**Table S1. Oligonucleotide DNA primers and templates.**

| No. | Name | DNA primer and template |
|---|---|---|
| 1 | FL_1f | 5'-CCGGAATTCTAATACGACTCACTATAGGAACACATCAGATTTC-3' |
| 2 | FL_2r | 5'-GAAGCACTTAAAAAATTCGTTACACCAGGAAATCTGATGTGTT-3' |
| 3 | FL_3f | 5'-ATTTTTTAAGTGCTTCTTGCTTAAGCAAGTTTCATCCCGACCC-3' |
| 4 | FL_4r | 5'-CTAGTCTAGAAAAATCCCGACCCTGAGGGGGTCGGGATGAA AC-3' |
| 5 | FL_5tem | 5'- GAAATTAATACGACTCACTATAGGAACACATCAGA-3' |
| 6 | FL_3tem | 5'-AAAAATCCCGACCCTGAGGGGG-3' |
| 7 | SL12 | 5'-AAACTTGCTTAAGCAAGAAGCACTTAAAAAATTCGTTACACCAGGAAATCTGATGTGTTCCTATAGTGAGTCGTATTAATTTC-3' |
| 8 | SL23 | 5'-AAATCCCGACCCTGAGGGGGTCGGGATGAAACTTGCTTAAGCAAGAAGCACTTCCTATAGTGAGTCGTATTAATTTC-3' |
| 9 | $SL1_{+4}$ | 5'-GGACACCAGGAAATCTGATGTCCTATAGTGAGTCGTATTAATTTC-3' |
| 10 | $SL2_{+4}$ | 5'-GGAAACTTGCTTAAGCAAGAAGCACTTCCTATAGTGAGTCGTATTAATTTC-3' |
| 11 | $SL3_{+4}$ | 5'-GGATCCCGACCCTGAGGGGGTCGGGATCCTATAGTGAGTCGTATTAATTTC-3' |
| 12 | DsrA32 | 5'-AAAATTCGTTACACCAGGAAATCTGATGTGTTCCTATAGTGAGTCGTATTAATTTC-3' |
| 13 | rpoS26 | 5'-CGGATTTCCCCTTGTAACGAATTTCCTATAGTGAGTCGTATTAATTTC-3' |
| 14 | R58 RNA | 5'-GGCGGATTTCCCCTTGTAACGAATTTCTTCCAAAATTCGTTACACCAGGAAATCTGCCTATAGTGAGTCGTATTAATTTC-3' |

| | | |
|---|---|---|
| 15 | Domain2 | 5’-GAAACTTGCTTAAGCAAGAAGCACTTAAAAAATTCGTTCCTATAGTGAGTCGTATTAATTTC-3’ |
| 16 | Domain2$_{35nt}$ | 5’-GAAACTTGCTTAAGCAAGAAGCACTTAAAAAATCCTATAGTGAGTCGTATTAATTTC-3’ |
| 17 | Domain2$_{30nt}$ | 5’-GAAACTTGCTTAAGCAAGAAGCACTTAAAAAATCCTATAGTGAGTCGTATTAATTTC-3’ |
| 18 | Domain2$_{25nt}$ | 5’-GAAACTTGCTTAAGCAAGAAGCACCTATAGTGAGTCGTATTAATTTC-3’ |
| 19 | Domain2$_{20nt}$ | 5’-GAAACTTGCTTAAGCAAGCCTATAGTGAGTCGTATTAATTTC-3’ |
| 20 | SL2$_{+4}$ C53Δ | 5’-GGAAACTTCTTAAGCAAGAAGCACTTCCTATAGTGAGTCGTATTAATTTC-3’ |
| 21 | SL12 C53Δ | 5’-GAAACTTCTTAAGCAAGAAGCACTTAAAAAATTCGTTACACCAGGAAATCTGATGTGTTCCTATAGTGAGTCGTATTAATTTC-3’ |
| 22 | R58L | 5’-GGCGGATTTCCCTTCCAGGAAATCTGCCTATAGTGAGTCGTATTAATTTC-3’ |
| 23 | R58M | 5’-GGTCCCCTTGTATTCCTACACCAGGACCTATAGTGAGTCGTATTAATTTC-3’ |
| 24 | R58U | 5’-GGTGTAACGAATTTCTTCCAAAATTCGTTACACCTATAGTGAGTCGTATTAATTTC-3’ |

