

## **Supplementary Material**

### **Solution structure and RNA-binding of a minimal ProQ-homolog from *Legionella pneumophila* (Lpp1663)**

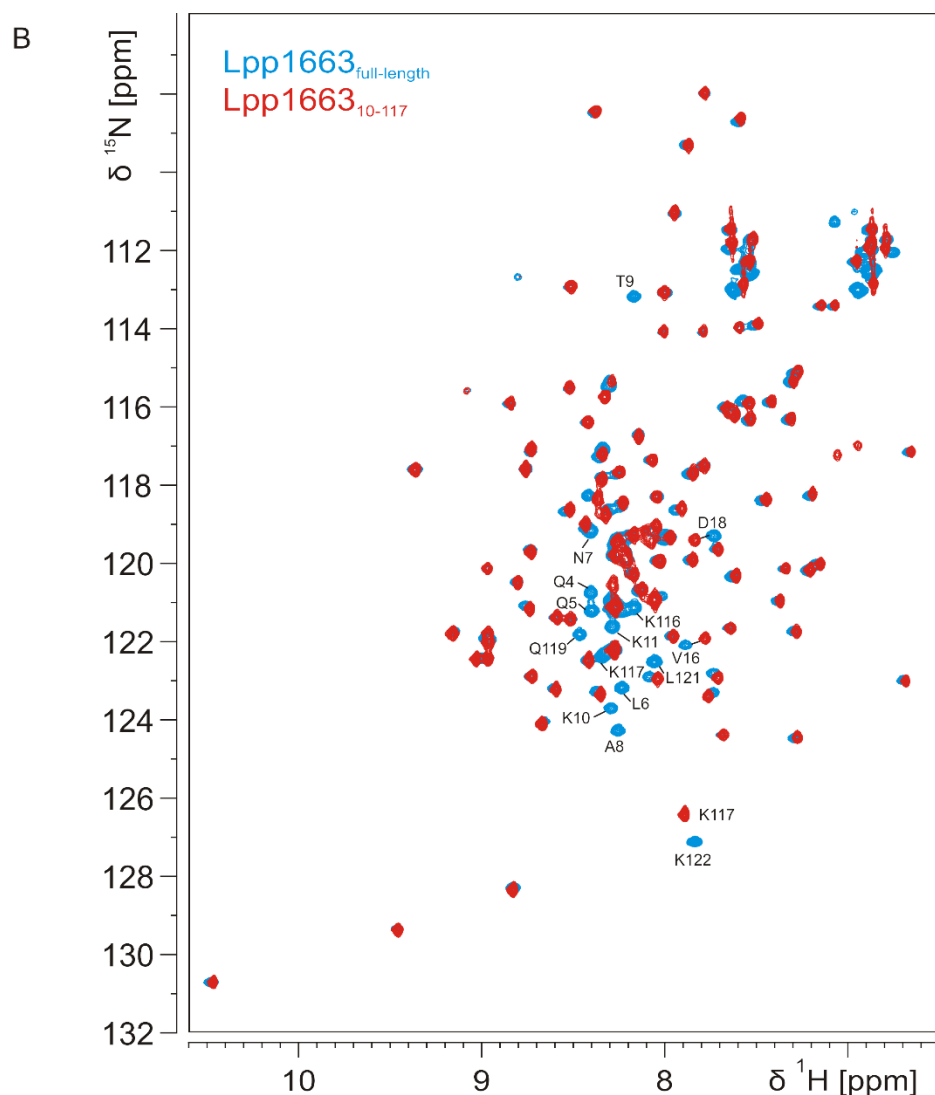
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Johann-Wolfgang-Goethe-University Frankfurt/Germany.

