Supplementary Information

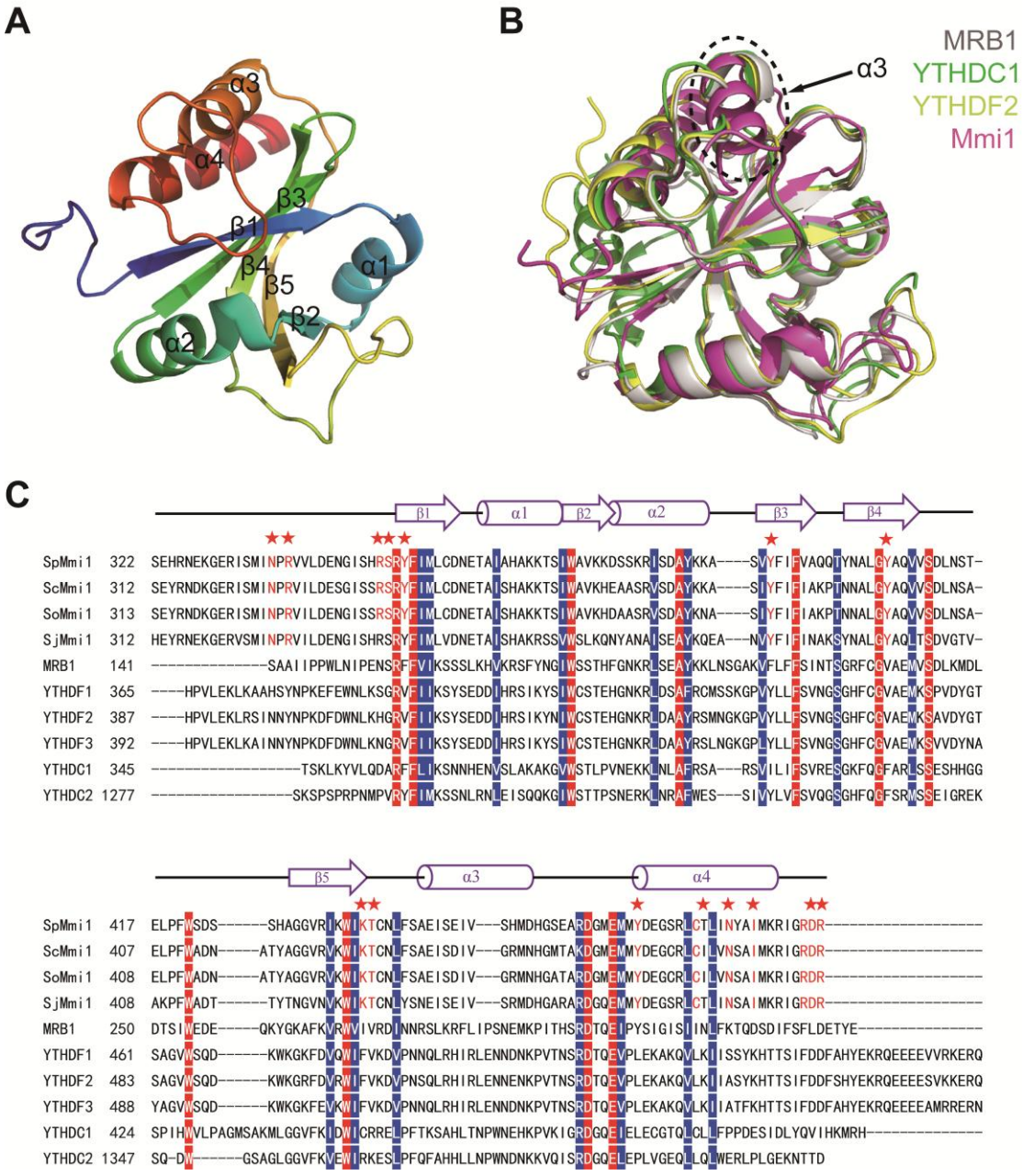
A novel RNA-binding model of the YTH domain reveals the mechanism for recognition of determinant of selective removal by Mmi1

