

## Supplementary materials

**Table S1** Patient demographics and clinicopathological features

Characteristics	Intra-hepatic CCA	Extra-hepatic CCA
Number	<i>N</i> = 18 (52.0%)	<i>N</i> = 20 (48.0%)
Age (years, median and range)	61 (45–79)	59 (33–86)
Sex (%)		
Male	10 (55.6)	11 (55.0)
Female	8 (44.4)	9 (45.0)
HBV+ (%)	9 (50.0)	2 (10.0)
Tumor size (cm, median and range)	5.0 (1.2–12.0)	2.7 (1.2–5.0)
CA19-9 (U/mL, median and range)	213.2 (0.6–17929.9)	160.6 (0.69–3925)
Differentiation stage (%)		
Well	5 (27.7)	6 (30.0)
Moderately	6 (33.3)	7 (35.0)
Moderately-poorly	1 (5.6)	0 (0.0)
Poorly	3 (16.7)	4 (20.0)
N/A <sup>a</sup>	3 (16.7)	3 (15.0)

<sup>a</sup>Not applicable.

**Table S2** Sequences for primers, siRNAs, and shRNAs used in this study

Primers	Sequences (5' → 3')
qPCR primers	
qMETTL3-forward	TTGTCTCCAACCTCCG TAGTG
qMETTL3-reverse	CCAGATCAGAGAGGTGGTGTAG
qMETTL14-forward	AGAAACTTGCAGGGCTTCCTAT
qMETTL14-reverse	TCTTCTCATATGGCAAATTTTCT
qWTAP-forward	CCTCTCCCAAGAAGGTTTCGATT
qWTAP-reverse	TGCAGACTCCTGCTGTTGTTG
qGAPDH-forward	GTCTCCTCTGACTTCAACAGCG
qGAPDH-reverse	ACCACCCTGTTGCTGTAGCCAA
qIGF2BP2-forward	GTTGGTGCCATCATCGAAAGG
qIGF2BP2-reverse	TGGATGGTGACAGGCTTCTCTG
qCTNNB1-forward	CATCTACACAGTTTGATGCTGCT
qCTNNB1-reverse	GCAGTTTGTGAGTTCAGGGA
qSOX4-forward	GCACTAGGACGTCTGCCTTT
qSOX4-reverse	ACACGGCATATTGCACAGGA

