Supplementary

Table S1 Clinical Characteristics of 293 HCC patients in Cohort 2

Variable	Number	Number
Age (years, >50; ≤50)	150	143
Gender (male/female)	265	28
Child-Pugh grade (A; B+C)	268	25
AFP (μg/L, >20; ≤20)	186	107
HBsAg (positive/negative)	256	37
Edmonson's grade (III+IV; I+II)	238	55
Tumor size (cm, >5; ≤5)	145	148
Tumor number (multiple/solitary)	59	234
Sub-foci (present/absent)	190	103
Microvascular invasion (present/absent)	155	138
HBeAg (positive/negative)	60	233
BCLC stage (B+C; A)	174	119
All cases	293	

Table S2 Clinical Characteristics of 90 HCC patients in Cohort 3

Variable	Number	Number
Age (years, >50; ≤50)	80	10
Gender (male/female)	53	37
Child-Pugh grade (A; B+C)	86	4
AFP (μg/L, >20; ≤20)	54	36
HBsAg (positive/negative)	71	19
Edmonson's grade (III+IV; I+II)	44	36
Tumor size (cm, >5; ≤5)	28	62
Tumor number (multiple/solitary)	11	79
Sub-foci (present/absent)	63	27
Microvascular invasion (present/absent)	68	22
HBeAg (positive/negative)	-	-
BCLC stage (B+C; A)	80	10
ALT (U/L; >40;<40)	53	37
TBil(umol/l;>17.1;<17.1)	25	65
GGT(U/L;>40;<40)	60	30
Encapsule(present/absent)	42	48
Cirrhosis nodule(multiple/solitary)	81	9
All cases	90	

Table S3 Sequences of primers for quantitative real-time PCR

Primer	Sequences (5'-3')
hum-YTHDF1-F	ACCTGTCCAGCTATTACCCG
hum-YTHDF1-R	TGGTGAGGTATGGAATCGGAG
hum-YTHDF2-F	AGCCCCACTTCCTACCAGATG
hum-YTHDF2-R	TGAGAACTGTTATTTCCCCATGC
hum-18S-F	GGAGAGGGAGCCTGAGAAACG
hum-18S-R	TTACAGGGCCTCGAAAGAGTCC
hum-YTHDF3-F	TCAGAGTAACAGCTATCCACCA
hum-YTHDF3-R	GGTTGTCAGATATGGCATAGGCT
hum-ACTB-F	CATGTACGTTGCTATCCAGGC
hum-ACTB-R	CTCCTTAATGTCACGCACGAT

Table S4 The details of antibodies used in the study

Antibody names	Brand	Product code	Usage
Anti-YTHDF1	Abcam	Ab230330	IHC, 1:200
Anti-YTHDF1	Abcam	Ab220162	WB, 1:1000 IP, 1:20
Anti-YTHDF1	Proteintech	17479-1-AP	WB, 1:1000
Anti-YTHDF2	Proteintech	24744-1-AP	WB, 1:1000 IHC, 1:200
Anti-YTHDF3	Proteintech	25537-1-AP	WB, 1:1000
Anti-β-actin	Servicebio	GB15001	WB, 1:1000
IRdye680 Goat anti-mouse IgG	Licor	926-32220	WB, 1:10000
IRdye800 Goat anti-rabbit IgG	Licor	926-32211	WB, 1:10000
Goat anti-Rabbit IgG HRP	Abcam	Ab205718	IHC, 1:2000

