Supplementary Information

Differential roles of YTHDF1 and YTHDF3 in embryonic stem cell-derived cardiomyocyte differentiation

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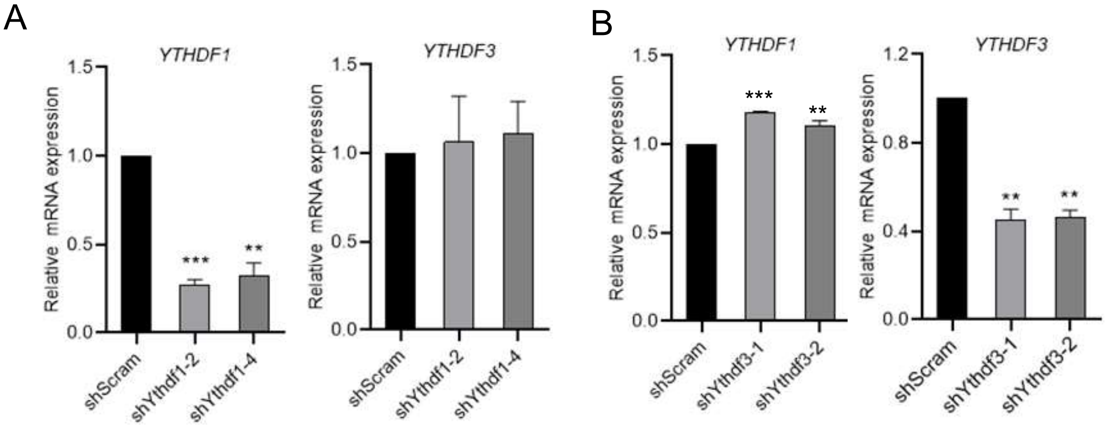

Figure S1. Detection of *YTHDF* mRNA levels in *Ythdf*-knockdown cells. (A) Detection of *YTHDF1* and *YTHDF3* mRNA levels in *Ythdf1*-knockdown ESCs by qRT-PCR. (B) Detection of *YTHDF1* and *YTHDF3* mRNA levels in *Ythdf3*- knockdown ESCs by qRT-PCR. Error bars, mean ± s.e.m.; $^{**}P < 0.01$, $^{***}P < 0.001$, unpaired two tailed *t*-test; $n = 4$ independent experiments.

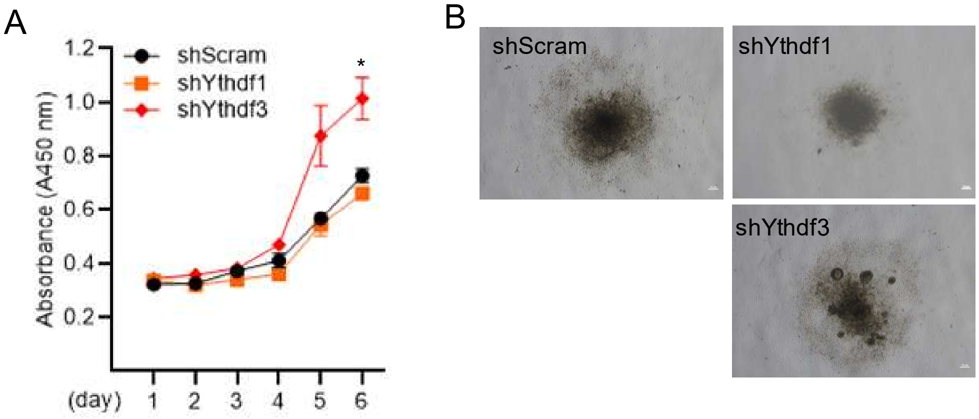

Figure S2. Cell growth and embryonic bodies (EBs) formation of *Ythdf*-knockdown cells. (A) Measurement of cell growth rate in ESCs lacking either YTHDF1 or YTHDF3. Error bars, mean ± s.e.m.; $*P < 0.05$, unpaired two tailed *t*-test; $n = 3$ independent experiments. (B) Representative morphologic images of *Ythdf1*- and *Ythdf3*-knockdown EBs compared with shScram EBs at day 4.