Chongyuan Wang, Yuwei Zhu, Hongyu Bao, Yiyang Jiang, Chao Xu, Jihui Wu* and Yunyu Shi*

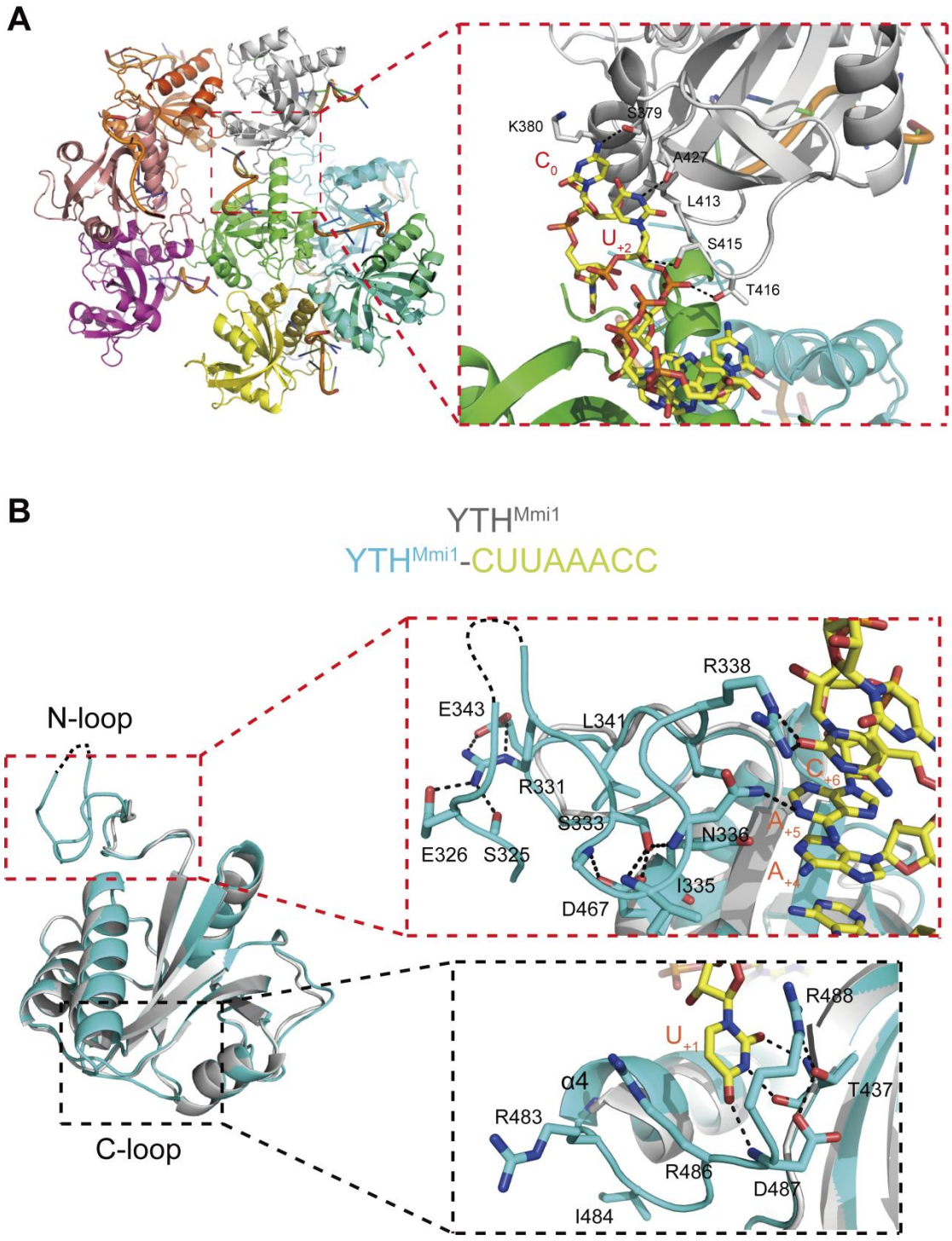
Hefei National Laboratory for Physical Sciences at Microscale and School of Life Sciences, University of Science and Technology of China, Anhui 230027, China