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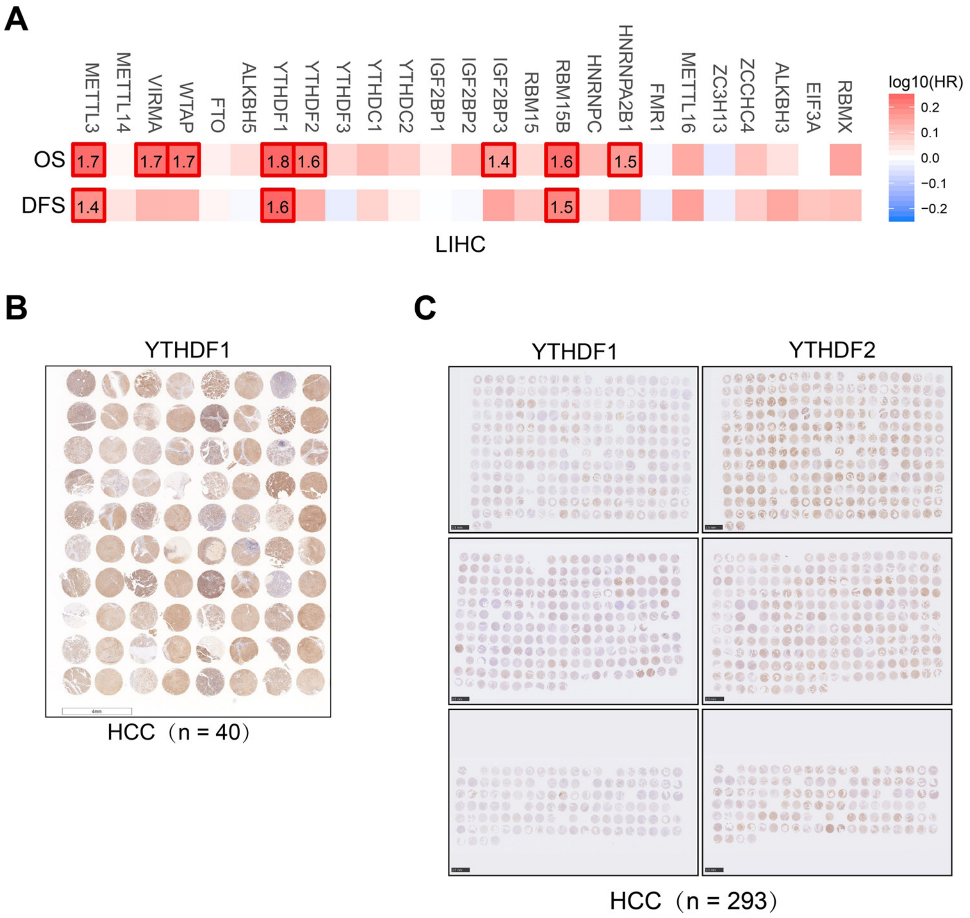

Figure S1 The association between m6A-related enzymes or proteins and the prognosis in HCC. (A) Correlations between m6A-related genes and OS/DFS in liver cancer from TCGA database. Color from red to blue represents risk factor to protective factor and solid-line boxes represent statistical significance, and the number inside the box represents the index of hazard ratio (HR). (B) Overview of immunohistochemical staining of YTHDF1 in HCC tissue array from Cohort 1 (n=40). (C) Overview of immunohistochemical staining of YTHDF1 and YTHDF2 in HCC tissue arrays from Cohort 2 (n=293).

