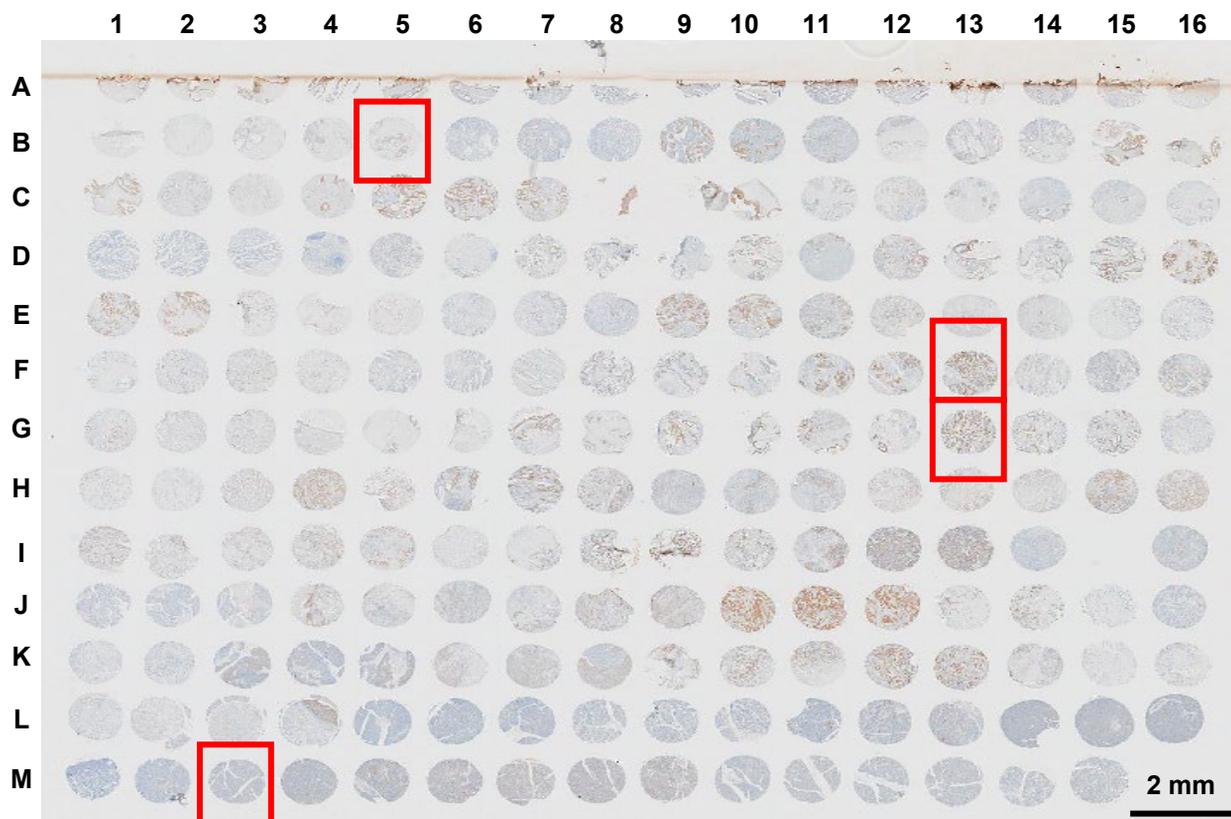
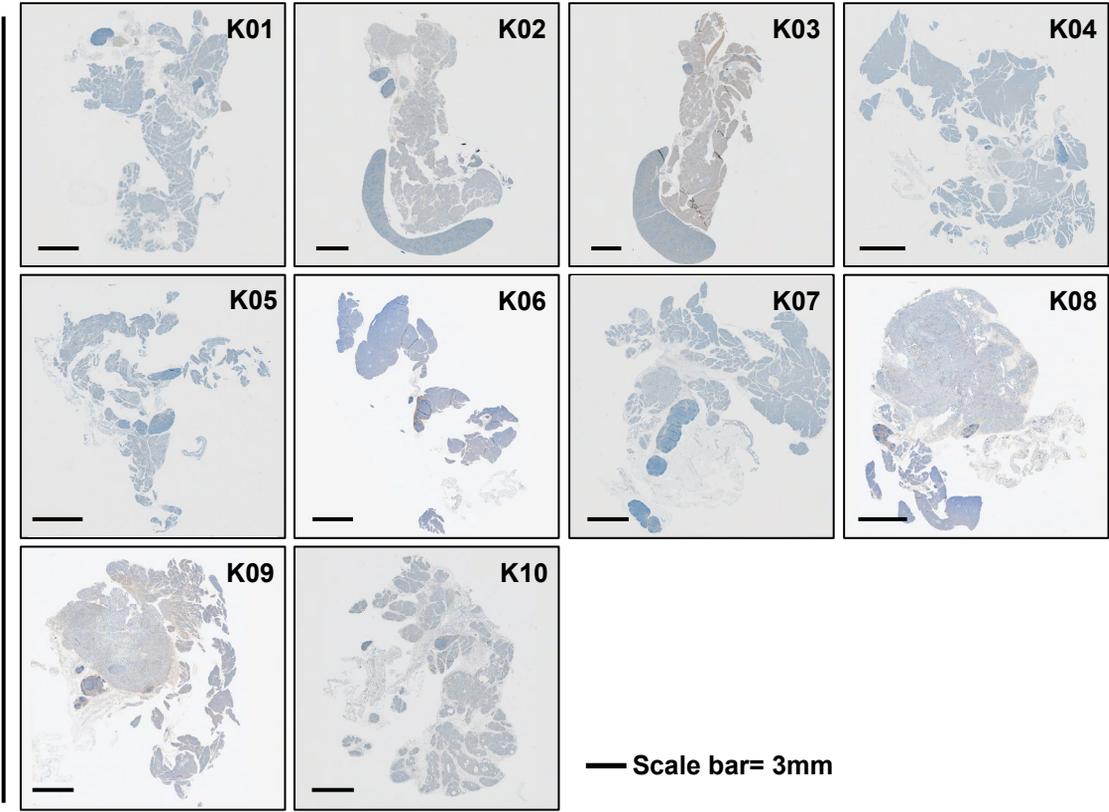
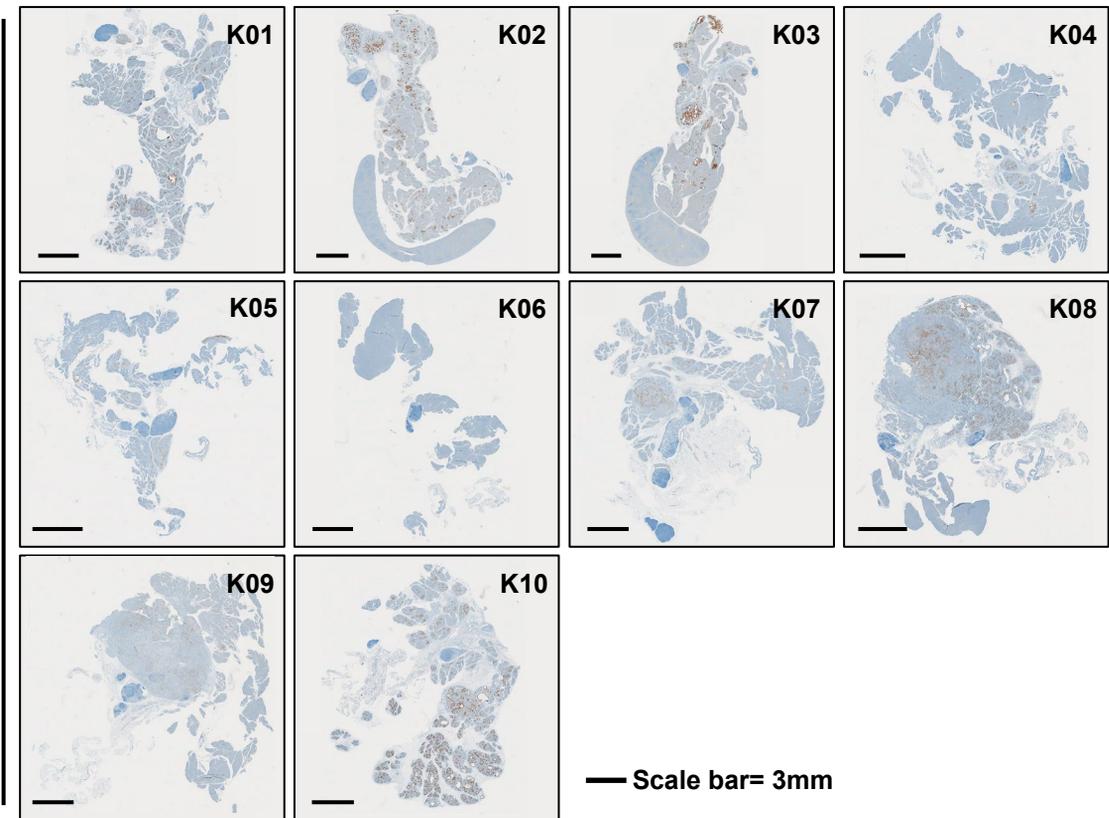
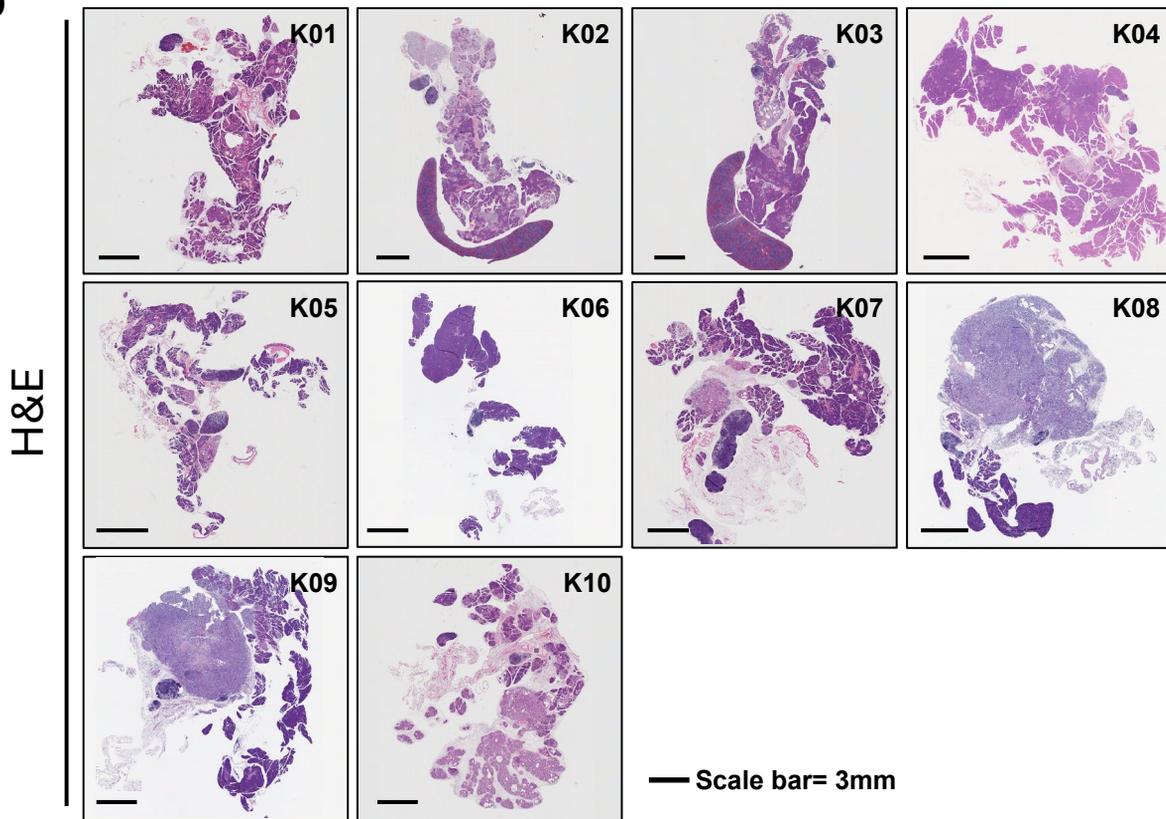


