldrich), Rabbit Anti-Amylase (A8273; AB_258380; Sigma-Aldrich), Rabbit Anti-cytokeratin19 (TROMA-III; RRID: AB_2133570; DHSB), Rabbit Anti-Glutaminase (ab93434; RRID: AB_10561964; Abcam), Rabbit Anti-Glutamate Dehydrogenase, (12793; RRID: AB_2750880; Cell Signaling Technology), Mouse Anti-β-actin (JLA20; RRID: AB_528068; DHSB), Rabbit Anti-GOT1 (HPA072629; RRID: AB_2686540; Sigma-Aldrich), Rabbit Anti-GOT1 (14886-1-AP, RRID:AB_2113630; Thermo Fisher), Rabbit Anti-GOT2 (ab171739; Abcam), Rabbit Anti-Cleaved Caspase3 (9664; RRID: AB_2070042; Cell Signaling Technology), Rabbit Anti-Ki67 (12202; RRID: AB_2620142; Cell Signaling Technology), Rabbit Pan anti-succinyllysine (PTM401; RRID: AB_2687628; PTM Biolabs), Rabbit Pan anti-malonyllysine (PTM901; RRID: AB_2687947; PTM Biolabs), Rabbit Pan anti-glutaryllysine (PTM1151; PTM Biolabs), Rabbit Pan anti-acetylated-lysine (9441; RRID: AB_331805; Cell Signaling Technology), Pierce Anti-HA Magnetic Beads (Cat#88837; Thermo

Fisher Scientific), Pierce Protein A/G Magnetic Beads (88803; Thermo Fisher Scientific), Donkey Anti-Rabbit Af594 (711-585-152; RRID: AB_2340621; Jackson Immunoresearch), Donkey Anti-Rat Af488 (712-545-153; RRID: AB_2340684; Jackson Immunoresearch), IgG Fraction, mouse anti-rabbit, light chain specific (211-002-171;RRID: AB_2339146; Jackson Immunoresearch), HA antibody (MMS-101R; RRID: AB_291262; Covance), Peroxidase AffiniPure Goat Anti-Mouse IgG, light chain specific (115-035-174; AB_2338512; Jackson Immunoresearch).

Immunoblotting

Cell lysates were extracted from cell culture dishes in RIPA lysis buffer (50mM Tris-HCl pH 8.0 containing 150mM NaCl, 1% Nonidet P-40, 5mM EDTA and 1mM phenylmethylsulphonyl fluoride). Protein samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% skim milk and then probed overnight at 4°C with indicated primary antibodies. After incubation with horseradish peroxidase (HRP) conjugated secondary antibodies, the specific protein bands were detected using Pierce™ ECL Western Blotting Substrate and ChemiDoc Imaging System (Bio-Rad; Hercules, CA, USA). Immunoblotting assays to detect acetylation levels of purified GOT1 protein used the light-chain specific secondary antibody.

Immunoprecipitation (IP) and Proteomic Analysis

The indicated PDAC cells transfected with HA-GOT1 for 48h were lysed in Pierce™ IP Lysis Buffer (87787, Thermo Fisher). Cell lysates were pre-cleared by Pierce™ Protein A/G Magnetic Beads and then incubated with anti-HA Magnetic Beads overnight at 4°C. After washing with IP Lysis Buffer five times and subsequently boiling with 2X sample buffer for 10 minutes, the GOT1-IP samples were then purified and resolved by SDS-PAGE. Following the Coomassie Brilliant Blue staining and in-gel digestion, the GOT1 peptide mixture was processed for proteomic analysis by LC-MS/MS at the Mass Spectrometry and Proteomics Core at UNMC. For cells expressing wild-type GOT1, K276R, K290R, and K369R mutant GOT1, cells were lysed in IP Lysis Buffer and then pre-cleared by Pierce™ Protein A/G Magnetic Beads. After incubating the pre-cleared lysates with anti-HA Magnetic Beads overnight at 4°C, the immune complexes were resolved by SDS-PAGE, followed by immunoblotting analysis. For endogenous IP, control and *SIRT5*-knockdown PDAC cells were lysed in Pierce™ IP Lysis Buffer. The IP-grade GOT1 antibody (14886-1-AP, Thermo Fisher) was conjugated to Pierce™ Protein A/G Magnetic Beads at 4°C for four hours before IP experiment. Cell lysates were pre-cleared by Pierce™ Protein A/G Magnetic Beads and then incubated with GOT1 antibody-Magnetic Beads mixture overnight at 4°C. The immune

complexes were washed with IP Lysis Buffer for five times and subsequently boiled with 2X sample buffer for 10 minutes, the GOT1-IP samples were then resolved by SDS-PAGE. The endogenous acetylation level of GOT1 was determined by western blotting using an anti-Acetylation antibody (CST, 9441S).

Histology

Pancreatic tissues were fixed with 4% paraformaldehyde, embedded in paraffin and then analyzed with hematoxylin and eosin (H&E), immunofluorescence, immunohistochemistry, or Alcian blue staining on 5μm sections. For immunofluorescence, paraffin-embedded tissue slides were deparaffinized by xylene and hydrated using decreasing ethanol gradients. Next, tissue slides were probed with primary antibodies diluted in PBST with 5% BSA at 4°C overnight, followed by incubation with 1:100 secondary antibodies conjugated with Alexa Fluor® 488 donkey anti-rat IgG and Alexa Fluor® 594 donkey anti-rabbit IgG. After being mounted using Anti-fade Fluorescence Mounting Media with DAPI, images were captured using an inverted fluorescent microscope. Alcian blue staining was performed according to manufacturer's instructions. In brief, the paraffin-embedded tissue sections were deparaffinized by xylene, hydrated using gradient ethanol and then incubated at room temperature for 30 minutes with Alcian Blue solution. The tissue sections were washed with running tap water, followed by counterstaining with eosin.

Immunohistochemistry

For immunohistochemical analysis of SIRT5, Ki67 and Cleaved Caspase3, antigen retrieval was performed by boiling the tissue slides in the Epitope Retrieval Solution (pH 6). After blocking with 5% BSA solution containing 1% goat serum, slides were incubated with indicated primary antibodies overnight at 4°C. Next, the slides were incubated by VECTASTAIN® Elite® ABC HRP Kit (PK-6100, VECTOR, USA) and ImmPACT® DAB Peroxidase (HRP) Substrate (SK-4105, VECTOR, USA) according to the manufacturer's protocol. The stained slides were scanned using Roche Ventana iScan HT System (Tissue Science Facility Core, UNMC). The Ki67 positive staining cells were counted from three random 20X fields in a section. For SIRT5 IHC score, two pathologist-trained blinded observers evaluated the IHC results independently according to an established semi-quantitative approach, which was evaluated by both the staining intensity and the percentage of positive staining cells in tumor cells. The staining intensity of the malignant cells was scored as 0, 1, 2, or 3 for negative, weak, intermediate, and strong staining respectively. The proportion represents the percentage of positively stained cells (0 = none, 1 =< 5%, 2 = 5 - 25%, 3 = 26 - 50%, 4 = 51 - 75%, 5 >= 75%). The overall protein expression level in every IHC sample is given as a histoscore, which was

calculated by multiplying the proportion (0–5) and intensity scores (0–3). The value of the histoscore ranges from 0 to 15, with a maximum of 15 [3]. For the survival analysis in Figure 1H-I, low SIRT5 expression is defined as SIRT5 IHC histoscore < 7.5 and high SIRT5 expression is defined as SIRT5 IHC histoscore ≥ 7.5.

Plasmids

The pcDNA3.1-hSIRT5-Flag, pcDNA3.1-hSIRT5 H158Y-HA plasmids were kindly provided by Dr. Eric Verdin (Addgene plasmid # 13816). The catalytically inactive mutant SIRT5 H158Y-Flag and pLX304-SIRT5 H158Y were generated based on pcDNA3.1-hSIRT5-Flag and pLX304-SIRT5 plasmid using KOD-Plus-Mutagenesis Kit (SMK-101, Toyobo, Japan). The human GOT1-HA Lentiviral Vector was purchased from Applied Biological Materials Inc. (LV791704). GOT1 mutant construct K276R, K290R, and K369R were generated by PCR amplification using KOD-Plus-Mutagenesis Kit based on the above GOT1-HA vector according to the manufacturer's protocol. The primers used for plasmid construction are listed in Supplementary Table S4.

Cell Growth and Clonogenic Assays

For the cell growth assay, 1×10^5 PDAC cells were seeded in 6 cm culture dishes (Corning, USA). These cells were trypsinized, stained with trypan blue (Gibco, USA) and counted using BioRad TC20 automated cell counter at indicated time points after inoculation. For the clonogenic assay, 200 PDAC cells were seeded in six-well cell culture plates (Corning, USA), and cell culture media was replaced every three days. Colonies were fixed with methanol and then stained with 0.2% crystal violet in 80% methanol. The soft agar cell colony formation assay was conducted according to the protocol of CytoSelect 96-Well Cell Transformation Assay (Cell Biolabs, Inc.; San Diego, CA USA). In brief, 5,000 cells were suspended in 75 µL 0.4% agar DMEM solution and seeded over 50 µL of bottom agar in 96-well plates. Images of colonies were captured after 10 days using the Cytation 3 Cell Imaging Multi-Mode Reader (BioTek Instruments, Inc.; Winooski, VT, USA). For 3D anchorage-free colony formation assay, 5,000 cells were seeded in 200 µL DMEM supplemented with 10% FBS in 96-well ultra-low attachment microplate (7007; Corning, USA). Cell culture media was refreshed every three days, and images of 3D colonies were captured using Cytation 3 Cell Imaging Multi-Mode Reader. The volume of 3D colonies was calculated using the formula: Volume = $4/3\pi R^3$

ROS Assay

The intracellular ROS levels were measured as described previously [4]. In brief, the oxidation-sensitive fluorescent dye 2',7'–dichlorofluorescein diacetate

(DCFDA) was used to detect the ROS level. The control and SIRT5 knockdown PDAC cells were seeded in a clear-bottom black 96-well plate (3×10^4 cells/per well). The cell culture medium was refreshed by the DMEM medium containing 20 μM DCFDA. H_2O_2 combined with DCFDA was used as the positive control, while 2',7'-Bis(2-carboxyethyl)-5(6)- carboxyfluorescein (CDCFDA) served as the negative control and utilized for data normalization. All these cells were incubated at 37°C for 30 min before testing. The cells were washed with 1X PBS solution, and next the emission of DCFDA was detected using the BioTek Cytation 3 plate reader (BioTek Instruments, Inc.; Winooski, VT, USA). The DCFDA fluorescence signals were measured at the 495nm excitation wavelength and 529nm emission wavelength.