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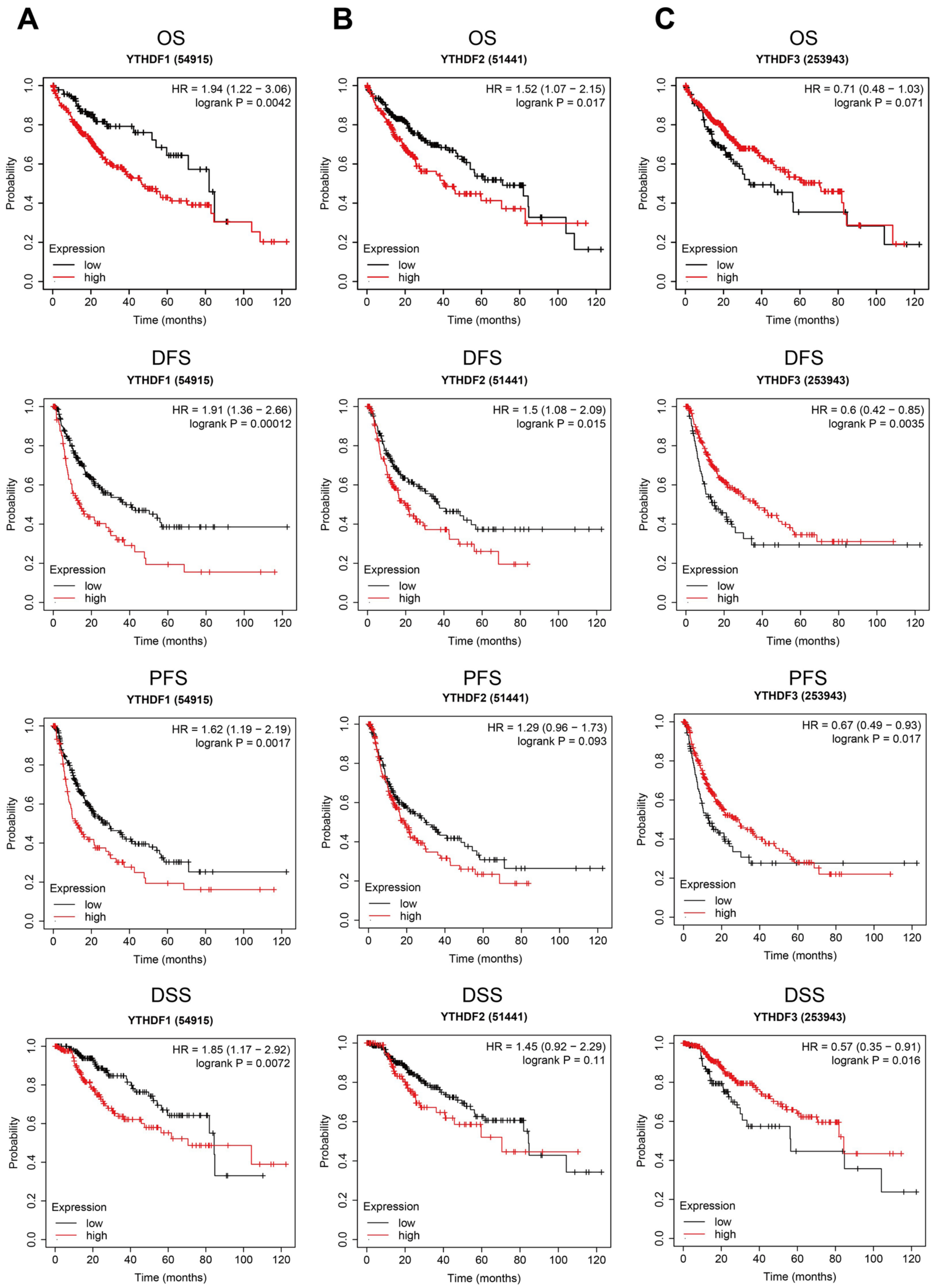

Figure S2 The association between YTHDF paralogs and the prognosis in HCC. (A) Kaplan-Meier survival curves showing the correlation between the expression of YTHDF1 and overall survival (OS), disease-free survival (DFS), progression-free survival (PFS), and disease-specific survival (DSS) using the Kaplan-Meier Plotter database. (B) Kaplan-Meier survival curves showing the correlation between the expression of YTHDF2 and the above four prognostic indicators using the Kaplan-Meier Plotter database. (C) Kaplan-Meier survival curves showing the correlation between the expression of YTHDF3 and the above four prognostic indicators using the Kaplan-Meier Plotter database.

