Supplemental Information

YTHDF1 Facilitates the Progression of Hepatocellular Carcinoma by Promoting FZD5 mRNA Translation in an m6A-Dependent Manner

Xiangxiang Liu, Jian Qin, Tianyi Gao, Chenmeng Li, Bangshun He, Bei Pan, Xueni Xu, Xiaoxiang Chen, Kaixuan Zeng, Mu Xu, Chengbin Zhu, Yuqin Pan, Huiling Sun, Li Sun, Tao Xu, and Shukui Wang
Fig. S1 YTHDF1 is upregulated in HCC and associated with prognosis of patients, related to Figure 1. 

- **a** H-score of YTHDF1 in HCC tissues and ANTs.
- **b** Detection of YTHDF1 protein levels in HCC cell lines and LO2 cells by western blot.
- **c** Kaplan-Meier survival curves of OS, RFS, PFS, and DSS of HCC patients based on YTHDF1 expression in Kaplan-Meier Plotter database.
Fig. S2 c-MYC and USF1 promote YTHDF1 expression, related to Figure 2. 

a qRT-PCR analysis of the effect of transfecting c-MYC and USF1 overexpression vectors in HepG2 and MHCC-LM3 cells. b qRT-PCR analysis of inhibitory effect of si-c-MYC and si-USF1 in HepG2 and MHCC-LM3 cells. c c-MYC/MAX and USF1 expression was positively correlated with YTHDF1 expression in HCC based on TCGA database.
Table S1: The correlations between YTHDF1 expression and clinical characteristics of HCC patients \((n = 84)\).

| Clinicopathologic parameters | Number | YTHDF1 expression | P value |
|------------------------------|--------|-------------------|---------|
|                              |        | High | Low   |        |
| Gender                       |        |      |       |       |
| Male                         | 53     | 28   | 25    | 0.65   |
| Female                       | 31     | 14   | 17    | 0.65   |
| Age (years)                  |        |      |       |       |
| \(\geq 60\)                  | 47     | 24   | 23    | 0.91   |
| \(<60\)                      | 37     | 18   | 19    | 0.91   |
| HBV surface antigen          |        |      |       |       |
| Negative                     | 27     | 12   | 15    | 0.61   |
| Positive                     | 57     | 30   | 27    | 0.61   |
| Cirrhosis                    |        |      |       |       |
| Absent                       | 27     | 11   | 16    | 0.36   |
| Present                      | 57     | 31   | 26    | 0.36   |
| Tumor size (cm)              |        |      |       |       |
| \(\geq 5\)                   | 45     | 28   | 17    | 0.028  |
| \(<5\)                       | 39     | 14   | 25    | 0.028  |
| Differentiation              |        |      |       |       |
| High + moderate              | 41     | 19   | 22    | 0.67   |
| Low                          | 43     | 23   | 20    | 0.67   |
| TNM stage                    |        |      |       |       |
| I+II                         | 65     | 27   | 36    | 0.042  |
| III+IV                       | 19     | 15   | 6     | 0.042  |
| Barcelona stage              |        |      |       |       |
| A+B                          | 60     | 26   | 34    | 0.09   |
| C+D                          | 24     | 16   | 8     | 0.09   |
| Microvascular invasion       |        |      |       |       |
| Absent                       | 40     | 14   | 26    | 0.016  |
| Present                      | 44     | 28   | 16    | 0.016  |
Table S2: RNA sequences used in this study.

| Name           | Sequence (5’- 3’)               | Application          |
|----------------|---------------------------------|----------------------|
| si-myc#1       | GCGAGGATATCTGGGAAGAA            | siRNA target site    |
| si-myc#2       | CGATGTTGTCTGAGAA               | siRNA target site    |
| si-myc#3       | GGCGAACAACACAAACGUCUU          | siRNA target site    |
| si-USF1#1      | GCTGGACAATGACGTGCTT            | siRNA target site    |
| si-USF1#2      | GACGACTCGGGATGAGAAA            | siRNA target site    |
| si-USF1#3      | CGCCGAGACAAGATCAACA            | siRNA target site    |
| si-METTL3#1    | GCACITTGGATCTACGGGAAT          | siRNA target site    |
| si-METTL3#2    | CGACTACAGTAGCTGCCTT            | siRNA target site    |
| si-METTL3#3    | CAGTGGATCTGTGTGTGATA           | siRNA target site    |
| sh-YTHDF1      | CGAAAGAGTTTGAGTGGAA            | shRNA target site    |
| sg-YTHDF1      | CACGCCACGTTGACTTC             | CRISPR/dcas9 target site |
Table S3: Primer sequences used in this study.