# Supplementary Figure S1

A

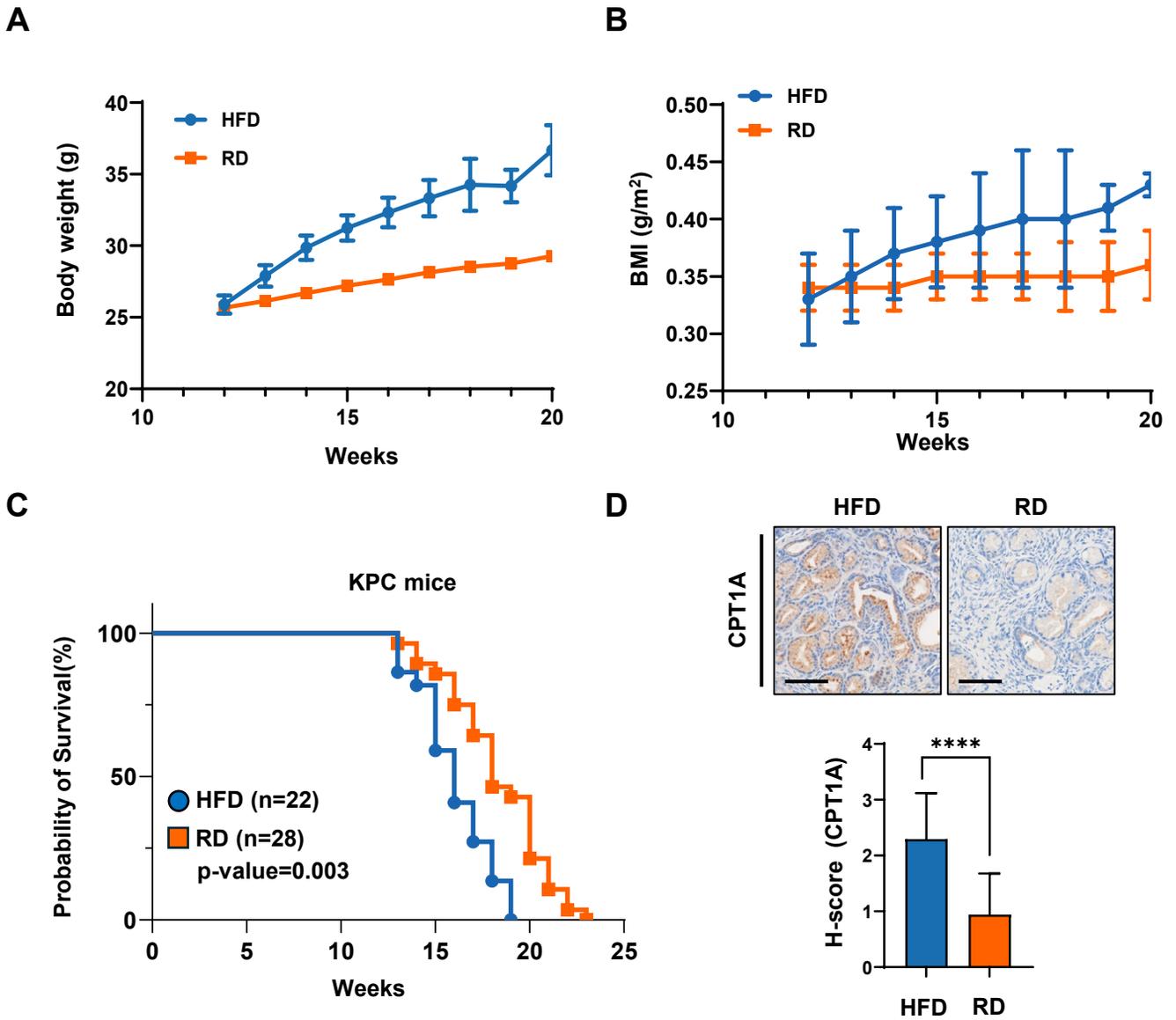


**B****CPT1A****C****CK-19**

**D**

**Supplementary Figure S1.** CPT1A expression in PDAC TMA. (A) Pancreatic carcinoma tissue microarray (TMA) containing 60 adenocarcinoma cases and 9 normal tissue samples, with triplicate cores per case (PA2072a) were purchased from Tissue Array (Derwood, MD, US). IHC images of TMA (PA2072a) stained with anti-CPT1A (ab234111). Red boxes highlight the images shown in Figure 1A. (Scale bar = 2 mm). (B-D) H&E and IHC staining images of KPC mouse tissue shown in Figure 1F-H. (B) Immunohistochemistry (IHC) image highlights CPT1A expression, (C) IHC image shows CK19 expression, and (D) Hematoxylin and eosin (H&E) staining image illustrates the histological architecture within the same tissues.

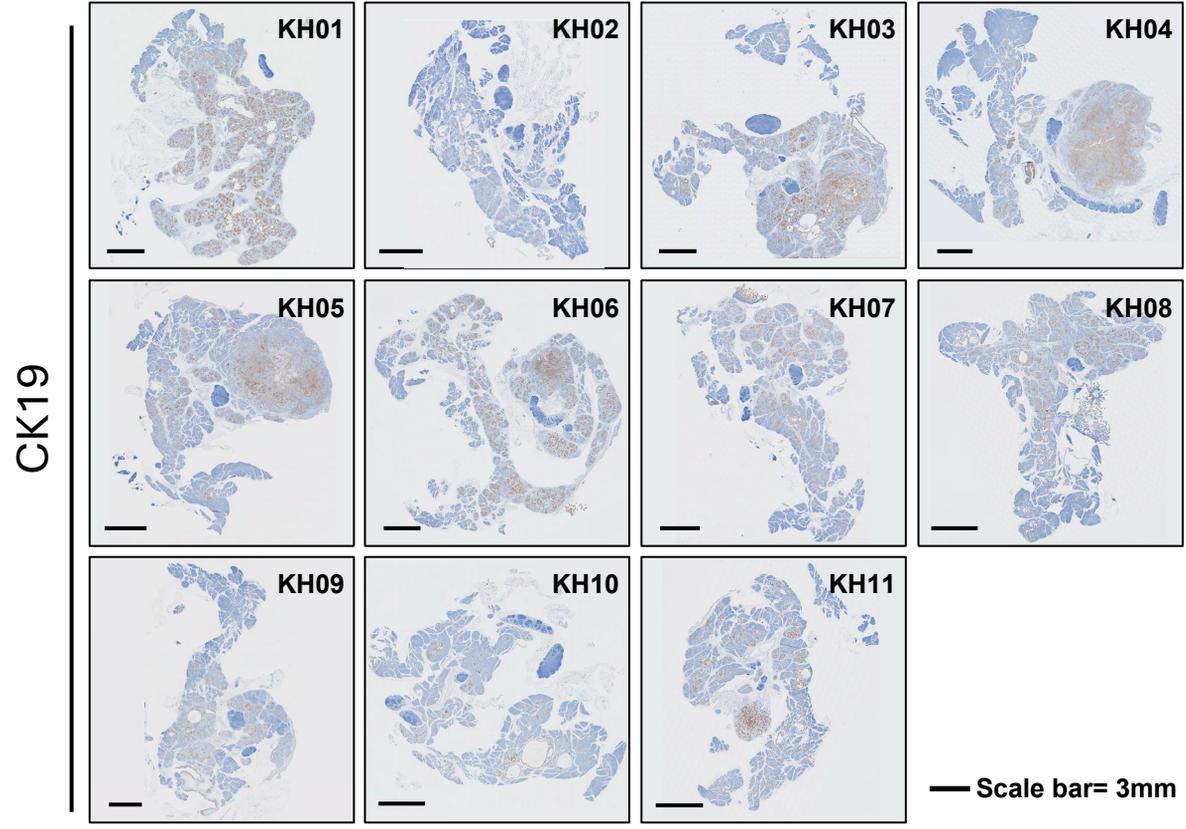
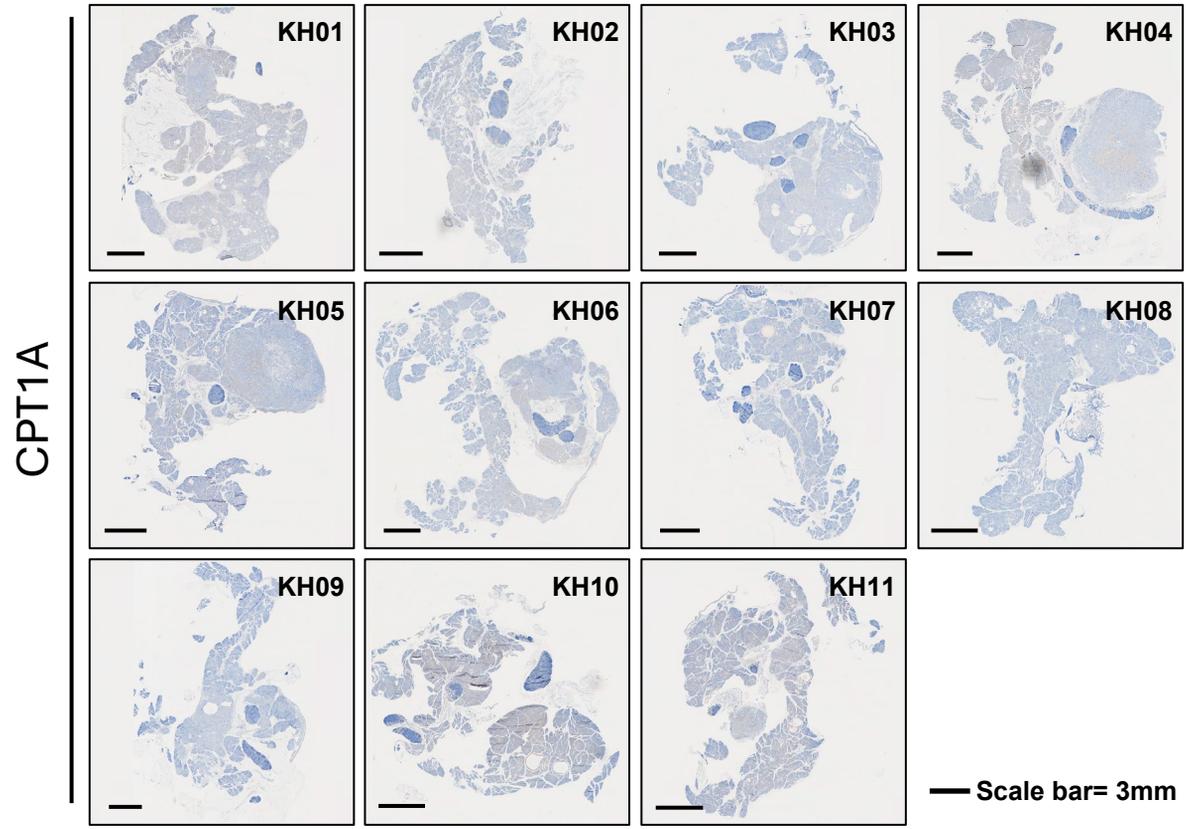
# Supplementary Figure S2