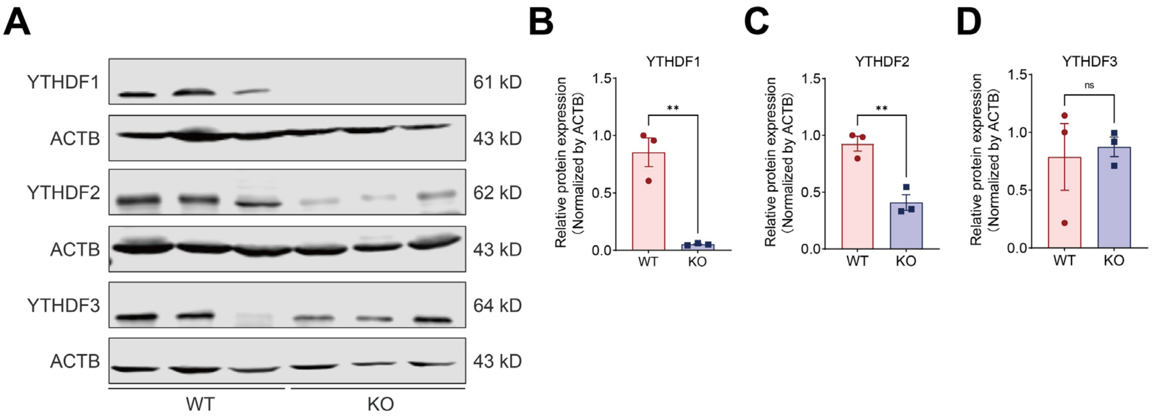

Figure S3 The expression of YTHDF paralogs in *Ythdf1* hepatocyte-specific knockout mice. (A) Western blotting showing the expression of YTHDF paralogs in the wild-type and *Ythdf1* hepatocyte-specific knockout mice. (B) Relative quantitative analysis of YTHDF1 in WT and KO mice (n=3). (C) Relative quantitative analysis of YTHDF2 in WT and KO mice (n=3). (D) Relative quantitative analysis of YTHDF3 in WT and KO mice (n=3). Data are represented as mean ± SEM. NS not significant, **, P<0.01.

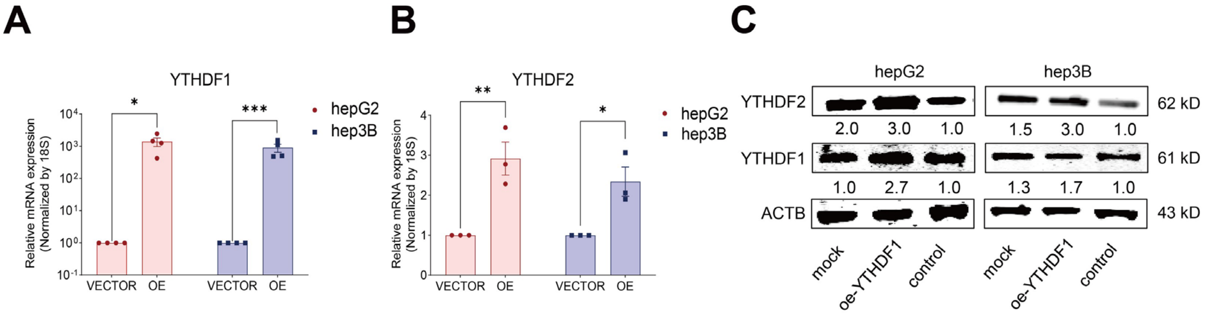

Figure S4 YTHDF1 regulates the mRNA and protein expression of YTHDF2. (A) The mRNA levels of YTHDF1 after overexpressing YTHDF1 in HepG2 and Hep3B cells using plasmid vector. (B) The mRNA levels of *YTHDF2* after overexpressing YTHDF1 in HepG2 and Hep3B cells using plasmid vector. (C) The protein levels of YTHDF1 and YTHDF2 after overexpressing YTHDF1 in HepG2 and Hep3B cells using plasmid vector. Data are represented as mean ± SEM. NS not significant, *, P<0.05, **, P<0.01, ***, P<0.001.

