

Supplementary Figures and Tables

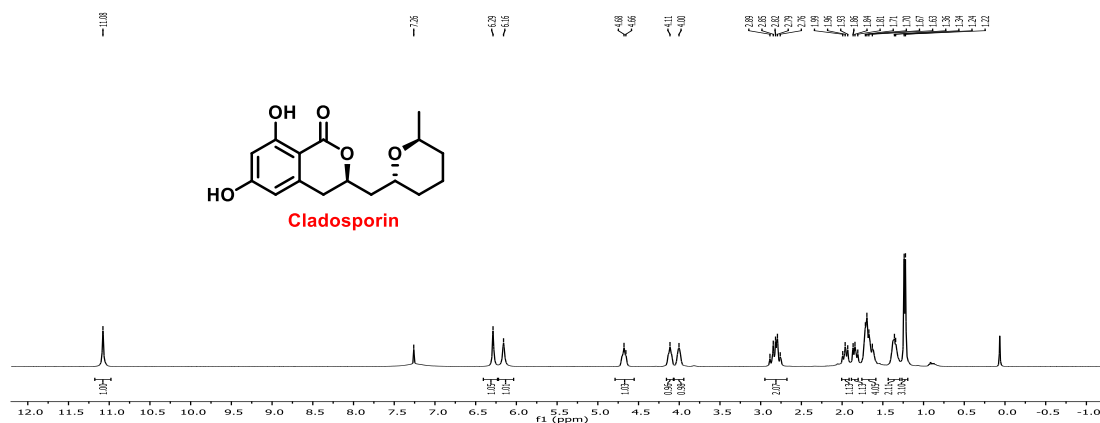
The biological process of lysine-tRNA charging is therapeutically targetable in liver cancer

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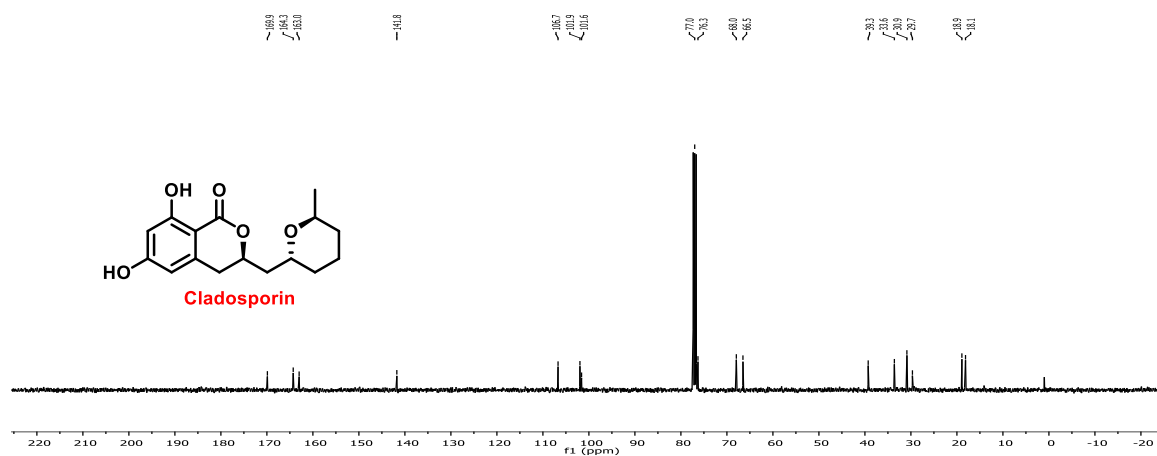
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^1H NMR of Cladosporin in 400 MHz