*To whom correspondence should be addressed. Tel: +86 551 3607464; Fax: +86 551 3603745; Email: yyshi@ustc.edu.cn.

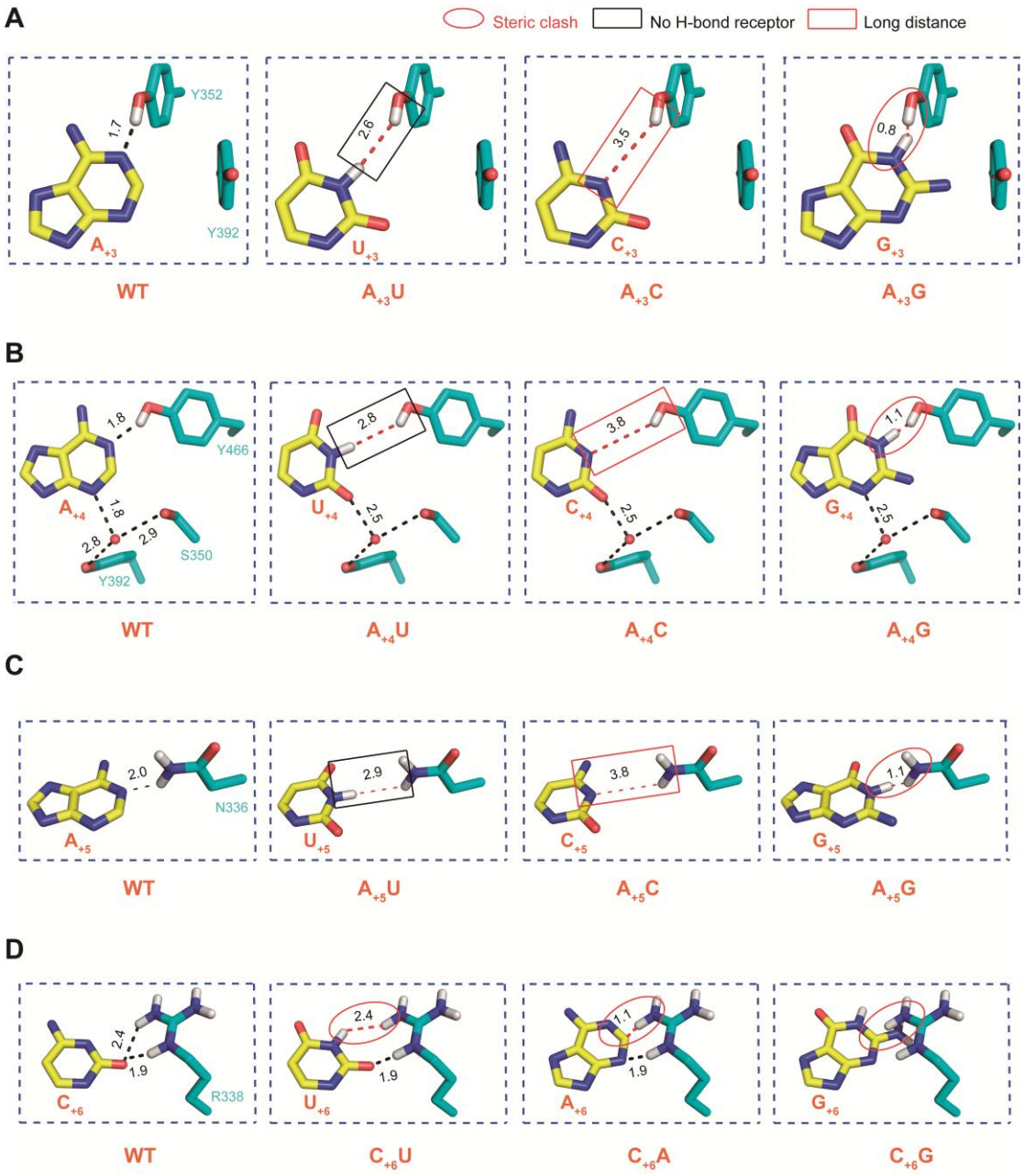
Correspondence may also be addressed to Jihui Wu. Tel: +86 551 3603745; Email: wujihui@ustc.edu.cn



