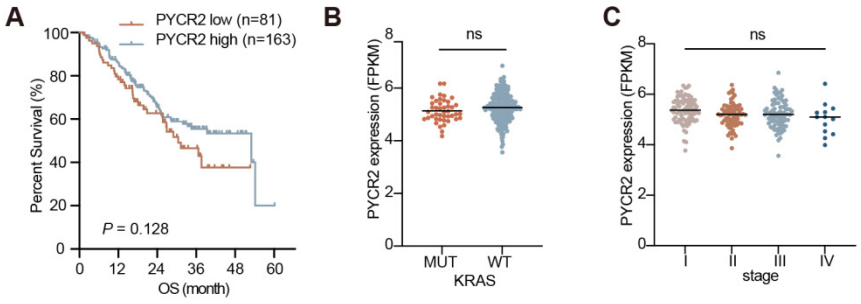
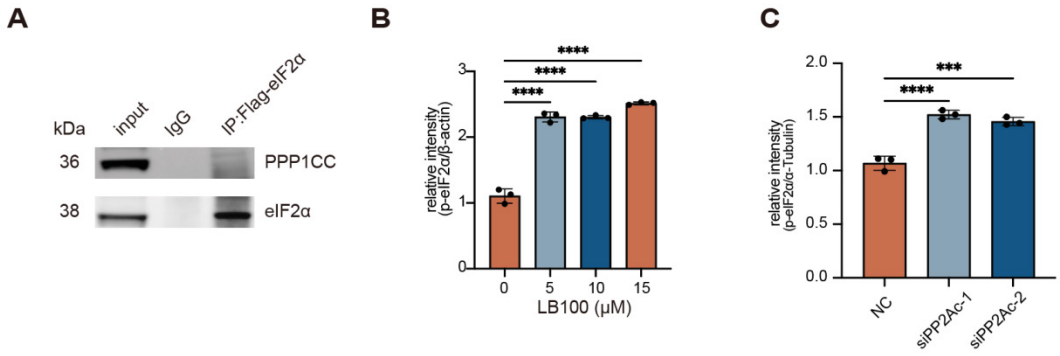
Supplementary Figure 1



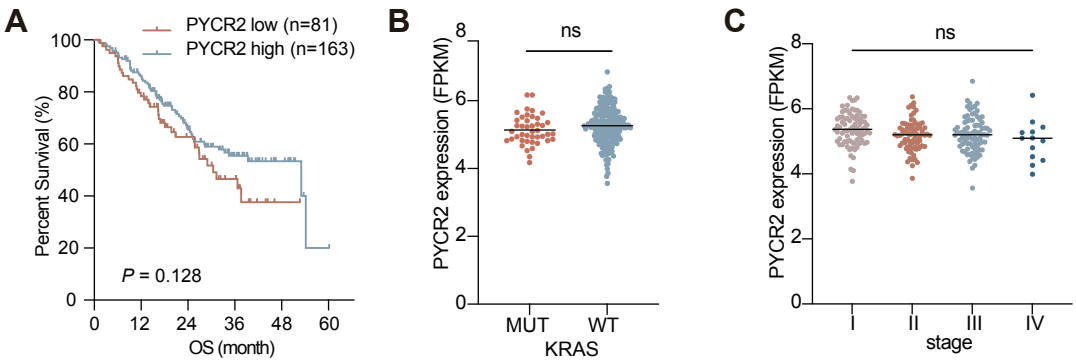
Supplementary Figure S1. Clinical relevance of PYCR2 in cholangiocarcinoma. (A) Kaplan-Meier analysis of overall survival in patients with CCA stratified by PYCR2 expression levels. (B) Comparison of PYCR2 expression between KRAS-mutant (MUT) and KRAS wild-type (WT) CCA samples using the Mann-Whitney U test. (C) Comparison of PYCR2 expression among different pathological stages of CCA using the Kruskal-Wallis test. Data are presented as individual samples with mean ± SD. ns: not significant.