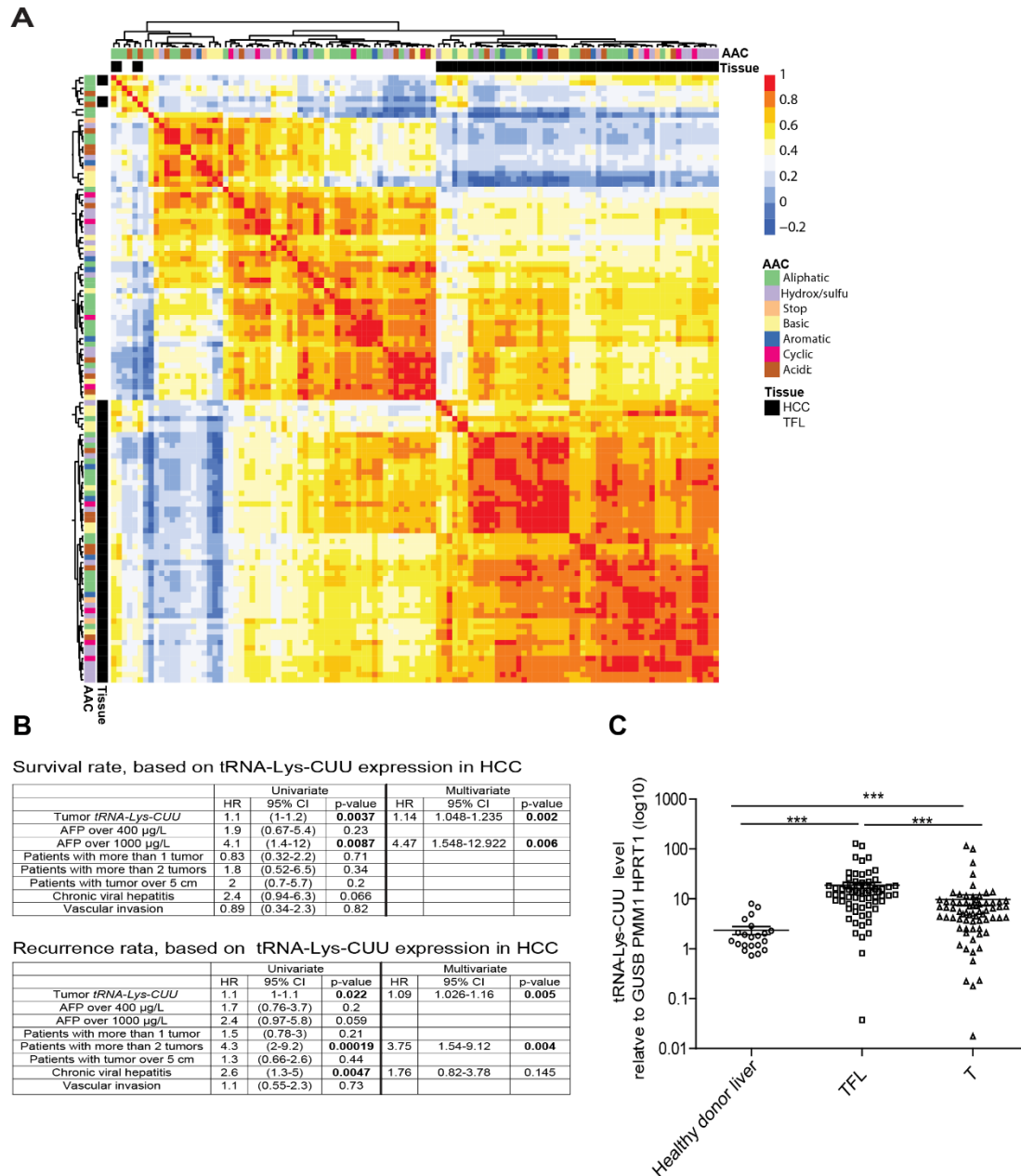
^1H NMR (400 MHz, CDCl_3) δ 11.08 (s, 1H), 6.29 (s, 1H), 6.16 (s, 1H), 4.68-4.66 (m, 1H), 4.11 (s, 1H), 4.00 (s, 1H), 2.89 – 2.76 (m, 2H), 1.90 (dt, $J = 23.0, 12.8$ Hz, 2H), 1.68 (dd, $J = 21.9, 12.2$ Hz, 4H), 1.36 -1.34 (m, 2H), 1.23 (d, $J = 6.4$ Hz, 3H).

^{13}C NMR of Cladosporin in 100 MHz

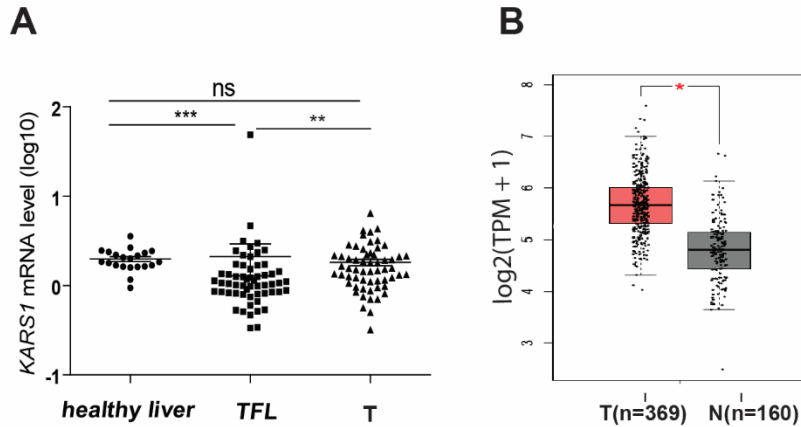


^{13}C NMR (100 MHz, CDCl_3) δ 169.9, 164.3, 163.0, 141.8, 106.7, 102.0, 101.6, 76.3, 68.0, 66.5, 39.3, 33.6, 30.9, 29.7, 18.9, 18.1

Supplementary Figure 1 NMR for Cladosporin



Supplementary Figure 2 (A) Heatmap showing the correlation patterns of 57 tRNA species in 69 pairs of human HCC and TFL tissues. (B) The expression of tRNA-Lys-CUU in organ donor liver tissues (n = 21), TFL and paired HCC tissues (n = 69), respectively. (C) Univariate and multivariate analysis of associations between clinicopathological risk factors and tRNA-Lys-CUU expression in tumor tissue and HCC-specific patient survival rate and cancer recurrence rate (n=69) in our HCC patient cohort. ***P <0.001, by the Mann-Whitney test. TFL: tumor free liver; T:HCC tumor.