**Table S2. NMR restraints and structure statistics for the 20 lowest energy structures of DsrA-SL1$_{+4}$**

| Cyana[1] | |
|---|---|
| NOE-derived restraints | 397 |
| Intraresidue | 245 |
| Interresidue | 152 |
| H-bond restraints | 92 |
| NOE restraints/residue | 17.26 |
| Total restraints/residue | 21.26 |
| Upper distance viol. (Å) | 0.0032 ± 0.0001 |
| Lower distance viol. (Å) | 0.0013 ± 0.0001 |
| Sum VDW viol. (Å) | 0.6 ± 0 |
| RMSD (Å)[2] | 1.65 ± 0.32 |
| RMSD stem (Å) (G-2-G10, C16-C+2) | 0.35 ± 0.07 |
| **Amber[3]** | |
| Amber energy (kcal mol$^{-1}$) | -5214.88 (5.55) |
| Distance (kcal mol$^{-1}$) | 1.718 (0.258) |
| Torsion (kcal mol$^{-1}$) | 0.128 (0.015) |
| RMSD (Å)[4] | 1.872 |
| RMSD stem (Å) (G-2-G10, C16-C+2) | 0.168 |
| **MolProbity analysis[5]** | |
| Clashscore | 0.14 (0.43) |
| Probably wrong sugar pucker (%) | 0 (0) |
| Bad backbone conformation (%) | 14.54 (3.62) |
| Bad bonds (%) | 0.40 (0) |
| Bad angles (%) | 0.01 (0.03) |

[1] Statistics for the 20 structures with lowest target function.

[2] Mean standard deviation for all heavy atoms, relative to mean coordinates, calculated with Cyana.

[3] Statistics for the 20 lowest energy structures.

[4] Mean standard deviation for all heavy atoms, relative to the average structure, calculated using suppose over the residues listed in parentheses.

[5] The 20 amber-refined structures were evaluated using the MolProbity webserver.

