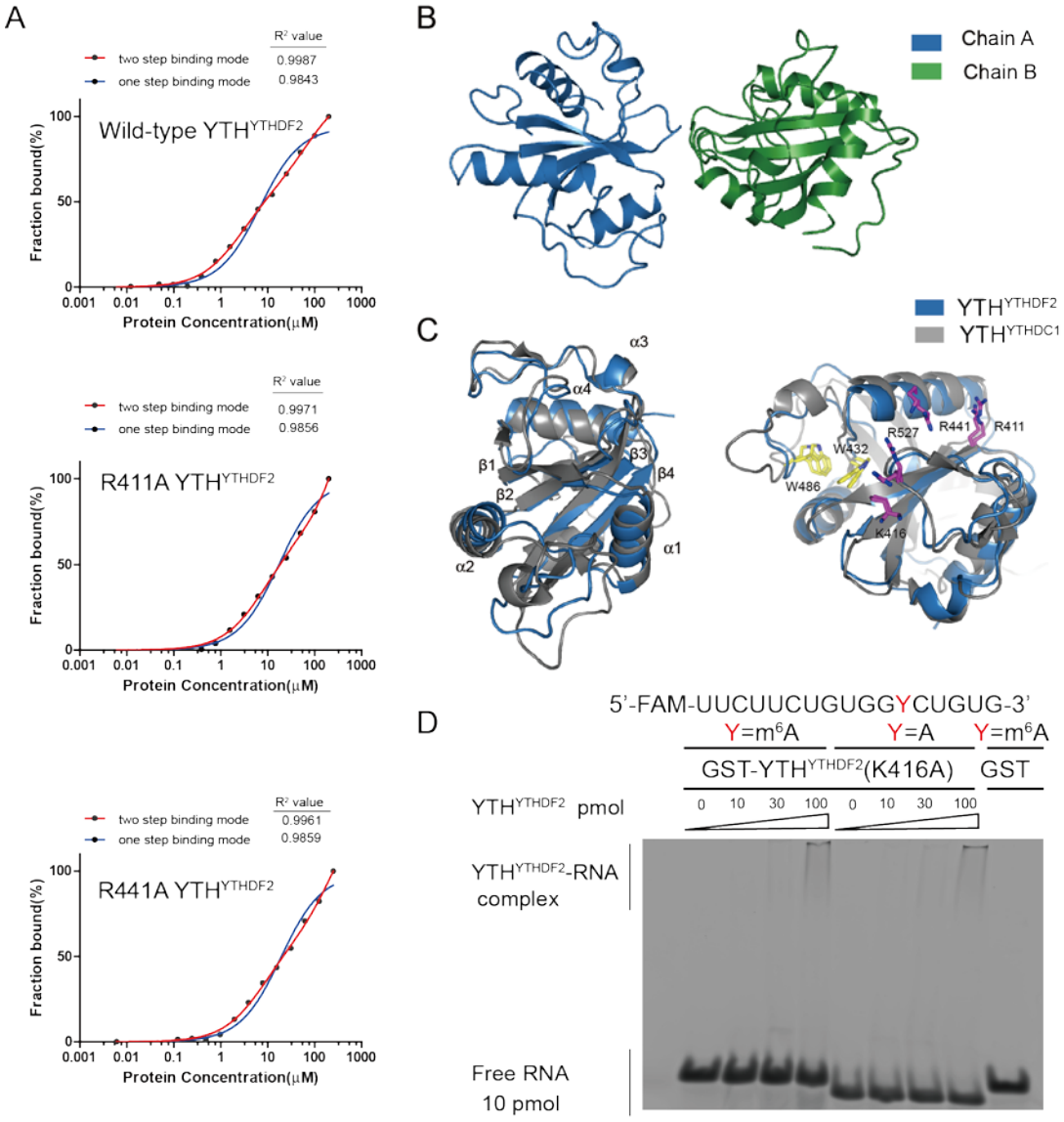
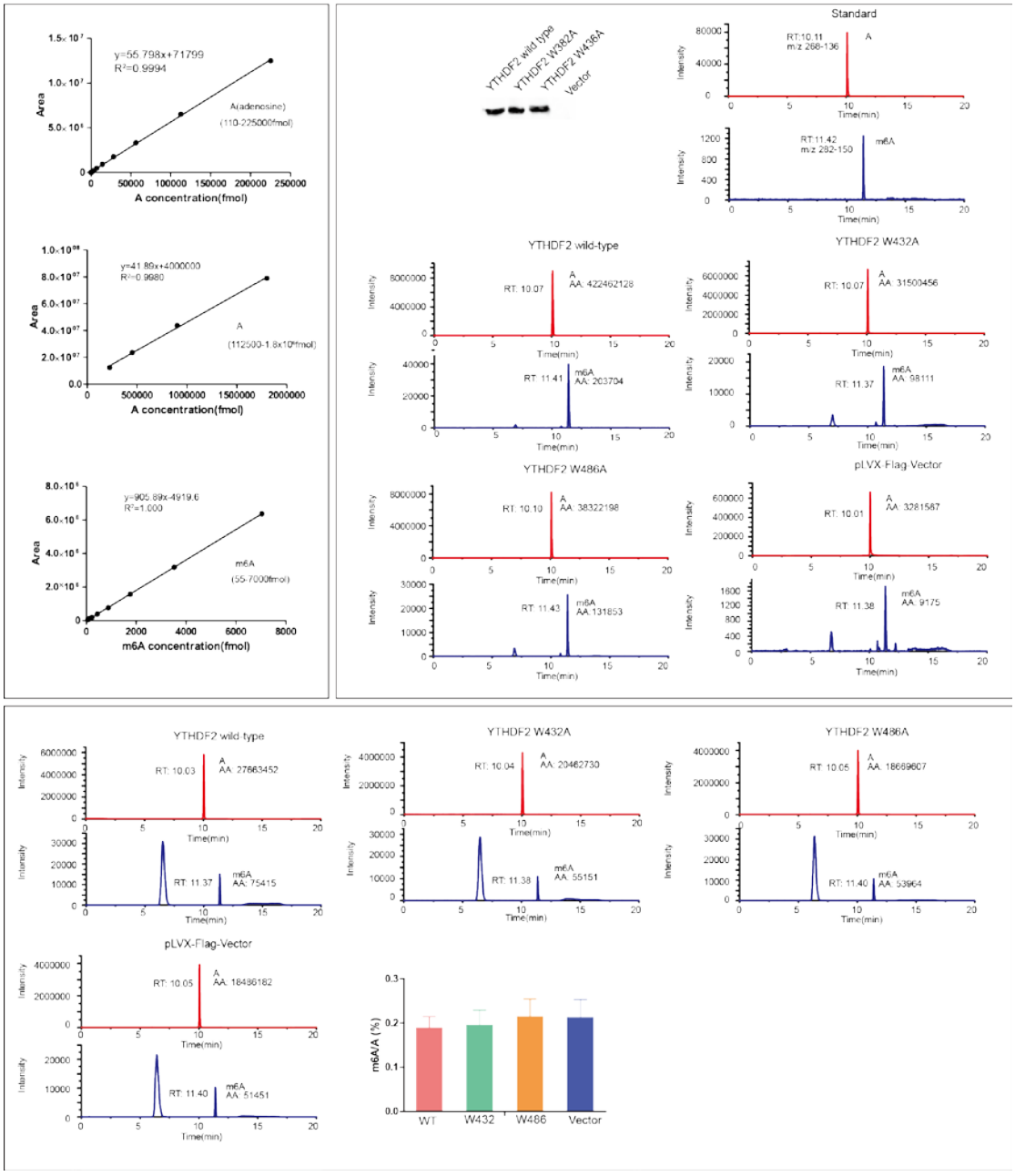
