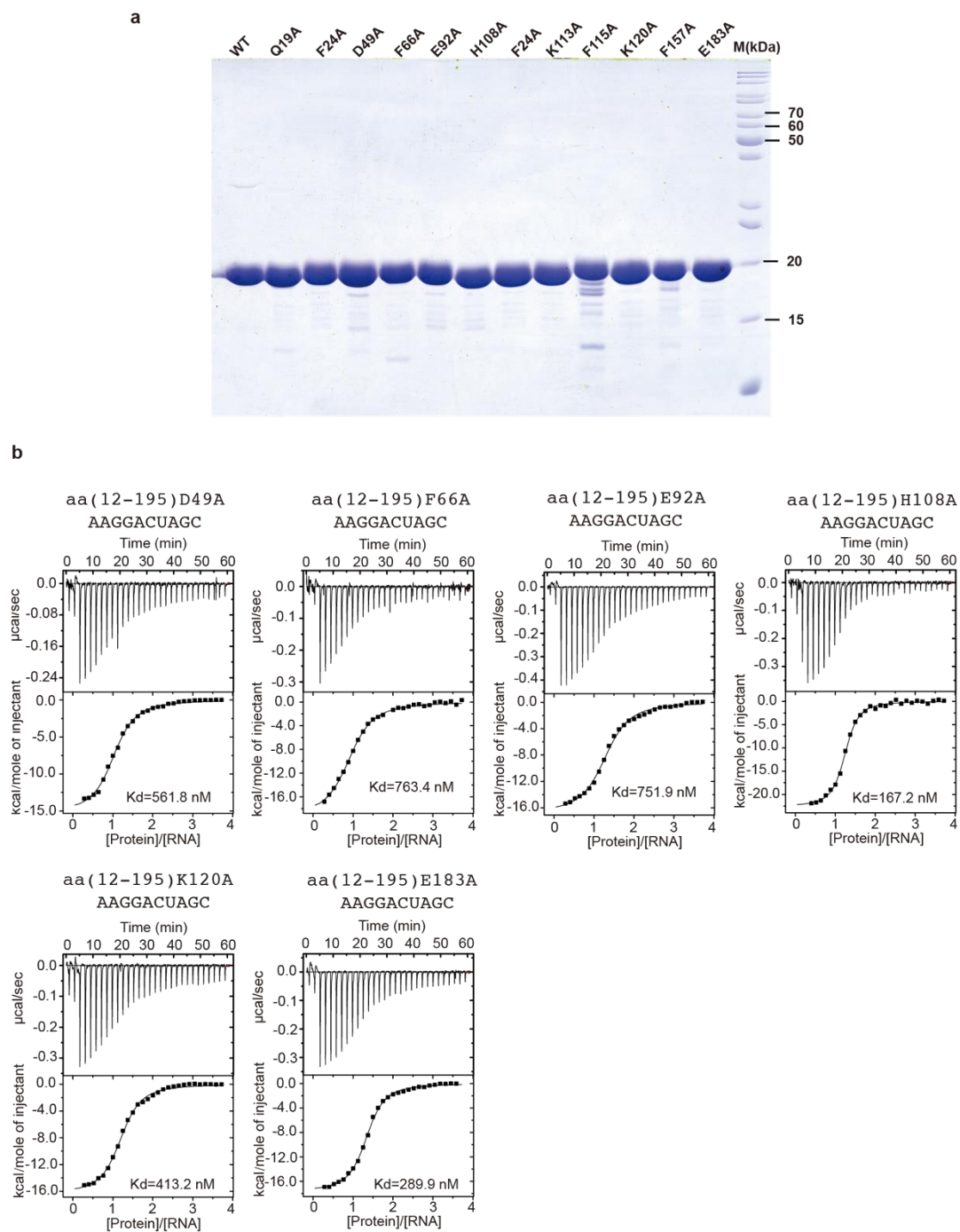


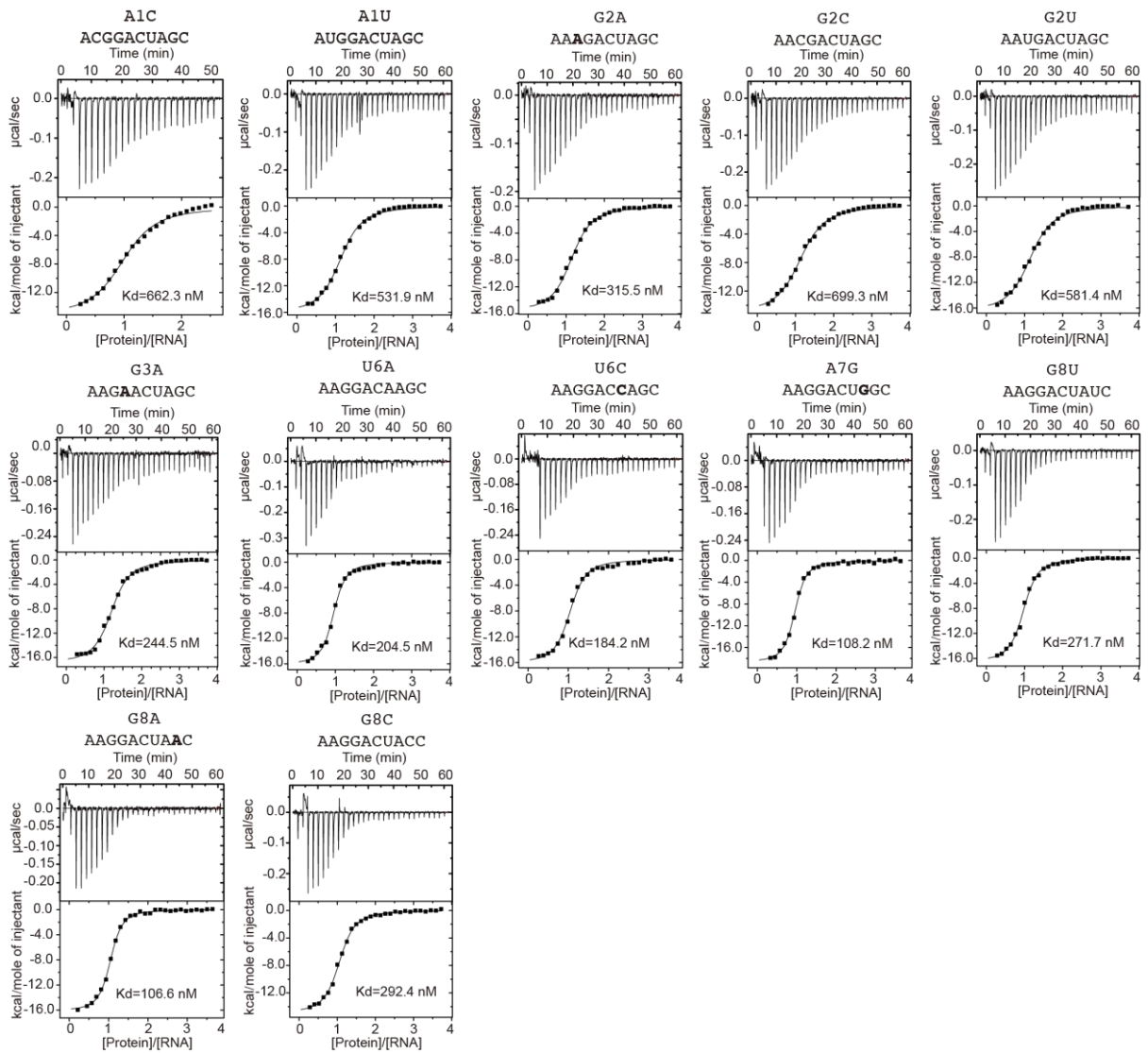
**Supplementary Figure 1. Superimposed of RRM1 and RRM2 in 10mer RNA complex**

(a) Sequence alignment between RRM1 and RRM2, the secondary structures are colored in purple-blue and green, respectively above the sequences. (b) Superposition of RRM1(12-110)-AAGG and RRM2(111-195)-ACUAGC.(c) Superposition of residues participated in RNA recognition in RRM1 and RRM2. The RNA chains are shown by cartoon.

