Supplementary Information

microRNA-122 target sites in the Hepatitis C Virus RNA NS5B coding region and 3´untranslated region: function in replication and influence of RNA secondary structure

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Running head: miR-122/Ago2 binding in coding region and 3´UTR of HCV

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Supplementary Figure S1 (next page), related to Figure 1

miR-122 and the conserved miR-122 target site 5B.2 in 106 selected HCV NS5B sequences (Fricke et al. 2015). The miR-122 sequence with the seed sequence (nts 2 – 8) and the supplementary binding region (nts 14 – 20) are marked in red. Gaps were introduced to align seed target sequences. HCV genotype and NCBI nucleotide database accession numbers are given on the left, nucleotide numbers are shown for each isolate. The miR-122 seed target site (A)CACUCC is 100 % conserved in 99 of 106 isolates, the supplementary binding site is conserved in all isolates. Below the sequences, the histogram shows the conservation of each residue.

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                        supplemental                  seed
                           region                    region
              miR-122  3'-GU     GGUAACAG              U-5'
                             UUGU            UGUGAGG
1a_EF621489.1       8776 UCGCGCGGGCCGCGUGGGAGACAGCAAGACACACUCC--AGUCAAUUCCUGGCUAGGCAACA 8835
1a_AB520610.1       8776 UCGCGAGAGCCGCGUGGGAGACAGCAAGACACACUCC--AGUCAAUUCCUGGCUAGGCAACA 8835
1b_AJ238799.1 Con1  8773 UUGCGCGGGCUGCGUGGGAGACAGCUAGACACACUCC--AGUCAAUUCCUGGCUAGGCAACA 8832
1b_AY460204.1       8776 UCGCGCGGGCUGCGUGGGAGACAGCUAGGCACACUCC--AGUCAACUCCUGGCUAGGCAACA 8835
1c_AY651061.1       8776 UCGCGCGUGCCGCUUGGGAGACAGCAAGACACACUCC--AGUGAACUCCUGGCUAGGCAACA 8835
1c_HPCCGS           8776 UCGCGCGUGCCGCUUGGGAAACAGCAAGACACACUCC--AGUGAACUCCUGGCUAGGCAACA 8835
1e_KC248194.1       8787 UCGCGCGCGCUGCGUGGGAAACAGCUAGACACACUCC--AAUCAACUCCUGGCUUGGCAACA 8846
1g_AM910652.2       8760 UCGCGCGCGCUGCGUGGGAAACAGCUAGACACACUCC--UGUUAACUCCUGGCUUGGCAACA 8819
1h_KC248198.1       8779 UCGCGCGAGCCGCGUGGGAAACAGCAAGACACACUCC--AGUGAACUCCUGGCUGGGCAACA 8838
1h_KC248199.1       8779 UCGCGCGAGCCGCGUGGGAAACAGCAAGACACACUCC--AGUAAACUCCUGGCUAGGCAACA 8838
1l_KC248196.1       8795 UCGCGCGAGCCGCGUGGGAGACAGCAAGACACACUCC--AGUUAACUCCUGGCUAGGCAACA 8854
2a_AF177036.1       8841 UCGCCCGGGCUGCCUGGGAAACAGUUAG--ACACUCCCCUGUCAAUUCAUGGCUGGGAAACA 8900
2a_AB047639.1 JFH1  8841 UCGCCCGGGCUGCCUGGGAAACAGUUAG--ACACUCCCCUAUCAAUUCAUGGCUGGGAAACA 8900
2b_AB030907.1       8842 UCACCCGAGCUGCUUGGGAAACAGUAAG--ACACUCCCCUGUCAAUUCUUGGCUGGGCAACA 8901
2b_JQ745651.1       8842 UCACCCGAGCUGCUUGGGAAACAGUAAG--ACACUCCCCUGUCAAUUCUUGGCUGGGCAACA 8901
2c_D50409.1         8853 UCGCCAGGGCUGCCUGGGAGACAGCAAG--ACACUCCCCUGUCAAUUCAUGGCUGGGAAACA 8912
2c_JX227965.1       8764 UCGCCAGGGCAGCCUGGGAGACAGCAAG--ACACUCCCCUGUCAAUUCAUGGUUGGGCAACA 8823
2d_JF735114.1       8841 UCGCCAGGGCCGCCUGGGAGACAGCACG--ACACUCCCCUGUCAAUUCAUGGCUGGGAAACA 8900
2e_JF735120.1       9057 UCGCCAGGGCUGCCUGGGAAACAGUGAA--GCACUCCCCUGUCAAUUCAUGGCUGGGAAACA 9116
6g_DQ314806.1       8806 UGGCCCGGGCUGCUUGGGAAACUGCUCGACACACUCC--AGUCAAUUCCUGGUUGGGCAACA 8865
6g_HPCJK046E2       8806 UAGCCCGGGCUGCUUGGGAAACUGCUCGACACACUCC--CGUGAAUUCCUGGUUGGGCAACA 8865
2i_DQ155561         8811 UCGCCAGGGCUGCCUGGGAGACCGCAAG--ACACUCCCCUGUCAAUUCAUGGCUGGGAAACA 8870
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2j_HM777359.1       8761 UCGCCAGAGCAGCCUGGGAAACUGUAAA--GCACUCCCCUGUCAAUUCAUGGCUGGGAAACA 8820
2k_AB031663.1       8842 UCGCCAGGGCUGCCUGGGAGACAGCUAG--ACACUCCCCUGUCAAUUCAUGGCUGGGAAACA 8901
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2m_JF735111.1       8844 UAGCCAGGGCUGCCUGGGAAACAGCUAG--ACACUCCCCUGUCAAUUCAUGGUUGGGAAACA 8903
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2q_FN666429         8814 UCGCCAGGGCUGCCUGGGAGACAGUCAG--ACACUCCCCUGUCAAUUCAUGGCUGGGAAACA 8873
2q_FN666428         8814 UCGCCAGGGCUGCCUGGGAGACAGUCAG--ACACUCCCCUGUCAAUUCAUGGCUGGGAAACA 8873
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3a_AB792683.1       8804 UGGCCCGCGCGGCUUGGGAAACAGCUCGUCACACUCC--AGUUAACUCCUGGUUGGGUAACA 8863
3b_HPCFG            8810 UAGCCCGUGCGGCUUGGGAGACUGCUCGCCACACUCC--AGUUAACUCCUGGUUGGGAAAUA 8869
3g_JF735123.1       8813 UGGCCCGAGCUGCUUGGGAAACCGCACGCCACACUCC--AGUUAACUCCUGGUUGGGUAACA 8872
3g_JX227954.1       8732 UGGCCCGUGCGGCUUGGGAAACAGCACGUCACACUCC--AGUUAACUCCUGGUUGGGAAACA 8791
3h_JF735121.1       8798 UUGCCCGUGCUGCGUGGGAAACAGCUAGACACACUCC--GGUGAAUUCCUGGCUUGGCAACA 8857
3h_JF735124.1       8804 UCGCCCGCGCGGCGUGGGAGACCGCCAGACACACACC--AGUCAACUCUUGGUUGGGCAACA 8863
3i_FJ407092.1       8805 UCGCCCGUGCGGCCUGGGAGACUGCUCGCCACACUCC--GGUAAACUCCUGGUUGGGGAACA 8864
3i_JF735125.1       8805 UGGCCCGUGCGGCUUGGGAGACUGCUCGCCACACUCC--GGUAAACUCCUGGUUGGGGAACA 8864
3k_HPCJK049E1       8798 UGGCCCGCGCGGCCUGGGAGACUGCCCGCCACACUCC--AGUUAACUCCUGGUUGGGAAAUA 8857
3k_JF735122.1       8798 UAGCCCGCGCGGCCUGGGAGACUGCUCGCCACACUCC--AGUUAACUCCUGGUUGGGAAAUA 8857
4a_AB795432.1       8767 UGGCGCGAGCCGCAUGGGAGACAGUCCGACACACUCC--AGUCAAUUCCUGGUUGGGAAACA 8826
4a_Y11604           8705 UGGCGCGAGCUGUAUGGGAGACAGUCCGACACACUCC--AGUCAAUUCCUGGUUGGGAAACA 8764
4b_FJ025856         8625 UGGCUAGAGCCGCGUGGGAGACAGUCCGACACACUCC--AGUGAACUCCUGGUUGGGGAACA 8684
4b_FJ462435.1       8775 UCGCGCGGGCUGCUUGGGAAACAGUACGACACACUCC--GGUUAACUCCUGGCUGGGUAACA 8834
4c_FJ462436.1       8808 UGGCGCGAGCUGCGUGGGAGACAGUCCGACACACUCC--AGUCAAUUCCUGGUUGGGAAACA 8867
4d_DQ516083         8699 UGGCACGGGCUGCCUGGGAGACAGCUCGACACACUCC--AGUCAACUCCUGGCUUGGGAACA 8758
4d_FJ462437.1       8760 UGGCACGGGCUGCCUGGGAGACAGCUCGACACACUCC--AGUCAACUCUUGGCUUGGGAACA 8819
4f_EF589160         8704 UCGCCAGAGCUGCGUGGGAGACAGCCAGACACACUCC--AGUCAACUCCUGGUUGGGAAACA 8763
4f_EF589161         8706 UCGCCAGAGCCGCGUGGGAAACAGCCAGACACACUCC--AGUCAACUCCUGGUUGGGAAACA 8765
4g_FJ462432.1       8767 UGGCACGAGCCGCCUGGGAAACAGCUCGACACACUCC--AGUCAAUUCCUGGUUGGGCAACA 8826
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4k_FJ462438.1       8776 UGGCACGAGCCGCCUGGGAAACAGUGCGACACACUCC--AGUCAACUCCUGGUUGGGAAAUA 8835
4k_EU392173         8698 UGGCACGAGCCGCCUGGGAAACAGUGCGACACACUCC--AGUCAACUCCUGGUUGGGAAAUA 8757
4L_JX227957.1       8689 UGGCACGGGCUGCUUGGGAGACAGUCAGACACACUCC--GGUAAAUUCCUGGCUAGGGAACA 8748
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4n_JX227970.1       8685 UGGCACGAGCAGCCUGGGAGACAGUCCGACACACUCC--AGUCAAUUCCUGGUUGGGUAAUA 8744
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4o_FJ462440.1       8758 UGGCACGAGCUGCCUGGGAAACAGUCCGACACACUCC--AGUCAACUCCUGGUUGGGCAACA 8817
4o_JX227977.1       8668 UGGCACGAGCUGCCUGGGAAACAGUCCGACACACUCC--GGUCAAUUCCUGGUUGGGCAACA 8727
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4r_JX227962.1       8700 UGGCCAGAGCUGCCUGGGAGACAGCUCGACACACUCC--AGUCAAUUCCUGGUUGGGGAACA 8759
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4v_HQ537009         8682 UGGCUCGCGCCGCUUGGGAAACAGUUCGACACACUCC--AGUUAAUUCUUGGCUAGGGAACA 8741
5a_NC_009826        8723 UUGCGAAGGCUGCGUGGGAAACUGCCAA--ACACUCCCCGGUUAAUUCCUGGCUAGGGAACA 8782
5a_AF064490         8690 UUGCGAGGGCCGCGUGGGAGACUGCUAA--ACACUCCCCAGUUAACUCCUGGUUGGGGAACA 8749
6a_AY859526.1       8754 UUGCGCGGGCCGCUUGGGAGACAGCCCGCCACACUCC--AGUCAAUUCGUGGUUGGGCAACA 8813
6a_DQ480524.1       8760 UAGCGCGGGCCGCCUGGGAGACAGCCCGCCACACUCC--AGUCAAUUCGUGGCUGGGCAACA 8819
6b_NC_009827.       8801 UUGCGCGAGCUGCUUGGGAGACAGCUCGCCAUACCCC--UGUCAAUUCGUGGUUGGGCAACA 8860
6b_D84262.2         8801 UUGCGCGAGCUGCUUGGGAGACAGCUCGCCAUACCCC--UGUCAAUUCGUGGUUGGGCAACA 8860
6c_EF424629.1       8786 UCUCACGAGCUGCUUGGGAAACAGCUCGCCACACUCC--AGUUAACUCAUGGUUGGGCAACA 8845
6d_D84263.2         8779 UGUCUCGGGCUGCCUGGGAAACAGCCCGACACACUCC--CGUUAACUCAUGGCUGGGCAACA 8838
6e_EU408326         8806 UUUCUCGAGCGGCCUGGGAAACCGCCCGACACACUCC--GGUGAAUUCCUGGUUGGGUAACA 8865
6e_DQ314805.1       8806 UUUCUCGGGCGGCCUGGGAAACCGCCCGACACACUCC--GGUGAAUUCCUGGUUGGGCAAUA 8865
6f_DQ835764.1       8794 UGACGCGAGCAGCUUGGGAAACAGCCCGCCACACUCC--AGUGAAUUCAUGGCUGGGCAACA 8853
6f_DQ835760.1       8794 UGUCGCGAGCAGCCUGGGAAACAGCCCGCCACACUCC--AGUGAAUUCAUGGCUGGGCAACA 8853
6h_D84265.2         8785 UGGCGAGGGCGGCGUGGGAGACCGCUCGUCACACUCC--UGUGAAUUCAUGGUUAGGGAACA 8844
6i_DQ835762.1       8788 UUGCCAGAGCAGCUUGGGAGACUGCCCGGCACACUCC--AGUCAACUCGUGGCUGGGAAACA 8847
6i_DQ835770.1       8788 UUGCCAGAGCAGCCUGGGAGACUGCCCGGCACACUCC--AGUUAACUCGUGGCUGGGGAACA 8847
6j_DQ835769.1       8788 UUGCUCGAGCAGCUUGGGAGACUGCUCGUCACACUCC--AGUAAACUCGUGGCUGGGGAACA 8847
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6k_D84264.2         8788 UGUCACGAGCUGCGUGGGAGACAGCUCGGCACACUCC--AGUAAAUUCCUGGUUGGGGAACA 8847
6l_JX183554.1       8788 UAGCCCGAGCUGCGUGGGAGACAGCUAGGCACACUCC--AGUGAACUCAUGGUUGGGAAACA 8847
6l_EF424628.1       8788 UAGCUCGAGCUGCAUGGGAGACAGCUAGACACACUCC--AGUGAACUCCUGGUUGGGGAAUA 8847
6m_DQ835765.1       8788 UAGCCCGCGCAGCAUGGGAGACUGCGCGGCAUACUCC--AGUCAACUCCUGGUUGGGGAACA 8847
6m_DQ835767.1       8788 UAGCCCGCGCAGCAUGGGAGACCGCGCGGCACACUCC--AGUCAACUCCUGGUUGGGGAACA 8847
6n_DQ835768.1       8785 UGGCACGAGCAGCAUGGGAGACUGCCCGGCACACUCC--AGUGAAUUCCUGGUUGGGGAACA 8844
6n_EU246937         8760 UCUCACGGGCUGCUUGGGAAACAGCCCGCCACACUCC--AGUGAAUUCAUGGUUGGGGAACA 8819
6o_EF424627.1       8788 UUUCACGGGCUGCUUGGGAAACAGCCCGUCACACUCC--AGUGAAUUCAUGGUUGGGAAACA 8847
6o_EU408327         8788 UUUCACGGGCUGCUUGGGAAACAGCCCGCCACACUCC--AGUGAACUCAUGGUUGGGAAACA 8847
6p_EF424626.1       8789 UGACACGUGCUGCCUGGGAAACAGCACGCCACACUCC--AGUGAAUUCAUGGUUGGGCAACA 8848
6q_EF424625.1       8786 UGGUCCGAGCUGCUUGGGAAACAGCGCGGCAUACUCC--AGUCAAUUCGUGGUUGGGUAACA 8845
6r_EU408328         8788 UGACACGAGCAGCUUGGGAAACAGCCCGGCACACUCC--AGUGAACUCGUGGCUGGGCAACA 8847
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6v_FJ435090.1       8788 UUGCCCGCGCCGCUUGGGAGACGGCCAGGCACUCUCC--AGUAAACUCAUGGUUGGGUAACA 8847
6v_EU798760         8788 UUGCCCGCGCCGCUUGGGAGACGGCCAGGCACUCUCC--AGUAAACUCAUGGUUGGGUAACA 8847
7a_EF108306         8749 UAUCCAGGGCAGCUUGGGAGACCGCCCGUCACUCACC--UGUAAACUCCUGGGUGGGCAACA 8808

