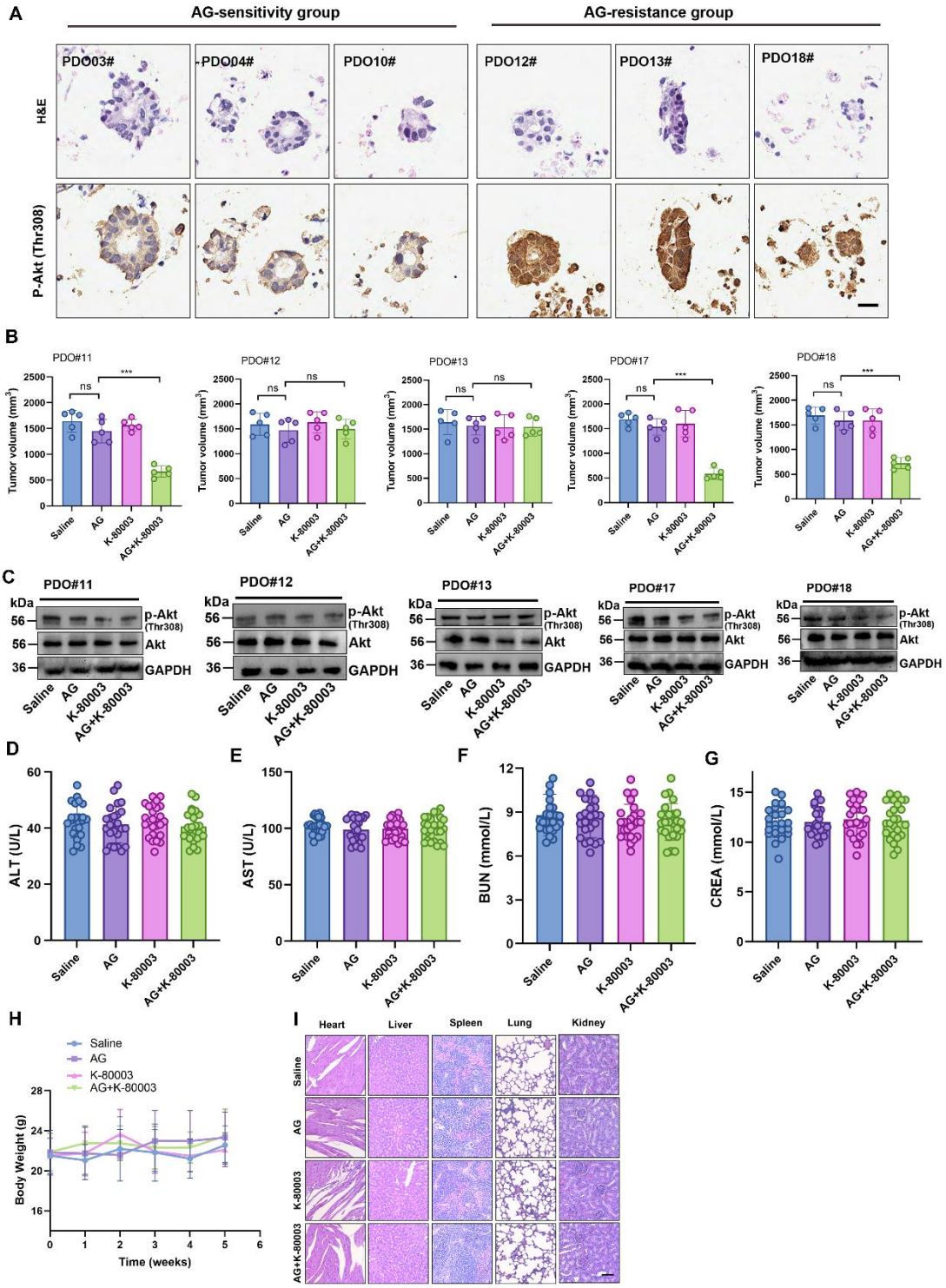
Supplementary Information (SI)

Sulindac (K-80003) with Nab-Paclitaxel and Gemcitabine Overcomes Drug-resistant Pancreatic Cancer.