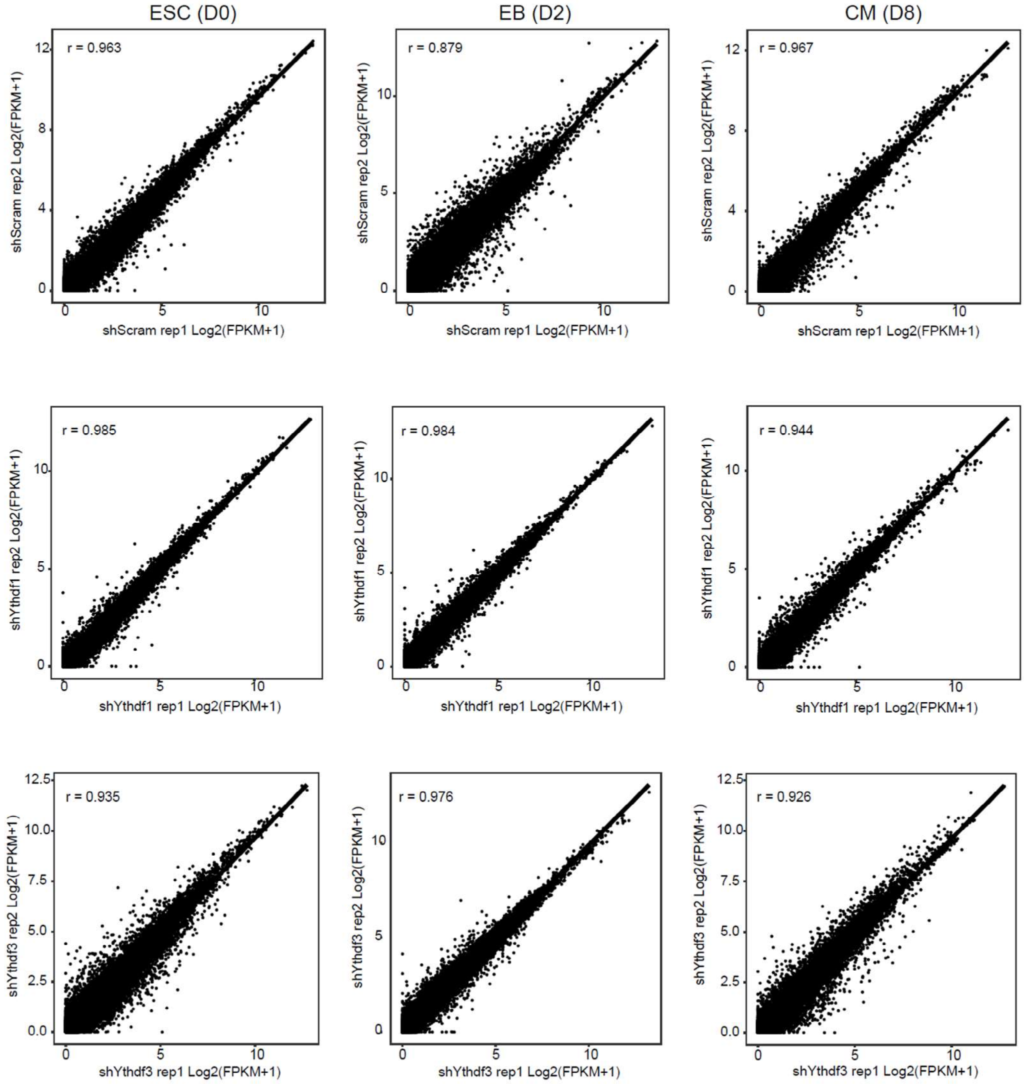

Figure S3. Reproductively of RNA-sequencing data. Pearson's correlation of reads enrichment as $\log_2$(FPKM+1) between two biological replicates in control and *Ythdf*-KD ESC (day 0), EB (day 2) and CM (day 8).