                         .........10........20........30........40........50........60.
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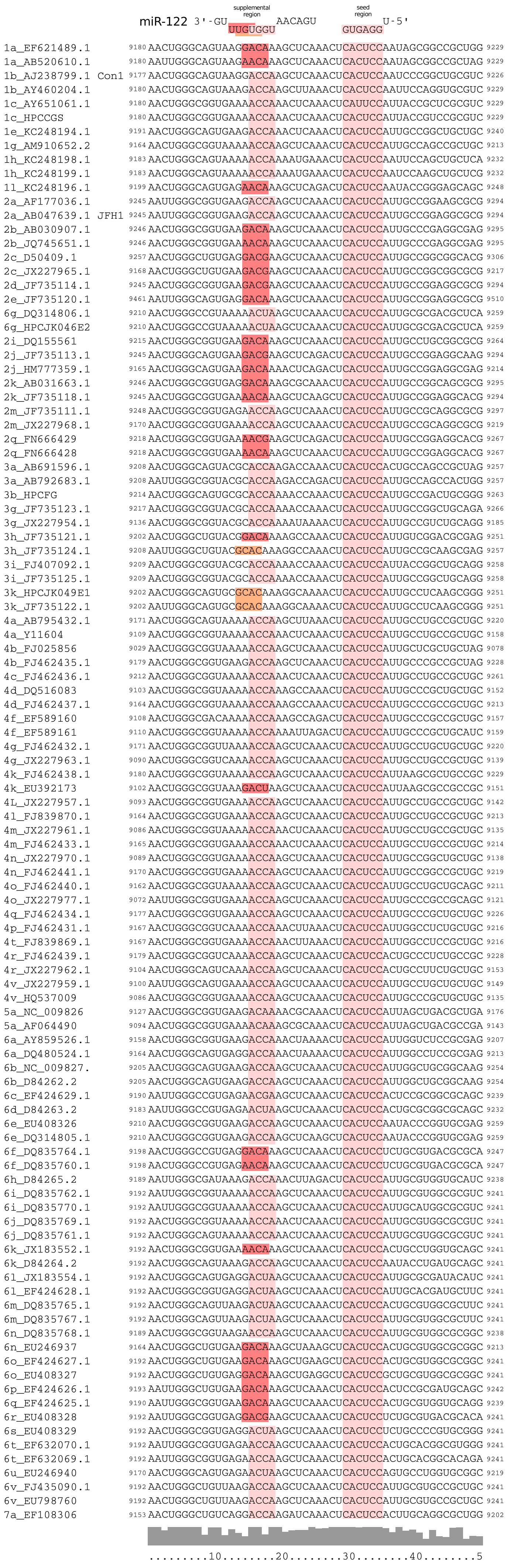
Supplementary Figure S2 (next page), related to Figure 1

miR-122 and the conserved miR-122 target site 5B.3 in 106 selected HCV NS5B sequences (Fricke et al. 2015). The miR-122 seed sequence (nts 2 – 7) is marked in light red. Nucleotides of the supplementary binding region (involving nucleotides 14 – 20) are marked in light red, dark red or orange, respectively, as the corresponding HCV target sequences. HCV genotype and NCBI nucleotide database accession numbers are given on the left, nucleotide numbers are shown for each isolate. Both the miR-122 seed target site and the supplementary binding site are conserved in all isolates. Below the sequences, the histogram shows the conservation of each residue.


supplemental region
seed region
miR-122 3'-GU UUGUGGU AACAGU GUGAGG U-5'
1a_EF621489.1 9180 AACUGGGCAGUAAGGACAAAGCUCAAACUCACUCCAAUAGCGGCCGCUGG 9229
1a_AB520610.1 9180 AAUUGGGCAGUAAGAACAAAGCUCAAACUCACUCCAAUAGCGGCCGCUAG 9229
1b_AJ238799.1 Con1 9177 AACUGGGCAGUAAGGACCAAGCUCAAACUCACUCCAAUCCCGGCUGCGUC 9226
1b_AY460204.1 9180 AACUGGGCAGUAAAGACCAAGCUUAAACUCACUCCAAUUCCAGGUGCGUC 9229
1c_AY651061.1 9180 AACUGGGCGGUGAAAACCAAACUCAAACUCAUUCCAUUACCGCUCGCGUC 9229
1c_HPCCGS 9180 AACUGGGCGGUGAAAACCAAACUCAAACUCACUCCAUUACCGUCCGCGUC 9229
1e_KC248194.1 9191 AACUGGGCAGUGAAGACCAAACUCAAACUCACUCCAUUGCCGGCUGCUGC 9240
1g_AM910652.2 9164 AACUGGGCGGUAAAAACCAAGCUCAAACUCACUCCAUUGCCAGCCGCUGC 9213
1h_KC248198.1 9183 AACUGGGCAGUAAAAACCAAAAUGAAACUCACUCCAAUUCCAGCUGCUCA 9232
1h_KC248199.1 9183 AACUGGGCAGUAAAAACCAAAAUGAAACUCACUCCAAUCCAAGCUGCUCG 9232
1l_KC248196.1 9199 AACUGGGCAGUGAGAACAAAGCUCAGACUCACUCCAAUACCGGGAGCAGC 9248
2a_AF177036.1 9245 AAUUGGGCGGUGAAGACCAAGCUCAAACUCACUCCAUUGCCGGAAGCGCG 9294
2a_AB047639.1 JFH1 9245 AAUUGGGCGGUGAAGACCAAGCUCAAACUCACUCCAUUGCCGGAGGCGCG 9294
2b_AB030907.1 9246 AACUGGGCGGUGAAGACAAAGCUCAAACUCACUCCAUUGCCCGAGGCGAG 9295
2b_JQ745651.1 9246 AACUGGGCGGUGAAAACAAAGCUCAAACUCACUCCAUUGCCCGAGGCGAG 9295
2c_D50409.1 9257 AACUGGGCUGUGAGGACGAAGCUCAAACUCACUCCAUUGCCGGCGGCACG 9306
2c_JX227965.1 9168 AACUGGGCUGUGAAGACGAAGCUCAAACUCACUCCAUUGCCGGCGGCGCG 9217
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2k_AB031663.1 9246 AACUGGGCGGUGAGGACAAAGCGCAAACUCACUCCAUUGCCGGCAGCGCG 9295
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2m_JF735111.1 9248 AACUGGGCGGUGAGAACCAAGCUCAAACUCACUCCAUUGCCGCAGGCGCG 9297
2m_JX227968.1 9170 AACUGGGCGGUGAAAACCAAGCUCAAACUCACUCCAUUGCCGCAGGCGCG 9219
2q_FN666429 9218 AACUGGGCGGUGAAAACGAAGCUCAGACUCACUCCAUUGCCGGAGGCACG 9267
2q_FN666428 9218 AACUGGGCGGUGAAAACAAAGCUCAAACUCACUCCAUUGCCGGAGGCACG 9267
3a_AB691596.1 9208 AACUGGGCAGUACGCACCAAGACCAAACUCACUCCACUGCCAGCCGCUAG 9257
3a_AB792683.1 9208 AAUUGGGCGGUACGCACCAAGACCAGACUCACUCCAUUGCCAGCCACUGG 9257
3b_HPCFG 9214 AACUGGGCAGUGCGCACCAAAACCAAACUCACUCCAUUGCCGACUGCGGG 9263
3g_JF735123.1 9217 AACUGGGCGGUACGCACCAAAACCAAACUCACUCCAUUGCCGGCUGCAGA 9266
3g_JX227954.1 9136 AACUGGGCGGUACGCACCAAAAUAAAACUCACUCCAUUGCCGUCUGCAGG 9185
3h_JF735121.1 9202 AACUGGGCUGUACGGACAAAAGCCAAACUCACUCCAUUGUCGGACGCGAG 9251
3h_JF735124.1 9208 AAUUGGGCUGUACGCACAAAGGCCAAACUCACUCCAUUGCCGCAAGCGAG 9257
3i_FJ407092.1 9209 AACUGGGCGGUACGCACCAAAACCAAACUCACUCCAUUACCGGCUGCAGG 9258
3i_JF735125.1 9209 AACUGGGCGGUACGCACCAAAACCAAACUCACUCCAUUGCCGGCUGCAGG 9258
3k_HPCJK049E1 9202 AACUGGGCAGUGCGCACAAAGGCAAAACUCACUCCAUUGCCUCAAGCGGG 9251
3k_JF735122.1 9202 AAUUGGGCAGUGCGCACAAAGGCAAAACUCACUCCAUUGCCUCAAGCGGG 9251
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4a_Y11604 9109 AACUGGGCGGUAAAAACCAAACUCAAACUCACUCCAUUGCCUGCCGCUGC 9158
4b_FJ025856 9029 AACUGGGCGGUAAAAACCAAGCUCAAACUCACUCCAUUGCUCGCUGCUAG 9078
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4d_FJ462437.1 9164 AACUGGGCGGUAAAAACCAAAGCCAAACUCACUCCAUUGCCCGCCGCUGC 9213
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4f_EF589161 9110 AACUGGGCGGUAAAAACCAAAAUUAGACUCACUCCAUUGCCCGCUGCAUC 9159
4g_FJ462432.1 9171 AACUGGGCGGUUAAAACCAAGCUCAAACUCACUCCAUUGCCUGCUGCUGC 9220
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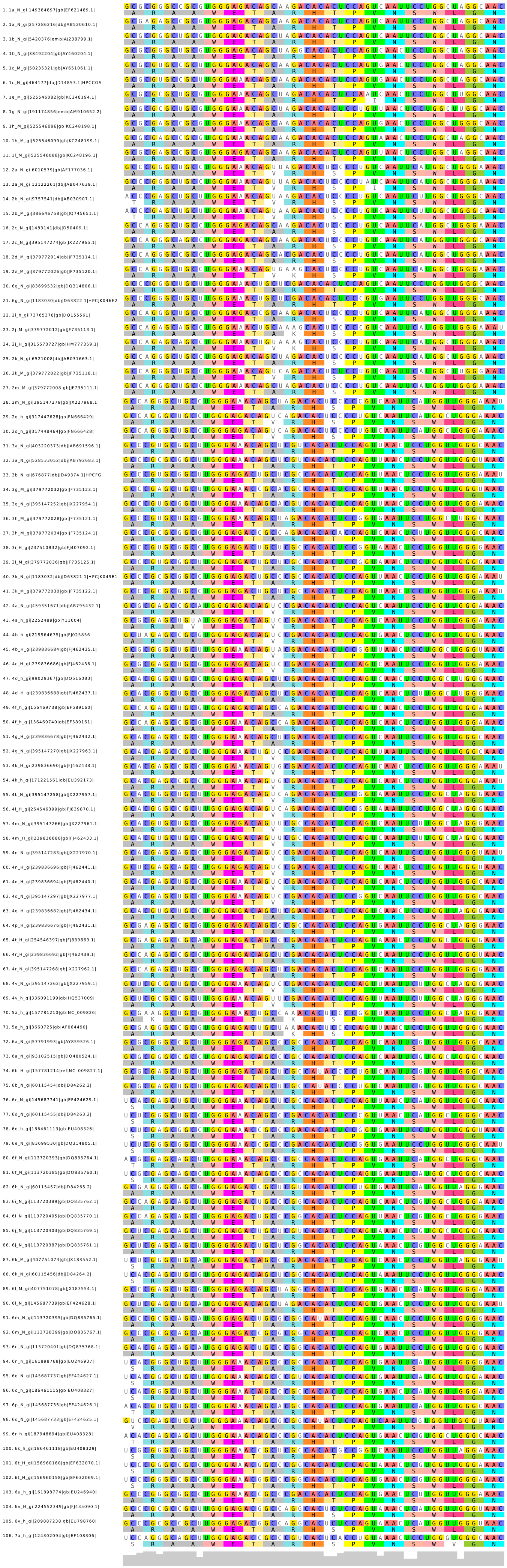
Supplementary Figure S3 (next page), related to Figure 1 and Supplementary Figure S1

Sequences with miR-122 site 5B.2. Nucleotide sequences were aligned according to amino acid sequences. Amino acids have been colored individually. Nucleotides are individually colored if more than 50 % conserved. Please note that the miR-122 seed target sequence is shifted by 2 nucleotides in some isolates (compare Supplementary Figure S1). The histogram shows the fraction of nucleotides actually used at each position relative to the number of nucleotides allowed by codon usage.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46. 4c_H_gi|239836686|gb|FJ462436.1|
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66. 4r_H_gi|239836692|gb|FJ462439.1|
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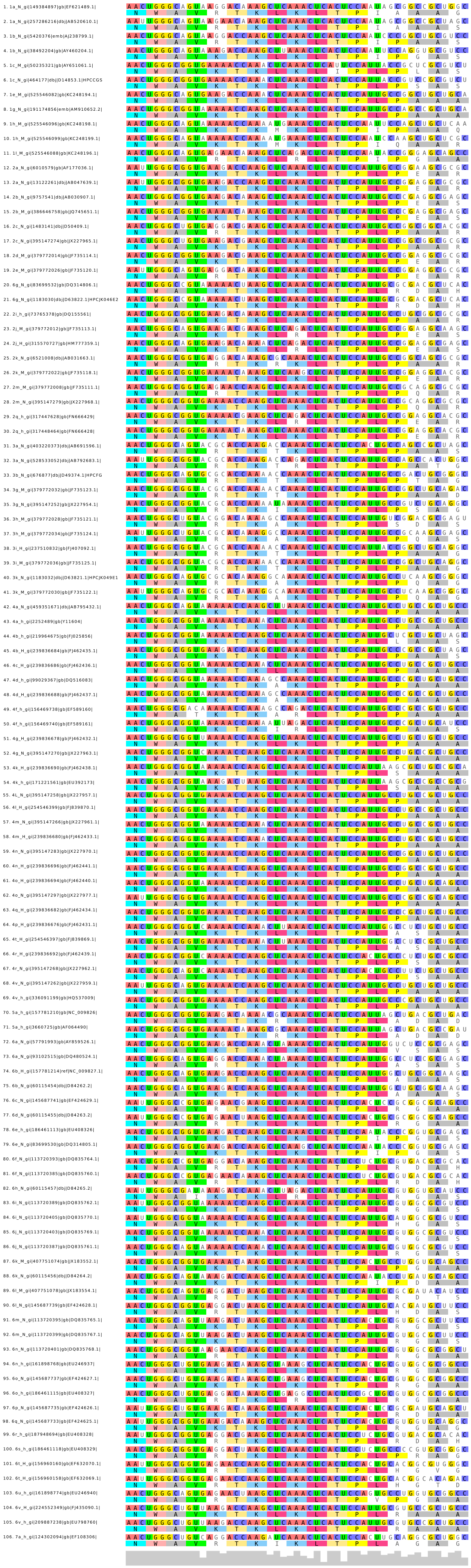
106. 7a_h_gi|124302094|gb|EF108306|