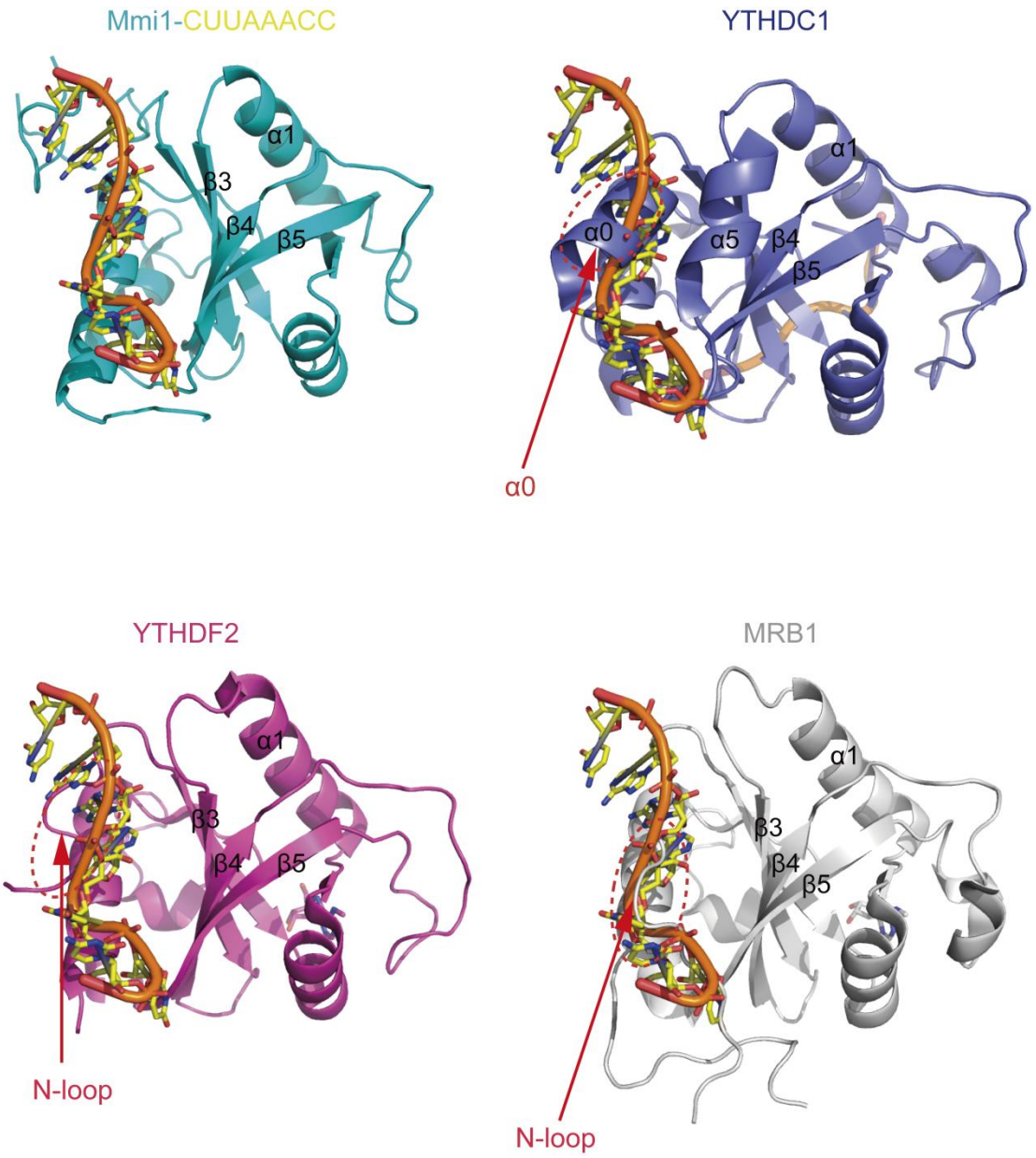
Supplementary Figure S1: Overall structure of Mmi1 YTH domain. (A) Structure of apo YTH^{Mmi1} is shown in cartoon. (B) Comparison of the YTH domains of YTHDC1, YTHDF2, MRB1 and Mmi1. YTH domains of human YTHDC1 (PDB: 4R3H), human YTHDF2 (PDB: 4RDO) and MRB1 (PDB: 4RCM) were aligned to apo structure of the Mmi1 YTH domain. The significant structural difference is indicated by arrow. (C) Sequence alignment of YTH domains of human YTHDC1, human YTHDF2, *Saccharomyces cerevisiae* MRB1 and Mmi1 homologues in fission yeast (*Schizosaccharomyces pombe*, *S. japonicus*, *S. octosporus*, and *S. cryophilus*). Conserved and Similar residues are highlighted in blue and red, respectively. The Mmi1 residues binding RNA are indicated by red asterisks, which are strictly conserved in fission homologues (colored in red). The secondary structure elements of YTH^{Mmi1} is shown at the top.