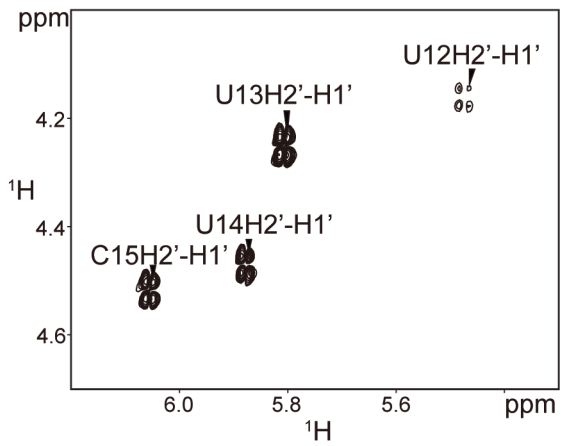


**Figure S1. Portion of 2D $^{1}$H-$^{1}$H DQF-COSY.**

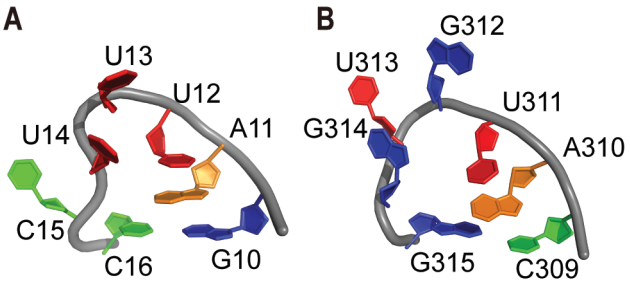


**Figure S2. Comparative structural alignment of DsrA-SL1 pentaloop (A) and 7SK-SL4 pentaloops (B)** (1).

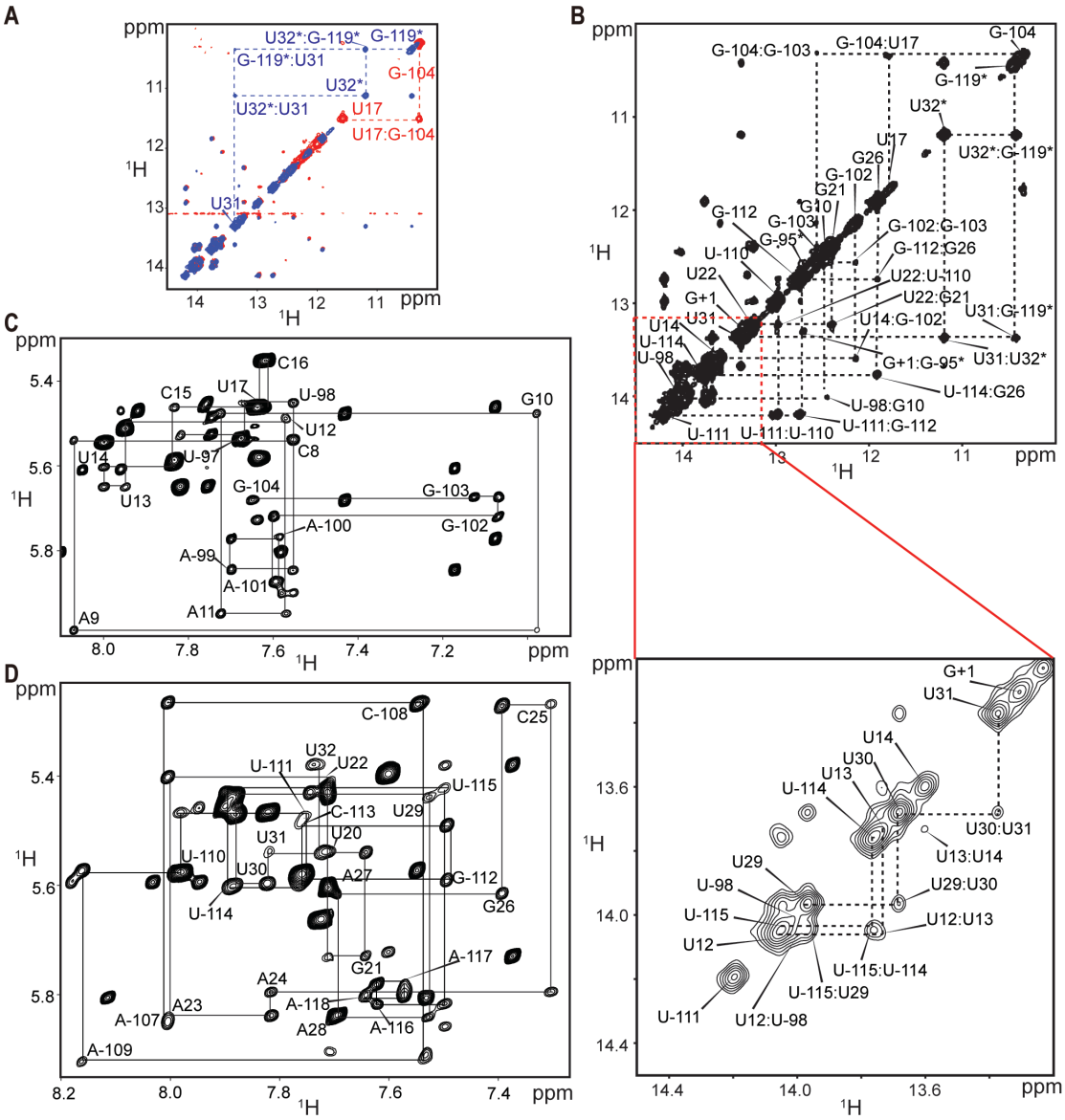


**Figure S3. NMR studies of DsrA/*rpoS* complex.** (A) 2D $^{1}$H-$^{1}$H NOESY spectra (in 90% $H_2O$/10% $D_2O$) of DsrA32/rpoS25 complex recorded at 900 MHz spectrometer (in red) and R58 RNA recorded at 700 MHz spectrometer (in blue). (B) Assignment of imino proton resonances of 2D NOESY spectrum of R58 RNA. Portions of 2D NOESY spectra obtained for R58L (C) and R58U (D) showing cross-peaks between aromatic H6/H8 protons and ribose H1' protons. Sequential NOE connectivities are indicated with lines.