C Survival rate, based on *KARS1* expression in HCC

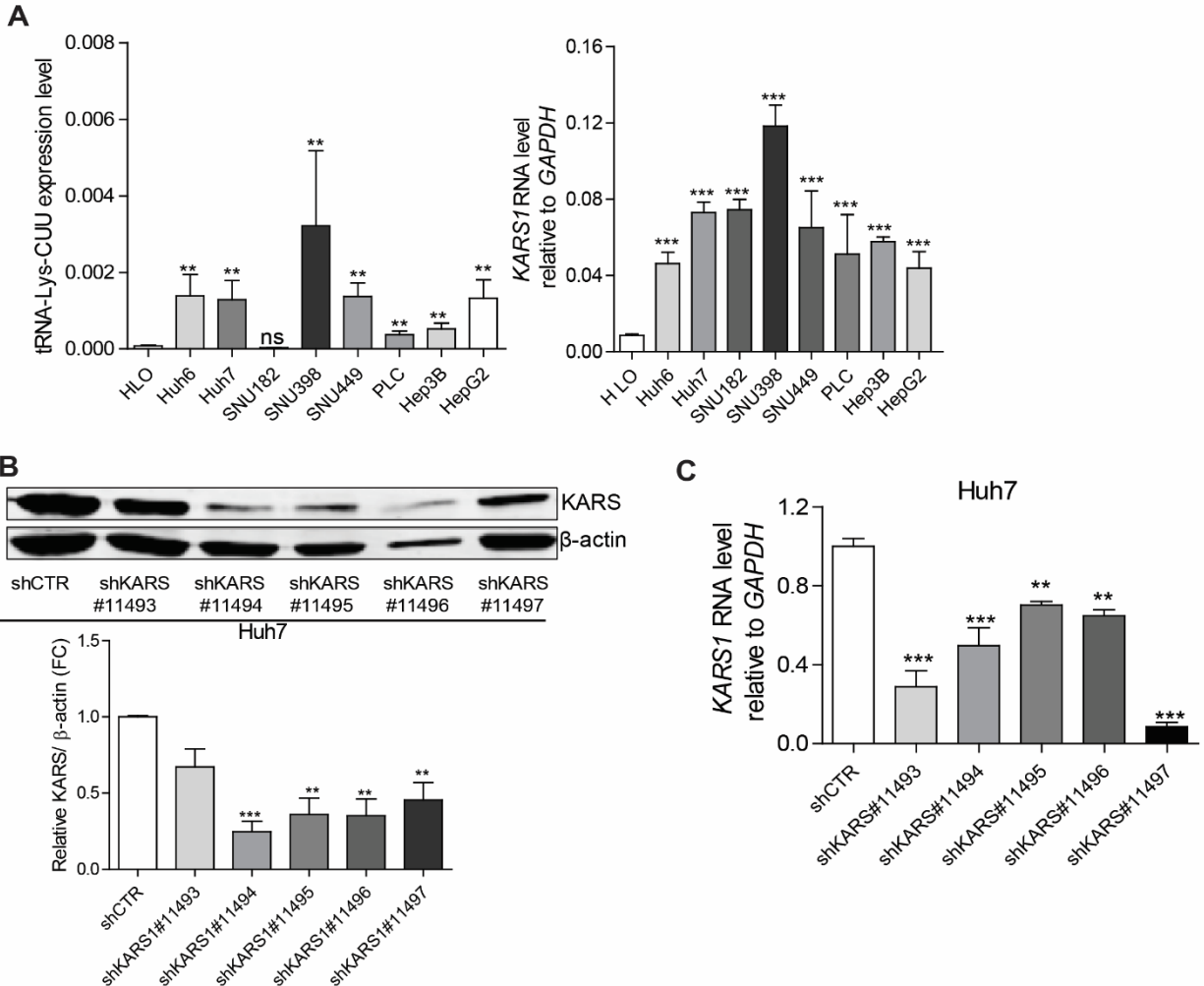
	Univariate			Multivariate		
	HR	95% CI	p-value	HR	95% CI	p-value
Tumor <i>KARS1</i>	1.4	0.96-2.1	0.078	1.66	1.08-2.55	0.021
AFP over 400 µg/L	1.9	(0.67-5.4)	0.23			
AFP over 1000 µg/L	4.1	1.4-12	0.008	5.77	1.86-17.94	0.002
Patients with more than 1 tumor	0.78	(0.3-2.1)	0.62			
Patients with more than 2 tumors	1.8	(0.5-6.3)	0.38			
Patients with tumor over 5 cm	2.1	(0.74-6)	0.16			
Chronic viral hepatitis	2.4	(0.92-6.2)	0.073			
Vascular invasion	1.2	(0.48-3.2)	0.66			

Recurrence rata, based on *KARS1* expression in HCC

	Univariate		
	HR	95% CI	p-value
Tumor <i>KARS1</i>	0.95	(0.66-1.3)	0.76
AFP over 400 µg/L	1.6	(0.7-3.8)	0.26
AFP over 1000 µg/L	2.3	(0.88-6.1)	0.09
Patients with more than 1 tumor	1.4	(0.68-2.8)	0.38
Patients with more than 2 tumors	3.6	(1.6-8.3)	0.0025
Patients with tumor over 5 cm	1.4	(0.68-2.9)	0.37
Chronic viral hepatitis	2.5	(1.2-5)	0.011
Vascular invasion	0.97	(0.48-2)	0.94

