The conserved 3'X terminal domain of hepatitis C virus genomic RNA forms a two-stem structure that promotes viral RNA dimerization

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SUPPLEMENTARY FIGURE LEGENDS AND SUPPLEMENTARY FIGURES

Figure S1. RNA sequences studied by NMR spectroscopy and gel electrophoresis experiments. The self-complementary DLS nucleotides are highlighted in red.

Figure S2. Impact of Mg^{2+} ions on the conformation of 3'X domain sequences analysed by native gel electrophoresis experiments. (A) Constructs SL2', SL2+3 and X98 (full-length 3'X domain); (B) constructs SL1, SL2 and SL3. Experiments (A) and (B) compare the mobility of 3'X domain sequences previously folded in the absence or presence of 1mM $MgCl_2$, relative to a tRNA control. Both experiments used a running buffer containing Mg^{2+} ions. Conditions: 20 μM RNA, TBM running buffer (1 mM $MgCl_2$).

Figure S3. NOESY analysis of subdomain SL1. The image shows the assignment of the imino-aromatic (top) and imino-imino (bottom) regions of a watergate-NOESY spectrum acquired at 16 ^oC with 150 ms mixing time. Sequential crosspeaks between G and U imino protons are indicated with solid lines, sequential contacts between A H2 and G H1 protons are marked with dashed lines, and NOE interactions across base pairs are labelled with colons. Assignments of C amino protons have been omitted for clarity, and crosspeaks marked with asterisks are visible at a lower contour level. Conditions: 240 μM SL1, 0 mM NaCl/$MgCl_2$.

Figure S4. NOESY analysis of subdomain SL2'. The image shows the assignment of the imino-aromatic (top) and imino-imino (bottom) regions of a watergate-NOESY spectrum acquired at 16 ^oC with 150 ms mixing time. Sequential crosspeaks between G and U imino protons are indicated with solid lines, sequential contacts between A H2 and G H1 protons are marked with dashed lines, and NOE interactions across base pairs are labelled with colons. Assignments of C amino protons have been omitted for clarity. Conditions: 240 μM SL2', 0 mM NaCl/$MgCl_2$.

Figure S5. NMR spectroscopy analysis of construct SL2+3. (A) ^1H-^{15}N HSQC spectrum of SL2+3 at low ionic strength (black), superposed with that of construct SL2' (red) acquired under the same temperature and ionic conditions. The assignments of SL2' crosspeaks are in red (orange for DLS nucleotides), and tentative SL2+3 assignments are indicated in black and between parentheses. Red arrows mark SL2' crosspeaks of

common nucleotides that are clearly not present in the SL2+3 spectrum (specifically U7 and G48), and black arrows mark SL2+3 crosspeaks that are clearly not matched in the SL2' spectrum. Conditions: 44 μM SL2+3, 0 mM NaCl/$MgCl_2$, 27 ^{o}C. (B) Superposition of exchangeable ^{1}H spectra of constructs SL2, SL3 and SL2+3 acquired at low ionic strength. Conditions: 73, 105 and 224 μM SL2, SL3 and SL2+3, respectively, 0 mM NaCl/$MgCl_2$, 27 ^{o}C. (C) ^{1}H-^{15}N HSQC spectrum of SL2+3 acquired in the presence of 100 mM NaCl (green), superposed with that of SL2' (red) acquired under the same temperature and ionic conditions. The assignments of SL2' crosspeaks are marked in red (orange for DLS nucleotides), and tentative SL2+3 assignments are indicated in green. Additional, weak SL2+3 crosspeaks that appeared at higher ionic strength and clearly matched SL2' DLS signals (U30, U34 and U36) are indicated with green circles. Conditions: 44 μM SL2+3, 100 mM NaCl, 27 ^{o}C. (D) Superposition of the exchangeable ^{1}H spectra of construct SL2 in the absence and presence of 100 mM NaCl. Notice the stabilization of imino resonances at higher ionic strength. Conditions: 73 μM SL2, 27 ^{o}C. (E) Models of SL2+3 monomeric and dimeric structures.

