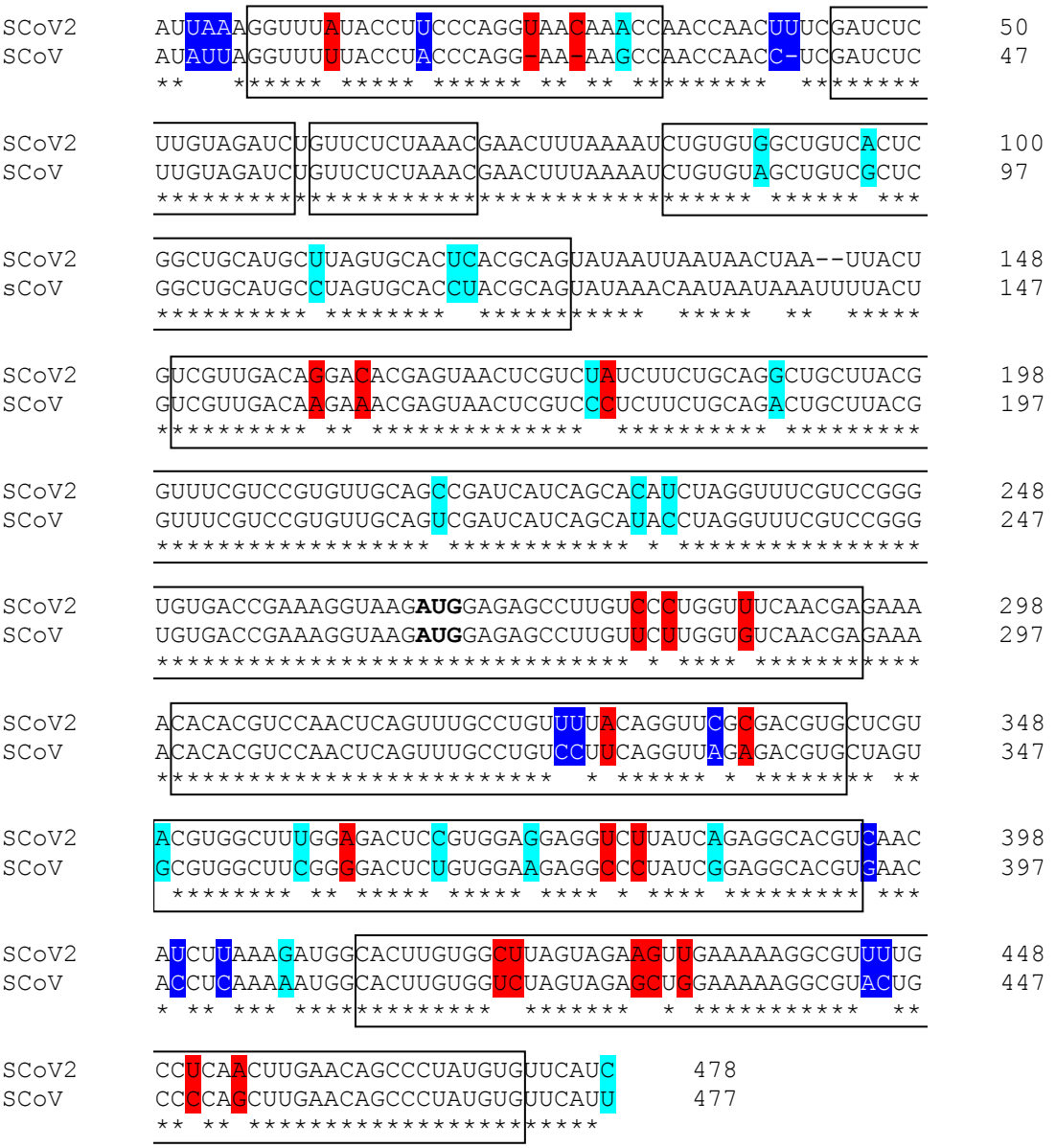
Supplementary Information: Secondary structure determination of conserved SARS-CoV-2 RNA elements by NMR spectroscopy

5’-genomic end:



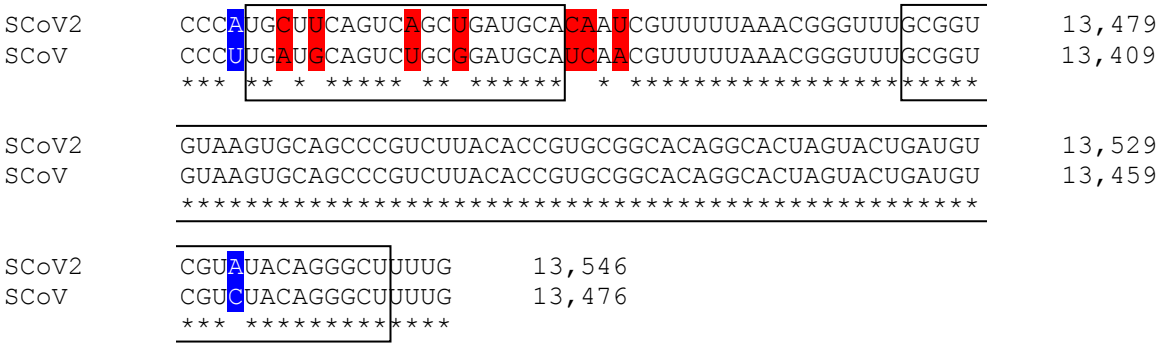
SL1 to SL8 (in this order) are boxed. The start codon of ORF1a is highlighted in bold.

N = compensatory mutation in helical region

N = structure-neutral mutation in single stranded region

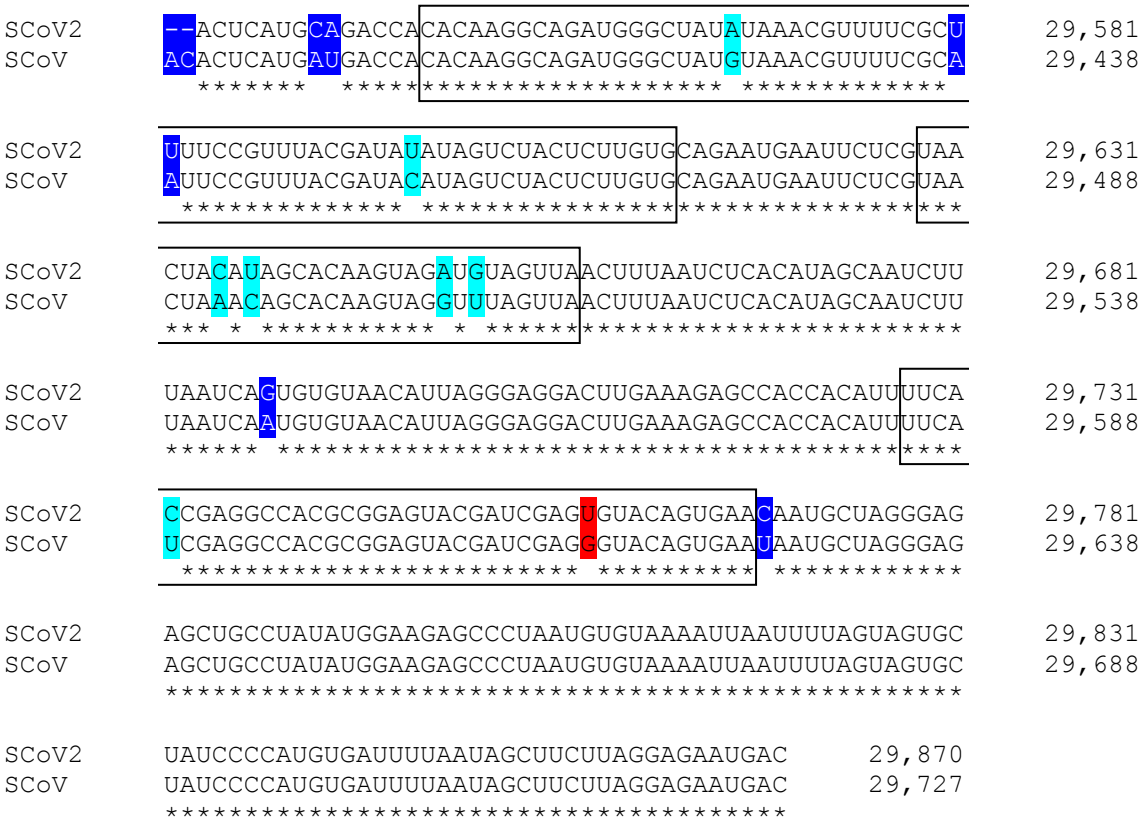
N = structure altering mutation

Frameshifting region:



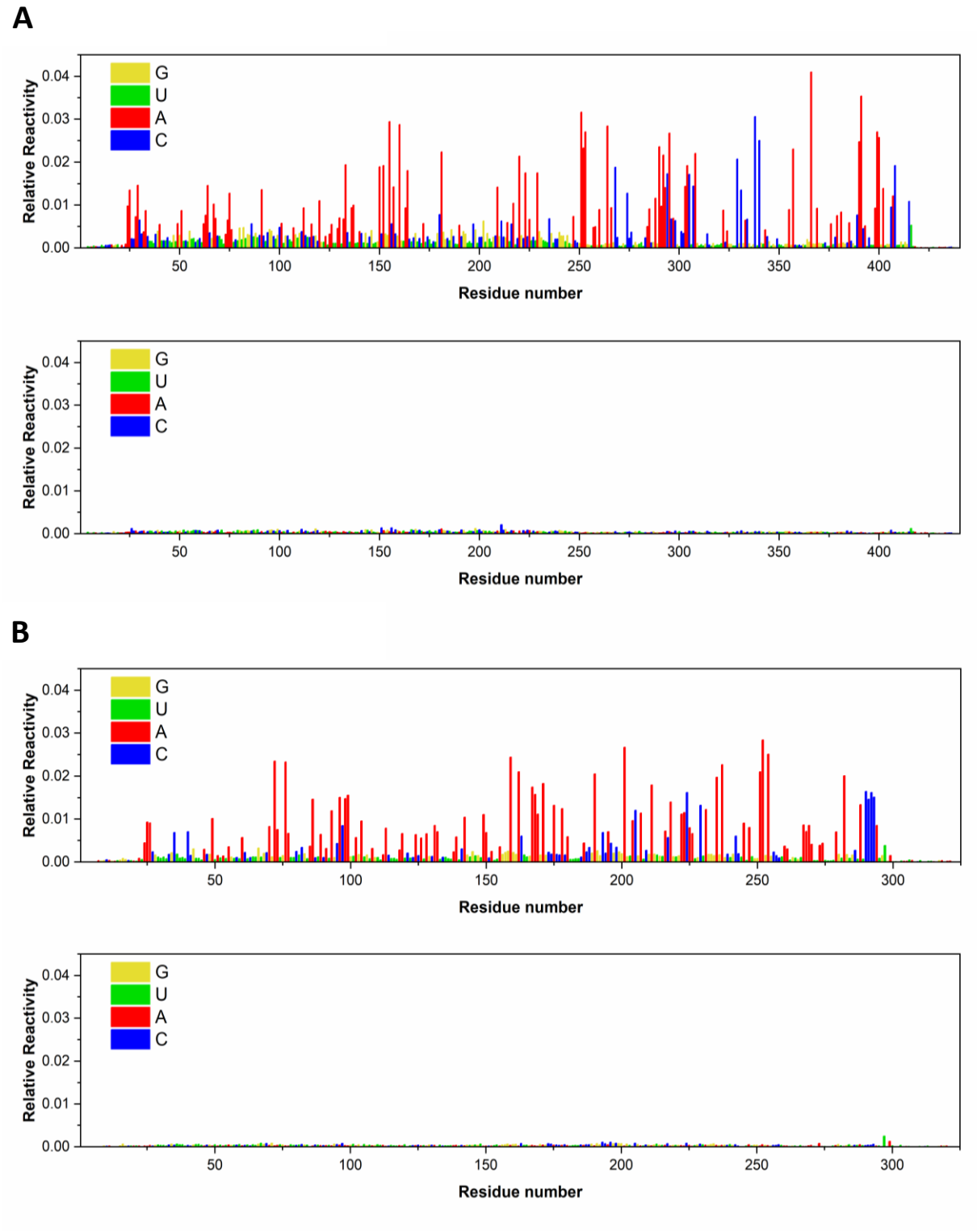
The att HP and the PK (in this order) are boxed.

