SUPPLEMENTARY INFORMATION

A cell-permeable peptide-based PROTAC against the oncoprotein CREPT proficiently inhibits pancreatic cancer

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1. Abbreviations

AHX, 6-aminohexanoic acid

CCK-8, cell counting kit

CCT, coiled-coil terminus

CD, circular dichroism

CL, CREPT ligand

CREPT, cell-cycle-related and expression-elevated protein in tumor

DA, degrading arm

DAPI, 4′, 6-diamidino-2-phenylindole

DC_{50}, half maximal degradation concentration

DMEM, Dulbecco's modified Eagle's medium

EDTA, ethylenediaminetetraacetic acid

FBS, fetal bovine serum

FITC, fluorescein isothiocyanate

GST, glutathione S-transferase

HPLC, high performance liquid chromatography

IHC, immunohistochemistry

IP, immunoprecipitation

Kd, dissociation constant

MS, mass spectrum

MST, microscale thermophoresis

OD, optical density

PAAD, pancreatic adenocarcinoma

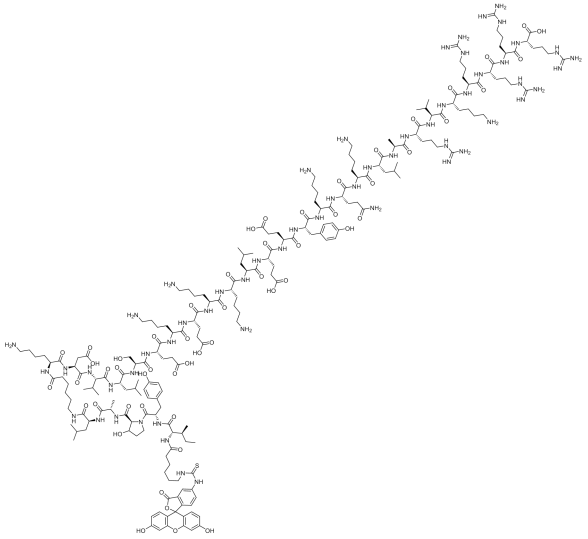
PBS, phosphate buffer saline

PFA, paraformaldehyde

PROTAC, proteolysis targeting chimeras
PRTC, a PROTAC against CREPT
qPCR, quantitative PCR
RNAPII, RNA polymerase II
RNAi, RNA interference
RPR, regulation of nuclear pre-mRNA
RT-PCR, reverse transcriptase PCR
TA, targeting arm
TCGA database, the cancer genome atlas database
TRITC, tetramethylrhodamine
TSA, thermal shift assay
VHL, von hippel-lindau

2. Evaluation of synthesized peptides

1) FITC-PRTC: 5-FITC-(Ahx)-IY-(Hyp)-AL-(Ahx)-KDVLSEKEKKLEEYKQKLARVKRRRR



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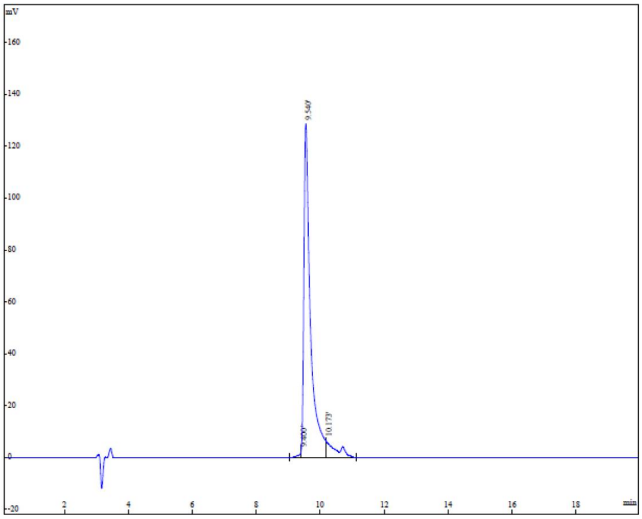
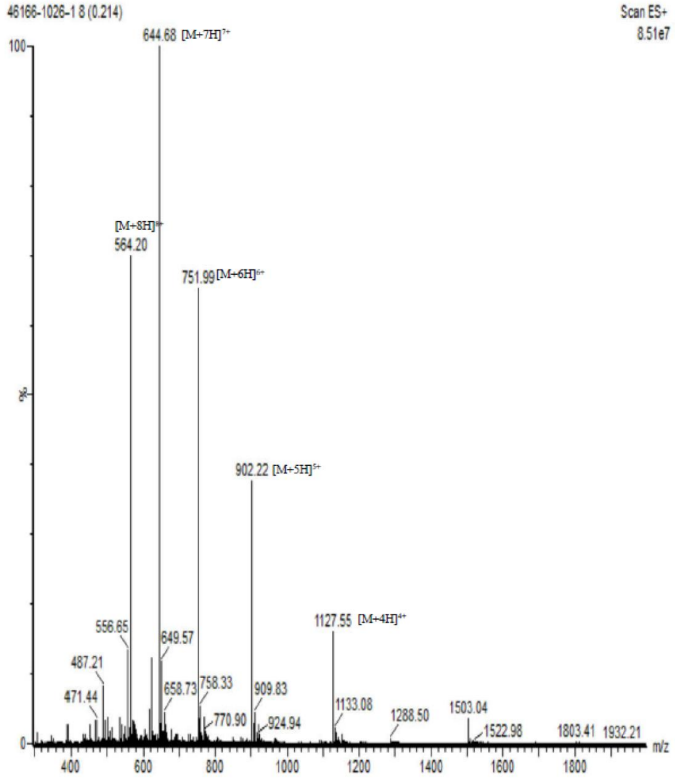


