SUPPLEMENTARY INFORMATION

NMR titrations of TIA-1-RRM2 and -RRM3 with U9 RNA

RNA binding of individual RRMs of TIA-1 was analyzed by adding known amounts of U9 to 0.1 mM of ^{15}N-labelled RRM, and ^{1}H,^{15}N HSQC spectra were recorded after each titration to measure the chemical shift of each amide resonance. U9 in small volumes with highly concentrated solutions were titrated into the protein samples leading to negligible dilution. At least 10 different protein:RNA ratios were used for each interaction, ranging from 1:0.1 to 1:1.8 ratio for RRM3 and 1:2 ratio for RRM2. The ligand binding occurred in the fast-exchange regime, and peaks in the NMR spectra were manually traced and checked by NMRView. Binding constants for each residue (without severely overlapped resonances) were determined by fitting with non-linear regression analysis to the change in chemical shift versus additional U9 concentrations by the following equation:

$$\Delta_{obs} = \Delta_{\max} \cdot \frac{(K_D + [L] + [P]) - \sqrt{(K_D + [L] + [P])^2 - 4[P][L]}}{2[P]}$$

where Δ_{obs} is the observed change in chemical shift, Δ_{max} is the total change in chemical shift at saturation, [L] and [P] are titrated RNA and total protein concentrations respectively. Given that a single stranded RNA with ~4-7 nucleotides has been recognized frequently by a single RRM (1,2), a Hill coefficient was included to consider the possibility of binding in multiple registers to the U9 RNA. Generally speaking, a Hill coefficient equal to one (n = 1) indicates binding of RRM and RNA with a stoichiometry of 1:1 binding without any cooperativity. A Hill coefficient greater than one (n > 1) suggests multiple binding sites with positive cooperativity. Titration profiles for several amide groups of RRM2 and RRM3 were fitted with the software package GraphPad Prism (version 6.00 for Mac, GraphPad Software, La Jolla California USA, www.graphpad.com). Global fits of five selected residues from RRM2 and RRM3 are shown in the right panel of **Suppl. Fig. S3** with (red line) or without considering a Hill coefficient (grey dashed line). The global fit results of five selected residues of RRM2 (K_D = 36.70 ± 1.28 μM, n = 1.58 ± 0.08 with R^2 = 0.9956) and RRM3 (K_D = 32.97 ± 0.56 μM, n = 1.43 ± 0.03 with R^2 = 0.9992) suggest the presence of multiple binding registers.