Enzyme Activity Assays

The enzyme activities of GOT were measured using a Glutamate-Oxaloacetate Transaminase Activity Assay Kit (MAK055, Sigma-Aldrich; St. Louis, MO, USA) based on the manufacturer's protocol. In brief, 1×10^6 cells were homogenized in 200 μL ice-cold AST Assay Buffer. After removing the insoluble material by centrifuging at 13000g for 10 minutes, the samples were incubated at 37°C for 2 minutes while protected from light. The absorbance at 450 nm was monitored every five minutes using the Cytation 3 Cell Imaging Multi-Mode Reader. To determine the GOT activity in MC3138-treated tumor tissues, 50 mg tumor tissues from control and MC3138-treated groups were ground into powder under with liquid nitrogen. Then the tumor tissue powder was homogenized in 200 μL of ice-cold AST Assay Buffer by vortex. Samples were centrifuged at 13,000g for 10 minutes to remove the insoluble material. 20 μL of samples were used for the GOT activity test as above. The enzyme activity of GLS and GLUD1 were determined by the Glutaminase Assay Kit (catalog #E-133, Biomedical Research Service Center; Buffalo, NY, USA) and Glutamate Dehydrogenase Assay Kit (catalog #E-123, Biomedical Research Service Center; Buffalo, NY, USA) respectively according to the manufacturer's instructions. Briefly, 1×10^6 cells were homogenized and then mixed with 100 μL ice-cold 1X cell lysis Solution. Then the lysate was incubated on ice for five minutes with gentle agitation. After removing the insoluble material by centrifuging at 14,000 rpm for five minutes, the supernatant was incubated at 37°C for one hour, protected from light. The optical density values at 492 nm were measured by the Cytation 3 Cell Imaging Multi-Mode Reader. The indicated enzyme activity was calculated using the above optical density value data according to the manufacturer's instruction.

Mass Spectrometric Metabolomics Analysis

For metabolite extraction, PDAC cells were cultured in 6 cm cell culture dishes (Corning, USA) and fresh cell culture medium was added two hours prior

to the metabolite extraction. Cells were washed twice with ice-cold PBS, and then polar metabolites were extracted by adding cold 80% methanol/water (v/v, -80°C). The lysates were centrifuged for 10 minutes at 13,500g. Next, the supernatant was evaporated using the SpeedVac Vacuum Concentrator (Thermo Fisher Scientific; Waltham, MA, USA). Lyophilized concentrates were dissolved using equal volume LC-MS-grade water and then analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) using the selected reaction monitoring (SRM) method [5]. Peak areas were integrated using Skyline software [6] and normalized by cell number from parallel plates and the internal control. The normalized peak areas values were subjected to pathway enrichment and relative quantification analyses using Metaboanalyst 3.0 [7]. For uniform ^{13}C-labeled glutamine ([U-$^{13}C_5$] glutamine) based flux analysis, PDAC cells were grown to around 80% confluence in complete cell culture media. Next, the complete medium was replaced by the glutamine/glucose-free DMEM supplemented with 10% dialyzed FBS, 25 mM unlabeled glucose (Sigma-Aldrich; St Louis, MO, USA), and 2 mM [U-$^{13}C_5$] glutamine (Cambridge Isotope Laboratories; Tewksbury, MA, USA) at indicated time point. Metabolites were extracted and analyzed by LC-MS/MS analysis using SRM method.

Lentivirus Transfection

Stable short hairpin RNA (shRNA) targeting SIRT5, GOT1, and GOT2 were obtained from Sigma-Aldrich. The plasmids overexpressing SIRT5 and the corresponding empty-vector pLX304 were purchased from DNASU Plasmid Repository (Arizona State University, Tempe, AZ, USA). CRISPR sgRNA targeting control or different regions of GOT1 were purchased from Applied Biological Materials Inc (Richmond, BC, Canada). Lentivirus was produced by transfecting HEK293T cells with the desired plasmid and packaging constructs using Turbofect. Cells were infected by the lentivirus with polybrene and then selected with the indicated antibiotics. For CRISPR sgRNA-mediated knockout, a single colony was isolated and propagated.

Sirtuins Deacetylase Activity Assay

Sirtuins deacetylase activity was measured using the SIRT1, SIRT3, and SIRT5 Deacetylase Fluorimetric Activity Assay Kit (#ab156065, #ab156067, #ab210074, Abcam) according to the manufacturer's guidelines. In brief, the indicated sirtuin activators were co-incubated with Fluoro-Substrate Peptide, NAD^+, developer, and recombinant sirtuin enzymes at 37°C for 30 min. The fluorescent intensity was detected using a BioTek Cytation 3 plate reader (BioTek Instruments; Winooski, VT, USA) at 350 nm/450 nm (for SIRT1 and SIRT3 activity assay) and 480 nm/530 nm (SIRT5 activity assay).

Synthesis of MC3138

The compound MC3138 (compound “7o” in previous literature) was prepared in the Mai’s lab. The detailed synthetic procedure has been previously described [8].

Docking of MC3138 with SIRT5

Sirt5 (PDB ID: 2B4Y) was retrieved from Protein Data Bank and MC3138 was prepared by downloading the 2D structure (PubChem CID 155524917) in SDF format from Pubchem followed by conversion to 3D. The docking study was carried out with AutoDock 4.2.6 (Scripps Research Institute). Visual analysis of complex of MC3138 in the active centers of the Sirt5 (PDB ID: 2B4Y) was performed using the Discovery Studio Visualizer (Figure 7a). The complex showed a binding energy of –7.1 kcal/mol.

MC3138 Pharmacokinetics

For pharmacokinetics analysis of MC3138, C57BL/6J mice were intraperitoneally injected with a single dose of MC3138 [diluted in DMSO (1): corn oil (9) solution, 200 mg/kg]. Mice were euthanized at indicated time points (0.5 h, 1 h, 3 h, 7 h, 12 h, 24 h, 48 h) to collect plasma and organs with n=4 for each time point. For MC3138 extraction, 50 μL plasma was mixed with 2 mL acetone and vortexed thoroughly for 3 min. Samples were then centrifuged at 3500 rpm for 10 min. The supernatant was transferred into a new tube and evaporated using a SpeedVac. The dried extract was reconstituted in 50 μL methanol and diluted with water into a final composition of 10% methanol, then purified by strata-x polymeric reverse phase columns (88-S100-UBJ Phenomenex), using an activation procedure consisting of consecutive washes with 3 mL of 100% methanol followed by 3 mL of water. After the samples were loaded into the column, 2 mL 50% methanol was used to wash out the contamination, and 2.5 mL methanol was then used to elute the drug. The eluent was transferred into autoinjector sample vials for LC-MS/MS analysis. LC-MS/MS analysis was performed with Waters ACQUITY UPLC systems connected to a Xevo TQ-S mass spectrometry using Multiple Reaction Monitoring (MRM) transitions 436.1888->104.8490 and 436.1888->390.1448. Chromatographic separation was performed on a Waters UPLC BEH C18 column (2.1×100 mm, 1.7 μm) with a gradient of solvents A (methanol) and solvents B (water). The gradient was as follows: 0 min, 50% A, 10 min, 80% A, 15 min, 80% A, 16 min 50% A, 20 min, and 50% A.

To determine the concentration of MC3138 in tumor tissues, MC3138 was extracted from 50 mg tumor tissues from MC3138-treated and control groups. In brief, tumor tissues were ground into powder while cooling in liquid nitrogen. Then the powder was mixed with 2 mL acetone and vortexed thoroughly for 3 min. Samples were then centrifuged at 3500 rpm for 10 min. The supernatant was transferred into a new tube and evaporated using a SpeedVac. The dried

extract was reconstituted in 50 μL methanol and diluted with water into a final composition of 10% methanol, then purified by strata-x polymeric reverse phase columns (88-S100-UBJ Phenomenex), using an activation procedure consisting of consecutive washes with 3 mL of 100% methanol followed by 3 mL of water. After the samples were loaded into the column, 2 mL 50% methanol was used to wash out the contamination, and 3 mL methanol was then used to elute the drug. The eluent was transferred into autoinjector sample vials for LC-MS/MS analysis. LC-MS/MS analysis was performed with Waters ACQUITY UPLC systems connected to a Xevo TQ-S mass spectrometry using Multiple Reaction Monitoring (MRM) transitions 436.1888->104.8490 and 436.1888->390.1448. Chromatographic separation was performed on a Waters UPLC BEH C18 column (2.1×100 mm, 1.7 μm) with a gradient of solvents A (methanol) and solvents B (water). The gradient was as follows: 0 min, 50% A, 10 min, 80% A, 15 min, 80% A, 16 min 50% A, 20 min, and 50% A.

Statistical Analysis

All data are shown as mean ± standard deviation (SD) from at least three independent experiments except when indicated. Log-rank tests and Kaplan-Meier method were used to perform survival analysis. The χ^2 test or Fisher's Exact test was used to analyze the qualitative variables of clinicopathological data. Univariate and multivariate Cox proportional hazards regression were used to evaluate the prognostic factors in the PDAC patients. The statistical analyses between the two groups were performed by two-tailed Student's t-test. The statistical difference between multiple groups was conducted using one-way ANOVA with Bonferroni's post hoc analysis or Tukey's post hoc analysis. The correlation analysis was conducted using the Pearson's correlation test. All statistical analysis was performed using GraphPad Prism (Version 6) and SPSS 22.0. Statistical analyses were two-tailed and used an alpha of 0.05 for these tests. All quantitative *in vitro* experiments were repeated at least three times in triplicates.

Supplementary References

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Author names in bold designate shared co-first authorship.

Supplementary Figure Legends.