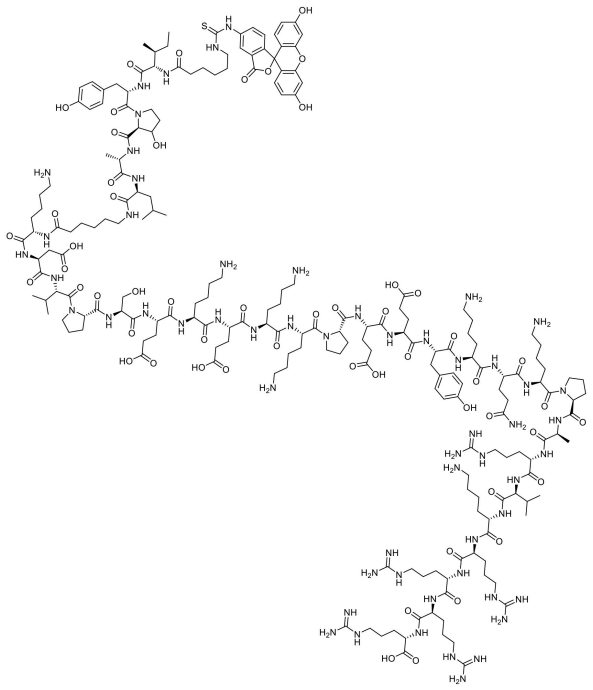
Peak No.	Ret Time	Area	Conc.
1	9.400	15367	0.7737
2	9.540	1819411	91.6

3	10.173	151321	7.619
Total:		**1986099**	**100.0000**

MS report:



2) FITC-PRTC-m: 5-FITC-(Ahx)-IY-(Hyp)-AL-(Ahx)-KDVPSEKEKKPEEYKQKPARVKRRRR



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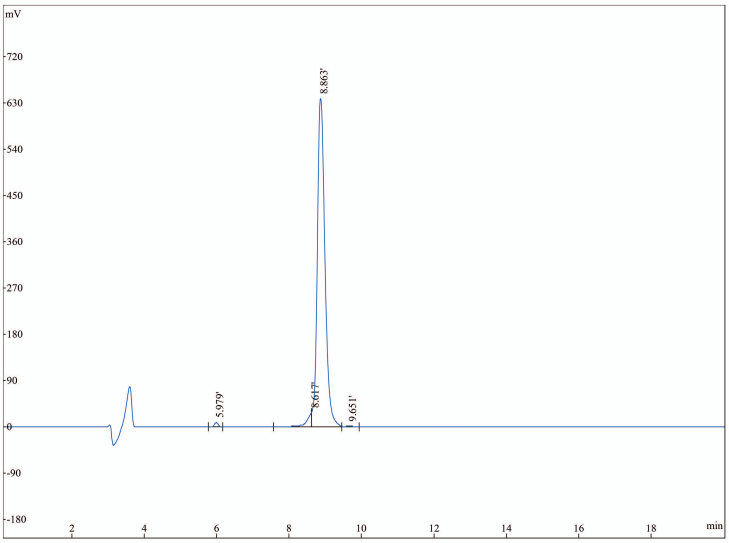
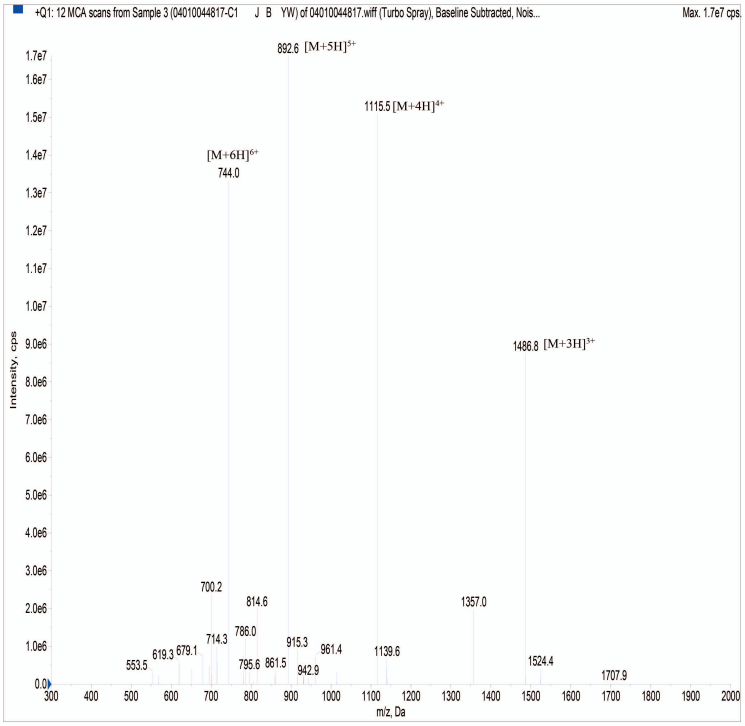
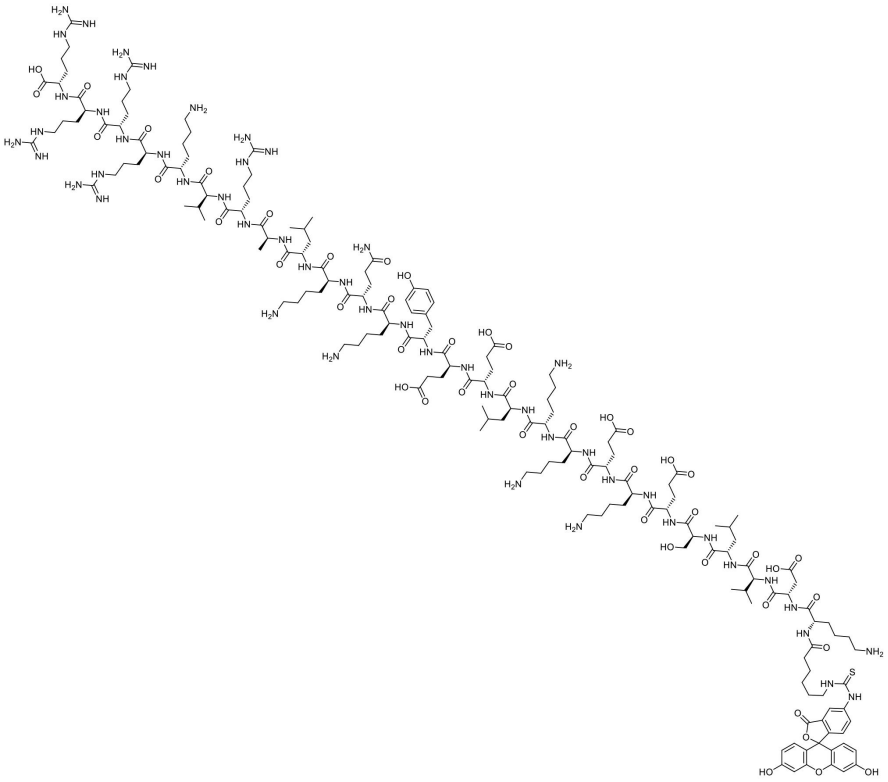


Peak No.	Ret Time	Area	Conc.
1	5.979	66676	0.7223
2	8.617	286971	3.109
3	8.863	8838102	95.74
4	9.651	39716	0.4302
Total:		**9231465**	**100.0000**

MS report:



3) FITC-PRTC-v: FITC-(Ahx)-KDVLSEKEKKLEEYKQKLARVKRRRR



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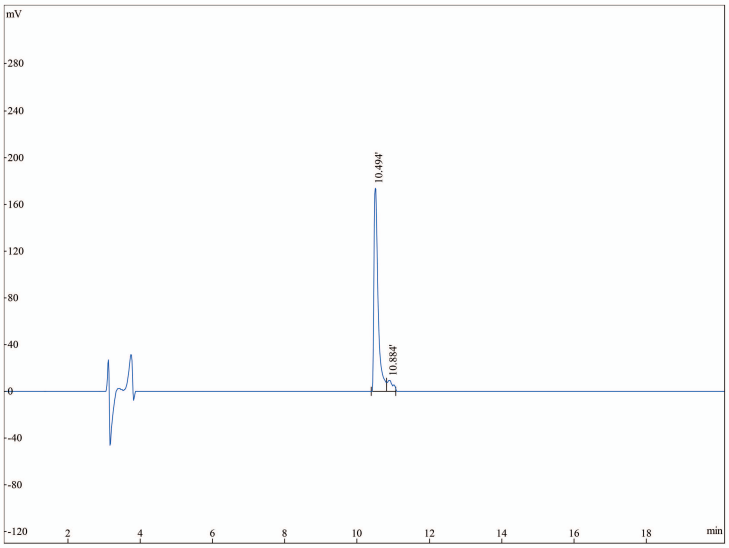
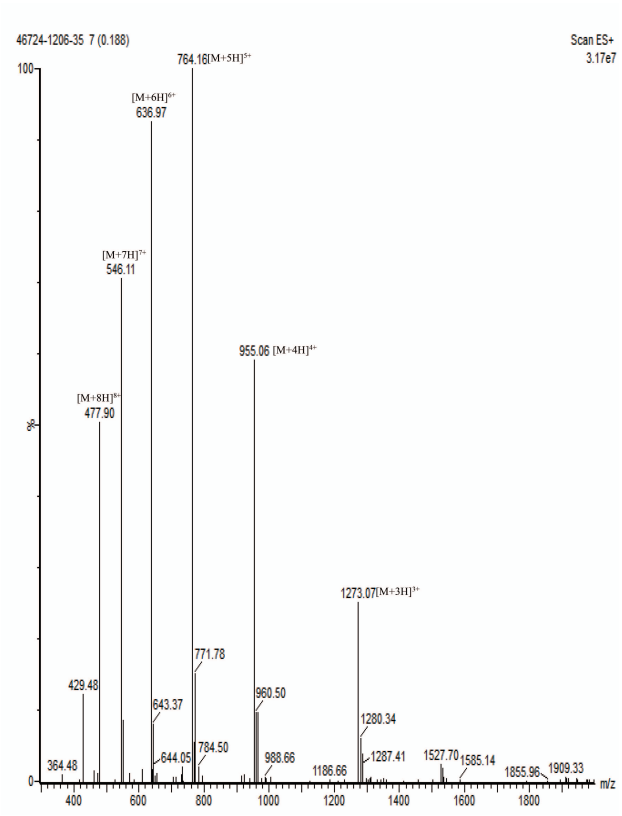
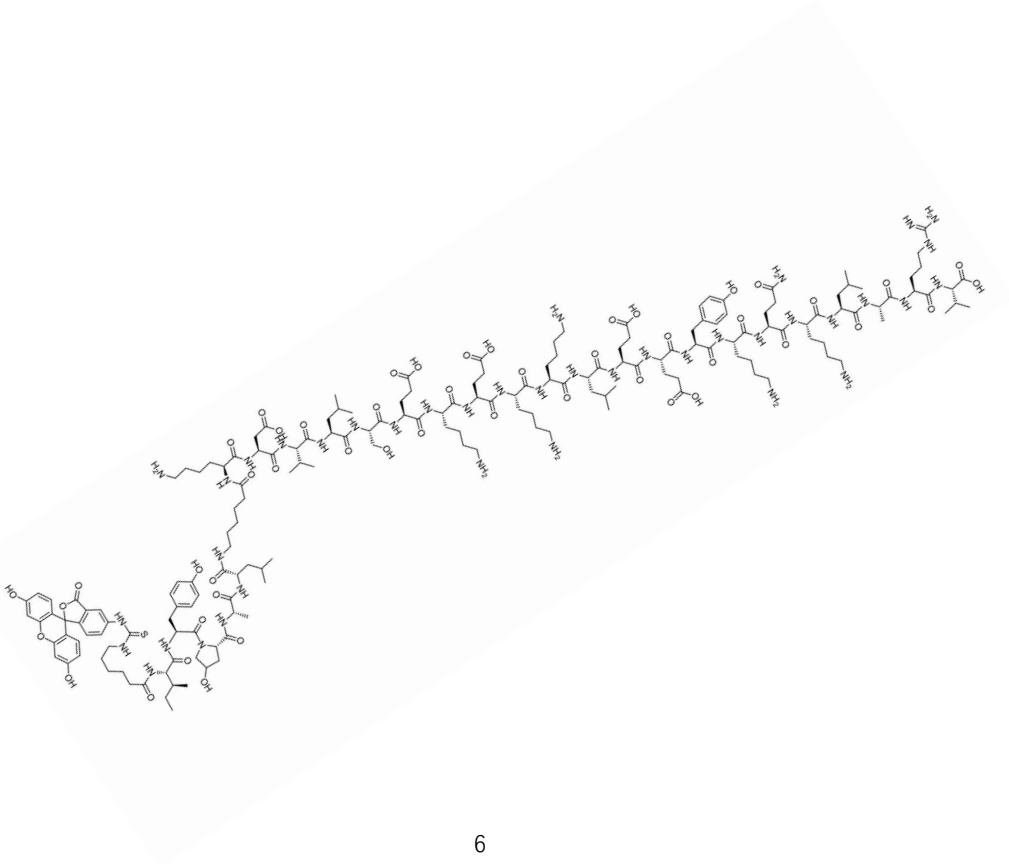


Peak No.	Ret Time	Area	Conc.
1	10.494	1263172	92.73
2	10.884	99063	7.272
Total:		**1362235**	**100.0000**

MS report:



4) FITC-PRTC-r : FITC-(Ahx)-IY-(Hyp)-AL-(AHX)-KDVLSEKEKKLEEYKQKLARV



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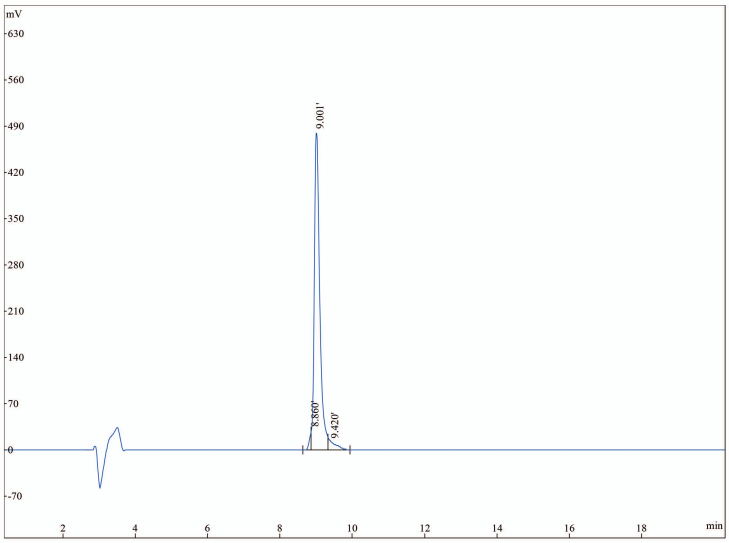