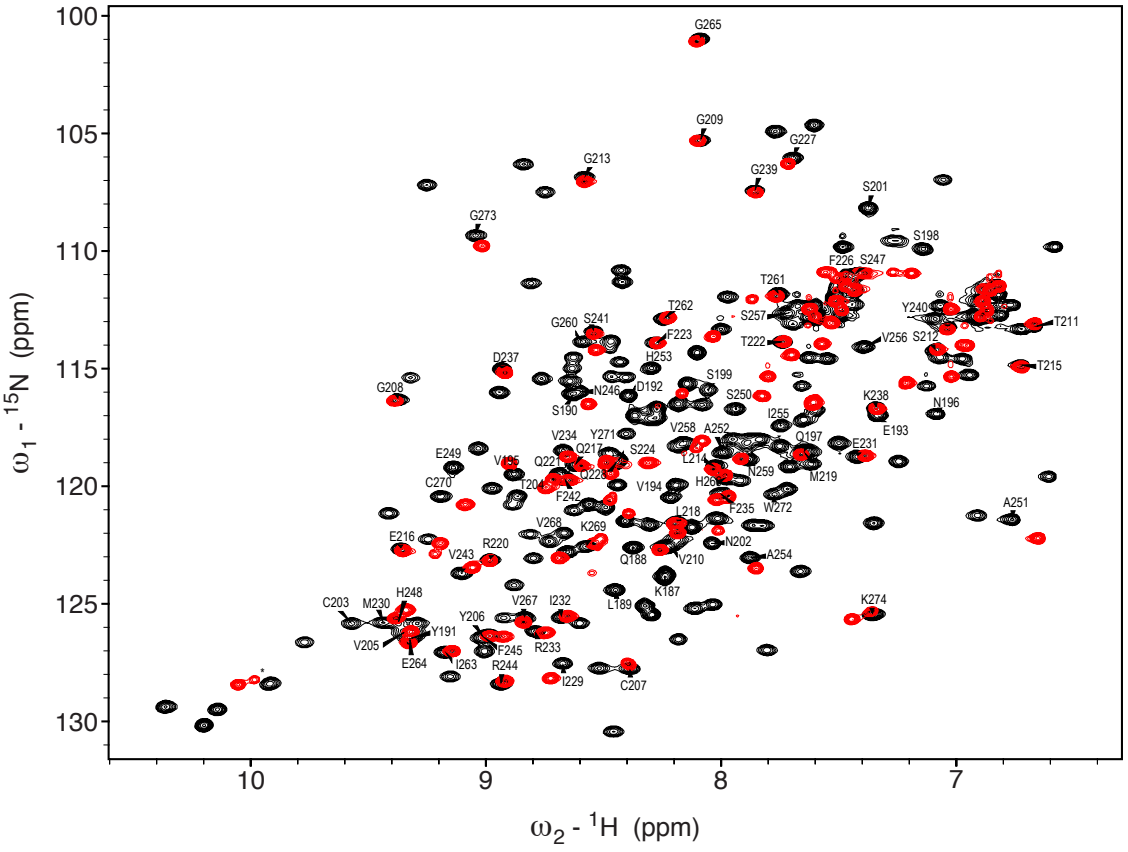

Fig. S1

Superposition of $^{1}H,^{15}N$-HSQC spectra of truncated RRM3 without the N-terminal helix (195-274) in red and RRM2,3 in black. Amide signals of RRM3 are annotated.