Figure S6. Impact of NaCl on the structure of the full-length 3'X domain analysed by NMR spectroscopy. (A) Superposition of ^{1}H-^{15}N HSQC spectra of construct X98 acquired in the absence (green) and presence (red) of 100 mM NaCl. Conditions: 34 μM X98, 27 ^{o}C. The assignments are based on comparisons with HSQC data of constructs SL1 and SL2' acquired under the same temperature and ionic conditions, and appear in blue and red for crosspeaks of subdomains SL1' and SL2', respectively. The two crosspeaks detected at 100 mM NaCl but absent at lower ionic strength are labelled in parentheses. (B) Model of a full-length 3'X dimer consistent with the NMR data. The NMR signals of each of the two symmetrical halves of the dimer (separated by a dashed line) are equivalent.

Figure S7. Impact of Mg^{2+} on the structure of constructs SL2' and X98 (full-length domain) analysed by NMR spectroscopy. (A) Superposition of ^{1}H-^{15}N HSQC spectra of SL2' acquired in the absence (green) and presence (blue) of 2 mM $MgCl_2$. Conditions: 61 (green) and 49 (blue) μM SL2', 27 ^{o}C. (B) ^{1}H-^{15}N HSQC spectrum of X98 acquired in the presence of 2 mM $MgCl_2$ (green), superposed with those of SL1 (blue) and SL2' (red) obtained under the same temperature and ionic conditions. The assignments appear in blue and red for crosspeaks of nucleotides belonging to subdomains SL1' and SL2', respectively. The SL1 G53, U55, G97 and G98 HN crosspeaks that do not match X98 signals are marked with black assignments. Conditions: 34 μM X98, 2 mM $MgCl_2$, 27 ^{o}C. To favour monomeric species, all samples were snap-cooled in the absence of NaCl or $MgCl_2$, and then incubated with 2 mM $MgCl_2$ for 150 minutes at 25 ^{o}C. In (A) and (B), additional crosspeaks that appeared with Mg^{2+} and were not visible at low ionic strength or with 100 mM NaCl are marked with asterisks.

X98 5’ GGUGGCUCCA UCUUAGCCCU AGUCACGGCU AGCUGUGAAA GGUCCGUGAG CCGCUUGACU GCAGAGAGUG CUGAUACUGG CCUCUCUGCA GAUCAAGU 3’

SL1 5’ GCUUGACUGC AGAGAGUGCU GAUACUGGCC UCUCUGCAGA UCAAGU 3’

SL2+3 5’ GGUGGCUCCA UCUUAGCCCU AGUCACGGCU AGCUGUGAAA GGUCCGUGA 3’