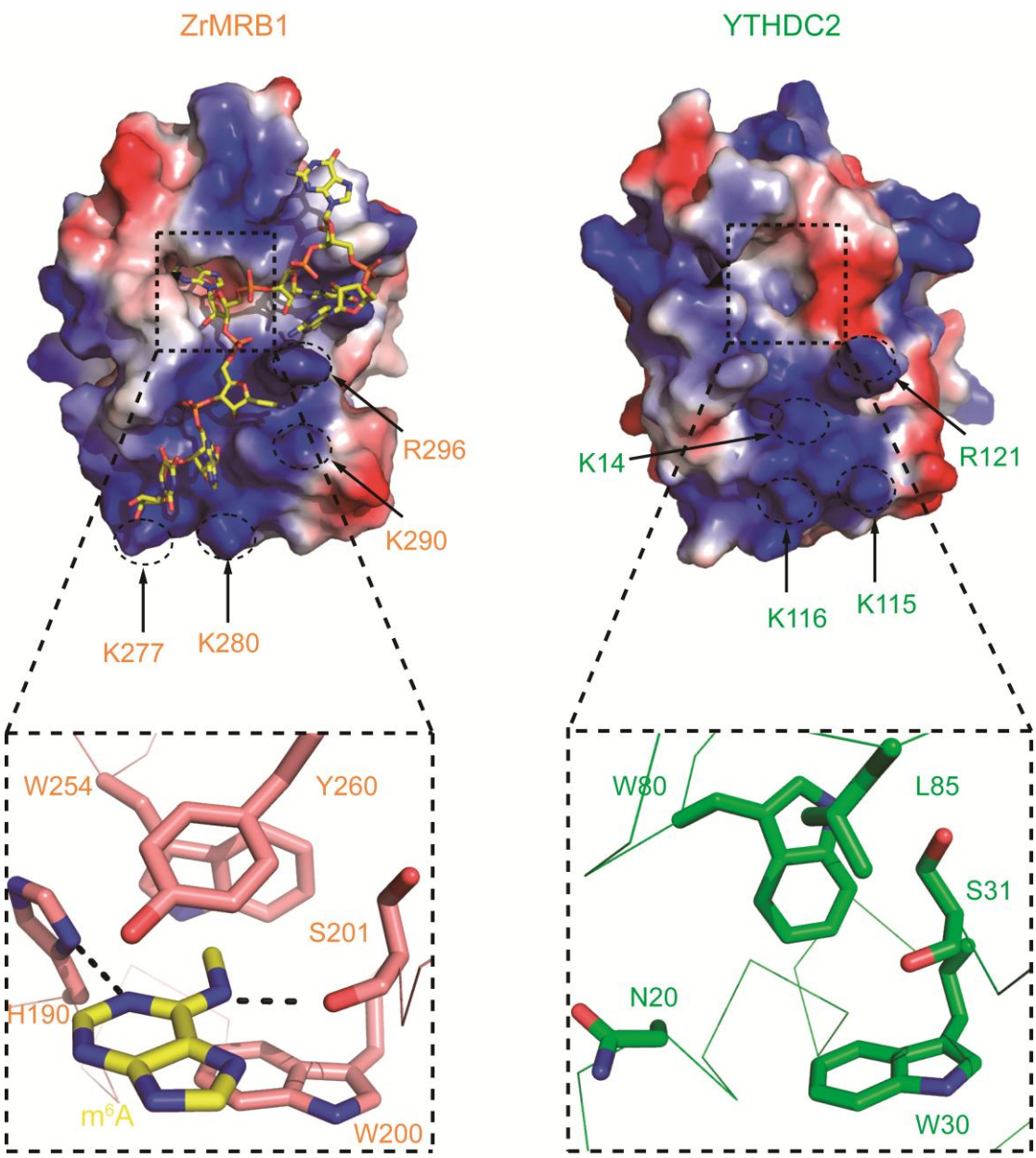
Supplementary Figure S2: The crystallization packing of YTH^{Mmi1}-RNA complex and comparison of apo (grey) and RNA-bound (cyan) structures of YTH^{Mmi1}. (A) C_0 and U_{+2} participate in crystallization packing via their contacts with another YTH^{Mmi1} molecule. Residues involved in crystallization packing are shown in stick and labled. Black dashed lines indicate the hydrogen bonds. (B) Residues with substantial conformational changes are shown and labeled. Hydrogen bonds are indicated by black dashed lines. Residues 326-329 of YTH^{Mmi1} in the RNA-bound structure are also invisible in the density map and indicated with the black dash line.