Supplementary Figure S4 (next page), related to Figure 1 and Supplementary Figure S2

Sequences with miR-122 site 5B.3. Nucleotide sequences were aligned according to amino acid sequences. Nucleotides are colored individually when more than 50 % conserved. Amino acids are colored individually. The histogram shows the fraction of nucleotides actually used at each position relative to the number of nucleotides allowed by codon usage.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Supplementary Figure S5 (next page), related to Figure 1

miR-122 and the conserved miR-122 target site S3 in 93 selected HCV NS5B sequences (Fricke et al. 2015). The miR-122 sequence with the seed sequence (nts 2 – 8) and the supplementary binding region (nts 17 – 20) are marked in red. Gaps were introduced to align seed target sequences. HCV genotype and NCBI nucleotide database accession numbers are given on the left, nucleotide numbers are shown for each isolate. The miR-122 seed target site (A)CACUCC is 100 % conserved in 85 of 94 isolates, the supplementary binding site is conserved in 92/94 isolates. Below the sequences, the histogram shows the conservation of each residue.

```
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                                               UUGU                UGUGAGG
1a_EF621489.1        9360 CUCCUCCCCAACCGAUGAAGGUUGGGGUAA-----ACACUCCGGCCUCUUAGGCC 9409
1a_AB520610.1        9360 CUCCUCCCCAACCGAUGAAGGUUGGGGUAA-----ACACUCCGGCCUCUUAGGCC 9409
1b_AJ238799.1 Con1   9357 CUACUCCCCAACCGAUGAACGGGGAGCUAA-----ACACUCCAGGCCAAUAGGCC 9406
1b_AY460204.1        9360 CUGCUCCCCAACCGGUGAACGGGGAGCUAA-----ACACUCCGGCCCACUAGGCC 9409
1c_AY651061.1        9360 CUCCUUCCCAACCGAUAGACGGUUGGGCAA-----CCACUCCAGGCCUUUAGGCC 9409
1c_HPCCGS            9360 CUCCUUCCCAACCGGUAGACGGUUGGGCAA-----CCACUCCAGGCCUUUAGGCC 9409
1e_KC248194.1        9371 CUGCUCCCCAACCGAUAGGUGGGGAGCUAA-----ACACUCCAGCCUCAUAGGCU 9420
1g_AM910652.2        9344 UUGCUCCCCAACCGAUAGGUGGGGAGUUAA-----ACACUCCAGCCUCAUAGGCU 9393
1h_KC248198.1        9363 CUGCUCCCCAAUCGAUGAUUGGGGAGCUAA-----ACACUCCGGCCCUUUAGGCC 9412
1h_KC248199.1        9363 CUGCUCCCCAAUCGAUGAUUGGGGAGCUAA-----ACACUCCGGCCCUUUAGGCC 9412
1l_KC248196.1        9379 UUGCUCCCCAACCGAUGAAGGGGAGCAUAA-----CCACUCCGGCCUCUUAGGCC 9428
2a_AF177036.1        9425 CUACUCCCCGCUCGGUAGAGCGGCACACAUUAGCUACACUCCAU-----AGCUAA 9474
2a_AB047639.1 JFH1   9425 CUACUCCCCGCUCGGUAGAGCGGCACACACUAGGUACACUCCAU-----AGCUAA 9474
2b_AB030907.1        9426 UUACUCCCCGCUCGGUAGAGCGGCAAACCCUAGCUACACUCCAU-----AGCUAG 9475
2b_JQ745651.1        9426 UUACUCCCCGCUCGGUAGAGCGGCAAACCCUAGCUACACUCCAU-----AGCUAG 9475
2c_D50409.1          9437 CUACUCCCCGCUCGGUAGAGCGGCACAU-UUAGCUACACUCCAU-----AGCUAA 9485
2c_JX227965.1        9348 CUACUCCCCGCUCGGUAGAGCGGC-----------ACACACU-------AGCUAC 9384
2d_JF735114.1        9425 UUACUCCCCGCUCGGUAGAGCGGCACACAUUAGCUACACUCCAU-----AGCUAA 9474
2e_JF735120.1        9641 UUACUCCCCGCUCGGUAGAGCGGCACACCUUAGCUACACUCCAU-----AGCUAA 9690
6g_DQ314806.1        9390 UUGCUCCCCGCCCGAUAAACGGGCGGCCUUAAGCAACACUCCAU-----UUCCGC 9439
6g_HPCJK046E2        9390 UUUCUCCCCCCCCGAUAAACGGGCGGCCUCAAGCAACACUCCAU-----UUGCCC 9439
2j_JF735113.1        9425 UUACUCCCCGCUCGAUAGAGCGGCACAC-UUAGCUACACUCCAU-----AGCUAA 9473
2k_AB031663.1        9426 UUACUCCCCGCUCGGUAGAGCGGCACACCUUAGCUACACUCCAU-----AGCUAA 9475
2k_JF735118.1        9425 UUACUCCCCGCCCGAUAGAGCGGCACACUAUAGCUACACUCCAU-----AGCUAA 9474
2m_JF735111.1        9428 UUACUCCCCGCUCGGUAGAGCGGCACAACCUAGCUACACUCCAU-----AGCUAA 9477
2m_JX227968.1        9350 UUACUCCCCGCUCGGUAGAGCGGC-----------ACACACU-------AGCCUA 9386
2q_FN666428          9398 UUACUCCCCGCUCGGUAGAGCGGCACACCUUAGCUACACUCCAU-----AGCUAA 9447
3a_AB691596.1        9388 CUCCUGCCAGCACGAUAAGCGGGUAGGAUA-----ACACUCCAUUCCUUUUCCCU 9437
3a_AB792683.1        9388 CUCCUGCCAGCACGAUGAGCUGGUAGGAUA-----ACACUCCAUUUGUUUUUUAU 9437
3b_HPCFG             9394 CUCCUACCUGCUCGGUGAGCUGGUAGGUUA-----ACACCCCAACCCUGUGUUUU 9443
3g_JF735123.1        9397 UUGCUGCCUGCUCGGUAG----GCGGC-UA-----ACACUCCGACCUUAGGGUCC 9441
3g_JX227954.1        9316 CUCCUGCCAGCUCGGUGAGCUGGUA-AGUUA----ACACCCC-A----------- 9353
3h_JF735121.1        9382 UUGCUCCCCGCUCGGUAGACGGGCGGCCU-UAGCAACACUCCAUCGUUCUUUUUU 9435
3h_JF735124.1        9388 UUGCUGCCUGCUCGGUAG----GCGGC-UA-----ACACUCCGACCUUAGGGUCC 9432
3i_FJ407092.1        9389 UUACUACCGGCCGAUUAGGCUGGUAGGAUA-----ACACUCCAUUCCUUU----- 9433
3i_JF735125.1        9389 UUGCUCCCCGCUCGGUAGACGGGCGGCCU-UAGCAACACUCCAUCGUUCUUUUUU 9442
3k_HPCJK049E1        9382 CUGUUACCAGCCCGAUAGGCUGGUAAC-UA-----ACACUCCAUUUUUAACUUAU 9430
3k_JF735122.1        9382 CUGUUACCAGCUCGAUAGGCUGGUAAU-UU-----ACACUCCAUAUUCUGUUUUU 9430
4a_AB795432.1        9351 CUGCUGCCUGCUCGGUAG----GCAGCUUA-----ACACUCCGACCUUAGGGUCC 9396
4a_Y11604            9289 CUGCUGCCUGCUCGGUAG----GCAGCUUA-----ACACUCCGACCUUAGGGUCC 9334
4b_FJ025856          9209 UUACUCCCCGCCCGGUAG-----GGGU--A-----ACACUCCGACCUUAGGGUCA 9251
4b_FJ462435.1        9359 CUGCUGCCCGCCCGGUAG----GCGGGU-A-----ACACUCCGACCUUAGGGUCA 9403
4c_FJ462436.1        9392 CUGCUGCCUGCUCGAUAG----GCAGCUUA-----ACACUCCGACCUUAGGGUCC 9437
4d_FJ462437.1        9344 CUGCUGCCCGCUCGGUAG----GCAGCUUA-----ACACUCCGACCUUAGGGUCC 9389
4g_FJ462432.1        9351 CUGCUGCCUGCCCGAUAG----GCAGGCUA-----ACACUCCGACCUUAGGGUCA 9396
4g_JX227963.1        9270 CUGCUGCCUGCUCGAUAG----GCAGCUUA-----ACACUCAGACCCUAGGGUC- 9314
4k_FJ462438.1        9360 UUGCUGCCUGCUCGAUAG----GCAGCUUA-----ACACUCCGACCUUAGGGUCA 9405
4k_EU392173          9282 UUGCUGCCUGCUCGAUAG----GCAGCUUA-----ACACU--------------- 9312
4L_JX227957.1        9273 CUGCUGCCUGCUCGAUAG----GCAGCUCA-----ACACUCAGACCCUAGGGUC- 9317
4l_FJ839870.1        9344 CUGCUGCCUGCUCGAUAG----GCAGCUUA-----ACACUCCGACCUUAGGCUC- 9388
4m_JX227961.1        9266 UUGCUGCCUGCUCGCUAG----GCAGCUUA-----ACACAAAGACCCUAGGGUC- 9310
4m_FJ462433.1        9345 UUGCUGCCUGCUCGGUAG----GCAGCUUA-----ACACUCCGACCUUAGGGUCC 9390
4n_JX227970.1        9269 CUGCUGCCUGCUCGAUAG----GCA-GCUUA----ACACUCC------------- 9301
4n_FJ462441.1        9350 UUGCUGCCUGCCCGAUAG----GCAGCUUA-----ACACUCCGACCUUAGGGUCC 9395
4o_FJ462440.1        9342 CUGCUGCCUGCACGGUAG----GCAGCUUA-----ACACUCCGACCUUAGGGUCC 9387
4o_JX227977.1        9252 UUGCUGCCUGCUCGGUAG----GCA-GCUUA----ACACUCCGA----------- 9286
4q_FJ462434.1        9357 UUGCUGCCUGCUCGGUAG----GCGGC-UA-----ACACUCCGACCUUAGGGUCC 9401
4p_FJ462431.1        9347 CUGCUGCCUGCUCGGUAG----GCAGC-CA-----ACACUCCGACCUUAGGGUCU 9391
4t_FJ839869.1        9347 CUGCUUCCCGCUCGGUAG----GCAGGUUA-----ACACUCCGACCUUAGGGUCC 9392
4r_FJ462439.1        9359 CUGCUGCCUGCUCGGUAG----GCAGUAUA-----ACACUCCGGCCUUAGGCCCU 9404
4r_JX227962.1        9284 CUGCUGCCUGCUCGGUAG----GCAGCUUA-----ACACUCCGACCCUAGGGUC- 9328
4v_JX227959.1        9280 UUGCUGCCUGCUCGGUAG----GCAGCU-A-----ACACUCAGACCCUAGGGUC- 9323
5a_NC_009826         9307 UUACUCCCCGCUCGGUGAGCUGGGA-GCUAA----ACACUCC------------- 9343
5a_AF064490          9274 UUACUCCCUGCUCGGUAAGCUGGGAGCUAA-----ACACUCCAUAACUCCUGUUU 9323
6b_NC_009827.        9385 UUACUCCCCGCUCGGUAGACGAGCGGCCCUAAACAACACUCCAU-----AACAUU 9434
6b_D84262.2          9385 UUACUCCCCGCUCGGUAGACGAGCGGCCCUAAACAACACUCCAU-----AACAUU 9434
6c_EF424629.1        9370 UUACUUCCCGCUCGAUAGACGGGGAGCAUAAAGCUACACUCCAU-----UAGGCU 9419
6d_D84263.2          9363 UUGCUCCCUGCUCGGUAAGCGGGGAGCUUAAAGCAACACUCCAU-----UAGUUU 9412
6e_EU408326          9390 CUUCUUCCCGCUCGGUAAGCGGGAAGCCCAAAGCUACACUCCAU-----UAAUGU 9439
6e_DQ314805.1        9390 CUUCUUCCCGCUCGGUAAGGGGGAAGCCUAAAGCUACACUCCAU-----UAAUGC 9439
6f_DQ835764.1        9378 CUGCUUCCUGCUCGAUAGACAGGGGGCAUAAA-UAACACUCCAU-----UUUCAU 9426
6f_DQ835760.1        9378 CUGCUUCCUGCUCGAUAGACAGGGAGCAUAAA-UAACACUCCAU-----UUUUGG 9426
6h_D84265.2          9369 CUGCUCCCAGCCCGGUAGGCUGAGAGCUAAACACUACACUCCGU-----UUUUUU 9418
6i_DQ835762.1        9372 UUGCUCCCAGCUCGGUAGGCUGAGAGCUUA-----ACACUCCAAACAUCUGUUUU 9421
6i_DQ835770.1        9372 UUGCUCCCAGCUCGAUAGGCUGAGAGCUUA-----ACACUCCAAACAUCUACUGU 9421
6j_DQ835769.1        9372 CUGCUCCCAGCCCGGUAGGCUGAGAGCCCC-----ACACUCCACACAAUUUUGCC 9421
6j_DQ835761.1        9372 CUGCUCCCAGCCCGGUAGGCUGAGAGCCCC-----ACACUCCACACAGUUUUUUU 9421
6k_JX183552.1        9372 UUGCUGCCUACCCGAUAG----GCAGCUUA-----ACACUCCGACCUUAGGGUCC 9417
6k_D84264.2          9372 UUACUACCCGCUCGGUAGACGGGUAGCUUA-----ACACUCCAUUUCUGUUUUUU 9421
6l_JX183554.1        9372 UUGCUCCCUGCUCGAUAGGCAGGCAGCAUA-----ACACUCCAUUUCCAUAACUU 9421
6l_EF424628.1        9372 CUGCUCCCUGCCCGGUAGGCAGGGAGCAUA-----ACACUCCAUUUCCAUAACUU 9421
6m_DQ835765.1        9372 CUACUCCCUGCUCGGUAGACAGGGGGCUUA-----ACACUCCACUUCCAUAGGUU 9421
6m_DQ835767.1        9372 CUGCUCCCUGCUCGGUAGACAGGGGGCUUA-----ACACUCCACUUCCAUAGUUU 9421
6n_DQ835768.1        9369 CUGCUCCCCGCCCGAUAGGCGGGGAGCCUA-----ACACUCCAUAUCUAUAGUUU 9418
6o_EF424627.1        9372 UUGCUUCCCGCUCGGUAGGCGGGAGGCCCAUAGCAACACUCCAU-----UUCCGC 9421
6o_EU408327          9372 UUGCUUCCCGCCCGGUAGGCGGGGGGCCCAUAGCAACACUCCAU-----UAGCUU 9421
6p_EF424626.1        9373 UUGCUUCCCGCUCGGUAGACGGGAAGCCCACUGCUACACUCCAU-----UAACUG 9422
6q_EF424625.1        9370 UUGCUCCCUGCUCGGUAGGCAGGGGGCCUAAAGCUACGCUCCAU-----UAGCUU 9419
6r_EU408328          9372 CUGCUCCCUGCCCGAUAGGCGGGGAGGAGAAA-UAACACUCCAC-----UUUUUU 9420
6s_EU408329          9372 UUGCUCCCUAACCGGUGA-GCAAGAAACUAAGGCAACACUCCAC-----UGUUUU 9420
6t_EF632070.1        9372 CUGCUCCCCGCUCGAUAAGCGAGGAGCCUAACGCAACACUCCAA-----UGAUUU 9421
6t_EF632069.1        9372 CUGCUUCCCGCUCGGUAAGCGGGAAGCCUAAAGCAACACUCCAA-----UAAUUC 9421
6v_FJ435090.1        9372 CUGCUUCCAGCCCGAUAGGCUGGAAGCUAA-----ACACUCCAUACCAUUAGGUC 9421
6v_EU798760          9372 CUGCUUCCAGCCCGAUAGGCUGGAAGCUAA-----ACACUCCAUACCAUUAGGUC 9421
                          ..((((((...............)))))).........................

                          .........10........20........30........40........50....
```

Supplementary Figure S6

RNAs used in binding experiments

Structures of RNAs used in binding experiments were predicted using the Vienna ***RNAfold*** WebServer (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi) (Gruber et al., 2008; Lorenz et al., 2011).