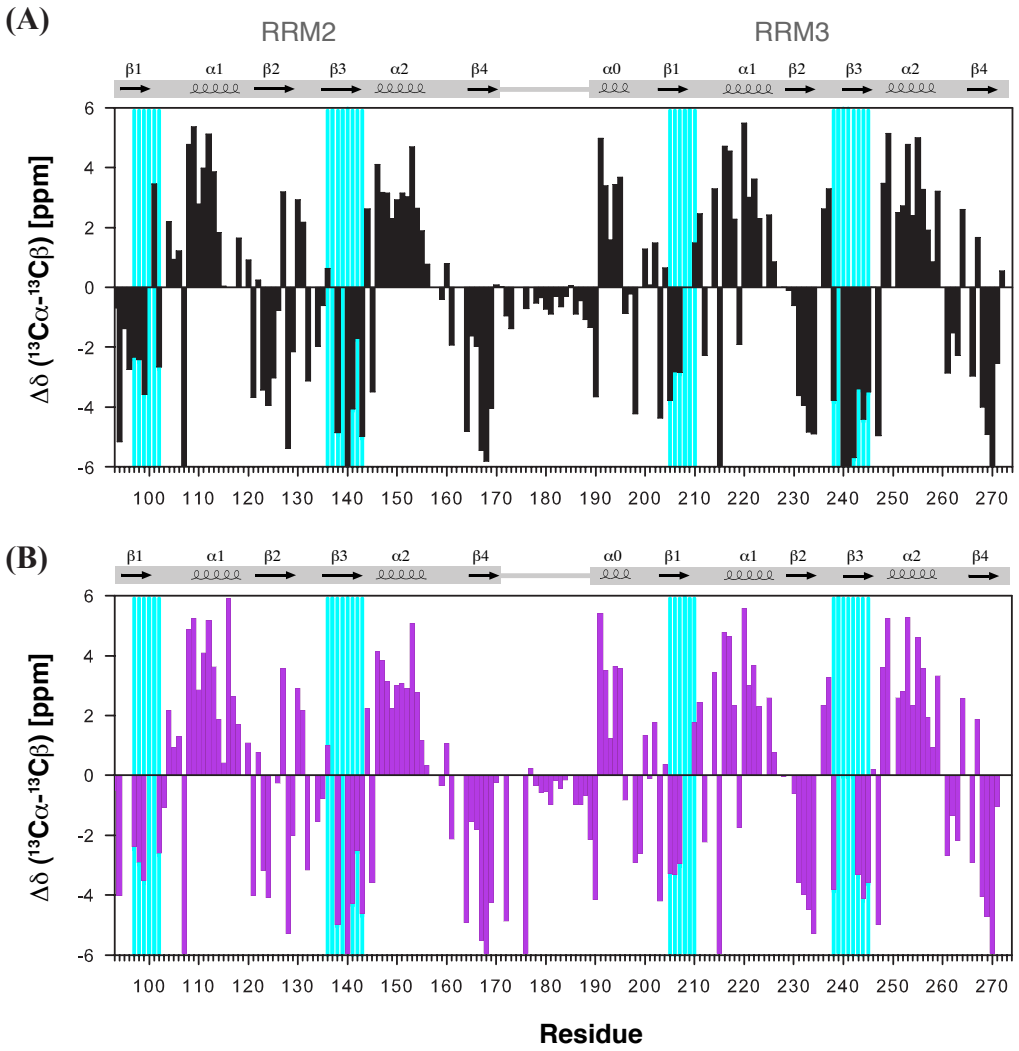

Fig. S2

Secondary ^{13}C chemical shifts $\Delta\delta(^{13}C\alpha\text{-}^{13}C\beta)$ of TIA-1-RRM2,3 (A) free (black) and (B) bound to fas_16 RNA (purple) predicted from Hα, Cα, Cβ, and C' chemical shifts by TALOS+. Residues with negative CSI indicate β-strands while positive values indicate turn or α-helical conformations. The CSI of residue 191-196 indicates the presence of an additional α-helix. Comparison of (A) and (B) indicates that the secondary structure is not altered RNA binding by TIA-1 RRM2,3.

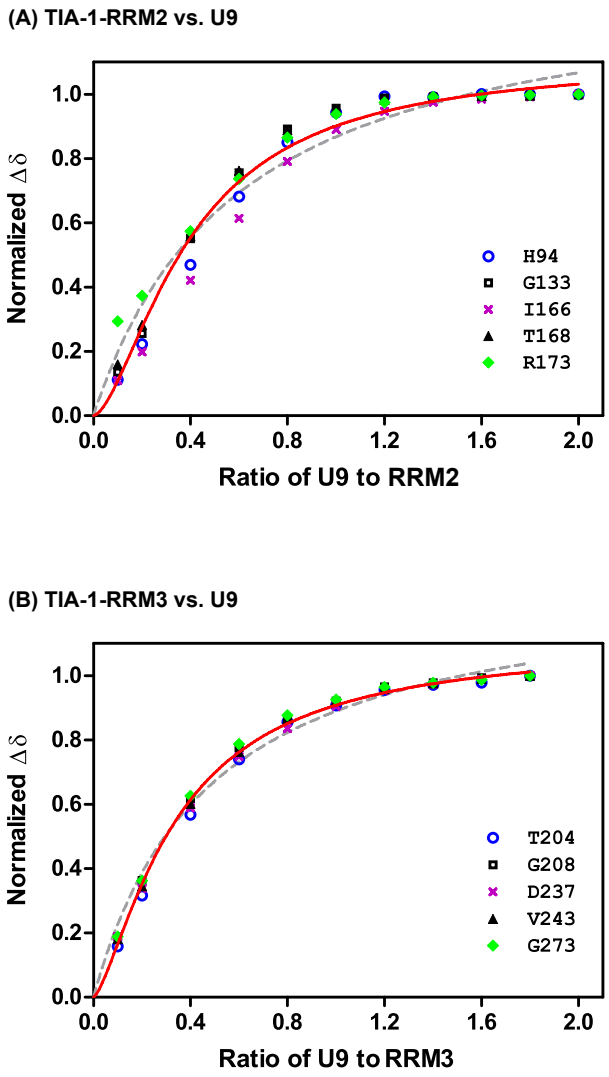

Fig. S3

Analysis of NMR titrations of TIA-1-RRM2 and -RRM3 with U9 RNA. Titration profiles for selected amide groups of each RRM (Fig. 4D, E) were normalized and analyzed by global fitting with (red line) or without (grey dashed line) including a Hill coefficient for a "one site-specific binding" model using the program Prism. Peaks showing intermediate exchange behaviors, e.g. Gly[137], were not applied to the fitting.