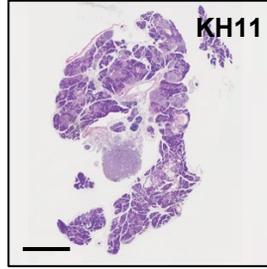
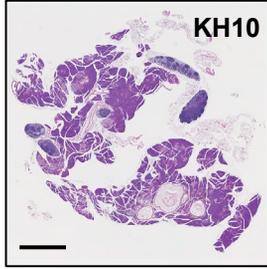
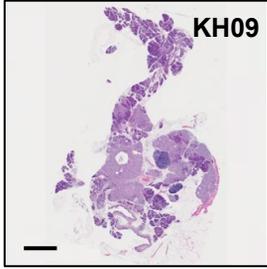
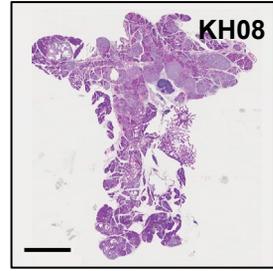
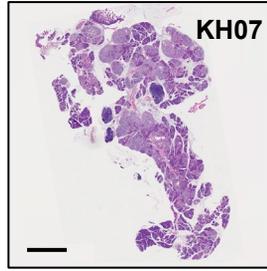
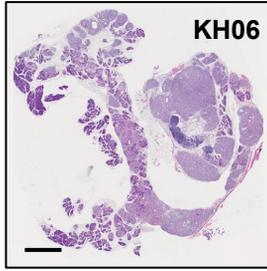
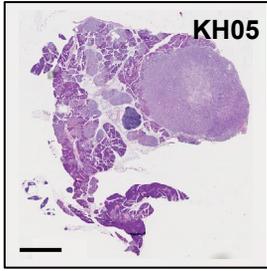
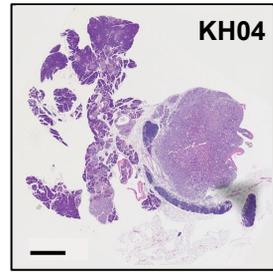
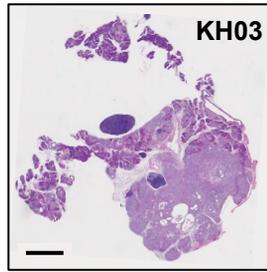
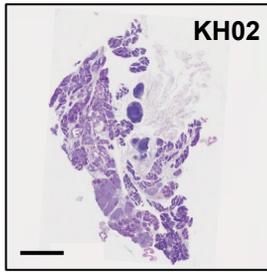
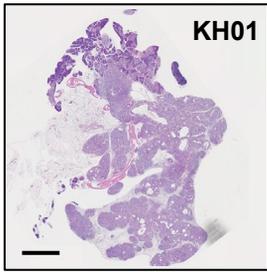
**Supplementary Figure S2. HFD promotes the growth of pancreatic cancer and reduces the survival rates of xenografted mice.** (A) The body weight change curve of the KPC mice fed an HFD (blue, 60% fat, n = 22) or a regular diet (orange, RD, 18% fat, n = 28). (B) BMI of KPC mice fed an HFD (blue) or an RD (orange). (C) Kaplan-Meier survival curves showed the HFD group (blue) survived for 2 weeks less than the RD group (orange). The difference in survival rates was significant (p-value=0.003). (D) IHC images of CPT1A in KPC mice with HFD (n=11) or with an RD (n=10). \*\*\*\*p < 0.0001.