3'-UTR:

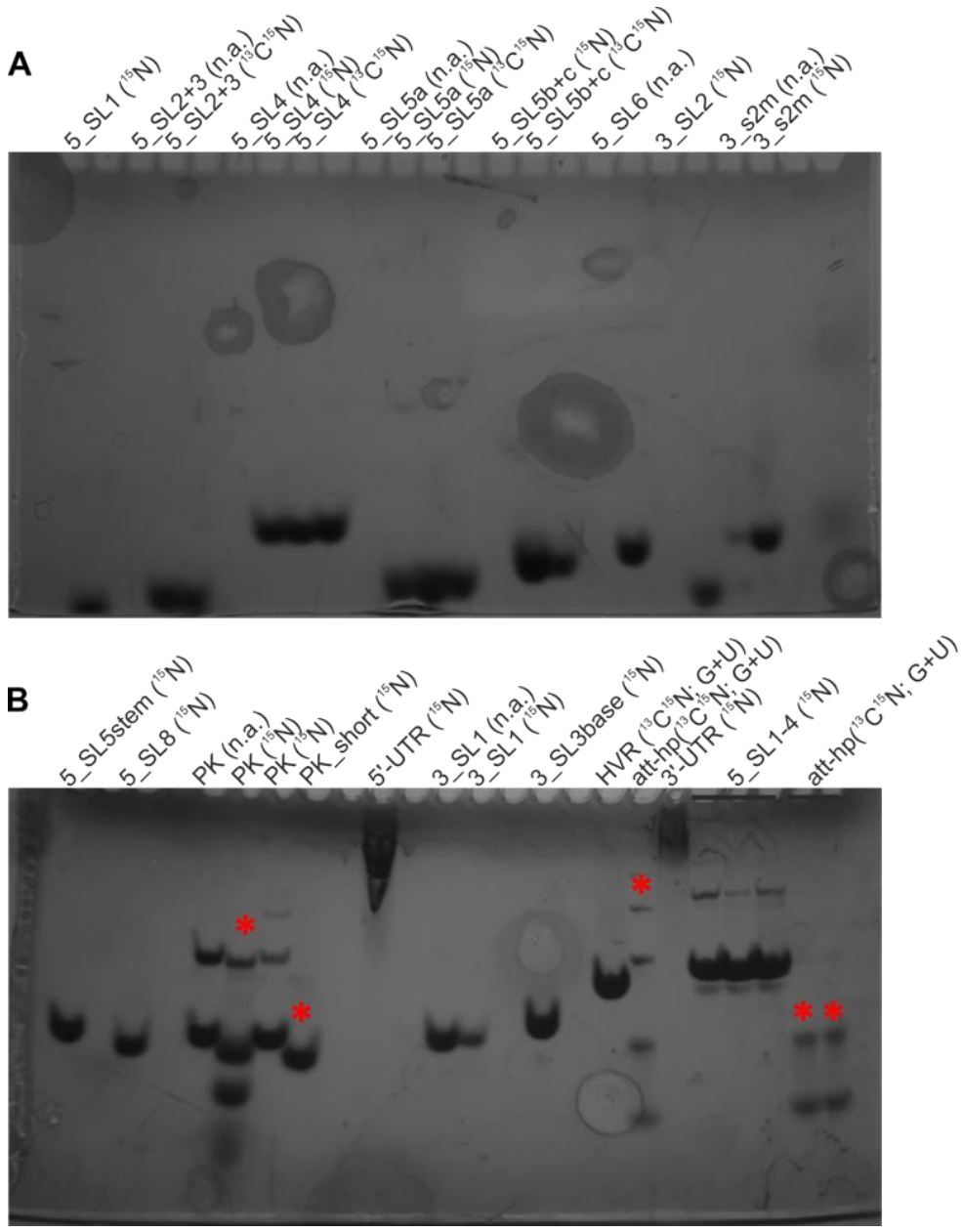


SL1, SL2 and s2m (in this order) are boxed.

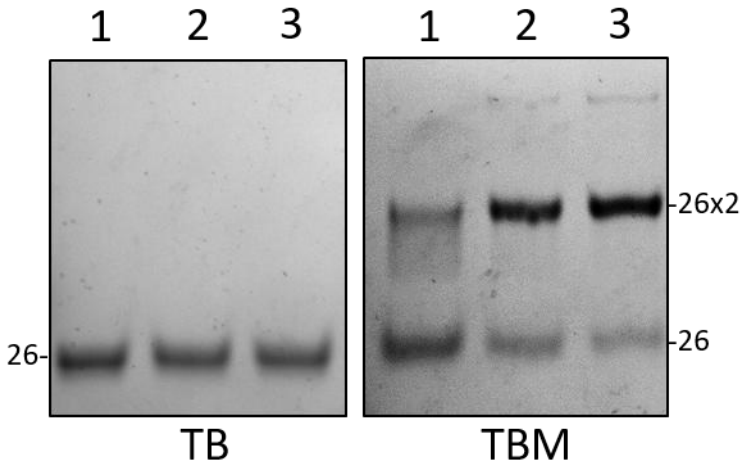
Supplementary Figure 1: Sequence and structure conservation between SCoV2 and SCoV of the 5'-genomic end, the frameshifting region and the 3'-UTR. Individual stem-loops investigated by NMR are boxed. Mutations are color-coded, with compensatory mutations highlighted in cyan, mutations in single stranded regions without effect on the predicted 2D structure highlighted in blue and mutations predicted to alter base pairing patterns highlighted in red.



Supplementary Figure 2: Raw data showing the reactivity profile of **(A)** the 5'-genomic end and **(B)** the 3'-UTR. Shown are DMS treated (top) and untreated (bottom) samples.



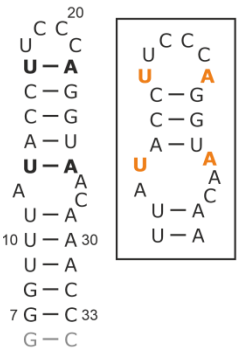
Supplementary Figure 3: Representative native PAGE of NMR-samples after the final buffer exchange step. RNA bands were visualized by UV-shadowing. The photographs show the entire gels. The labeling scheme is given, where unl. abbreviates unlabeled. The most distinct bands represent the monomeric form of the respective RNA constructs. Slower migrating bands indicate dimeric or oligomeric RNAs, while faster migrating bands arise from degradation. 500 pmol RNA were loaded onto the gel. For RNAs that showed degradation on the gel, sample preparation was repeated. **(A)** 10% native PAA gel of shorter constructs. **(B)** 10% native PAA gel of longer constructs. The constructs annotated with a red asterisk were not used in this study.



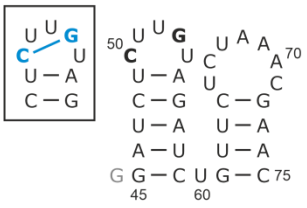
Supplementary Figure 4: Folding analysis of the att HP by native PAGE. Left panel: TB gel (no Mg^{2+}); right panel: TBM gel (2 mM Mg^{2+}). RNA samples were prepared as follows: 1: 0 mM Mg^{2+}, 2: 2 mM Mg^{2+} after heating treatment, 3: 2 mM Mg^{2+} before heating treatment. All treatments were carried out with RNA in consortium buffer (25 mM Kpi, 50 mM KCl).

