

Supplemental Figure Legends

Supplemental Figure 1. Additional contacts within RRM12 domain contribute to the unique conformation of IMP3 RRM12. (A) IMP3 RRM12 (with C-terminal His₆ tag) is a monomer showed by size exclusion column coupled with multi-angle light scattering (MALS). (B) Hydrophobic contacts in the interface of RRM1 and RRM2. (C) A dense network of interactions stabilizes the linker conformation.

Supplemental Figure 2. Sequence alignment of RRM12 domains of IMP1-3. Identical residues (white), highly conserved (red) and not conserved (black) are depicted. Residues involved in hydrophobic contacts within RRM1 and RRM2 interface are marked in dark gray stars. The linker amino acids, which are important for the unique conformation of linker and IMP3 RRM12, are marked in light gray stars. The ones labeled in black circles are involved in RRM1 and RNA interaction.

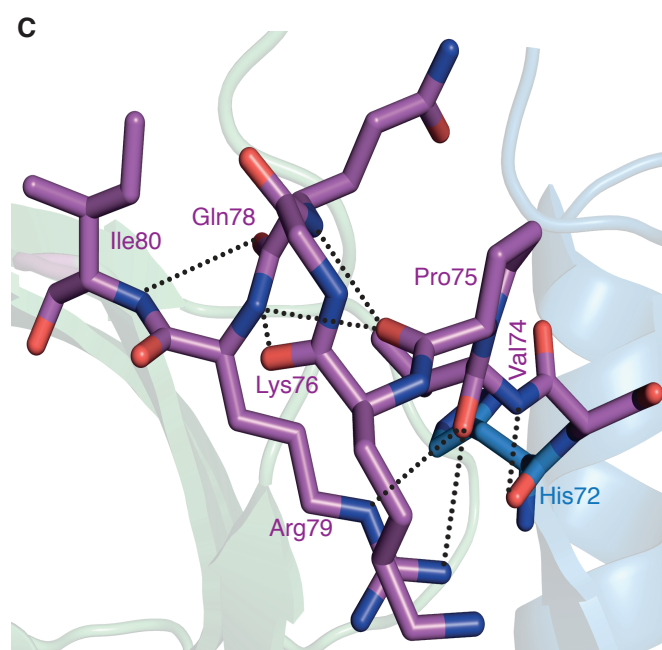
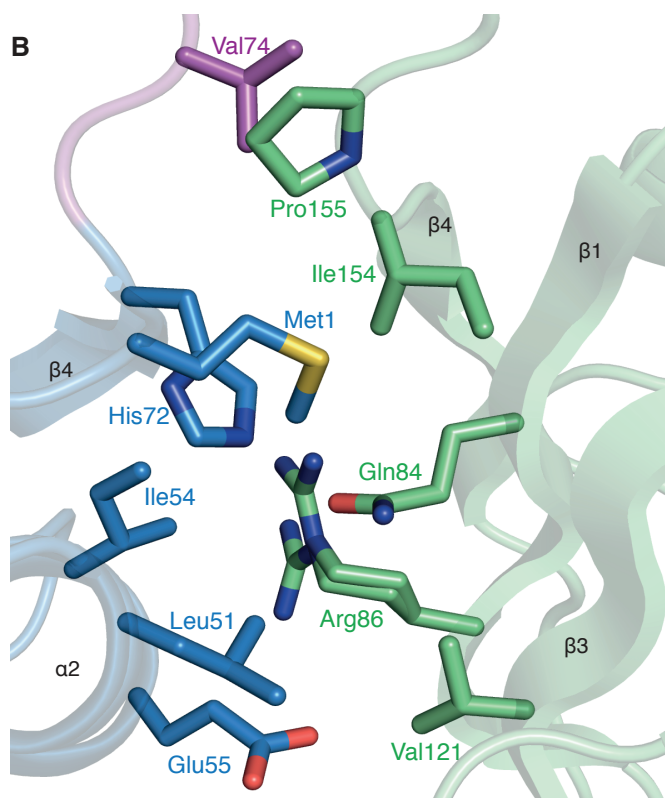
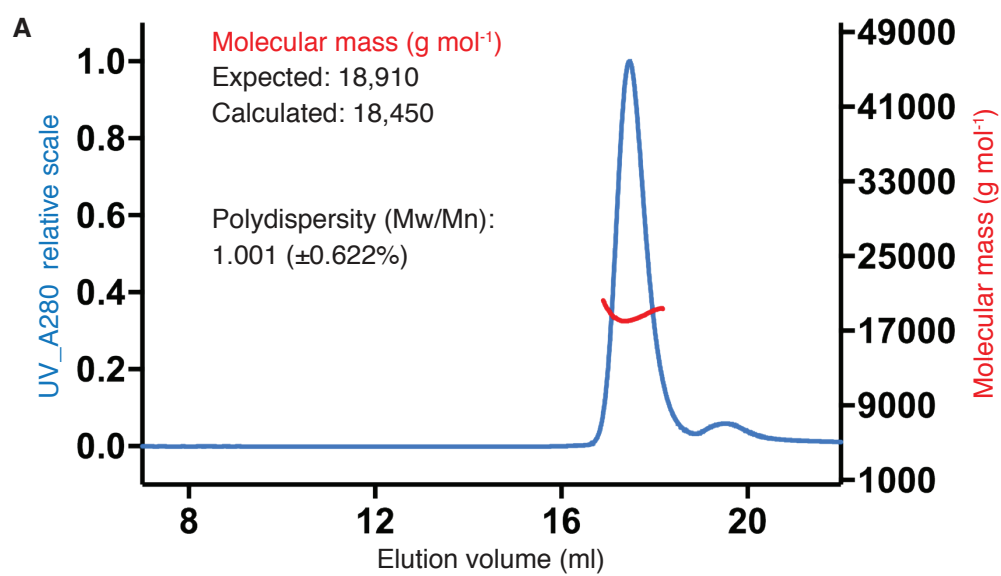
Supplemental Figure 3. IMP3 RRM12 domain orientation represents a novel tandem RRM domain arrangement. Overlay of IMP3 RRM12 (green) with FIR RRM12 (pink, PDB 2KXF), hnRNPL RRM34 (wheat, PDB 3TO8), hnRNPA1 RRM12 (grey, PDB 1HA1), Prp24 RRM12 (cyan, PDB 2GO9) and CPEB1 RRM12 (yellow, PDB 2MKH), aligned on one of their two RRMs.