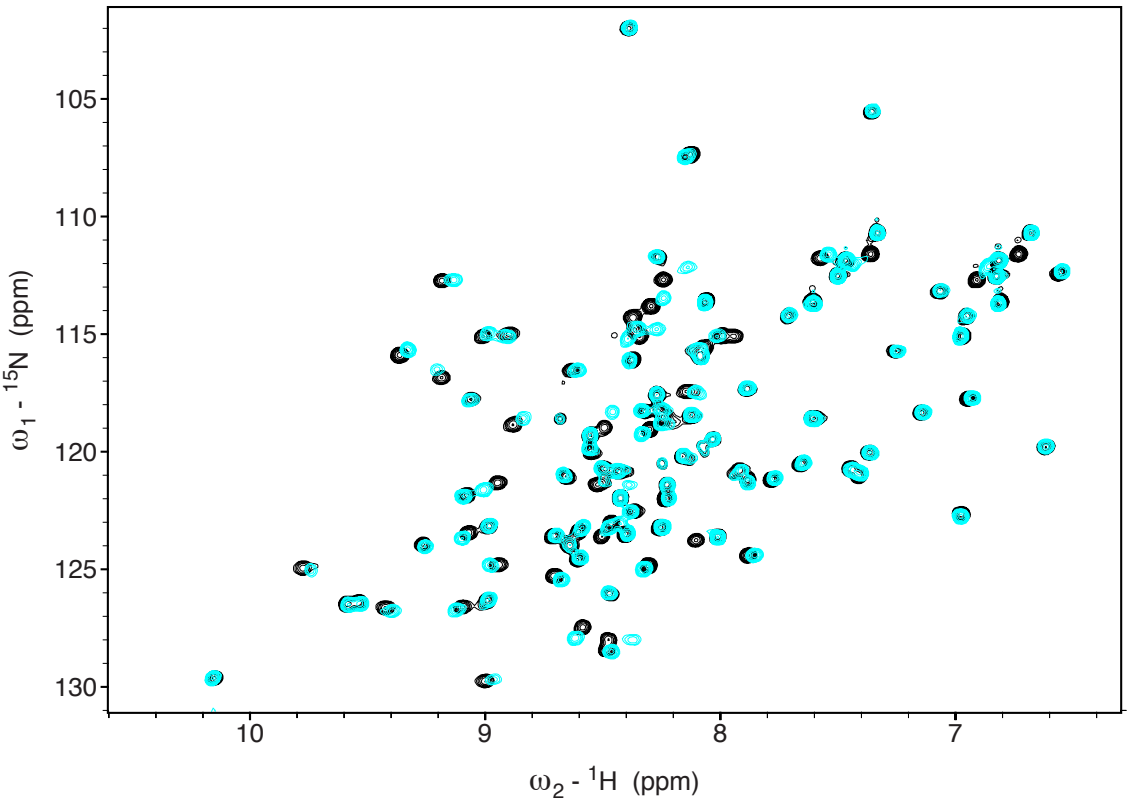

Fig. S4
Superposition of ^{1}H,^{15}N-HSQC spectra of RRM1 free (black) and bound to U9 RNA (cyan) at 1:2 molar ratio.