5'-genomic end

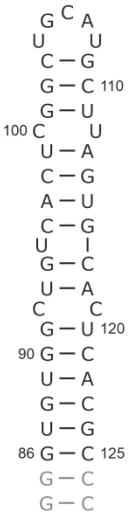
5_SL1



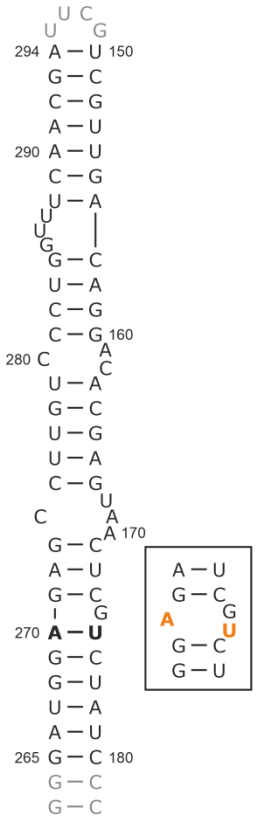
5_SL2+3



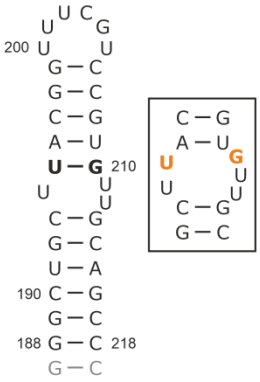
5_SL4



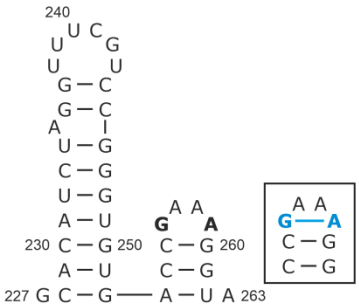
5_SL5stem



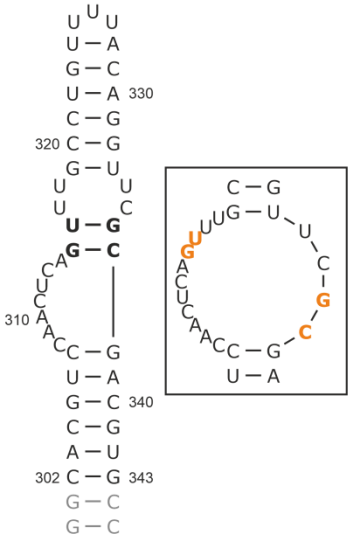
5_SL5a



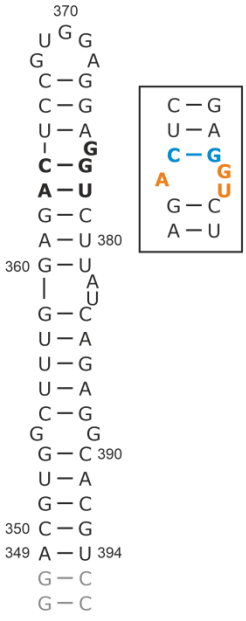
5_SL5b+c



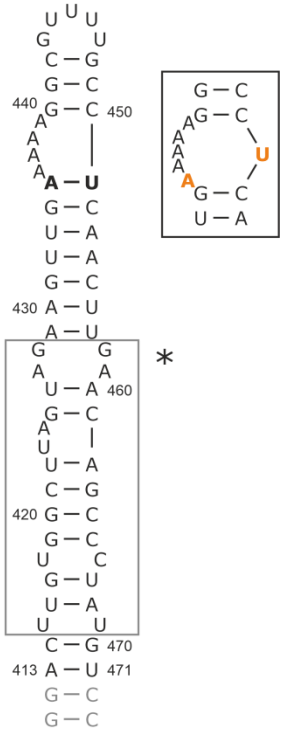
5_SL6

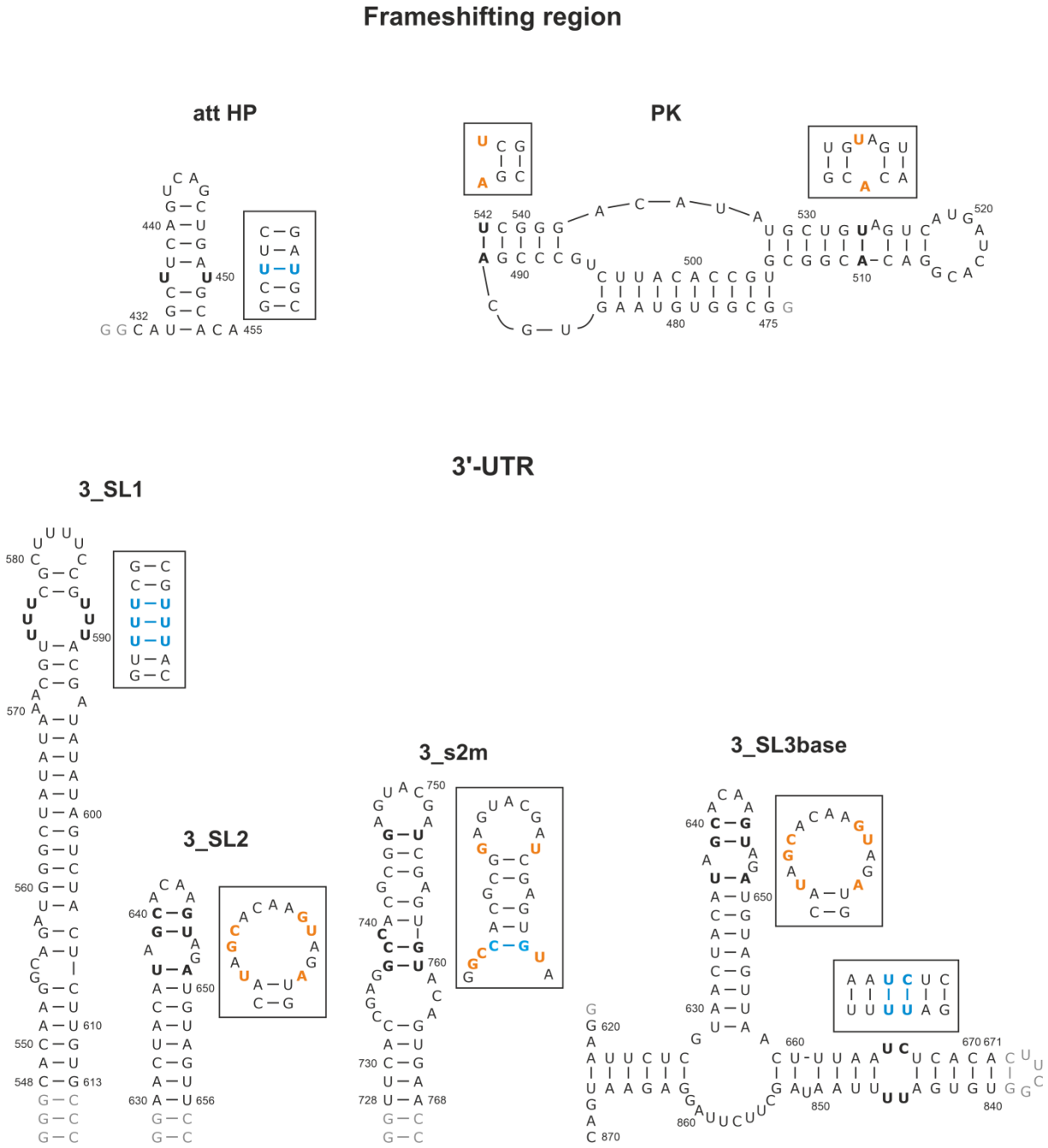


5_SL7

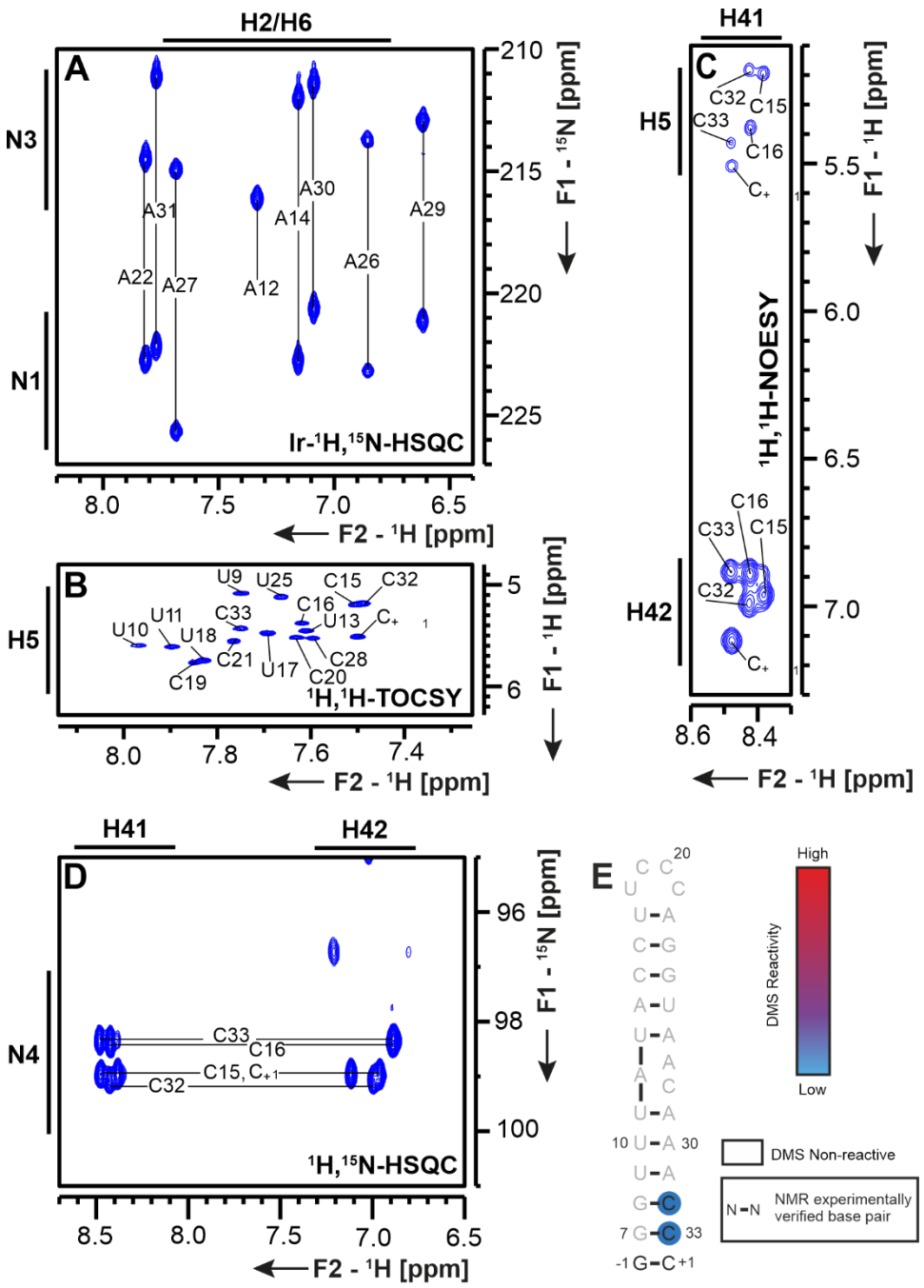


5_SL8

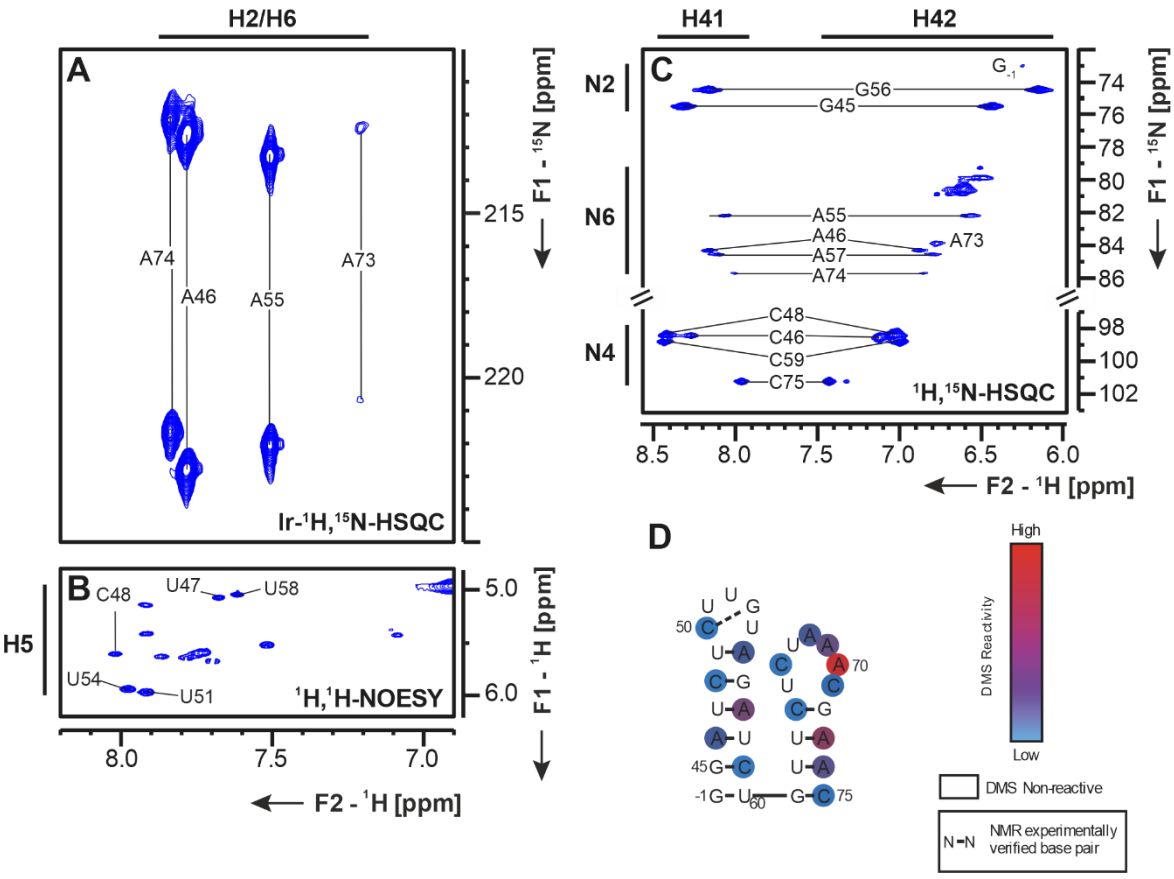




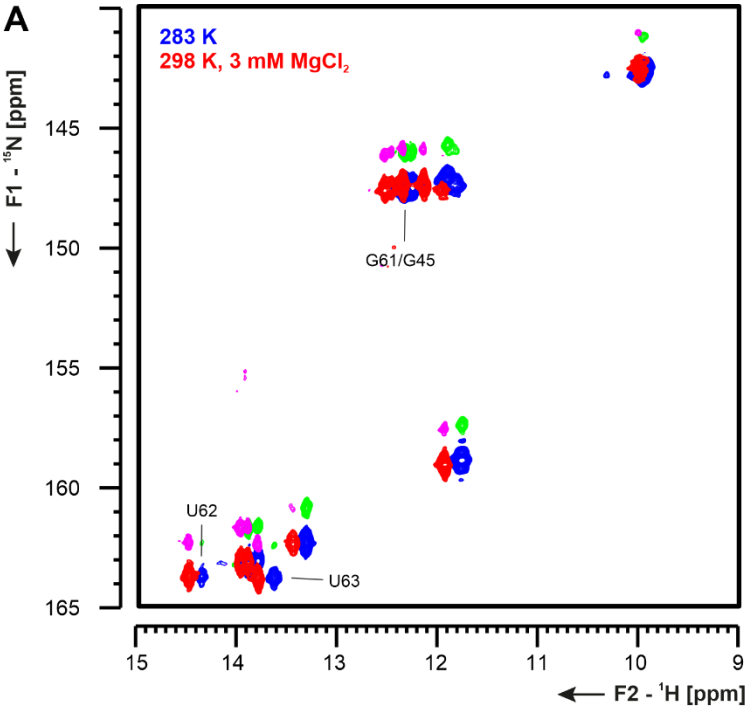
Supplementary Figure 5: Structure prediction by RNAstructure v6.0.1 for the investigated stem-loop constructs (https://rna.urmc.rochester.edu/RNAstructureWeb/) and by pKiss for the PK (https://bibiserv.cebitec.uni-bielefeld.de/pkiss). Differences found in the experimental data (NMR and DMS) are shown next to each construct. Differences in base pairing patterns are highlighted in blue. Predicted base pairs that were found to be open are highlighted in orange. Genomic numbering shifted for convenience by 13,000 from 5' for the frameshifting region and 29,000 from 5' for the 3'-UTR. * The middle region of 5_SL8 could not be unambiguously assigned by NMR and was not examined by DMS footprinting.



Supplementary Figure 6: Assignment of the aromatic protons of 5_SL1 encompassing nucleotides 7 to 33. Observed atoms are annotated with bars next to the spectra. **(A)** Ir-^{1}H,^{15}N-HSQC spectrum for the adenosine H2-N1/N3 correlations. **(B)** ^{1}H,^{1}H-TOCSY spectrum with annotated cytidine H6-H5 cross peaks. **(C)** ^{1}H,^{1}H-NOESY spectrum with intra-nucleobase cytidine amino proton correlations to the corresponding H5 protons. **(D)** Cytidine amino group region of the ^{1}H,^{15}N-HSQC spectrum with annotated amino group resonances. **(E)** Secondary structure of 5_SL1 as derived from NMR and DMS with genomic numbering. 5'- and 3'-terminal base pairs ("additional closing base pairs") introduced to allow for transcription and for stabilization of stem elements are annotated with G_{-1} and C_{+1}. Nucleotides that constitute the DMS primer site are held in gray. DMS reactivity is represented by circles from blue (low reactivity) to red (high reactivity) as shown in legend. The nucleobases of guanosine and uridine nucleotides as well as the closing base pairs were not tested by DMS probing. Base pairs confirmed by NMR are indicated by horizontal black lines between base pairing partners.

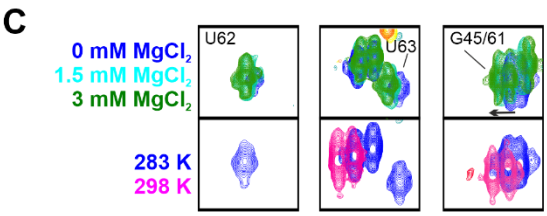


Supplementary Figure 7: Assignment of the aromatic and amino protons of 5_SL2+3 encompassing nucleotides 45 to 75. Observed atoms are annotated with vertical bars next to the spectra. **(A)** Ir-^{1}H,^{15}N-HSQC spectrum for the adenosine H2-N1/N3 correlations. **(B)** ^{1}H,^{1}H-NOESY spectrum with annotated pyrimidine H6-H5 correlations. **(C)** ^{1}H,^{15}N-HSQC spectrum showing resonances of the cytidine, adenosine and guanosine exocyclic amino groups. **(D)** DMS reactivity is represented by circles from blue (low reactivity) to red (high reactivity) as shown in the legend. The additional guanosine introduced to allow for transcription is annotated with G_{-1}.The nucleobases of guanosines and uridines as well as the closing base pairs were not tested by DMS probing. Base pairs confirmed by NMR are indicated by horizontal black lines between base pairing partners.