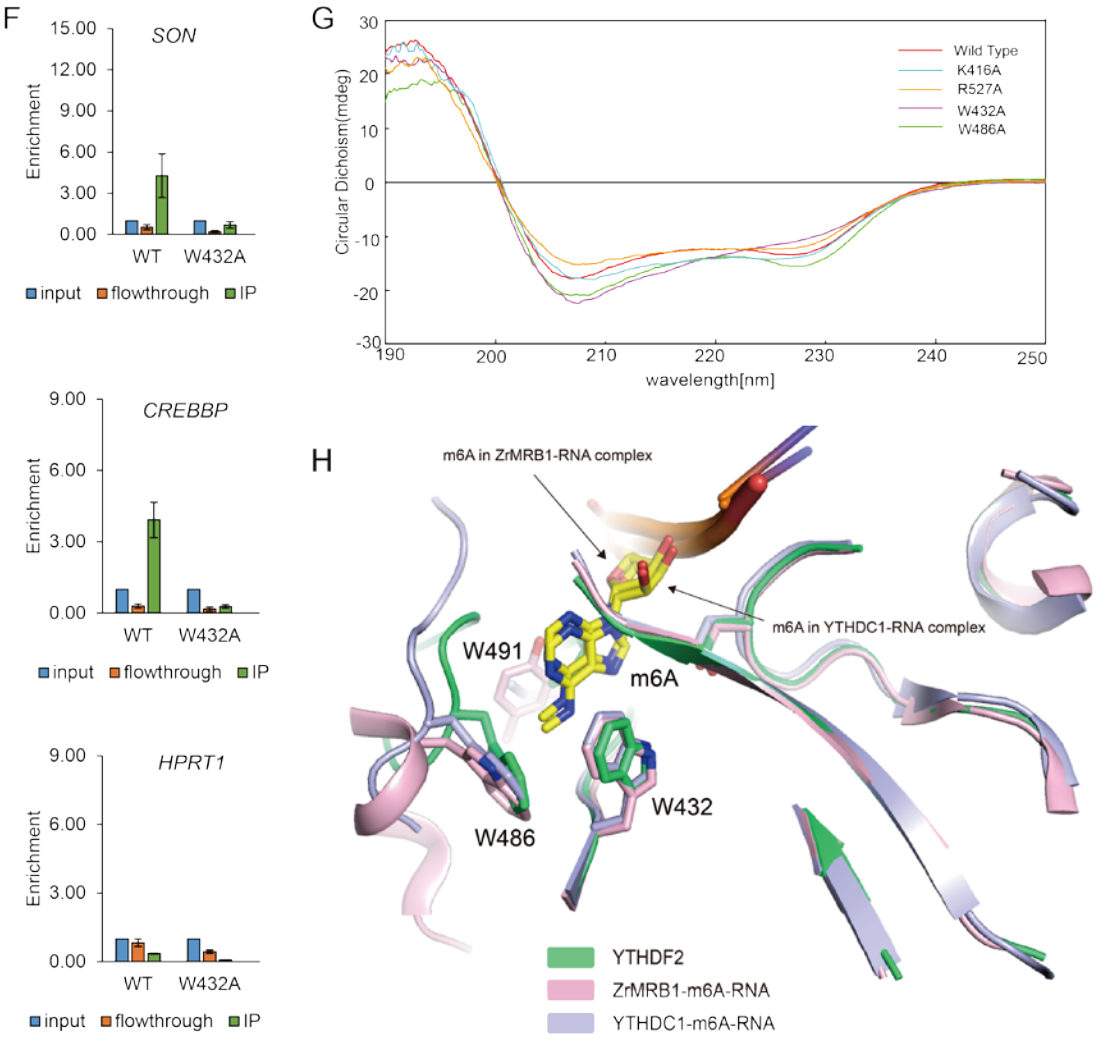