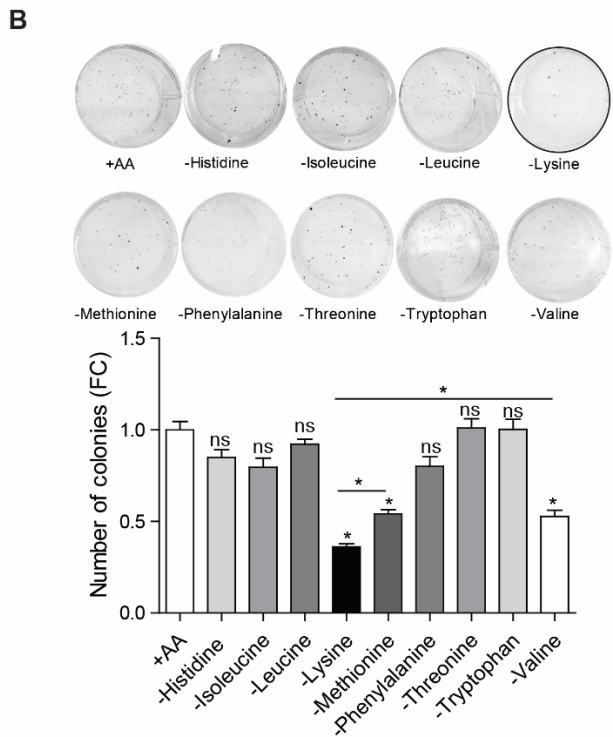
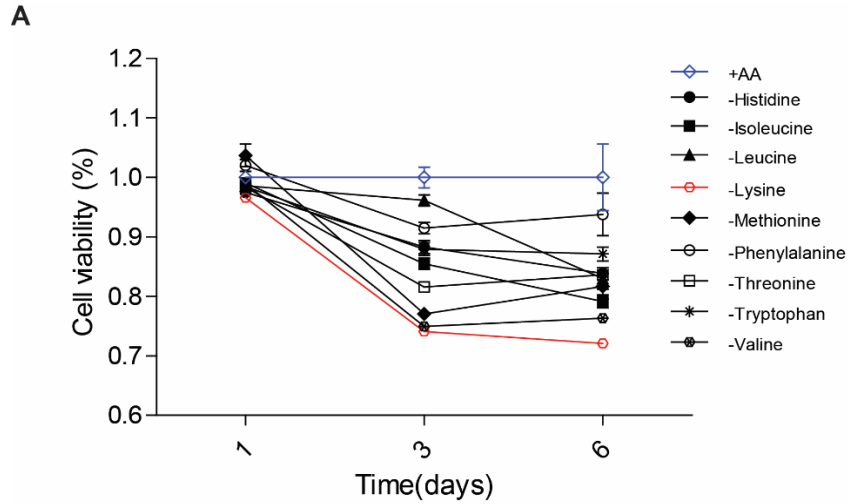
Supplementary Figure 3 The mRNA expression levels of *KARS1*, and their relation with clinical outcomes.

(A) The mRNA expression levels of *KARS1* in organ donor liver tissues (n = 21), TFL and paired HCC tissues(n = 59).(B)The mRNA expression data of *KARS1* from GEPIA database, and red boxplot showed HCC tumor samples (n = 369), black boxplot showed Normal healthy liver samples (n = 160). (C) Univariate and multivariate analysis of associations between clinicopathological risk factors and *KARS1* expression in tumor tissue and HCC-specific patient survival rate and cancer recurrence rate (n=59) in our HCC patient cohort. *P<0.05; **P<0.01; ***P<0.001, by the Mann-Whitney test. TFL: tumor free liver; T:HCC tumor.