1. Supplemental Figures

2. Supplemental Tables

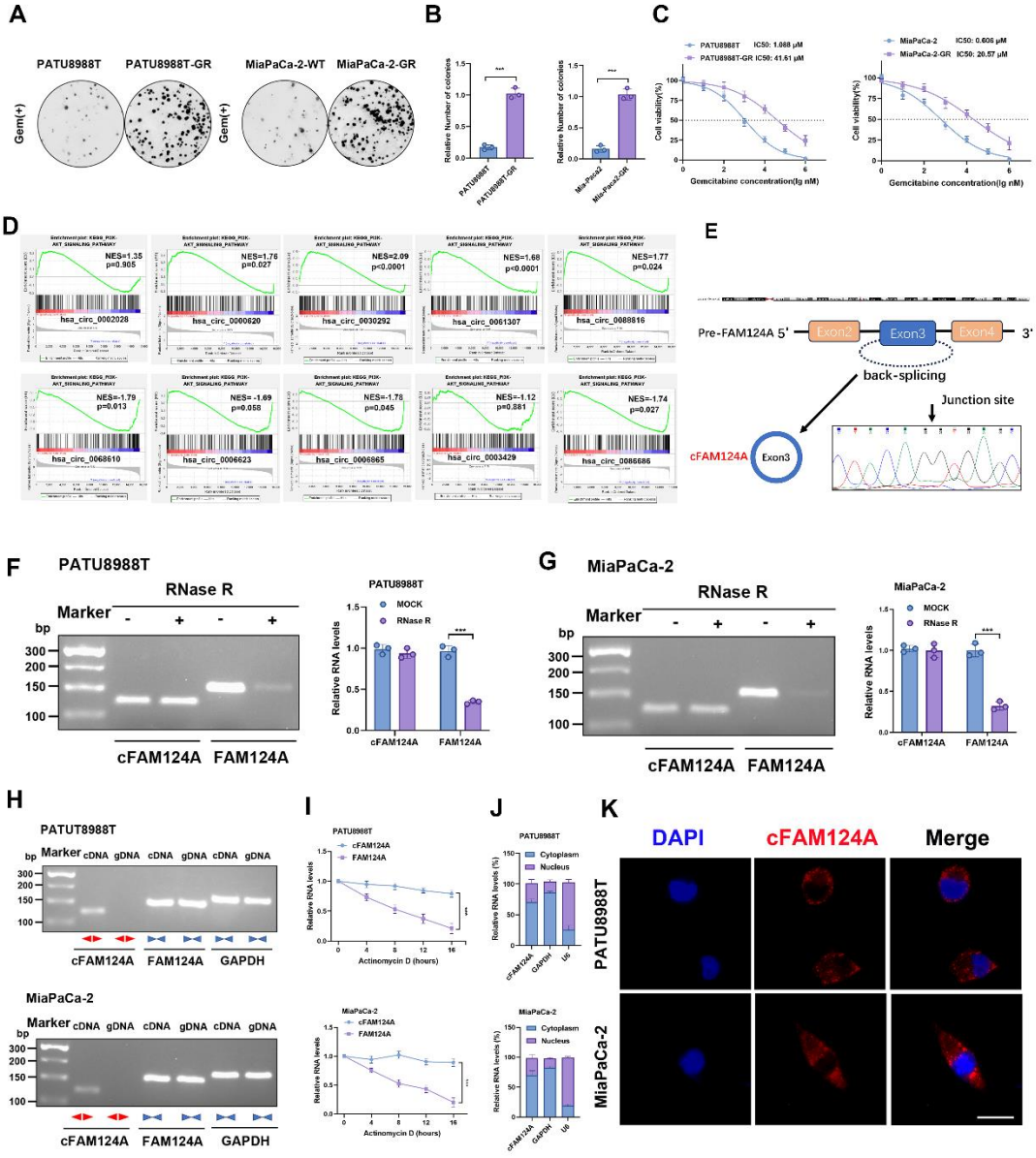
Fig. S1 Biosafety of sulindac K-80003 in vivo.



A, Representative images of H&E and IHC staining for p-Akt (Thr308) in PDOs from PDO-AGS and PDO-AGR groups. **B**, Tumors weight quantification from different groups of PDOXs received different treatments. **C**, Western blot analysis of Akt and p-