Options were set as follows: Basic options: "avoid isolated base pairs". Advanced folding options were: "no dangling end energies" with the Turner model, 2004.

The Vienna RNA structure predicion output is shown in dot-bracket form. Seed binding sites and supplemental binding sites for microRNA-122 are labelled in yellow.

The origin of the templates for PCR and those nucleotides of the PCR primers that are contained in the in vitro-transcribed RNA are listed below each sequence. Here we show only those sequences of the primers which became part of the RNA; upstream of the forward primer there was always a T7 promoter included in the primer. With most forward primers, three G residues were added to the sequence to improve T7 RNA polymerase-mediated in vitro transcription of the RNAs.

RNAs for Site 5B.2 - Secondary structure predictions

```
RNA_5B.2a_Con1_227_nts
GGGGUGGGAGACAGCUAGACACACUCCAGUCAAUUCCUGGCUAGGCAACAUCAUCAUGUAUGCGCCCACCUUGUGGGCAAGG
AUGAUCCUGAUGACUCAUUUCUUCUCCAUCCUUCUAGCUCAGGAACAACUUGAAAAAGCCCUAGAUUGUCAGAUCUACGGGG
CCUGUUACUCCAUUGAGCCACUUGACCUACCUCAGAUCAUUCAACGACUCCAUGGCCUUAGCG
((((((.............))))))..((((((((((((((((((((((....)))).)))((((((...))))))((((
(((.....((.((......)).))..)))))))))))))).))))))....))))....((((((((((....)))))..))))
).(((((..((((.(((....((((...............))))...))).))))...)))))

Template: HCV genotype 1b Con1 (AJ238799.1)
Primer fwd: 5´-GGGGTGGGAGACAGCTAGACACACTCCAG-3´
Primer rev: 5´-CGCTAAGGCCATGGAGTCGTTGAATGATCTGAGG-3´
```

```
RNA_5B.2a*_JFH1_267 nts
GGGAAACAGUUAGACACUCCCCUAUCAAUUCAUGGCUGGGAAACAUCAUCCAGUAUGCUCCAACCAUAUGGGUUCGCAUGGU
CCUAAUGACACACUUCUUCUCCAUUCUCAUGGUCCAAGACACCCUGGACCAGAACCUCAACUUUGAGAUGUAUGGAUCAGUA
UACUCCGUGAAUCCUUUGGACCUUCCAGCCAUAAUUGAGAGGUUACACGGGCUUGACGCCUUUUCUAUGCACACAUACUCUC
ACCACGAACUGACGCGGGUGG
((((((..(((((...(((.(((.((((((.((((((((((..((((((..((((((((....((((((((((((...((((
((...((.......((((..((((....))))...))))))....))))))))))))............))))))...))))
))))..))))......))....))))))))))))))))).))).......))).)))))...))))))..............((
(((.((........)))))))

Template: HCV genotype 2a JFH-1 (AB047639.1)
Primer fwd: 5´-GGGAAACAGTTAGACACTCCCC-3´
Primer rev: 5´-CCACCCGCGTCAGTTCGTGGTGAGAGTATGTGTGC-3´
```

```
RNA_5B.2b_Con1_148_nts
GGGUGCGUGGGAGACAGCUAGACACACUCCAGUCAAUUCCUGGCUAGGCAACAUCAUCAUGUAUGCGCCCACCUUGUGGGCA
AGGAUGAUCCUGAUGACUCAUUUCUUCUCCAUCCUUCUAGCUCAGGAACAACUUGAAAAAGCCCUA
(((((.((.((.....))..)).)))))...((((((((((((((((((((((....)))).)))((((((...))))))(
((((((.....((.((......)).))..)))))))))))).))))))....))))..........

Template: HCV genotype 1b Con1 (AJ238799.1)
Primer fwd: 5´-GGGTGCGTGGGAGACAGCTAGAC-3´
Primer rev: 5´-TAGGGCTTTTTCAAGTTGTTCCTGAG-3´
```

```
RNA_5B.2b*_JFH1_291_nts
GGGAAACAGUUAGACACUCCCCUAUCAAUUCAUGGCUGGGAAACAUCAUCCAGUAUGCUCCAACCAUAUGGGUUCGCAUGGU
CCUAAUGACACACUUCUUCUCCAUUCUCAUGGUCCAAGACACCCUGGACCAGAACCUCAACUUUGAGAUGUAUGGAUCAGUA
UACUCCGUGAAUCCUUUGGACCUUCCAGCCAUAAUUGAGAGGUUACACGGGCUUGACGCCUUUUCUAUGCACACAUACUCUC
ACCACGAACUGACGCGGGUGGCUUCAGCCCUCAGAAAACUUGGGG
.............((((((.((((((.((((((((((..((((((..((((((((....((((((((((((...((((
((...((.......((((..((((....))))...))))))....))))))))))))............))))))...))))
))))..))))......))....))))))))))))))))).))).......(((((.((.(((......................
(((.((........)))))))).))))))).......... .))))

Template: HCV genotype 2a JFH-1 (AB047639.1)
Primer fwd: 5´-GGGAAACAGTTAGACACTCCCC-3´
Primer rev: 5´-CCCCAAGTTTTCTGAGGGCTGAAGCCACCC-3´
```

```
RNA_5B.2c_Con1_152_nts
GGGCGUGGGAGACAGCUAGACACACUCCAGUCAAUUCCUGGCUAGGCAACAUCAUCAUGUAUGCGCCCACCUUGUGGGCAAG
GAUGAUCCUGAUGACUCAUUUCUUCUCCAUCCUUCUAGCUCAGGAACAACUUGAAAAAGCCCUAGAUUGU
...........((((((((.......((.(((...((((((((((((((((((....)))).)))((((((...))))))(((
((((.....((.((......)).))..)))))))))))).))))))..))).)).......)))).))))
```

Template: HCV genotype 1b Con1 (AJ238799.1)
Primer fwd: 5´-GGGCGTGGGAGACAGCTAGACACAC-3´
Primer rev: 5´-ACAATCTAGGGCTTTTTCAAGTTGTTCC-3´

RNA_5B.2d_JFH1_160_nts
GGGCCACUCGCCCGGGCUGCCUGGGAAACAGUUAGACACUCCCCUAUCAAUUCAUGGCUGGGAAACAUCAUCCAGUAUGCUC
CAACCAUAUGGGUUCGCAUGGUCCUAAUGACACACUUCUUCUCCAUUCUCAUGGUCCAAGACACCCUGGACCAGAACC
((((((..(((((((((...(((((.....(((......((((((((......))))..)))))))....)))))...))))
.........)))..))..)))))).......................((.(((((((........)))))))))...

Template: HCV genotype 2a JFH-1 (AB047639.1)
Primer fwd: 5´-GGGCCACTCGCCCGGGCTGCCTGG-3´
Primer rev: 5´-GGTTCTGGTCCAGGGTGTC-3´

RNAs for Site 5B.2 - Alignment

```
CLUSTAL format alignment by MAFFT (v7.304b)

RNA_5B.2a_Con1_ -----------------GGGGUGGGAGACAGCUAGACACACUCC--AGUCAAUUCCUGGC
RNA_5B.2a_JFH1_ ----------------------GGGAAACAGUUAG--ACACUCCCCUAUCAAUUCAUGGC
RNA_5B.2b_Con1_ --------------GGGUGCGUGGGAGACAGCUAGACACACUCC--AGUCAAUUCCUGGC
RNA_5B.2b_JFH1_ ----------------------GGGAAACAGUUAG--ACACUCCCCUAUCAAUUCAUGGC
RNA_5B.2c_Con1_ ----------------GGGCGUGGGAGACAGCUAGACACACUCC--AGUCAAUUCCUGGC
RNA_5B.2d_JFH1_ GGGCCACUCGCCCGGGCUGCCUGGGAAACAGUUAG--ACACUCCCCUAUCAAUUCAUGGC
                                      ****.**** ***  *******    ******* ****

RNA_5B.2a_Con1_ UAGGCAACAUCAUCAUGUAUGCGCCCACCUUGUGGGCAAGGAUGAUCCUGAUGACUCAUU
RNA_5B.2a_JFH1_ UGGGAAACAUCAUCCAGUAUGCUCCAACCAUAUGGGUUCGCAUGGUCCUAAUGACACACU
RNA_5B.2b_Con1_ UAGGCAACAUCAUCAUGUAUGCGCCCACCUUGUGGGCAAGGAUGAUCCUGAUGACUCAUU
RNA_5B.2b_JFH1_ UGGGAAACAUCAUCCAGUAUGCUCCAACCAUAUGGGUUCGCAUGGUCCUAAUGACACACU
RNA_5B.2c_Con1_ UAGGCAACAUCAUCAUGUAUGCGCCCACCUUGUGGGCAAGGAUGAUCCUGAUGACUCAUU
RNA_5B.2d_JFH1_ UGGGAAACAUCAUCCAGUAUGCUCCAACCAUAUGGGUUCGCAUGGUCCUAAUGACACACU
                *.** *********  ****** ** *** *.****   * ***.****.***** ** *

RNA_5B.2a_Con1_ UCUUCUCCAUCCUUCUAGCUCAGGAACAACUUGAAAAAGCCCUAGAUUGUCAGAUCUACG
RNA_5B.2a_JFH1_ UCUUCUCCAUUCUCAUGGUCCAAGACACCCUGGACCAGAACCUCAACUUUGAGAUGUAUG
RNA_5B.2b_Con1_ UCUUCUCCAUCCUUCUAGCUCAGGAACAACUUGAAAAAGCCCUA----------------
RNA_5B.2b_JFH1_ UCUUCUCCAUUCUCAUGGUCCAAGACACCCUGGACCAGAACCUCAACUUUGAGAUGUAUG
RNA_5B.2c_Con1_ UCUUCUCCAUCCUUCUAGCUCAGGAACAACUUGAAAAAGCCCUAGAUUGU----------
RNA_5B.2d_JFH1_ UCUUCUCCAUUCUCAUGGUCCAAGACACCCUGGACCAGAACC------------------
                ********** **  *.*  **.**    ** **  *.. **

RNA_5B.2a_Con1_ GGGCCUGUUACUCCAUUGAGCCACUUGACCUACCUCAGAUCAUUCAACGACUCCAUGGCC
RNA_5B.2a_JFH1_ GAUCAGUAUACUCCGUGAAUCCUUUGGACCUUCCAGCCAUAAUUGAGAGGUUACACGGGC
RNA_5B.2b_Con1_ ------------------------------------------------------------
RNA_5B.2b_JFH1_ GAUCAGUAUACUCCGUGAAUCCUUUGGACCUUCCAGCCAUAAUUGAGAGGUUACACGGGC
RNA_5B.2c_Con1_ ------------------------------------------------------------
RNA_5B.2d_JFH1_ ------------------------------------------------------------


RNA_5B.2a_Con1_ UUAGCG------------------------------------------------------
RNA_5B.2a_JFH1_ UUGACGCCUUUUCUAUGCACACAUACUCUCACCACGAACUGACGCGGGUGG---------
RNA_5B.2b_Con1_ ------------------------------------------------------------
RNA_5B.2b_JFH1_ UUGACGCCUUUUCUAUGCACACAUACUCUCACCACGAACUGACGCGGGUGGCUUCAGCCC
RNA_5B.2c_Con1_ ------------------------------------------------------------
RNA_5B.2d_JFH1_ ------------------------------------------------------------


RNA_5B.2a_Con1_ ---------------
RNA_5B.2a_JFH1_ ---------------
RNA_5B.2b_Con1_ ---------------
RNA_5B.2b_JFH1_ UCAGAAAACUUGGGG
RNA_5B.2c_Con1_ ---------------
RNA_5B.2d_JFH1_ ---------------
```

RNAs for Site 5B.3 - Secondary structure predictions

```
RNA_5B.3a_Con1_226_nts
GGGGGCUACUGUCCCAGGGGGGGAGGGCUGCCACUUGUGGCAAGUACCUCUUCAACUGGGCAGUAAGGACCAAGCUCAAACU
CACUCCAAUCCCGGCUGCGUCCCAGUUGGAUUUAUCCAGCUGGUUCGUUGCUGGUUACAGCGGGGGAGACAUAUAUCACAGC
CUGUCUCGUGCCCGACCCCGCUGGUUCAUGUGGUGCCUACUCCUACUUUCUGUAGGGGUAGG
.((..((((((.(((((..((((((((((((((....)))).))).)))))))..))))))))).))..))...........
((((.((...(((((.(((..(((((((((....)))))))))..))).)))))...((((((((((((((...........
.))))))........)))))))).....)).))))(((((((((((.....)))))))))))

Template: HCV genotype 1b Con1 (AJ238799.1)
Primer fwd: 5´-GGGGGCTACTGTCCCAGGG-3´
Primer rev: 5´-CCTACCCCTACAGAAAGTAGGAGTAGGCAC-3´
```

```
RNA_5B.3a*_JFH1_200_nts
GGGCGGUGAAGACCAAGCUCAAACUCACUCCAUUGCCGGAGGCGCGCCUACUGGACUUAUCCAGUUGGUUCACCGUCGGCGC
CGGCGGGGGCGACAUUUUUCACAGCGUGUCGCGCGCCCGACCCCGCUCAUUACUCUUCGGCCUACUCC
UACUUUUCGUAGGGGUAGGCCUCUUCCUACUCCCCGCUCGGUAGAGCGGC
(((((((....)))..)))).............((((((.((...(((.((((((....)))))).)))...)).))))))).
.((((((((((((((..........)))))))........)))))))...........(((((((((((((.....))))))
)))))))............((((((....))))))).

Template: HCV genotype 2a JFH-1 (AB047639.1)
Primer fwd: 5´-GGGCGGTGAAGACCAAGCTCAAACTCACTCCATTG-3´
Primer rev: 5´-GCCGCTCTACCGAGCGGGGAGTAGG-3´
```

```
RNA_5B.3b_JFH1_196_nts
GGGCGGUGAAGACCAAGCUCAAACUCACUCCAUUGCCGGAGGCGCGCCUACUGGACUUAUCCAGUUGGUUCACCGUCGGCGC
CGGCGGGGGCGACAUUUUUCACAGCGUGUCGCGCGCCCGACCCCGCUCAUUACUCUUCGGCCUACUCCUACUUUUCGUAGGG
GUAGGCCUCUUCCUACUCCCCGCUCGGUAGAG
(((((((....)))..))))..............(((((.((...(((.((((((....)))))).)))...)).)))))((
(((((((((((((((..........)))))))(((........)))............(((((((((((((.....))))))
)))))))..........)))))).))))....

Template: HCV genotype 2a JFH-1 (AB047639.1)
Primer fwd: 5´-GGGCGGTGAAGACCAAGCTCAAACTCACTCCATTG-3´
Primer rev: 5´-CTCTACCGAGCGGGGAGTAGGAAGAGGCC-3´
```

```
RNA_5B.3c_JFH1_224_nts
GGGUCAUCUCCCGUGGAGGGAAAGCGGCCGUUUGCGGCCGAUAUCUCUUCAAUUGGGCGGUGAAGACCAAGCUCAAACUCAC
UCCAUUGCCGGAGGCGCGCCUACUGGACUUAUCCAGUUGGUUCACCGUCGGCGCCGGCGGGGGCGACAUUUUUCACAGCGUG
UCGCGCGCCCGACCCCGCUCAUUACUCUUCGGCCUACUCCUACUUUUCGUAGGGGUAGGC
((.(((((.((((((((((((...((((((....))))))...))))))))..)))).)))))...)).............
.....((((((.((...(((.((((((....)))))).)))...)).))))))..((((((((((((((..........)))
))))........)))))))............((((((((((((.....))))))))))))

Template: HCV genotype 2a JFH-1 (AB047639.1)
Primer fwd: 5´-GGGTCATCTCCCGTGGAGG-3´
Primer rev: 5´-GCCTACCCCTACGAAAAGTAGGAGTAGGCCG-3´
```