Peak No.	Ret Time	Area	Conc.
1	8.860	101984	2.052
2	9.001	4647112	93.49
3	9.420	221760	4.461
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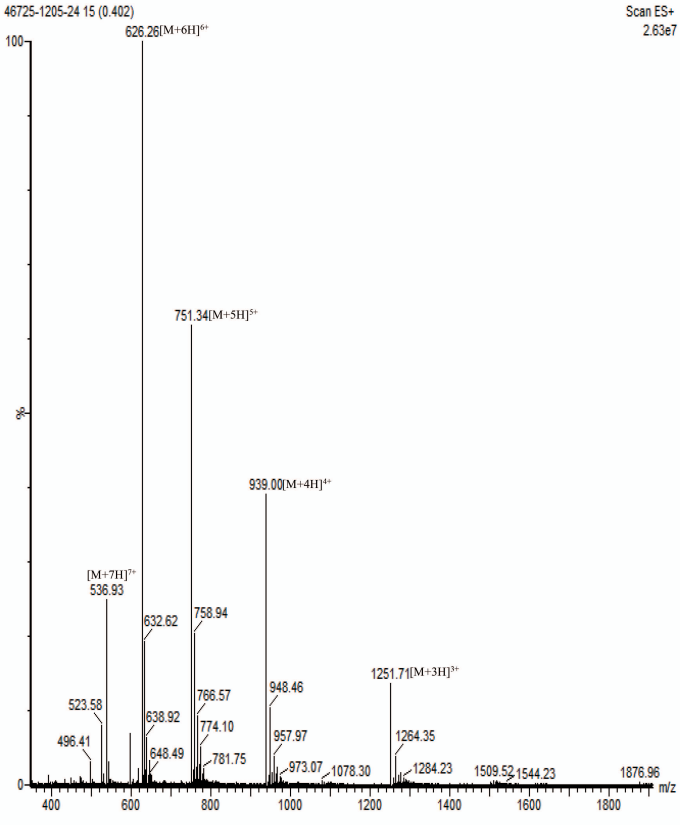
MS report:



3. Supplemental table

Table S1. Analytical data of synthesized peptides

Entry	Chemical formula	Purity	MS (calculated)	MS (found)
FITC-PRTC	$C_{206}H_{333}N_{57}O_{54}S$	91.60%	4504.26	4505.76
FITC-PRTC-m	$C_{203}H_{321}N_{57}O_{54}S$	95.74%	4456.14	4458.00
FITC-PRTC-v	$C_{171}H_{279}N_{51}O_{46}S$	92.73%	3817.51	3815.80
FITC-PRTC-r	$C_{176}H_{273}N_{39}O_{49}S$	93.49%	3751.46	3751.56