Supplemental Figure S1

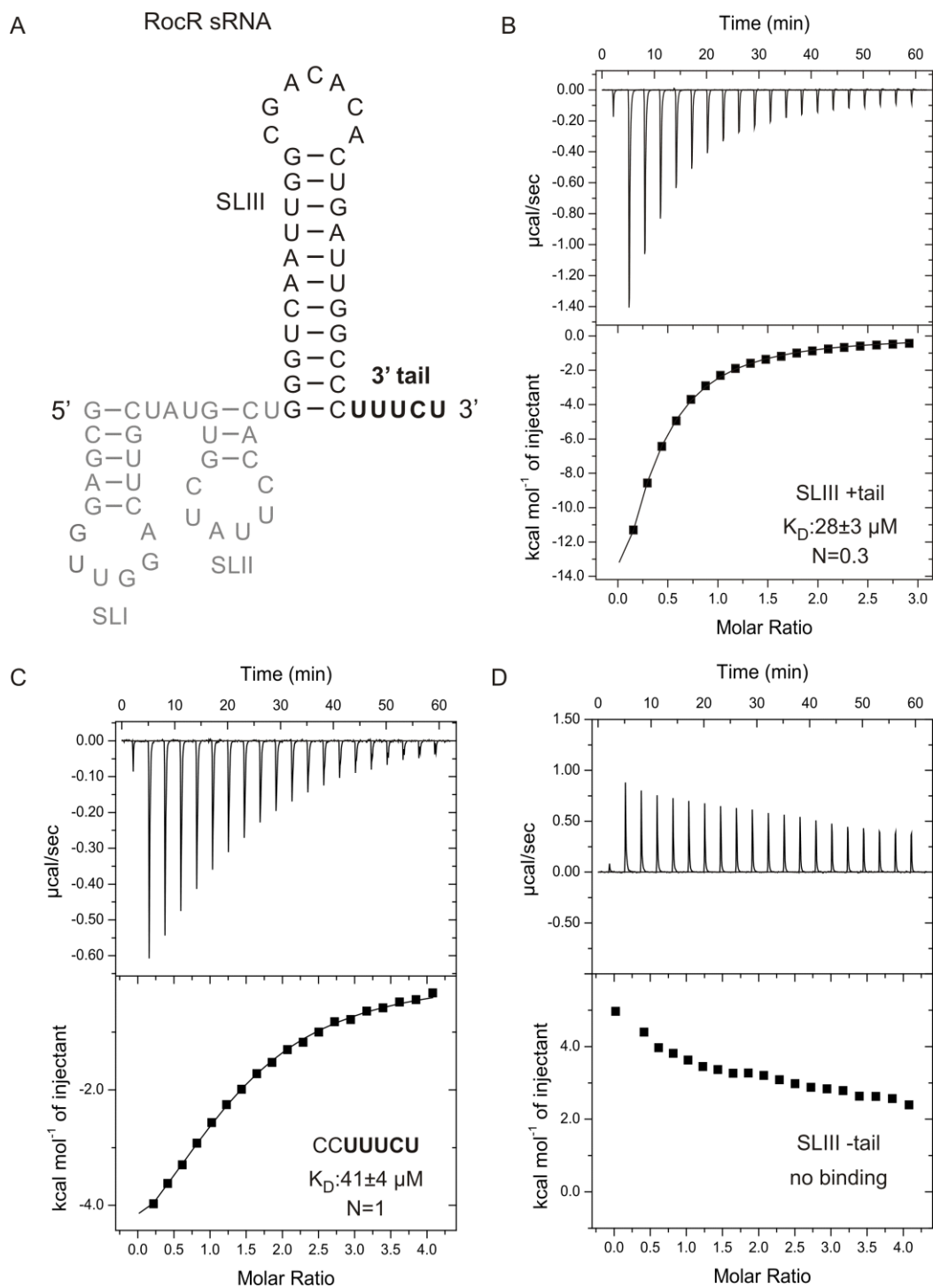
A

EcProQ	-----ME-	2
SeProQ	-----ME-	2
EcFinO	---MTEQKRPVLTILKRRTEGET-PTRSRTIINVTTTPKWKVKQKLA---EKAAREA	51
SeFopA	MGKNQEERKTPVIVVKKRRTFSLPSLSEKTDIIA---FVFTEQTAESAPAGINSSAVET	56
NmProQ	-----MT---QETALG-	8
LpRocC	-----MRKQALH---PRTAVI-	13
LpLpp1663	-----	0
EcProQ	-----NQPK-----LNSSKEVIAFLAERFPHCF-SAEGEARPLKIGIFQD	41
SeProQ	-----NQPK-----LNSSKEVIAFLAERFPHCF-SAEGEARPLKIGIFQD	41
EcFinO	ELTA---KKAQA--RQALSIYLNLPDLDEAVNTLKPWWPGLF--DGDTPRLLACGIRDV	103
SeFopA	HIPEAPARKKKKKRHRFRPSPHWTRREYTHECVEKIKALFPHLR-AEGGGFIPLKIGINND	115
NmProQ	-----AALKSAVQTMSSKKKQTEMIADHIYGKY-DVFKRFKPLALGIDQD	51
LpRocC	-----NKAQK--NQ-----SKRARSALLWLAANFPEAF-DNSLRIRPLKIGIMSD	56
LpLpp1663	-----GSNQQL--NA-----TKKDKLQVIDWLIENFPNAFFKKGNOVKPLKIGIFDD	45
	: * * *	
EcProQ	LVDRVA--GEMNLSKTQLRSALRLYTSSWRVLYG-VKPGATRVLDLGNPCGELDEQHVHE	98
SeProQ	LVERVG--GEMNLSKTQLRSALRLYTSSWRVLYG-VKPGATRVLDLGNPCGELDEQHVHE	98
EcFinO	LLEDVA-QRNIPLSHKKLRRALKAITRSESYLCA-MKAGACRYDTEGYVTEHISQEEVY	161
SeFopA	ISAFLAHEHPETELTMDDEWLCAVSCITSRVYLQRTAVAGVPRYGLDGHKQVSDSEAQS	175
NmProQ	LIAALPQ----YDAAL IARVLANHCRPRYLKA-LARGGKRFDLNRFKGEVTPPEQAI	105
LpRocC	ILQHAEKAEQVGVSKSKLREAVVLFTRRLDYLAC-LKAREVRIDLHGNPVAEVTETEEAEN	115
LpLpp1663	LIDFYERLDTPPFSKKSLEALSYYSSAPAYLSC-QKPD <del>T</del> ARVDIYGNEVDVVTPEQAKY	104
	: * * *	
EcProQ	ARKQLEEKARVQAQRAEQQAKKREAAATAGEKED-APR-----RERKPRPTTPR	147
SeProQ	ARKQLEEKARVQAQRAEQQAKKREAAAAAGEKED-APR-----RERKPRPVA-R	146
EcFinO	AAERLDKIRRQNRKAEIQA-----VLDEQ-----	186
SeFopA	AGRRLATLEQKWLRMQAQQENIS-----GQ-----	200
NmProQ	AQNHFFV-----QQALQQQSAQ-AAAETLSVEAAEAESSAAE-----	141
LpRocC	ASMKIKKRVEKSVKNARKQVNAK---AANHSYVNN-QPSTVSSVKPMNSFDSHPEPLLP	171
LpLpp1663	AYQRYQERYGNKKSQDLK-----	122
	* :	
EcProQ	---RKEGAERKPRAQKPVKAPKTV---KAPREEQHTPVSDI SALTVGQALKVKAGQNAM	201
SeProQ	---RKEGAERKPRADKPTTKAP-----RAPREEKHTPVSDI SVLTVGQSLKVKAGNNAM	197
EcFinO	-----	186
SeFopA	-----	200
NmProQ	-----	141
LpRocC	YPLRSSTYASQNVAM-QSAKSPSVVVKHAKPKQYDPAV---AR-----LKEKLGLSRK	221
LpLpp1663	-----	122
EcProQ	DATVLEITKDGVRVQLNSGMSLIVRAEHLVF	232
SeProQ	DATVLEITKDGVRVQLNSGMSLIVRAEHLVF	228
EcFinO	-----	186
SeFopA	-----	200
NmProQ	-----	141
LpRocC	AEDKKETTE-----	230
LpLpp1663	-----	122



**Figure S1: Sequence conservation and stability of the Lpp1663 ProQ domain. A** Sequence alignment of the ProQ/FinO homologs used in Figure 1. The central domain that harbours several conserved residues is highlighted in grey. Lpp1663 is numbered according to the construct used in this study. Residues important for RNA-binding are indicated in red. **B** The overlay of  $^1\text{H}$ ,  $^{15}\text{N}$  HSQCs of  $^{15}\text{N}$ -labelled Lpp1663 (blue) and a truncated version of the protein lacking the flexible N- and C-termini (Lpp1663tr, residues 10-117, red) show that the truncated protein is correctly folded. Residues that disappeared due to the truncation or changed their position are indicated by their residue number.