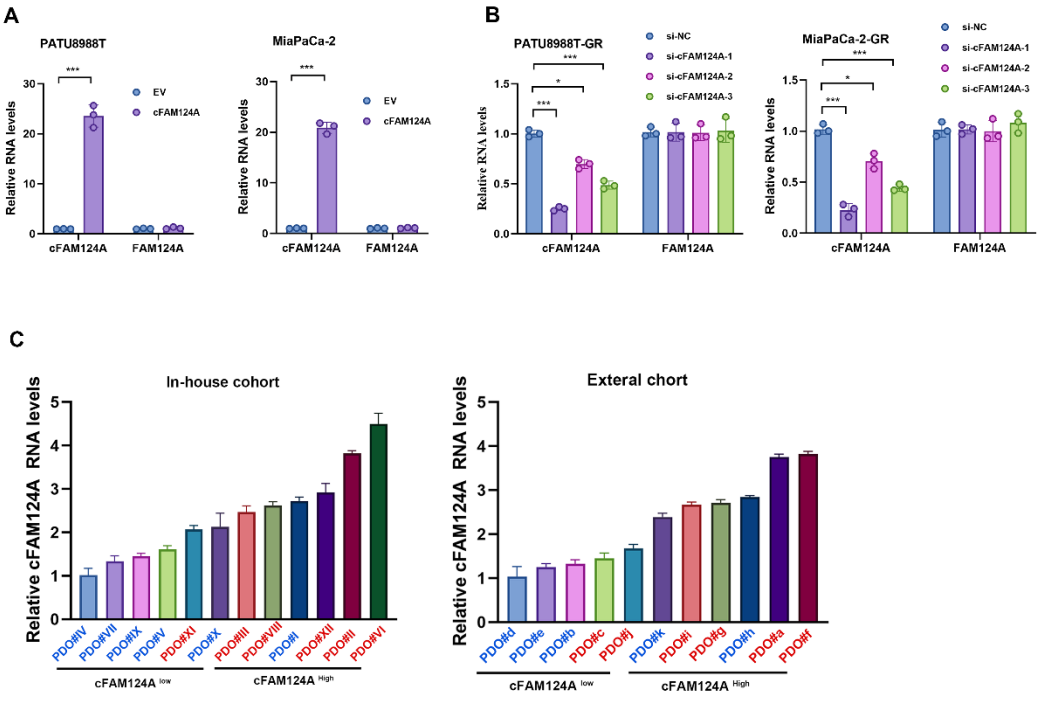
Akt (Thr308) in tumor tissue that derived from orthotopic PDOX mice model received different treatments. **D-G**, Kidney function (CREA and BUN levels) and liver function (AST and ALT levels) in subcutaneous tumor-bearing mice in the indicated groups (n=25). **H**, Body weights of subcutaneous tumor-bearing mice in the indicated groups (n=25). **I**, H&E staining of the main organs of subcutaneous tumor-bearing mice in the indicated groups. ns, $P>0.05$; **$P<0.01$; ***$P<0.001$.

Fig. S2 cFAM124A has a central position in the PI3K/Akt signaling pathway.