4. Supplemental figures and figure legends

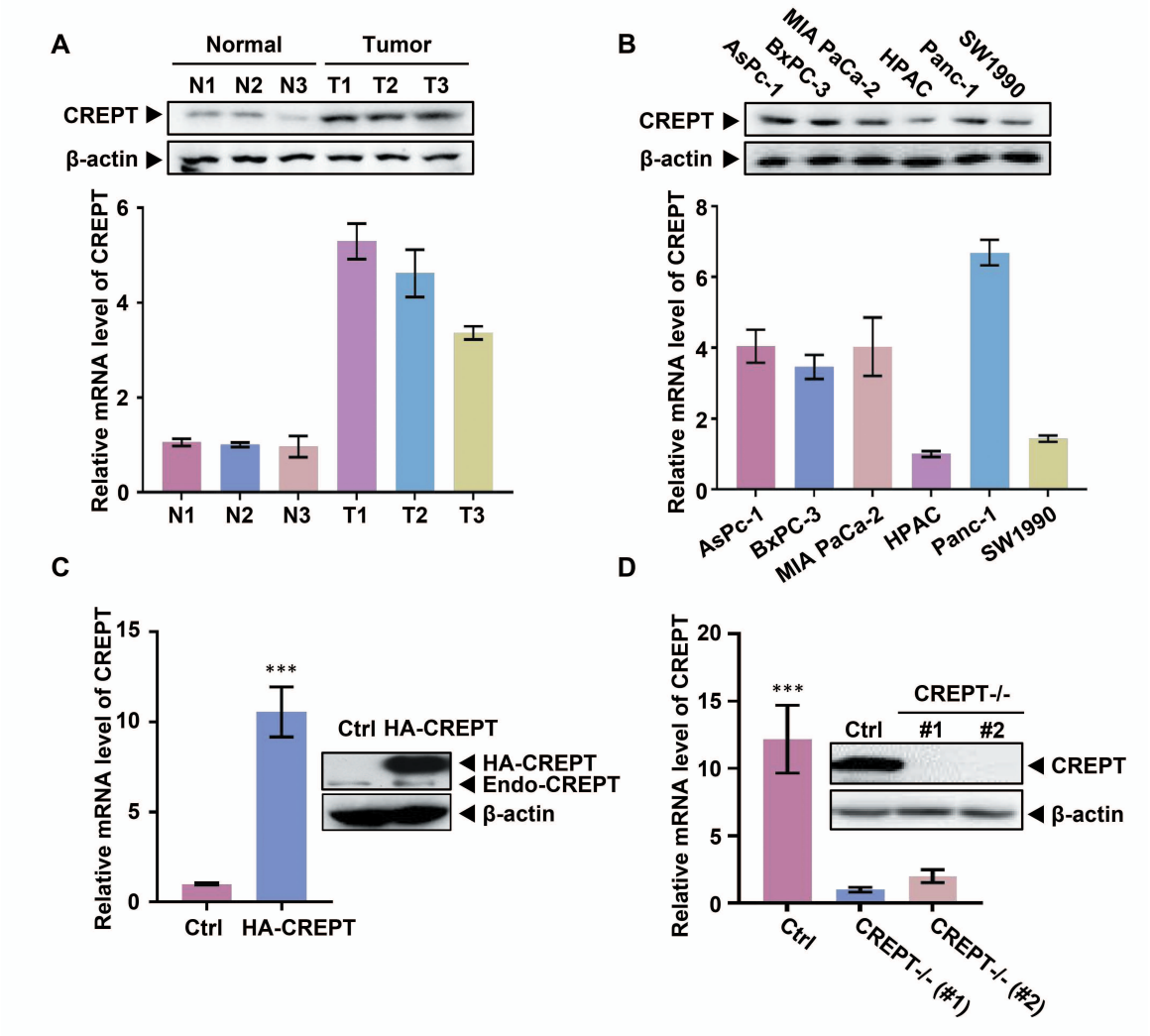


Figure S1. Overexpression of CREPT in pancreatic cancer promotes cell proliferation and tumorigenesis. (A) CREPT protein and mRNA level of mouse normal pancreatic tissues and K-ras/p53-driven pancreatic cancer tissues. N1, N2, N3 are wild type pancreatic tissues. T1, T2, T3 are tumors from KPC mouse model. **(B)** Western blot and quantitative PCR analysis of six pancreatic cell lines. **(C-D)** Identification of CREPT overexpression and CREPT deletion cell lines.