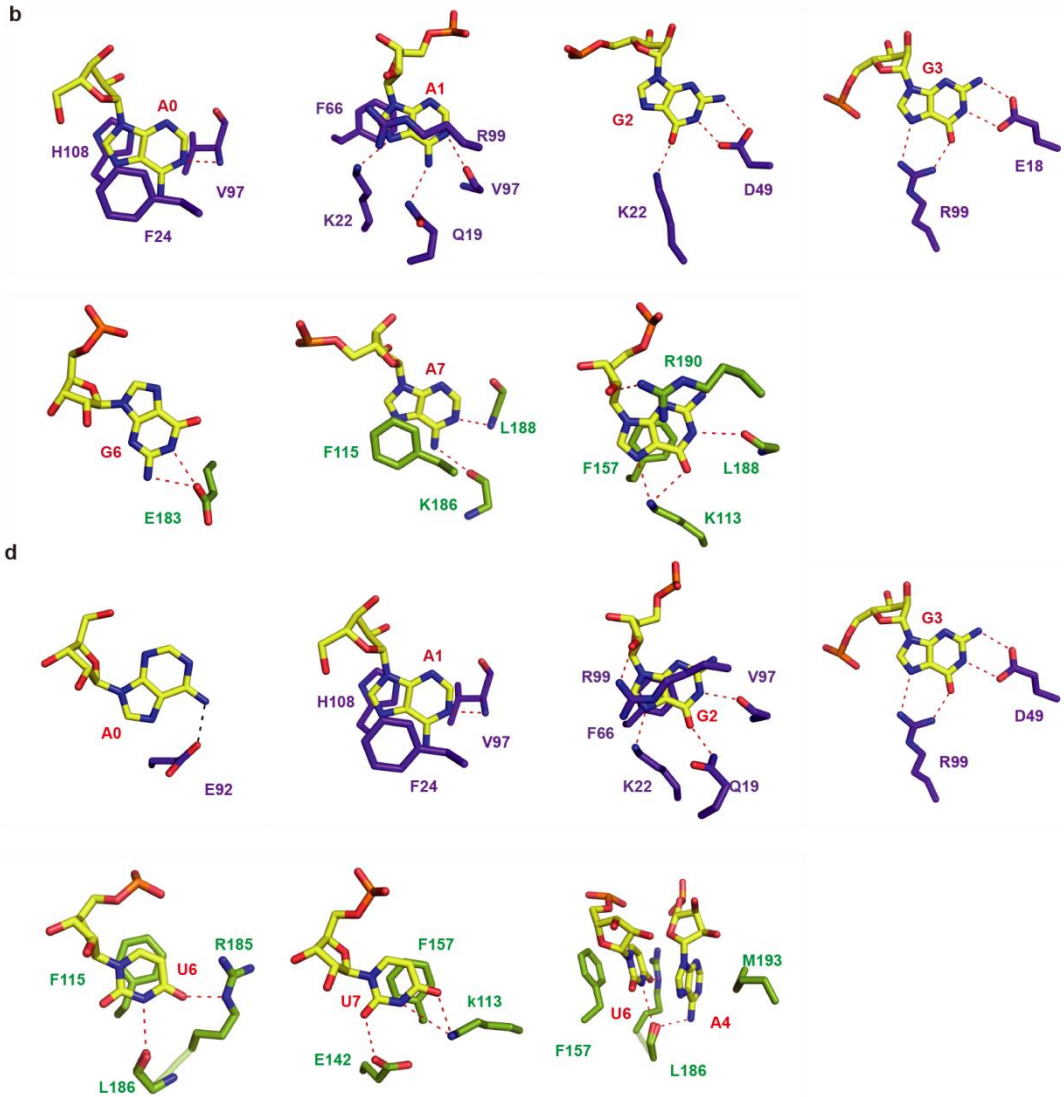
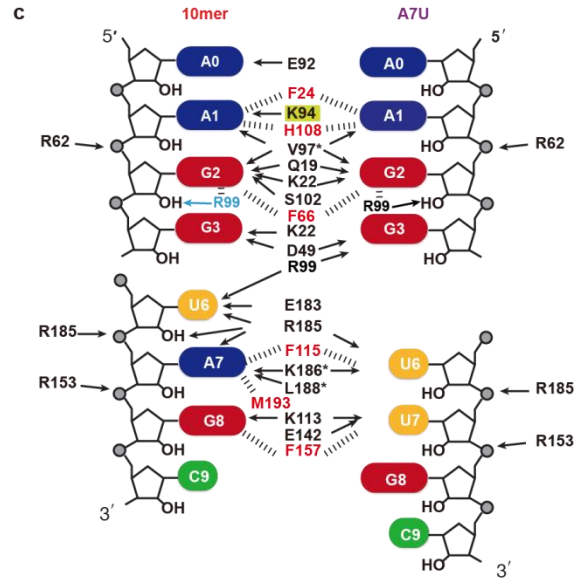
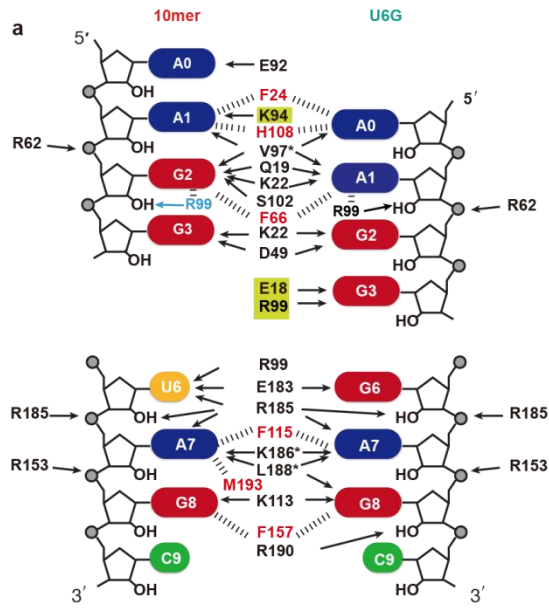