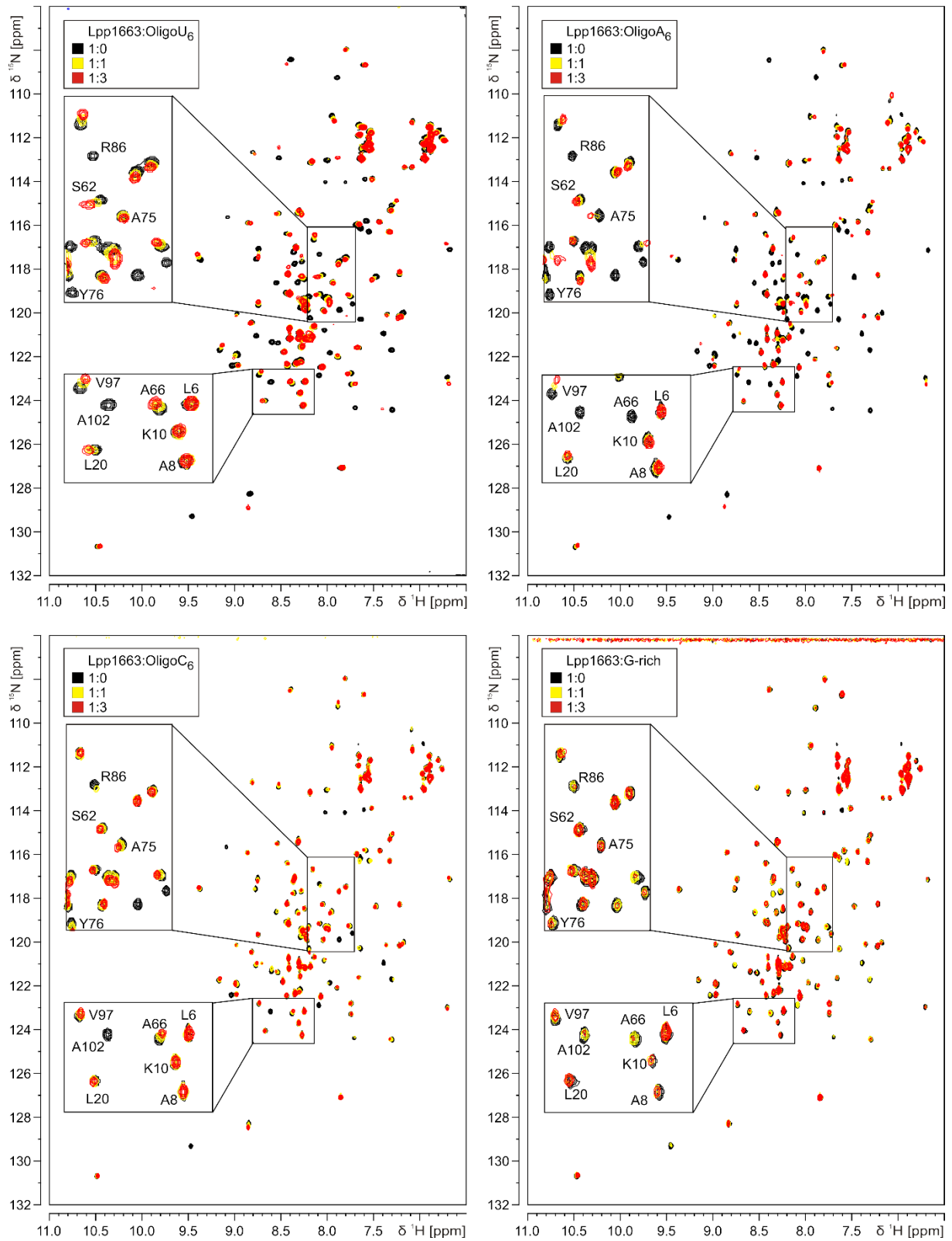
Supplemental Figure S2



**Figure S2: Binding of Lpp1663 to RocR sRNA.** **A** Secondary structure of RocR sRNA according to Attaiech *et al.*, 2016. SLIII indicated in black, the 3' extension depicted in bold. **B** **C** **D**) ITC thermograms of Lpp1663 titrated with RocR SLIII with or without its 3'-single stranded extension or the isolated extension.