# Supplementary Figure S3

## A High-Fat Diet



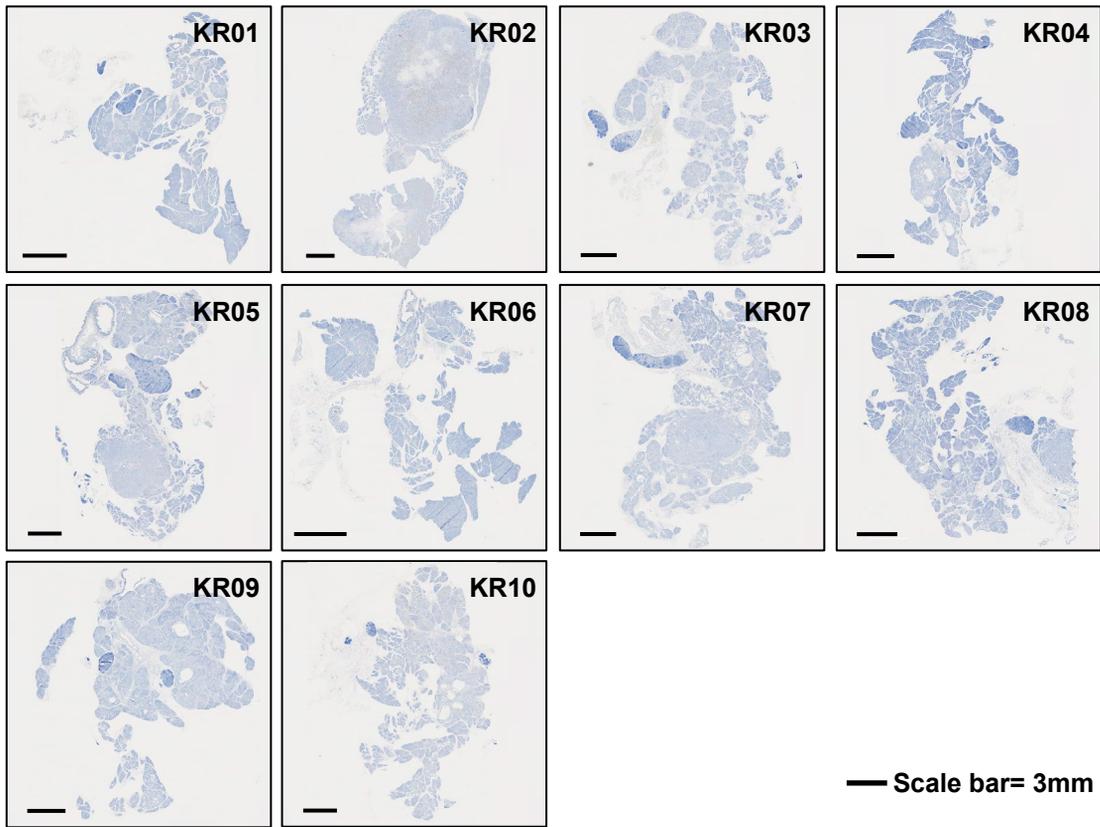
H&E



— Scale bar= 3mm

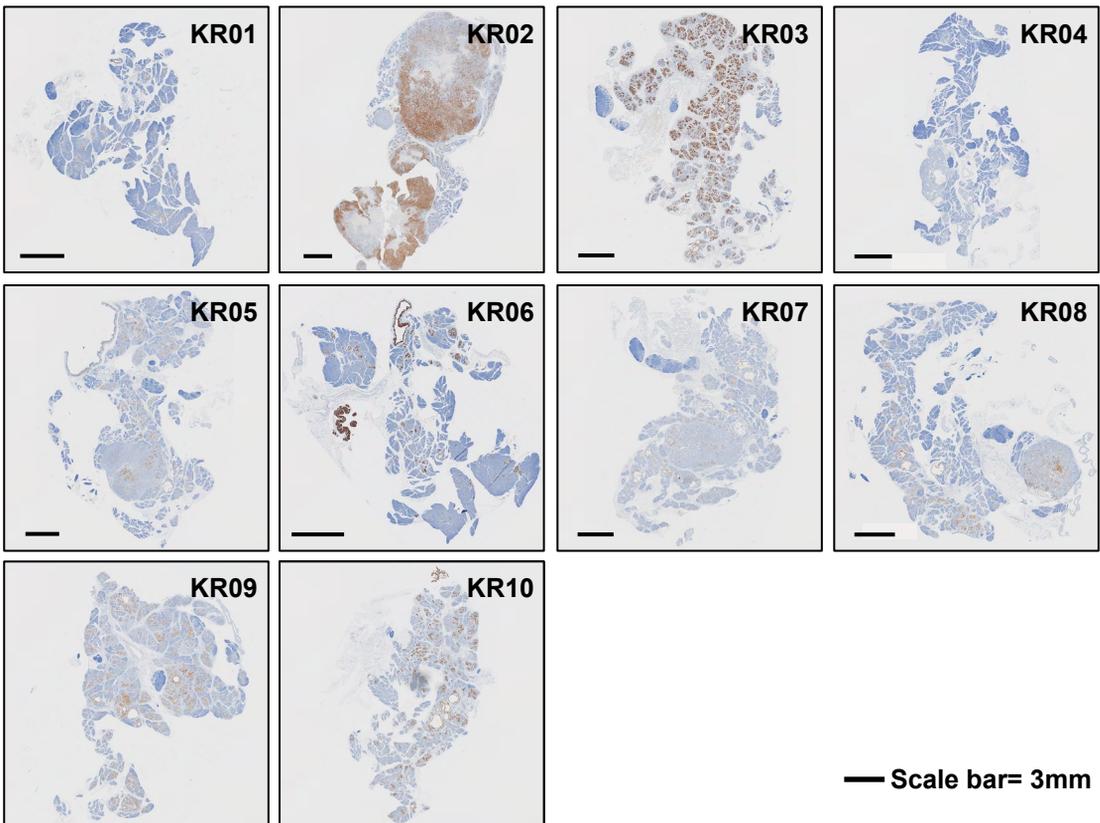
**B** Regular Diet

CPT1A

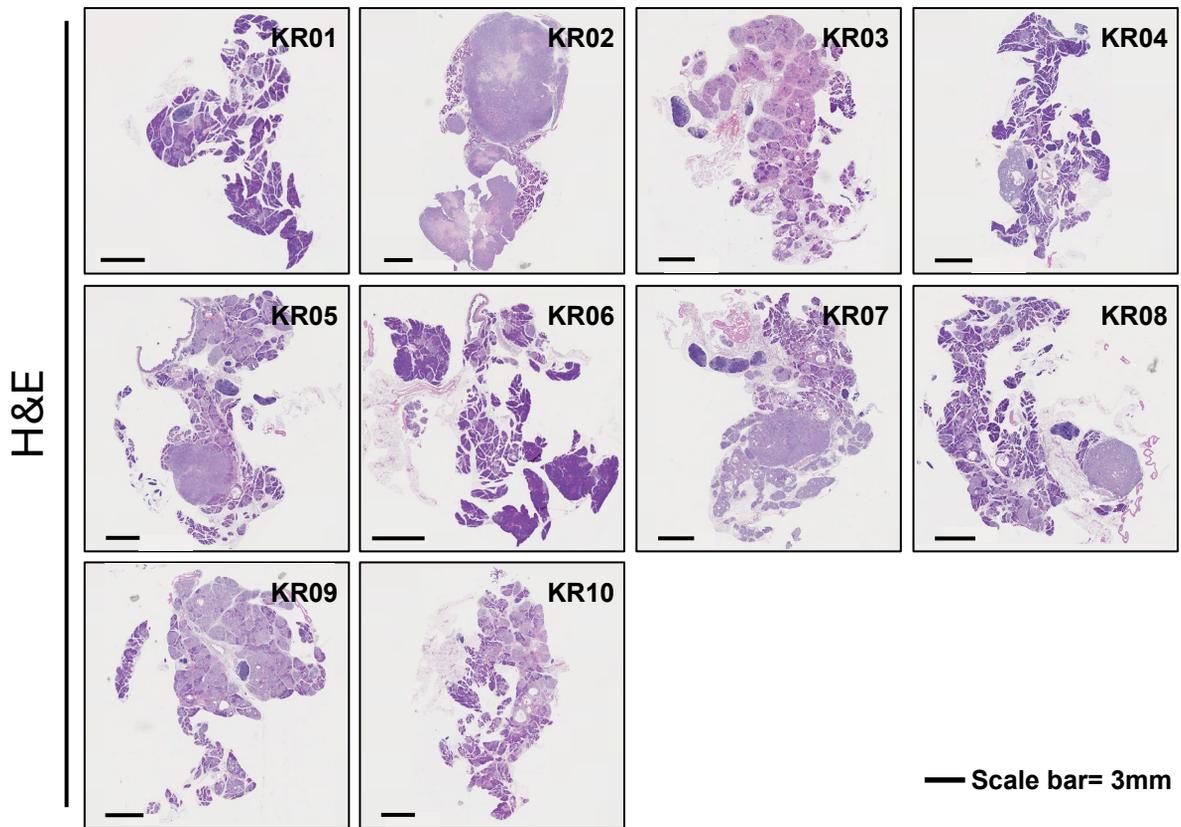


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CK19

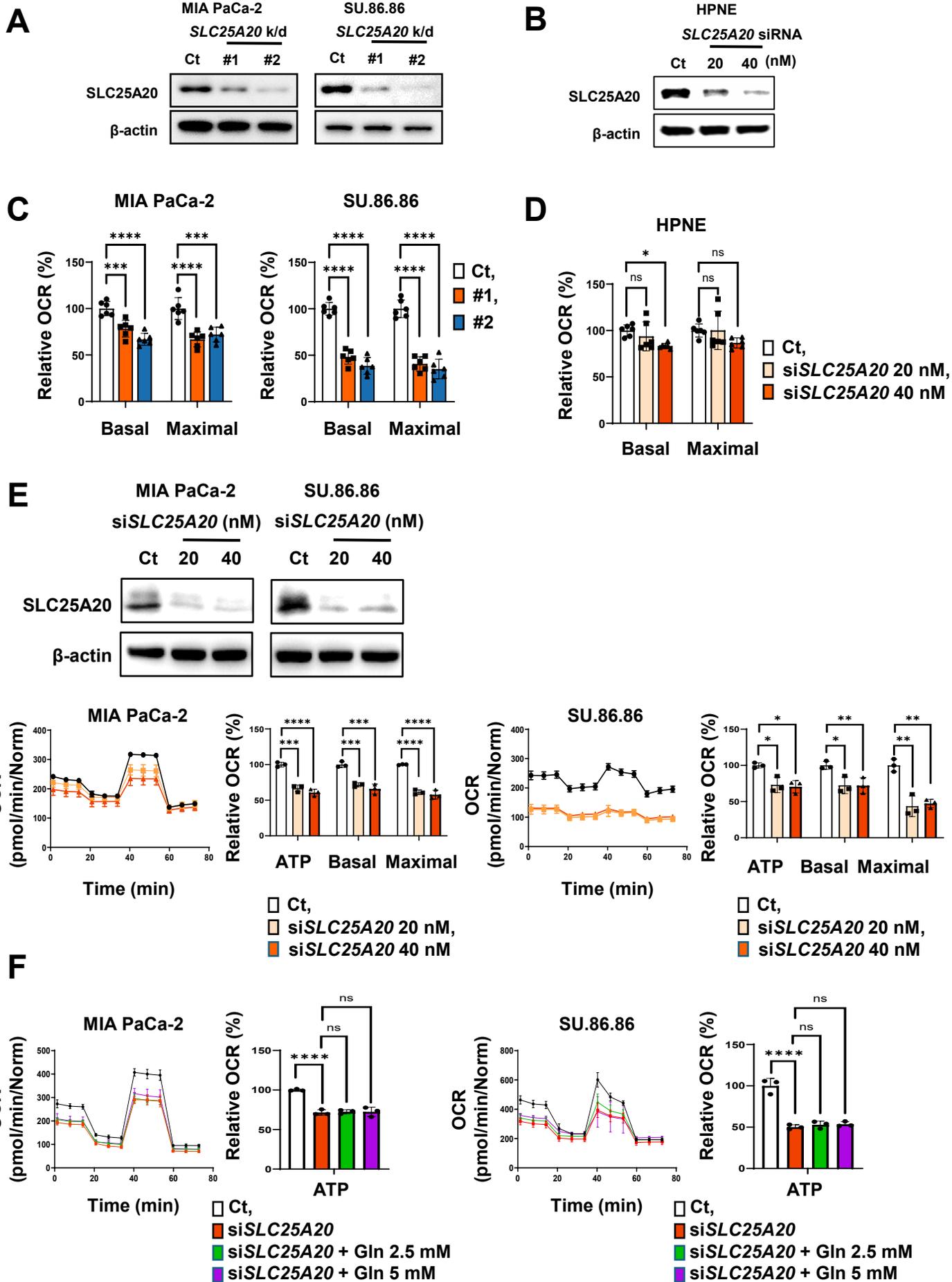


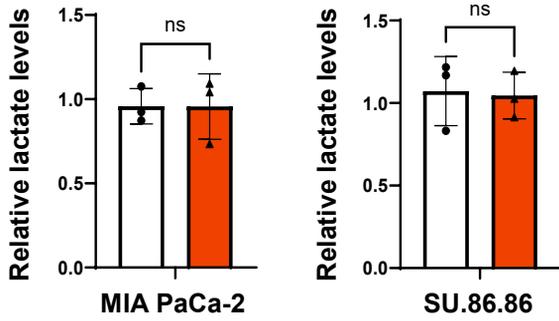
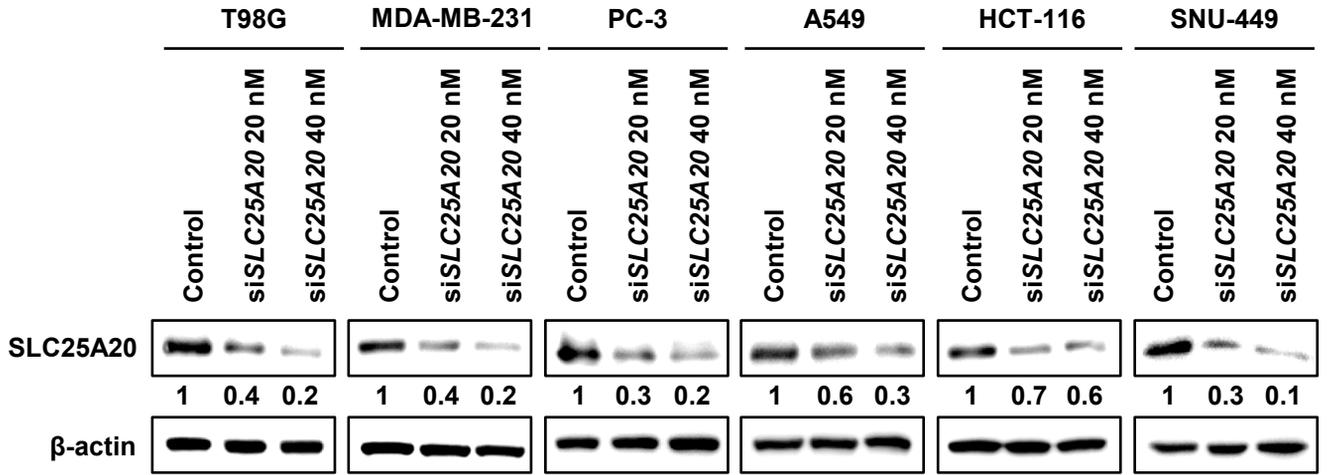
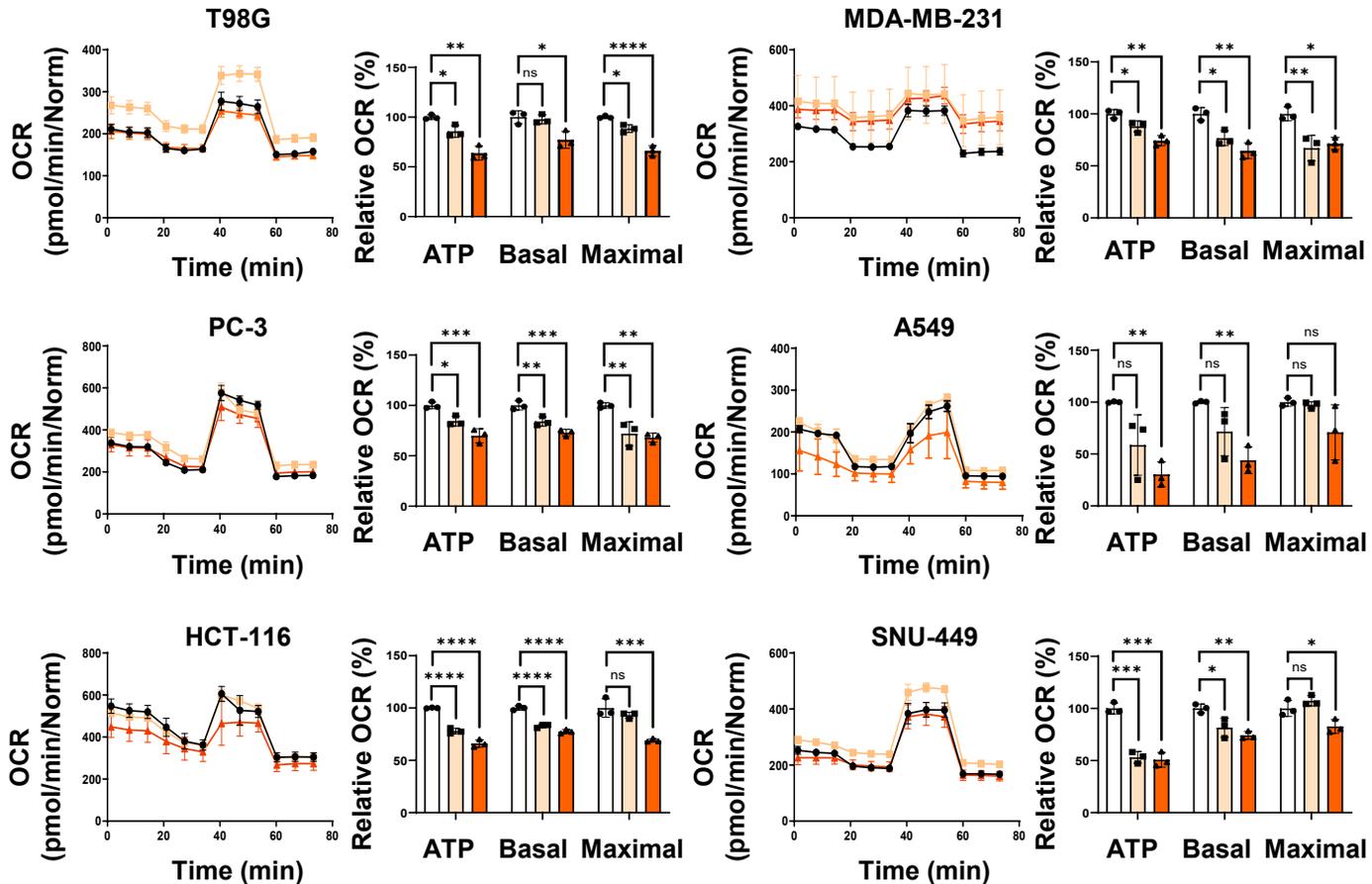
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**Supplementary Figure S3.** Immunohistochemistry and H&E staining of the pancreas from KPC mice. (A) Fed by High-Fat Diet, (B) Fed by Regular Diet.