RNAs for Site 5B.3 - Alignment

```
CLUSTAL format alignment by MAFFT (v7.304b)


RNA_5B.3a_Con1_ GGGGGCUACUGUCCCAGGGGGGGAGGGCUGCCACUUGUGGCAAGUACCUCUUCAACUGGG
RNA_5B.3a_JFH1_ ---------------------------------------------------------GGG
RNA_5B.3b_JFH1_ ---------------------------------------------------------GGG
RNA_5B.3c_JFH1_ ---GGGUCAUCUCCCGUGGAGGGAAAGCGGCCGUUUGCGGCCGAUAUCUCUUCAAUUGGG
                                                                         ***

RNA_5B.3a_Con1_ CAGUAAGGACCAAGCUCAAACUCACUCCAAUCCCGGCUGCGUCCCAGUUGGAUUUAUCCA
RNA_5B.3a_JFH1_ CGGUGAAGACCAAGCUCAAACUCACUCCAUUGCCGGAGGCGCGCCUACUGGACUUAUCCA
RNA_5B.3b_JFH1_ CGGUGAAGACCAAGCUCAAACUCACUCCAUUGCCGGAGGCGCGCCUACUGGACUUAUCCA
RNA_5B.3c_JFH1_ CGGUGAAGACCAAGCUCAAACUCACUCCAUUGCCGGAGGCGCGCCUACUGGACUUAUCCA
                *.**.*.********************** * ****  ***  ** . **** *******

RNA_5B.3a_Con1_ GCUGGUUCGUUGCUGGUUACAGCGGGGGAGACAUAUAUCACAGCCUGUCUCGUGCCCGAC
RNA_5B.3a_JFH1_ GUUGGUUCACCGUCGGCGCCGGCGGGGGCGACAUUUUUCACAGCGUGUCGCGCGCCCGAC
RNA_5B.3b_JFH1_ GUUGGUUCACCGUCGGCGCCGGCGGGGGCGACAUUUUUCACAGCGUGUCGCGCGCCCGAC
RNA_5B.3c_JFH1_ GUUGGUUCACCGUCGGCGCCGGCGGGGGCGACAUUUUUCACAGCGUGUCGCGCGCCCGAC
                * ******.  *  **   *.******* ***** * ******* **** ** *******

RNA_5B.3a_Con1_ CCCGCUGGUUCAUGUGGUGCCUACUCCUACUUUCUGUAGGGGUAGG--------------
RNA_5B.3a_JFH1_ CCCGCUCAUUACUCUUCGGCCUACUCCUACUUUUCGUAGGGGUAGGCCUCUUCCUACUCC
RNA_5B.3b_JFH1_ CCCGCUCAUUACUCUUCGGCCUACUCCUACUUUUCGUAGGGGUAGGCCUCUUCCUACUCC
RNA_5B.3c_JFH1_ CCCGCUCAUUACUCUUCGGCCUACUCCUACUUUUCGUAGGGGUAGGC-------------
                ****** .**  * *   ***************  ***********

RNA_5B.3a_Con1_ -----------------
RNA_5B.3a_JFH1_ CCGCUCGGUAGAGCGGC
RNA_5B.3b_JFH1_ CCGCUCGGUAGAG----
RNA_5B.3c_JFH1_ -----------------
```

RNAs for Site S3 - Secondary structure predictions

```
RNA_S3a_Con1_250_nts
GGGCCGAUGAACGGGGAGCUAAACACUCCAGGCCAAUAGGCCAUCCUGUUUUUUUCCCUUUUUUUUUUUCUUUUUUUUUUUU
UUUUUUUUUUUUUUUUUUUUUUCUCCUUUUUUUUUCCUCUUUUUUUCCUUUUCUUUCCUUUGGUGGCUCCAUCUUAGCCCUA
GUCACGGCUAGCUGUGAAAGGUCCGUGAGCCGCUUGACUGCAGAGAGUGCUGAUACUGGCCUCUCUGCAGAUCAAGU
(((((...(((.((((((..(((((.....((((....))))....)))))..)))))).)))...................
...........................................................((((.((((.......)))))))
)((((((....))))))..))))).......((((((((((((((((.(((......))))))))))))).)))))).....
....
```

Template: HCV genotype 1b Con1 (AJ238799.1)
Primer fwd: 5´-GGGCCGATGAACGGGGAGCTAAACACTCCAGGC-3´
Primer rev: 5´-ACTAGTAGTACTTGATCTGCAGAGAGGCC-3´

```
RNA_S3b_JFH1_252_nts
GGGUCCCCGCUCGGUAGAGCGGCACACACUAGGUACACUCCAUAGCUAACUGUUCCUUUUUUUUUUUUUUUUUUUUUUUUUU
UUUUUUUUUUUUUUUUUCUUUUUUUUUUUUUUCCCUCUUUCUUCCCUUCUCAUCUUAUUCUACUUUCUUUCUUGGUGGCUCC
AUCUUAGCCCUAGUCACGGCUAGCUGUGAAAGGUCCGUGAGCCGCAUGACUGCAGAGAGUGCCGUAACUGGUCUCUCUGCAG
AUCAUG
......((((((....))))))........(((.(((.............))).)))..........................
..........................................................................(((((((.
((....(((....((((((....))))))..)))..)))))))))((((((((((((((.((((....))))))))))))))))))
.)))).
```

Template: HCV genotype 2a JFH-1 (AB047639.1)
Primer fwd: 5´GGGTCCCCGCTCGGTAGAGCGGCACACACTAGGTACACTCCATAGCTAAC--3´
Primer rev: 5´-CATGATCTGCAGAGAGACCAGTTACGGCAC-3´

```
RNA_S3b*_Con1_153_nts
GGGCAGCGGGGGAGACAUAUAUCACAGCCUGUCUCGUGCCCGACCCCGCUGGUUCAUGUGGUGCCUACUCCUACUUUCUGUA
GGGGUAGGCAUCUAUCUACUCCCCAACCGAUGAACGGGGAGCUAAACACUCCAGGCCAAUAGGCCAUCCUG
((.((((((((((((((............))))))........)))))))).)).....(((((((((((((((.....)))
))))))))))))......((((((...........))))))............((((....))))......
```

Template: HCV genotype 1b Con1 (AJ238799.1)
Primer fwd: 5´-GGGCAGCGGGGGAGACATATATCACAGC-3´
Primer rev: 5´-CAGGATGGCCTATTGGCCTGGAG-3´

```
RNA_S3c_JFH1_149_nts
GGGUCGGCGCCGGCGGGGGCGACAUUUUUCACAGCGUGUCGCGCGCCCGACCCCGCUCAUUACUCUUCGGCCUACUCCUACU
UUUCGUAGGGGUAGGCCUCUUCCUACUCCCCGCUCGGUAGAGCGGCACACACUAGGUACACUCCAUA
(((((((((((((((...((.............)).))))).))).)))))))...............(((((((((((((.
....)))))))))))))....((((....((((((....)))))).......))))...........
```

Template: HCV genotype 2a JFH-1 (AB047639.1)
Primer fwd: 5´-ggGTCGGCGCCGGCGGGGGCGA-3´
Primer rev: 5´-TATGGAGTGTACCTAGTGTGTGCCGCTCTACCGAG-3´

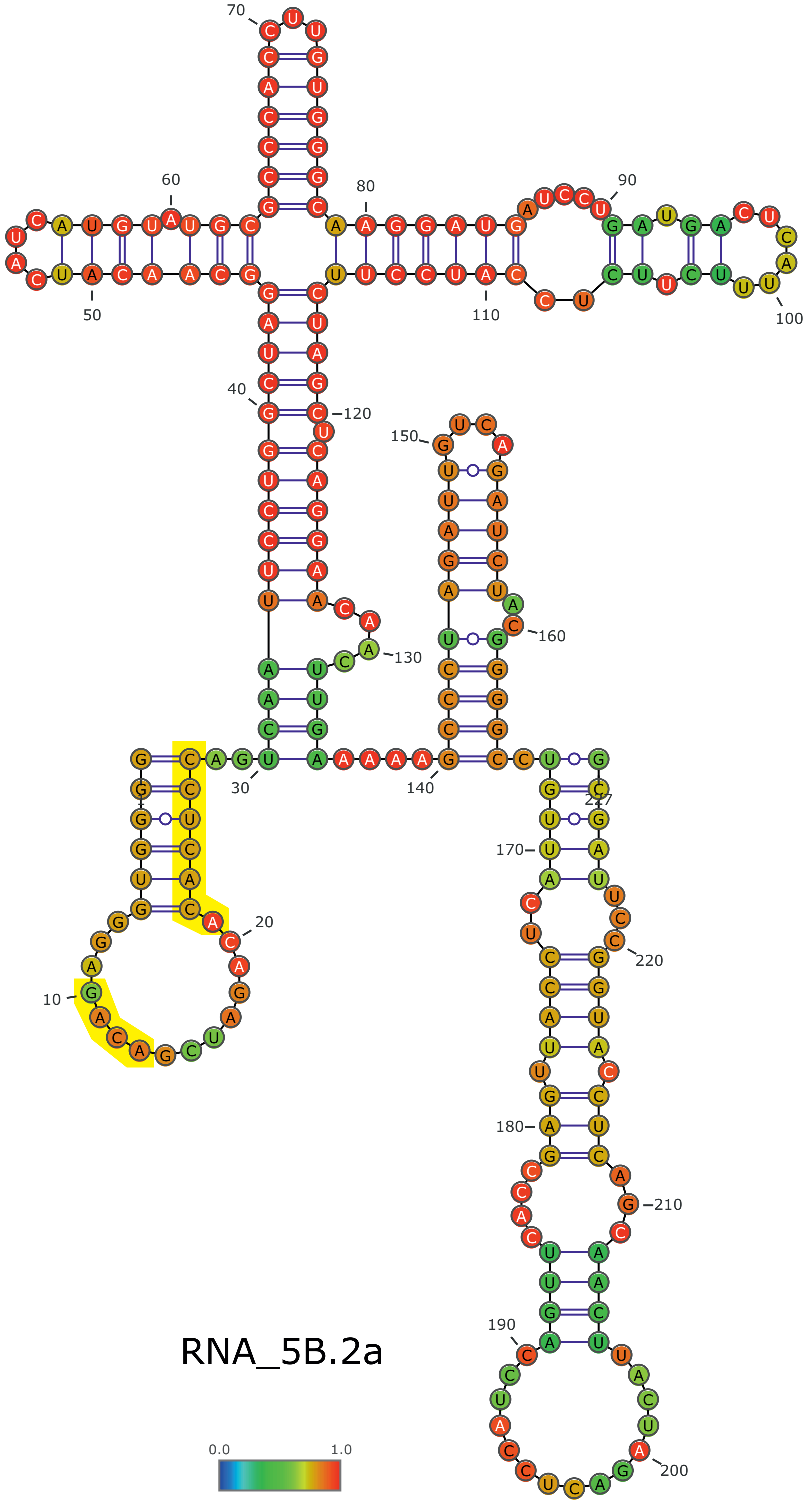
RNAs for Site S3 - Alignment

```
CLUSTAL format alignment by MAFFT (v7.304b)


RNA_S3a_Con1_25 GGG---------------------------------------------------------
RNA_S3b_JFH1_25 GGG---------------------------------------------------------
RNA_S3b_        GGG-------CAGCGGGGGAGACAUAUAUCACAGCCUGUCUCGUGCCCGACCCCGCUGGU
RNA_S3c_JFH1_14 GGGUCGGCGCCGGCGGGGGCGACAUUUUUCACAGCGUGUCGCGCGCCCGACCCCGCUCAU
                ***

RNA_S3a_Con1_25 -------------------------------------------------------CCGAU
RNA_S3b_JFH1_25 ------------------------------------------------UCCCCGCUCGGU
RNA_S3b_        UCAUGUGGUGCCUACUCCUACUUUCUGUAGGGGUAGGCAUCUAUCUACUCCCCAACCGAU
RNA_S3c_JFH1_14 UACUCUUCGGCCUACUCCUACUUUUCGUAGGGGUAGGCCUCUUCCUACUCCCCGCUCGGU
                                                                        **.*

RNA_S3a_Con1_25 GAACGGGGAGCUAA-----ACACUCCAGGCCAAUAGGCCAUCCUGUUUUUUUCCCUUUUU
RNA_S3b_JFH1_25 AGAGCGGCACACACUAGGUACACUCCAUAGCUAACUGUUCCUUUUUUUUUUUUUUUUUUU
RNA_S3b_        GAACGGGGAGCUAA-----ACACUCCAGGCCAAUAGGCCAUCCUG---------------
RNA_S3c_JFH1_14 AGAGCGGCACACACUAGGUACACUCCAUA-------------------------------
                ..*  ** *   *      ******** .

RNA_S3a_Con1_25 UUUUUUCUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUCUCCUUUUUUUUUCCUCUU
RNA_S3b_JFH1_25 UUUUUUUUUUUUUUUUUUUUUUUUCUUUUUUUUUUUUUUCCCUCUUUCUUCCCUUCUCAU
RNA_S3b_        ------------------------------------------------------------
RNA_S3c_JFH1_14 ------------------------------------------------------------


RNA_S3a_Con1_25 UUUUUCCUUUUCUUUCCUUUGGUGGCUCCAUCUUAGCCCUAGUCACGGCUAGCUGUGAAA
RNA_S3b_JFH1_25 CUUAUUCUACUUUCUUUCUUGGUGGCUCCAUCUUAGCCCUAGUCACGGCUAGCUGUGAAA
RNA_S3b_        ------------------------------------------------------------
RNA_S3c_JFH1_14 ------------------------------------------------------------


RNA_S3a_Con1_25 GGUCCGUGAGCCGCUUGACUGCAGAGAGUGCUGAUACUGGCCUCUCUGCAGAUCAAGU
RNA_S3b_JFH1_25 GGUCCGUGAGCCGCAUGACUGCAGAGAGUGCCGUAACUGGUCUCUCUGCAGAUCAUG-
RNA_S3b_        ----------------------------------------------------------
RNA_S3c_JFH1_14 ----------------------------------------------------------
```