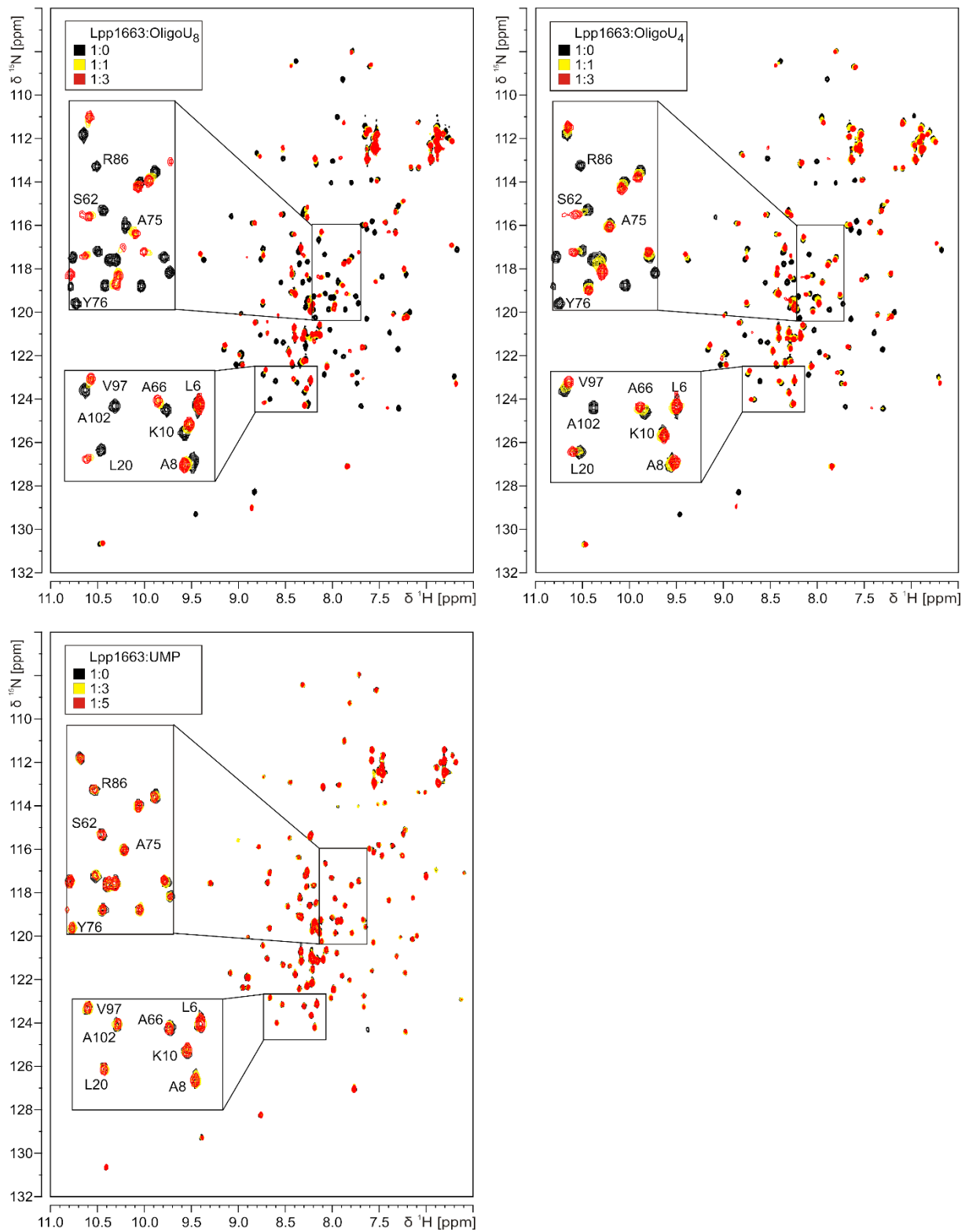
# Supplemental Figure S3

S3 A



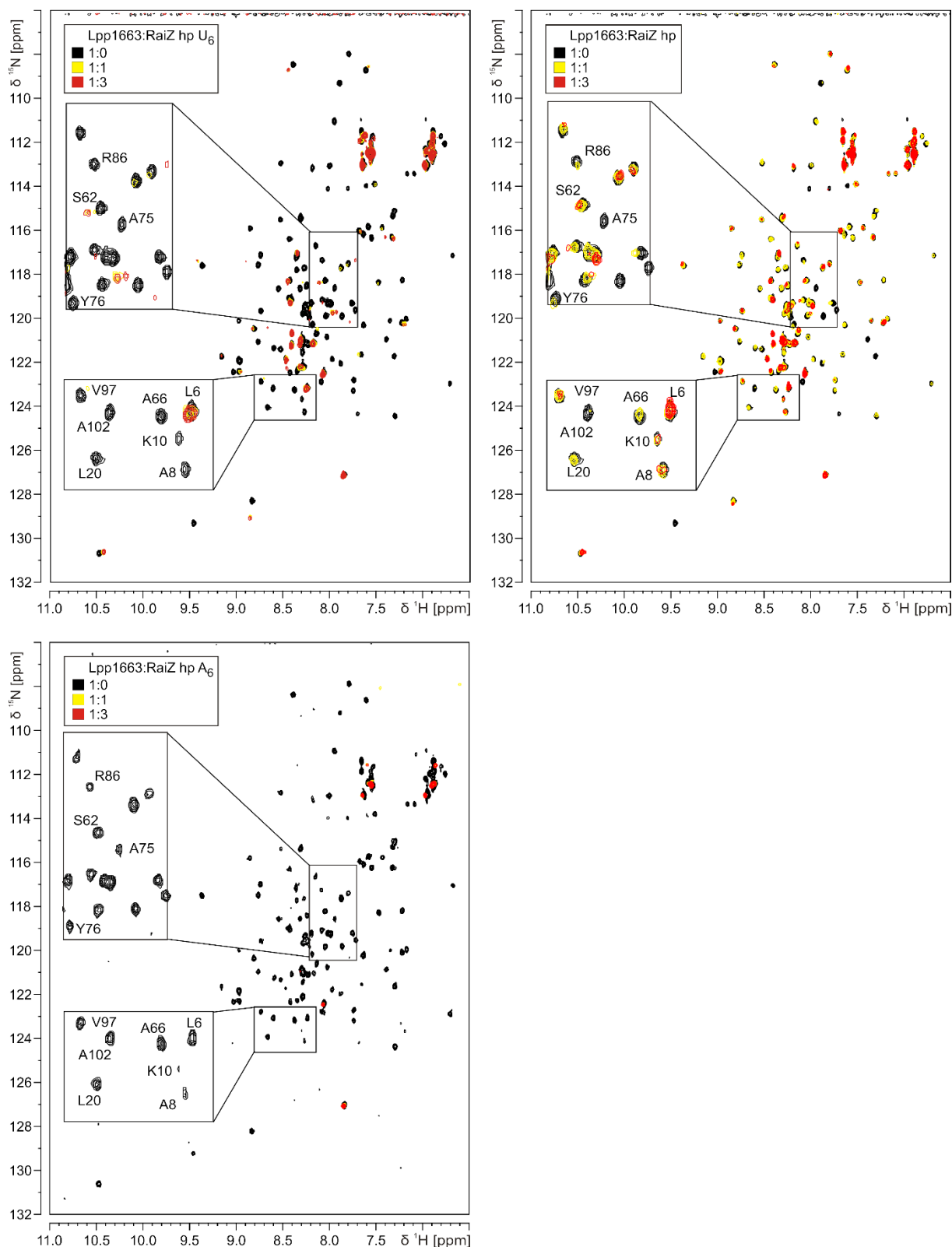
# Supplemental Figure S3

## S3 B

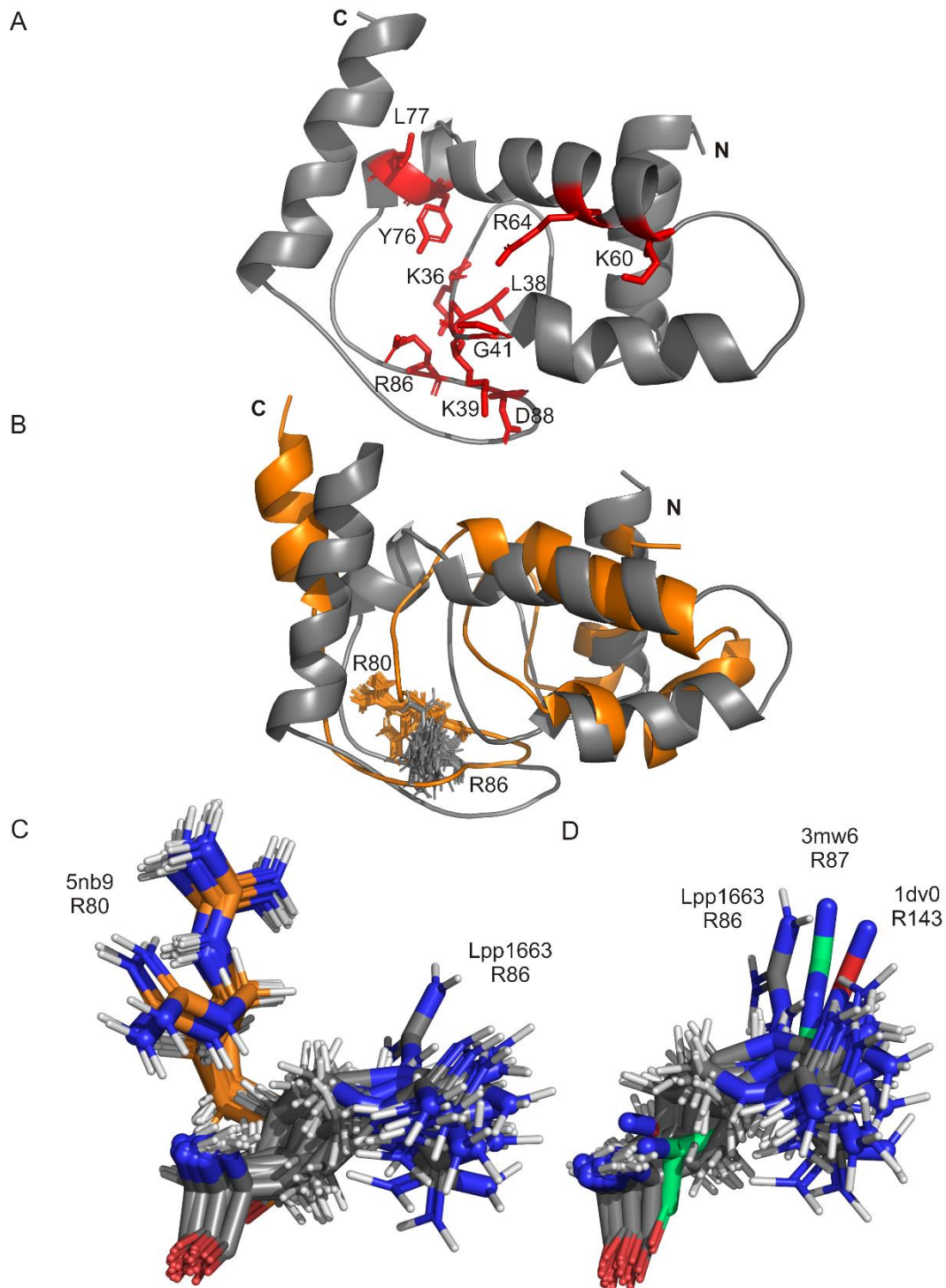


Supplemental Figure S3

S3 C



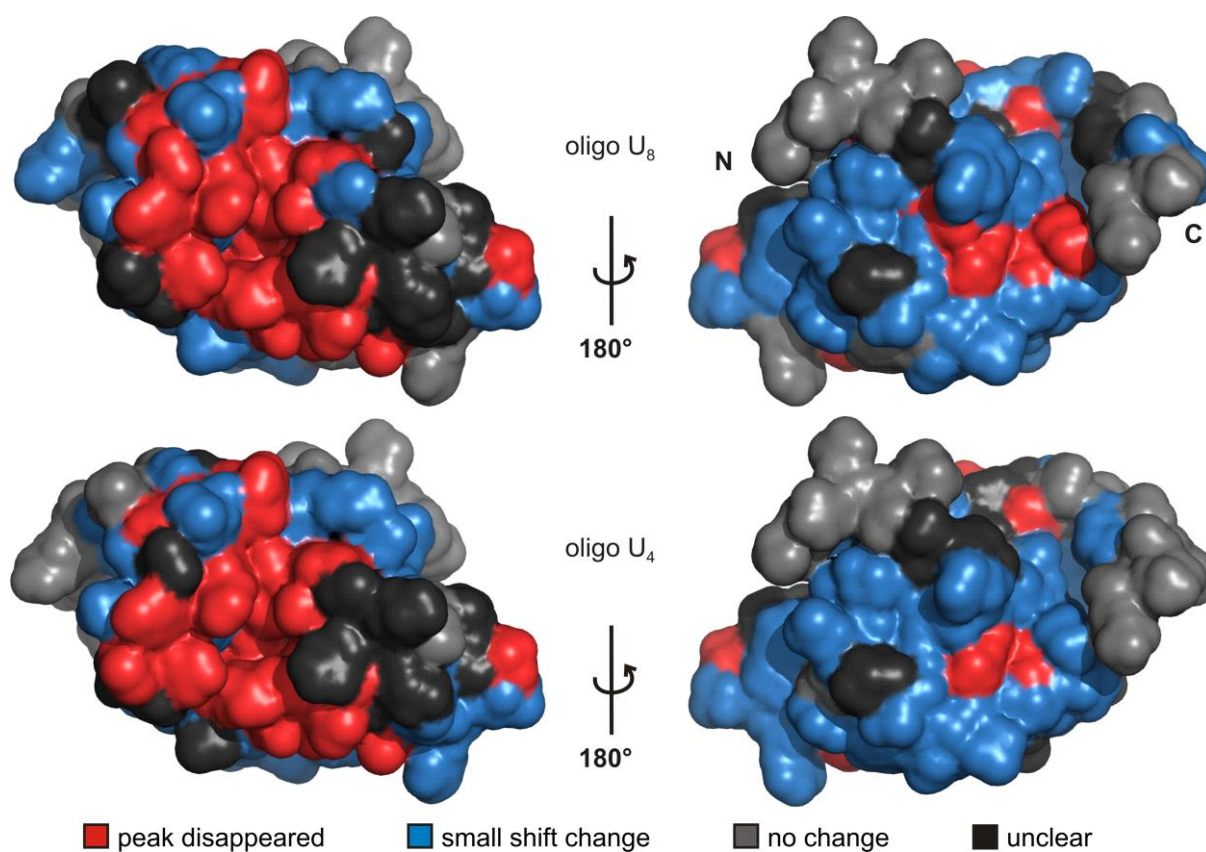
**Figure S3 A-C: Overlays of the  $^1\text{H}$ ,  $^{15}\text{N}$  HSQC spectra of  $^{15}\text{N}$ -labelled Lpp1663 titrated with RNA.** 80  $\mu\text{M}$  Lpp1663 was titrated with equivalents of RNA at 298K as indicated by the coloured boxes. Changes in the chemical shift of the corresponding residues were categorized and plotted on the structure or surface of Lpp1663. For all ligands spectra with a 3fold or a 5fold excess are virtually identical. Thus, in most cases only the spectrum at a 3:1 ligand-protein ratio is shown.