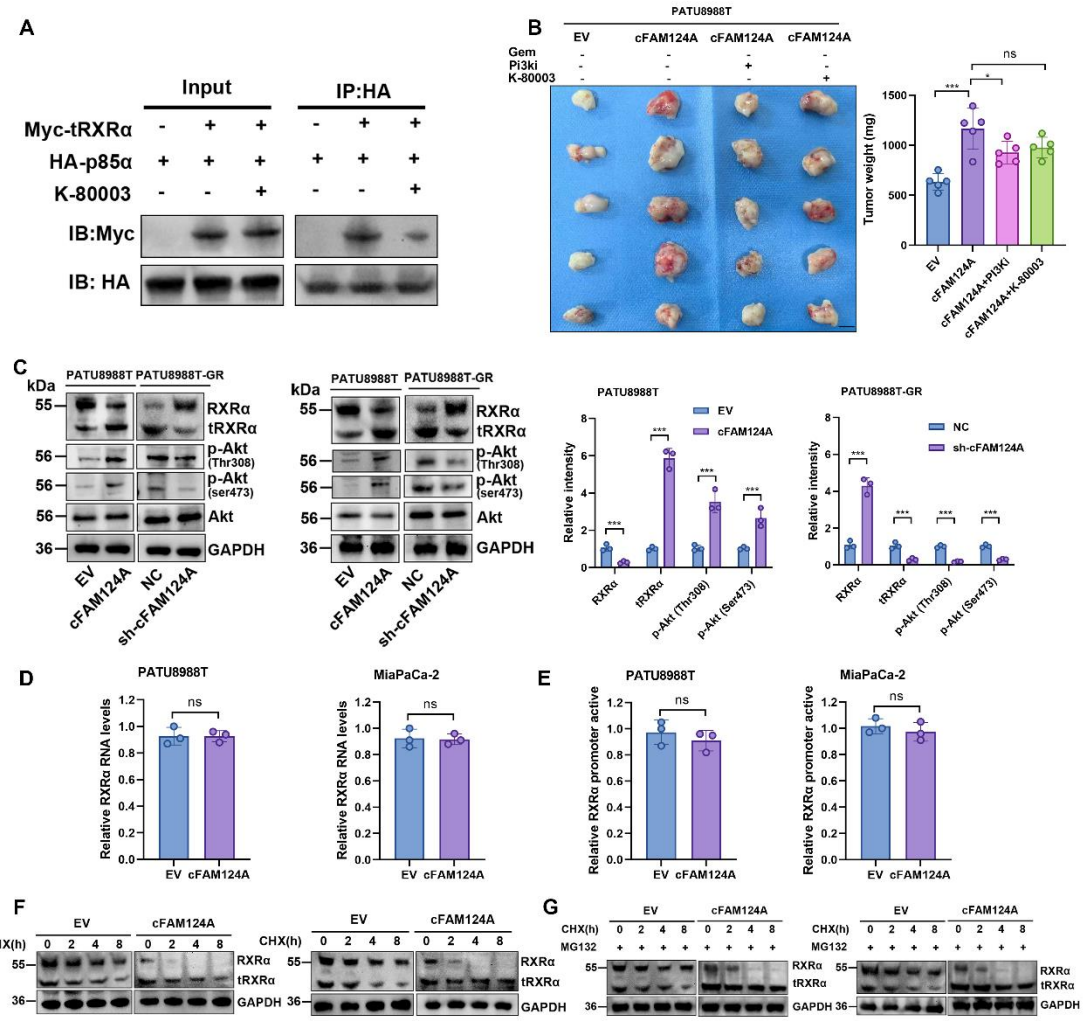
A, Colony formation assay for PATU8988T and PATU8988T-GR cells with GEM treatment in 6-well dishes (800 cells/well) for 2 weeks. Quantitative data are shown on the right. **B**, IC50 values for GEM in PATU8988T cells and PATU8988T-GR cells. **C**, Colony formation assay for MiaPaCa-2 and MiaPaCa-2-GR cells with GEM treatment in 6-well dishes (800 cells/well) for 2 weeks. Quantitative data are shown on the right. **D**, GSEA of 10 circRNAs demonstrating enrichment of DEGs in the PI3K/Akt pathway. **E,** Schematic of the genomic region of cFAM124A and its cyclization (top). The connection point of cFAM124A as identified by Sanger sequencing (bottom) **F-G**, Expression of cFAM124A and FAM124A mRNA expression after RNase R treatment by RT-qPCR and agarose gel electrophoresis. **H**, Agarose gel electrophoresis images showing amplification of cFAM124A and FAM124A mRNA in gDNA and cDNA from PATU8988T and MiaPaCa-2 cells. **I**, Relative expression levels of cFAM124A and FAM124A mRNA after actinomycin D treatment by RT-qPCR at the indicated time points. **J**, Detection of cFAM124A expression in cytoplasmic and nuclear fractions of RNAs extracted from PDAC cells. **K**, Subcellular localization of cFAM124A in PATU8988T and MiaPaCa-2 cells by FISH. Scale bar, 50 μm. ns, $P>0.05$; **$P<0.01$; ***$P<0.001$.