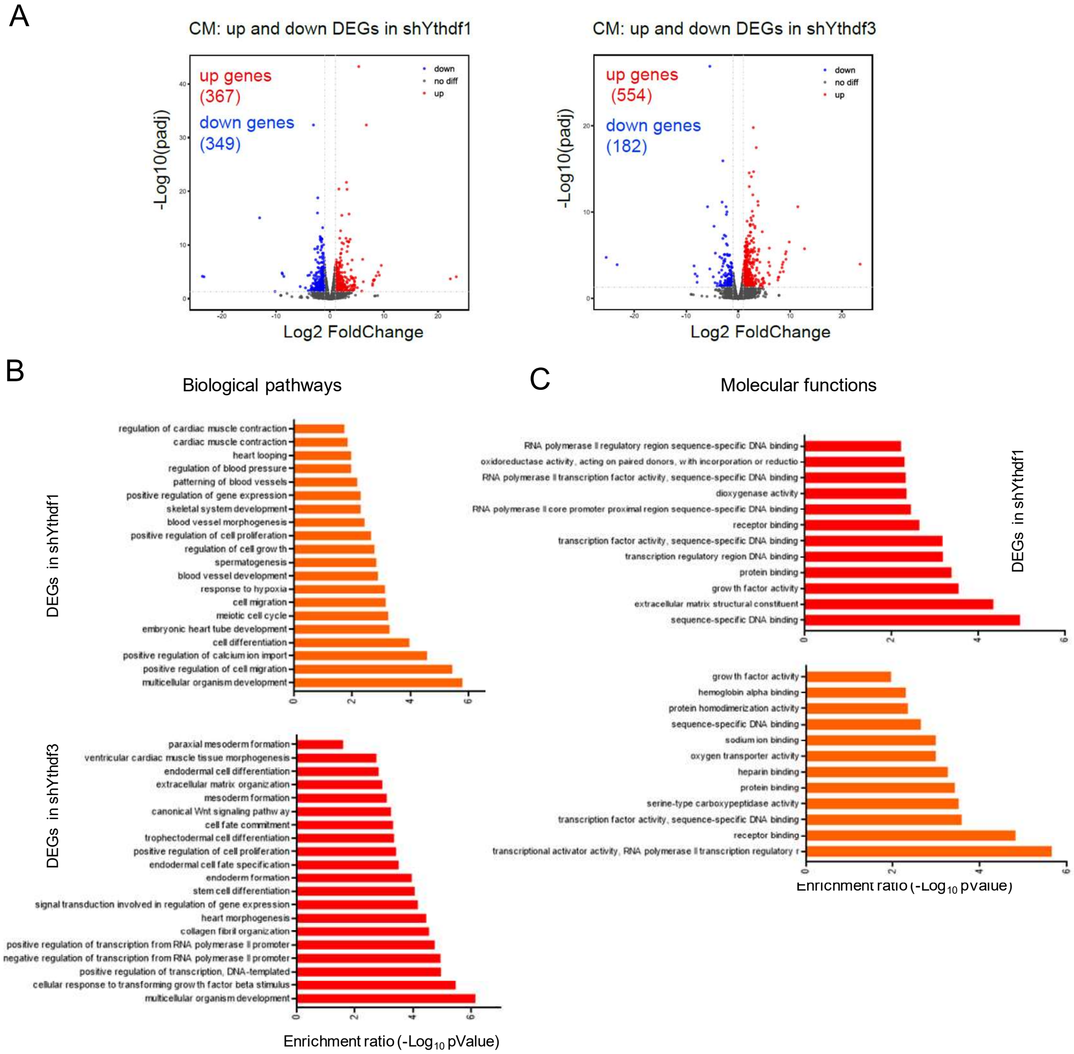

Figure S4. GO analysis of significantly differentially expressed genes in *Ythdf*-KD CMs. (A) Volcano plot of differentially expressed genes between the control and *Ythdf*-KD CMs. (B-C) GO analysis of significantly differentially expressed genes in *Ythdf*-KD CMs versus control CMs by RNA-seq. The *P*-value for the enrichment of biological pathways (B) and molecular functions (C) GO-term is shown.