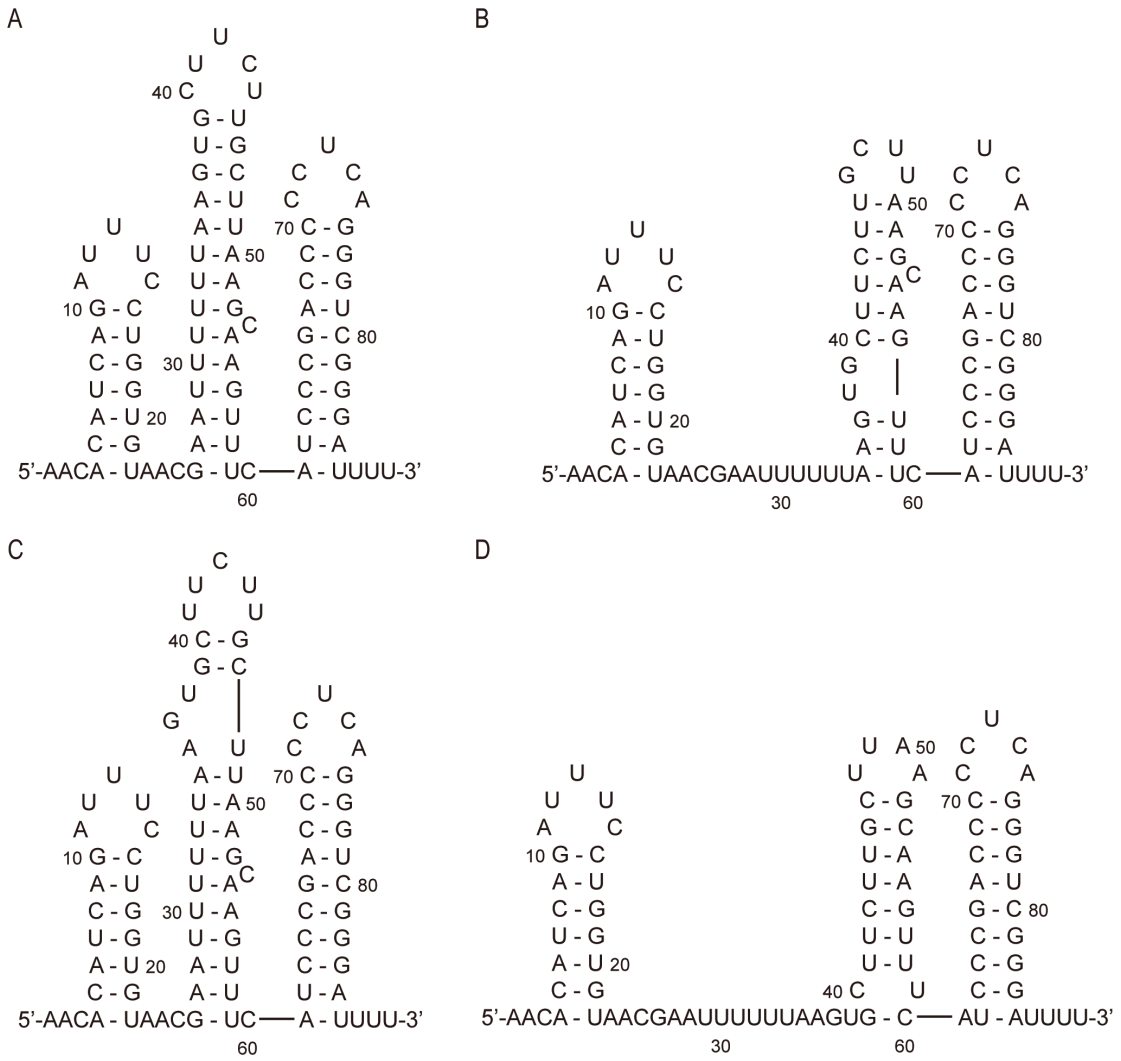


**Figure S4. The secondary structure models of DsrA sRNA.** (A) Model A was based on thermodynamic calculations and mutation analysis (2). (B) Model B was based on specific nuclease cleavage experiments (3). (C) Model C was based on nuclease footprinting analysis (4). (D) Model D was based on isoenergetic microarray mapping method (5).