FASTA compatible format

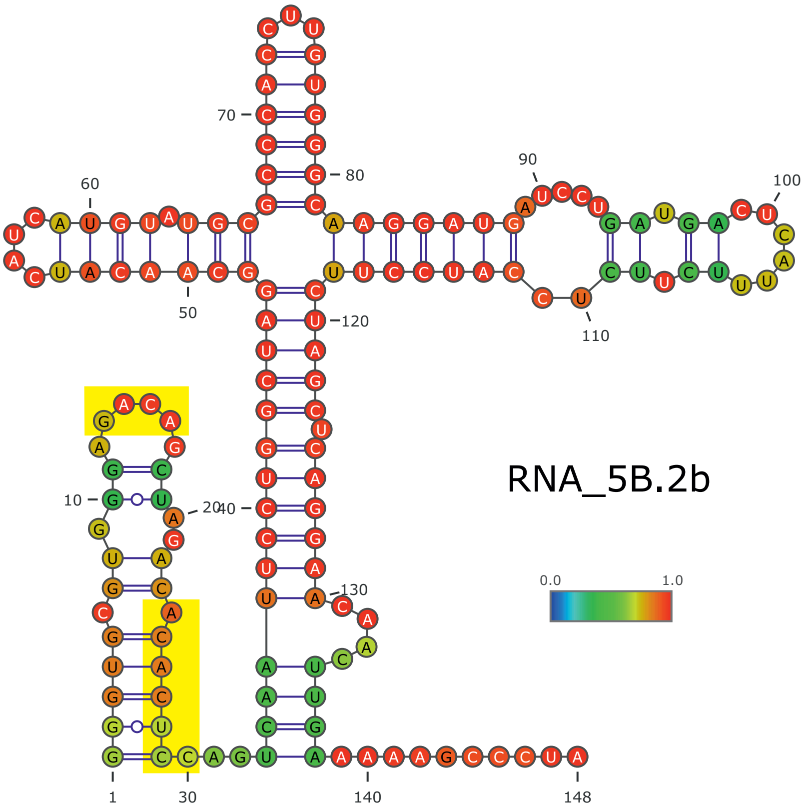
```
>RNA_5B.2a_Con1_227_nts
GGGGUGGGAGACAGCUAGACACACUCCAGUCAAUUCCUGGCUAGGCAACAUCAUCAUGUAUGCGCCCACCUUGUGGGCAAGG
AUGAUCCUGAUGACUCAUUUCUUCUCCAUCCUUCUAGCUCAGGAACAACUUGAAAAAGCCCUAGAUUGUCAGAUCUACGGGG
CCUGUUACUCCAUUGAGCCACUUGACCUACCUCAGAUCAUUCAACGACUCCAUGGCCUUAGCG

>RNA_5B.2a*_JFH1_267 nts
GGGAAACAGUUAGACACUCCCCUAUCAAUUCAUGGCUGGGAAACAUCAUCCAGUAUGCUCCAACCAUAUGGGUUCGCAUGGU
CCUAAUGACACACUUCUUCUCCAUUCUCAUGGUCCAAGACACCCUGGACCAGAACCUCAACUUUGAGAUGUAUGGAUCAGUA
UACUCCGUGAAUCCUUUGGACCUUCCAGCCAUAAUUGAGAGGUUACACGGGCUUGACGCCUUUUCUAUGCACACAUACUCUC
ACCACGAACUGACGCGGGUGG

>RNA_5B.2b_Con1_148_nts
GGGUGCGUGGGAGACAGCUAGACACACUCCAGUCAAUUCCUGGCUAGGCAACAUCAUCAUGUAUGCGCCCACCUUGUGGGCA
AGGAUGAUCCUGAUGACUCAUUUCUUCUCCAUCCUUCUAGCUCAGGAACAACUUGAAAAAGCCCUA

>RNA_5B.2b*_JFH1_291_nts
GGGAAACAGUUAGACACUCCCCUAUCAAUUCAUGGCUGGGAAACAUCAUCCAGUAUGCUCCAACCAUAUGGGUUCGCAUGGU
CCUAAUGACACACUUCUUCUCCAUUCUCAUGGUCCAAGACACCCUGGACCAGAACCUCAACUUUGAGAUGUAUGGAUCAGUA
UACUCCGUGAAUCCUUUGGACCUUCCAGCCAUAAUUGAGAGGUUACACGGGCUUGACGCCUUUUCUAUGCACACAUACUCUC
ACCACGAACUGACGCGGGUGGCUUCAGCCCUCAGAAAACUUGGGG

>RNA_5B.2c_Con1_152_nts
GGGCGUGGGAGACAGCUAGACACACUCCAGUCAAUUCCUGGCUAGGCAACAUCAUCAUGUAUGCGCCCACCUUGUGGGCAAG
GAUGAUCCUGAUGACUCAUUUCUUCUCCAUCCUUCUAGCUCAGGAACAACUUGAAAAAGCCCUAGAUUGU

>RNA_5B.2d_JFH1_160_nts
GGGCCACUCGCCCGGGCUGCCUGGGAAACAGUUAGACACUCCCCUAUCAAUUCAUGGCUGGGAAACAUCAUCCAGUAUGCUC
CAACCAUAUGGGUUCGCAUGGUCCUAAUGACACACUUCUUCUCCAUUCUCAUGGUCCAAGACACCCUGGACCAGAACC

>RNA_5B.3a_Con1_226_nts
GGGGGCUACUGUCCCAGGGGGGGAGGGCUGCCACUUGUGGCAAGUACCUCUUCAACUGGGCAGUAAGGACCAAGCUCAAACU
CACUCCAAUCCCGGCUGCGUCCCAGUUGGAUUUAUCCAGCUGGUUCGUUGCUGGUUACAGCGGGGGAGACAUAUAUCACAGC
CUGUCUCGUGCCCGACCCCGCUGGUUCAUGUGGUGCCUACUCCUACUUUCUGUAGGGGUAGG

>RNA_5B.3a*_JFH1_200_nts
GGGCGGUGAAGACCAAGCUCAAACUCACUCCAUUGCCGGAGGCGCGCCUACUGGACUUAUCCAGUUGGUUCACCGUCGGCGC
CGGCGGGGGCGACAUUUUUCACAGCGUGUCGCGCGCCCGACCC

>RNA_5B.3b_JFH1_196_nts
GGGCGGUGAAGACCAAGCUCAAACUCACUCCAUUGCCGGAGGCGCGCCUACUGGACUUAUCCAGUUGGUUCACCGUCGGCGC
CGGCGGGGGCGACAUUUUUCACAGCGUGUCGCGCGCCCGACCCCGCUCAUUACUCUUCGGCCUACUCCUACUUUUCGUAGGG
GUAGGCCUCUUCCUACUCCCCGCUCGGUAGAG

>RNA_5B.3c_JFH1_224_nts
GGGUCAUCUCCCGUGGAGGGAAAGCGGCCGUUUGCGGCCGAUAUCUCUUCAAUUGGGCGGUGAAGACCAAGCUCAAACUCAC
UCCAUUGCCGGAGGCGCGCCUACUGGACUUAUCCAGUUGGUUCACCGUCGGCGCCGGCGGGGGCGACAUUUUUCACAGCGUG
UCGCGCGCCCGACCCCGCUCAUUACUCUUCGGCCUACUCCUACUUUUCGUAGGGGUAGGC

>RNA_S3a_Con1_250_nts
GGGCCGAUGAACGGGGAGCUAAACACUCCAGGCCAAUAGGCCAUCCUGUUUUUUUCCCUUUUUUUUUUUCUUUUUUUUUUUU
UUUUUUUUUUUUUUUUUUUUUUCUCCUUUUUUUUUCCUCUUUUUUUCCUUUUCUUUCCUUUGGUGGCUCCAUCUUAGCCCUA
GUCACGGCUAGCUGUGAAAGGUCCGUGAGCCGCUUGACUGCAGAGAGUGCUGAUACUGGCCUCUCUGCAGAUCAAGUACUAC
UAGU

>RNA_S3b_JFH1_252_nts
GGGUCCCCGCUCGGUAGAGCGGCACACACUAGGUACACUCCAUAGCUAACUGUUCCUUUUUUUUUUUUUUUUUUUUUUUUUU
UUUUUUUUUUUUUUUUUCUUUUUUUUUUUUUUCCCUCUUUCUUCCCUUCUCAUCUUAUUCUACUUUCUUUCUUGGUGGCUCC
AUCUUAGCCCUAGUCACGGCUAGCUGUGAAAGGUCCGUGAGCCGCAUGACUGCAGAGAGUGCCGUAACUGGUCUCUCUGCAG
AUCAUG
```

```
> RNA_S3b*_Con1_153_nts
GGGCAGCGGGGGAGACAUAUAUCACAGCCUGUCUCGUGCCCGACCCCGCUGGUUCAUGUGGUGCCUACUCCUACUUUCUGUA
GGGGUAGGCAUCUAUCUACUCCCCAACCGAUGAACGGGGAGCUAAACACUCCAGGCCAAUAGGCCAUCCUG

>RNA_S3c_JFH1_149_nts
GGGUCGGCGCCGGCGGGGGCGACAUUUUUCACAGCGUGUCGCGCGCCCGACCCCGCUCAUUACUCUUCGGCCUACUCCUACU
UUUCGUAGGGGUAGGCCUCUUCCUACUCCCCGCUCGGUAGAGCGGCACACACUAGGUACACUCCAUA
```



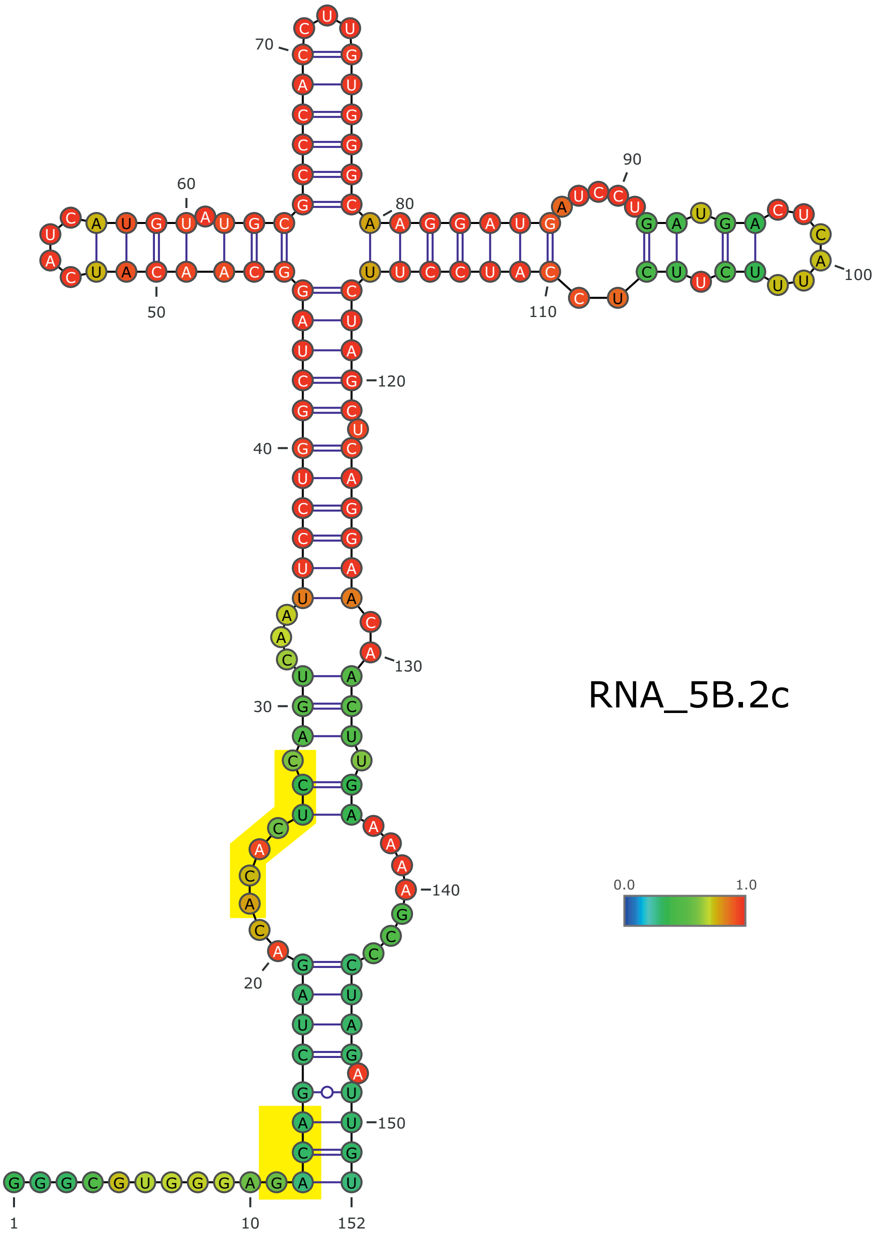
Supplementary Figure S7, related to Figure 3

Predicted target RNA minimum free energy (MFE) structures. Primers for transcription template production, RNA sequences and Vienna dot bracket outputs are shown in Supplementary Figure S6. Base pair probability (bpp) values were loaded into VARNAv3-92 (Darty et al. 2009) from the Vienna dot plot EPS file output (Gruber et al. 2008; Lorenz et al. 2011), and the bpp color code was set to Vienna style. The Vienna MFE output returns bpp values for bases predicted to pair, but single strand probabilities (ssp = 1 – bpp) for bases predicted to be unpaired.



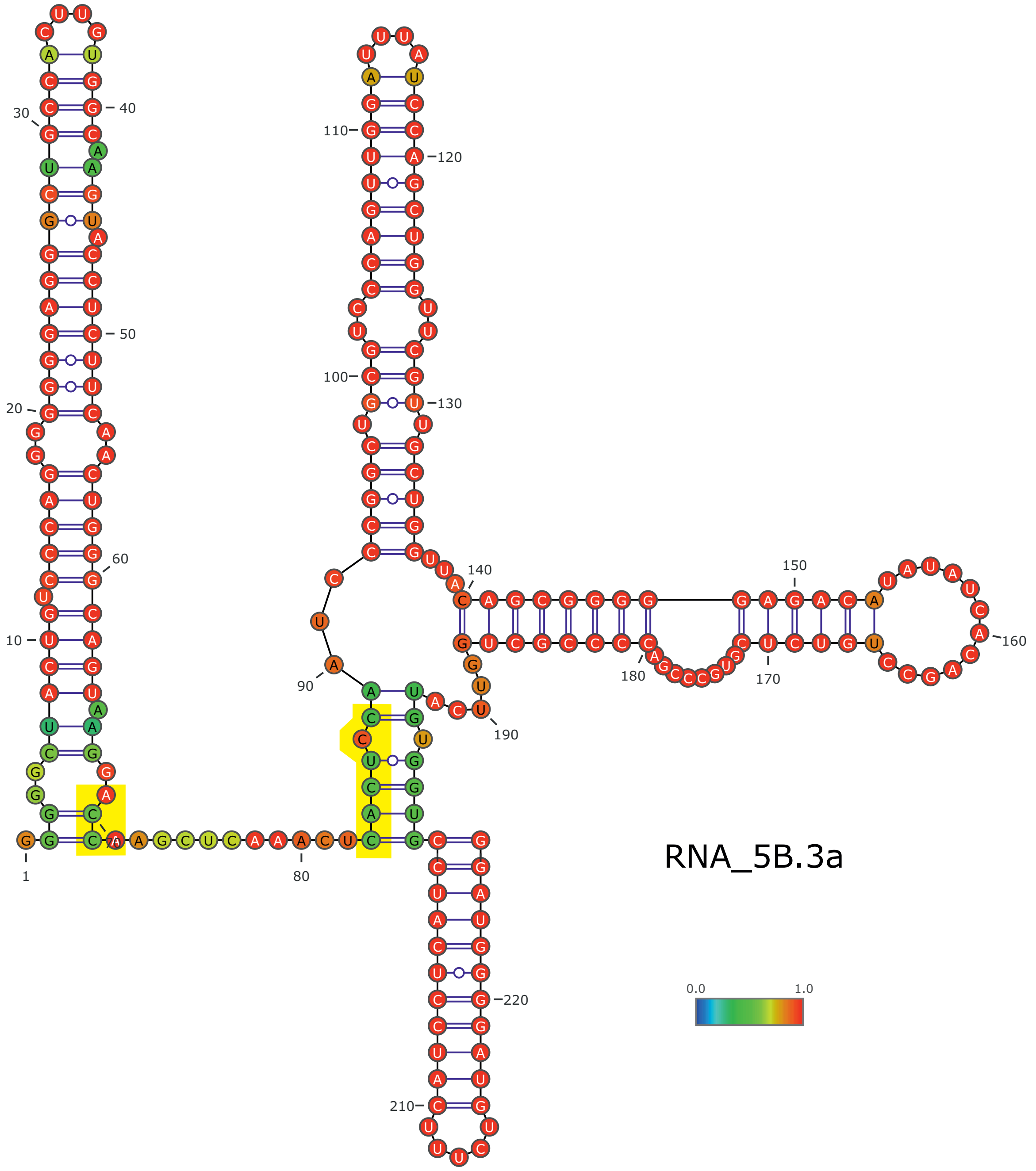
Supplementary Figure S8, related to Figure 3

Predicted target RNA minimum free energy (MFE) structures. Primers for transcription template production, RNA sequences and Vienna dot bracket outputs are shown in Supplementary Figure S6. Base pair probability (bpp) values were loaded into VARNAv3-92 (Darty et al. 2009) from the Vienna dot plot EPS file output (Gruber et al. 2008; Lorenz et al. 2011), and the bpp color code was set to Vienna style. The Vienna MFE output returns bpp values for bases predicted to pair, but single strand probabilities (ssp = 1 – bpp) for bases predicted to be unpaired.