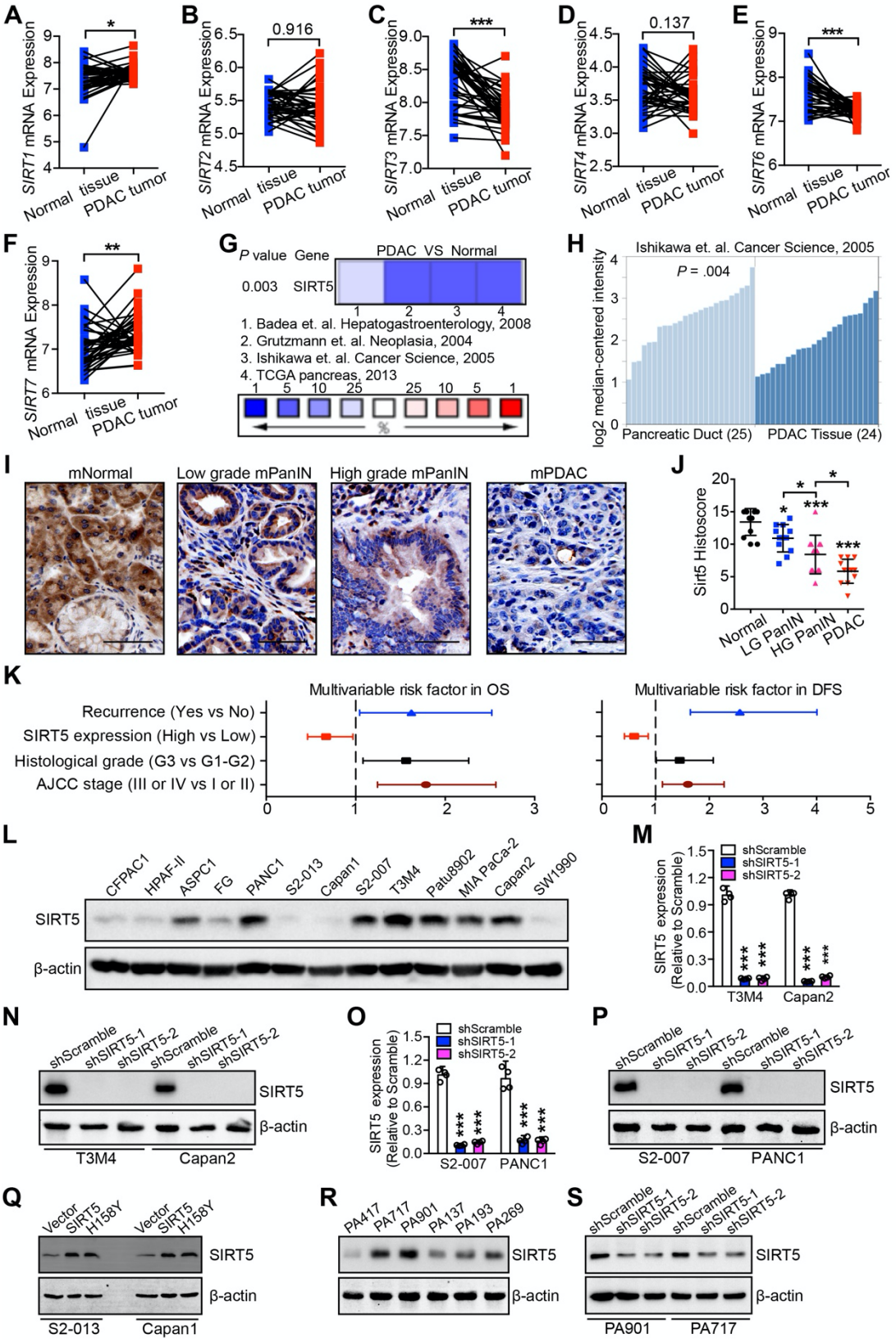


Figure S1. SIRT5 downregulation in PDAC correlates with disease progression, poor survival outcomes, and enhanced tumor cell growth.

(A-F). The mRNA levels of *SIRT1-7* in pancreatic cancer tissues and the paired adjacent normal tissues from GEO database (GDS4103, n = 39).
(G-H). *SIRT5* mRNA is downregulated in pancreatic cancer tissues among various cohorts from the Oncomine database (https://www.oncomine.org). The representative scatter plot of *SIRT5* mRNA level from the Ishikawa cohort (H).
(I-J). IHC staining (I) and quantification (J) for SIRT5 expression in normal acinar cells from control mice and low-grade (LG) PanINs, high-grade (HG) PanINs, and pancreatic tumors from KPC mice. Scale bar represents 100 μm.
(K). Multivariate regression analysis of the overall survival and disease-free survival in PDAC patient cohort from Shanghai Outdo Biotech Tissue Bank (Bars indicate 95% confidence intervals).
(L). Immunoblotting for SIRT5 expression in thirteen pancreatic cancer cell lines.
(M-P). qRT-PCR and immunoblotting for SIRT5 expression in control and *SIRT5*-knockdown PDAC cells.
(Q). Immunoblotting for SIRT5 expression in control, *SIRT5*-overexpressing and SIRT5H158Y-overexpressing PDAC cells.
(R). Immunoblotting for SIRT5 expression in six human PDAC organoids.
(S). Immunoblotting of SIRT5 expression levels in the control and *SIRT5*-knockdown PDAC organoids.
For all *in vitro* studies $n \geq 3$. The data are represented as mean ± SD. The statistics is calculated by paired Student's t-test (A-F). The cohorts were compared with the control group by one-way ANOVA with Bonferroni's post hoc analysis (M, O) or compared with every other group by one-way ANOVA with Tukey's post hoc analysis (J), $^{*}P<.05$, $^{**}P<.01$, and $^{***}P<.001$.

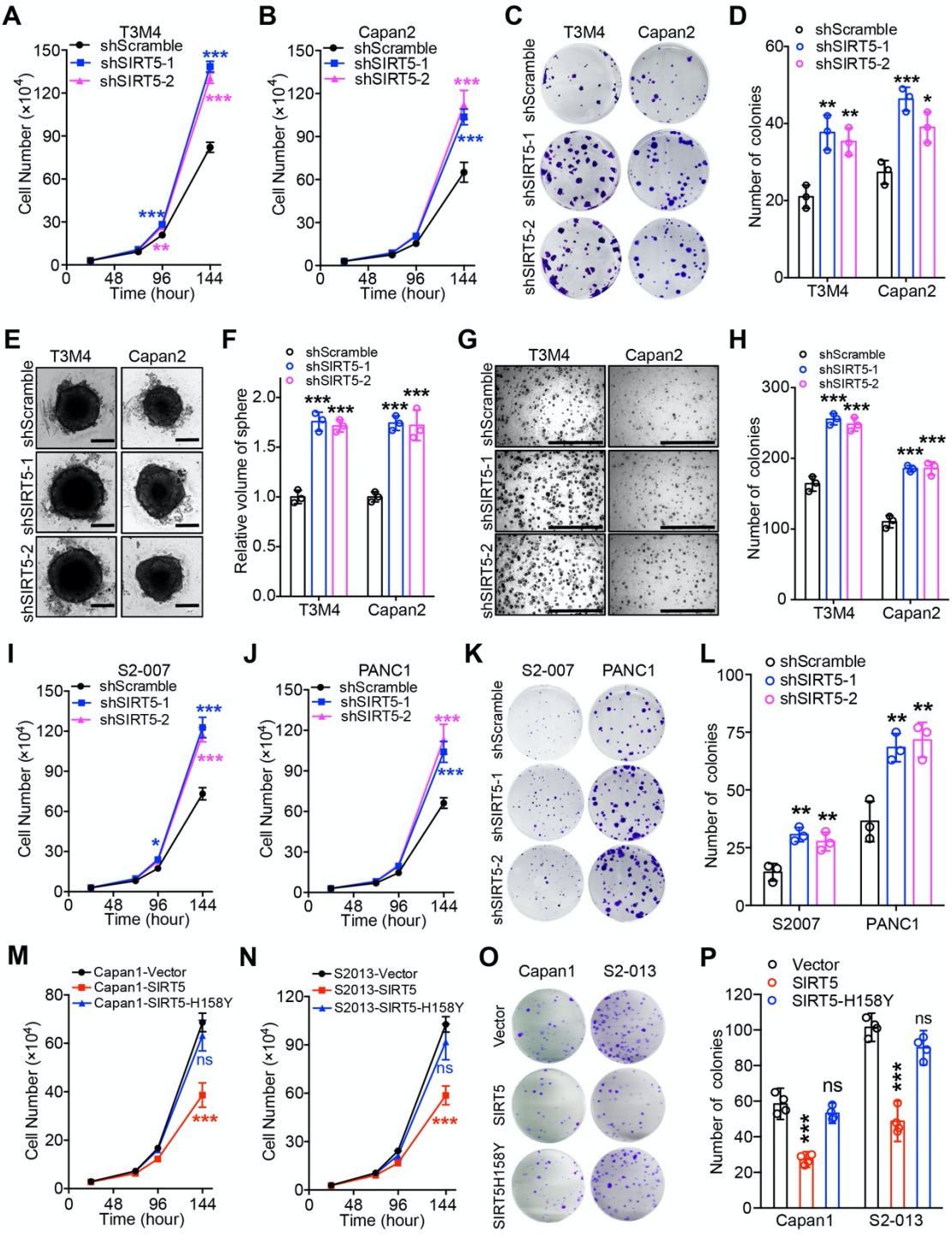


Figure S2. SIRT5 inhibits pancreatic cancer cell growth *in vitro*.

(A-B). The cell growth curve of control and *SIRT5*-knockdown T3M4 and Capan2 PDAC cells.

(C-D). Representative images and quantitation of colony formation assay in control and *SIRT5*-knockdown T3M4 and Capan2 PDAC cells.

(E-F). Sphere formation assay with control and *SIRT5*-knockdown T3M4 and Capan2 PDAC cells that were grown in 96-well low attachment plates for 15

days. Images were captured by Cytation 3 and sphere volume was calculated by $V=4*\pi r^3/3$. Scale bar represents 300 μm.
(G-H). Representative images and quantitation of colonies in soft agar colony formation assays for control and *SIRT5*-knockdown T3M4 and Capan2 PDAC cells. Scale bar represents 1000 μm.
(I-J). Growth rates of control and *SIRT5*-knockdown PANC1 and S2-007 PDAC cells.
(K-L). Representative images and quantitation of the colony formation assay in control and *SIRT5*-knockdown PANC1 and S2-007 PDAC cells.
(M-N). Growth rates of control, *SIRT5*-overexpressing and SIRT5H158Y-over expressing cells (Capan1 and S2-013).
(O-P). Representative images and quantitation of the colony formation assay in control, *SIRT5*-overexpressing and SIRT5H158Y-overexpressing cells (Capan1 and S2-013).
For all *in vitro* studies $n \geq 3$. The data are represented as mean ± SD. The cohorts were compared with the control group by one-way ANOVA with Bonferroni's post hoc analysis (D, F, H, L, P). Cohorts were compared with the control group by two-way ANOVA with Bonferroni's post hoc analysis (A, B, I, J, M, N), $^*P<.05$, $^{**}P<.01$, and $^{***}P<.001$.