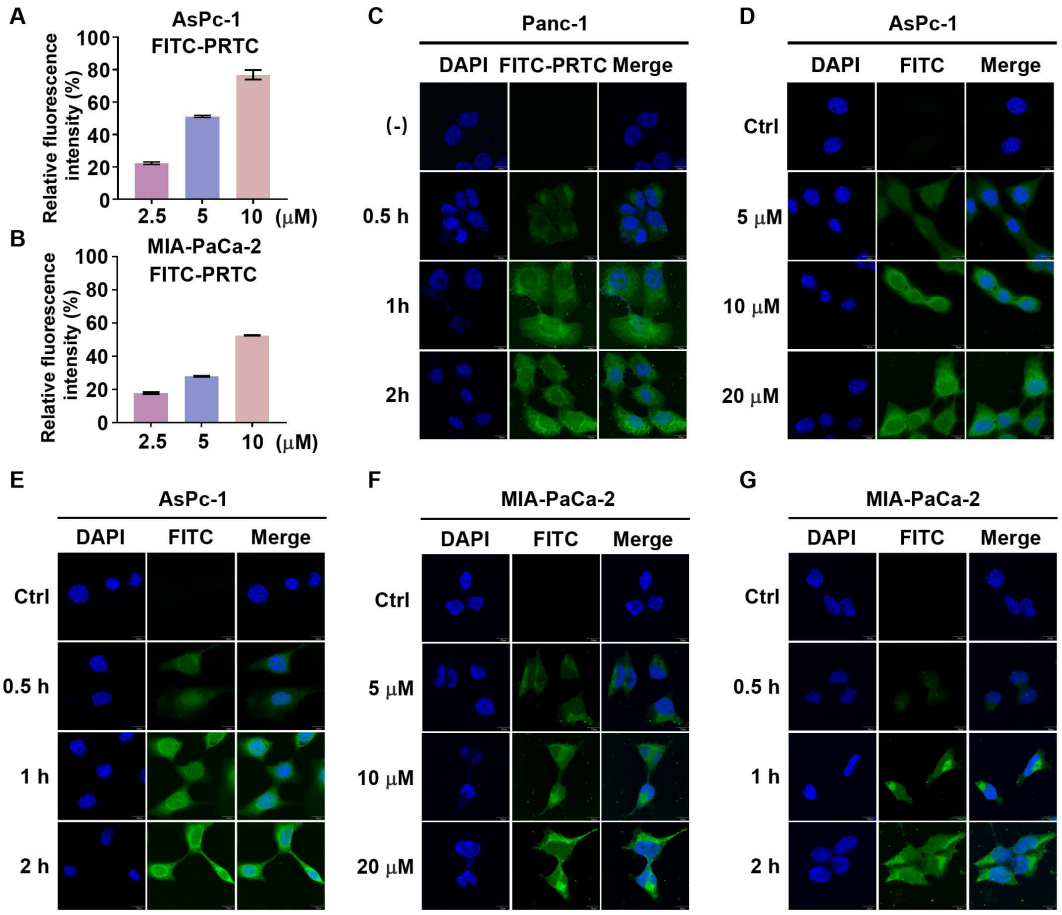


Figure S2. Identification of the permeability of PRTC in pancreatic cancer cells. (A) Quantification of cellular uptake of AsPc-1 cells treated with different concentrations of FITC-PRTC. **(B)** Quantification of cellular uptake of MIA PaCa-2 cells treated with different concentrations of FITC-PRTC. **(C)** Confocal microscope images of Panc-1 cells treated with 10 μM FITC-PRTC at different time points. Scale bars, 10 μm. **(D)** Confocal microscope images of AsPc-1 cells treated with different concentrations of FITC-PRTC. Scale bars, 10 μm. **(E)** Confocal microscope images of AsPc-1 cells treated with 10 μM FITC-PRTC at different time points. Scale bars, 10 μm. **(F)** Confocal microscope images of MIA PaCa-2 cells treated with different concentrations of FITC-PRTC. Scale bars, 10 μm. **(G)** Confocal microscope images of MIA PaCa-2 cells treated with 10 μM FITC-PRTC at different time points. Scale bars, 10 μm.

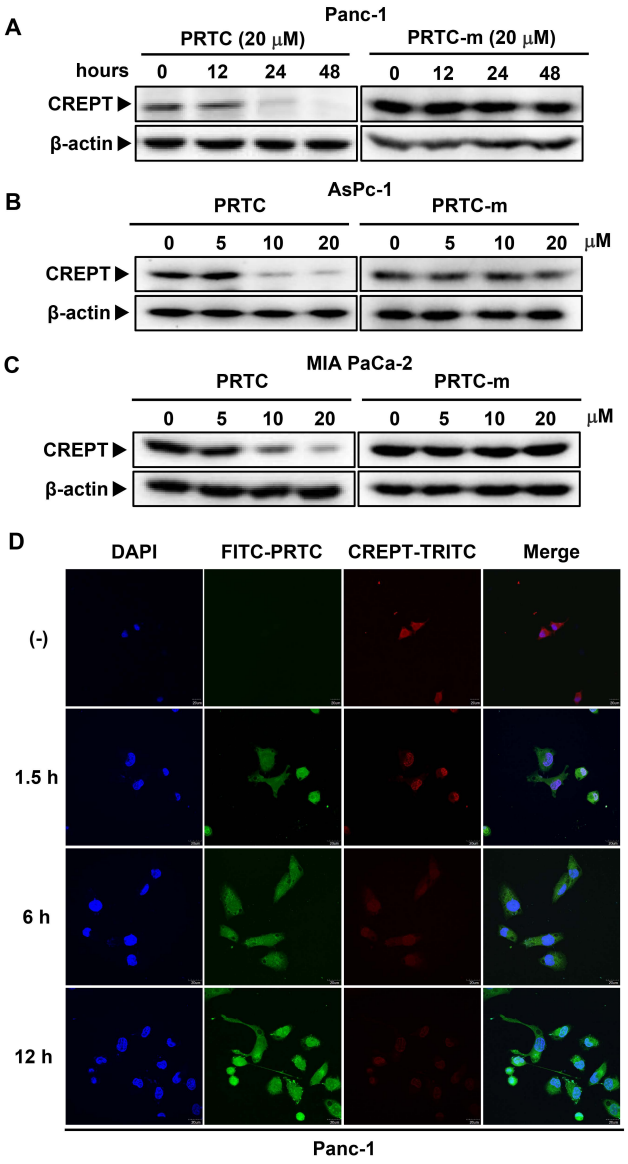


Figure S3. PRTC induces CREPT degradation. (A) Immunoblot of CREPT exposed to 20 μM PRTC or PRTC-m for different times in Panc-1 cells. **(B)** Immunoblot of CREPT following 24 h incubation with different concentrations of PRTC or PRTC-m in AsPc-1 cells. **(C)** Immunoblot of CREPT following 24 h incubation with different concentrations of PRTC or PRTC-m in MIA PaCa-2 cells. **(D)** Immunofluorescence visualization of Panc-1 cells after treatment with PRTC for 1.5 h, 6 h and 12 h. Scale bars, 20 μm.