Fig. S3



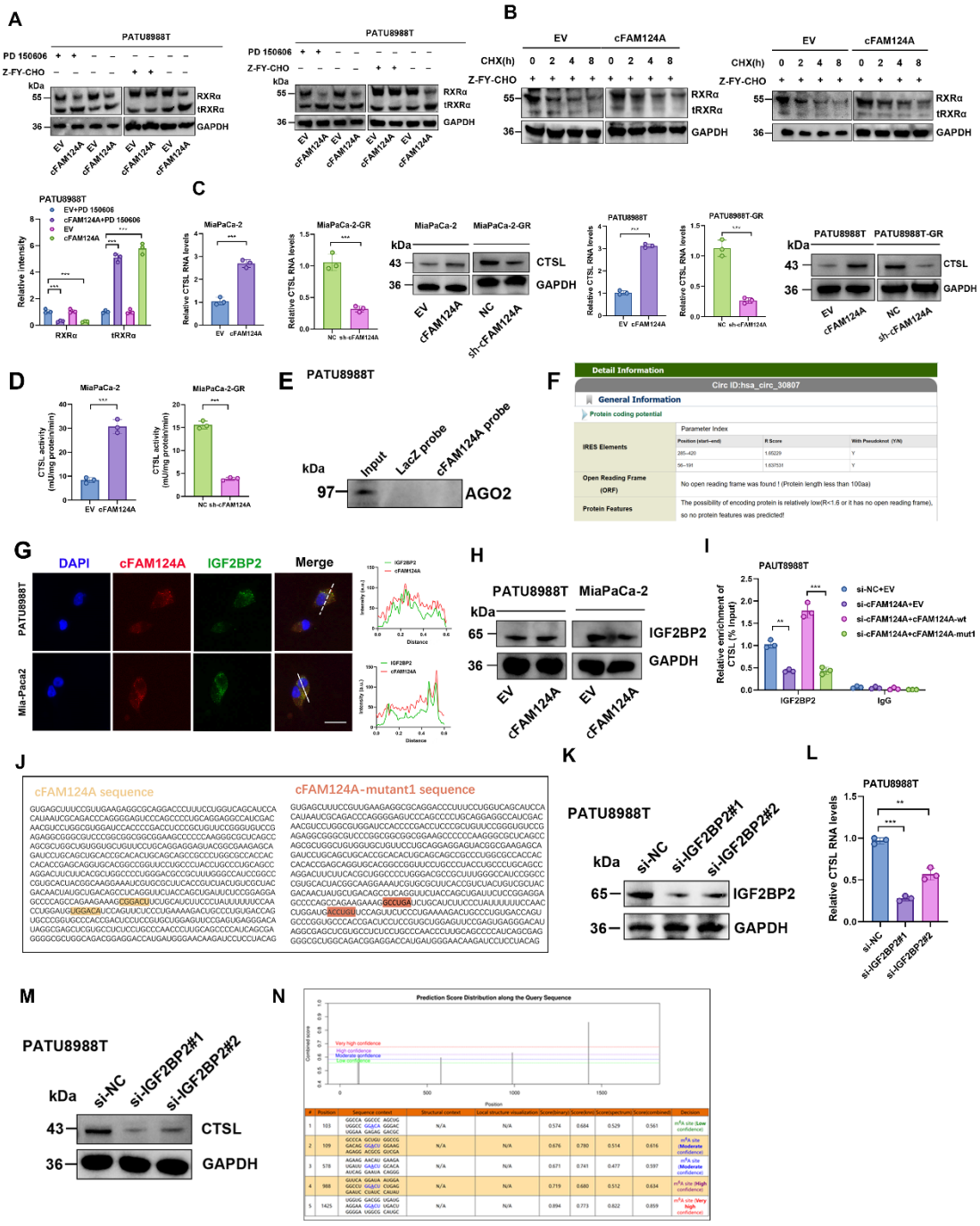
A-B, Relative RNA expression of cFAM124A and FAM124A in PDAC cells with cFAM124A overexpression or knockdown by qRT-PCR. C, cFAM124A expression in in both the In-house cohort and External cohort PDOs by RT-qPCR. P>0.05; **P<0.01; ***P<0.001. **C**, cFAM124A expression in In-house cohort and external cohort PDOs (Red: Response; Blue: Non-response)

Fig. S4. PDAC cells overexpressing cFAM124A leads to an increase in tRXRα protein.