# Supplementary Figure S4

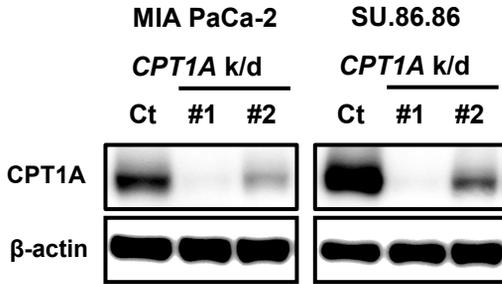


**G****H****I**

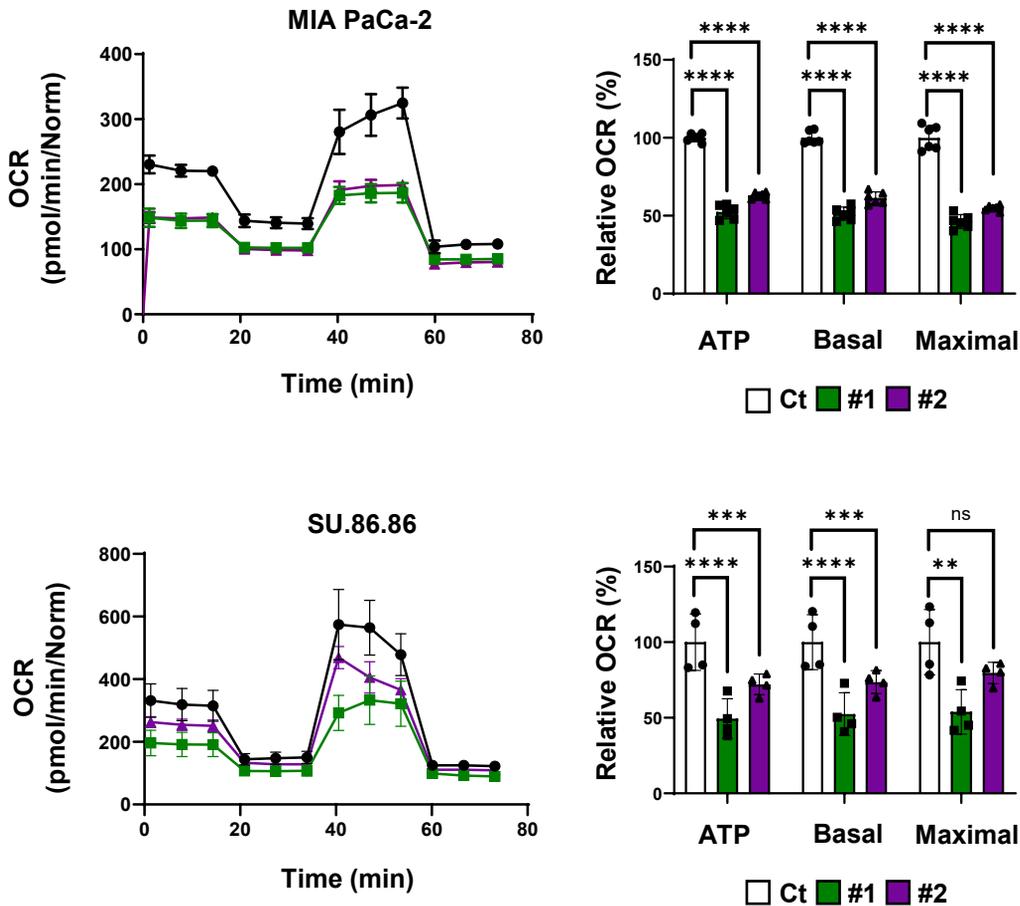
**Supplementary Figure S4.** Effect of *SLC25A20* knockdown on OCR between normal and pancreatic cancer cells. (A) Immunoblot analysis reveals a reduction in *SLC25A20* levels in MIA PaCa-2 and SU.86.86 cells in which *SLC25A20* was knocked down using two different shRNAs (#1 and #2). (B) Immunoblot analysis reveals a reduction in *SLC25A20* levels in HPNE in a dose dependent manner of shRNAs (#1). (C) OCRs were analyzed by Seahorse XFe96 analysis. All data were normalized by SRB quantification. MIA PaCa-2 and SU.86.86 with knockdown of *SLC25A20* by two different shRNAs of #1 and #2. (D) OCR of HPNE with *SLC25A20* knockdown were analyzed by Seahorse XFe96 analysis. (E) Immunoblotting analysis showed the decrease of *SLC25A20* in MIA PaCa-2, SU.86.86 with *SLC25A20* knockdown by two doses of siRNA for 48 h. OCR analysis were performed by siRNA of *SLC25A20* treatment. (F) OCRs were analyzed by Seahorse XFe 96 analyzer in control and 40 nM siRNA-mediated *SLC25A20* knockdown PDAC cells following treatment with 2.5 or 5 mM glutamine for 24 h. Glutamine supplementation did not rescue ATP depletion induced by *SLC25A20* knockdown. (G) Lactate levels were measured using a lactate assay kit in cultured cells under *SLC25A20* knockdown for 48 h. (H) Immunoblotting analysis showed the decrease of *SLC25A20* in T98G, MDA-MB-231, PC-3, A549, HCT-116 and SNU-449 with *SLC25A20* knockdown by two dose of siRNA. (I) ATP levels, basal respiration, and maximal respiration were analyzed by Seahorse XFe96 analysis. All data were normalized by SRB quantification. Data are presented as the mean  $\pm$  SD from at least three experiments. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

# Supplementary Figure S5

**A**

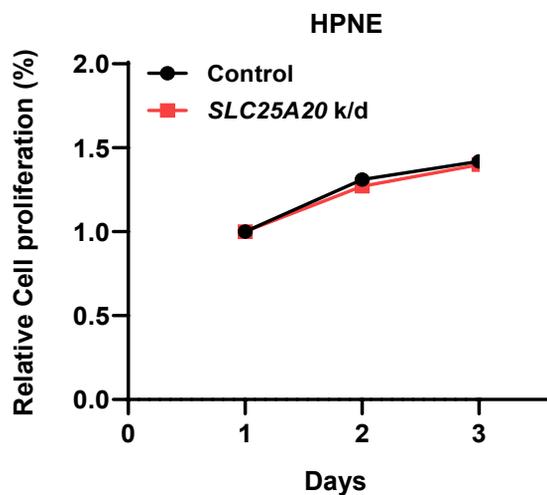


**B**



**Supplementary Figure S5.** Effect of *CPT1A* knockdown on ATP production in the PDAC cells. (A) Immunoblotting analysis showed the decrease of *CPT1A* in MIA PaCa-2 and SU.86.86 with *CPT1A* knockdown by two different shRNAs of #1 and #2. (B) ATP levels, basal respiration, and maximal respiration were analyzed by Seahorse XFe96 analysis in MIA PaCa-2 and SU.86.86. All data were normalized by SRB quantification.