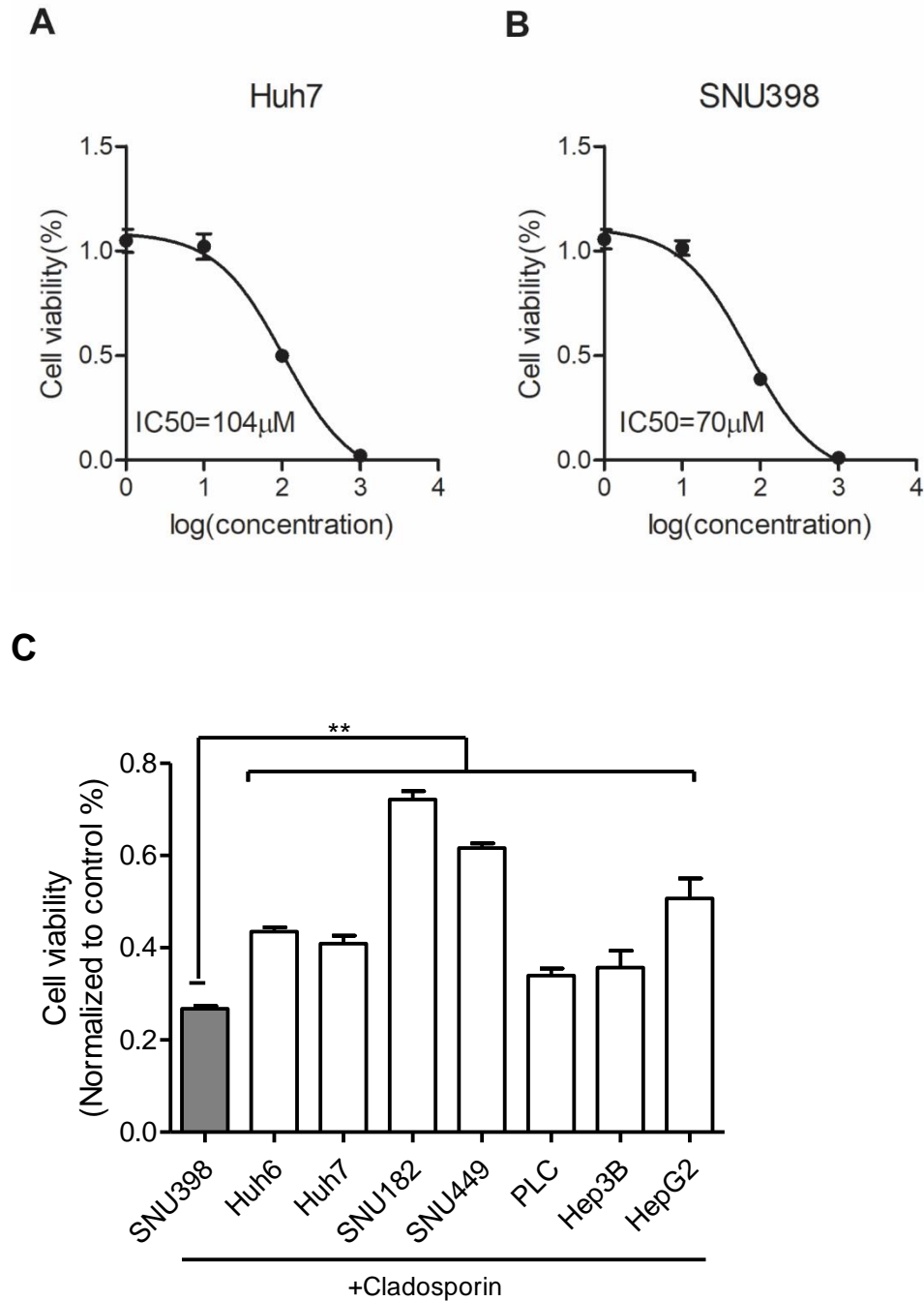


Supplementary Figure 4 The expression level of tRNA-Lys-CUU and *KARS1*, and KARS knockdown efficiency.

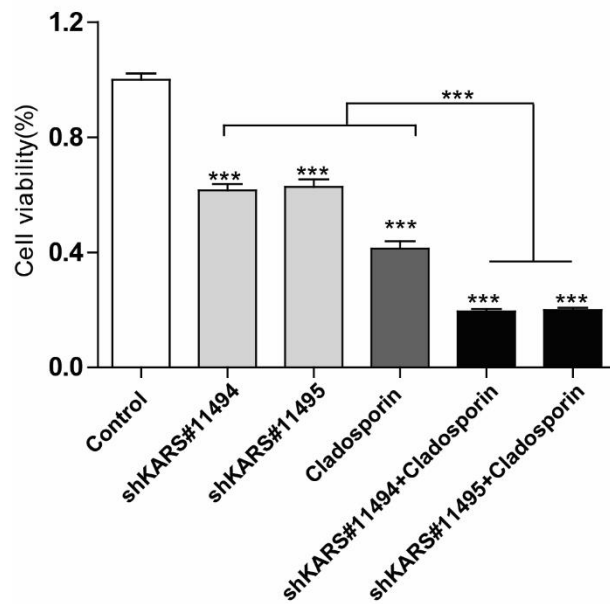
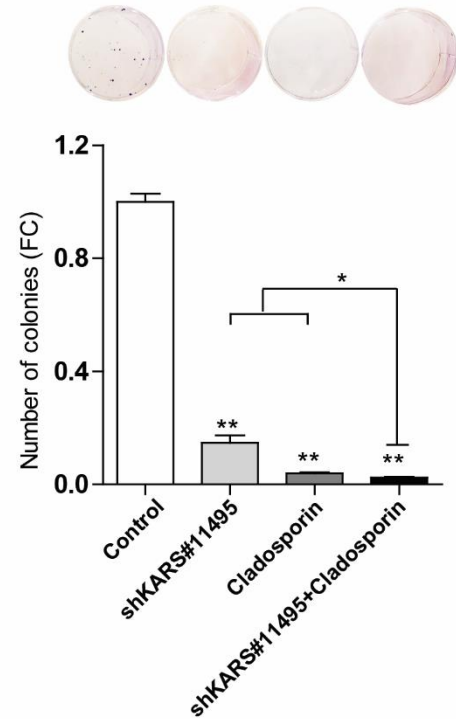
(A) The expression level of tRNA-Lys-CUU (left graph) and *KARS1* (right graph) respectively, relative to GAPDH in HCC cell lines compared to normal healthy liver organoids (n = 4-7). (B-C) Knockdown in Huh7 and SNU398 was analyzed in (B) by western blot (upper graph), and the intensity was quantified relative to β-actin (lower graph) by image studio software. In (C) the expression of *KARS1* mRNA upon knockdown was determined by qRT-PCR (mean ± SEM, n = 4-9). **P<0.01; ***P<0.001, by Mann-Whitney test. HLO: healthy liver organoids.



Supplementary Figure 5 Lysine deprivation exerts the strongest inhibitory effects on HCC cell lin growth.(A) The effects of nine essential amino acid deprivation on SNU398 cell growth measured by MTT assay following 1, 3 and 6 days of culture (n = 8). (B) The effects of amino acid deprivation in SNU398 single cell-derived colony formation as assayed 2 weeks after seeding (n = 4). *P<0.05, by the Mann-Whitney test. AA: amino acid; “-” means deprivation.