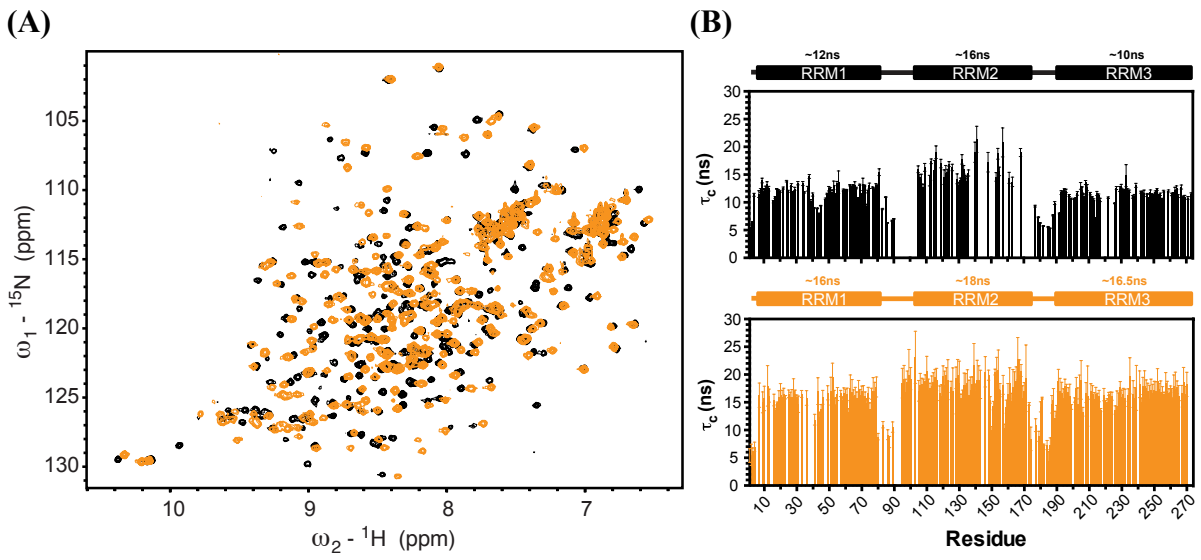

Fig. S5

The $^{1}H,^{15}N$ HSQC NMR spectra of RRM1,2,3 free (black) and bound with U15 RNA (orange) are shown in (A) and the corresponding rotational correlation times (τ_c) estimated from the ratio of ^{15}N R_2/R_1 relaxation rates of RRM1,2,3 free (black) and U15 bound form (orange) as a function of residue number are represented in (B). The average τc values for each RRM domain are indicated above.