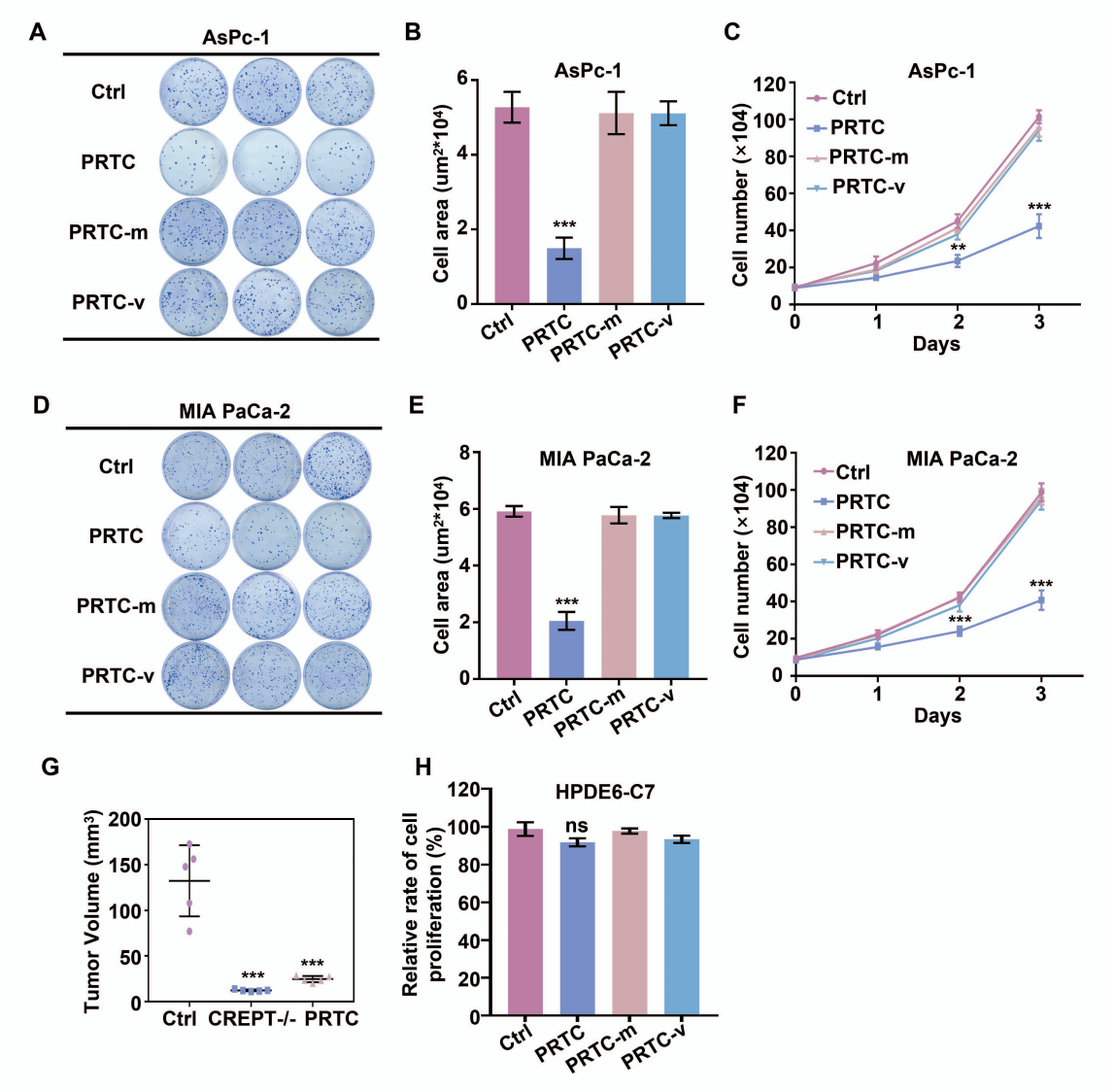


Figure S4. CREPT degradation by PRTC treatment inhibits cell proliferation and tumorigenesis in AsPc-1 and MIA PaCa-2 cells. (A-B) Colony formation of AsPc-1 respectively treated with deionized H_2O (Ctrl), 10 μM PRTC, 10 μM PRTC-m and 10 μM PRTC-v. Cells were seeded into 6-well plates (500 cells per well) and cultured for 8 days.**(C)** Cell proliferation assay of AsPc-1 cells treated with deionized water (Ctrl), 10 μM PRTC, 10 μM PRTC-m and 10 μM PRTC-v. **(D-E)** Colony formation of MIA PaCa-2 respectively treated with deionized H_2O (Ctrl), 10 μM PRTC, PRTC-m and PRTC-v. **(F)** Cell proliferation assay of MIA PaCa-2 cells treated with deionized H_2O (Ctrl), 10 μM PRTC, PRTC-m and PRTC-v. **(G)** Tumor volumes of xenograft tumors, which were measured by calipers. $V = (a \times b^2)/2$ (V is volume, a is the length of the tumor, b is the width of the tumor). **(H)** Cell proliferation assay of HPDE6-C7 cells. PRTC failed to influence the cell proliferation of HPDE6-C7 cells. HPDE6-C7 is a normal pancreatic epithelial cell line.