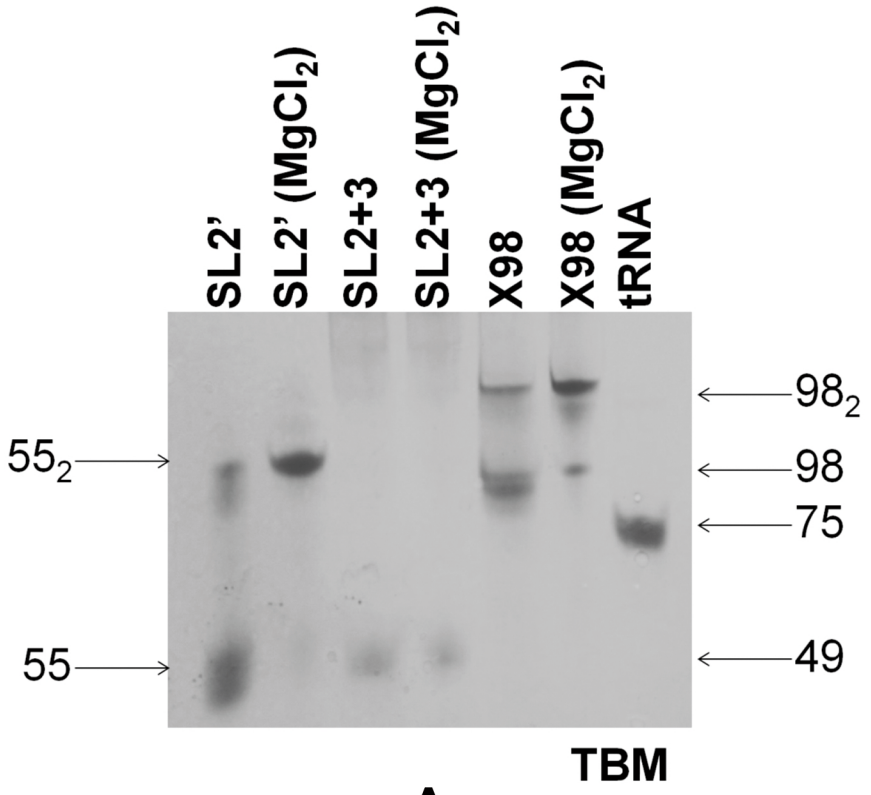
SL2’ 5’ GGUGGCUCCA UCUUAGCCCU AGUCACGGCU AGCUGUGAAA GGUCCGUGAG CCGCU 3’