Supplementary Figure 2

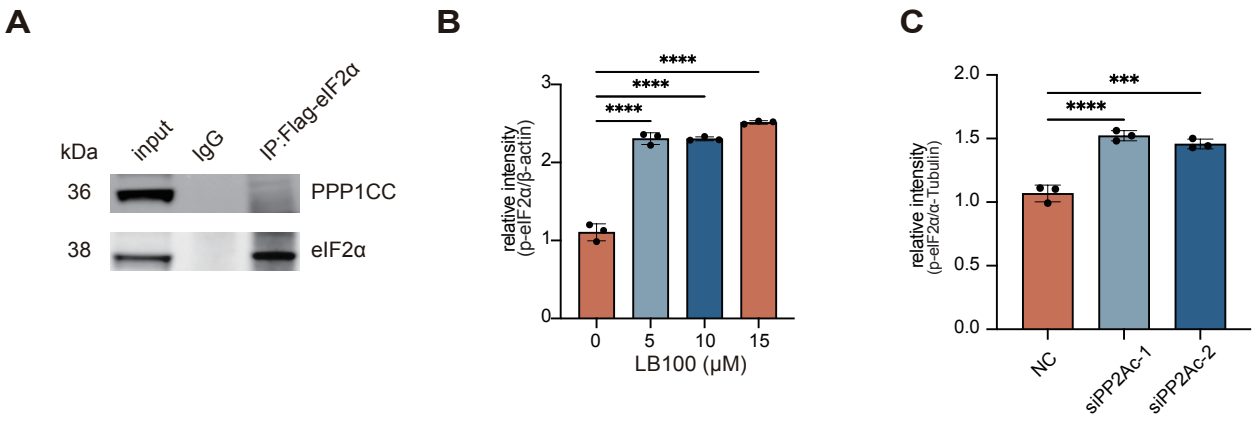


Supplementary Figure S2. Validation of PP2Ac-mediated regulation of eIF2α phosphorylation. (A) Co-IP assays in HCC3975-R cells evaluating the interaction between PPP1CC and eIF2α. (B) Quantification of p-eIF2α protein levels in HCC3975-R cells treated with the indicated concentrations of LB100, normalized to β-actin (n = 3 biological replicates, one-way ANOVA). (C) Quantification of p-eIF2α protein levels in HCC3975-R cells following PP2Ac knockdown, normalized to α-tubulin (n = 3 biological replicates, one-way ANOVA). Values are expressed as mean ± SD, ***$P < 0.001$, ****$P < 0.0001$.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Table S1. Sequences of siRNAs, shRNAs, and qPCR primers used in this study.

siRNA	Sense(5'-3')
PYCR2 #1	GCAACAAGGAGACGGUGAAGC
PYCR2 #2	GGGAAGCUCCUCACAAGAAGC
eIF2α #1	CAUUAAGAUUAAUCUAAUAGC
eIF2α #2	GAUUCUUCUUAGUGAAUUAUC
PP2Ac #1	AAGCTGCAATCATGGAACTTG
PP2Ac #2	CCGTGAACGCATCACCATT

shRNA	Sequence (5'-3')
shPYCR2	GCACAGCGACGTCCTGTTTCT

primer	Sequence (5'-3')
PYCR2-F	GAACCTGACACGCAGCAACAAG
PYCR2-R	GTCATGCAGCGAATCACTTTGG

Supplementary Table S3

Data corresponding to Figure. 1E

Group	Log_2(GI50, μM)
NC	4.648
PANK4-1	4.592
PANK4-2	4.472
PYCR2-1	4.545
PYCR2-2	4.675
PTPN20B-1	4.658
PTPN20B-2	4.234

Group	Log_2(GI50, μM)
NC	4.648
PTPN11-1	4.393
PTPN11-2	4.276
SGK3-1	4.604
SGK3-2	4.579
VAMP2-1	4.554
VAMP2-2	4.321

Group	Log_2(GI50, μM)
NC	4.648
NDUFC2-1	4.907
NDUFC2-2	4.674
NDUFA1-1	4.702
NDUFA1-2	4.624

Data corresponding to Figure. 1F

Group	Log_2(GI50, μM)
NC	6.047
PYCR2-1	5.627
PYCR2-2	4.714
PTPN11-1	5.735
PTPN11-2	5.973
PANK4-1	5.446
PANK4-2	5.932

Data corresponding to Figure. 2B

Group	Log_2(GI50, μM)
HCC3975-R	9.264
HCC3975	4.629

Data corresponding to Figure. 2I

Group	Log_2(GI50, μM)
NC	5.184
siPYCR2-1	4.162
siPYCR2-2	3.917

Data corresponding to Figure. 4E

Group	Log_2(GI50, μM)
DMSO	5.095
NAM	5.016
FK866	5.157

Supplementary Table S4. Antibodies used for Western blotting and immunohistochemistry.

Target	Application	Manufacturer	Catalog No.	Dilution
PYCR2	WB	Abclonal	A15155	1:1000
p-eIF2α (Ser51)	WB/IHC	Abclonal	AP0692	1:1000/1:400
eIF2α	WB	Abclonal	A0764	1:1000
p-ERK1/2	WB/IHC	CST	#4370	1:1000/1:400
ERK1/2	WB	CST	#4695	1:1000
PP2Ac	WB	Proteintech	67809-1-Ig	1:1000
Ki67	IHC	Abcam	ab15580	1:400
PCNA	IHC	Proteintech	10205-2-AP	1:3000
Puromycin	WB	Abclonal	A23031	1:1000
Phospho-ATM (Ser1981)	WB	CST	#5883	1:1000
Alpha Tubulin	WB	Proteintech	66031-1-Ig	1:5000
β-actin	WB	Proteintech	66009-1-Ig	1:5000
PPP1CC	WB	Proteintech	11082-1-Ig	1:1000