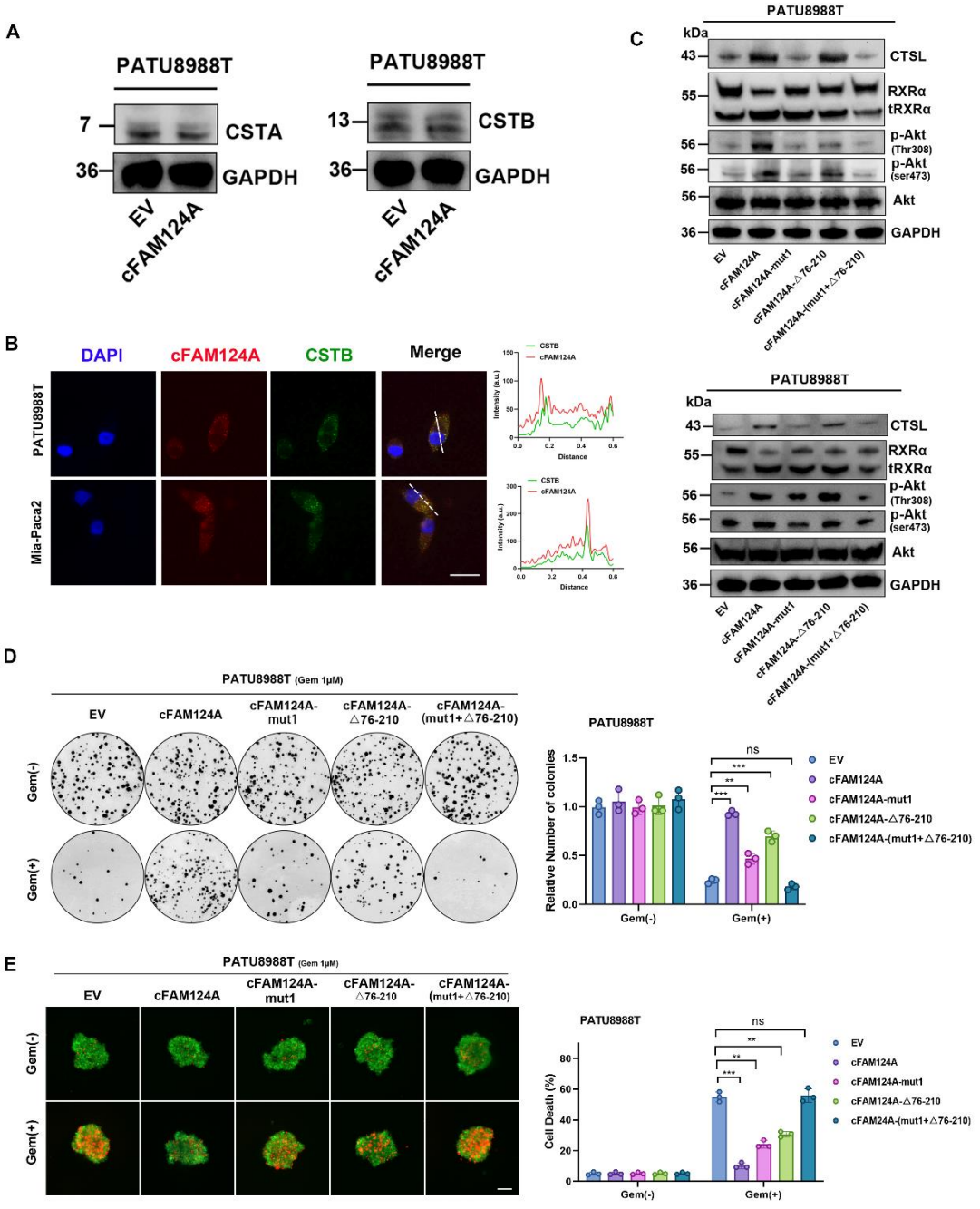
A, HA-p85α was transfected with Myc-tagged tRXRα into HEK293T cells, and their interaction was analyzed by coIP using anti-HA antibody. **B**, Subcutaneous xenograft model of mice in the different groups. Copanlisib (1 mg/kg, iv. 2×/week for 4 weeks), or K-80003 (20 mg/ kg i.p. 2×/week for 4 weeks) at 2 weeks after subcutaneous injection of 5×10^6 cells overexpressing cFAM124A and control cells. Representative images of tumors are shown (n=5). **C**, Western blot analysis of RXRα, Akt, p-Akt (Ser473), and p-Akt (Thr308) expression in PDAC cells overexpressing cFAM124A or with cFAM124A knockout and control cells. **D**, Relative RNA expression of RXRα in PDAC cells overexpressing cFAM124A by RT-qPCR. **E**, Promoter activity of RXRα genes in PDAC cells overexpressing cFAM124A. **F**, cFAM124A increased RXRα protein degradation: Indicated PDAC cell lines were incubated with CHX for indicated time periods before western blot analysis of RXRα and GAPDH expression. Representative images are shown. **G**, Indicated PDAC cell lines were incubated with MG132 and then with CHX for indicated time periods before western blot analysis of RXRα and GAPDH expression. Representative images are shown.

Fig. S5 cFAM124A acts as a scaffold for interaction between CTSL mRNA and IGF2BP2 to promote CTSL mRNA stability.