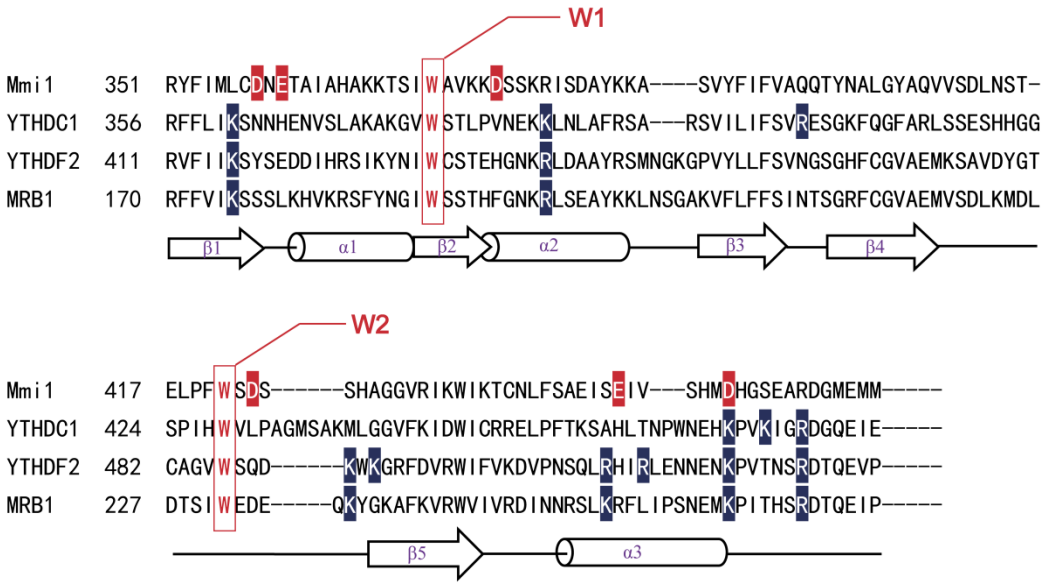
Supplementary Figure S3: Models for A_{+3}, A_{+4}, A_{+5}, and C_{+6} mutants. (A-D) The polar hydrogen atoms in the binding interface are shown in grey sticks. Black dashed lines indicate the hydrogen bonds. Red dashed lines indicate the distances between atoms without hydrogen bonding interactions. The steric clash is highlighted with red ovals, the loss of hydrogen bonds is highlighted within black or red rectangles.