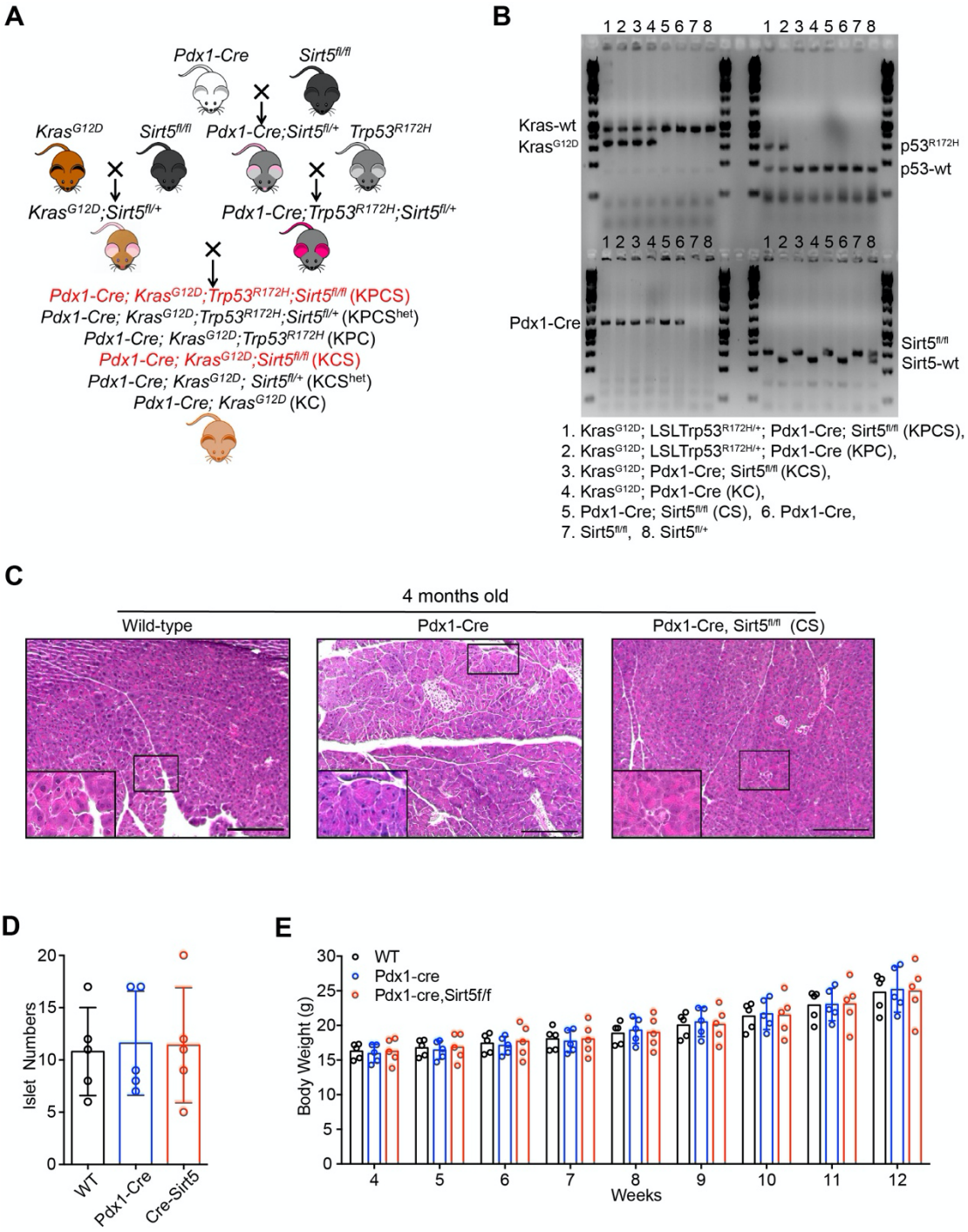


Figure S3. SIRT5 deficiency in murine pancreas does not display distinct abnormalities.

All panels shown are age-matched wild-type C57 mice, *Pdx1-Cre* (Cre), and *Pdx1-Cre, Sirt5$^{fl/fl}$* (CS) littermate mice (n = 5, each group).

(A). *LSL-Kras^{G12D}*, *LSL-Trp53$^{R172H/+}$*, and *Pdx1-Cre* mice were crossed with *Sirt5$^{fl/fl}$* mice to obtain mice with *Kras^{G12D}; Sirt5$^{fl/+}$* (KS^{het}) and *LSL-Trp53$^{R172H/+}$; Pdx1-Cre; Sirt5$^{fl/fl}$* (PCS^{het}) genotypes. Intercrossing of the KS^{het} and PCS^{het} mice generated *LSL-Kras^{G12D}; LSL-Trp53$^{R172H/+}$; Pdx1-Cre; Sirt5$^{fl/fl}$* (KPCS) and *LSL-Kras^{G12D}; LSL-Trp53$^{R172H/+}$; Pdx1-Cre* (KPC) mice.

(B). Representative images depicting the genotyping results of tissues extracted from KPCS (lane 1), KPC (lanes 2), KCS (lane 3), KC (lane 4), CS (lane 5), Pdx1-Cre (lane 6), *Sirt5*$^{fl/fl}$ (lane 7), and *Sirt5*$^{fl/+}$ (lane 8) mice.
(C). Representative image of H&E stained pancreatic tissues from mice of indicated genotypes at 16 weeks of age. Scale bar is 200 μm.
(D). Quantitation of the islet numbers in 16-week-old wild-type, *Pdx1-Cre*, and CS mice.
(E). Body weight in age-matched wild-type, *Pdx1-Cre*, and CS mice from age 4 to 16 weeks.
The data are represented as mean ± SD. The cohorts were compared with the wild type group by one-way ANOVA with Bonferroni's post hoc analysis (D). Cohorts were compared with every other group by two-way ANOVA with Tukey's post hoc analysis (E). Differences between each group (D-E) are not significant.

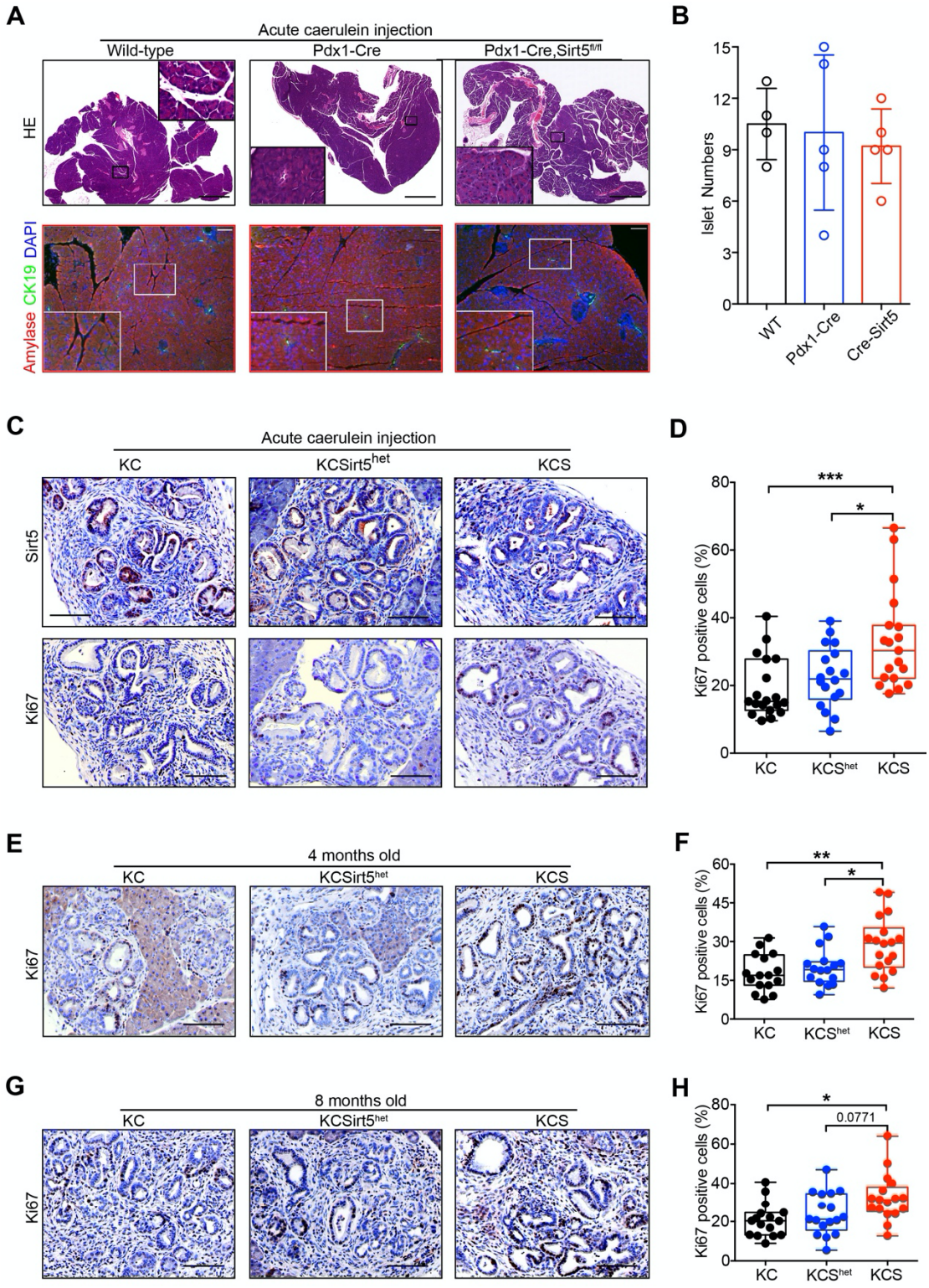


Figure S4. Sirt5 deficiency accelerates acinar-to-ductal metaplasia, PanIN formation, and PDAC progression cooperated with oncogenic mutations. (A). Representative image of H&E staining and CK19/Amylase immunofluorescence staining of pancreatic tissue from the indicated genotype mice receiving two-days caerulein-injection. Scale bars: H&E 2000 μm, immunofluorescence 100 μm.

(B). Statistical analysis of the islet number in the age-matched wild-type, *Pdx1-Cre*, and CS mice receiving two-days caerulein-injection.
(C). IHC staining images of SIRT5 and Ki67 in pancreatic tissue from caerulein-injected KC, KCS^{het}, and KCS mice. Scale bars are 100 μm.
(D). Quantification of Ki67 positive cells in pancreatic tissue from caerulein-injected KC, KCS^{het}, and KCS mice.
(E-F). IHC staining and quantification of Ki67 positive cells in pancreatic tissue from 4-month-old KC, KCS^{het}, and KCS mice. Scale bars are 100 μm.
(G-H). IHC staining and quantification of Ki67 positive cells in pancreatic tissue from 8-month-old KC, KCS^{het}, and KCS mice. Scale bars are 100 μm.
The data are represented as mean ± SD. The cohorts were compared with every other group by one-way ANOVA with Tukey's post hoc analysis (B, D, F, H), $*P<.05$, $**P<.01$, and $***P<.001$.