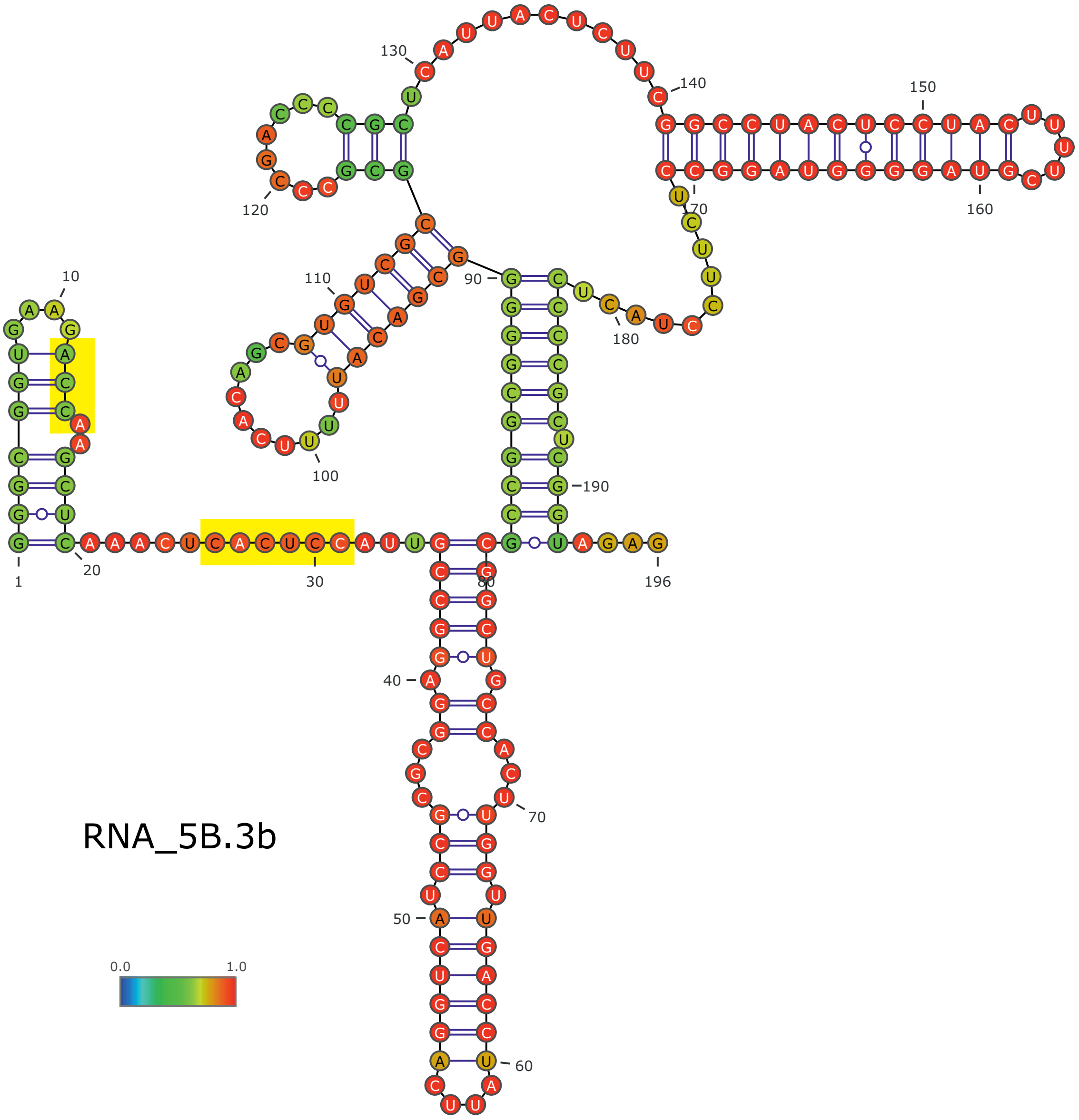
Supplementary Figure S9, related to Figure 3

Predicted target RNA minimum free energy (MFE) structures. Primers for transcription template production, RNA sequences and Vienna dot bracket outputs are shown in Supplementary Figure S6. Base pair probability (bpp) values were loaded into VARNAv3-92 (Darty et al. 2009) from the Vienna dot plot EPS file output (Gruber et al. 2008; Lorenz et al. 2011), and the bpp color code was set to Vienna style. The Vienna MFE output returns bpp values for bases predicted to pair, but single strand probabilities (ssp = 1 – bpp) for bases predicted to be unpaired.



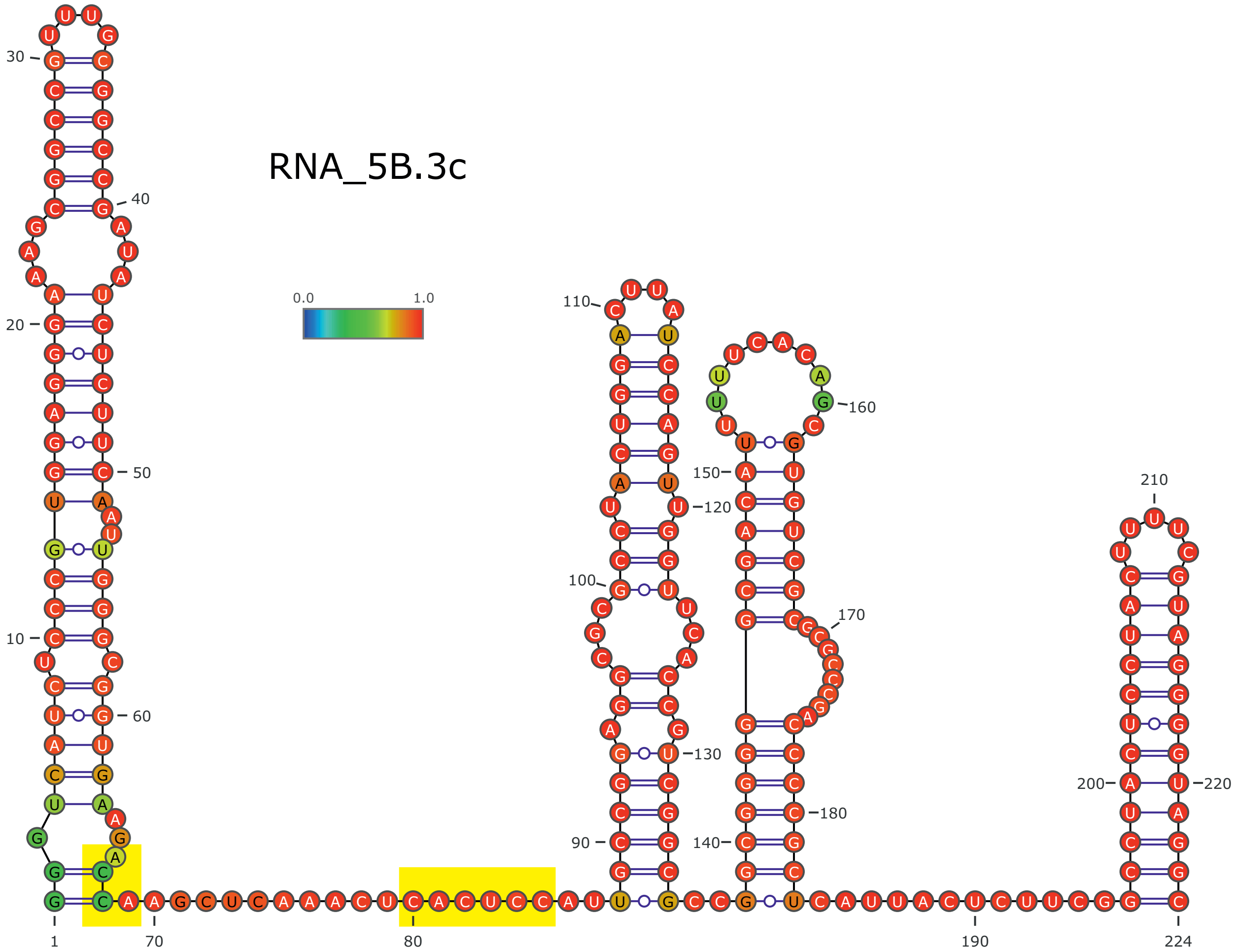
Supplementary Figure S10, related to Figure 4

Predicted target RNA minimum free energy (MFE) structures. Primers for transcription template production, RNA sequences and Vienna dot bracket outputs are shown in Supplementary Figure S6. Base pair probability (bpp) values were loaded into VARNAv3-92 (Darty et al. 2009) from the Vienna dot plot EPS file output (Gruber et al. 2008; Lorenz et al. 2011), and the bpp color code was set to Vienna style. The Vienna MFE output returns bpp values for bases predicted to pair, but single strand probabilities (ssp = 1 – bpp) for bases predicted to be unpaired.



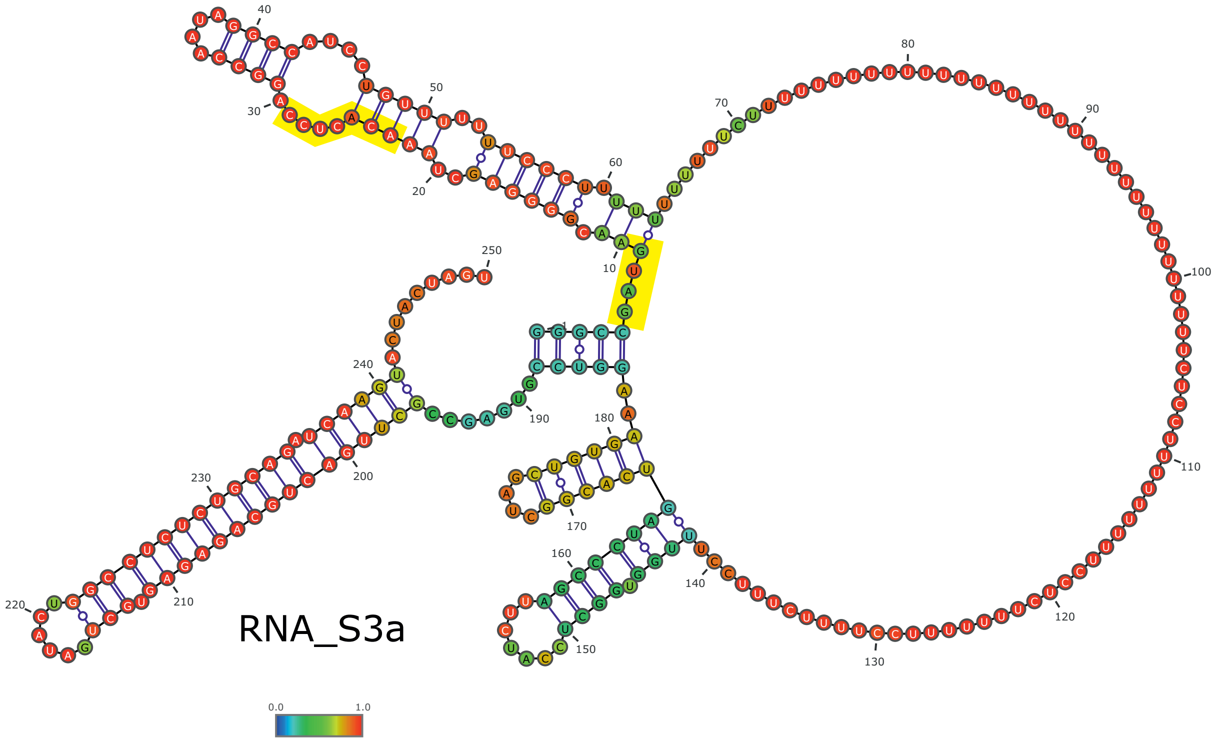
Supplementary Figure S11, related to Figure 4

Predicted target RNA minimum free energy (MFE) structures. Primers for transcription template production, RNA sequences and Vienna dot bracket outputs are shown in Supplementary Figure S6. Base pair probability (bpp) values were loaded into VARNAv3-92 (Darty et al. 2009) from the Vienna dot plot EPS file output (Gruber et al. 2008; Lorenz et al. 2011), and the bpp color code was set to Vienna style. The Vienna MFE output returns bpp values for bases predicted to pair, but single strand probabilities (ssp = 1 – bpp) for bases predicted to be unpaired.



