Supplementary information, Data S1 Materials and Methods

Protein Expression, Purification and Crystallization

The YTH domain (residues 383-553) of human YTHDF2 (NP_057342.2) was subcloned into a modified pGEX-6p-1 vector. Mutations of YTH domain were generated by the Quick-Change mutagenesis protocol (Stratagene). All plasmids were verified by DNA sequencing and transformed into *Escherichia coli* strain BL21(DE3). Recombinant proteins were overexpressed in 2xYT medium at 20 °C overnight and induced by adding isopropyl-β-D-thiogalactopyranoside to 1 mM. Cell expressing YTH domain were harvested and lysed in the buffer containing 20 mM HEPES (pH 7.4), 200 mM NaCl, 1 mM DTT supplemented with protease inhibitors and DNase. The supernatant was loaded to Ni-NTA affinity column (GE healthcare) and the fusion proteins were digested with PreScission protease overnight at 4 °C. The eluted proteins were further purified by cation exchange chromatography and gel filtration chromatography (GE Healthcare, Superdex 200, 10/300 GL). The peak fractions were collected and concentrated to 20 mg/ml for crystallization.

The crystals for YTH^{YTHDF2} were obtained at 4°C by the hanging-drop, vapor-diffusion method by mixing 1 µl protein solution (20 mg/ml) with 1 µl reservoir solution containing 0.1 M phosphate-citric pH 4.3, 0.2 M Li_2SO_4, 10% PEG8000.

Data Collection and Structure Determination

Crystals were mounted in nylon loops and flash-cooled in a cold nitrogen stream at 100 K. The data were collected at single wavelength at beamline BL17U of Shanghai Synchrotron Radiation Facility in China (SSRF). Data were indexed, integrated and scaled using the

HKL2000 program[1]. The structure of YTH domain was determined by molecular replacement using the solution structure of YTH containing protein 2 (PDB:2YU6) as a searching model[2]. The Matthews coefficients were calculated to estimate the the number of molecule in one asymmetry unit. Rotation and translation searches were performed using PHASER[3]. The structural model was manually built using Coot[4]. All refinements were performed using the refinement module phenix.refine of PHENIX package[5]. The model quality was checked with the PROCHECK program[6], which shows a good stereochemistry according to the Ramachandran plot. All figures were generated using PyMOL[7].

Electrophoretic Mobility Shift Assay

The FAM-17mer-RNA (FAM-UUCUUCUGUGG(m6)ACUGUG) (10 pmol) was mixed with increasing amount of YTH^{YTHDF2} (0, 10, 30, and 100 pmol) in a buffer containing 20 mM HEPES pH 8.2, 50 mM NaCl, 0.05% Triton X-100, 5% v/v Glycerol at 4 °C for 20 min. The samples were subjected to 8% native polyacrylamide gel. The gels were scanned by Typhoon FLA 9500 (GE Healthcare).

Fluorescence Polarization

Fluorescence Polarization experiments were performed in buffer containing 20 mM HEPES pH8.2, 100 mM NaCl, and 3 mM DTT. Wild-type or mutant YTH^{YTHDF2} proteins was incubated with FAM-17mer-RNA (50 nM) at 4 °C, 30 min. Fluorescence polarization measurements were performed at 25 °C on a Synergy 4 Microplate Reader (BioTek). The data were fitted using GraphPad Prism 5.

Circular Dichroism

Far UV (from 190 nm to 250 nm) circular dichroism spectra were recorded using a Jasco 715 CD spectralpolarimeter at 25 °C. 0.2 mg/ml of purified wild-type or mutant YTH^{YTHDF2} proteins were measured in 350 μl buffer containing 1 mM HEPES pH 7.0 in a quartz cuvette.

Relative m6A-RNA-binding Affinity of YTHDF2 in HEK293T Cells

HEK293T or HeLa cells (10 cm dish) were transfected with pLVX-N-Flag-YTHDF2 wild-type and mutants (W432A and W486A) and empty vector. The cells were harvested 24 h after transfections. Cell pellet was washed with cold PBS, and resuspended with 400 μl lysis buffer containing 150 mM KCl, 10 mM HEPES pH7.6, 2 mM EDTA, 0.5% NP-40, 0.5 mM DTT, protease inhibitors, and 400 U/ml RNAse inhibitor. The supernatant was added to 20 μl anti-Flag M2 agarose and incubated overnight at 4°C. The beads were washed three times with 1 ml wash buffer containing 200 mM NaCl, 50 mM HEPES pH7.6, 2 mM EDTA, 0.05% NP-40, 0.5 mM DTT. Part of the bound proteins and RNAs were subjected to immunoblotting for protein concentration determination using a Tanon-5200 Chemiluminescent Imaging System (Tanon Science & Technology Co., Ltd.). Most bound proteins and RNAs were incubated with digestion buffer (50 μl) containing 30 mM Tris-HCl pH8.0 and 1U Proteinase K (Sigma Aldrich) at 55°C for 1.5 h. The sample was centrifuged at 4000 rpm for 30 s. The supernatant was further digested to nucleosides using 1U Nuclease P1 (Sigma Aldrich) at 37°C for 16 h and 1U CIP (NEB) at 37°C for 1.5 h. The products were subjected to LC-MS/MS using a Shimadzu LC (LC-20AB pump)

system.

Total RNAs were isolated from HEK293T cells (10 cm dish) after 24 h transfection. The isolated RNAs were dissolved in RNase-free water and digested to nucleosides as mentioned above. The products were subjected to LC-MS/MS using a Shimadzu LC (LC-20AB pump) system.

Relative m6A nucleoside level was calculated with the amount of m6A nucleoside divided by that of adenosine (A) nucleoside. Relative m6A-binding affinity of YTHDF2 was calculated with relative m6A nucleoside level divided by corresponding protein concentration. Relative m6A-binding affinity of wild-type YTHDF2 was normalized to 1.

RT-PCR was performed using Platinum one-step kit using 50 ng RNA template. Primers used for RT-PCR are listed below:

SON: TGACAGATTTGGATAAGGCTCA; GCTCCTCCTGACTTTTTAGCAA.

CREBBP: CTCAGCTGTGACCTCATGGA; AGGTCGTAGTCCTCGCACAC.

HPRT1: TGACACTGGCAAAACAATGCA; GGTCCTTTTCACCAGCAAGCT.

Reference:

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