**Supplementary Figure 2. Mutagenesis study of RRM1 by ITC experiments**

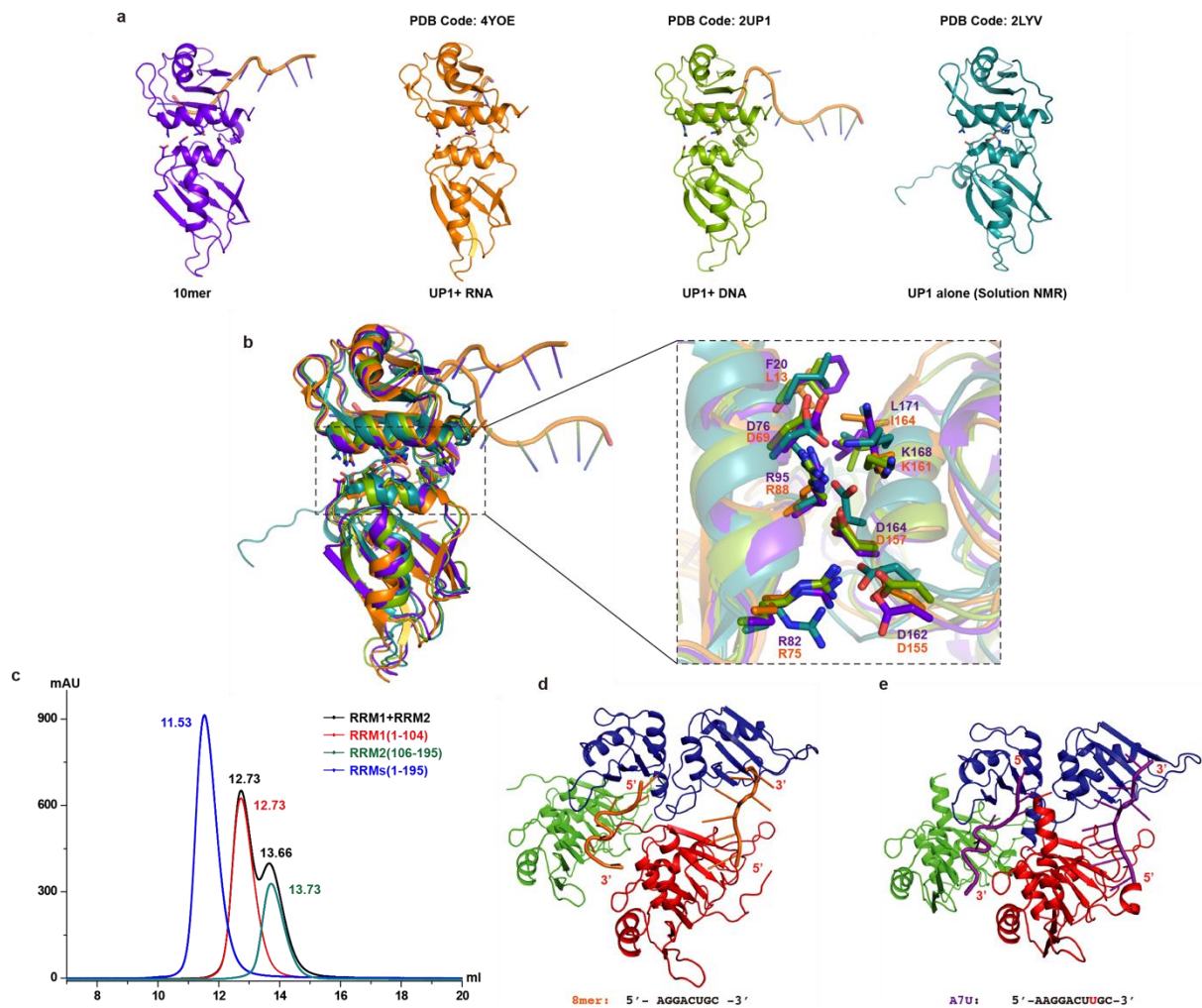
(a) SDS-PAGE of wildtype and protein mutants. (b) ITC results of hnRNP A2/B1 mutants with 10mer RNA.