A
R² value
two step binding mode 0.9987
one step binding mode 0.9843
Wild-type YTH^YTHDF2
Fraction bound(%)
Protein Concentration(μM)
R² value
two step binding mode 0.9971
one step binding mode 0.9856
R411A YTH^YTHDF2
Fraction bound(%)
Protein Concentration(μM)
R² value
two step binding mode 0.9961
one step binding mode 0.9859
R441A YTH^YTHDF2
Fraction bound(%)
Protein Concentration(μM)
B
Chain A
Chain B
C
YTH^YTHDF2
YTH^YTHDC1
α3
α4
β1
β3
β4
β2
α2
α1
R527
R441
R411
W432
W486
K416
D
5'-FAM-UUCUUCUGUGGYCUGUG-3'
Y=m⁶A
Y=A
Y=m⁶A
GST-YTH^YTHDF2(K416A)
GST
YTH^YTHDF2 pmol
0 10 30 100 0 10 30 100
YTH^YTHDF2-RNA complex
Free RNA 10 pmol

E


y=55.798x+71799
R²=0.9994
A(adenosine)
(110-225000fmol)
Area
A concentration(fmol)
y=41.89x+4000000
R²=0.9980
A
(112500-1.8x10⁶fmol)
y=905.89x-4919.6
R²=1.000
m6A
(55-7000fmol)
m6A concentration(fmol)
YTHDF2 wild type
YTHDF2 W382A
YTHDF2 W486A
Vector
Standard
RT:10.11
m/z 268-136
RT:11.42
m/z 282-150
m6A
YTHDF2 wild-type
RT: 10.07
AA: 422462128
RT: 11.41
AA: 263704
YTHDF2 W432A
AA: 31500456
RT: 11.37
AA: 98111
YTHDF2 W486A
RT: 10.10
AA: 38322198
RT: 11.43
AA:131853
pLVX-Flag-Vector
RT: 10.01
AA: 3281587
RT: 11.38
AA: 9175
Intensity
Time(min)
YTHDF2 wild-type
RT: 10.03
AA: 27663452
RT: 11.37
AA: 75415
YTHDF2 W432A
RT: 10.04
AA: 20462730
RT: 11.38
AA: 55151
YTHDF2 W486A
RT: 10.05
AA: 18669807
RT: 11.40
AA: 53964
pLVX-Flag-Vector
RT: 10.05
AA: 18486182
RT: 11.40
AA: 51451
m6A/A (%)
WT
W432
W486
Vector