A, Western blot analysis of RXRα expression after 24 h of treatment with PD150606 (200 nM, m-calpain inhibitor) or ZFY-CHO (10 μM, CTSL inhibitor). Representative images are shown. **B**, Indicated PDAC cell lines were incubated with ZFY-CHO and then with CHX for indicated time periods before western blot analysis of RXRα and GAPDH expression. Representative images are shown. **C**, Relative RNA expression of CTSL in PDAC cells with cFAM124A overexpression or knockdown by RT-qPCR. CTSL protein expression in PDAC cells with cFAM124A overexpression or knockdown by western blotting. **D**, CTSL level by ELISA in PDAC cells with cFAM124A overexpression or knockdown. **E**, AGO2 pulldown by the LacZ probe (control) or cFAM124A probe. **F**, Coding potential of cFAM124A predicted based on the circRNADb database. **G**, Subcellular localization of IGF2BP2 (green) and cFAM124A (red) detected by FISH. Scale bar, 50 μm. **H**, IGF2BP2 protein expression in PATU8988T with cFAM124A overexpression by western blotting. **I**, CTSL mRNA enrichment by IGF2BP2 on RIP assay in the indicated groups. **J**, Sequences of wild-type cFAM124A and mutated cFAM124A. **K**, Protein expression of IGF2BP2 in PATU8988T cells with IGF2BP2 knockdown. **L-M**, mRNA (L) and protein (M) expression of CTSL in PATU8988T cells with IGF2BP2 knockdown. **N**, Prediction of m6A methylation of CTSL mRNA at one site based on SRAMP software analysis. ns, $P>0.05$; **$P<0.01$; ***$P<0.001$.

Fig. S6 cFAM124A competes with CSTB through bait effect and enhances CTSL enzyme activity.



A, CSTA and CSTB expression in PATU8988T cells overexpressing cFAM124A by western blotting. **B**, Subcellular localization of CSTB (green) and cFAM124A (red) detected by FISH. Scale bar, 50 μm. **C**, Western blot analysis of CTSL, RXRα, Akt, p-Akt (Ser473), and p-Akt (Thr308) protein expression in the indicated groups. **D**, Colony formation assay in the indicated groups with GEM treatment in 6-well dishes (800 cells/well) for 2 weeks. E, Necrosis in 3D tumor spheroids based on PI staining (red) and its quantification in the indicated groups after treatment with GEM. Scale bar, 100 μm. Quantitative data are shown on the right. ns, $P>0.05$; **$P<0.01$; ***$P<0.001$.

Table S1. Baseline characteristics of 20 PDAC Patients.

				Pre-treatment				Post-treatment					
PDAC Patients	**Group**	**Location**	**Differentiation**	**Vascular invasion (PV/SMV/SMA)**	**Liver metastasis**	**CA199 (U/ml)**	**Tumor diameter (cm)**	**Pre-existing liver metastasis changes**	**Newly developed liver metastasis**	**CA199 (U/ml)**	**Tumor diameter (cm)**	**Progression of tumor**	**RECIST1.1**
P01	AG-S	Body/tail	poor	SMA	No	324	3.7*3.2	No	no	29	2.3*2.1	no	PR
P02	AG-S	Head	well/moderate	No	Single	68	3.2*2.3	Shrinkage	no	57	2.1*2.0	no	PR
P03	AG-S	Body/tail	poor	SMA	Single	433	3.2*2.9	Shrinkage	no	327	2.4*1.9	no	PR
P04	AG-S	Body/tail	poor	No	Single	341	3.7*3.3	Shrinkage	no	322	2.2*1.7	no	PR
P05	AG-S	Head	well/moderate	PV	No	32	2.5*2.4	No	no	34	1.3*1.1	no	PR
P06	AG-S	Body/tail	poor	NO	Multiple	567	4.2*3.4	Shrinkage	no	325	2.7*2.1	no	PR
P07	AG-S	Head	well/moderate	SMV	No	35	3.6*2.7	No	no	34	2.5*1.8	no	PR
P08	AG-S	Head	well/moderate	SMV	Multiple	165	4.1*3.2	Shrinkage	no	67	3.2*2.2	no	PR
P09	AG-S	Body/tail	well/moderate	No	Single	131	2.8*2.6	No	no	35	2.2*1.5	no	PR
P10	AG-S	Body/tail	poor	No	Single	133	3.4*2.9	Shrinkage	no	65	2.8*2.1	no	PR
P11	AG-R	Head	well/moderate	SMV	Multiple	232	3.3*3.1	Increase	no	423	3.6*3.2	yes	PD
P12	AG-R	Body/tail	poor	No	Single	272	3.6*3.2	No	yes	341	3.6*3.5	yes	PD
P13	AG-R	Head	poor	SMV	No	34	2.7*2.5	No	no	274	4.2*3.1	yes	PD
P14	AG-R	Head	poor	PV	No	242	3.8*2.7	No	yes	323	3.2*2.1	yes	PD
P15	AG-R	Body/tail	well/moderate	No	Single	30	3.7*2.4	Increase	no	332	3.7*2.9	yes	PD
P16	AG-R	Body/tail	poor	No	Single	32	2.8*2.5	Increase	no	127	3.1*2.7	yes	PD
P17	AG-R	Head	poor	PV	NO	673	3.2*2.1	Increase	no	931	4.0*3.7	yes	PD
P18	AG-R	Head	poor	SMV	Multiple	433	3.7*3.4	No	yes	523	3.4*3.4	yes	PD
P19	AG-R	Body/tail	poor	No	Multiple	321	3.4*3.1	No	yes	453	4.1*3.2	yes	PD
P20	AG-R	Body/tail	well/moderate	SMA	Single	432	4.3*3.5	Increase	no	464	4.2*3.9	yes	PD