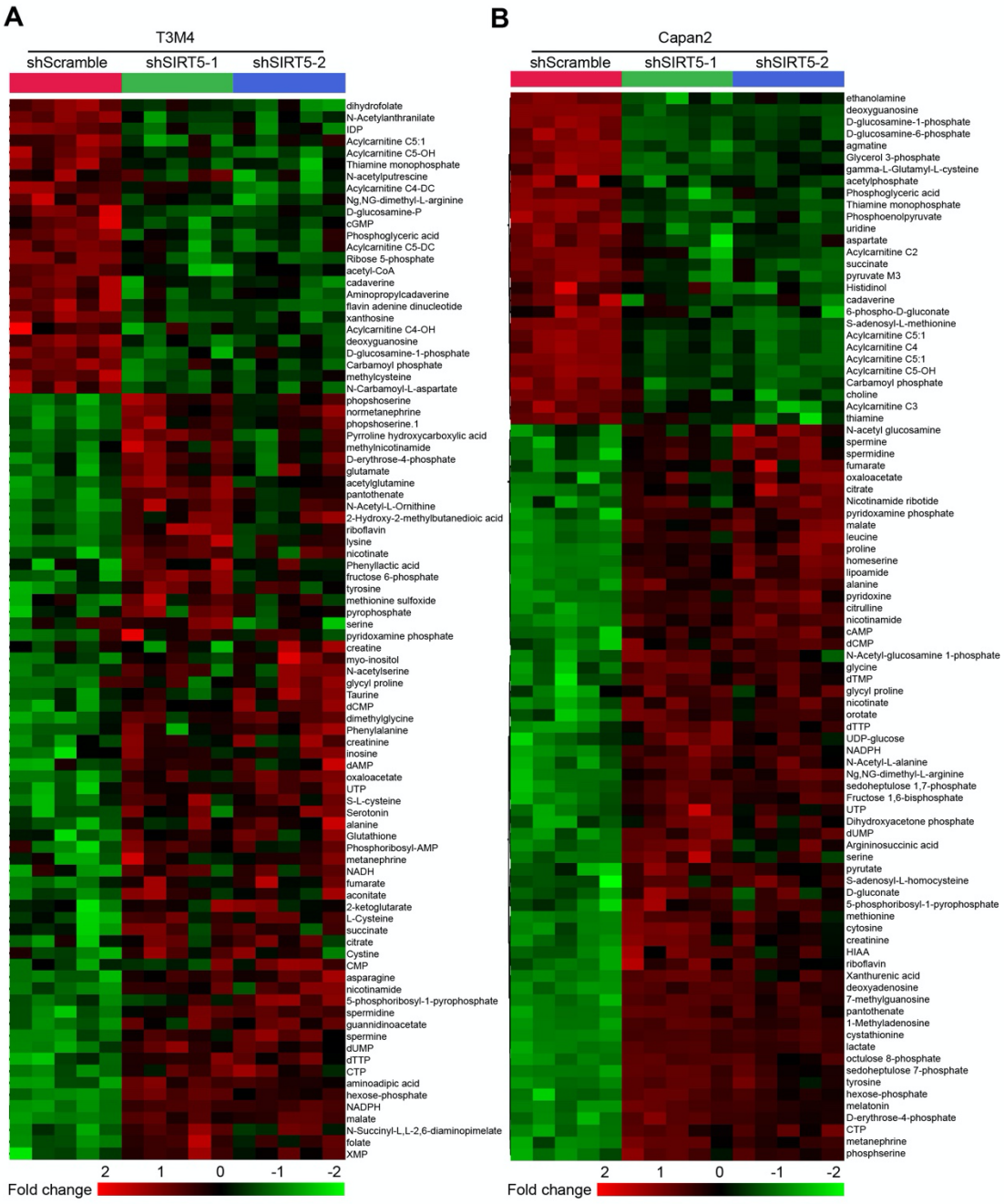
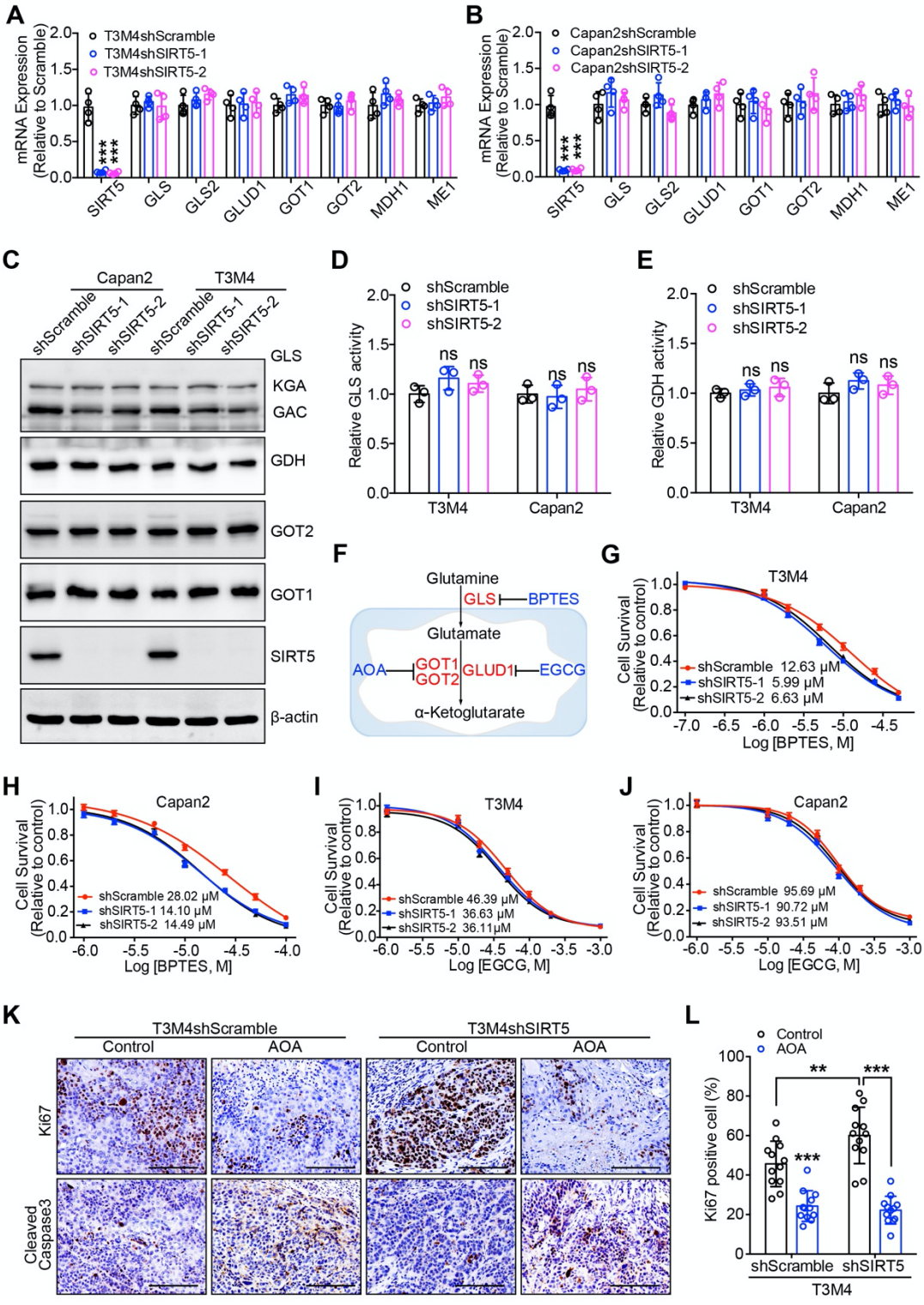


Figure S5 related to Figure 4. SIRT5 suppresses glutamine and glutathione metabolism, and regulates cellular redox homeostasis.

(A-B). The heatmap of significantly deregulated metabolites between control and *SIRT5*-knockdown PDAC cells (n= 5 samples for each group).


A
mRNA Expression (Relative to Scramble)
T3M4shScramble
T3M4shSIRT5-1
T3M4shSIRT5-2
SIRT5
GLS
GLS2
GLUD1
GOT1
GOT2
MDH1
ME1
B
Capan2shScramble
Capan2shSIRT5-1
Capan2shSIRT5-2
C
Capan2
T3M4
shScramble
shSIRT5-1
shSIRT5-2
GLS
KGA
GAC
GDH
GOT2
GOT1
SIRT5
β-actin
D
Relative GLS activity
ns
T3M4
Capan2
E
Relative GDH activity
F
Glutamine
GLS
BPTES
Glutamate
AOA
GOT1
GOT2
GLUD1
EGCG
α-Ketoglutarate
G
T3M4
Cell Survival (Relative to control)
shScramble 12.63 μM
shSIRT5-1 5.99 μM
shSIRT5-2 6.63 μM
Log [BPTES, M]
H
Capan2
shScramble 28.02 μM
shSIRT5-1 14.10 μM
shSIRT5-2 14.49 μM
I
T3M4
shScramble 46.39 μM
shSIRT5-1 36.63 μM
shSIRT5-2 36.11μM
Log [EGCG, M]
J
Capan2
shScramble 95.69 μM
shSIRT5-1 90.72 μM
shSIRT5-2 93.51 μM
K
T3M4shScramble
T3M4shSIRT5
Control
AOA
Ki67
Cleaved Caspase3
L
Control
AOA
Ki67 positive cell (%)
shScramble
shSIRT5
T3M4