Table S2 Continued

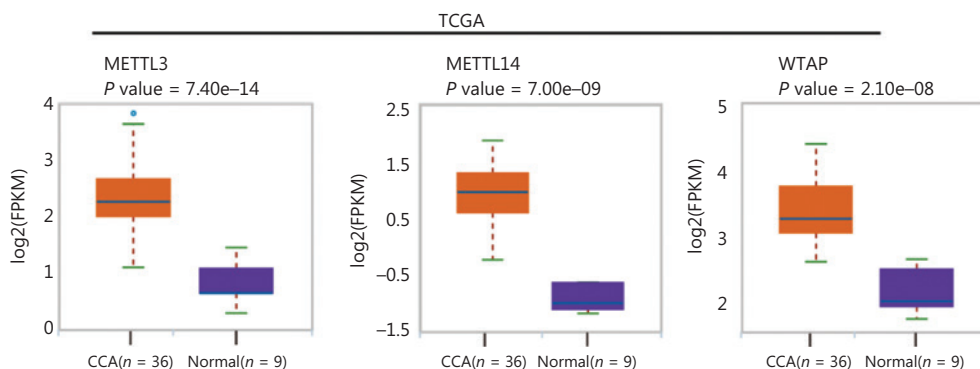
Primers	Sequences (5' → 3')
qSOX6-forward	CAACACCAGGAAGAGACTTACGA
qSOX6-reverse	TCAAAGCGCGTTCTTTCCTTT
qCD133-forward	AGTCGGAAACTGGCAGATAGC
qCD133-reverse	GGTAGTGTGTACTGGGCAAT
qNanog-forward	AAGGTCCCGGTCAAGAAACAG
qNanog-reverse	CTTCTGCGTCACACCATTGC
qHPRT1-forward	CATTATGCTGAGGATTTGGAAAGG
qHPRT1-reverse	CTTGAGCACACAGAGGGCTAC
ChIP primers for qPCR	
ChIP-METTL3-forward	GAGAGAGCGGGTATGAGGACTAT
ChIP-METTL3-reverse	GCTGCTTCTTGTGGCCTGGAT
ChIP-METTL14-forward	GATGAGATTGCAGCACCTCGATCA
ChIP-METTL14-reverse	AGCACCATCTTACCACTCTTCCAA
meRIP primers for qPCR	
meRIP-CTNNB1-forward	TCTGAACTTGCATTGTGATTGGC
meRIP-CTNNB1-reverse	GTCGTTCCGCAACAAGTCC
meRIP-SOX6-forward	GGCAGCAAATGGACCTTG
meRIP-SOX6-reverse	GGGAAGAGGAATCCCTGTTGG
meRIP-SOX4-forward	AGTTTTCTAGAGACTGAAGGAGTCTCC
meRIP-SOX4-reverse	CCTGCGTGGAGTCTCTCAGT
SiRNA/shRNA	
Si-METTL3-1-S	5'CUGCAAGUAUGUUCACUAUGA dTdT 3'
Si-METTL3-1-AS	3'dTdT GACGUUCAUACAAGUGAUACU 5'
Si-METTL3-2-S	5'AGGAGCCAGCCAAGAAAUCA dTdT 3'
Si-METTL3-2-AS	3'dTdT UCCUCGGUCGGUUCUUUAGUU 5'
Si-METTL14-1-S	5'AAGGAUGAGUUAUAGCUAAA dTdT 3'
Si-METTL14-1-AS	3'dTdT UCCUACUCAAUUAUCGAUUU 5'
Si-METTL14-2-S	5'UGGUGCCGUGUAAAUAAGCAA dTdT 3'
Si-METTL14-2-AS	3' dTdT ACCACGGCACAAUUUAUCGUU 5'
Si-WTAP-1-S	5'AAGGUUCGAUUGAGUGAAACA dTdT 3'
Si-WTAP-1-AS	3' dTdT UCCAAGCUAACUCACUUUGU 5'
Si-WTAP-2-S	5'GGGCAAGUACACAGAUCUUAA dTdT 3'
Si-WTAP-2-AS	3' dTdT CCCGUUCAUGUGUCUAGAAUU 5'
Si-IGF2BP2-S	5'GCAAUUUAUACGAGGUUGU dTdT 3'
Si-IGF2BP2-AS	3' dTdT CGUUAAAUAUGCUCCAACA 5'