SL2 5’ GUCACGGCUA GCUGUGAAAG GUCCGUGAG

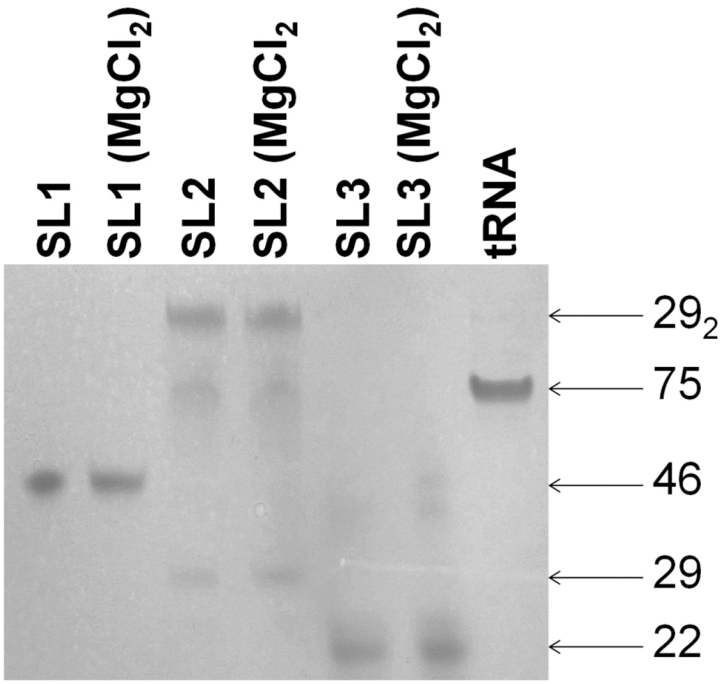
SL3 5’ UGGUGGCUCC AUCUUAGCCC UA

cre26 5’ GGAGACAUAU AUCACAGCCU GUCUCC

Fig. S1


SL2'
SL2' (MgCl2)
SL2+3
SL2+3 (MgCl2)
X98
X98 (MgCl2)
tRNA
55
55
98
98
75
49
TBM

A


SL1
SL1 (MgCl2)
SL2
SL2 (MgCl2
SL3
SL3 (MgCl2)
tRNA
29
75
46
29
22
TBM

B

Fig. S2

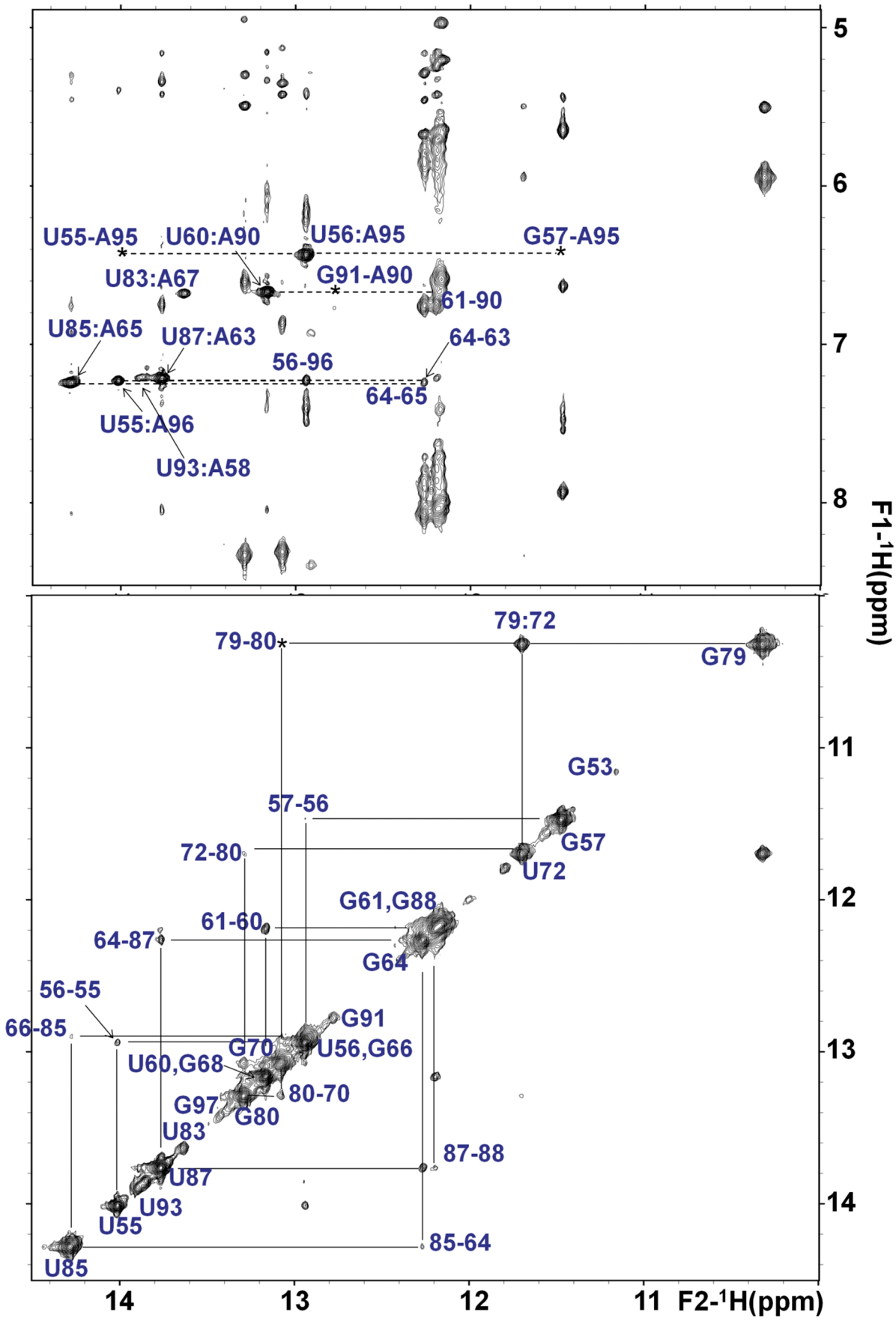

U55-A95
U60:A90
U56:A95
G57-A95
U83:A67
G91-A90
61-90
U85:A65
U87:A63
64-63
56-96
64-65
U55:A96
U93:A58
79-80
79:72
G79
G53
57-56
G57
72-80
U72
G61,G88
61-60
64-87
G64
56-55
66-85
G91
G70
U56,G66
U60,G68
80-70
G97
G80
U83
87-88
U87
U93
U55
85-64
U85
5
6
7
8
11
12
13
14
F1-1H(ppm)
14
13
12
11
F2-1H(ppm)