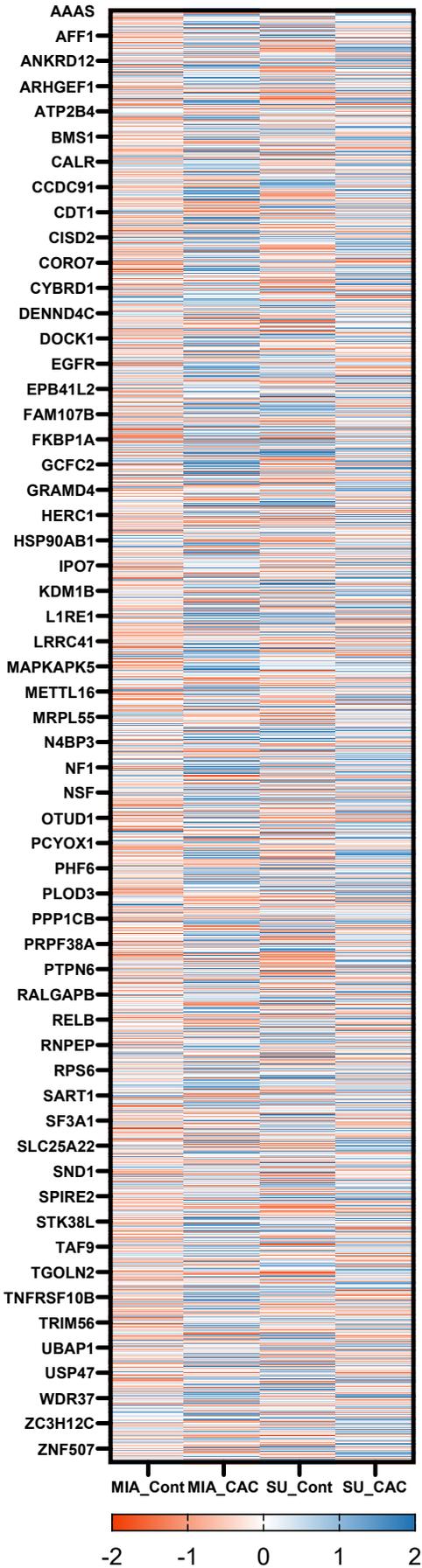
## Supplementary Figure S6

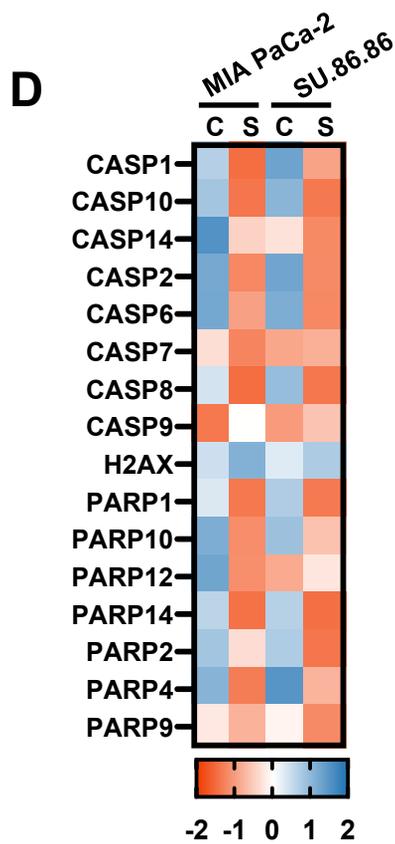
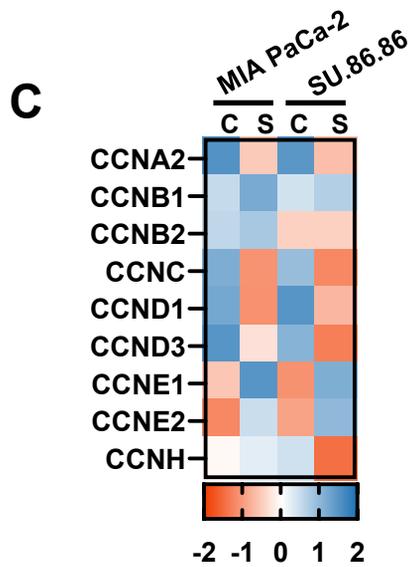
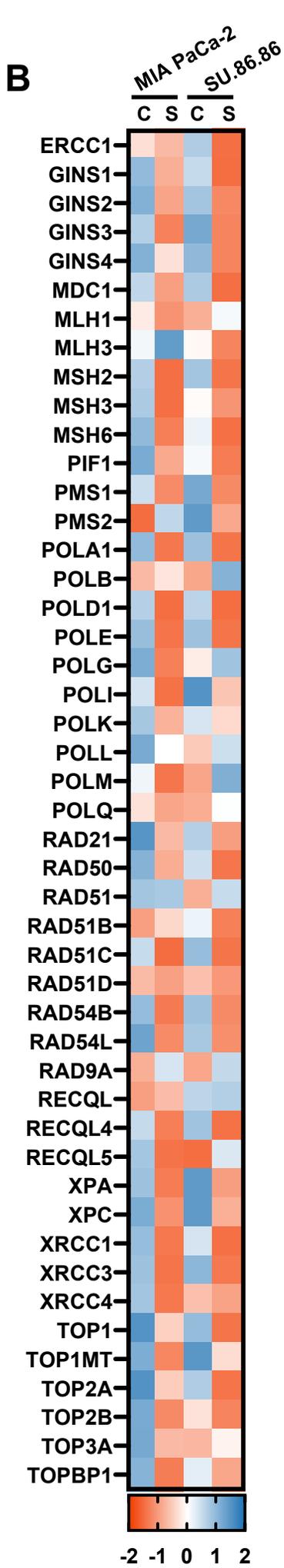


**Supplementary Figure S6.** Knockdown of *SLC25A20* in pancreatic normal ductal epithelial cells (HPNE) did not affect cell proliferation. HPNE cells were transfected with Scramble siRNA or *SLC25A20* siRNA for 3 days. SRB assay was performed, and the absorbance was measured at 515 nm to assess relative cell proliferation.

# Supplementary Figure S7

A

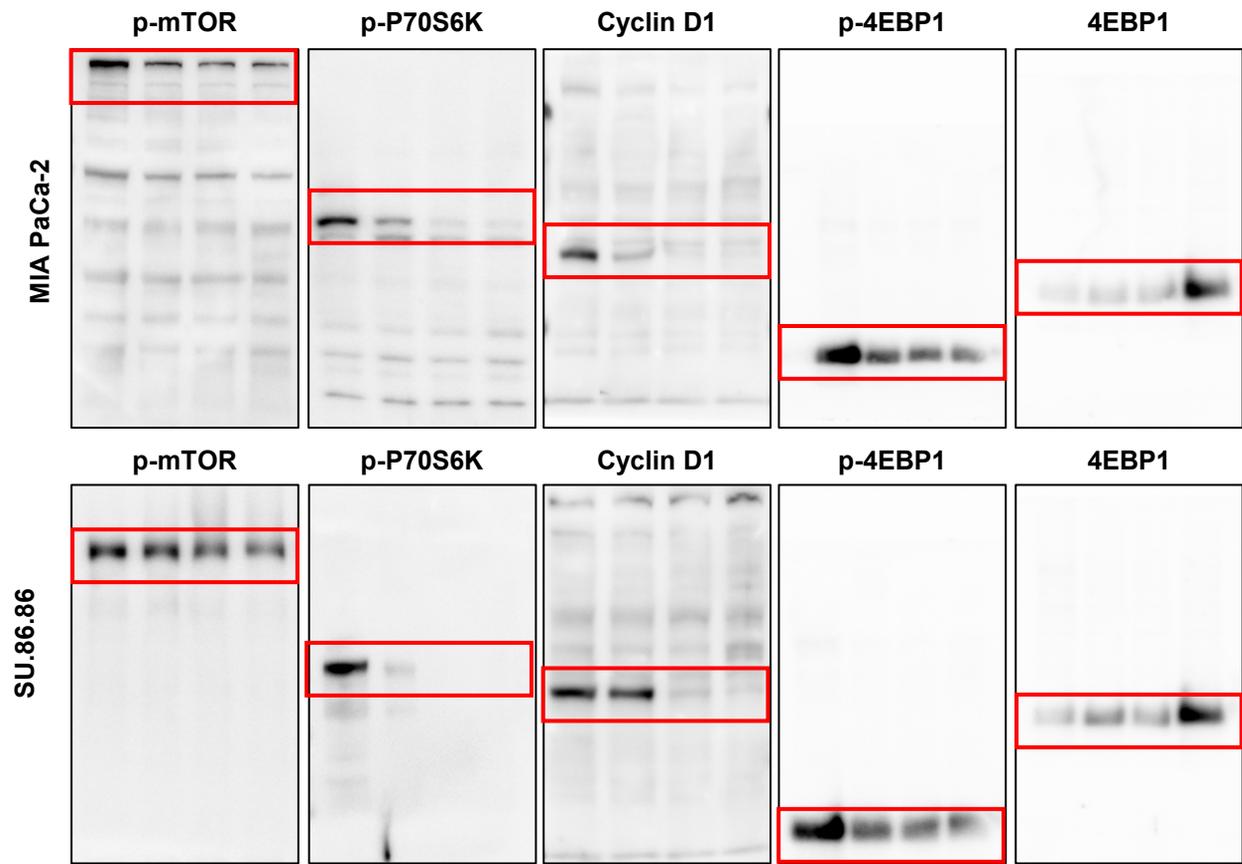




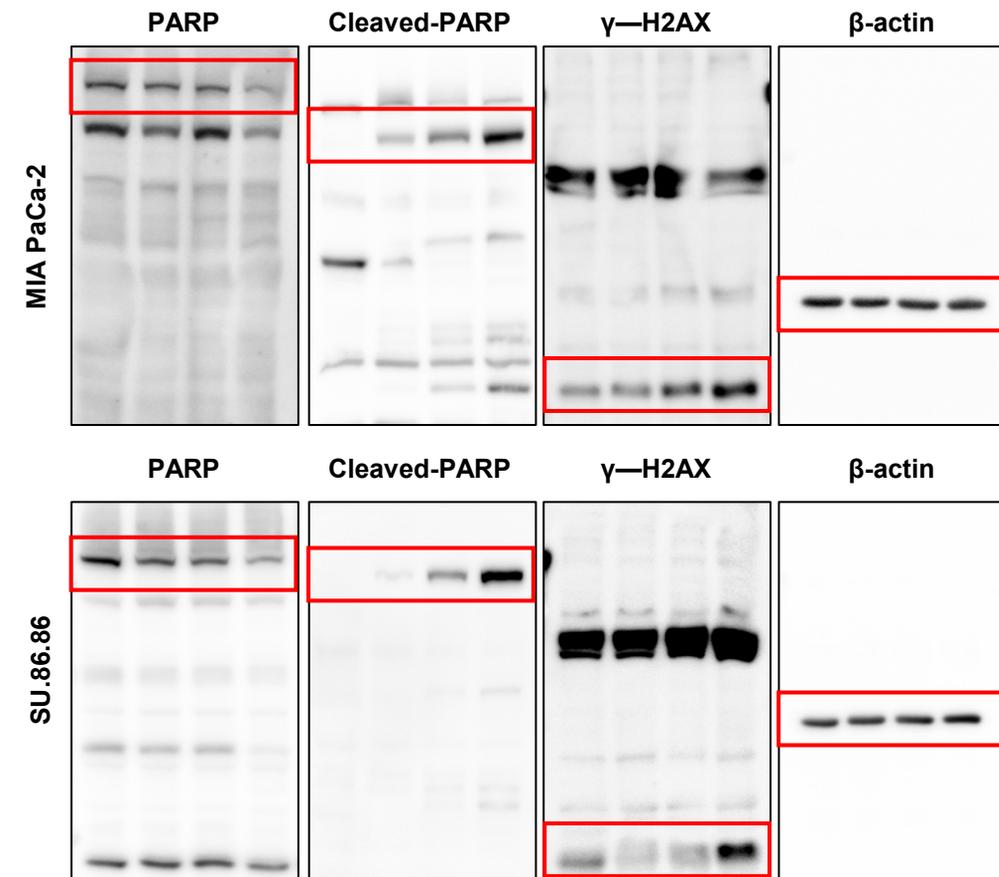
**Supplementary Figure S7.** Protein Phosphorylation Changes Induced by *SLC25A20* Inhibition. (A) MIA PaCa-2 and SU.86.86 cells were treated with siRNA (40 nM) for the *SLC25A20* gene for 48 h, and total protein phosphorylation was analyzed using LC-MS/MS. (B-C) After knocking down the *SLC25A20* gene in pancreatic cancer cell lines for 72 hours, proteomics analysis was performed. (B) DNA damage repair-related proteins (C) cell cycle checkpoint proteins (D) apoptotic cell death related proteins. All graph represent the z-score.