Supplementary Figure S4: The N-terminal segments occupy the potential DSR-binding grooves in YTHDC1, YTHDF2 and MRB1. The YTH^{YTHDC1}-GGm^{6}ACU complex (PDB: 4R3I), YTH^{YTHDF2}-m^{6}A complex (PDB: 4RDN) and YTH^{MRB1}-m^{6}A complex (PDB: 4RCM) are aligned to YTH^{Mmi1}-CUUAAACC complex. The potential steric clashes are highlighted with red dashed ovals.



Supplementary Figure S5: The aromatic cages and the surrounding grooves of YTH domains of ZrMRB1 and YTHDC2. YTH^{ZrMRB1} complex (PDB: 4U8T) and YTH^{YTHDC2} (PDB: 2YU6) were aligned to YTH^{mmi1}, as in Figure 4. The upper pictures are the electrostatic potential surface, in which positive charged residues in the m^6A RNA-binding interfaces of ZrMRB1 and YTHDC2 are indicated. The lower pictures are enlarged views of the aromatic cages.



Supplementary Figure S6: Sequence alignment of the YTH domains. The positively and negatively charged residues in Figure 8A are highlighted in blue and red, respectively. A schematic representation of the secondary structure elements of YTH^{Mmi1} is shown below the sequences.

Supplementary Table S1. The thermodynamic parameters of the ITC experiments.