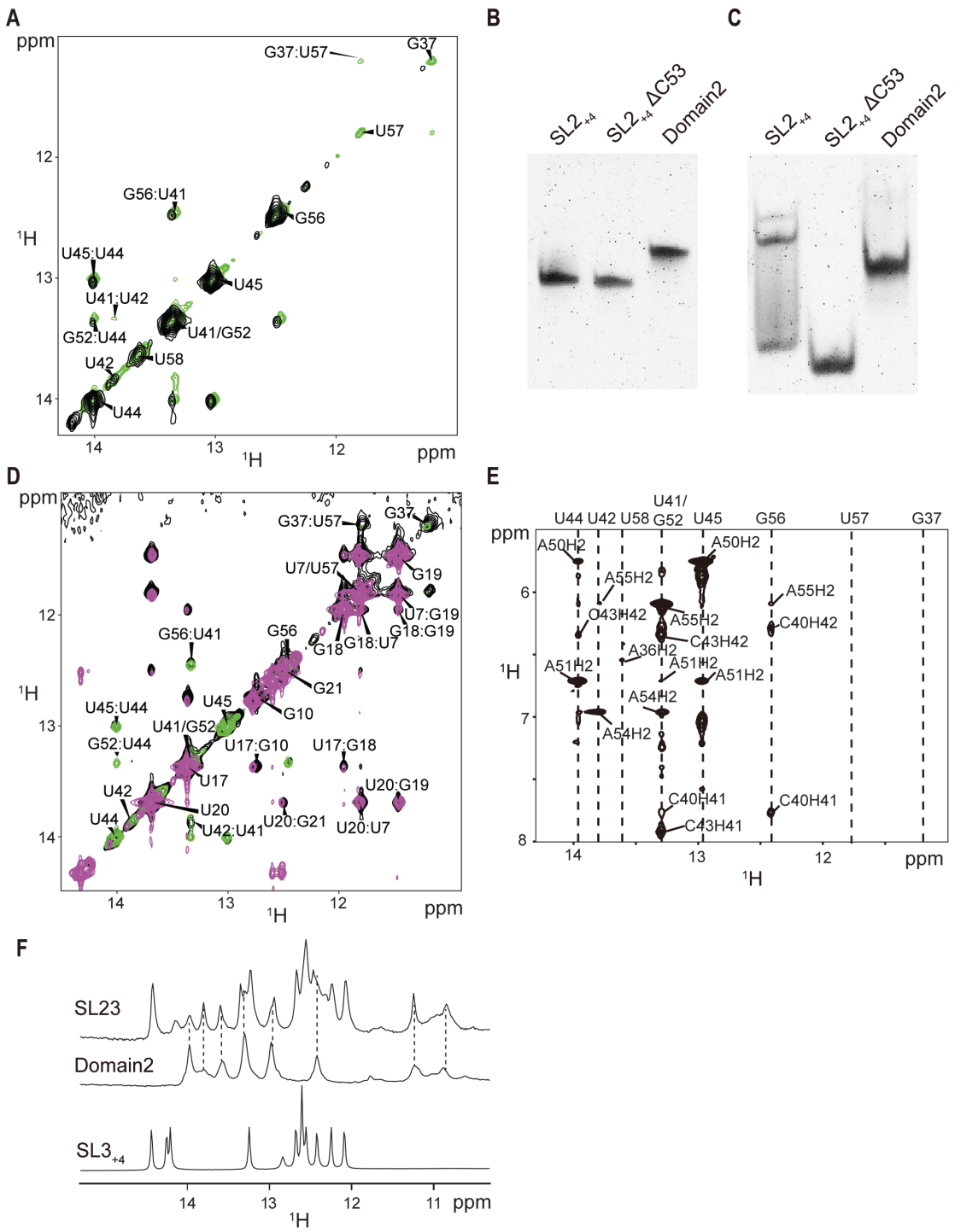


**Figure S5. Secondary structure of SL2.** (A) Overlap of imino-to-imino regions of 2D $^{1}$H-$^{1}$H NOESY in $H_2O$ of $SL2_{+4}$ (black) and Domain2 (green). (B) 16% denaturing PAGE results for $SL2_{+4}$, $SL2_{+4}$ ΔC53 and Domain2 RNAs. $SL2_{+4}$ ΔC53 RNA has a single buldge C53 deletion. (C) 16% native PAGE results for $SL2_{+4}$, $SL2_{+4}$ ΔC53 and Domain2 RNAs. (D) Overlap of imino-to-imino regions of $SL1_{+4}$ (magenta), Domain2 (green) and SL12 (black). (E) Portion of the 2D NOESY spectrum obtained for Domain2 showing NOEs from the imino protons to H2 protons of Adenine and amino protons of cytosine. (F) One-dimensional imino spectra of SL23

(top), Domain2 (middle) and $SL3_{+4}$ (bottom). Resonance assignments belonging to Domain2 are indicated by the dash line. All spectra above were recorded at 10 °C and pH 6.5.