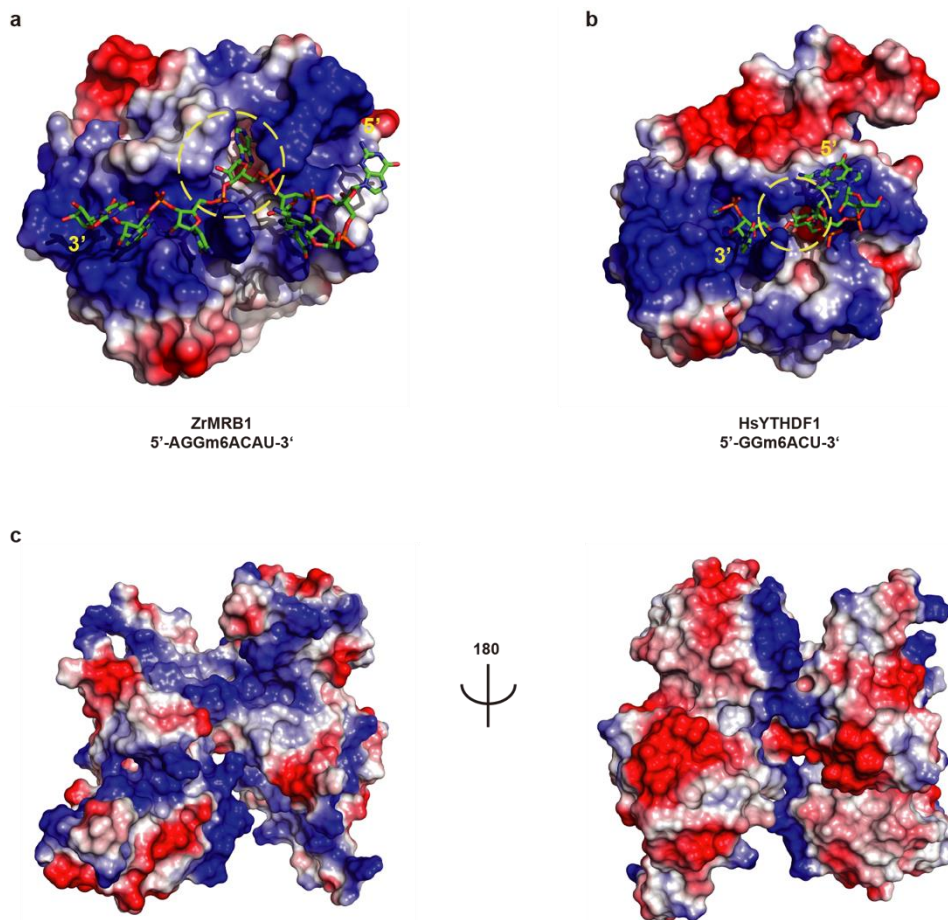
**Supplementary Figure 3. Mutagenesis study of RNA by ITC experiments.**