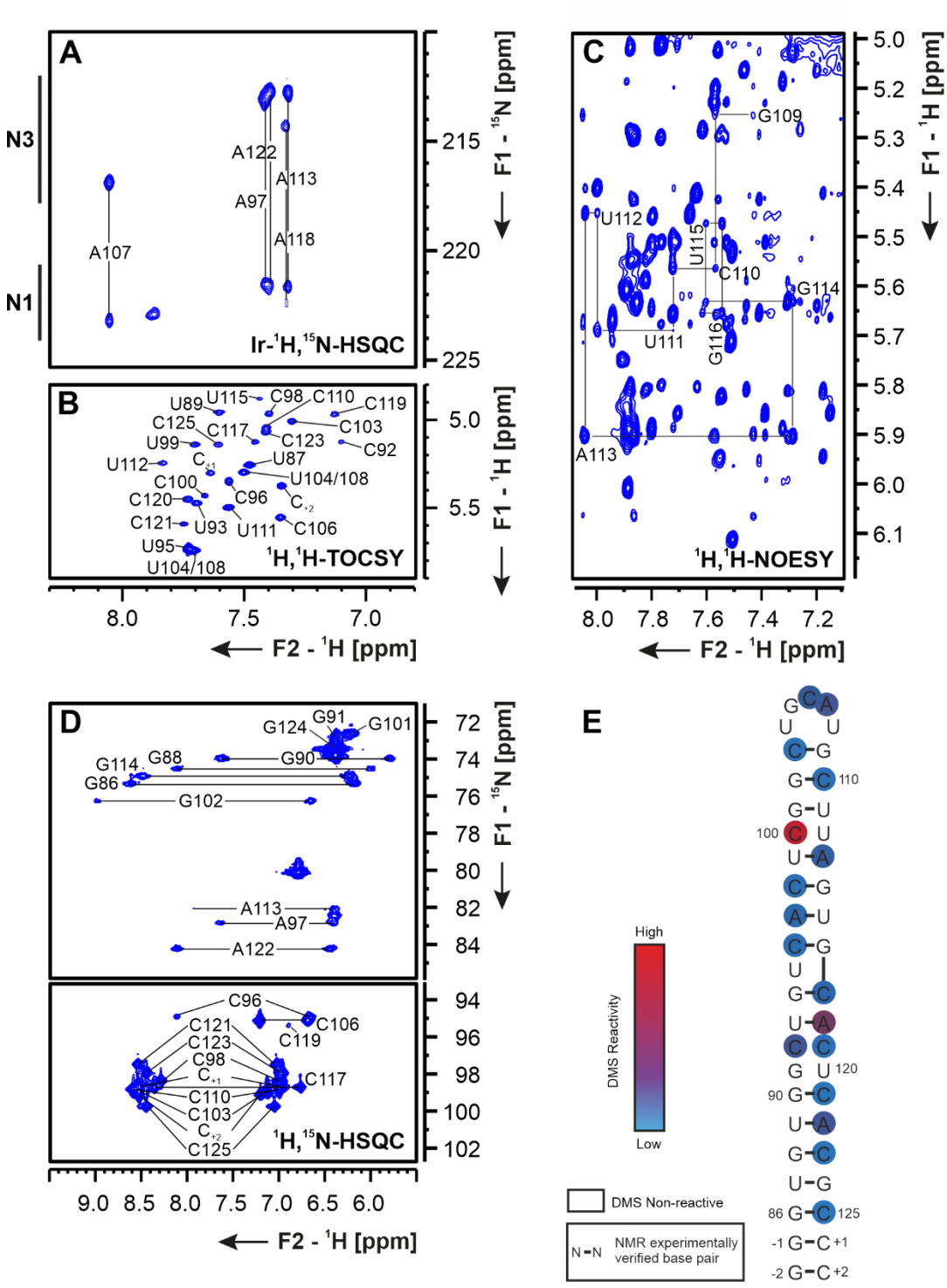


B

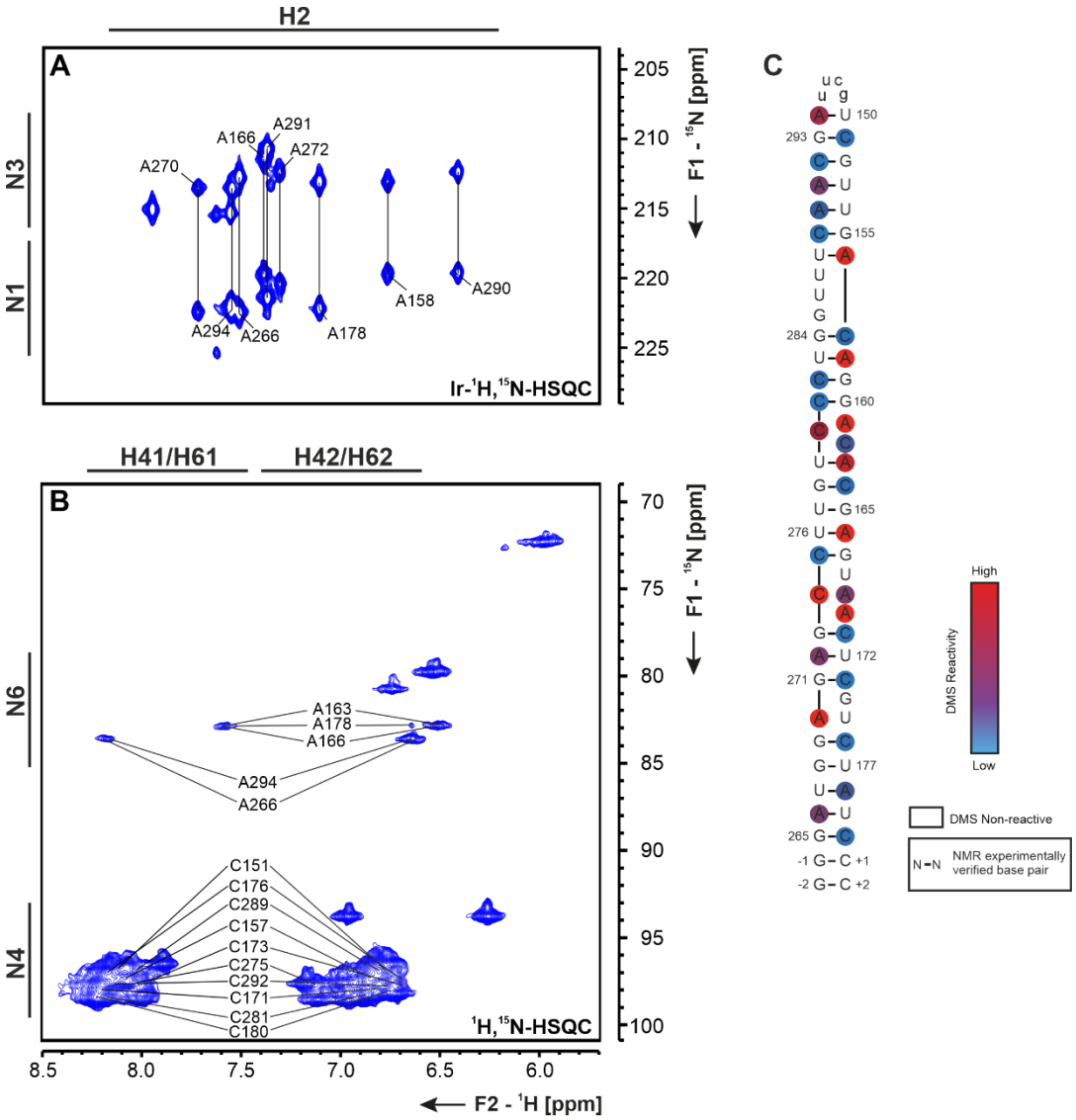
Residue	NMR-observed change between conditions
U62	Mg^{2+} stabilization
U63	Mg^{2+} stabilization
G61/G45	Strong CSPs (>75/>180 Hz δ ^{1}H)



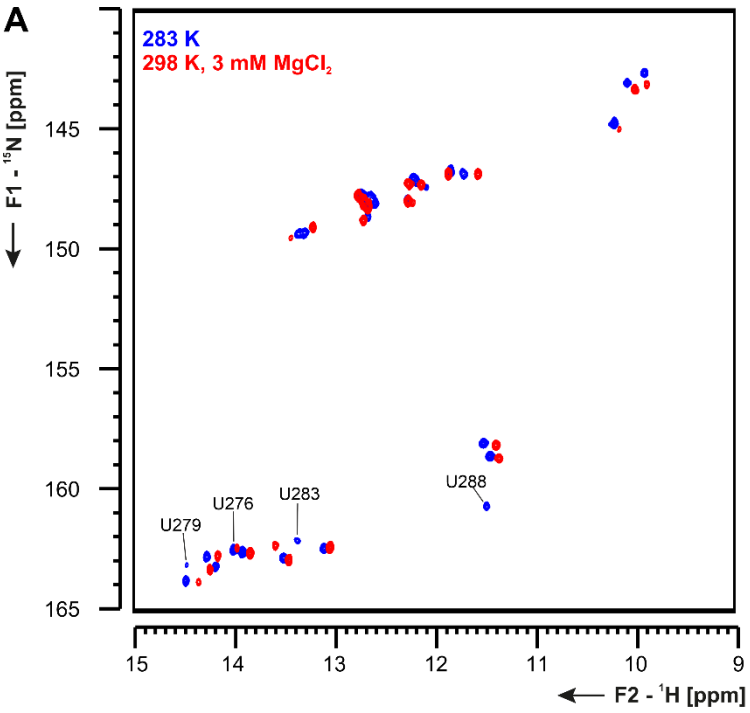
Supplementary Figure 8. Effects of magnesium and temperature on 5_SL2+3. **(A)** Overlay of ^{1}H,^{15}N-TROSY spectra recorded in conditions as used for NMR-based determination of secondary structure shown in **Figure 2** and **Supplementary Figure 7D** (blue) or at RT and after addition of 3 mM $MgCl_2$ (red). Relevant, affected imino group signals are denoted with their assignments. **(B)** Summary of effects observed in the spectral comparison of panel **A** for the labelled residues. **(C)** Zoom-ins of residues labelled in panel **A** showing overlays of spectra at 283 K during titration of $MgCl_2$ (upper row) or comparing the two temperatures in the absence of $MgCl_2$ (lower row). The color code of concentrations and temperatures is given.



Supplementary Figure 9: Assignment of the aromatic and amino protons of 5_SL4 encompassing nucleotides 86 to 125. Observed atoms are annotated with bars next to the spectra. **(A)** Ir-^{1}H,^{15}N-HSQC experiment correlating adenosine H2 protons to the adenosine N1 and N3 nitrogen atoms. **(B)** ^{1}H,^{1}H-TOCSY spectrum with annotated cytidine and uridine H6-H5 cross peaks. **(C)** Exemplary sequential walk consisting of H1'-H6/H8 NOEs in the ^{1}H,^{1}H-NOESY spectrum for nucleotides G109 - G116. **(D)** Amino group region of the ^{1}H,^{15}N-HSQC spectrum with annotated amino group resonances. **(E)** Experimentally observed secondary structure of 5_SL4 with genomic numbering. Additional closing base pairs are annotated with G_{-1}, C_{+1}. DMS reactivity is represented by circles from blue (low reactivity) to red (high reactivity) as shown in the legend. The nucleobases of guanosines and uridines as well as the closing base pairs were not tested by DMS probing. Base pairs confirmed by NMR are indicated by horizontal black lines between base pairing partners.

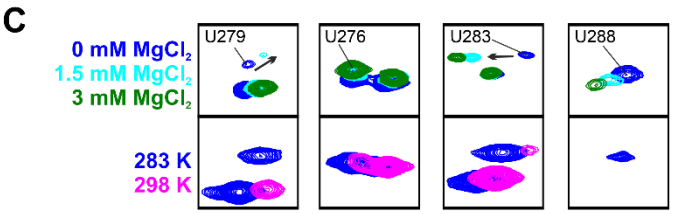


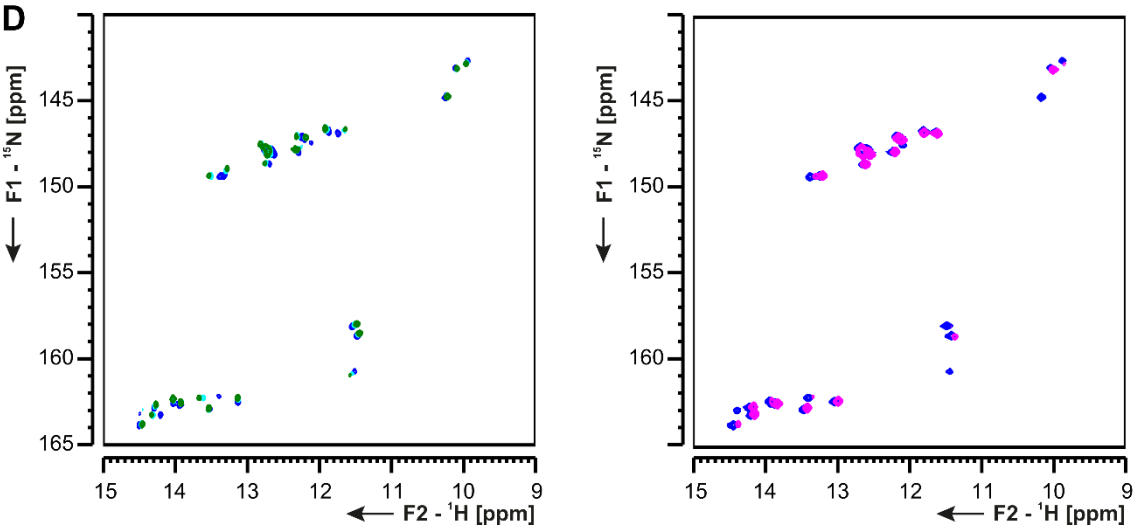
Supplementary Figure 10: Assignment of the aromatic and amino protons of 5_SL5stem encompassing nucleotides 150 to 180 and 265 to 294. The construct is capped with a UUCG tetraloop between nucleotides 294 and 150. Observed atoms are annotated with bars next to the spectra. **(A)** lr-^{1}H,^{15}N-HSQC spectrum for the adenosine H2-N1/N3 correlations. **(B)** ^{1}H,^{15}N-HSQC highlighting the amino region with assignable resonances labelled. **(C)** Depiction of the NMR-experimentally observed secondary structure of 5_SL5stem with genomic numbering. All identified base pairs according to main text **Figure 6** are shown with black bars. The additional tetraloop bases are annotated in lower-case letters and additional closing base pairs at the termini are annotated with G_{-2}, G_{-1}, C_{+1}, C_{+2}. For direct comparison with the DMS reactivity, relevant cytidines and adenosines of the 5_5SLstem natural part of the underlying sequence are color-coded as depicted. DMS reactivity is represented by circles from blue (low reactivity) to red (high reactivity) as shown in the legend. The nucleobases of guanosines and uridines as well as the closing base pairs were not tested by DMS probing. Base pairs confirmed by NMR are indicated by horizontal black lines between base pairing partners.