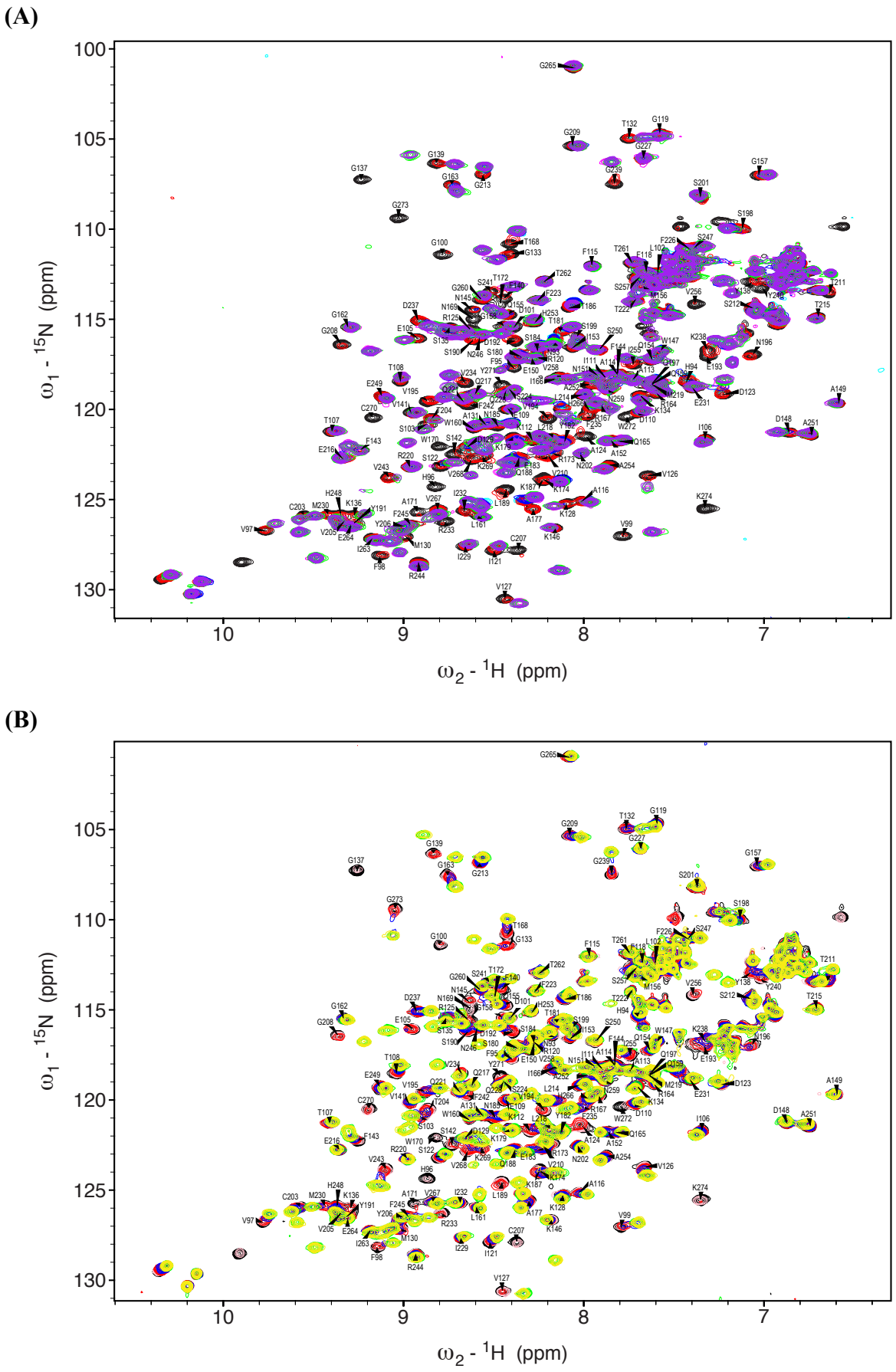

Fig. S6

NMR titration of TIA-1-RRM2,3 with fas_16 (A) and U9 RNA (B). (A) Spectra are colored as: free protein (black), with 0.2 (cyan), 0.4 (red), 0.6 (magenta), 0.8 (blue), 1.0 (green) and 2.0 (purple) molar equivalents of fas_16 RNA, and (B) with U9 RNA: 0.1 (pink), 0.2 (red), 0.4 (blue), 0.8 (green) and 1.25 (yellow) molar equivalents.

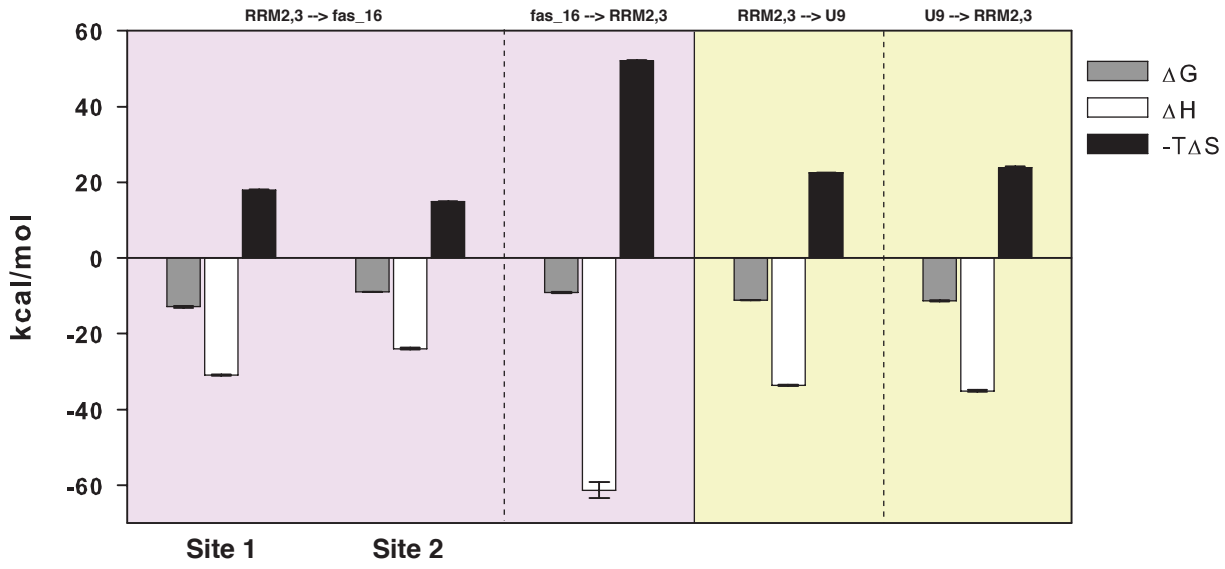

Fig. S7
Thermodynamic parameters for TIA-1-RRM2,3 binding to fas_16 and U9 RNAs determined from isothermal titration calorimetry measurements. All interactions are enthalpically favored with negative ΔH.