**Figure S4: Conserved residues of Lpp1663 involved in RNA-binding and position of R86 in different ProQ/FinO structures.** **A** Conserved residues on the concave site of the Lpp1663 structure involved in RNA binding, shown as red sticks. N- and C- termini indicated as N and C, respectively. **B** Superposition of the Lpp1663 NMR bundle (grey) and *E. coli* ProQ NMR bundle (5nb9, orange), R86 shown as sticks. N- and C-terminus are indicated as N and C, respectively. **C** Overlays of R86 of the Lpp1663 NMR bundle (grey) and corresponding R80 of the 17 lowest energy structures of *E. coli* ProQ (5nb9, orange). **D** Overlay of the arginine residues corresponding to R86 of the twenty final structures of the Lpp1663 NMR bundle (grey) and the crystal structures of NMB1681 (3mw6, green) and FinO (1dvo, red).

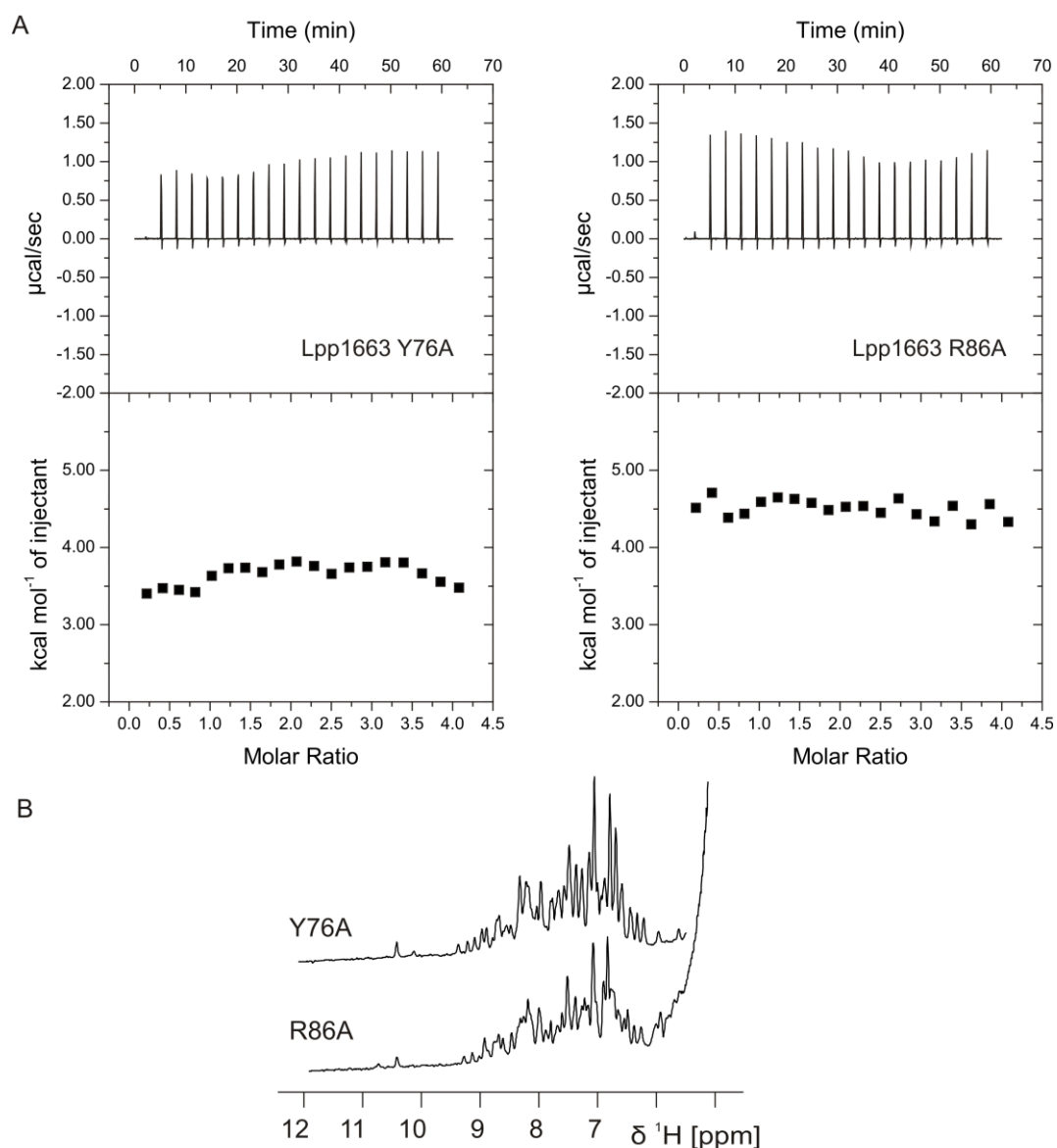


Supplemental Figure S5



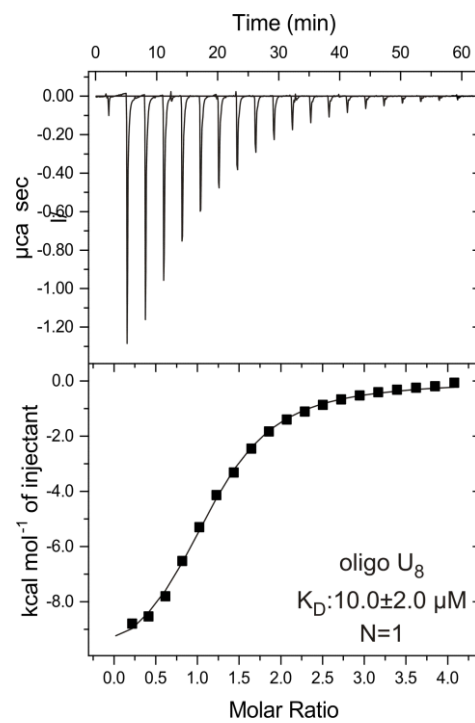
**Figure S5, supplementary to Figure 6.** In NMR experiments, the chemical shift changes upon oligo U<sub>8</sub> or oligo U<sub>4</sub> RNA titration have very similar patterns as for oligo U<sub>6</sub> (color scheme as described in Figure 5). The most dramatic changes occur on the concave surface patch including the N-terminal half of helix  $\alpha_2$ , helices  $\alpha_3$ ,  $\alpha_4$  and  $\alpha_5$ , as well as loop L1. N- and C-terminus are indicated as N and C, respectively.