# Supplementary Figure S8

## A. Figure 5D

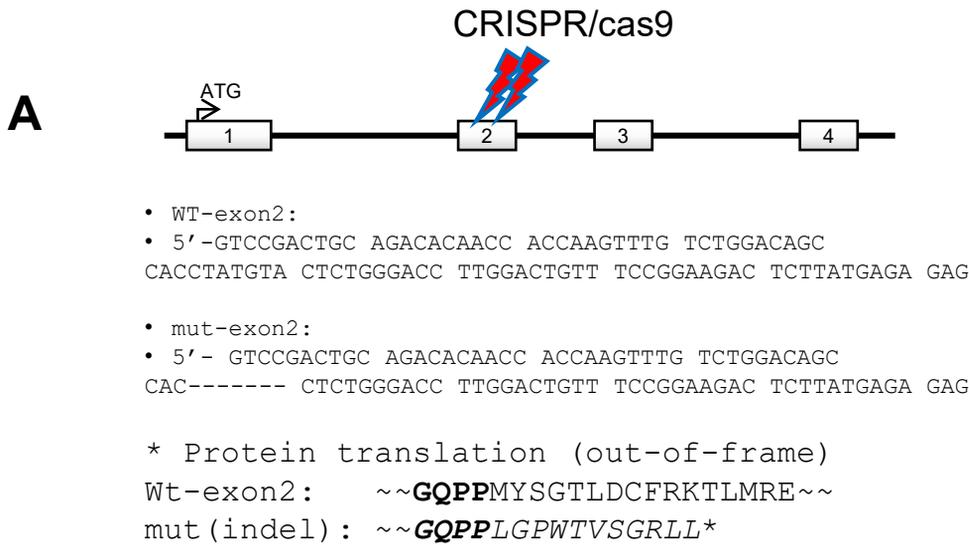


## B. Figure 5E



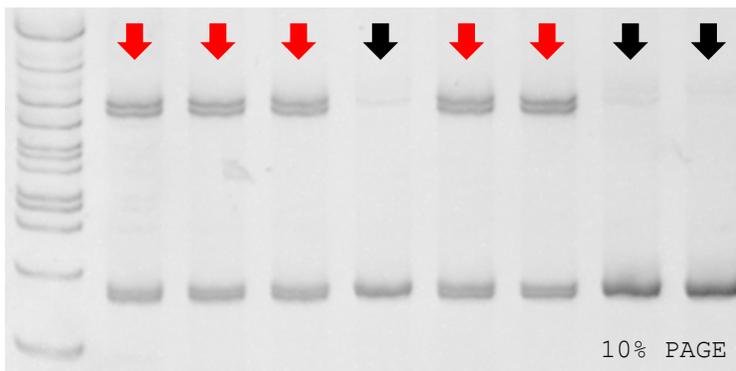
**Supplementary Figure S8.** *SLC25A20* knockdown reduces cell proliferation and increases cell death. Time-dependent treatment with *SLC25A20* siRNA was performed in MIA PaCa-2 and SU.86.86 cells. (A) The key markers of cell proliferation, mTOR, P70S6K, and 4EBP1, were analyzed by immunoblotting. (B) In the same samples, cell death markers such as PARP, cleaved PARP, and  $\gamma$ -H2AX were examined by immunoblotting.

# Supplementary Figure S9



**B**

- Genotype PCR Primer
- Slc25a20\_For: TG GAT CGA AGG ATG AGG AGC AG
- Slc25a20\_Rev: AT CTC ACA GCT ACC TTC TTC AC

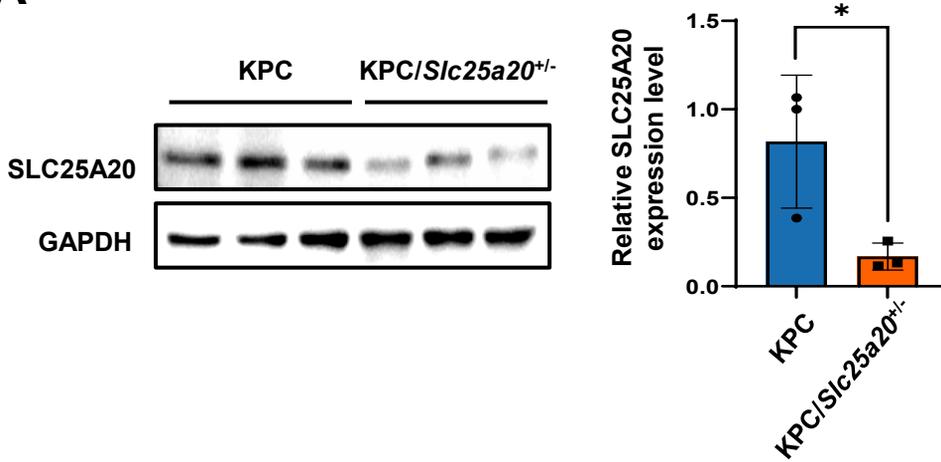


■ *Slc25a20*<sup>+/-</sup>  
■ WT

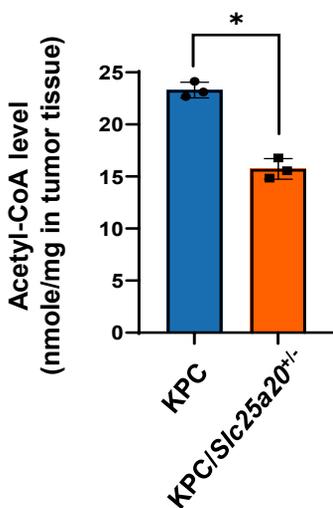
**Supplementary Figure S9.** Generation of *Slc25a20*-null mouse carnitine acylcarnitine carrier (7nt deletion). (A) Schematic of *Slc25a20* knockout mouse generation. The *SLC25A20* gene was targeted for knockout using CRISPR/Cas9. (B) Genotyping PCR primers and 10% polyacrylamide gel electrophoresis of genotyping PCR products. Representative PAGE gel showed that the red arrow indicates the band corresponding to the *Slc25a20* knockout allele, and the black arrow indicates the wild-type allele.