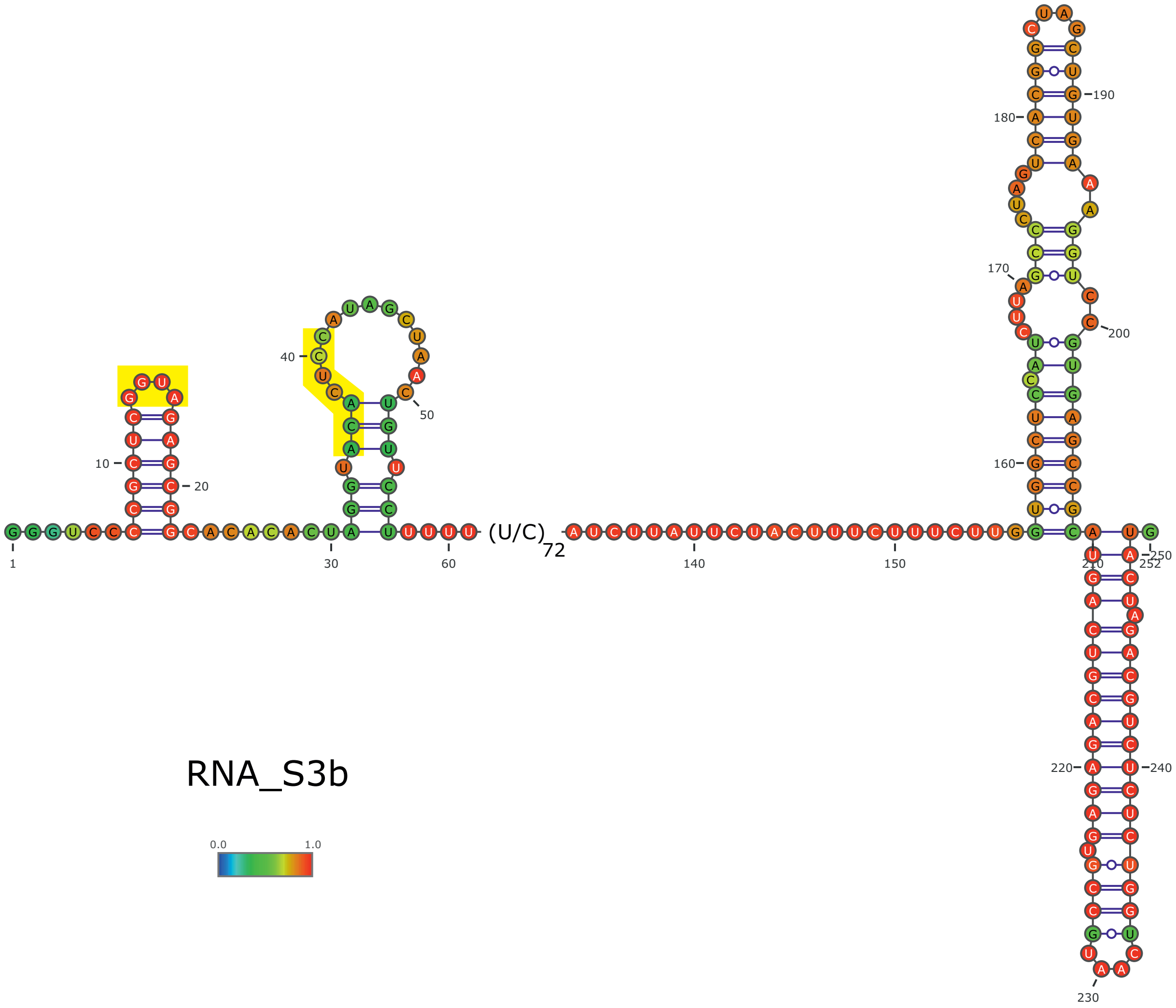
Supplementary Figure S12, related to Figure 4

Predicted target RNA minimum free energy (MFE) structures. Primers for transcription template production, RNA sequences and Vienna dot bracket outputs are shown in Supplementary Figure S6. Base pair probability (bpp) values were loaded into VARNAv3-92 (Darty et al. 2009) from the Vienna dot plot EPS file output (Gruber et al. 2008; Lorenz et al. 2011), and the bpp color code was set to Vienna style. The Vienna MFE output returns bpp values for bases predicted to pair, but single strand probabilities (ssp = 1 – bpp) for bases predicted to be unpaired.



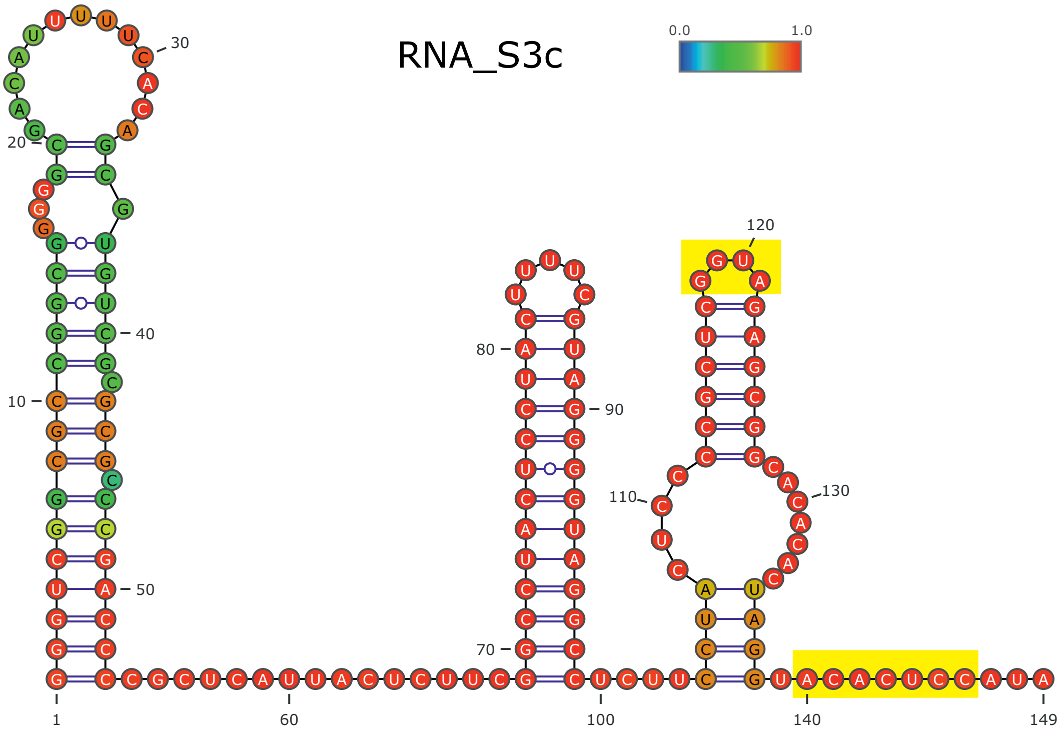
Supplementary Figure S13, related to Figure 5

Predicted target RNA minimum free energy (MFE) structures. Primers for transcription template production, RNA sequences and Vienna dot bracket outputs are shown in Supplementary Figure S6. Base pair probability (bpp) values were loaded into VARNAv3-92 (Darty et al. 2009) from the Vienna dot plot EPS file output (Gruber et al. 2008; Lorenz et al. 2011), and the bpp color code was set to Vienna style. The Vienna MFE output returns bpp values for bases predicted to pair, but single strand probabilities (ssp = 1 – bpp) for bases predicted to be unpaired.



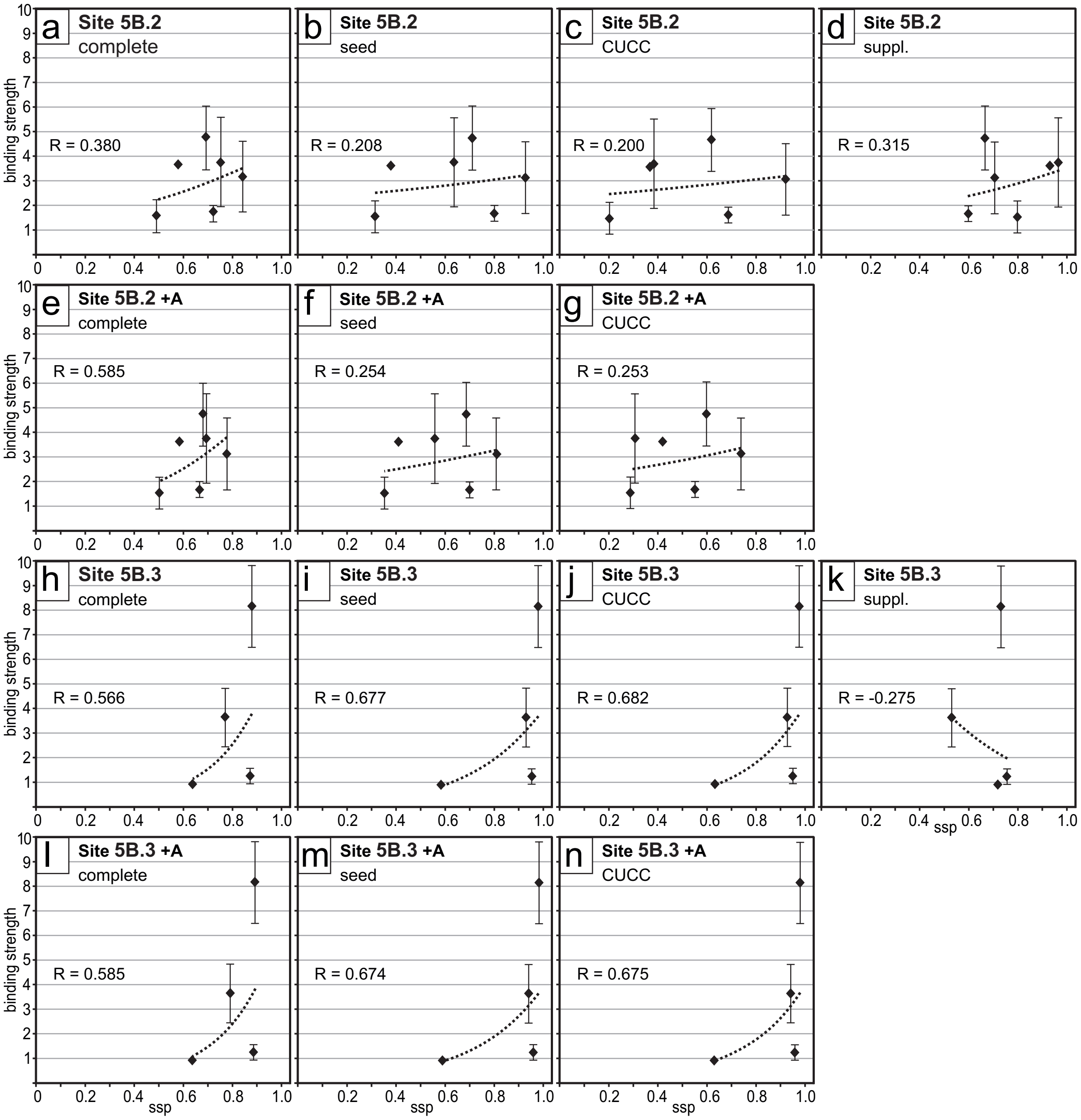
Supplementary Figure S14, related to Figure 5

Predicted target RNA minimum free energy (MFE) structures. Primers for transcription template production, RNA sequences and Vienna dot bracket outputs are shown in Supplementary Figure S6. Base pair probability (bpp) values were loaded into VARNAv3-92 (Darty et al. 2009) from the Vienna dot plot EPS file output (Gruber et al. 2008; Lorenz et al. 2011), and the bpp color code was set to Vienna style. The Vienna MFE output returns bpp values for bases predicted to pair, but single strand probabilities (ssp = 1 – bpp) for bases predicted to be unpaired.



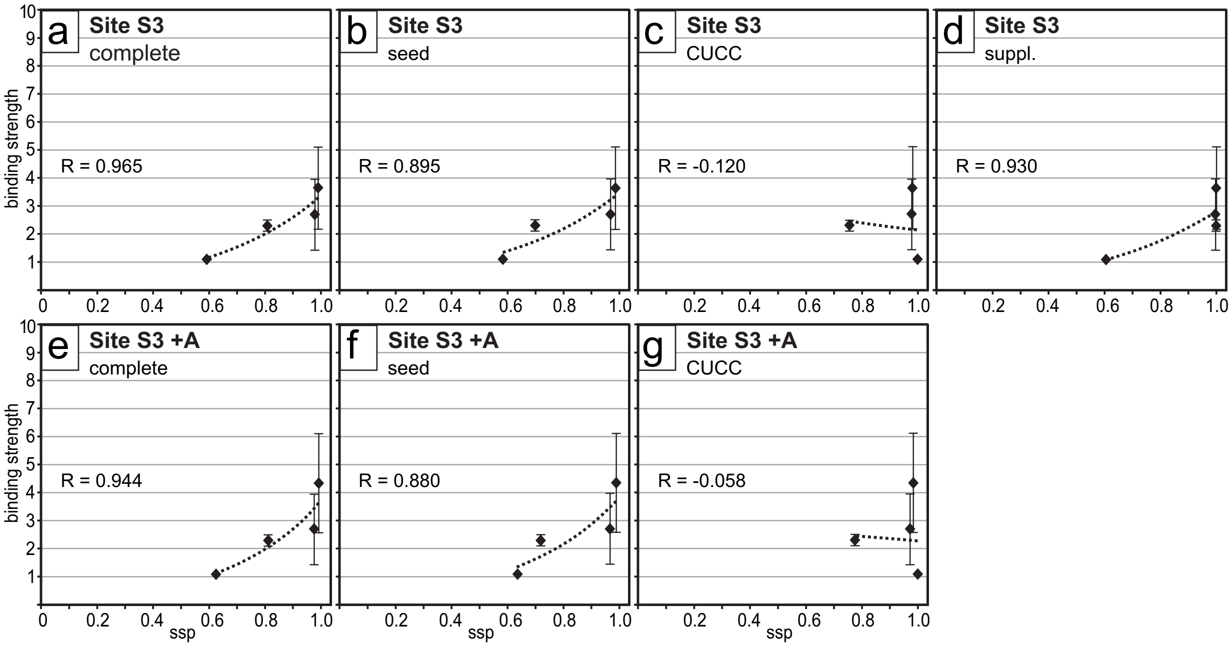
Supplementary Figure S15, related to Figure 5

Predicted target RNA minimum free energy (MFE) structures. Primers for transcription template production, RNA sequences and Vienna dot bracket outputs are shown in Supplementary Figure S6. Base pair probability (bpp) values were loaded into VARNAv3-92 (Darty et al. 2009) from the Vienna dot plot EPS file output (Gruber et al. 2008; Lorenz et al. 2011), and the bpp color code was set to Vienna style. The Vienna MFE output returns bpp values for bases predicted to pair, but single strand probabilities (ssp = 1 – bpp) for bases predicted to be unpaired.



Supplementary Figure S16, related to Figures 3 - 5

The intensity of miR-122/Ago2 complex binding to NS5B target sites plotted against target site accessibility. The intensity of miR-122/Ago2 complex binding to each target site was calculated from the phosphorimager scans (examples shown in Figures 2, 3 and 4) and several additional experiments using all RNAs listed in Supplementary Figure 6. Binding strength was plotted against single strand probability (ssp) values (calculated as described in Materials and Method). Error bars indicate standard deviations. "R" values are correlation coefficients for regression curves. Panels show plots against average single strand probabilities of the complete target site and separately for seed sequence, nucleotides opposite nucleotides 2 – 5 of the miRNA ("CUCC") and for the supplementary site as indicated. The A residue was additionally included in the calculations in plots labeled "+A".



Supplementary Figure S17, related to Figures 3 - 5

The intensity of miR-122/Ago2 complex binding to 3´UTR S3 target sit plotted against target site accessibility. The intensity of miR-122/Ago2 complex binding to each target site was calculated from the phosphorimager scans (examples shown in Figures 2, 3 and 4) and several additional experiments using all RNAs listed in Supplementary Figure 6. Binding strength was plotted against single strand probability (ssp) values (calculated as described in Materials and Method). Error bars indicate standard deviations. "R" values are correlation coefficients for regression curves. Panels show plots against average single strand probabilities of the complete target site and separately for seed sequence, nucleotides opposite nucleotides 2 – 5 of the miRNA ("CUCC") and for the supplementary site as indicated. The A residue was additionally included in the calculations in plots labeled "+A".

REFERENCES

Darty, K, Denise, A and Ponty, Y (2009) VARNA: Interactive drawing and editing of the RNA secondary structure. *Bioinformatics*, 25:1974-1975.

Fricke, M, Dünnes, N, Zayas, M, Bartenschlager, R, Niepmann, M and Marz, M (2015) Conserved RNA secondary structures and long-range interactions in Hepatitis C Viruses. *RNA*, 21:1219-1232.

Gruber, AR, Lorenz, R, Bernhart, SH, Neubock, R and Hofacker, IL (2008) The Vienna RNA websuite. *Nucleic Acids Res.*, 36, W70-74.

Lorenz, R, Bernhart, SH, Honer Zu Siederdissen, C, Tafer, H, Flamm, C, Stadler, PF and Hofacker, IL (2011) ViennaRNA Package 2.0. *Algorithms for Molecular Biology: AMB*, 6:26.