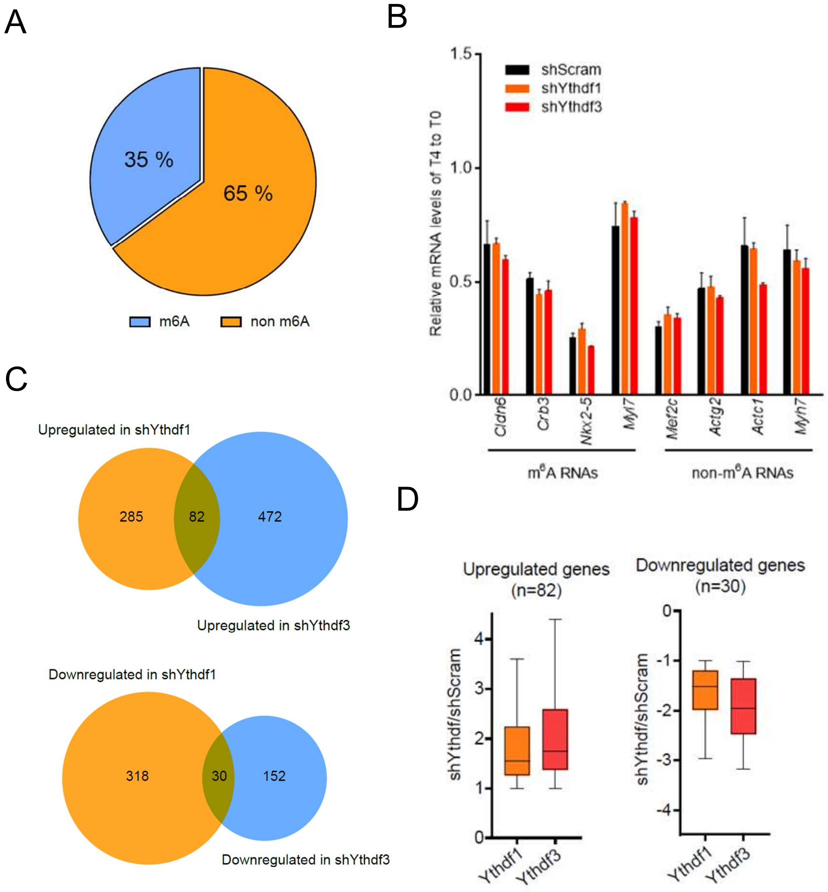

Figure S5. Stability of m^6A-bearing and non-m^6A transcripts in *Ythdf*-depleted CMs. (A) A total of 238 CM-specific genes showed simultaneously downregulated in *Ythdf1*-KD cells and upregulated in *Ythdf3*-KD cells, among which 155 transcripts are not m^6A-modified, others contains m^6A peaks in either ESC, EB or CM samples. (B) Measurement of mRNA levels of CM-specific transcripts bearing m^6A or not in *Ythdf*-KD and control CMs after transcription inhibitor actinomycin D treatment for 4 h. (C) Overlapped the upregulated (*upper*) or dowregulated (*down*) genes of *Ythdf1*-KD and *Ythdf3*-KD CMs (day 8) in comparison with control CMs. (D) Box plot showing the fold changes (shYthdf/shScram) of CM RNA-seq FPKM in overlapping upregulated (*left*) and downregulated (*right*) genes shown in A. CM-specific genes indicates the genes with $\log_2$(CM FPKM/ESC FPKM)>1.