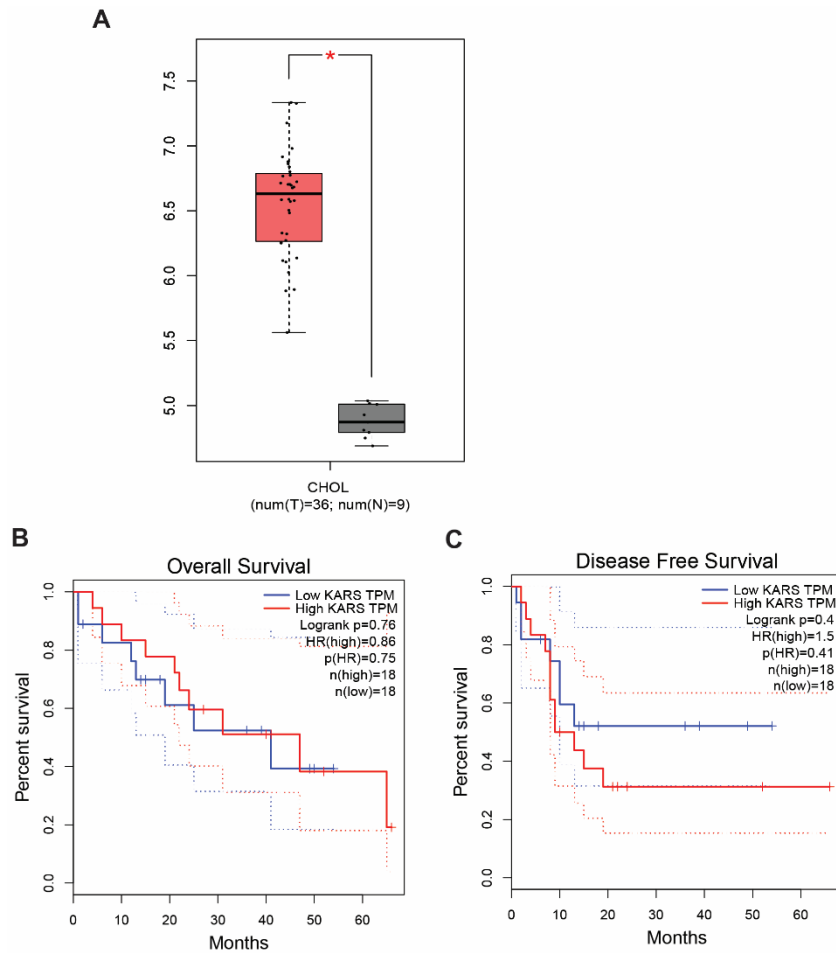


Supplementary Figure 6 The 50% inhibitory concentration of cladosporin was determined by MTT for Huh7 (A) and SNU398 (B) cells following 3 days of treatment. The drug concentrations were 1, 10, 100 and 1000 μ M. Data are presented as mean \pm SEM. (C) SNU398 cells are more sensitive than other HCC cell lines to treatment of 100 μ M cladosporin (n = 6). **P<0.01, by the Mann-Whitney test.

A**B**

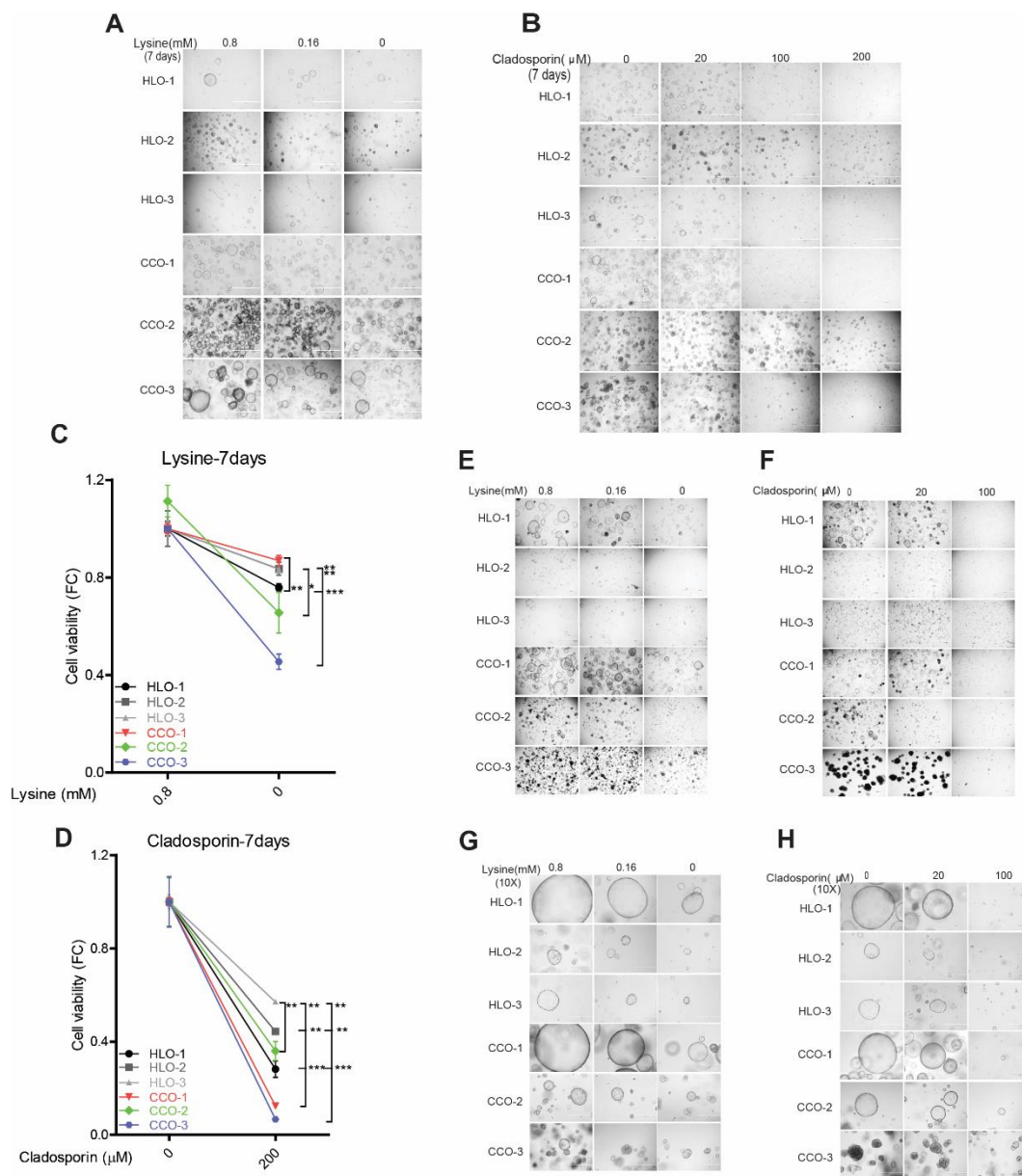
Supplementary Figure 7 The effects of combining cladosporin treatment with KARS silencing in SNU398 cells.