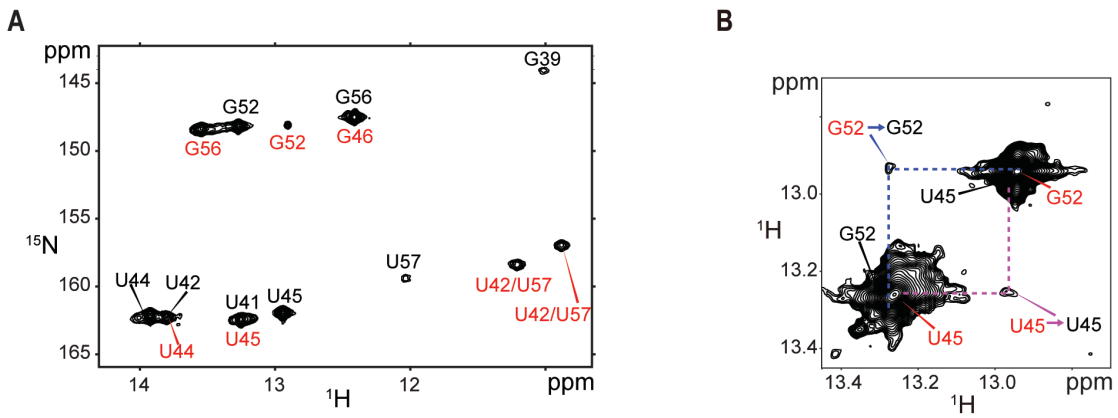


**Figure S6. NMR studies of $^{15}$N-labelled Domain2$_{25nt}$ RNA (2 mM) at 293 K.** (A) Portion of $^{1}$H-$^{15}$N HSQC spectrum. (B) Portion of 2D $^{1}$H-detected $^{15}$N EXSY spectrum recorded with a mixing time $\tau_m$ = 1.2 s. Pulse sequence of EXSY comes from previous report (6). Nucleotides of Fold A are coloured and numbered in black, and Fold B are in red. Cross peaks from G52 and U45 are labelled in blue and magenta, respectively.

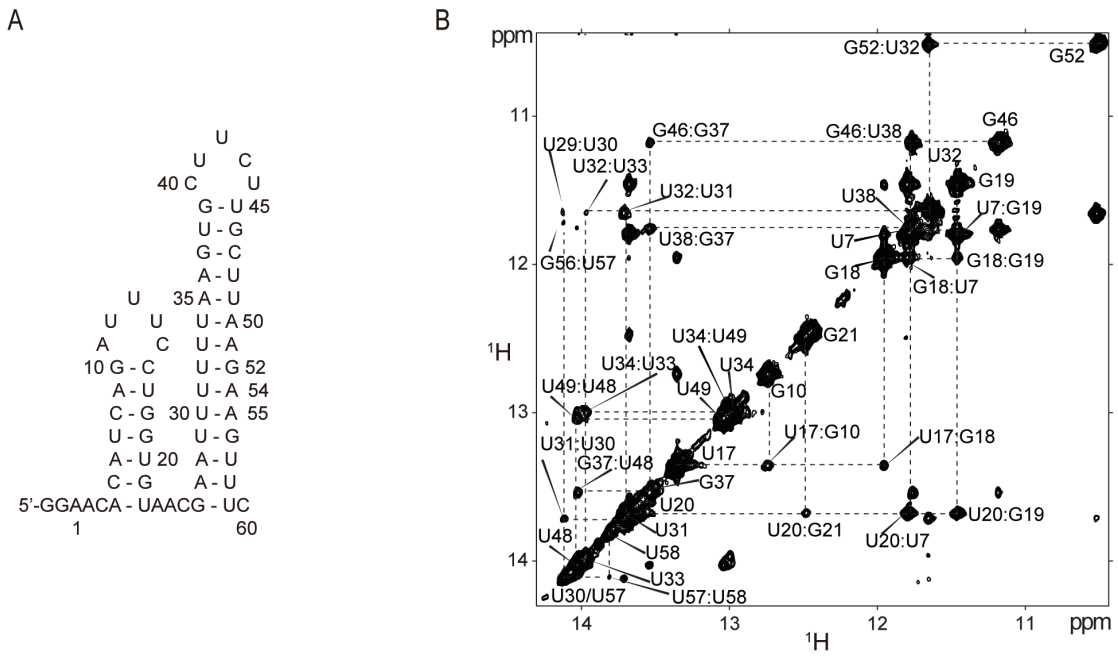


**Figure S7. C53-deleted mutant causes an obvious structural change in DsrA.** (A) Secondary structure of SL12 ΔC53 mutant. (B) Imino proton region of the NOESY spectrum of SL12 ΔC53 mutant. Imino proton resonances are labelled by the one-letter nucleotide code and the residue number, and their sequential NOE assignments are indicated by bash lines.