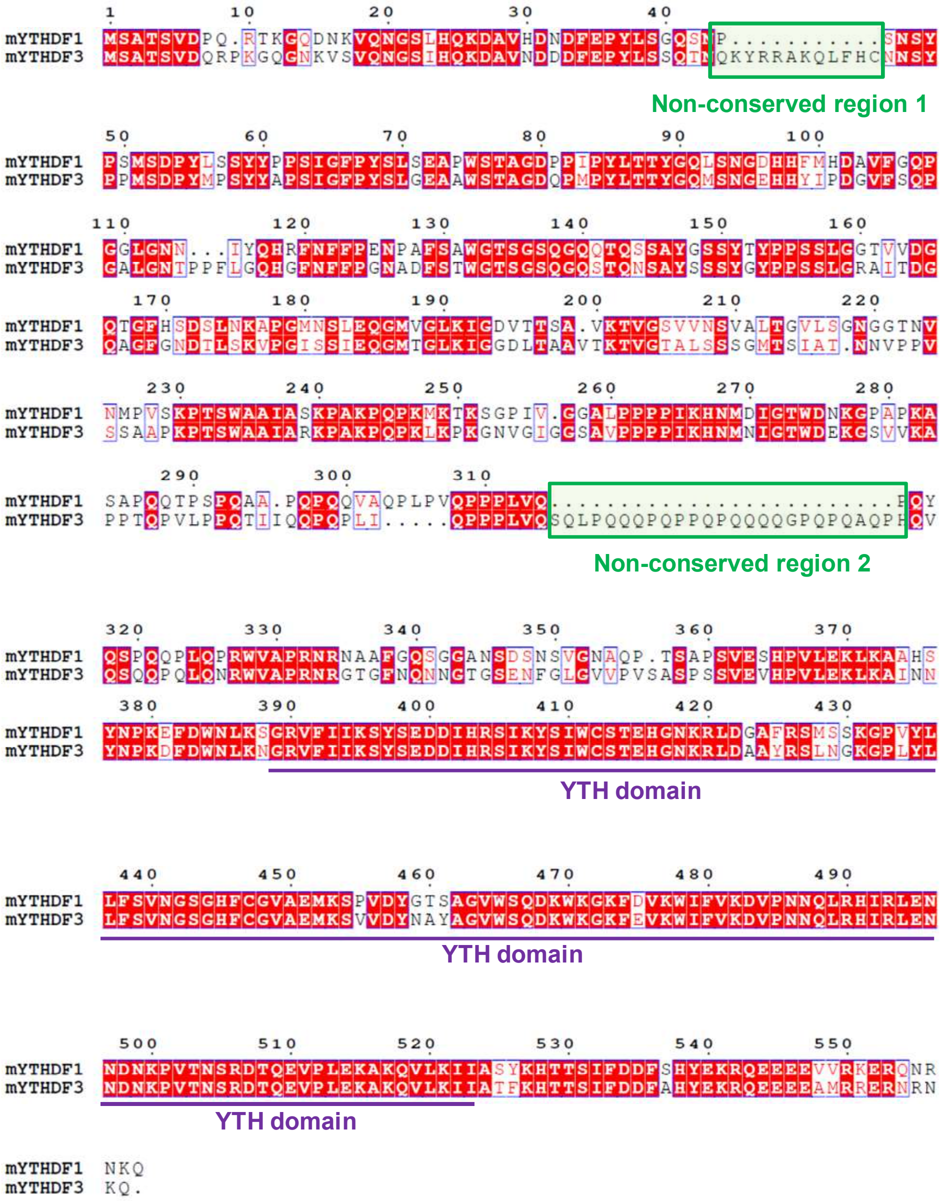

Figure S6. Protein sequence alignment of mouse YTHDF1 and YTHDF3. Conserved YTH domain and non-conserved regions are highlighted in green and purple, respectively.

Table S1. Primers for qPCR used in this study.

Gene name	Forward	Reverse
Ythdf1	5′-GCCCACAGCTATAACCCTAAA-3′	5′-GTGGATGTCGTCCTCAGAATAG-3′
Ythdf3	5′-GGGAAAGGCCCACTCTATTT-3′	5′-GAGACCAGACACCAGCATAAG-3′
Nanog	5′-CAGCATCCATTGCAGCTATCC-3′	5′-CTGCCCCACATGGAAAGG-3′
Pou5f1	5′-TCGGACCAGGCTCAGAGGTA-3′	5′- ATCCCTCCGCAGAACTCGTA-3′
Sox2	5′-AAACCAAGACGCTCATGAAGAAG-3′	5′- CGCTCGCCATGCTGTTC-3′
Dppa3	5′- AGACTTGTTCGGATTGAGCAG-3′	5′-TTTCGCATTCTCAGAGGGATC-3′
Klf2	5′- CCTATCTTGCCGTCCTTTGC-3′	5′- CAGACCGTCCAATCCCATG-3′
Brachyury	5′- TCCCGAGACCCAGTTCATAG-3′	5′- TTCTTTGGCATCAAGGAAGG-3′
Nkx2-5	5′-CTTTAGGAGAAGGGCGATGAC-3′	5′-AGGCTACGTCAATAAAGTGGG-3′
Gata4	5′-CACTTAGGGATATGGGTGTTCC-3′	5′-GGCAGGTGGAGAATAAGGAAG-3′
Mef2c	5′-TTCCACTCCCCCATTGGA-3′	5′-TGCGCTTGACTGAAGGACTTT-3′
Actc1	5′- GCTGTCTTCCCGTCCATC-3′	5′- ACTTCAGAGTCAGGATACCTCG-3′
Actg2	5′- CCTAAACCCCAAAGCAAACAG-3′	5′- CCCCGAATCCAGAACGATG-3′
Sox7	5′- CTTCAGGGGACAAGAGTTCG-3′	5′- GGGTCTCTTCTGGGACAGTG-3′
Cldn6	5′- TTTGCAGGCTCGGATATCAG-3′	5′- TTGACCCAGCCAAGCAG-3′
Crb3	5′- AGACGCTGGAGTCAGGTCC-3′	5′- TTAGGCGACGAGTTGGTGAC-3′
Myh7	5′- CCATCTCTGACAACGCCTATC-3′	5′- GGATGACCCTCTTAGTGTTGAC-3′
Myl7	5′- CTGAGTGCCTTCCGCATGT-3′	5′- GCAAACAGTTGCTCTACCTCAG-3′
Tnnt2	5′- ACCCTCAGGCTCAGGTTCA-3′	5′- GTGTGCAGTCCCTGTTCAGA-3′
β-actin	5′-TTGCTGACAGGATGCAGAAG-3′	5′-ACTCCTGCTTGCTGATCCACAT-3′