| Name                     | Sequence (5’-3’)                     | Application     |
|--------------------------|--------------------------------------|-----------------|
| YTHDF1- forward          | GACATTGGGCACCTGGGATA                 | qRT-PCR         |
| YTHDF1-Reverse           | GCTCTGATACTGCGTTGAG                  | qRT-PCR         |
| FZD5- forward            | GTCACACCCGCTCTACAACA                 | qRT-PCR         |
| FZD5-Reverse             | GGACGTGGAGATGAAGCACA                 | qRT-PCR         |
| USF1- forward            | GTGATCCAGGGGTCTTTTCAC                | qRT-PCR         |
| USF1-Reverse             | CCTCTGAGCCCTGGGTAGTA                 | qRT-PCR         |
| c-MYC- forward           | CCACGAAACTTTGCCCATAG                 | qRT-PCR         |
| c-MYC-Reverse            | TGCAAGGAGAGCCTTTTCAGAG               | qRT-PCR         |
| METTL3- forward          | CTATCTCCTGGCACTCGCAAGA               | qRT-PCR         |
| METTL3-Reverse           | GCTTGAAACCGTGCAACCACCATC             | qRT-PCR         |
| GAPDH- forward           | GAACGGGAAGCTCACTGG                   | qRT-PCR         |
| GAPDH-Reverse            | GCCTGCTTCACCACCTTCTT                | qRT-PCR         |
| FZD5 m6A- forward        | GGCGCTGAGCTCCGTGGAC                  | MeRIP-qRT-PCR   |
| FZD5 m6A-Reverse         | CGGCAGCCTCGCTCAATCC                  | MeRIP-qRT-PCR   |
| YTHDF1 promoter- forward 1 | CGCGACCTGGAAACACGC                 | ChIP-qRT-PCR   |
| YTHDF1 promoter- Reverse 1 | AGGTCCGAAGACTAAACGC          | ChIP-qRT-PCR   |
| YTHDF1 promoter- forward 2 | TGTGTTGGCGGCTGTA                   | ChIP-qRT-PCR   |
| YTHDF1 promoter- Reverse 2 | TCCAGTCTCGTGCGG                | ChIP-qRT-PCR   |
**Table S4:** Antibodies used in this study.

| Antibody   | Company                              | Application | Concentration |
|------------|--------------------------------------|-------------|---------------|
| YTHDF1     | Proteintech (17479-1-AP)             | WB          | 1:1000        |
|            |                                      | IHC         | 1:100         |
|            |                                      | RIP         | 2.0 μg        |
| FZD5       | Abcam (ab75234)                      | WB          | 1:500         |
|            |                                      | IHC         | 4.0 μg        |
| c-MYC      | Proteintech (10828-1-AP)             | WB          | 1:2000        |
|            |                                      | ChIP        | 2.0 μg        |
|            | Abcam (ab32072)                      | IHC         | 1:100         |
| USF1       | SANTA CRUZ (sc-390027)               | IHC         | 1:50          |
|            |                                      | ChIP        | 2.0           |
| β-catenin  | Proteintech (51067-2-AP)             | WB          | 1:2000        |
|            |                                      | IHC         | 1:1000        |
| GAPDH      | Proteintech (60004-1-Ig)             | WB          | 1:5000        |
| β-actin    | Proteintech (60008-1-Ig)             | WB          | 1:5000        |
| HRP-conjugated Affinipure Goat Anti-Mouse IgG(H+L) | Proteintech (SA00001-1) | WB | 1:10000 |
| HRP-conjugated Affinipure Goat Anti-Rabbit IgG(H+L) | Proteintech (SA00001-2) | WB | 1:10000 |