Cell viability (A) and single-colony formation (B) were quantified when combining *KARS1* knockdown and cladosporin treatment (n = 4 -16). *P<0.05; **P<0.01; ***P<0.001, by the Mann-Whitney test.



Supplementary Figure 8 The expression level of *KARS1* and association with patient survival in cholangiocarcinoma from the GEPIA database.

(A) The boxplot shows expression level of *KARS* in CHOL (red color, n = 36). (B) and (C) indicated overall survival and disease free survival in CHOL, respectively. *P<0.05; CHOL: cholangiocarcinoma.



Supplementary Figure 9 Lysine deprivation or treatment with cladosporin affects the growth of patient CC organoids.

(A-B) Images of organoids were treated with different concentrations of lysine (A) or cladosporin (B) for 7 days. (C-D) For cell viability of HLOs and CCOs were treated with lysine deprivation (C) or cladosporin 200 μ M (D), high expression of *KARS1*, CCO-3 more sensitive. (E-H) Images were taken after 14 days of culture. (G) and (H) show a 10x magnification highlighting the reduced organoid size after prolonged culture with lysine deprivation or cladosporin. CCO: cholangiocarcinoma organoid; HLO: human liver healthy organoid. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, by the Mann-Whitney test.

Supplementary Table 1 Patient characteristics

Characteristic	HCC patients (n=69)	Healthy controls (n=21)
Age at treatment (years)		
Mean ± SD	60 ± 16	51 ± 16.2
Median (range)	64 (11-82)	52 (13-88)
Sex – no. (%)		
Male	41 (59.4)	12 (57.1)
Female	28 (40.6)	9 (42.9)
Race – no. (%)		
White	58 (84.1)	-
African	6 (8.7)	-
Asian	4 (5.8)	-
Not reported	1 (1.4)	21 (100)
Etiology – no. (%)		
No known liver disease	20 (29.0)	NA
Alcohol	16 (23.2)	NA
NASH	8 (11.6)	NA
Hepatitis B	8 (11.6)	NA
Hepatitis C + Alcohol	5 (7.2)	NA
Hepatitis B + Alc/HepC/HepD/NASH	4 (5.8)	NA
Hepatitis C	4 (5.8)	NA
Fibrolamellar HCC	3 (4.4)	NA
Other	1 (1.5)	NA
Hepatitis status – no. (%)		
Hepatitis B or C positive	21 (30.4)	0 (0)
Chronic Hepatitis B	12 (17.4)	0 (0)
Chronic Hepatitis C	10 (14.5)	0 (0)
Cirrhosis – no. (%)		
Yes	50 (72.5)	0 (0)
No	19 (27.5)	21 (100)
Differentiation grade – no. (%)		
Good	8 (11.6)	NA
Moderate	35 (50.7)	NA
Poor	14 (20.3)	NA
Unknown	12 (17.4)	NA
Number of lesions – no. (%)		
1	38 (55.1)	NA
>1	31 (44.9)	NA
Median (range)	1 (1-11)	NA
Size of largest lesion (cm)		
Mean ± SD	7.5 ± 5.1	NA
Median (range)	6.2 (1-24)	NA
AFP level before resection (ug/l)		
Mean ± SD	46679.2 ± 375310.5	NA
Median (range)	9 (1-3118700)	NA

Supplementary Table 2 shRNA target sequences

id	symbol	targetTaxonId	targetSeq	geneDesc	oligoSeq	NCBI_genelid	cloneName	vectorId
11493	KARS	human	CGGCGAATCAACATGGTAGAA	lysyl-tRNA synthetase	CCGGCGGCGAATCAACATGGTAGAACTCGA GTTCTACCATGTTGATTCGCCGTTTTTG	3735 NM_005548.1-1214s1c1	pLKO.1	
11494	KARS	human	GCCTTTCATCACTTATCACAA	lysyl-tRNA synthetase	CCGGGCCTTTCATCACTTATCACAACTCGAG TTGTGATAAGTGATGAAAGGCTTTTTG	3735 NM_005548.1-883s1c1	pLKO.1	
11495	KARS	human	CCTGGAAGTGACTTGCATCAA	lysyl-tRNA synthetase	CCGGCCTGGAAGTGACTTGCATCAACTCGA GTTGATGCAAGTCACCTCCAGGTTTTTG	3735 NM_005548.1-1393s1c1	pLKO.1	
11496	KARS	human	CGTGGACCCAAATCAATACTA	lysyl-tRNA synthetase	CCGGCGTGGACCCAAATCAATACTACTCGA GTAGTATTGATTGGGTCCACGTTTTTG	3735 NM_005548.1-250s1c1	pLKO.1	
11497	KARS	human	CCAGAGATACTGGACTTGAT	lysyl-tRNA synthetase	CCGGCCAGAGATACTGGACTTGATCTCGA GATCAAGTCCAAGTATCTCTGTTTTTG	3735 NM_005548.1-724s1c1	pLKO.1	