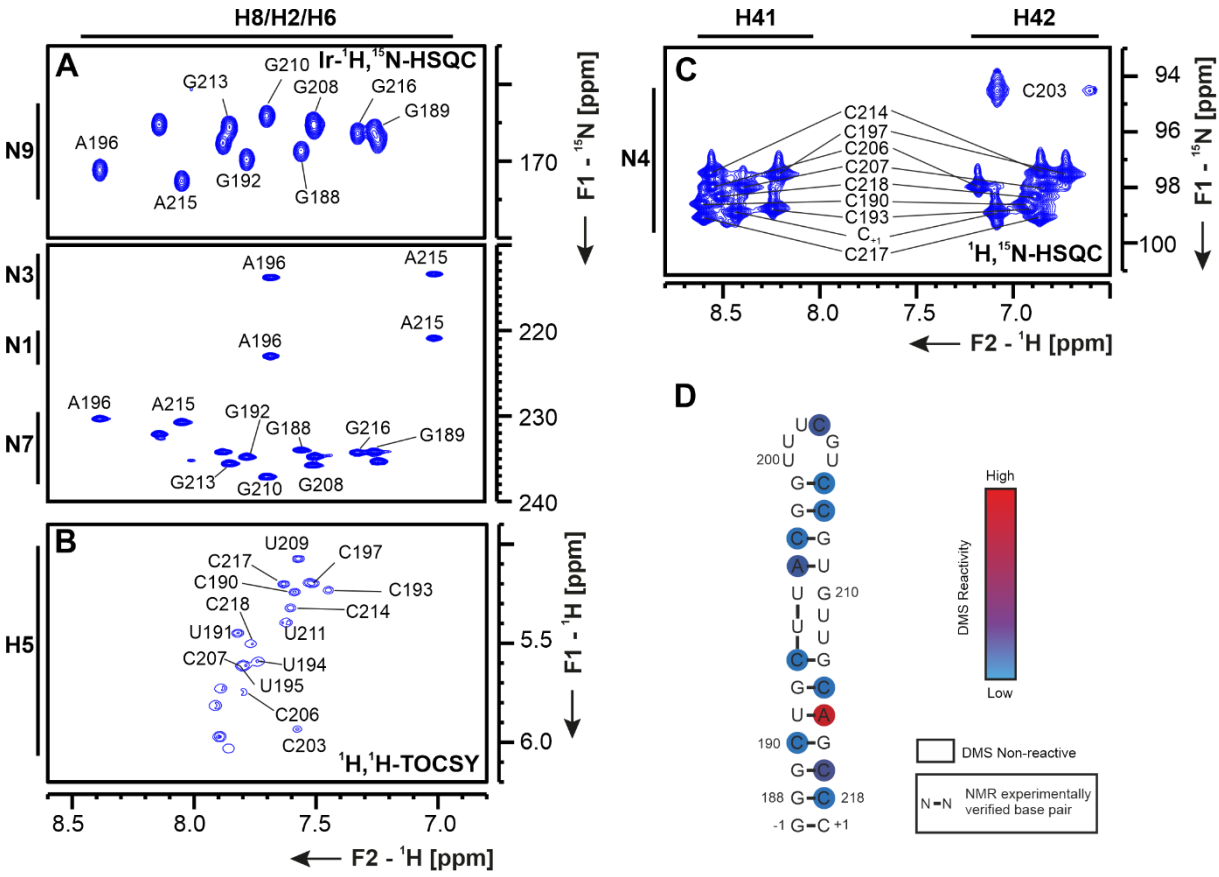
B

Residue	NMR-observed change between conditions
U276	Line-Broadening
U279	Strong Line-Broadening
U283	Strong CSPs (>150 Hz δ ^{1}H)
U288	Strong Line-Broadening

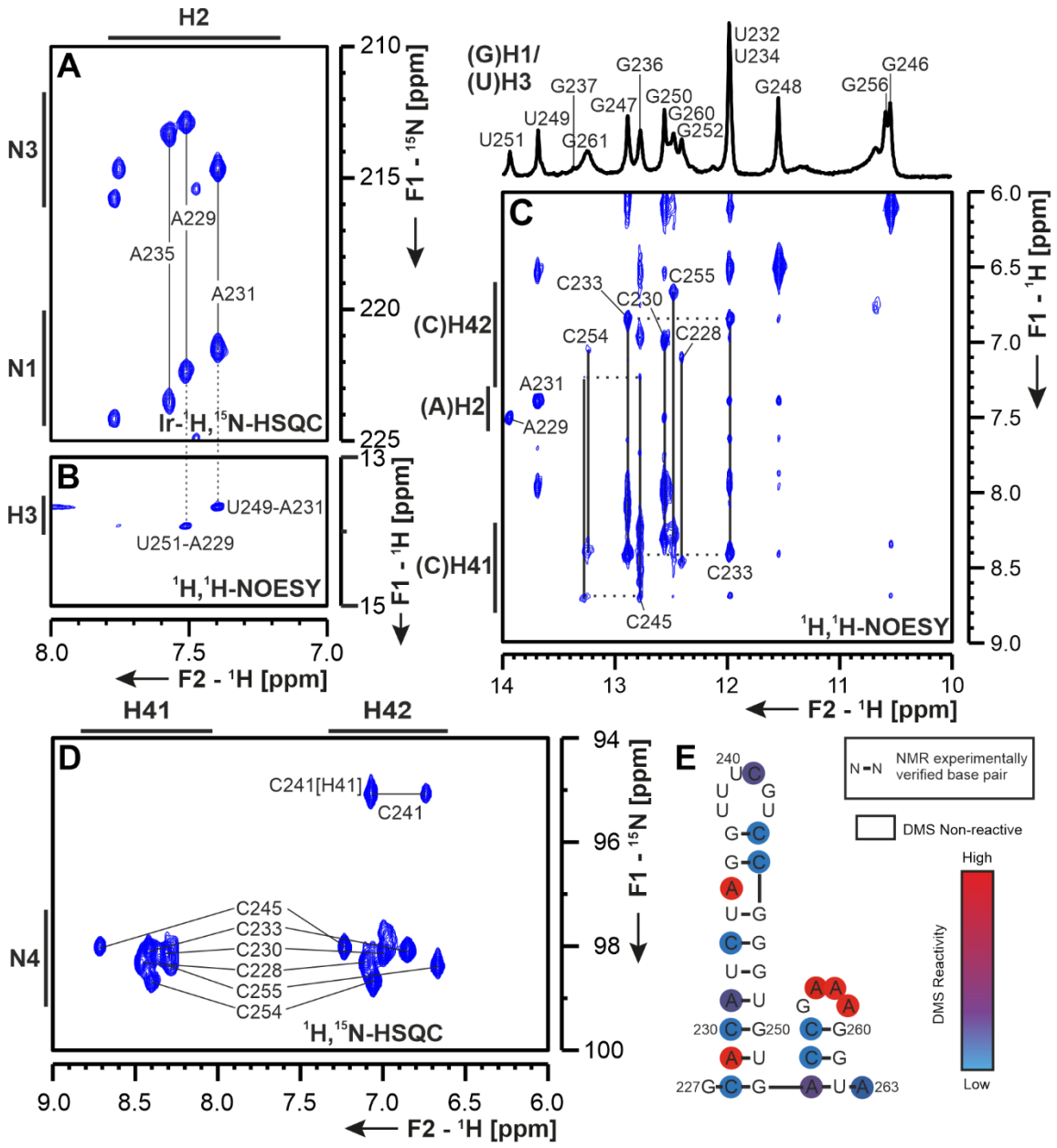




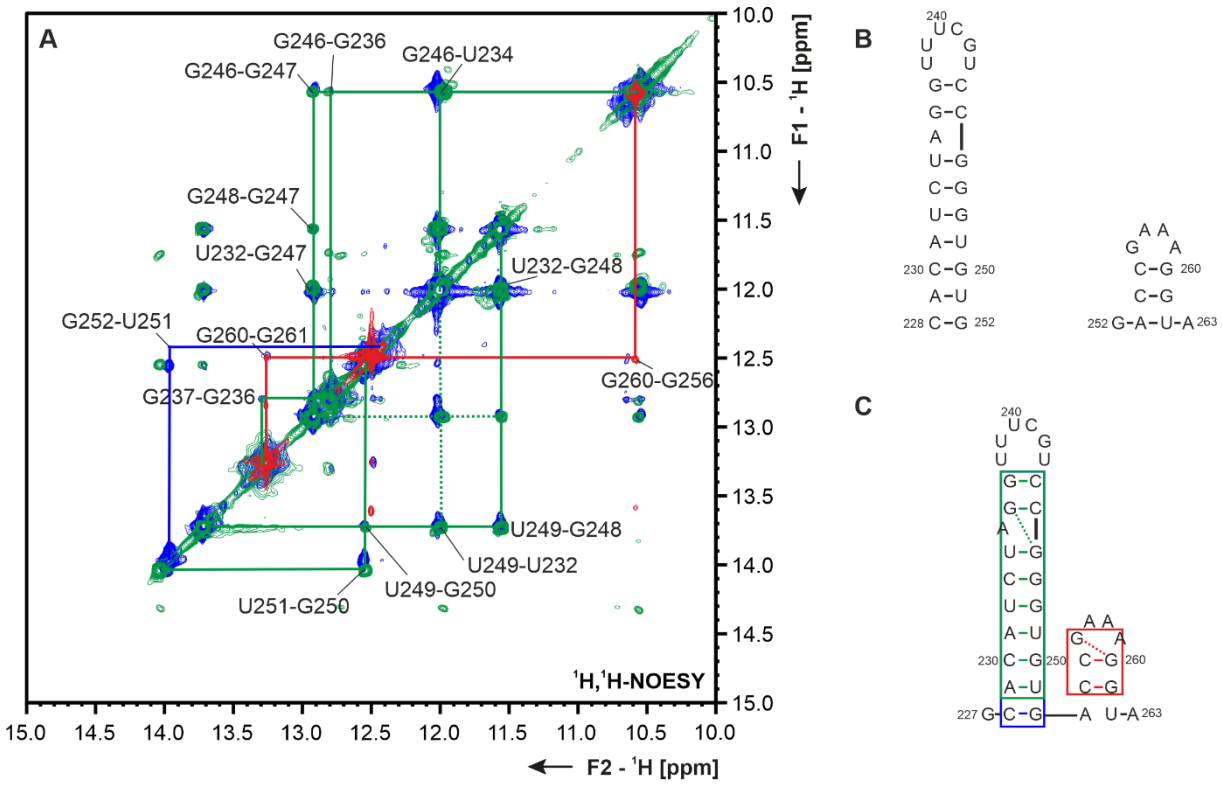
Supplementary Figure 11. Effects of magnesium and temperature on 5_SL5stem. **(A)** Overlay of ^{1}H,^{15}N-TROSY spectra recorded in conditions as used for NMR-based determination of secondary structure shown in **Figure 6** and **10** (blue) or at RT and after addition of 3 mM $MgCl_2$ (red). Relevant, affected imino group signals are denoted with their assignments. **(B)** Summary of effects observed in the spectral comparison of panel A for the labelled residues. **(C)** Zoom-ins of residues labelled in panel A showing overlays of spectra during titration of $MgCl_2$ at 283 K (upper row) or comparing the two temperatures in the absence of $MgCl_2$ (lower row). The color code of concentrations and temperatures is given. **(D)** Full spectral overlays for the magnesium titration (left panel) and temperature differences (right panel) as the basis for the zoom-ins in panel C using the same color code.



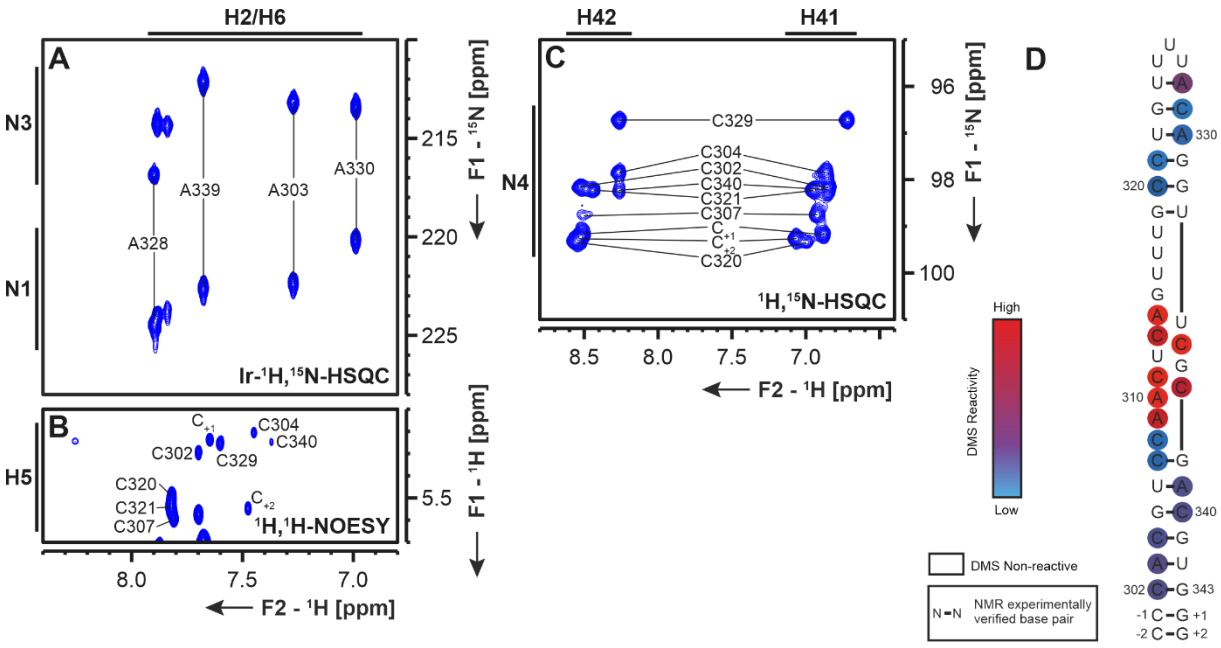
Supplementary Figure 12: Assignment of the aromatic and amino protons of 5_SL5a encompassing nucleotides 188 to 218. Observed atoms are annotated with bars next to the spectra. **(A)** Ir-^{1}H,^{15}N-HSQC experiment correlating purine-aromatic protons to nitrogen atoms for adenosine H2-N1/N3 or guanosine H8-N7/N9. **(B)** ^{1}H,^{1}H-TOCSY spectrum with annotated cytidine and uridine H6-H5 cross peaks. **(C)** Amino group region of the ^{1}H,^{15}N-HSQC spectrum with annotated amino group resonances for cytidine. **(D)** Combined NMR-DMS experimentally observed secondary structure of 5_SL5a with genomic numbering. Additional closing base pairs are annotated with G_{-1}, C_{+1}. DMS reactivity is represented by circles from blue (low reactivity) to red (high reactivity) as shown in the legend. The nucleobases of guanosines and uridines as well as the closing base pairs were not tested by DMS probing. Base pairs confirmed by NMR are indicated by horizontal black lines between base pairing partners.