Table S2 Continued

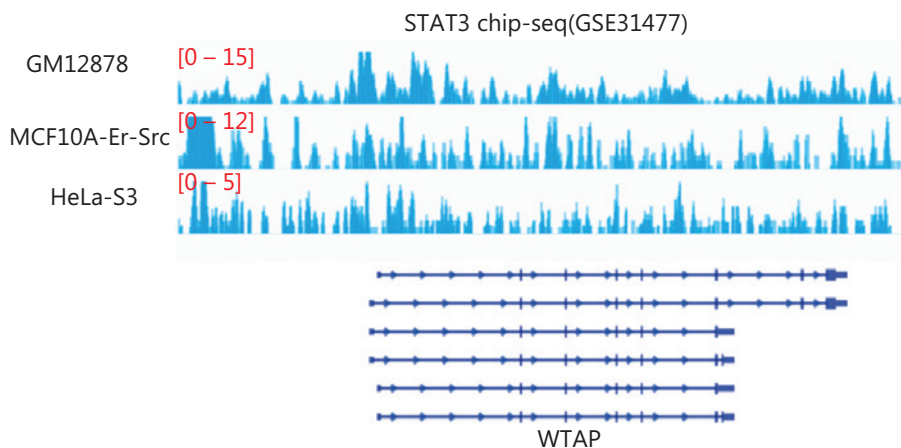
Primers	Sequences (5' → 3')
Sh-METTL3-1-S	5'-GATCCCTGCAAGTATGTTCACTATGACTTCCTGTCAGATCATAGTGAACATACTTGCGAG TTTTGG-3'
Sh-METTL3-1-AS	5'-AATTCAAAACTGCAAGTATGTTCACTATGATCTGACAGGAAGTCATAGTGAACATACTTGCGAGG-3'
Sh-METTL3-2-S	5'-GATCCAGGAGCCAGCCAAGAAATCAACTTCCTGTCAGATTGATTTCTTGGCTGGCTCCTTTTTTG-3'
Sh-METTL3-2-S	5'-AATTCAAAAAGGAGCCAGCCAAGAAATCAATCTGACAGGAAGTTGATTTCTTGGCTGGCTCCTG-3'
Sh-METTL14-1-S	5'-GATCCAAGGATGAGTTAATAGCTAACTTCCTGTCAGA TTAGCTATTAACATCATCCTT TTTTGG-3'
Sh-METTL14-1-AS	5'-AATTCAAAAAGGATGAGTTAATAGCTAACTTCCTGACAGGAAGTTAGCTATTAACATCATCCTT G-3'
Sh-METTL14-2-S	5'-GATCCTGGTGCCGTGTTAAATAGCAACTTCCTGTCAGATTGCTATTTAACACGGCACCA TTTTGG-3'
Sh-METTL14-2-AS	5'-AATTCAAAAATGGTGCCGTGTTAAATAGCAATCTGACAGGAAGTTGCTATTTAACACGGCACCAG-3'
Sh-WTAP-1-S	5'-GATCCAAGGTTGCGATTGAGTGAAACACTTCCTGTCAGATGTTTCACTCAATCGAACCTT TTTTGG-3'
Sh-WTAP-1-AS	5'-AATTCAAAAAGGTTGCGATTGAGTGAAACATCTGACAGGAAGTTTCACTCAATCGAACCTT G-3'
Sh-WTAP-2-S	5'-GATCCGGGCAAGTACACAGATCTTAATTCCTGTCAGATTAAGATCTGTGTAAGTACTGCCC TTTTGG-3'
Sh-WTAP-2-AS	5'-AATTCAAAAAGGGCAAGTACACAGATCTTAATCTGACAGGAAGTTAAGATCTGTGTAAGTACTGCCC G-3'
Sh-IGF2BP2-S	5'-GATCCGCAATTTATACGAGGTTGTCTTCTGTCAGAACCAACCTCGTATAAATTGC TTTTGG-3'
Sh-IGF2BP2-AS	5'-AATTCAAAAAGCAATTTATACGAGGTTGTCTGTCAGGAAGACAACCTCGTATAAATTGC G-3'

Table S3 All of the antibodies and reagents used in this study

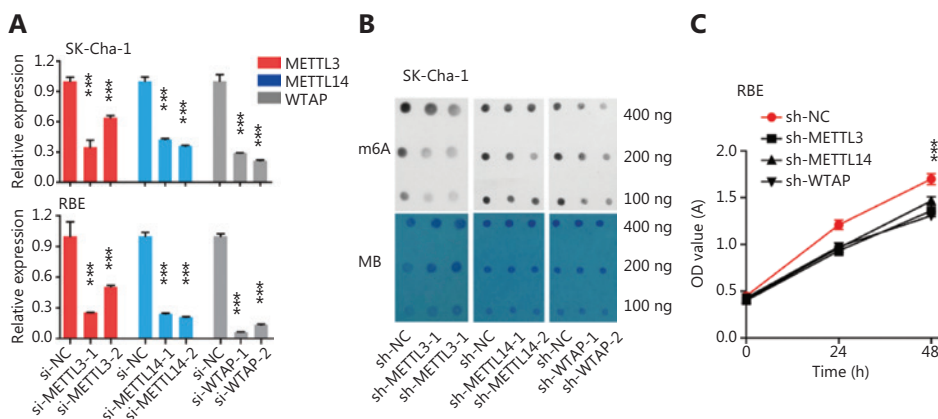
Name	Species	Company	NO.
CD133	Rabbit	Proteintech	18470-1-AP
P-STAT3	Rabbit	CST	9145S
T-STAT3	Rabbit	Santa Cruz	sc-8019
METTL3	Rabbit	Proteintech	15073-1-AP
METTL14	Rabbit	Proteintech	26158-1-AP
WTAP	Mouse	Proteintech	60188-1-Ig
CTNNB1	Rabbit	CST	8480S
GAPDH polyAb	Rabbit	Proteintech	10494-1-AP
β-actin	Mouse	Sigma-Aldrich	A2228
Goat Anti-Mouse IgG HRP	Mouse	Thermo Fisher	H10007
Goat Anti-Rabbit IgG HRP	Rabbit	Thermo Fisher	A18903
m6A	Rabbit	Synaptic System	202003
IGF2BP2	Mouse	Santa Cruz	sc-377014