F
SON
Enrichment
15.00
12.00
9.00
6.00
3.00
0.00
WT
W432A
input
flowthrough
IP
CREBBP
9.00
6.00
3.00
0.00
HPRT1
G
Circular Dichoism(mdeg)
30
20
10
0
-10
-20
-30
190
200
210
220
230
240
250
wavelength[nm]
Wild Type
K416A
R527A
W432A
W486A
H
m6A in ZrMRB1-RNA complex
m6A in YTHDC1-RNA complex
W491
m6A
W432
W486
YTHDF2
ZrMRB1-m6A-RNA
YTHDC1-m6A-RNA

Supplementary information, Figure S1.

A. FP assays of wild-type and mutants of YTH^{YTHDF2} toward m6A-RNA. One- and two-step binding modes were used for fitting the data. R^2 values for all the analyses are indicated above the curves. Two-step binding mode seems to be much better than one-step binding mode for fitting the experimental data. In the two-step binding mode, the binding affinity (Kd) of the first step is indicated. A lower limit of the Kd value is reported because saturation of the binding could not be achieved during FP assays.

B. Ribbon representation of YTH^{YTHDF2} dimer in one asymmetric unit of the crystals. The two molecules are colored in blue and green, respectively. The protein is monomer in solution (data not shown). The dimer may be resulted from crystal packing the crystals.

C. Structural comparison of YTH domains of YTHDF2 and YTHDC1. Two structures are shown in ribbon representation in two different views. YTH^{YTHDF2} and YTH^{YTHDC1} (PDB:2YUD) are colored in blue and grey, respectively. Secondary structures of YTH^{YTHDF2} are indicated and critical residues that are potentially involved in RNA-binding of YTH^{YTHDF2} are shown in stick representation.

D. EMSA assay using A-RNA or m6A-RNA with the increasing amount of YTH^{YTHDF2} mutant (K416A) as indicated. The experiments were performed as in Figure 1C.

E. Relative m6A-RNA-binding affinity of YTHDF2 in HEK293T cells. (Top left panel) Standard curves for adenosine (A) and m6A for quantification. Good linearity was obtained as indicated. Note that two standard curves were generated for low-concentration (110-225000 fmol) and high-concentration (112500-1800000 fmol) of adenosine. (Top right panel) Relative m6A-RNA-binding affinity of

YTHDF2 in HEK293T cells. Standard nucleosides were used for each set of experiments. The nucleosides were detected by LC-MS/MS immunoprecipitated by YTHDF2 proteins. Protein concentration was determined by immunoblotting followed by band densitometry. (Bottom panel) LC-MS/MS for detection the ratio of m6A/A from HEK293T that were transfected with wild-type and mutant YTHDF2 and empty vector. Error bars represent SD for triplicate experiment. Note that transfection of YHTDF2 did not affect the m6A/A ratio in HEK293T cell.

F. Relative target enrichment by RNA-IP from HeLa cells of wild-type and mutant YTH^{YTHDF2}. *SON* and *CREBBP* are targets and *HPRT1* serves as a negative control. The error bars represent ± SD for duplicate experiments.

G. The CD spectra of wild-type and mutants of YTH^{YTHDF2}. The CD curves show negative absorption peaks at 222 nm and 208 nm, which is similar to that of typical α helix and consistent with the observation in crystal structure. Color scheme is indicated. Although mutation W432A of YTH^{YTHDF2} showed a slight change compared to wild-type protein, the protein still possessed RNA-binding affinity, supporting that the mutant is well-folded.

H. Comparison of YTH domains in YTHDF2 structure and that in YTHDC1-m6A-RNA and ZrMRB1-m6A-RNA complex structures. The compared structures are shown in ribbon representation with m6A-binding residues shown in stick representation. YTHDC1-m6A-RNA (PDB:4R3I)[8] and ZrMRB1-m6A-RNA (PDB:4U8T)[9] complex structures were reported when our manuscript was under revision in Cell Research.