## Supplemental Figure S6



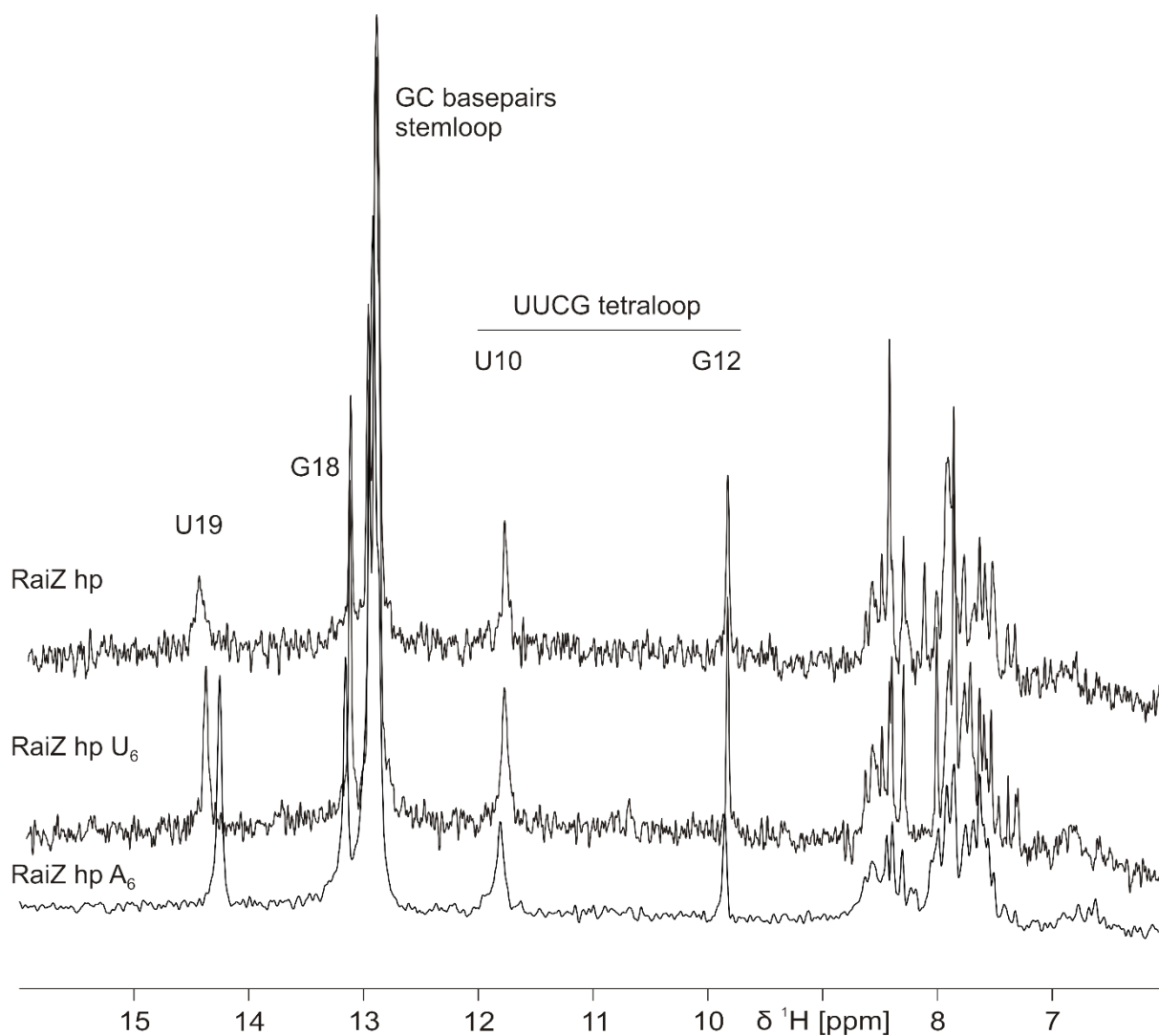
**Figure S6: Mutation of the conserved residues Y76 and R86 abolishes RNA binding. A** 1D <sup>1</sup>H NMR spectra of the amide region of Lpp1663 Y76A and R86A indicate well-folded proteins. The spectra were recorded at 298 K in NMR buffer with ~100 µM protein. **B** ITC thermograms of Y76A and R86A titrated with Oligo U<sub>8</sub> in NMR buffer. No binding could be observed.

Supplemental Figure S7



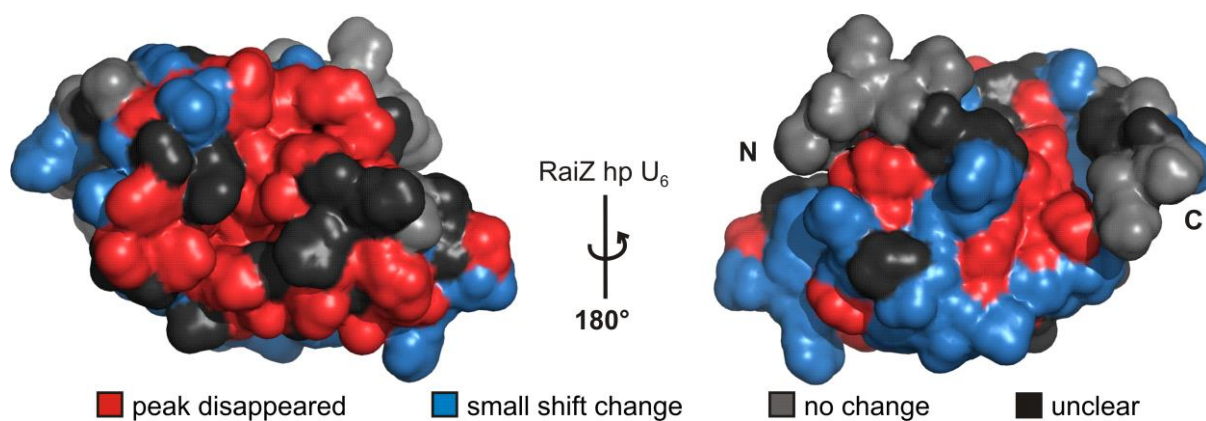
**Figure S7: Removal of the flexible N-and C-termini of Lpp1663 does not affect its RNA binding properties.** ITC thermograms of Lpp1663tr titrated with Oligo U<sub>8</sub>. The truncated protein has nearly the same affinity to the RNA as the full-length protein ( $\sim 8 \mu\text{M}$ ), indicating that the N-and C-terminus are not required for RNA-binding.