Supplemental Figure 4. Binding affinity measurements of IMP3 RRM12 with RNA UUCCCG (A), UUUU (B) and GGGG (C) using ITC. The continuous line shows the fit of the data to a one-site binding model.

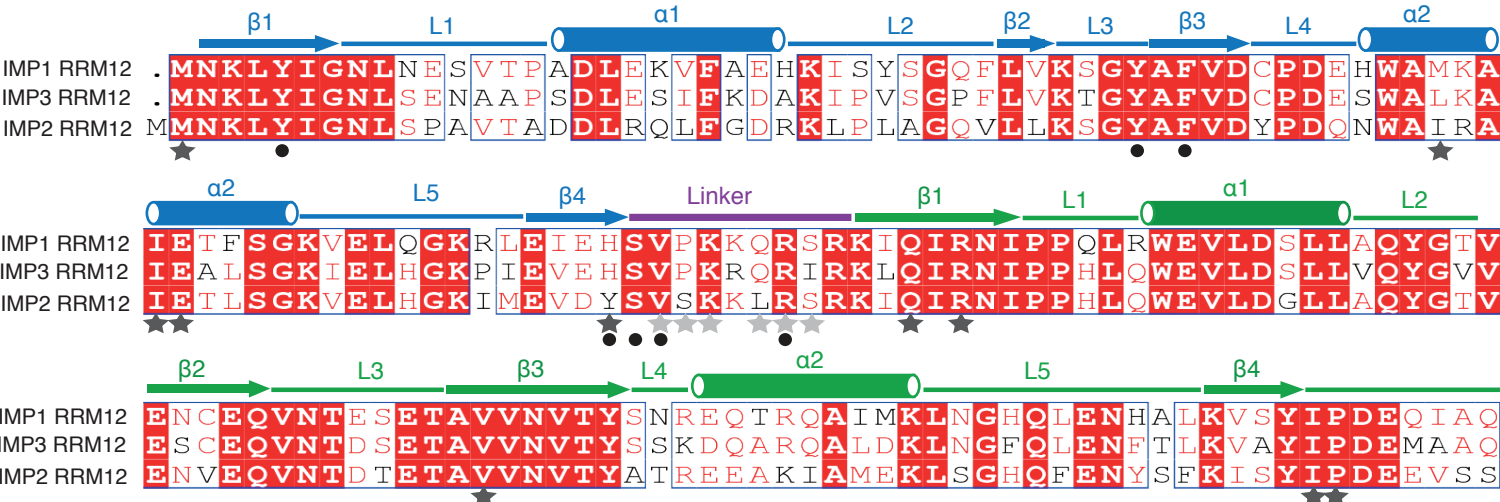
Supplemental Figure 5. Binding affinity measurements of IMP3 RRM12 with RNA ACACACA (A), CCCCCCCC (B), AAAAAAAAA (C) and UUUUUUUU (D) using ITC. The continuous line shows the fit of the data to a one-site binding model. (E) Statistics of the above measurements.