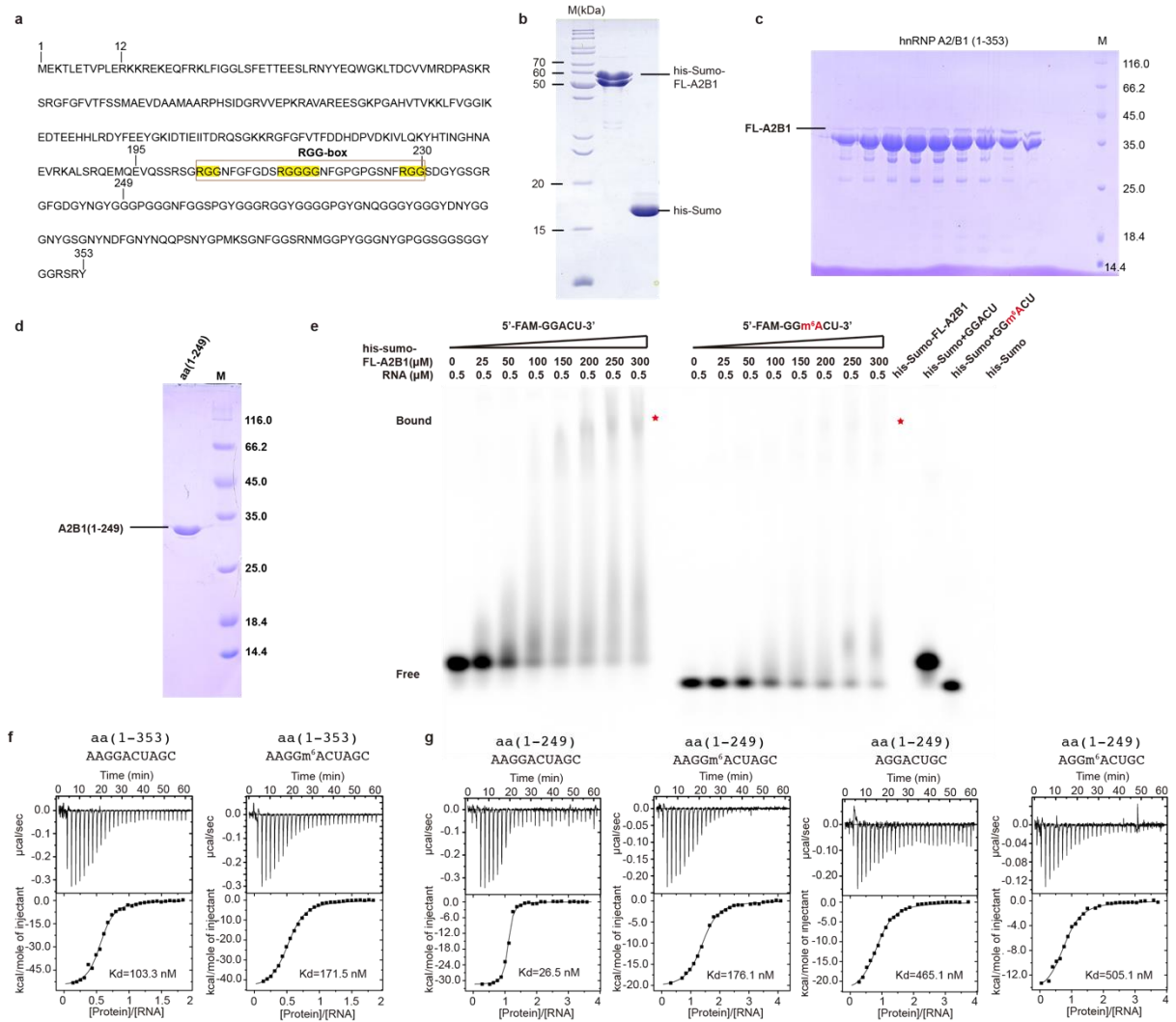
**Supplementary Figure 4. Schematic representations of RRMs interactions with RNA mutants.** (a) Close-up view showing the specific RNA recognition in U6G complex. (b) Comparison of detailed interactions between 10mer complex and U6G complex. (c) Close-up view showing the specific RNA recognition in A7U complex. (d) Comparison of detailed interactions between 10mer complex and A7U complex.



**Supplementary Figure 5. Crystal packing interactions.** (a) Structures of hnRNP A2/B1 with 10mer complex and hnRNP A1-RRMs (UP1) in different structural status. The UP1 in complex with RNA (PDB code: 4YOE) is colored in orange, the UP1 in complex with telomere DNA (PDB code: 2UP1) and solution structure of UP1 (PDB code: 2LYV) are colored in green and cyan, respectively. (b) Superposition of the structures listing in Supplementary Figure 5a reveals the conserve interactions between RRM1 and RRM2. The residues account for the interactions of inter-domain are shown as stick colored as above. (c) Gel-filtration binding assay for RRM1 and RRM2. Black curve: RRM1+RRM2; Red curve: RRM1; Green curve: RRM2; Blue curve: RRM1s(1-195). (d) Crystal packing interactions in 8mer. (e) Crystal packing interactions in A7U complex.



**Supplementary Figure 6. Surface charge comparison of *ZrMRB1*, *HsYTHDF1* and hnRNP A2/B1.** (a) Surface representation of *ZrMRB1* in complex with RNA containing m<sup>6</sup>A modification. The aromatic cage is circled with yellow dashline, RNA is shown by stick colored in green. (b) *HsYTHDF1* in complex with RNA containing m<sup>6</sup>A modification, RNA is shown by stick. (c) Surface representation of hnRNP A2/B1 RRMs in complex with 10mer RNA.



**Supplementary Figure 7. Full-length hnRNP A2/B1 does not specifically recognize m<sup>6</sup>A-modified RNA.** (a) Sequence of full-length hnRNP A2/B1 with RGG-box (203-230) colored in yellow enclosed with rectangle. (b) SDS-PAGE of full-length hnRNP A2/B1 with his-sumo tag. (c) SDS-PAGE of full-length hnRNP A2/B1 without tag. (d) SDS-PAGE of hnRNP A2/B1(aa 1-249) containing the RGG-box. (e) EMSA result of full-length hnRNP A2/B1 and 5'-FAM-labeled RNA substrates with or without m<sup>6</sup>A modification. (f) ITC results of full-length hnRNP A2/B1 with various RNA substrates. (g) ITC results of hnRNP A2/B1(aa 1-249) with various RNA substrates.