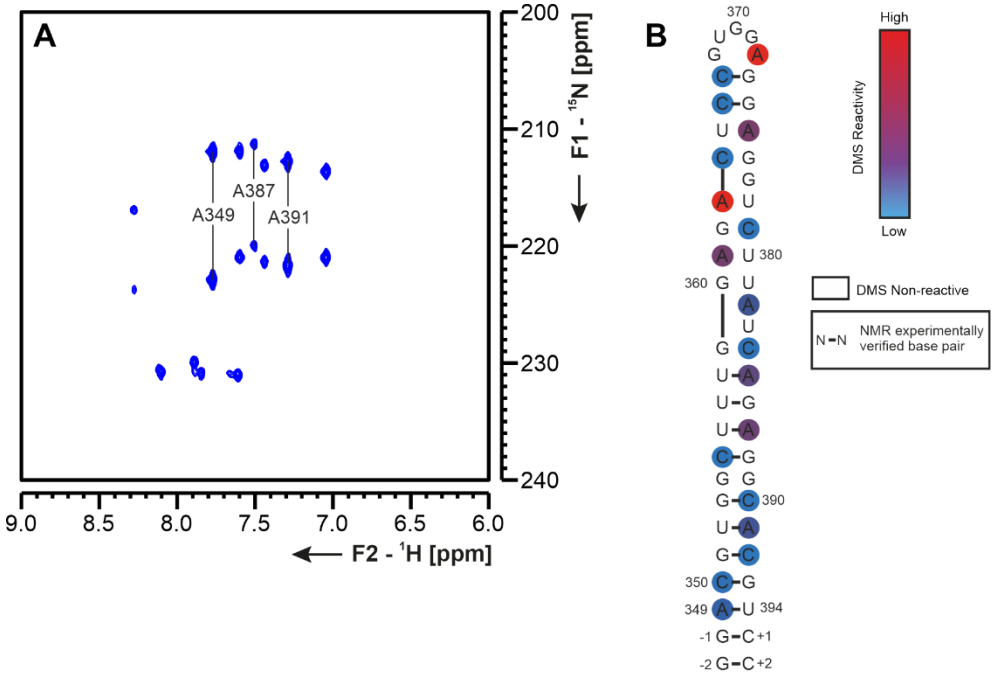
Supplementary Figure 13: Assignment of the aromatic and amino protons of 5_SL5b+c encompassing nucleotides 227 to 263. Observed atoms are annotated with bars next to the spectra. **(A)** Ir-^{1}H,^{15}N-HSQC experiment correlating adenosine H2 protons to the adenosine N1 and N3 nitrogen atoms. **(B)** ^{1}H,^{1}H NOESY showing the correlations between uridine H3 and adenosine H2. **(C)** ^{1}H,^{1}H NOESY showing further insight for the assignment of guanosine-H1 and uridine-H3 imino protons to corresponding cytidine amino protons H41 or H42 or aromatic H2 protons of adenosine. **(D)** Amino ^{1}H,^{15}N-HSQC spectrum showing the cytidine region. **(E)** Combined NMR-DMS experimentally observed secondary structure of 5_SL5b+c with genomic numbering. The DMS reactivity is represented by circles from blue (low reactivity) to red (high reactivity) as shown in the legend. Guanosine and uridine residues as well as the closing base pairs are not tested by the DMS method. NMR-spectroscopically confirmed base pairs are indicated by horizontal black lines between base pairing partners.



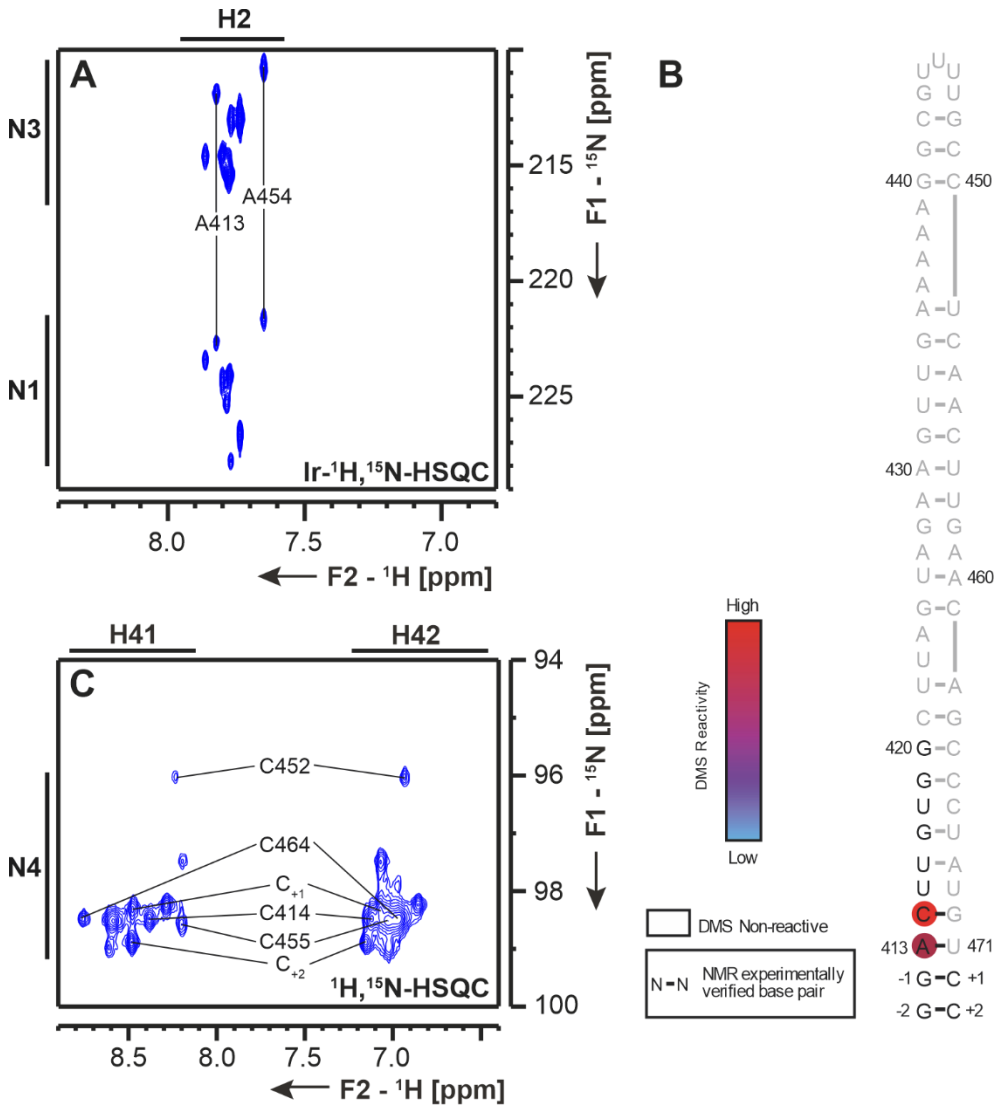
Supplementary Figure 14: Assignment strategy for 5_SL5b+c **(C)** by comparison to single hairpins 5_SL5b and 5_SL5c **(B)**. **(A)** Overlay of ^{1}H,^{1}H-NOESY spectra of 5_SL5b+c (blue contours), 5_SL5b (green contours) and 5_SL5c (red contours). The assigned imino proton walks are depicted in the same color code as the boxes in panel **(C)**.



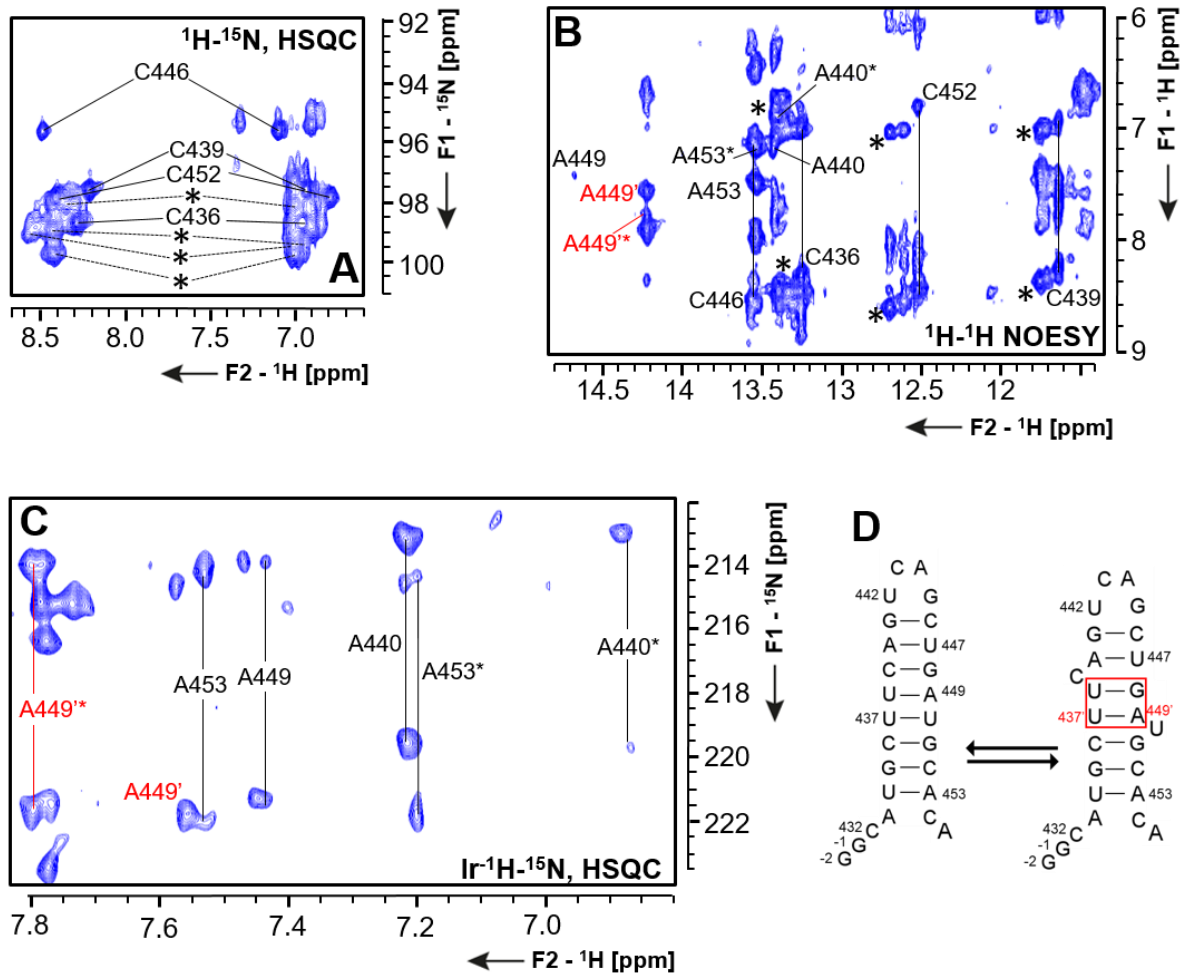
Supplementary Figure 15: Assignment of the aromatic and amino protons of 5_SL6 encompassing nucleotides 302 to 343. Observed atoms are annotated with bars next to the spectra. **(A)** Ir-^{1}H,^{15}N-HSQC spectrum correlating adenosine H2 protons to the adenosine N1 and N3 nitrogen atoms. **(B)** ^{1}H,^{1}H-NOESY spectrum with annotated cytidine H6-H5 cross peaks. **(C)** Cytidine amino group region of the ^{1}H,^{15}N-HSQC spectrum with annotated amino group resonances. **(D)** Experimentally observed secondary structure of 5_SL6 with genomic numbering. Additional closing base pairs are annotated with G_{-2}, G_{-1}, C_{+1}, C_{+2}. Nucleotides that constitute the DMS primer site are held in gray. DMS reactivity is represented by circles from blue (low reactivity) to red (high reactivity) as shown in the legend. The nucleobases of guanosines and uridines as well as the closing base pairs were not tested by DMS probing. Base pairs confirmed by NMR are indicated by horizontal black lines between base pairing partners.