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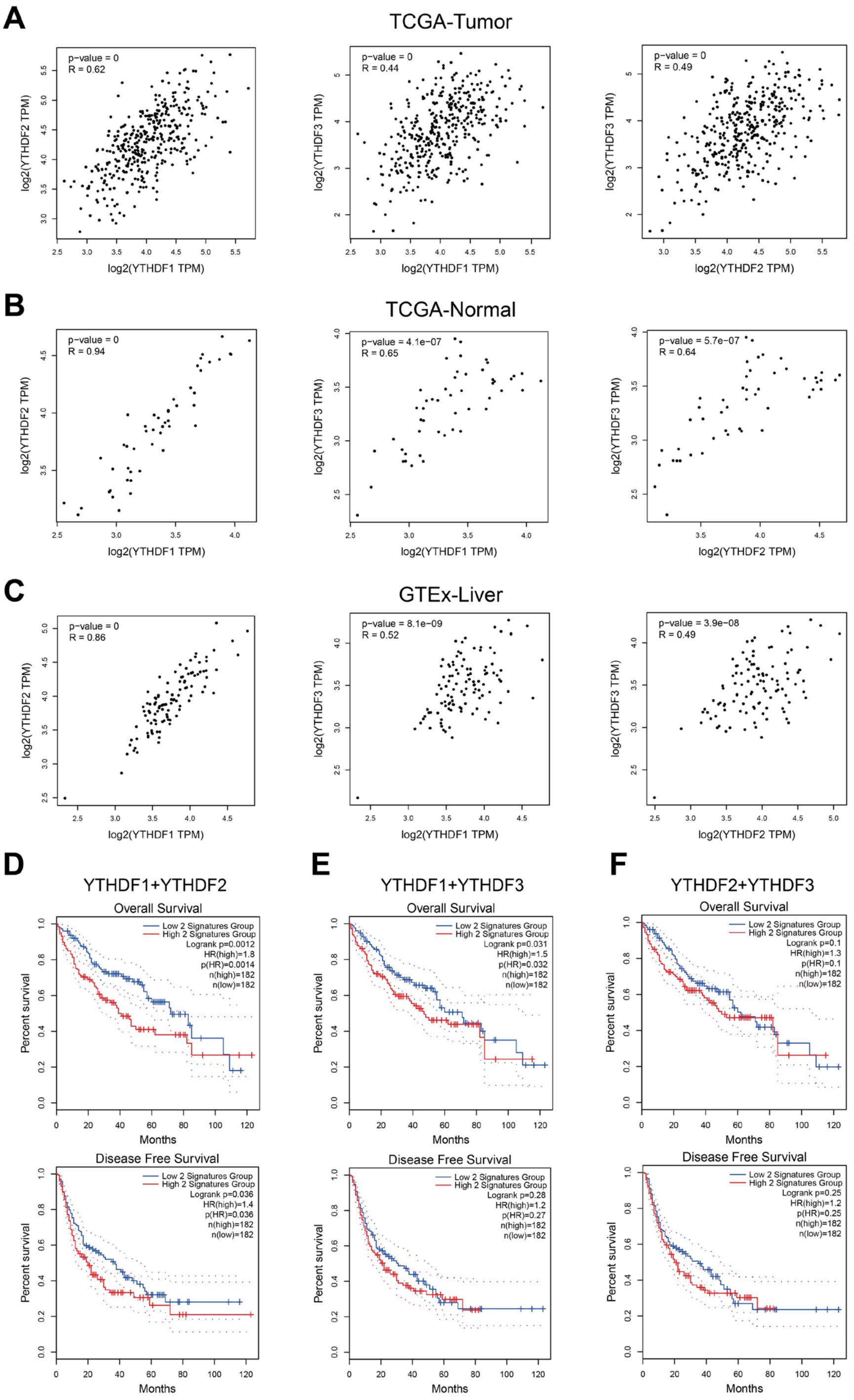

Figure S5 The correlations between YTHDF1 and YTHDF2 in TCGA Tumor/Normal/GTEx-liver database. (A) The correlation between YTHDF1 and YTHDF2 in the TCGA-Tumor database. (B) The correlation between YTHDF1 and YTHDF2 in the TCGA-Normal database. (C) The correlation between YTHDF1 and YTHDF2 in the TCGA GTEx-Liver database. (D) Kaplan-Meier survival curves showing the correlation between combined YTHDF1 and YTHDF2 expression and the prognosis in HCC patients using TCGA database. (E) Kaplan-Meier survival curves showing the correlation between combined YTHDF1 and YTHDF3 expression and the prognosis in HCC patients using TCGA database. (F) Kaplan-Meier survival curves showing the correlation between combined YTHDF2 and YTHDF3 expression and the prognosis in HCC patients using TCGA database.

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