**Supplementary Table 1.** Data collection and refinement statistics.

	aa (12-195) and 8mer AGGACUGC	aa (12-195) and 10mer AAGGACUAGC	aa (12-195) and A1G AGGGACUAGC	aa (12-195) and U6G AAGGACGAGC	aa (12-195) and G7U AAGGACUUGC
<b>Data collection</b>					
Space group	C2 <sub>1</sub>	C2 <sub>1</sub>	P4 <sub>3</sub>	P2 <sub>1</sub>	C2 <sub>1</sub>
Cell dimensions					
<i>a</i> , <i>b</i> , <i>c</i> (Å)	122.623, 46.38, 37.516	109.604, 38.959,70.442	62.591, 62.591, 53.043	49.351, 38.301, 107.931	132.810, 46.228, 33.994
$\alpha, \beta, \gamma$ , (°)	90, 96.02, 90	90, 116.26, 90	90, 90, 90	90, 92.43, 90	90, 102.22, 90
Wavelength(Å)	0.97776	0.97776	0.97776	0.97776	0.97776
Resolution (Å)	28.81-2.60(2.64- 2.6)	63.17-1.85 (1.917- 1.851)	30.00-2.40(2.44- 2.40)	30.00-2.15(2.23- 2.15)	30-2.03(2.082- 2.030)
<i>R</i> <sub>merge</sub>	0.119	0.057	0.104	0.137	0.131
<i>I</i> / $\sigma$ <i>I</i>	7.9 (1.5)	18.4 (2.1)	32.75(2.33)	11.0(2.25)	15.81(2.4)
Completeness (%)	93.4 (82.0)	92.43(55.36)	100.0(100)	96.8(87.9)	97.2(94.0)
Redundancy	2.5	2.9	13.4	4.7	4.9
<b>Refinement</b>					
Resolution (Å)	2.6	1.85	2.4	2.15	2.03
No. reflections	15240 (461)	21311(1255)	8124(392)	21583(1917)	14087(1172)
<i>R</i> <sub>work</sub> / <i>R</i> <sub>free</sub>	0.1916/0.2362	0.1839/0.2227	0.22436/.27434	0.20255/0.25772	0.20145/0.25628
No.atoms	1652	1796	1637	3399	1734
Protein	1493	1470	1433	2903	1522
RNA	149	193	198	436	169
Water	10	134	6	60	33
Average <i>B</i> -factor (Å)	45.5	23.57	68.279	29.476	42.795
<b>R.m.s. deviations</b>					
Bond lengths (Å)	0.012	1.018	0.012	0.013	0.016
Bond angles (°)	1.7	1.95	1.581	1.757	1.831
<b>Ramachandran plot</b>					
Favored/allowed/ disallowed(%)	96.00/4.00/0.00	99.00/1.00/0.00	94.51/2.44/3.05	98.21/0.9/0.9	98.27/0.58/1.16