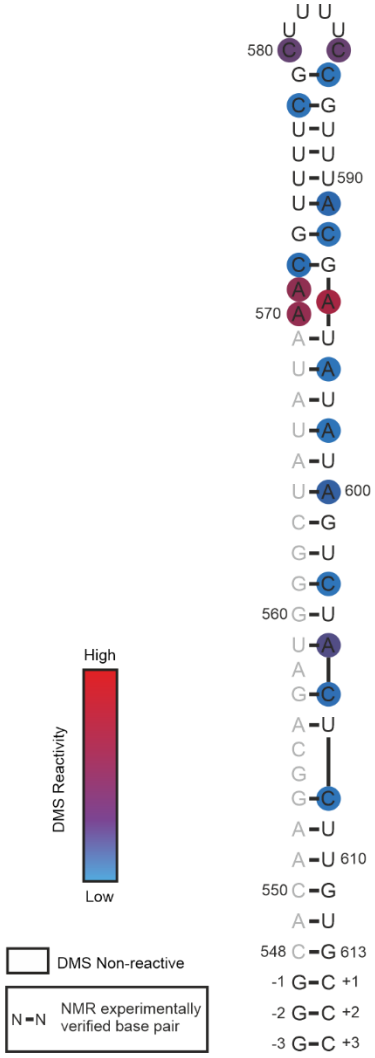
Supplementary Figure 16: Assignment of the aromatic protons of 5_SL7 encompassing nucleotides 349 to 394. Observed atoms are annotated with bars next to the spectra. **(A)** Ir-^{1}H,^{15}N-HSQC spectrum for the adenosine H2-N1/N3 correlations. **(B)** Experimentally observed secondary structure of 5_SL7 with genomic numbering. Experimentally observed secondary structure of 5_SL7 with genomic numbering. Additional closing base pairs are annotated with G_{-2}, G_{-1}, C_{+1}, C_{+2}. DMS reactivity is represented by circles from blue (low reactivity) to red (high reactivity) as shown in the legend. The nucleobases of guanosines and uridines as well as the closing base pairs were not tested by DMS probing. Base pairs confirmed by NMR are indicated by horizontal black lines between base pairing partners.



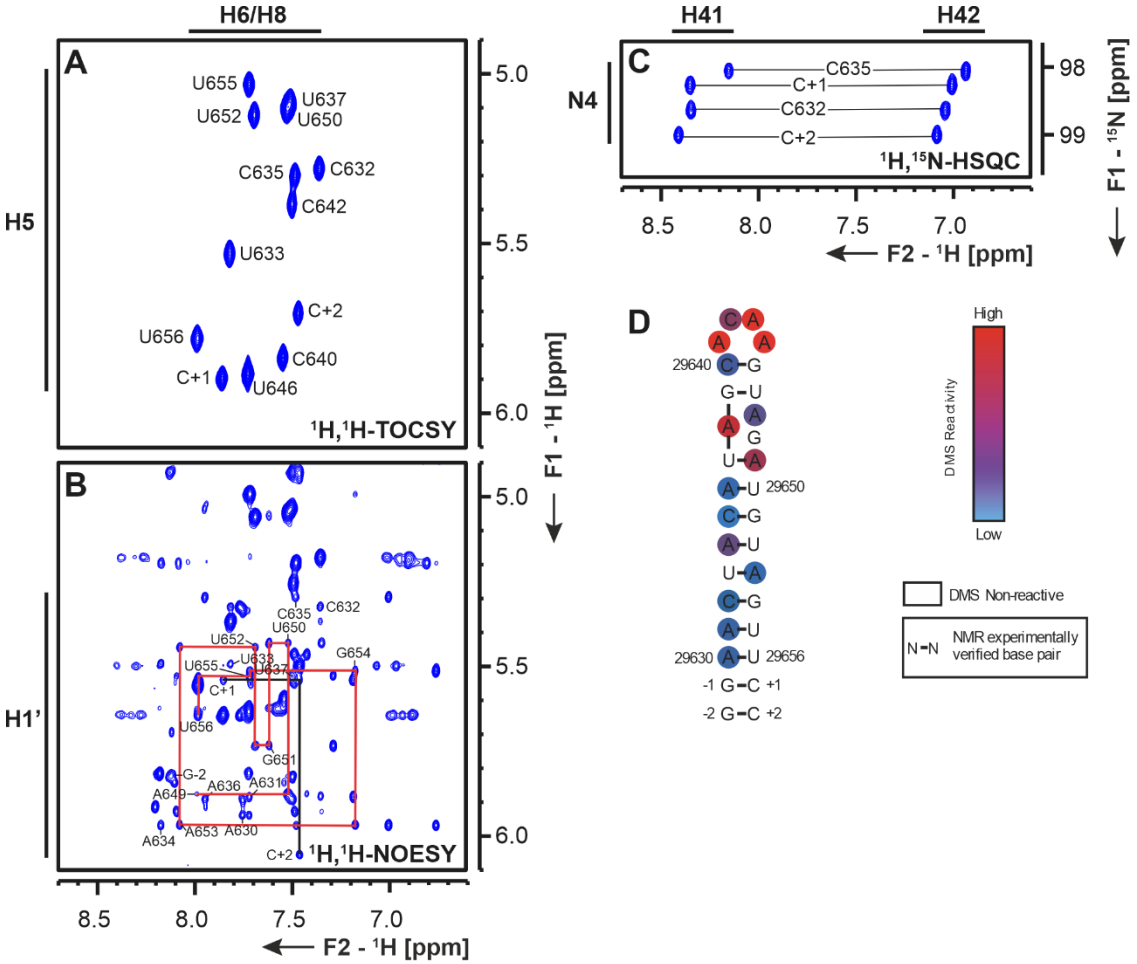
Supplementary Figure 17: Assignment of the aromatic and amino protons of 5_SL8 encompassing nucleotides 413 to 471. Observed atoms are annotated with bars next to the spectra. **(A)** Ir-^{1}H,^{15}N-HSQC spectrum correlating adenosine H2 hydrogen atoms to the adenosine N1 and N3 nitrogen atoms. **(B)** Possible secondary structure of 5_SL8 with genomic numbering, which are in agreement with experimental data. Additional closing base pairs are annotated with G_{-2}, G_{-1}, C_{+1}, C_{+2}. Nucleotides that constitute the DMS primer site are held in gray. DMS reactivity is represented by circles from blue (low reactivity) to red (high reactivity) as shown in the legend. The nucleobases of guanosines and uridines as well as the closing base pairs were not tested by DMS probing. Base pairs confirmed by NMR are indicated by horizontal black lines between base pairing partners. **(C)** Cytidine amino group region of the ^{1}H,^{15}N-HSQC spectrum with annotated amino group resonances.



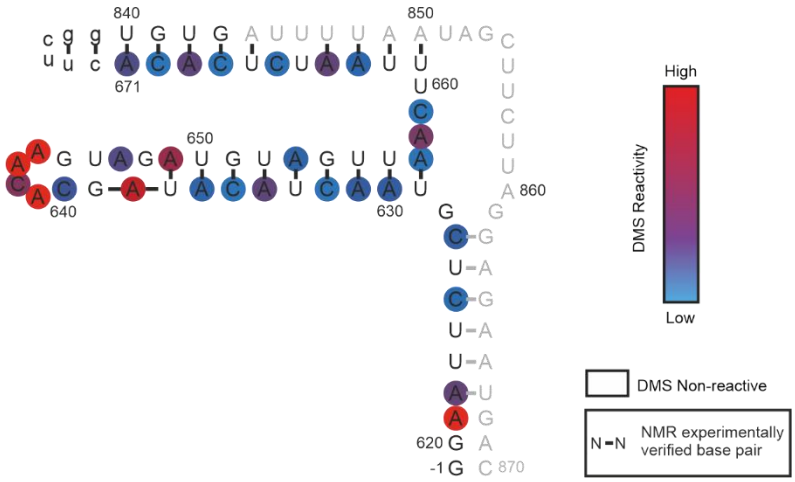
Supplementary Figure 18: Assignment of the aromatic and amino protons of the attenuator hairpin encompassing nucleotides 13432 to 13455. Annotations done with genomic numbering shifted for convenience by 13,000 from 5' (13,432-13,455). **(A)** ^{1}H,^{1N}-HSQC spectrum for cytidine amino correlations of H41 and H42 protons to N4 nitrogen. **(B)** ^{1}H,^{1}H-NOESY spectrum showing correlations of imino protons with adenosine H2 and cytidine H41/42 resonances. **(C)** Ir-^{1}H,^{15}N-HSQC spectrum showing adenosine H2-N1/N3 correlations. **(D)** Experimentally observed secondary structure of the attenuator hairpin with the assumed equilibrium of two conformations (see also Figure 13). Additional base pairs are annotated with G_{-2}, G_{-1}. NMR spectroscopically-confirmed base pairs are indicated by horizontal black lines between base pairing partners. Asterisks indicate secondary shifts due to conformational exchange.



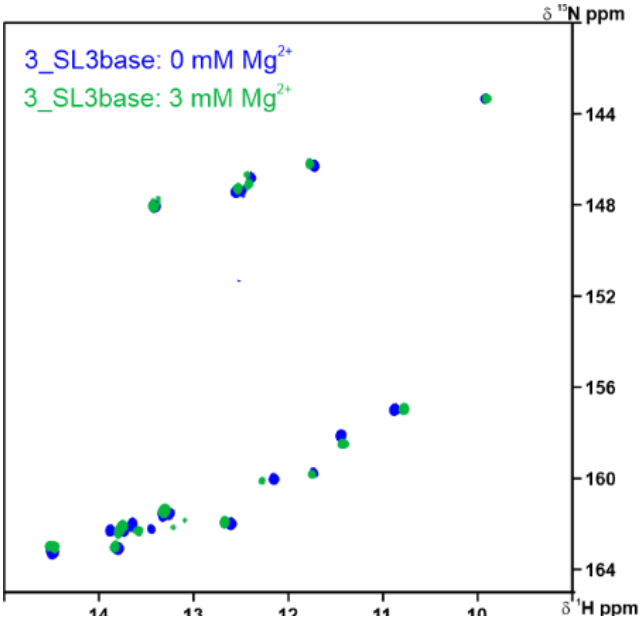
Supplementary Figure 19: Experimentally observed secondary structure of 3_SL1 with genomic numbering shifted for convenience by 29,000 from 5' (29548-29613). Additional closing base pairs introduced to allow for transcription and for stabilization of stem elements are annotated with G_{-3}, G_{-2}, G_{-1}, C_{+1}, C_{+2}, C_{+3}. DMS reactivity is represented by circles from blue (low reactivity) to red (high reactivity) as shown in the legend. The nucleobases of guanosines and uridines as well as the closing base pairs were not tested by DMS probing. Base pairs confirmed by NMR are indicated by horizontal black lines between base pairing partners.



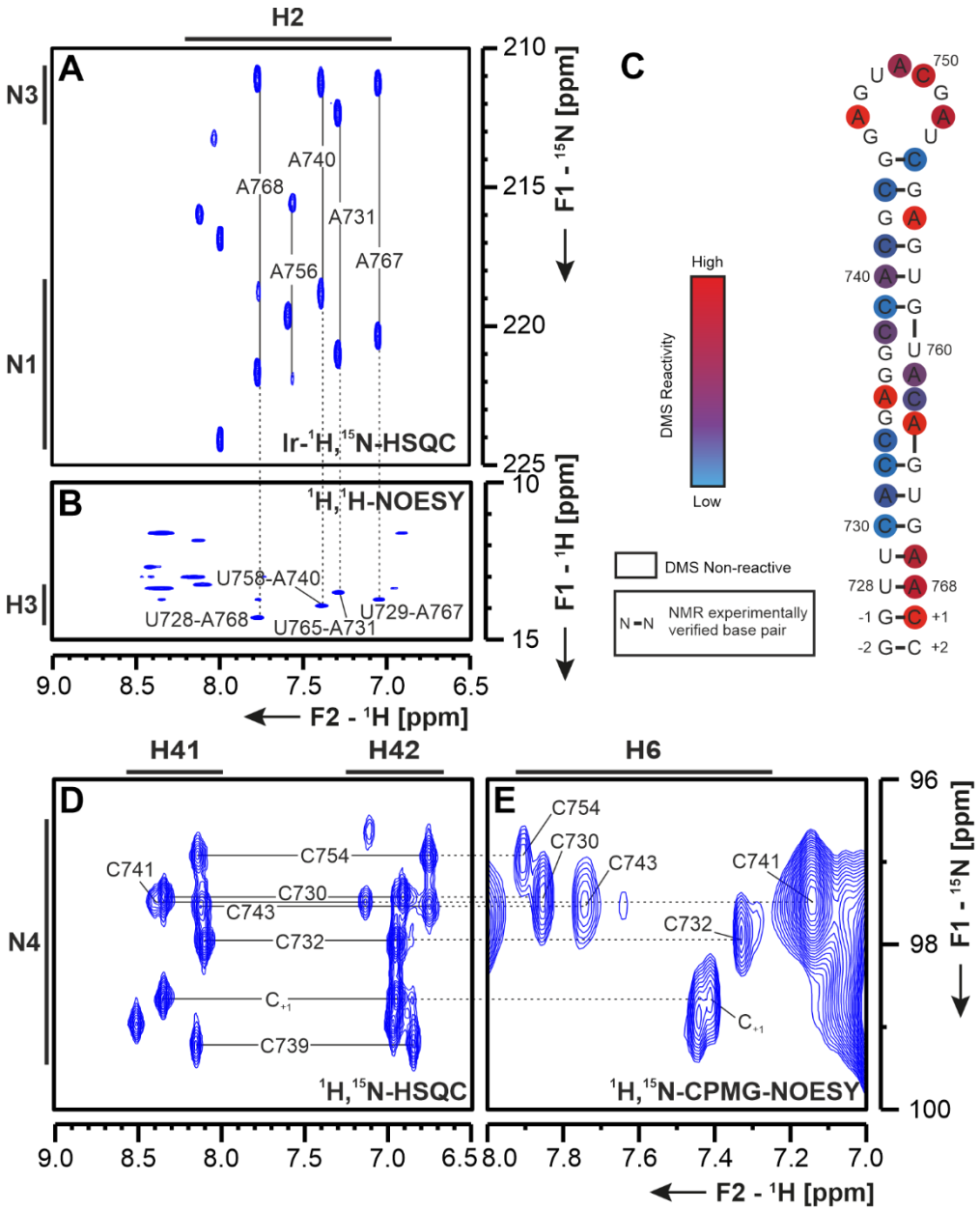
Supplementary Figure 20: Experimentally observed secondary structure of 3_SL2 with genomic numbering shifted for convenience by 29,000 from 5' (29,630-29,656). Additional closing base pairs are annotated with G_{-2}, G_{-1}, C_{+1}, C_{+2}. Observed atoms are annotated with bars next to the spectra. **(A)** ^{1}H,^{1}H-TOCSY spectrum correlating pyrimidine H5 and H6 protons. **(B)** ^{1}H,^{1}H-NOESY spectrum with an annotated exemplary H1'-H6/H8 walk. **(C)** Cytidine amino group region of the ^{1}H,^{15}N-HSQC spectrum with annotated amino group resonances. **(D)** DMS reactivity is represented by circles from blue (low reactivity) to red (high reactivity) as shown in the legend. The nucleobases of guanosines and uridines as well as the closing base pairs were not tested by DMS probing. Base pairs confirmed by NMR are indicated by horizontal black lines between base pairing partners.