**Figure S1** Expression of m6A genes in The Tumor Cancer Genome Atlas (TCGA) database. The expressions of METTL3, METTL14, and WTAP in TCGA database of cholangiocarcinomas.

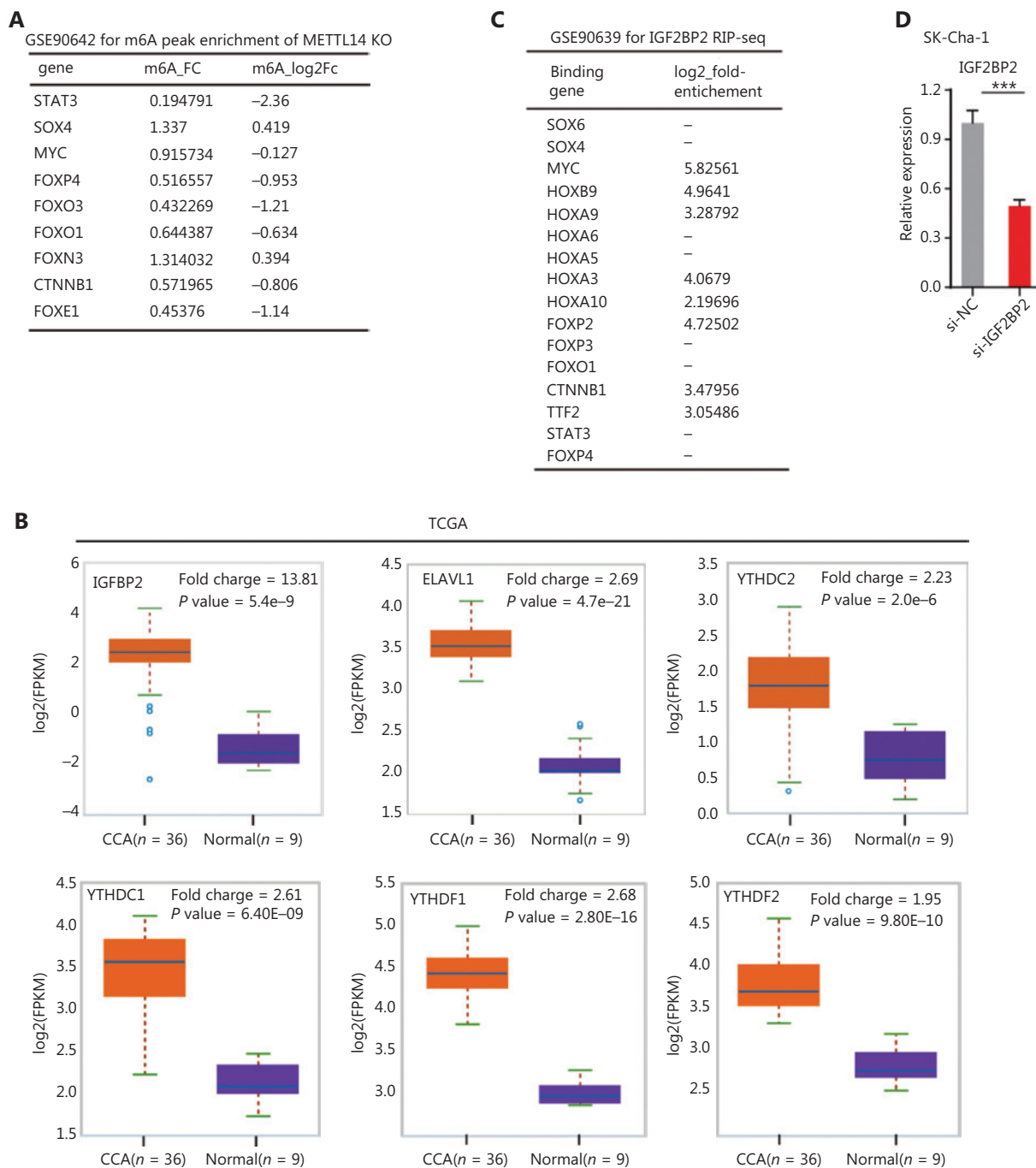


**Figure S2** STAT3 was located at the genetic locus of WTAP in the GES31477 data set.

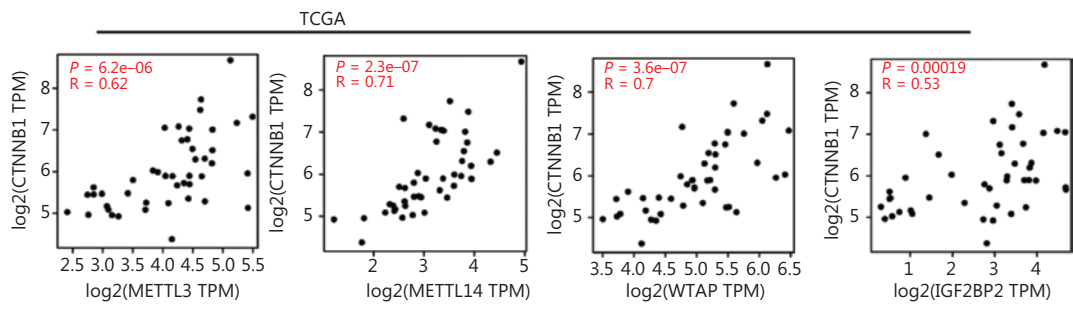


**Figure S3** Knocking down m6A writers inhibited cholangiocarcinoma (CCA) cell proliferation. (A) The qRT-PCR showing the knockdown efficiency of METTL3, METTL14, and WTAP in CCA cells. Error bars denote  $\pm$  SEM (\*\*\*)  $P < 0.001$  in 3 independent experiments. (B) Dot blots showing the global m6A levels after knocking-down m6A genes. (C) Cell proliferation was measured using a CCK-8 assay at 0, 24, and 48 h in METTL3-, METTL14-, and WTAP-silenced CCA cells. Error bars denote  $\pm$  SEM (\*\*\*)  $P < 0.001$  in 3 independent experiments.





**Figure S5** IGF2BP2 improved the RNA stability and translation of stemness-related genes. (A) The GSE90642 data set showing the enrichment of m6A modifications of some stemness-related genes between METTL14 silenced and control cells. (B) The expression patterns of IGF2BP2, ELAVL1, YTHDC2, YTHDC1, YTHDF1, and YTHDF2 in The Cancer Genome Atlas datasets consist of 36 CCA and 9 healthy samples. (C) The GSE90639 data set showing the IGF2BP2-enrichment of many stemness-related genes. (D) The qRT-PCR showing the expression levels of IGF2BP2 in the IGF2BP2 knockdown cells. Error bars denote  $\pm$  SEM (\*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ) in 3 independent experiments.



**Figure S6** The expression correlations of m6A genes and CTNNB1. Scatter plot showing the correlation of expression between m6A genes and CTNNB1 in the TCGA database of CCA.