Supplemental Figure 6. Electron density map of visible nucleotides in the complex of IMP3 RRM12 with RNA ACAC (A) and CCCC (B). The contour level is 1.0.

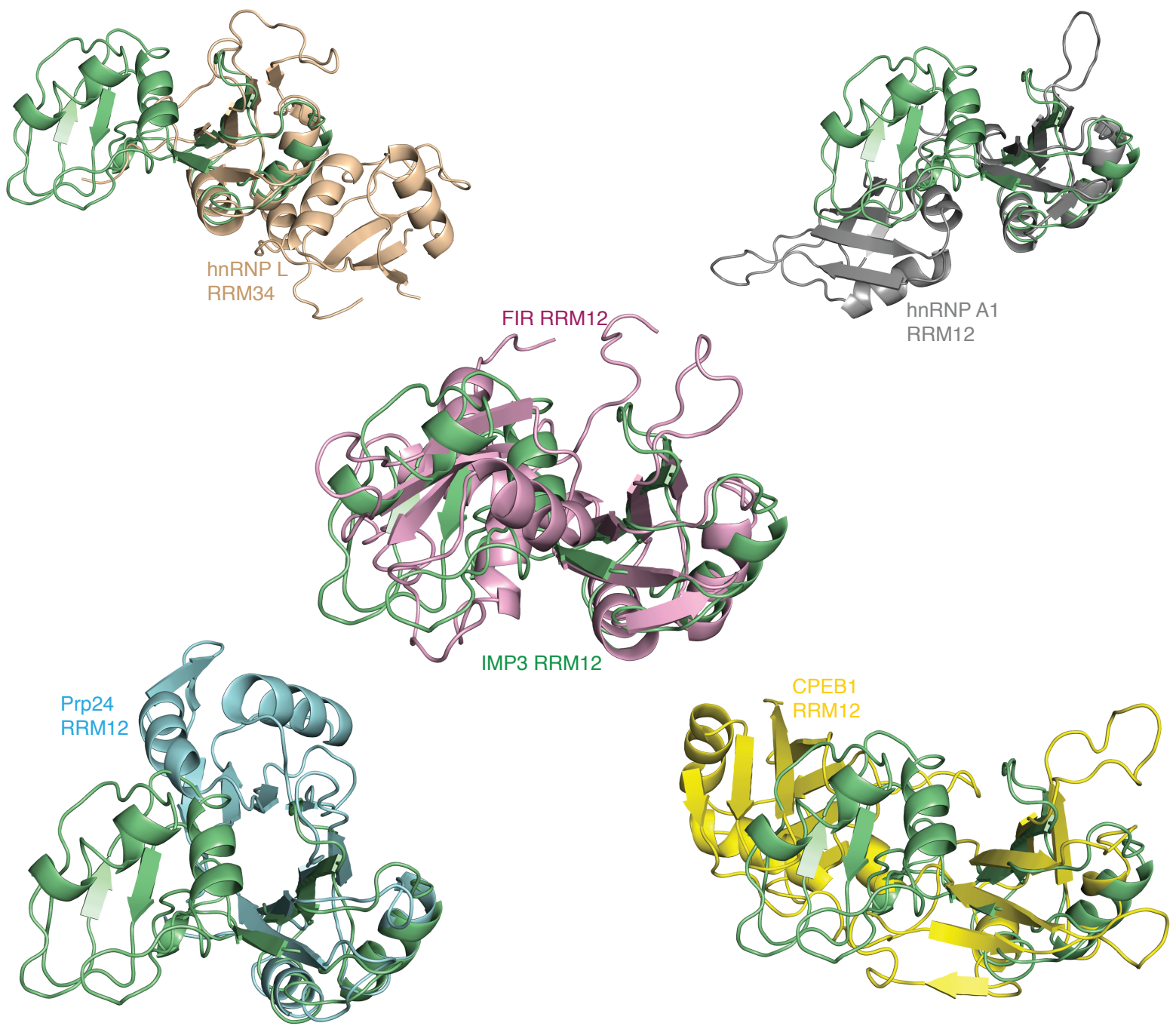
Supplemental Figure 1



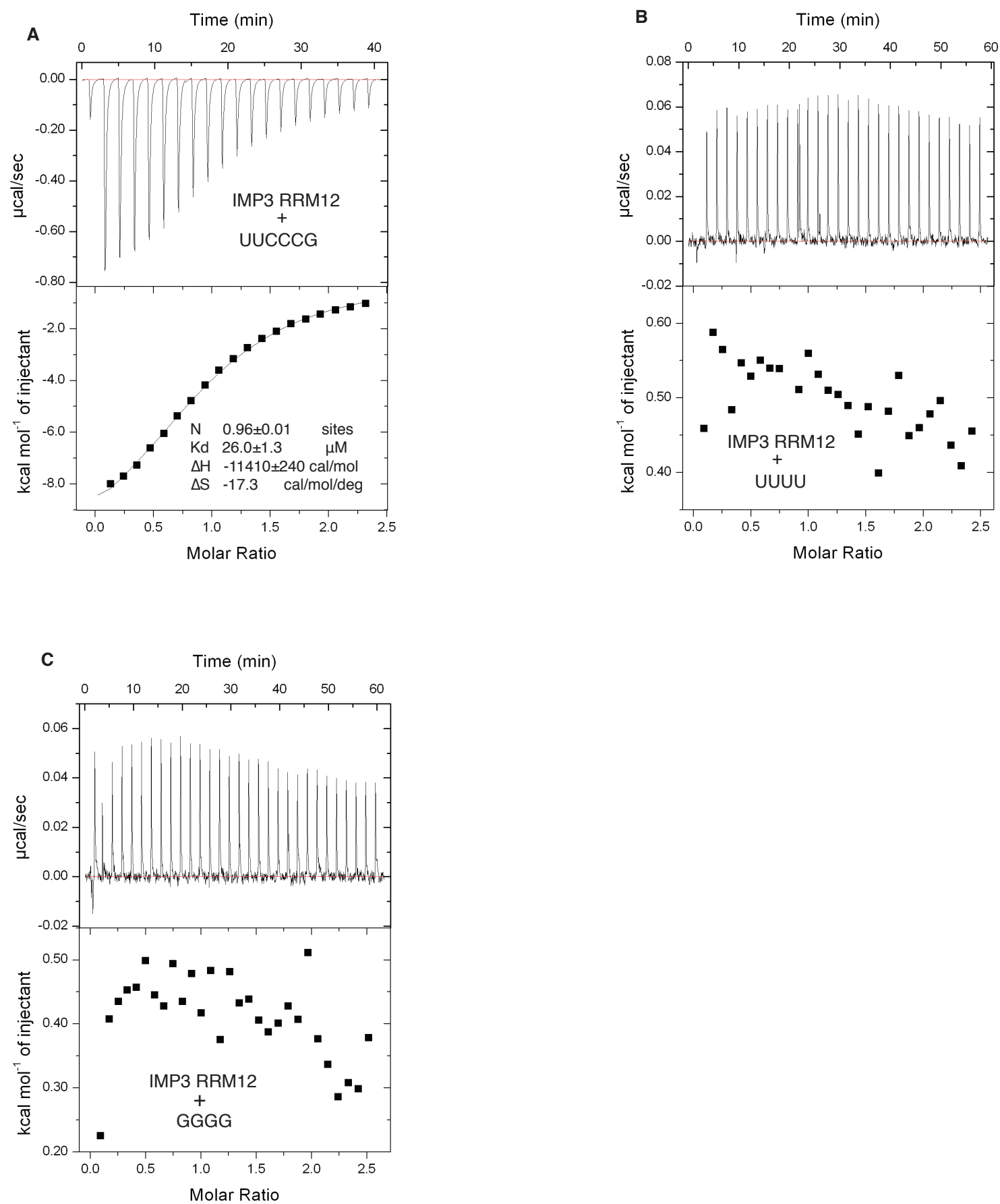
Supplemental Figure 2



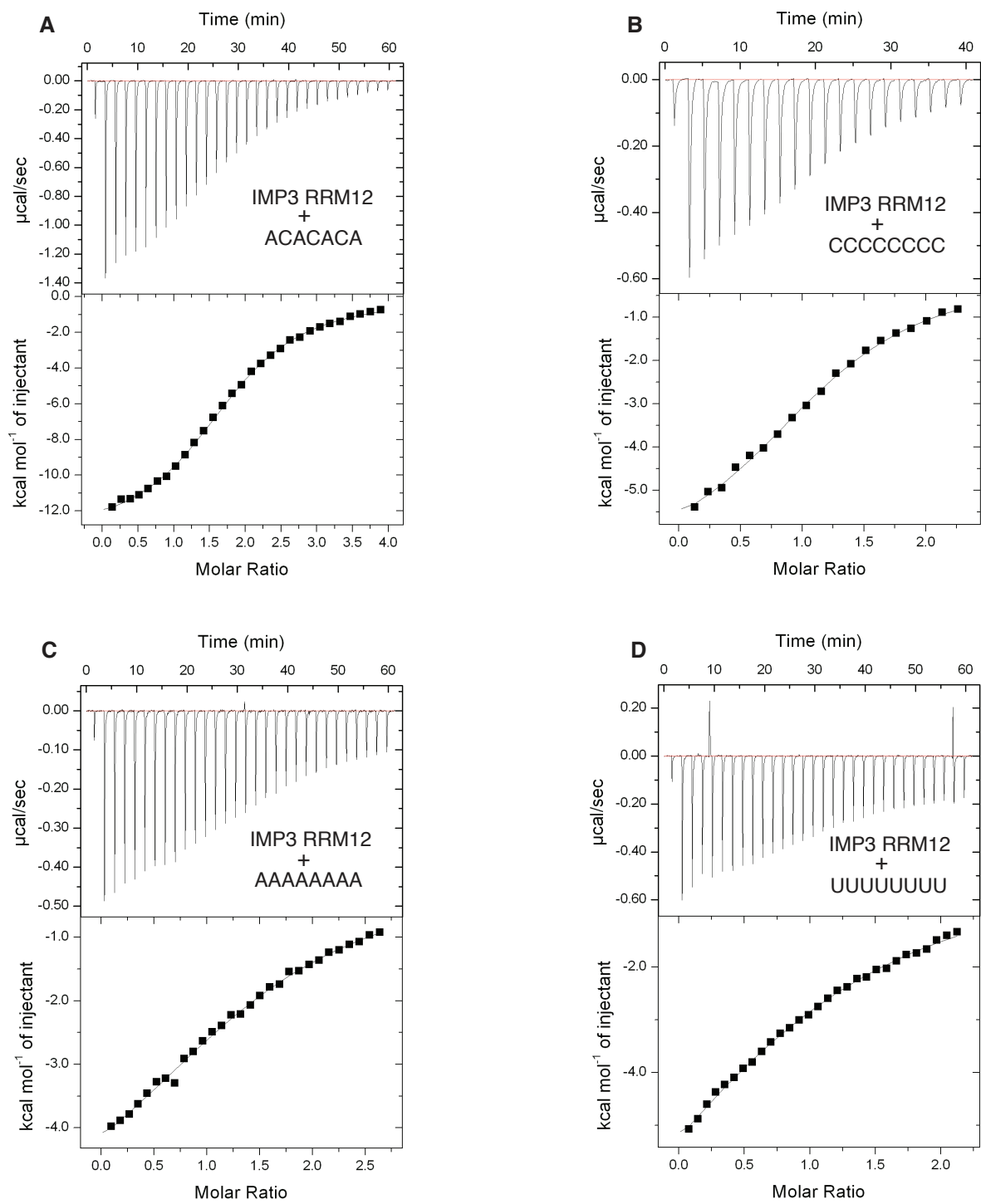
Supplemental Figure 3



Supplemental Figure 4



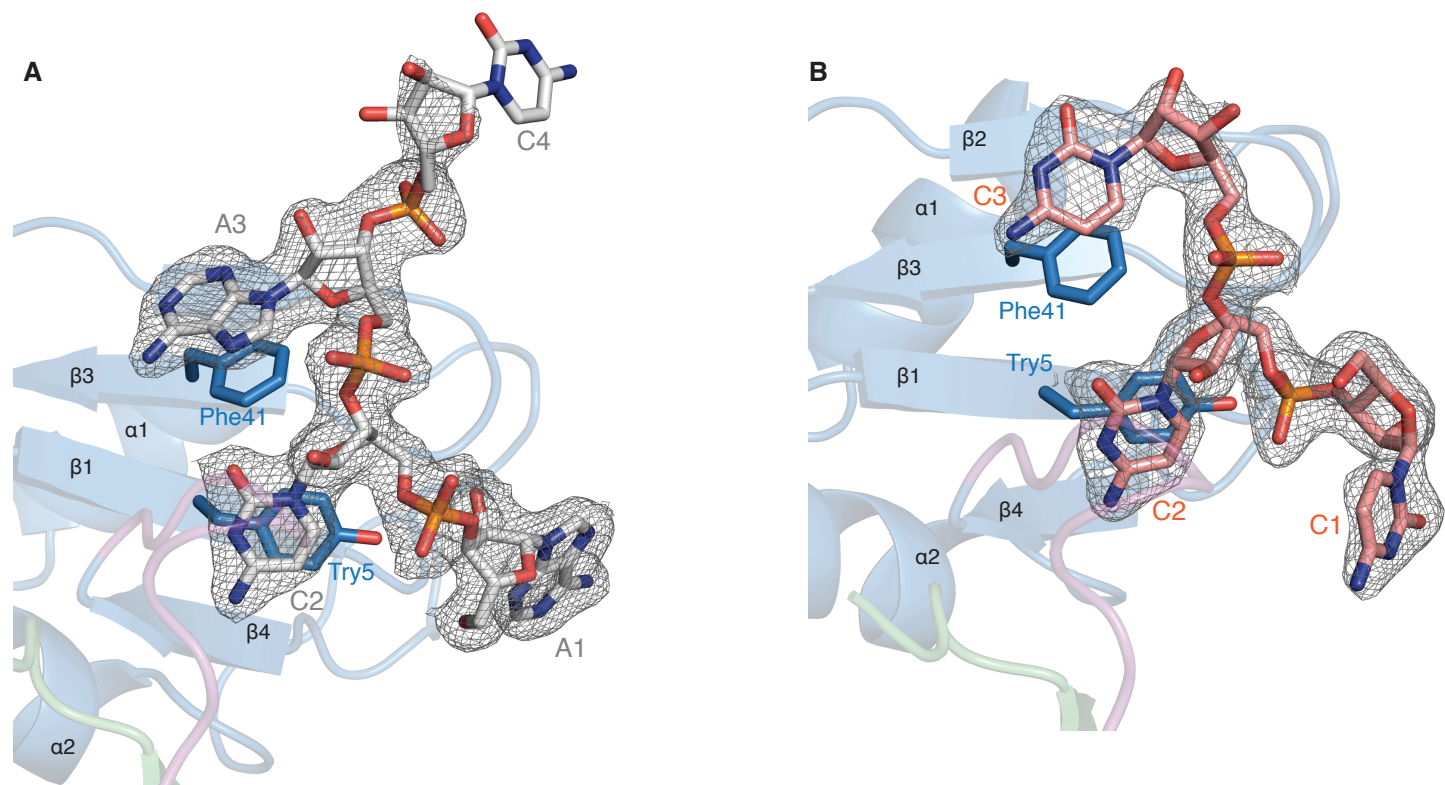
Supplemental Figure 5



E