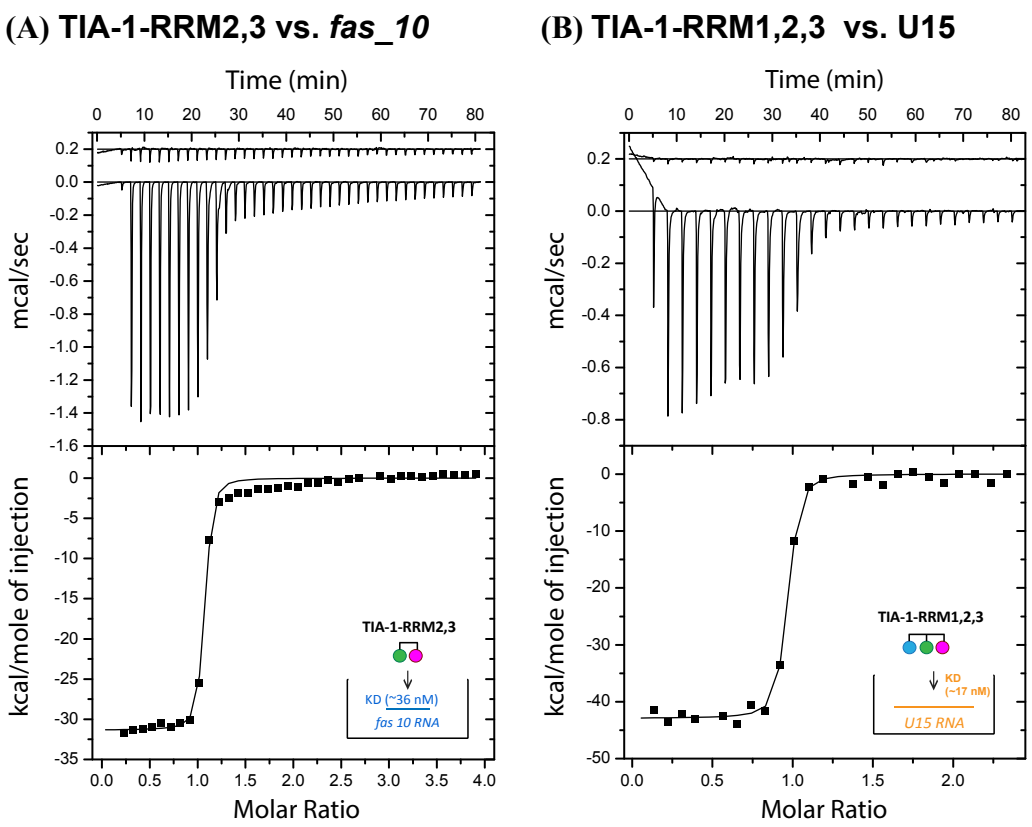

Fig. S8

(A) ITC measurements for the binding of TIA-1-RRM2,3 to *fas_10* RNA: by adding 577 µM RRM2,3 as titrant into 30 µM RNA. The dilution heat of the titrant is shown shifted by a value of 0.2 µcal/sec on top. (B) Binding of TIA-1-RRM1,2,3 to U15 RNA: 167 µM RM1,2,3 into 15 µM RNA. The fitted data for the titrations are reported in Table 2.

SUPPLEMENTARY REFERENCES:

1. Shamoo, Y., Abdul-Manan, N., Patten, A.M., Crawford, J.K., Pellegrini, M.C. and Williams, K.R. (1994) Both RNA-binding domains in heterogenous nuclear ribonucleoprotein A1 contribute toward single-stranded-RNA binding. *Biochemistry*, **33**, 8272-8281.
2. Varani, G. and Nagai, K. (1998) RNA recognition by RNP proteins during RNA processing. *Annu Rev Biophys Biomol Struct*, **27**, 407-445.

SUPPLEMENTARY TABLE 1. SAXS Data collection and scattering-derived parameters

	TIA-1 RRM2,3 free	TIA-1 RRM2,3 16mer bound
Data-collection		
Instrument	BioSAXS BM29 ESRF	BioSAXS BM29 ESRF
Beam geometry	10 mm slit	10 mm skit
Wavelength (Å)	1.008	1.008
q range ($Å^{-1}$)	0.004-0.45	0.004-0.45
Exposure time (s)[a]	2	2
Concentration range (mg ml^{-1})	2-10	2-3
Temperature (K)	298	298
Structural parameters[b]		
$I(0)$ (cm^{-1}) [from P(r)]	21.1 ± 0.018	25.4 ± 0.015
R_g (Å) [from P(r)]	24.4 ± 0.003	21.6 ± 0.003
$I(0)$ (cm^{-1}) [from Guinier]	21.1 ± 0.025	25.5 ± 0.021
R_g (Å) [from Guinier]	24.1 ± 0.096	22.0 ± 0.264
D_{max} (Å)	80	67
Porod volume estimate ($Å^3$)	29160	31740
Molecular mass determination[b]		
Molecular mass M_r [from $I(0)$]	19.9 kDa	22.5 kDa
Calculated monomeric M_r from sequence	20.2 kDa	25.3 kDa
Software employed		
Primary data reduction	BsxCuBE	BsxCuBE
Data processing	PRIMUS	PRIMUS

[a]10 frames were recorded for each sample.
[b]reported for a 2 mg/ml measurement