545

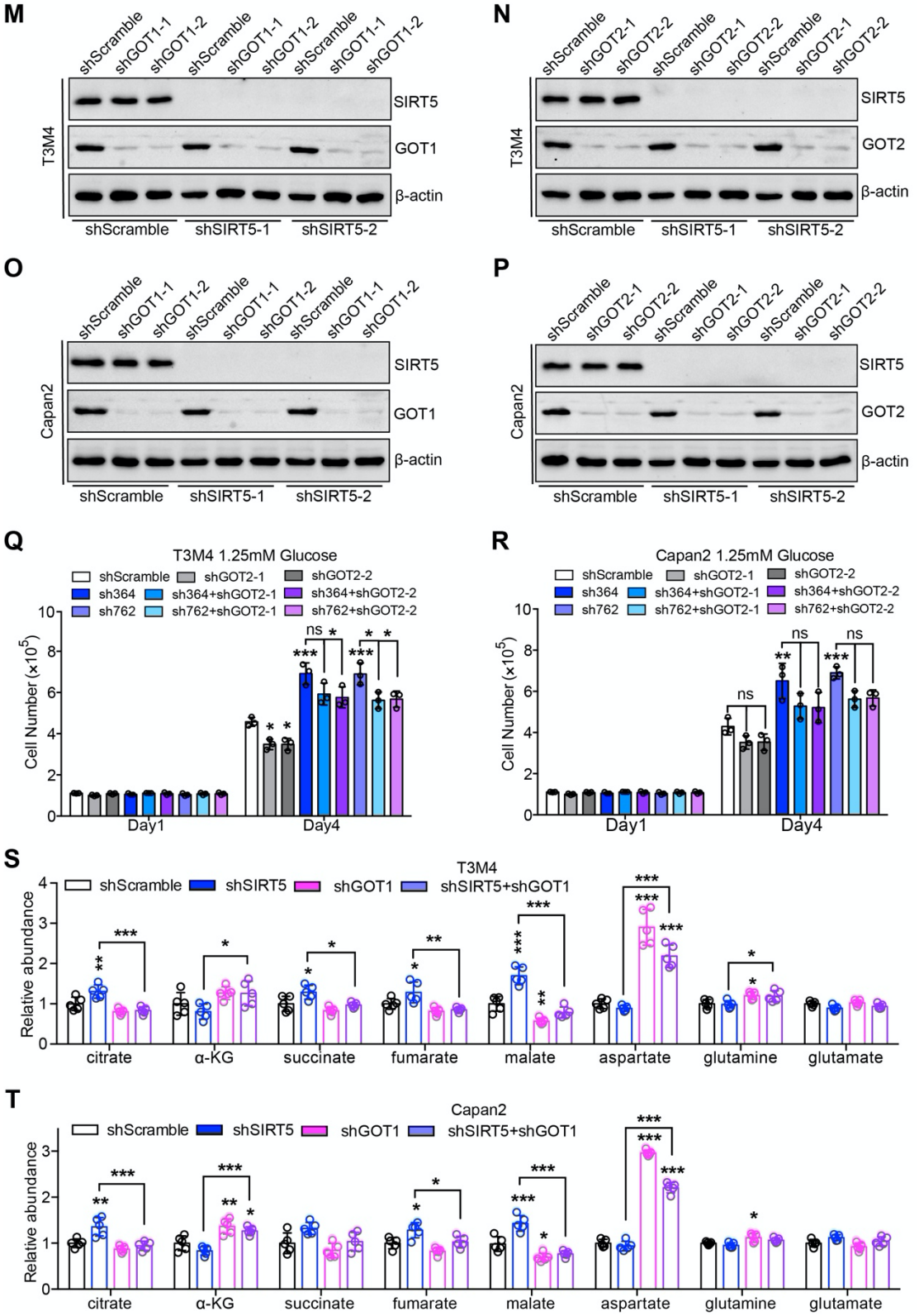


Figure S6. SIRT5 inhibits glutamine and glutathione metabolism by decreasing GOT1 enzyme activity.

(A-B). The mRNA levels of enzymes involved in glutamine and glutathione metabolism in control and *SIRT5*-knockdown PDAC T3M4 and Capan2 cells.

(C). The protein levels of enzymes involved in glutamine and glutathione metabolism from control and *SIRT5*-knockdown PDAC cells.
(D). The GLS enzyme activity was determined in T3M4 and Capan2 *SIRT5*-knockdown cells.
(E). The GDH (GLUD1) enzyme activity was determined in T3M4 and Capan2 *SIRT5*-knockdown cells.
(F). A schematic depiction of the key enzymes in glutamine metabolism and the corresponding inhibitors. Bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl) ethyl sulfide (BPTES), epigallocatechin gallate (EGCG), and aminooxyacetate (AOA).
(G-H). Relative cell survival of control and *SIRT5*-knockdown cells treated with GLS inhibitor BPTES. Data are presented relative to the respective untreated control and *SIRT5*-knockdown cells.
(I-J). Relative cell survival of control and *SIRT5*-knockdown cells treated with GLUD1 inhibitor EGCG. Data are presented relative to the respective untreated control and *SIRT5*-knockdown cells.
(K-L). Ki67 IHC staining (K) and quantitation (L) in tumor sections from orthotopically implanted scrambled control and *SIRT5*-knockdown cells treated with saline control or AOA. Scale bars are 100 μm.
(M-P). Western blotting to confirm *GOT1* and *GOT2* knockdown efficiency in scrambled control and *SIRT5*-knockdown cells.
(Q-R). Cell growth of scrambled control and *SIRT5*-knockdown cells transfected with scrambled control or *GOT2* shRNA under low glucose conditions (1.25 mM). Experiment for scrambled control and *SIRT5*-knockdown cells transfected with *GOT1* or *GOT2* shRNA were set up together.
(S-T). Relative levels of metabolites in glutamine and glutathione metabolism pathways from scrambled control and *SIRT5*-knockdown cells transfected with scrambled control or *GOT1* shRNA.
For all *in vitro* studies $n \geq 3$. The data are represented as mean ± SD. Cohorts were compared with the control group by one-way ANOVA with Bonferroni's post hoc analysis (A, B, D, E) or compared with every other group by one-way ANOVA with Tukey's post hoc analysis (L, Q-T), $^{*}P<.05$, $^{**}P<.01$, and $^{***}P<.001$.

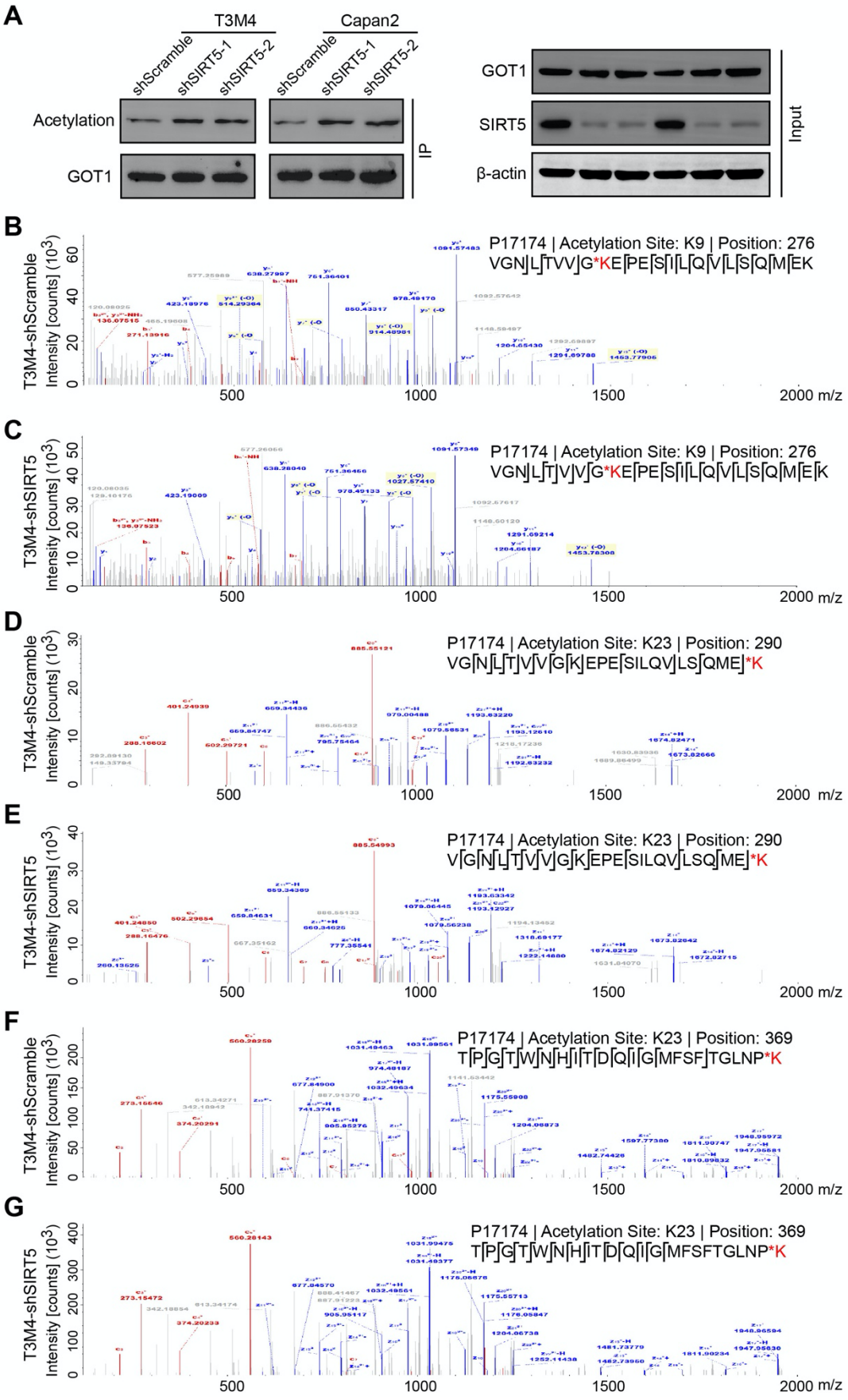
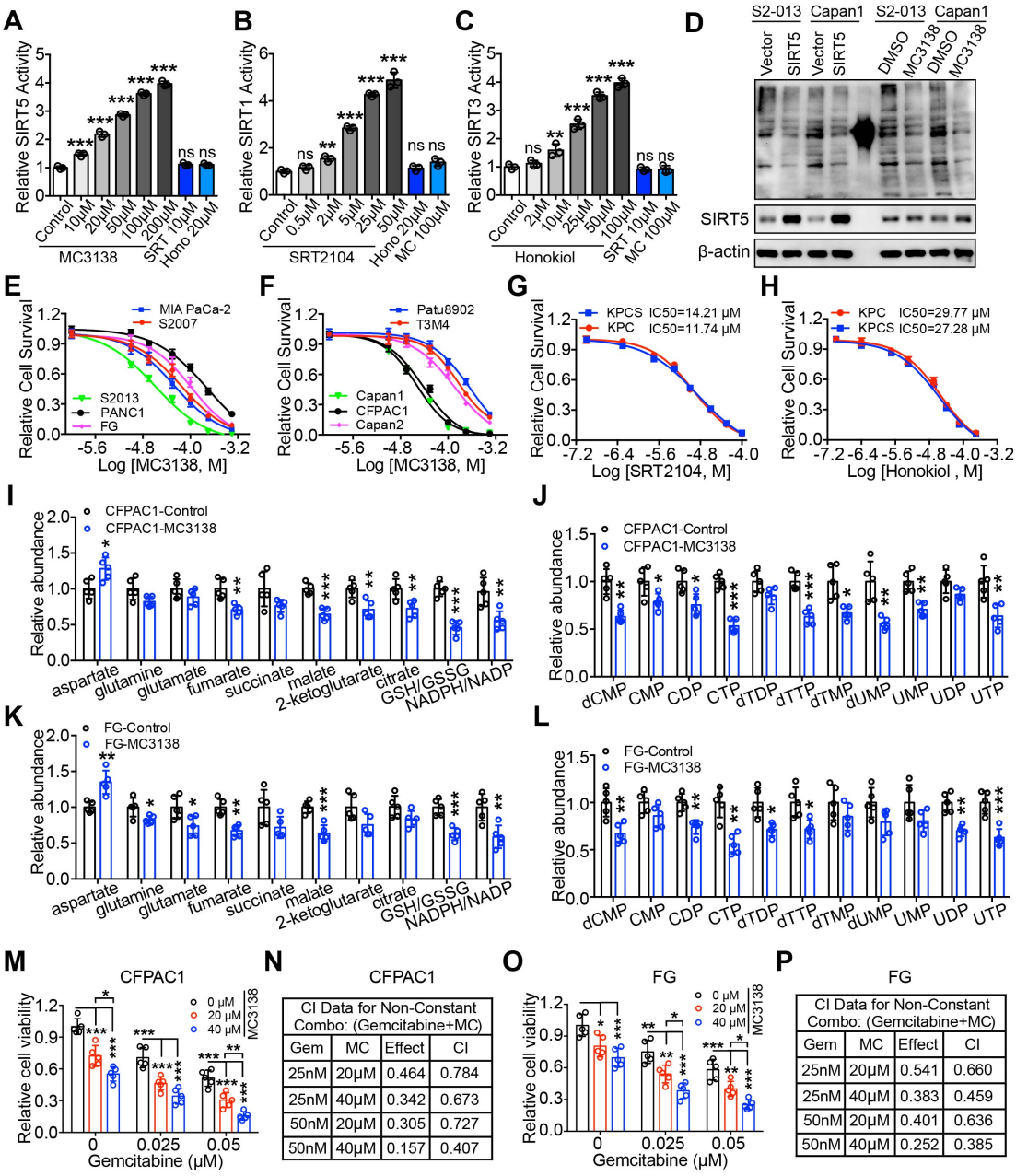