Supplementary Table 3 Primer sequences

Genes	Forward primers (5'-3')	Reverse primer (5'-3')
<i>KARS1</i>	GACGCACAATCCTGAGTTCACC	AGGTGACCTTGTAAGTGCCTGTA
<i>GUSB</i>	CAGGTGATGGAAGAAGTGG	GTTGCTCACAAAGGTCACAG
<i>HPRT1</i>	GCTATAAATTCTTTGCTGACCTGCTG	AATTACTTTTATGTCCCCTGTTGACTGG
<i>PMM1</i>	CGAGTTCTCCGAAGTGGAC	CTGTTTTTCAGGGCTTCCAC
<i>GAPDH</i>	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA

Supplementary Table 4 List of tRNA expression comparison

Differences of tRNA expression between tumor and TFL patient tissues (n=69) were analyzed using the Wilcoxon matched pairs test. *P<0.05, **P<0.01, ***P<0.001. TFL:tumor-free liver. Red: significant up-regulation in tumor; Green: significant down-regulation in tumor; Black: no significant difference.

Gene	Mean		Wilcoxon matched pairs test	Gene	Mean		Wilcoxon matched pairs test
	TFL	Tumor			TFL	Tumor	
tRNA-Leu-CAA	1.522	1.277	***	tRNA-Gly-GCC	197.2	603.3	***
tRNA-Leu-CAG	5.106	3.551	***	tRNA-Ser-AGA	2.547	2.894	***
tRNA-Ile-AAU	1.37	1.254	***	tRNA-Leu-AAG	6.162	8.093	**
tRNA-Ile-GAU	1.076	0.8677	***	tRNA-Leu-UAG	8.082	8.548	**
tRNA-iMet-CAU	13.49	12.49	***	tRNA-Pro-UGG	3.571	3.923	**
tRNA-Met-CAU	6.762	5.325	***	tRNA-Trp-CCA	4.311	5.186	**
tRNA-Stop-UUA	5.32	4.172	***	tRNA-Glu-CUC	2.303	3.733	*
tRNA-His-GUG	16.01	13.99	***	tRNA-Gly-UCC	1.371	3.731	*
tRNA-Phe-GAA	1.095	0.801	***	tRNA-Leu-UAA	4.735	5.492	ns
tRNA-Val-AAC	1.8	0.6382	***	tRNA-Ser-UGA	4.997	5.848	ns
tRNA-Val-CAC	2.248	0.7724	***	tRNA-Ser-GCU	3.642	4.028	ns
tRNA-Pro-GGG	0.6167	0.4805	***	tRNA-Arg-CCU	1.474	1.573	ns
tRNA-Pro-CGG	4.063	3.23	***				
tRNA-Thr-AGU	0.748	0.5531	***				
tRNA-Thr-UGU	2.64	1.922	***				
tRNA-Thr-CGU	1.107	0.7374	***				
tRNA-Ala-CGC	10.14	4.745	***				
tRNA-Ala-GGC	3.448	1.932	***				
tRNA-Ala-UGC	8.042	4.49	***				
tRNA-Ala-AGC	1.779	1.098	***				
tRNA-Tyr-AUA	1.429	1.227	***				
tRNA-Tyr-GUA	5.609	2.763	***				
tRNA-Stop-CUA	5.562	2.651	***				
tRNA-Asn-AUU	2.535	1.669	***				
tRNA-Lys-UUU	18.79	9.738	***				
tRNA-Lys-CUU	6.867	3.389	***				
tRNA-Asp-AUC	4.942	2.412	***				
tRNA-Asp-GUC	0.8559	0.6074	***				
tRNA-Glu-UUC	0.9868	0.753	***				
tRNA-Cys-GCA	4.178	3.506	***				
tRNA-Arg-ACG	0.8352	0.6351	***				
tRNA-Arg-CCG	2.109	1.783	***				
tRNA-Ser-ACU	1.497	1.283	***				
tRNA-seCys-UCA	3.942	3.471	***				
tRNA-Val-UAC	1.783	0.4871	**				
tRNA-Ser-CGA	5.424	5.389	**				
tRNA-Pro-AGG	1.317	1.246	**				
tRNA-Gln-CUG	18.07	12.53	**				
tRNA-Cys-ACA	0.8275	0.4544	**				
tRNA-Arg-UCG	0.682	0.5212	**				
tRNA-Ile-UAU	2.046	1.529	ns				
tRNA-Gln-UUG	18.85	14.06	ns				
tRNA-Asn-GUU	0.4828	0.3918	ns				
tRNA-Arg-UCU	0.7006	0.6279	ns				
tRNA-Gly-CCC	0.4193	0.3827	ns				