**Supplementary Table 2. Summary of ITC results**

RNA substrates	Protein	Kd	n
8mer: AGGACUGC	hnRNP A2/B1 (12-195)	276.2 ± 27.5 nM	0.826
10mer: AAGGACUAGC	hnRNP A2/B1 (12-195)	114.7 ± 11.5 nM	1.09
10mer-m <sup>6</sup> A: AAGG(m <sup>6</sup> A)CUAGC	hnRNP A2/B1 (12-195)	1.32 ± 0.143 μM	0.948
8mer-m <sup>6</sup> A: AGG(m <sup>6</sup> A)CUGC	hnRNP A2/B1 (12-195)	460.8 ± 47.4 nM	0.963
A1G: A <u>G</u> GGACUAGC	hnRNP A2/B1 (12-195)	534.8 ± 40.3 nM	1.01
A1C: A <u>C</u> GGACUAGC	hnRNP A2/B1 (12-195)	662.3 ± 54.3 nM	1.02
A1U: A <u>U</u> GGACUAGC	hnRNP A2/B1 (12-195)	531.9 ± 66.7 nM	1.14
G2A: AA <u>G</u> ACUAGC	hnRNP A2/B1 (12-195)	315.5 ± 37.0 nM	1.14
G2C: AA <u>C</u> ACUAGC	hnRNP A2/B1 (12-195)	699.3 ± 88.5 nM	1.17
G2U: AA <u>U</u> ACUAGC	hnRNP A2/B1 (12-195)	581.4 ± 58.1 nM	1.17
G3A: AAG <u>A</u> CUAGC	hnRNP A2/B1 (12-195)	244.5 ± 25.3 nM	1.19
G3C: AAG <u>C</u> CUAGC	hnRNP A2/B1 (12-195)	952.4 ± 1.14 nM	1.1
U6C: AAGGAC <u>C</u> AGC	hnRNP A2/B1 (12-195)	184.2 ± 16.3 nM	1.03
U6A: AAGGAC <u>A</u> AGC	hnRNP A2/B1 (12-195)	204.5 ± 19.9 nM	0.924
U6G: AAGGAC <u>G</u> AGC	hnRNP A2/B1 (12-195)	238.1 ± 42.7 nM	1.08
A7G: AAGGACU <u>G</u> GC	hnRNP A2/B1 (12-195)	108.2 ± 11.5 nM	0.919
A7U: AAGGACU <u>U</u> GC	hnRNP A2/B1 (12-195)	219.8 ± 21.6 nM	0.904
G8A: AAGGACUA <u>A</u> C	hnRNP A2/B1 (12-195)	106.6 ± 16.2 nM	1.01
G8C: AAGGACUA <u>C</u> C	hnRNP A2/B1 (12-195)	292.4 ± 44.2 nM	1.04
G8U: AAGGACUA <u>U</u> C	hnRNP A2/B1 (12-195)	271.7 ± 30.7 nM	0.954
10mer:AAGGACUAGC	hnRNP A2/B1 (12-195)Q19A	440.5 ± 42.0 nM	1.19
10mer:AAGGACUAGC	hnRNP A2/B1 (12-195)F24A	456.6 ± 35.5 nM	1.03
10mer:AAGGACUAGC	hnRNP A2/B1 (12-195)D49A	561.8 ± 69.4 nM	1.04
10mer:AAGGACUAGC	hnRNP A2/B1 (12-195)F66A	763.4 ± 10.2 nM	0.952
10mer:AAGGACUAGC	hnRNP A2/B1 (12-195)E92A	751.9 ± 85.5 nM	1.3
10mer:AAGGACUAGC	hnRNP A2/B1 (12-195)H108A	167.2 ± 19.2 nM	1.21
10mer:AAGGACUAGC	hnRNP A2/B1 (12-195)K113A	813.0 ± 1.17 nM	1.27
10mer:AAGGACUAGC	hnRNP A2/B1 (12-195)F115A	781.3 ± 87.7 nM	1.31
10mer:AAGGACUAGC	hnRNP A2/B1 (12-195)K120A	413.2 ± 50.5 nM	1.2
10mer:AAGGACUAGC	hnRNP A2/B1 (12-195)F157A	534.8 ± 97.1 nM	1.39
10mer:AAGGACUAGC	hnRNP A2/B1 (12-195)E183A	289.9 ± 47.8 nM	1.32
10mer: AAGGACUAGC	hnRNP A2/B1 (1-353)	103.3 ± 8.93 nM	0.555
10mer-m <sup>6</sup> A: AAGG(m <sup>6</sup> A)CUAGC	hnRNP A2/B1 (1-353)	171.5 ± 25.6 nM	0.523
10mer: AAGGACUAGC	hnRNP A2/B1 (1-249)	26.5 ± 1.8 nM	1.05
10mer-m <sup>6</sup> A: AAGG(m <sup>6</sup> A)CUAGC	hnRNP A2/B1 (1-249)	176.1 ± 25.3 nM	1.19
8mer: AGGACUGC	hnRNP A2/B1 (1-249)	465.1 ± 53.2 nM	0.866
8mer-m <sup>6</sup> A: AGG(m <sup>6</sup> A)CUGC	hnRNP A2/B1 (1-249)	505.1 ± 44.2 nM	0.795

**Supplementary Table 3. Potential targets for hnRNP A2/B1.**

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A2RE: 5'-GCCAAGGAGCCAGAGAGCAUG-3'	(JBC, 1999) <sup>1</sup>
A2RE11: 5'-GCCAAGGAGCC-3'	(JBC, 1999) <sup>1</sup>
CLIP-seq: 5'-GGUAGUAG-3',	(Cell Reports, 2012) <sup>2</sup>
CLIP-seq: 5'-AGG(A/U)U(A/C/U)G(A/G)	
5'-UAASUUUAU-3'	(Gene & Dev. 2016) <sup>3</sup>
5'-UAG(A/G)-3'	(Neuron, 2016) <sup>4</sup>
HOTAIR: Purine-rich	(RNA, 2016) <sup>5</sup>
5'-UAGGG-3'	(Nature, 2013) <sup>6</sup>
5'-GG(m6A)CU-3'	(Cell, 2015) <sup>7</sup>
5'-GAGG-3'	(Nat. Comms. 2013) <sup>8</sup>

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