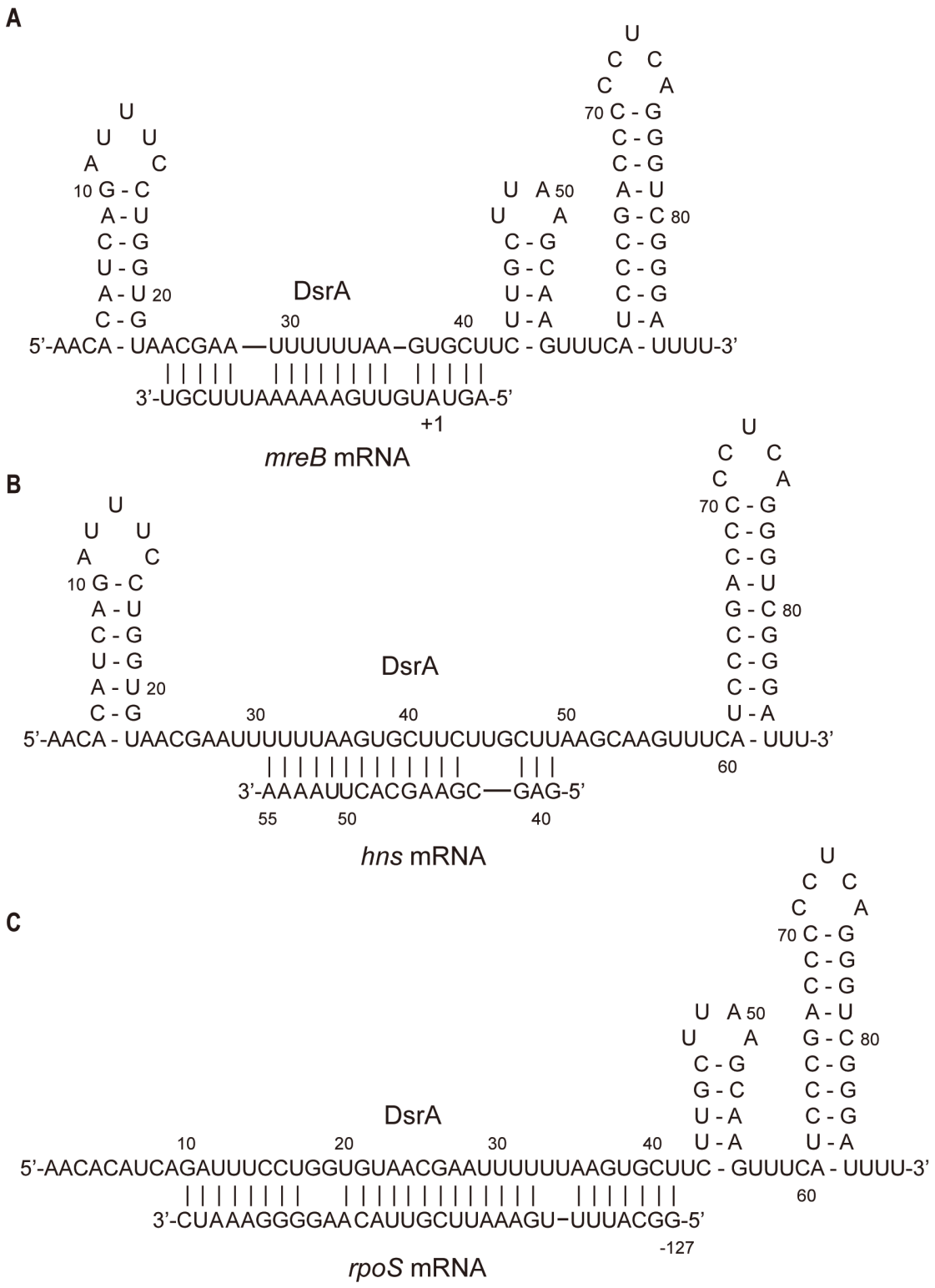


**Figure S8. Secondary structures of DsrA sRNA in complex with mRNAs.** (A) Secondary structure model for the DsrA/*MreB* RNA duplex predicted by using RNAfold (7). (B) Secondary structure model for the DsrA/*hns* RNA duplex predicted by the probing assays (8). (C) Secondary structure model for the DsrA/*rpoS* RNA duplex predicted by the isoenergetic microarray mapping method (5).

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