A
T3M4
Capan2
shScramble
shSIRT5-1
shSIRT5-2
shScramble
shSIRT5-1
shSIRT5-2
Acetylation
GOT1
IP
GOT1
SIRT5
β-actin
Input
B
T3M4-shScramble
Intensity [counts] (10^3)
P17174 | Acetylation Site: K9 | Position: 276
VGN[L[T]VV]G*KE[PE[S[I[L[Q[V[L[S[Q[M[EK
C
T3M4-shSIRT5
Intensity [counts] (10^3)
P17174 | Acetylation Site: K9 | Position: 276
VGN[L[T]V]V]G*KE[PE[S[I[L[Q[V[L[S[Q[M[E[K
D
T3M4-shScramble
Intensity [counts] (10^3)
P17174 | Acetylation Site: K23 | Position: 290
VG[N[L[T]V]V[G[K]EPE[SILQV]LS[QME]*K
E
T3M4-shSIRT5
Intensity [counts] (10^3)
P17174 | Acetylation Site: K23 | Position: 290
V[G[N[L[T]V]V]G[K]EPE[SILQV]LSQ[ME]*K
F
T3M4-shScramble
Intensity [counts] (10^3)
P17174 | Acetylation Site: K23 | Position: 369
T[P[G[T]W[N[H[I[T[D[Q[I[G[MFSF]TGLNP*K
G
T3M4-shSIRT5
Intensity [counts] (10^3)
P17174 | Acetylation Site: K23 | Position: 369
T[P[G[T]W[N[H[I[T[D[Q[I[G[MFSFTGLNP*K
m/z

Figure S7. SIRT5 inhibits GOT1 enzymatic activity by catalyzing its lysine deacetylation.
(A). Endogenous GOT1 protein was immunoprecipitated from control and *SIRT5*-knockdown T3M4/Capan2 cells. The lysine acetylation level of GOT1 was determined by western blotting. Input protein levels are shown on the right.
(B-G). Mass spectra demonstrating lysine acetylation sites in exogenously expressed GOT1 protein immunoprecipitated from scrambled control and *SIRT5*-knockdown T3M4 cells. The specific lysine acetylation sites were identified by the liquid chromatography-tandem mass spectrometry. The identified acetylated peptide ions in GOT1 protein are indicated.

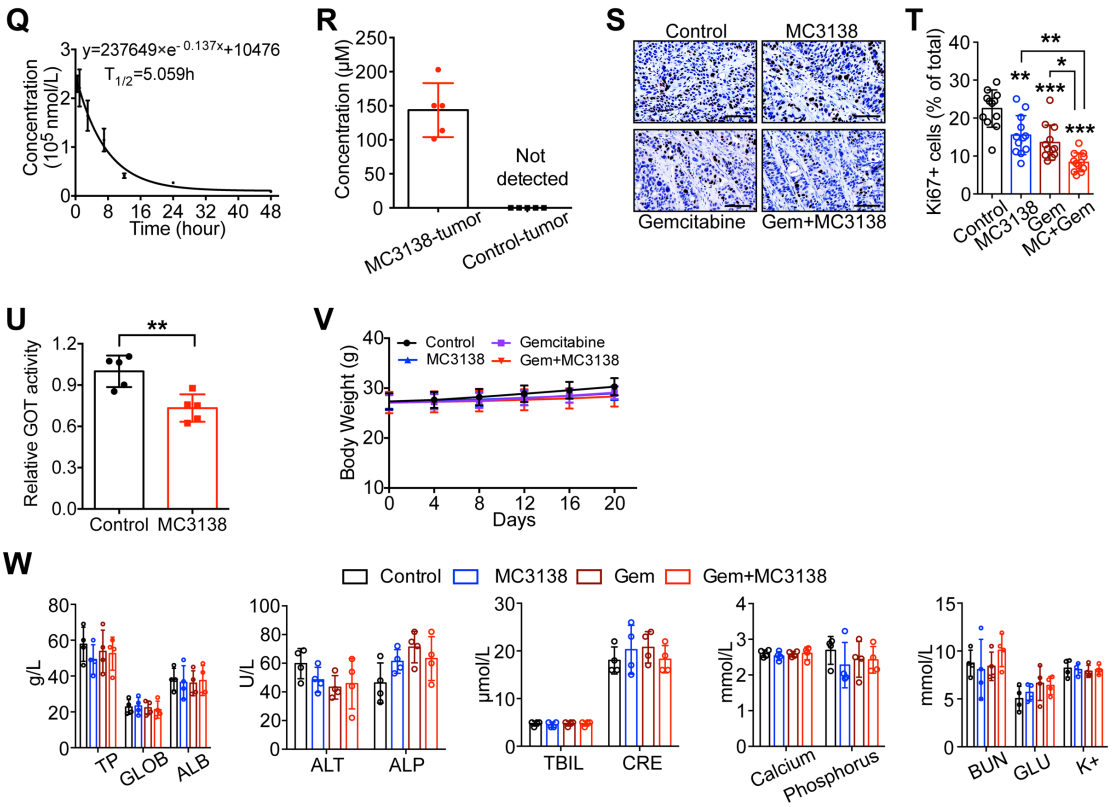


CI Data for Non-Constant Combo: (Gemcitabine+MC)

Gem	MC	Effect	CI
25nM	20μM	0.464	0.784
25nM	40μM	0.342	0.673
50nM	20μM	0.305	0.727
50nM	40μM	0.157	0.407

CI Data for Non-Constant Combo: (Gemcitabine+MC)

Gem	MC	Effect	CI
25nM	20μM	0.541	0.660
25nM	40μM	0.383	0.459
50nM	20μM	0.401	0.636
50nM	40μM	0.252	0.385



598

599 **Figure S8.** SIRT5 activator MC3138 exhibits anti-tumor effects and synergism
600 with gemcitabine in human PDAC cells, organoids, and PDX models.
601 (A). Increased SIRT5 deacetylase activity by MC3138. SIRT5 deacetylase
602 activity was measured under different concentrations of MC3138 using SIRT5
603 Deacetylase Fluorometric Assay Kit. The selective SIRT1 activator SRT2104
604 (SRT) and SIRT3 activator Honokiol (Hono) were used as negative controls.
605 (B-C). SIRT5 activator MC3138 does not increase SIRT1 and SIRT3
606 deacetylase activities. SIRT1 and SIRT3 deacetylase activities were detected
607 using SIRT1 and SIRT3 Deacetylase Fluorometric Assay Kits, respectively.
608 The selective SIRT1 activator SRT2104 and SIRT3 activator Honokiol were
609 used as positive controls.
610 (D). Immunoblotting to show the lysine acetylation level in control or *SIRT5*-
611 overexpressing PDAC cells and PDAC cells treated with DMSO solvent control
612 or 10 μM SIRT5 activator MC3138 for 24 h.
613 (E-F). Relative cell survival of ten PDAC cell lines treated with different doses
614 of MC3138.
615 (G-H). Relative cell survival of KPC cell line and *SIRT5*-knockout KPC cell line
616 (KPCS) treated with different doses of SIRT1 activator SRT2104 and SIRT3
617 activator Honokiol.
618 (I-L). Bar charts depicting the relative levels of metabolites in glutamine,
619 glutathione and pyrimidine metabolism pathways in CFPAC1 and FG cells
620 treated with MC3138 (CFPAC1: 20 μM; FG: 50 μM) for 24 h.

(M, O). Cell viability of CFPAC1 (M) and FG (O) cells treated with the indicated concentrations of gemcitabine and MC3138 for 72 h.
(N, P). Combination index (CI) of gemcitabine and MC3138 at indicated concentrations was calculated using Compusyn software.
(Q). MC3138 Pharmacokinetics: The plasma concentration-time curve of MC3138 after intraperitoneal injection of a single dose of MC3138 (200 mg/kg) (n = 4).
(R). The concentration of MC3138 in tumor tissues from MC3138-treated PA137 PDX tumors.
(S-W). Effect of MC3138 (MC) combined with Gemcitabine (Gem) on human PDX tumors. IHC staining of Ki67 (S) and quantitation (T). The relative GOT activity of control and MC3138-treated PA137 PDX tumors (U). The body weights of mice from each group at indicated time points (V). The blood biochemistry indices test results of mice from indicated groups by automatic biochemical analyzer (W). Scale bars are 100 μm.
For all *in vitro* studies $n \geq 3$. The data are represented as mean ± SD. The cohorts were compared with the control group by one-way ANOVA with Bonferroni's post hoc analysis (A, B, C) or compared with every other group by one-way ANOVA with Tukey's post hoc analysis (M, O, T, W). Cohorts were compared with every other group by two-way ANOVA with Tukey's post hoc analysis (V). The statistics was calculated by Student's t-test (I, J, K, L, R, U), $*P<.05$, $**P<.01$, and $***P<.001$.