Statistics IMP3 RRM12	Kd(μM)	N binding site	ΔH cal/mol	ΔS cal/mol/deg
ACACACA	9.3 $\pm$ 0.3	1.79 $\pm$ 0.01	-13400 $\pm$ 92.5	-21.9
8C	23.6 $\pm$ 1.6	1.20 $\pm$ 0.02	-6795 $\pm$ 145	-1.62
8A	44.8 $\pm$ 3.6	1.49 $\pm$ 0.03	-6057 $\pm$ 228	-0.40
8U	87.7 $\pm$ 6.4	1.10 $\pm$ 0.04	-10480 $\pm$ 612	-16.5

Supplemental Figure 6



Supplemental Table 1

Data collection and refinement statistics of IMP3 RRM12 and IMP3 RRM12+RNA.

Dataset	IMP3 RRM12	IMP3 RRM12 +CCCC	IMP3 RRM12 +ACAC
PDB	6FQ1	6FQR	6GX6
Space group	P1	P1	P3 ₁ 2 1
Cell dimensions			
<i>a</i> , <i>b</i> , <i>c</i> (Å)	30.13, 41.58, 72.49	30.06, 41.19, 72.27	75.44, 75.44, 66.26
α , β , γ (°)	92.01, 99.91, 108.94	92.14, 100.40, 108.65	90.0, 90.0, 120.0
Data Collection			
Wavelength (Å)	0.9795	0.9999	0.9999
Resolution range	50.0-1.31 (1.38-1.31)	50.0-2.10 (2.15-2.10)	66.26-2.0 (2.11-2.0)
No. of reflections	244,339	35,187	191,147
No. of unique reflection	75,254	17,886	15,116
<i>R</i> _{merge} (%)	4.4 (42.3)	7.7 (54.1)	9.2 (116.5)