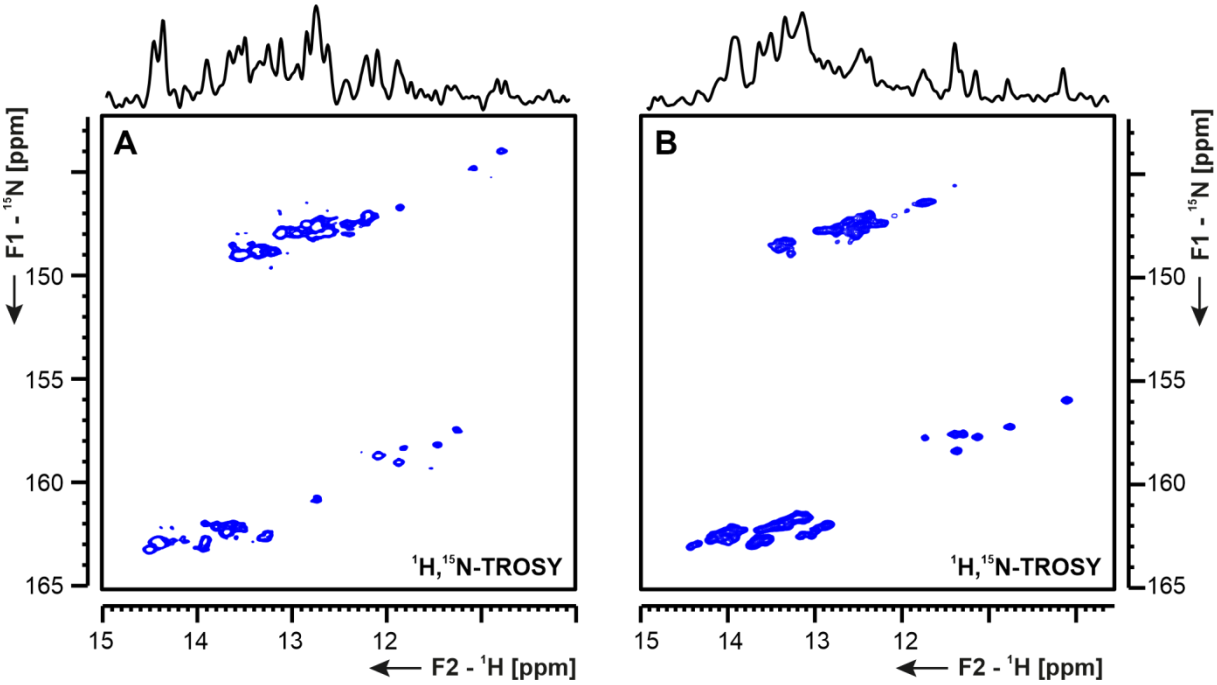
Supplementary Figure 21: Experimentally observed secondary structure of 3_SL3base with genomic numbering shifted for convenience by 29,000 from 5' (29,620-29,671 Δ29,840-29,870). Additional closing base pairs are annotated with G_{-1}. Nucleotides that belong to the cuucgg mutation are written in lowercase. Nucleotides that constitute the DMS primer site are held in gray. DMS reactivity is represented by circles from blue (low reactivity) to red (high reactivity) as shown in the legend. The nucleobases of guanosines and uridines as well as the closing base pairs were not tested by DMS probing. Base pairs confirmed by NMR are indicated by horizontal black lines between base pairing partners.



Supplementary Figure 22: Imino proton overlays of the $^{1}H,^{15}N$-HSQCs showing 3_SL3base at 0 (blue) and 3 mM Mg^{2+} (green). Spectra were recorded on a 200 µM RNA sample at 283 K. No additional resonances in the non-canonical regions of the spectrum are observed at 3 mM Mg^{2+}, which would have been indicative of the G-U base pair suggested by several secondary structure prediction programs (mfold, RNAfold, RNAstructure) (1–3).



Supplementary Figure 23: Assignment of the aromatic and amino protons of 3_s2m encompassing nucleotides 29,728 to 29,767. Annotations done with genomic numbering shifted for convenience by 29,000 from 5' (29,728-29,767). Observed atoms are annotated with bars next to the spectra. **(A)** Ir-^{1}H,^{15}N-HSQC spectrum for the adenosine H2-N1/N3 correlations. **(B)** ^{1}H,^{1}H-NOESY spectrum with annotated cross peaks of adenosine H2 and their pairing uridine H3. **(C)** Experimentally observed secondary structure of 3_s2m with genomic numbering. Additional closing base pairs are annotated with G_{-2}, G_{-1}, C_{+1}, C_{+2}. DMS reactivity is represented by circles from blue (low reactivity) to red (high reactivity) as shown in the legend. The nucleobases of guanosines and uridines as well as the closing base pairs were not tested by DMS probing. Base pairs confirmed by NMR are indicated by horizontal black lines between base pairing partners. **(D)** ^{1}H,^{15}N-HSQC spectrum for the cytidine H41/H42-N4 correlations. **(E)** ^{1}H,^{15}N-CPMG-NOESY for the cytidine correlations for H6-N4.



Supplementary Figure 24: $^{1}H,^{15}N$-TROSY spectra for imino-proton correlation of the **(A)** 5'-geRNA encompassing nucleotides 1 to 472 and **(B)** 3'-UTR encompassing the 337 terminal nucleotides upstream of the polyA-tail (nts 29,534 – 29,870).

Supplementary Table 1: Overview of the Reverse Transcription and PCR primers used for DMS footprinting (DMS-MaPseq).

Primer	#nt	genomic start	genomic end	Sequence 5’ to 3’
3F1	20	29,548	29,567	CACAAGGCAGATGGGCTATA
3F2	24	29,605	29,627	ACTCTTGTGCAGAATGAATTCTC
3R1	22	29,800	29,779	CTCTTCCATATAGGCAGCTCTC
3R2	28	29,870	29,843	GTCATTCTCCTAAGAAGCTATTAAAATC
5F1	22	7	28	GGTTTATACCTTCCCAGGTAAC
5F2	23	219	241	GATCATCAGCACATCTAGGTTTC
5R1	22	273	252	CTCTCCATCTTACCTTTCGGTC
5R2	24	446	423	AAACGGGTTTTTCAACTTCTACTA

Supplementary Table 2: Overview of RNA sequences.

construct	# nt	genomic start	genomic end	Sequence 5’ to 3’
5’-geRNA	472	1	472	AUUAAAGGUUUAUACCUUCCCAGGUAACAAACCA ACCAACUUUCGAUCUCUUGUAGAUCUGUUCUCU AAACGAACUUUAAAAUCUGUGUGGCUGUCACUC GGCUGCAUGCUUAGUGCACUCACGCAGUAUAAU UAAUAACUAAUUACUGUCGUUGACAGGACACGA GUAACUCGUCUAUCUUCUGCAGGCUGCUUACGG UUUCGUCCGUGUUGCAGCCGAUCAUCAGCACAU CUAGGUUUCGUCCGGGUGUGACCGAAAGGUAAG AUGGAGAGCCUUGUCCCUGGUUUCAACGAGAAA ACACACGUCCAACUCAGUUUGCCUGUUUUACAG GUUCGCGACGUGCUCGUACGUGGCUUUGGAGAC UCCGUGGAGGAGGUCUUAUCAGAGGCACGUCAA CAUCUUAAAGAUGGCACUUGUGGCUUAGUAGAA GUUGAAAAAGGCGUUUUGCCUCAACUUGAACAG CCCUAUGUG
5_SL1	29	7	33	GGGUUUAUACCUUCCCAGGUAACAAACCC
5_SL1-4	119	7	125	GGUUUAUACCUUCCCAGGUAACAAACCAACCAAC UUUCGAUCUCUUGUAGAUCUGUUCUCUAAACGA ACUUUAAAAUCUGUGUGGCUGUCACUCGGCUGC AUGCUUAGUGCACUCACGC
5_SL2+3	32	45	75	GGAUCUCUUGUAGAUCUGUU CUCUAAACGAAC
5_SL4	44	86	125	GGGUGUGGCUGUCACUCGGCUGCAUGCUUAGUG CACUCACGCCC
5_SL5stem	69	265-294 Δ 150-180		GGGAUGGAGAGCCUUGUCCCUGGUUUCAACGAU UCGUCGUUGACAGGACACGAGUAACUCGUCUAU CCC
5_SL5a	33	188	218	GGGCUGCUUACGGUUUCGUCCGUGUUGCAGCCC
5_SL5b+c	37	227	263	GCACAUCUAGGUUUCGUCCGGGUGUGACCGAAA GGUA
5_SL5b	25	227	251	CACAUCUAGGUUUCGUCCGGGUGUGG
5_SL5c	12	252	263	GACCGAAAGGUA
5_SL6	46	302	343	GGCACGUCCAACUCAGUUUGCCUGUUUUACAGG UUCGCGACGUGCC
5_SL7	50	349	394	GGACGUGGCUUUGGAGACUCCGUGGAGGAGGUC UUAUCAGAGGCACGUCC
5_SL8	63	413	471	GGACUUGUGGCUUAGUAGAAGUUGAAAAAGGCG UUUUGCCUCAACUUGAACAGCCCUAUGUCC
5_SL8loop	31	430	456	GGAGUUGAAAAAGGCGUUUUGCCUCAACUCC
attenuator hairpin (att HP)	26	13,432	13,455	GGCAUGCUUCAGUCAGCUGAUGCACA
Pseudoknot (PK)	69	13,475	13,542	GGCGGUGUAAGUGCAGCCCGUCUUACACCGUGC GGCACAGGCACUAGUACUGAUGUCGUAUACAGG GCU
3’-UTR	337	29,534	29,870	ACUCAUGCAGACCACACAAGGCAGAUGGGCUAUA UAAACGUUUUCGCUUUUCCGUUUACGAUAUAUA GUCUACUCUUGUGCAGAAUGAAUUCUCGUAACU ACAUAGCACAAGUAGAUGUAGUUAACUUUAAUC UCACAUAGCAAUCUUUAAUCAGUGUGUAACAUU

				AGGGAGGACUUGAAAGAGCCACCACAUUUUCACC GAGGCCACGCGGAGUACGAUCGAGUGUACAGUG AACAAUGCUAGGGAGAGCUGCCUAUAUGGAAGA GCCCUAAUGUGUAAAAUUAAUUUUAGUAGUGCU AUCCCCAUGUGAUUUUAAUAGCUUCUUAGGAGA AUGAC
3_HVR	115	29,698	29,806	GGGUUAGGGAGGACUUGAAAGAGCCACCACAUU UUCACCGAGGCCACGCGGAGUACGAUCGAGUGU ACAGUGAACAAUGCUAGGGAGAGCUGCCUAUAU G GAAGAGCCCUAACCC
3_s2m	45	29,728	29,768	GGUUCACCGAGGCCACGCGGAGUACGAUCGAGU GUACAGUGAACC
3_SL1	72	29,548	29,614	GGGCACAAGGCAGAUGGGCUAUAUAAACGUUUU CGCUUUUCCGUUUACGAUAUAUAGUCUACUCUU GUGCCC
3_SL2	31	29,630	29,656	GGAACUACAUAGCACAAGUAGAUGUAGUUCC
3_SL3base	90	29,620-29,671 Δ 29,840-29,870		GGAAUUCUCGUAACUACAUAGCACAAGUAGAUG UAGUUAACUUUAAUCUCACACUUCGGUGUGAUU UUAAUAGCUUCUUAGGAGAAUGAC

Supplementary References

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