Supplemental Figure S8



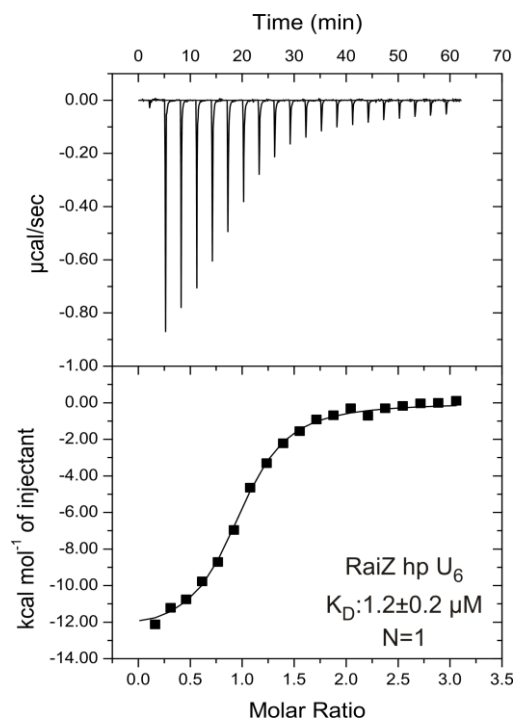
**Figure S8: The RaiZ constructs are folded according to the predicted secondary structure.** A 1D <sup>1</sup>H NMR spectra of 40-50  $\mu$ M RaiZ hp, RaiZ hp U<sub>6</sub> and RaiZ hp A<sub>6</sub> were recorded at 283 K in NMR buffer. Signals were assigned according to standard chemical shifts for RNA (Fürtig *et al.*, 2003).

Supplemental Figure S9



**Figure S9, supplementary to Figure 7:** Surface view of the chemical shift changes upon titration with RaiZ hp U<sub>6</sub>. The main RNA-binding region includes the same surface area on the concave face of Lpp1663 as for the oligo U-RNAs. However, the number of affected residues increases. N- and C-terminus are indicated as N and C, respectively.

Supplemental Figure S10



**Figure S10: Removal of the flexible N-and C-termini of Lpp1663 does not affect its RNA binding to RaiZ hp U<sub>6</sub>.** ITC thermogram of Lpp1663tr titrated with RaiZhp U<sub>6</sub>. The truncated protein has nearly the same affinity to the RNA as the full-length protein (~800 nM), indicating that the N-and C-terminus are not required for RNA-binding.

Table S1: Results of the ITC measurements

Protein	RNA	$K_D$ ( $\mu$ M)	Molar ratio / stoichiometry (N)	$\Delta H$ (cal/mol)	$\Delta S$ (cal/mol/deg)
Lpp1663	RocR SLIII -tail	No Binding			
Lpp1663	RocR SLIII +tail (1)	31.0	0.22	-36400	-101
Lpp1663	RocR SLIII +tail (2)	26.0	0.29	-28570	-74.8
Lpp1663	RocR SLIII +tail (3)	26.0	0.26	-23650	-58.3
	mean	<b>28.0<math>\pm</math>3.0</b>		<b>-29540<math>\pm</math>5250</b>	<b>-78<math>\pm</math>18</b>
Lpp1663	CCUUUCU (1)	46.0	1	-7917	-6.76
Lpp1663	CCUUUCU (2)	42.0	1	-7360	-4.66
Lpp1663	CCUUUCU (3)	36.2	1	-6422	-1.22
	mean	<b>41.0<math>\pm</math>5.0</b>		<b>-7233<math>\pm</math>617</b>	<b>-4<math>\pm</math>2</b>
Lpp1663	Oligo U <sub>6</sub> (1)	14.0	1	-4663	-6.56
Lpp1663	Oligo U <sub>6</sub> (2)	33.0	1	-8725	-8.76
Lpp1663	Oligo U <sub>6</sub> (3)	40.0	1	-10051	-15.2
	mean	<b>30.0<math>\pm</math>14.0</b>		<b>-7813<math>\pm</math>2292</b>	<b>-10<math>\pm</math>4</b>
Lpp1663	Oligo A <sub>6</sub> (1)	75.2	1	-1816	12.8
Lpp1663	Oligo A <sub>6</sub> (2)	70.4	1	-2028	12.2
Lpp1663	Oligo A <sub>6</sub> (3)	61.4	1	-1758	13.4
	mean	<b>70.0<math>\pm</math>7.0</b>		<b>-1867<math>\pm</math>116</b>	<b>12.8<math>\pm</math>0.5</b>
Lpp1663	Oligo C <sub>6</sub>	No Binding			
Lpp1663	G-rich	No Binding			
Lpp1663	Oligo U <sub>8</sub> (1)	7.0	1	-9703	-8.94
Lpp1663	Oligo U <sub>8</sub> (2)	8.8	1	-7882	-3.31
Lpp1663	Oligo U <sub>8</sub> (3)	7.2	1	-7697	-2.3
	mean	<b>8.0<math>\pm</math>1.0</b>		<b>-8427<math>\pm</math>905</b>	<b>-5<math>\pm</math>3</b>
Lpp1663	Oligo U <sub>4</sub> (1)	34.8	1	-10340	-14.2
Lpp1663	Oligo U <sub>4</sub> (2)	27.6	1	-8863	-8.87
Lpp1663	Oligo U <sub>4</sub> (3)	32.9	1	-10130	-13.5
	mean	<b>31.0<math>\pm</math>3.0</b>		<b>-9778<math>\pm</math>652</b>	<b>-12<math>\pm</math>2</b>
Lpp1663	RaiZ hp	No Binding			
Lpp1663	RaiZ hp U <sub>6</sub> (1)	0.6	1	-11290	-9.71
Lpp1663	RaiZ hp U <sub>6</sub> (2)	0.7	1	-11310	-9.85
Lpp1663	RaiZ hp U <sub>6</sub> (3)	1.0	1	-10780	-8.77
	mean	<b>0.82<math>\pm</math>0.18</b>		<b>-11127<math>\pm</math>245</b>	<b>-9.4<math>\pm</math>0.5</b>
Lpp1663	RaiZ hp A6	No Binding			
Lpp1663 Y76A	Oligo U <sub>8</sub>	No Binding			
Lpp1663 R86A	Oligo U <sub>8</sub>	No Binding			
Lpp1663tr	Oligo U <sub>8</sub> (1)	9.2	1	-10760	-13
Lpp1663tr	Oligo U <sub>8</sub> (2)	8.5	1	-8993	-6.97
Lpp1663tr	Oligo U <sub>8</sub> (3)	12.2	1	-13340	-22.3
	mean	<b>10.0<math>\pm</math>2.0</b>		<b>-11031<math>\pm</math>1785</b>	<b>-14<math>\pm</math>6</b>
Lpp1663tr	RaiZ hp U <sub>6</sub> (1)	1.4	1	-12480	-15.1
Lpp1663tr	RaiZ hp U <sub>6</sub> (2)	1.0	1	-12140	-13.3
Lpp1663tr	RaiZ hp U <sub>6</sub> (3)	1.2	1	-12680	-15.4
	mean	<b>1.2<math>\pm</math>0.2</b>		<b>-12433<math>\pm</math>223</b>	<b>-15<math>\pm</math>1</b>