Proteins	RNA 5'-3'	ΔH kcal/mol	ΔS cal/mol/K	K_D μM	N
Mmi1$^{316-488}$	CCUUAAACCU	-38.6	-100	0.39 ±0.01	1.1
Mmi1$^{338-488}$	CCUUAAACCU			>30	
Mmi1$^{345-488}$	CCUUAAACCU			ND	
YTH^{Mmi1}	CCUUAAACCU	-33.6	-83.6	0.44±0.03	1.0
	CCUUAAACC	-32.2	-80.7	1.1±0.02	1.1
	CUUAAACCU	-32.4	-81.5	1.2±0.03	1.1
	CUUAAACC	-31.4	-79.1	1.8±0.1	1.0
	UUAAAC	-14.3	-24.5	8.3±2	0.88
	CCUU**m^{6}A**AACCU	-28.3	-70.7	5.4±0.3	1.0
	CCUUA**m^{6}A**ACCU	-27.7	-67.6	2.8±0.09	0.94
	CCUUAA**m^{6}A**CCU	-29.3	-70.1	0.69±0.02	0.98
	CCU***G***AAACCU	-32.6	-81.3	0.70±0.04	0.88
	CCU***A***AAACCU	-30.5	-74.8	0.95±0.09	0.97
	CCU***C***AAACCU	-32.6	-81.0	1.0±0.04	0.97
	CC***G***UAAACCU	-28.2	-70.0	3.9±0.2	0.96
	CC***A***UAAACCU	-21.1	-46.1	4.4±0.3	0.85
	CC***C***UAAACCU	-14.5	-26.2	13±2	1.2
	CCUU***G***AACCU			>30	
	CCUU***C***AACCU	-23.0	-54.8	13±1	1.0
	CCUU***U***AACCU	-22.2	-50.4	5.6±0.4	1.1
	CCUUA***G***ACCU			ND	
	CCUUA***C***ACCU			>30	
	CCUUA***U***ACCU			ND	
	CCUUAA***G***CCU			>20	
	CCUUAA***C***CCU	-23.6	-54.3	3.9±0.1	0.98
	CCUUAA***U***CCU	-26.7	-66.3	6.0±0.4	1.1
	CCUUAAA***G***CU	-25.9	-62.2	4.1±0.4	1.1
	CCUUAAA***A***CU	-26.1	-61.3	1.9±0.01	1.1
	CCUUAAA***U***CU	-30.9	-77.8	2.1±0.1	0.92
	AUGGACUCC			ND	
R331A(YTH^{Mmi1})		-38.3	-102	1.9±0.05	0.98
S333A		-36.4	-98.4	6.2±0.02	1.3
N336A		-34.3	-91.8	8.9±0.2	1.1
R338A	CCUUAAACCU	-24.3	-58.6	9.6±0.7	0.99
Y352F		-28.8	-75.1	19±3	0.97
K436A		-18.2	-39.8	21±3	0.84
Y466F				>100	
N477A		-31.6	-82.9	8.7±0.7	1.0

YTH^{YTHDC1}		-13.1	-11.1	0.068 ±0.01	1.2
YTH^{YTHDF2}	AUGGm^{6}ACUCC	-9.8	-2.51	0.25 ±0.03	1.0
YTH^{MRB1}		-20.3	-39.3	0.56 ±0.02	1.1
YTH^{Mmi1}				ND	
YTH^{YTHDC1}				ND	
YTH^{YTHDF2}	CCUUAAACCU			ND	
YTH^{MRB1}				ND	

Bold and italic fonts are used to highlight the mutant nucleotides and the m^{6}A nucleotides in the 10-mer DSR RNAs.

Supplementary Table S2. Data collection and refinement statistics.

	YTH^{Mmi1}	YTH^{Mmi1}-CUUAAACC
PDB ID	5DNP	5DNO
Data Collection		
Space group	C2	$P6_5$
Cell dimensions		
a, b, c (Å)	100.5, 58.3, 54.3	77.5, 77.5, 65.5,
α, β, γ (˚)	90,108,90	90,90,120
Wavelength(Å)	0.9795	0.9795
Resolution* (Å)	49.79-2.30 (2.34-2.30)	38.76-1.80 (1.86-1.80)
Completeness (%)	99.3(96.7)	99.9(99.6)
Redundancy	3.5(3.5)	11.0(10.9)
R_{sym} or R_{merge} (%)	12.6(64.8)	17.0(59.5)
$I/\sigma I$	13.0(2.9)	12.9(4.6)
Refinement		
No. reflections used/free	13290/995	20917/1084
Resolution (Å)	49.79-2.30	38.76-1.80
R_{work}/R_{free}	21.2/25.6	17.6/21.6
R.m.s.deviations		
Bondslengths (Å)	0.008	0.008
Bond angles (˚)	1.2	1.3
B-factors ($Å^2$)		
Protein	30.9	13.4
RNA	N/A	20.2
Water	24.8	22.7
No. atoms		
Protein	2195	1304
RNA	N/A	143
Water	57	116
Ramachandran plot		
Favored/ allowed/ outlier(%)	98.0/2.0/0.0	100.0/0.0/0.0

* Values in parentheses are for highest-resolution shell.