Fig. S3

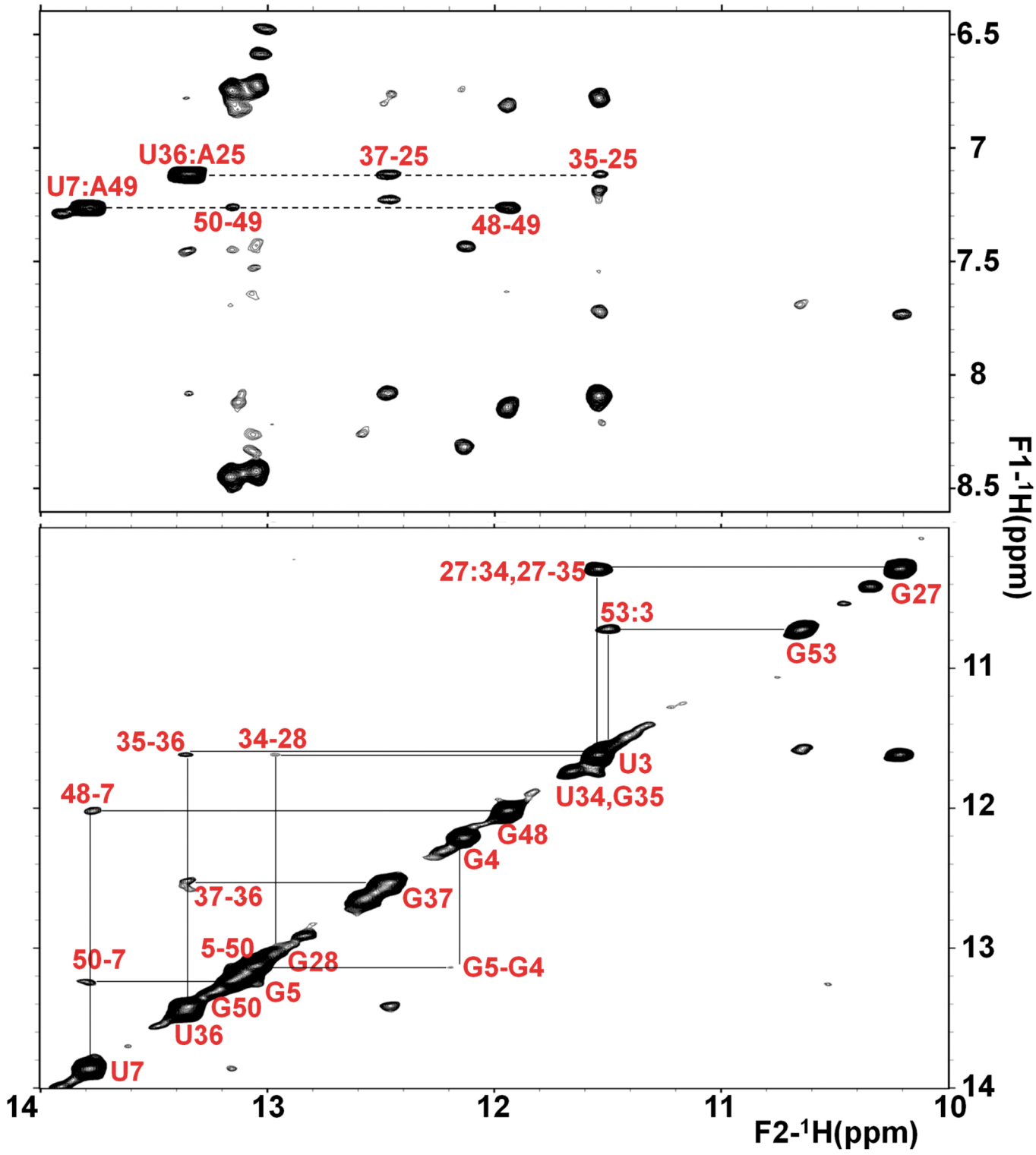

U36:A25
37-25
35-25
U7:A49
50-49
48-49
27:34,27-35
G27
53:3
G53
35-36
34-28
U3
48-7
U34,G35
G48
G4
37-36
G37
50-7
5-50
G28
G5-G4
G5
G50
U36
U7
6.5
7
7.5
8
8.5
11
12
13
14
14
13
12
11
10
F1-¹H(ppm)
F2-¹H(ppm)