# Supplementary Figure S10

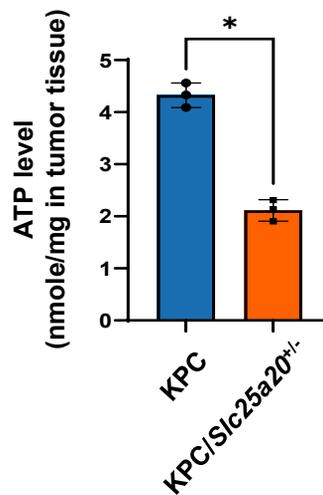
**A**



**B**



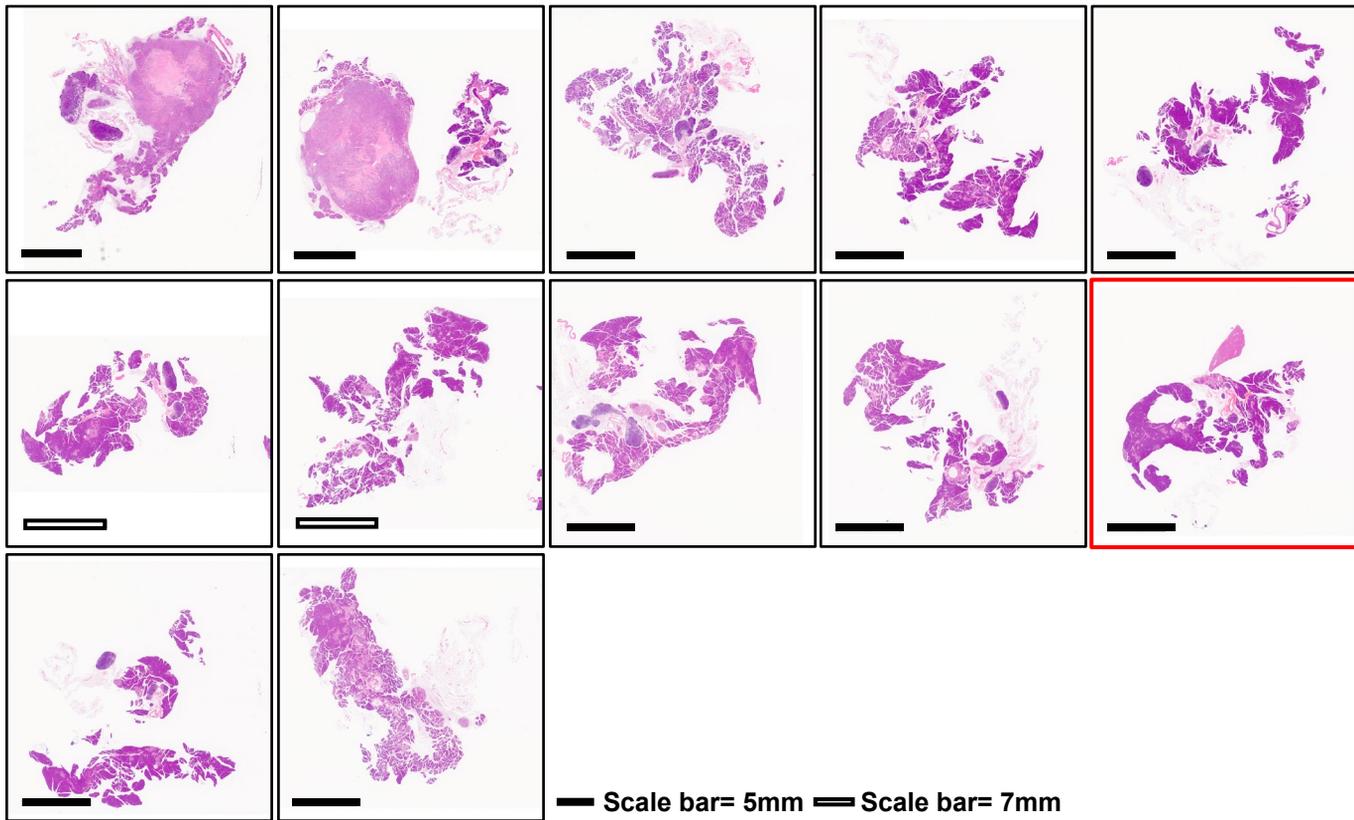
**C**



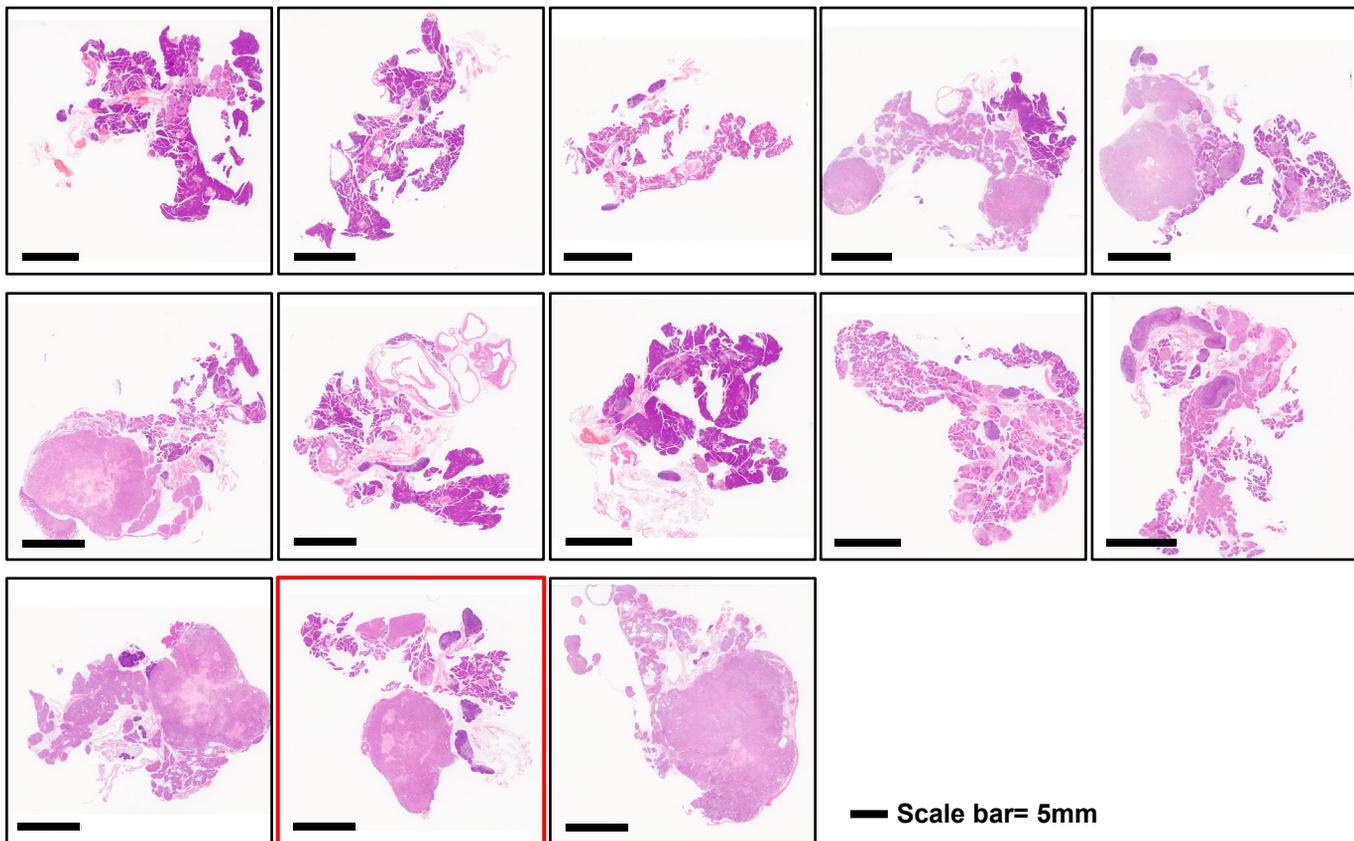
**Supplementary Figure S10.** *SLC25A20* knockdown in KPC mice reduces FAO metabolism. The final metabolite of FAO is acetyl-CoA, which is synthesized into ATP via TCA-OxPhos. Therefore, the decrease in acetyl-CoA and ATP in KPC/*Slc25a20*<sup>+/-</sup> mice indicates a decrease in FAO. (A) Immunoblot analysis revealed a reduction in SLC25A20 levels in pancreatic tumor tissues of KPC (n=3, age (weeks): 19, 12, 10) and KPC/*Slc25a20*<sup>+/-</sup> (n=3, age (weeks): 18, 16, 15) mouse. (B) Acetyl-CoA levels and (C) ATP levels in pancreatic tumor tissues. Data are presented as the mean  $\pm$  SD \*p < 0.05.

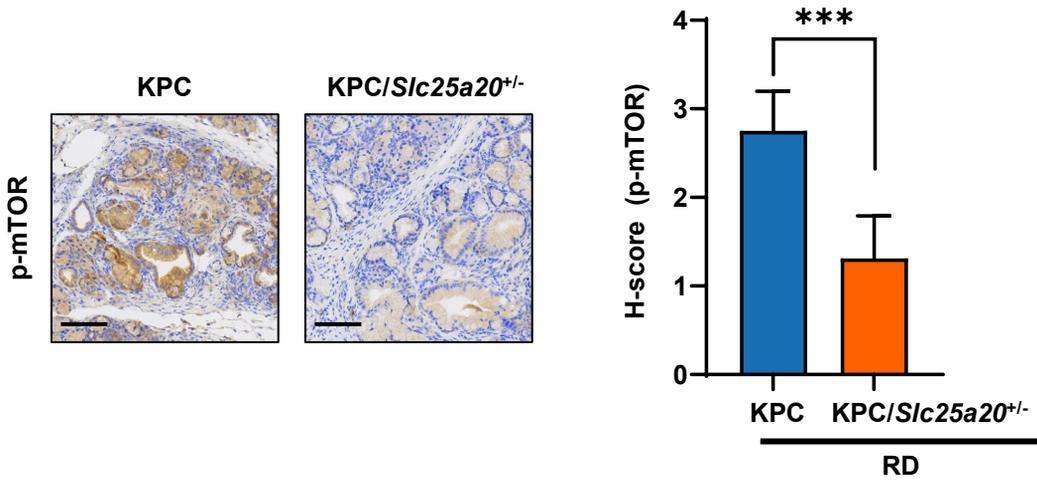
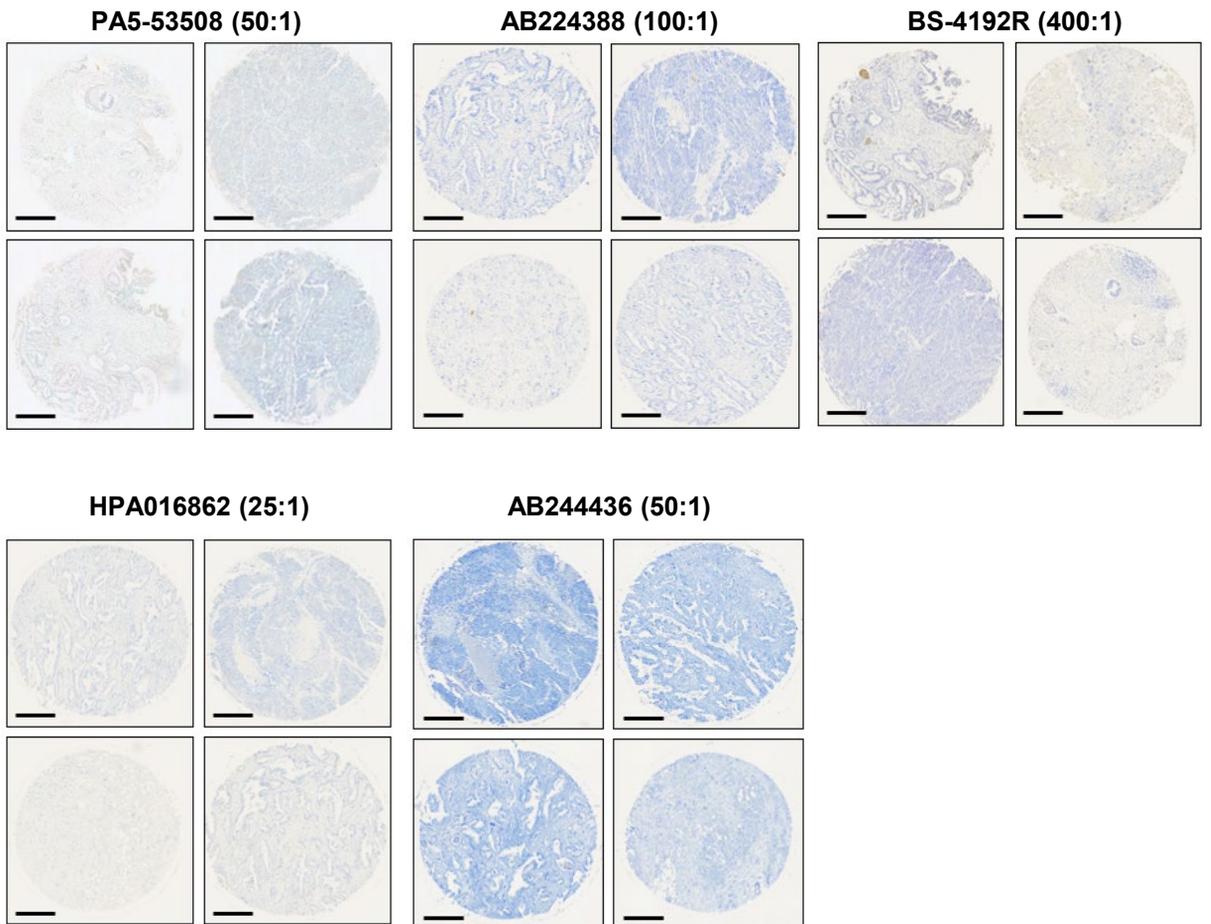
# Supplementary Figure S11

## A *KPC/Slc25a20*<sup>+/-</sup> mice



## B KPC mice



**C****D**

**Supplementary Figure S11.** H&E staining images of KPC and KPC/*Slc25a20*<sup>+/-</sup> mice shown in Figure 7. (A) Pancreas tissue from KPC/*Slc25a20*<sup>+/-</sup> mice, (B) Pancreas tissue from KPC mice. (C) Immunohistochemical (IHC) analysis of p-mTOR expression in 20-week-old KPC/*Slc25a20*<sup>+/-</sup> and KPC pancreatic cancer tissues. (Scale bar: 100  $\mu$ m) The median H-score for p-mTOR in KPC and KPC/*Slc25a20*<sup>+/-</sup> was 2.75 and 1.31, respectively. (D) Immunohistochemical (IHC) analysis of SLC25A20 expression in TMA (T142C) tissue using various anti-SLC25A20 antibodies. IHC staining was performed using the following anti-SLC25A20 antibodies: PA5-53508 (1:50 dilution, Invitrogen), AB224388 (1:100 dilution, Abcam), BS-4192R (1:400 dilution, Bioss Antibodies), HPA016862 (1:25 dilution, Atlas Antibodies), and AB244436 (1:50 dilution, Abcam). No specific staining for SLC25A20 was observed in the TMA (T142C) tissue sections with any of the antibodies tested. (Scale bar: 400  $\mu$ m)