644 **Table S1. Correlation between Expression of SIRT5 and**
645 **Clinicopathological Features in PDAC Patients from Figure 1.**
646

Variables	Low SIRT5	High SIRT5	*p* value
Numbers	73	70	
Gender			0.161
Male	49 (67.1)	39 (55.7)	
Female	24 (32.9)	31 (44.3)	
Median age			0.801
<62 years	37 (50.7)	34 (48.6)	
≥62 years	36 (49.3)	36 (51.4)	
pT stage			0.160
T1-T2	44 (60.3)	50 (71.4)	
T3-T4	29 (39.7)	20 (28.6)	
pN stage			0.145
N0	36 (49.3)	43 (61.4)	
N1-N2	37 (50.7)	27 (38.6)	
pM stage[a]			0.962
M0	71 (97.3)	67 (95.7)	
M1	2 (2.7)	3 (4.3)	
TNM stage			0.148
I- II	35 (47.9)	42 (60.0)	
III- IV	38 (52.1)	28 (40.0)	
Histological grade[b]			0.659
G1	7 (9.6)	11 (15.7)	
G2	40 (54.8)	34 (48.6)	
G3	26 (35.6)	25 (35.7)	
Tumor size (cm)			0.765
≤4	42 (57.5)	42 (60.0)	
>4	31 (42.5)	28 (40.0)	
Recurrence			0.033
YES	55 (75.3)	41 (58.6)	
NO	18 (24.7)	29 (41.4)	
CEA (ng/mL)			0.339
<5	38 (52.1)	42 (60.0)	
≥5	35 (47.9)	28 (40.0)	
CA199 (U/mL)			0.783
<37	39 (53.4)	39 (55.7)	
≥37	34 (46.6)	31 (44.3)	
Location			0.016
head	45 (61.6)	29 (41.4)	
body/tail	28 (38.4)	41 (58.6)	

Neural Invasion			0.923
No	38 (52.1)	37 (52.9)	
Yes	35 (47.9)	33 (47.1)	
Chemotherapy			0.771
No	32 (43.8)	29 (41.4)	
Yes	41 (56.2)	41 (58.6)	
Chemotherapy (Yes)			0.888
Gemcitabine	16 (39.0)	13 (31.7)	
Gemcitabine + Capecitabine	7 (17.1)	9 (22.0)	
Gemcitabine + nab-paclitaxel	12 (29.3)	12 (29.3)	
Gemcitabine + Tegafur	6 (14.6)	7 (17.1)	

Note: All data are shown as numbers and percentages--numbers (percentage%). Chi-square Analysis was used in these data.

a: The Yate's correction for continuity was used in data of this group.

b: The Kruskal Wallis Test was used in data of this group.

Table S2. Univariate and Multivariate Analysis for the Prognostic Factors of Overall Survival in PDAC Patients from Figure 1.

Variables	Univariate Analysis HR (95% CI)	*P* value	Multivariate Analysis HR (95% CI)	*P* value
Gender (male versus female)	1.529 (1.049-2.230)	0.027	1.415 (0.953-2.099)	0.085
Age (≥62 years vs <62 years)	1.246 (0.871-1.782)	0.229		
pT status (T3 or T4 vs T1 or T2)	0.857 (0.587-1.252)	0.425		
pN status (N1-2 vs N0)	1.753 (1.222-2.515)	0.002	1.116 (0.390-3.196)	0.838
pM status (M1 vs M0)	1.562 (0.636-3.840)	0.331		
AJCC stage (III or IV vs I or II)	1.793 (1.250-2.572)	0.002	1.786 (1.243-2.568)	0.002
Histological grade (G3 vs G1 or G2)	1.558 (1.079-2.250)	0.018	1.565 (1.082-2.265)	0.018
Tumor size (≤4 cm vs >4 cm)	0.840 (0.584-1.207)	0.345	-	-
Recurrence (Yes vs No)	1.786 (1.167-2.734)	0.008	1.623 (1.045-2.522)	0.031
CEA (≥5 ng/mL vs <5ng/mL)	0.973 (0.681-1.390)	0.881		
CA199 (≥37 U/mL vs <37U/mL)	1.237 (0.863-1.771)	0.247	-	-
Location (Head vs Body/Tail)	1.295 (0.899-1.867)	0.165	-	-
Neural Invasion (Yes vs No)	1.123 (0.784-1.608)	0.526	-	-
Chemotherapy (Yes vs No)	0.580 (0.403-0.853)	0.003	0.761 (0.524-1.105)	0.151
SIRT5 expression (High vs Low)	0.590 (0.411-0.847)	0.004	0.669 (0.462-0.970)	0.034

Univariate and multivariate Cox proportional hazards regression were used to calculate Hazard ratio (HR), 95% confidence intervals (95% CI) and *p* values in SPSS 22.0.

Table S3. Univariate and Multivariate Analysis for the Prognostic Factors of Disease-Free Survival in PDAC Patients from Figure 1.

Variables	Univariate Analysis HR (95% CI)	*P* value	Multivariate Analysis HR (95% CI)	*P* value
Gender (male versus female)	1.313 (0.920-1.875)	0.134		
Age (≥62 years vs <62 years)	1.190 (0.842-1.683)	0.324		
pT status (T3 or T4 vs T1 or T2)	0.932 (0.648-1.341)	0.706		
pN status (N1-2 vs N0)	1.580 (1.115-2.239)	0.010	1.398 (0.492-3.977)	0.529
pM status (M1 vs M0)	1.302 (0.531-3.195)	0.564		
AJCC stage (III or IV vs I or II)	1.593 (1.125-2.256)	0.009	1.605 (1.130-2.277)	0.008
Histological grade (G3 vs G1 or G2)	1.413 (0.990-2.017)	0.057	1.451 (1.014-2.076)	0.042
Tumor size (≤4 cm vs >4 cm)	0.858 (0.604-1.218)	0.392	-	-
Recurrence (Yes vs No)	2.790 (1.807-4.308)	<0.001	2.572 (1.651-4.008)	<0.001
CEA (≥5 ng/mL vs <5ng/mL)	0.985 (0.697-1.392)	0.931		
CA199 (≥37 U/mL vs <37U/mL)	1.139 (0.804-1.613)	0.464	-	-
Location (Head vs Body/Tail)	1.211 (0.852-1.721)	0.286	-	-
Neural Invasion (Yes vs No)	1.142 (0.807-1.618)	0.454	-	-
Chemotherapy (Yes vs No)	0.587 (0.413-0.836)	0.003	0.773 (0.540-1.108)	0.161
SIRT5 expression (High vs Low)	0.505 (0.355-0.719)	<0.001	0.605 (0.423-0.864)	0.006

Univariate and multivariate Cox proportional hazards regression were used to calculate Hazard ratio (HR), 95% confidence intervals (95% CI) and *p* values in SPSS 22.0.

Table S4. Primers used in this study.

Oligonucleotides		
Primers for qPCR		
hSIRT5-F	Eurofins MWG Operons	GCCATAGCCGAGTGTGAGAC
hSIRT5-R	Eurofins MWG Operons	CAACTCCACAAGAGGTACATCG
hGLS-F	Eurofins MWG Operons	AGGGTCTGTTACCTAGCTTGG
hGLS-R	Eurofins MWG Operons	ACGTTCGCAATCCTGTAGATTT
hGLS2-F	Eurofins MWG Operons	GCCTGGGTGATTTGCTCTTTT
hGLS2-R	Eurofins MWG Operons	CCTTTAGTGCAGTGGTGAACTT
hGLUD1-F	Eurofins MWG Operons	GGGCTTTTGTATCCGTTACAGC
hGLUD1-R	Eurofins MWG Operons	GGTCAAACGGCCATAGCTGA
hGOT1-F	Eurofins MWG Operons	ATGGCACCTCCGTCAGTCT
hGOT1-R	Eurofins MWG Operons	AGTCATCCGTGCGATATGCTC
hGOT2-F	Eurofins MWG Operons	AGCCTTACGTTCTGCCTAGC
hGOT2-R	Eurofins MWG Operons	AAACCGGCCACTCTTCAAGAC
hME1-F	Eurofins MWG Operons	GAGTGCTGACATCTGACATTGA

hME1-R	Eurofins MWG Operons	TTGGCTTCCGAAACACCAAAC
hMDH1-F	Eurofins MWG Operons	GGTGCAGCCTTAGATAAATACGC
hMDH1-R	Eurofins MWG Operons	AGTCAAGCAACTGAAGTTCTCC
h18S rRNA-F	Sigma-Aldrich	CTACCACATCCAAGGAAGCA
h18S rRNA-R	Sigma-Aldrich	TTTTTCGTCACTACCTCCCCG
Primer for genotyping		
Mice $Kras^{G12D}$-Y116	Sigma-Aldrich	TCCGAATTCAGTGACTACAGATG
Mice $Kras^{G12D}$-Y117	Sigma-Aldrich	CTAGCCACCATGGCTTGAGT
Mice $Kras^{G12D}$-Y118	Sigma-Aldrich	ATGTCTTTCCCCAGCACAGT
Mice $p53^{R172H}$-T35	Sigma-Aldrich	CTTGGAGACATAGCCACACTG
Mice $p53^{R172H}$-T36	Sigma-Aldrich	CTCTGGAATTCCGCAAGCTA
Mice $p53^{R172H}$-T37	Sigma-Aldrich	TTACACATCCAGCCTCTGTGG
Mice Pdx1-Cre-F	Sigma-Aldrich	CTGGACTACATCTTGAGTTGC
Mice Pdx1-Cre-R	Sigma-Aldrich	GGTGTACGGTCAGTAAATTTG
Mice $Sirt5^{flox/flox}$-F	Sigma-Aldrich	TGTGCTTGTACGTGCTGTGC
Mice $Sirt5^{flox/flox}$-R	Sigma-Aldrich	CCCCTCACTCAGCTCACAAA
Primers for site mutagenesis		
GOT1-sequence-F	Sigma-Aldrich (HPLC purified)	TGCCTGGGCCATTCGCTATTTTG
GOT1-sequence-R	Sigma-Aldrich (HPLC purified)	TCACTGGATTTTGGTGACTGC
GOT1-K276R-F	Sigma-Aldrich (HPLC purified)	CGA GAA CCT GAG AGC ATC CTG CAA G
GOT1-K276R-R	Sigma-Aldrich (HPLC purified)	TCCAACCACAGTCAGATTCCCGAC
GOT1-K290R-F	Sigma-Aldrich (HPLC purified)	CGG ATC GTG CGG ATT ACT TGG TC
GOT1-K290R-R	Sigma-Aldrich (HPLC purified)	CTC CAT CTG GGA AAG GAC TTG CAG
GOT1-K369R-F	Sigma-Aldrich (HPLC purified)	CGG CAG GTT GAG TAT CTG GTC AAT G
GOT1-K369R-R	Sigma-Aldrich (HPLC purified)	GGG GTT CAA CCC AGT GAA GCT GAA C
sgGOT1-resistant-F	Sigma-Aldrich (HPLC purified)	G AGA AAG GTC AAC CTG GGA GTG
sgGOT1-resistant-R	Sigma-Aldrich (HPLC purified)	GGGTCCGGATCCTCCCTGAAG
SIRT5-H158Y-F	Sigma-Aldrich (HPLC purified)	TAT GGTAGCT TATTTAAAAC TCG

SIRT5-H158Y-R	Sigma-Aldrich (HPLC purified)	GATCTCCAGAAGGTTCTTGGTG
H158Y-sequence-F	Sigma-Aldrich (HPLC purified)	A TGCGACCTCT CCAGATTGTC
H158Y-sequence-R	Sigma-Aldrich (HPLC purified)	GGAAGTTTCTCAACTGGGATGCT

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