<i>I</i> / σ <i>I</i>	12.3 (2.8)	8.1 (1.8)	18.2 (2.4)
Completeness (%)	95.1 (92.4)	95.3 (93.2)	100 (100)
Multiplicity	3.2 (3.2)	2.0 (2.0)	12.6 (12.0)
CC _{1/2}	99.9 (81.0)	99.4 (52.6)	99.9 (60.0)
Refinement			
Resolution range	19.44-1.31 (1.35-1.31)	38.82-2.1 (2.18-2.10)	46.52-2.0 (2.11-2.0)
<i>R</i> _{work} / <i>R</i> _{free} (%)	14.02 / 17.45	16.70 / 22.96	18.05 / 23.59
Wilson B-factor (Å ²)	13.02	27.15	36.4
Average B-factors (Å ²)	20.8	29.5	43.0
No. of atoms			
Protein	2787	2533	1261
RNA	0	57	81
Ligands	4	0	21
Stereochemistry			
R.m.s.d. bond lengths (Å)	0.009	0.007	0.007
R.m.s.d. bond angles (°)	1.4	1.2	1.02
Ramachandran favored (%)	98.0	98.0	97.5
Ramachandran outliers (%)	0.6	0.3	0.6

Supplemental Table 2

Sequence motifs enriched from SELEX by nine rounds of selection with IMP3

Sequences	Copies #	% of Total copies
-G/A/C/T- TTCCC -T/C/G/A-	17	37.78
CCC -G/A/C-	14	31.11
CC -G/A-	9	20
- TTCTC - - TGCCC - - TTTCC - - TCCCC - - TTCCT -	5	11.11