AG-sensitive, AG-S; AG-resistant, AG-R; PV, portal vein; SMV, superior mesenteric vein; SMA, superior mesenteric artery.

Table S2. Baseline characteristics of PDAC samples.

Characteristics	In-house cohort(n=12)			External cohort(n=11)			*p* (In house vs. External)
	Response	Non-Response	total	Response	Non-Response	total	
Age							0.667
<60	1(16.7)	2(33.3)	3(25.0)	1(20.0)	3(33.3)	4(36.4)	
≥60	5(83.3)	4(66.7)	9(75.0)	4(80.0)	3(66.7)	7(63.6)	
Sex							1.000
female	1(16.7)	2(33.3)	3(25.0)	1(20.0)	2(33.3)	3(27.3)	
male	5(83.3)	4(66.7)	9(75.0)	4(80.0)	4(66.7)	8(72.7)	
Tumor location							0.684
Body/tail	4(66.7)	3(50.0)	7(58.3)	1(20.0)	4(66.7)	5(45.6)	
Head	2(33.3)	3(50.0)	5(41.7)	4(80.0)	2(33.3)	6(54.4)	
Lymph node metastasis							1.000
no	1(16.7)	1(16.7)	2(16.7)	1(20.0)	0	1(9.1)	
yes	5(83.3)	5(83.3)	10(83.3)	4(80.0)	6(100)	10(90.9)	
CA199							1.000
<37	1(16.7)	2(33.3)	3(25.0)	1(20.0)	1(16.7)	2(18.2)	
≥37	5(83.3)	4(66.7)	9(75.0)	4(80.0)	5(83.3)	9(81.8)	
Differentiation							1.000
poor	4(66.7)	4(60.0)	8(66.7)	3(60.0)	4(66.7)	7(63.6)	
well/moderate	2(33.3)	2(40.0)	4(33.3)	2(40.0)	2(33.3)	4(36.4)	
Tumor size							1.000
⩽4cm	2(33.3)	2(33.3)	4(33.3)	2(40.0)	2(33.3)	4(36.4)	
> 4cm	4(66.7)	4(66.7)	8(66.7)	3(60.0)	4(66.7)	7(63.6)	
AJCC stage							1.000
II	2(33.7)	3(50.0)	5(41.7)	2(40.0)	2(33.3)	4(36.4)	
III	4(66.7)	3(50.0)	7(58.3)	3(60.0)	4(66.7)	7(63.6)	

Pancreatic ductal adenocarcinoma; PDAC; *p,* Two-sided Fisher's exact test

Table S3. Baseline characteristics of cFAM124A in clinical PDAC samples.

Characteristics	Low expression cFAM124A n=66	High expression cFAM124A n=66	*p*
Age			0.596
<60	26(39.4)	29(43.9)	
≥60	40(60.6)	37(56.1)	
Sex			0.856
female	23(34.8)	24(36.4)	
male	43(65.2)	42(63.6)	
Tumor location			1.000
Body/tail	38(57.6)	38(57.6)	
Head	28(42.4)	28(42.4)	
Lymph node metastasis			0.159
no	32(48.5)	24(36.4)	
yes	34(51.5)	42(63.6)	
CA199			0.024
<37	21(31.8)	10(15.2)	
≥37	45(68.2)	56(84.8)	
Differentiation			0.002
poor	25(37.9)	43(65.2)	
well/moderate	41(62.1)	23(34.8)	
AJCC stage			0.798
I	5(7.6)	3(4.5)	
II	12(18.2)	13(19.7)	
III	27(40.9)	24(36.4)	
IV	22(33.3)	26(39.4)	
PFS			0.008
<12 (Gem-R)	21(31.8)	36(54.5)	
≥12 (Gem-S)	45(68.2)	30(45.5)	

cFAM124A, Pancreatic ductal adenocarcinoma PDAC; *p*, Two-sided Pearson's $\chi 2$ test