Fig. S4

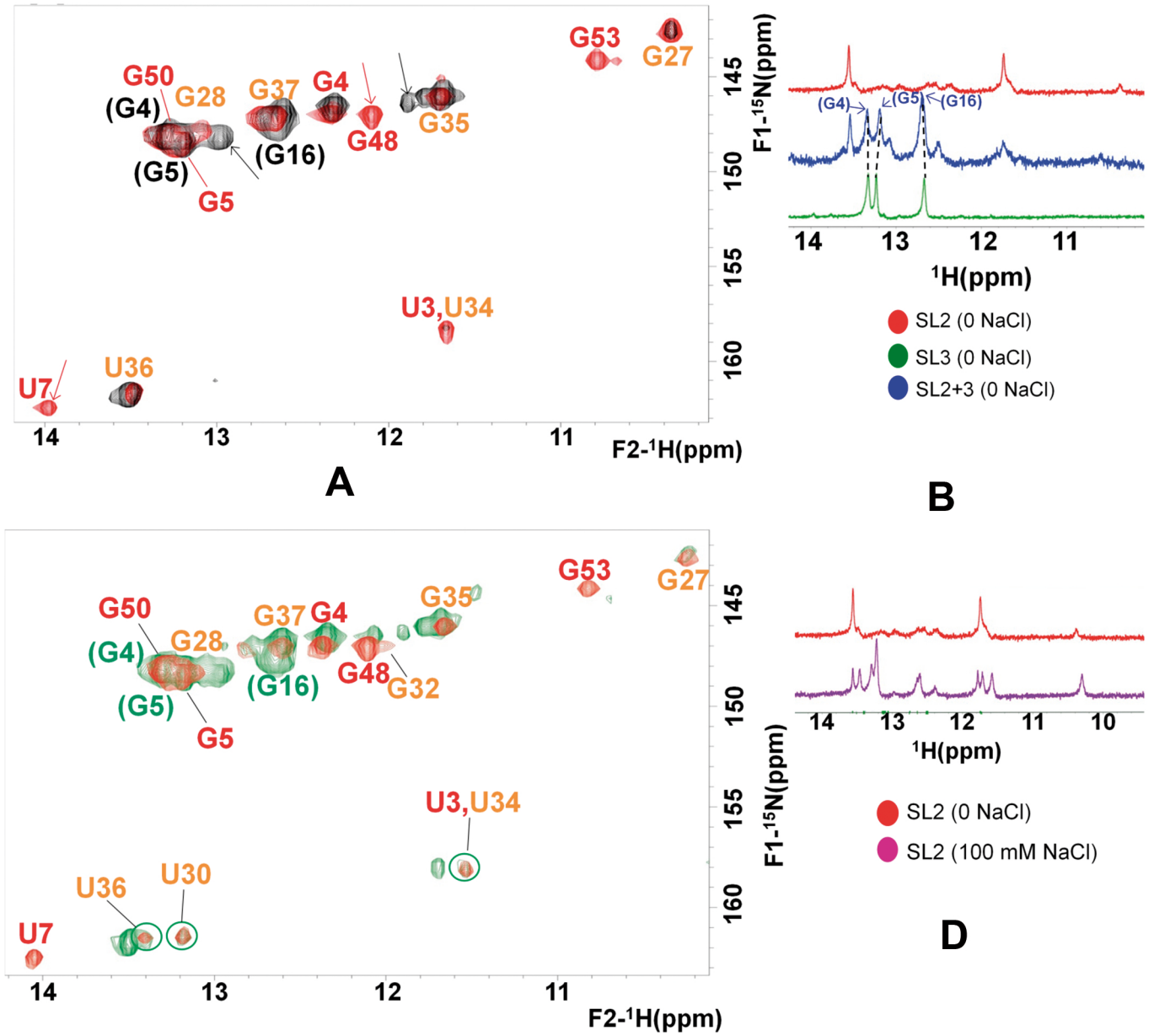
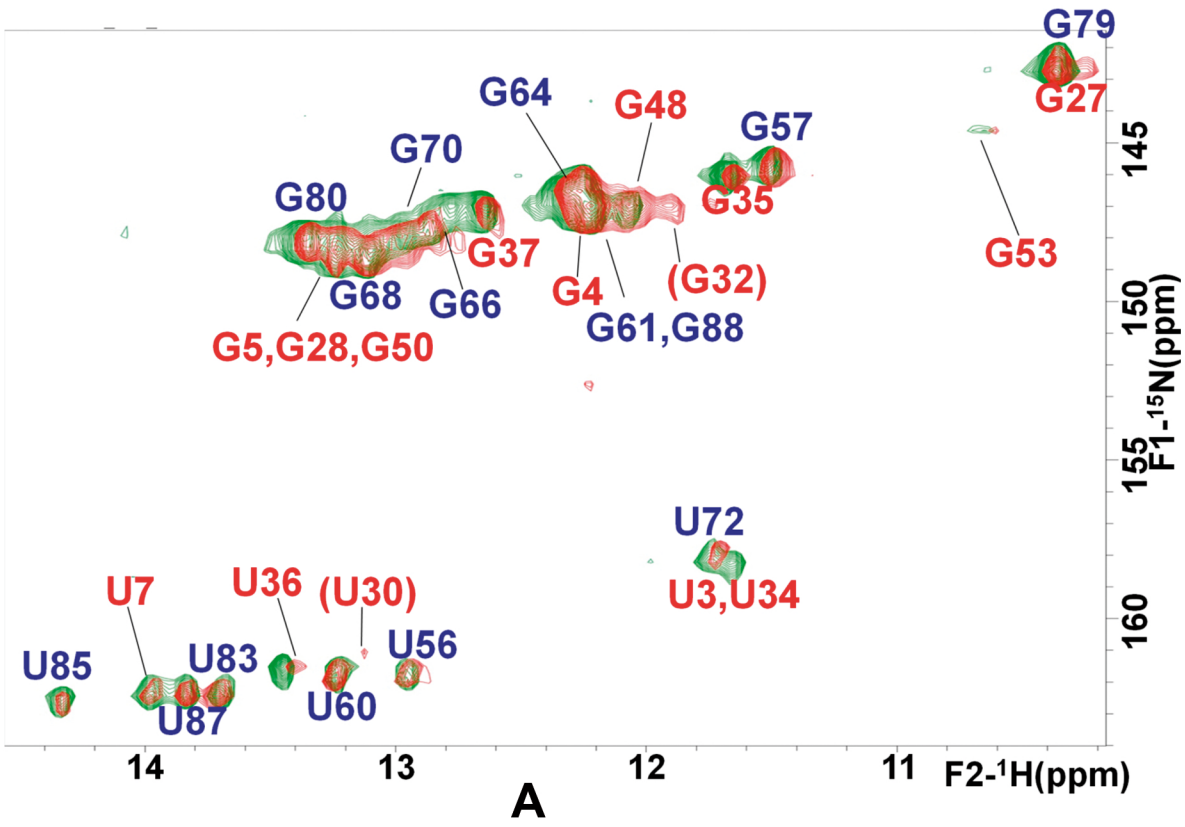
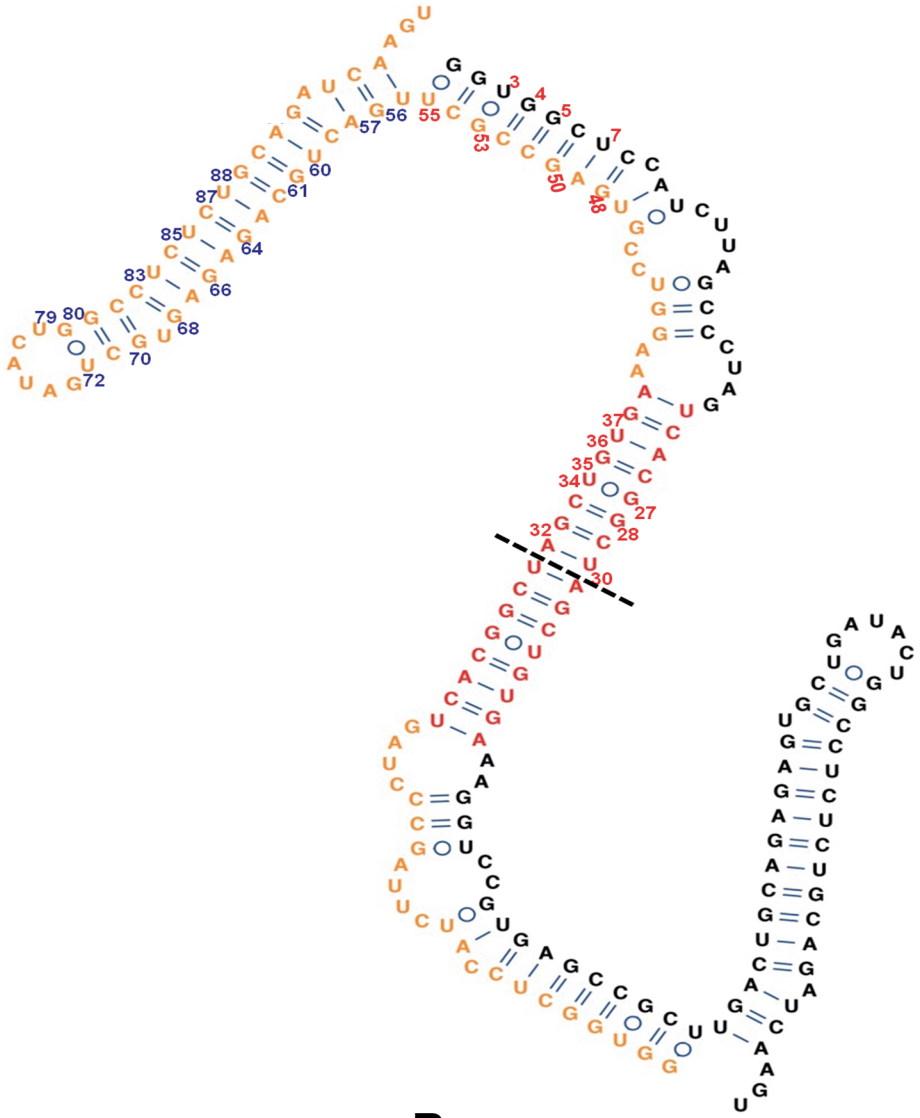

G53
G27
G50
(G4)
G28
G37
G4
G35
G48
(G5)
(G16)
G5
U3,U34
U7
U36
14
13
12
11
F2-¹H(ppm)
145
150
155
160
F1-¹⁵N(ppm)
(G4)
(G5)
(G16)
¹H(ppm)
SL2 (0 NaCl)
SL3 (0 NaCl)
SL2+3 (0 NaCl)
A
B
G53
G27
G50
(G4)
G28
G37
G4
G35
G48
G32
(G16)
(G5)
G5
U3,U34
U36
U30
U7
SL2 (0 NaCl)
SL2 (100 mM NaCl)
C
D
SL2
SL3
NaCl
E

Fig. S5

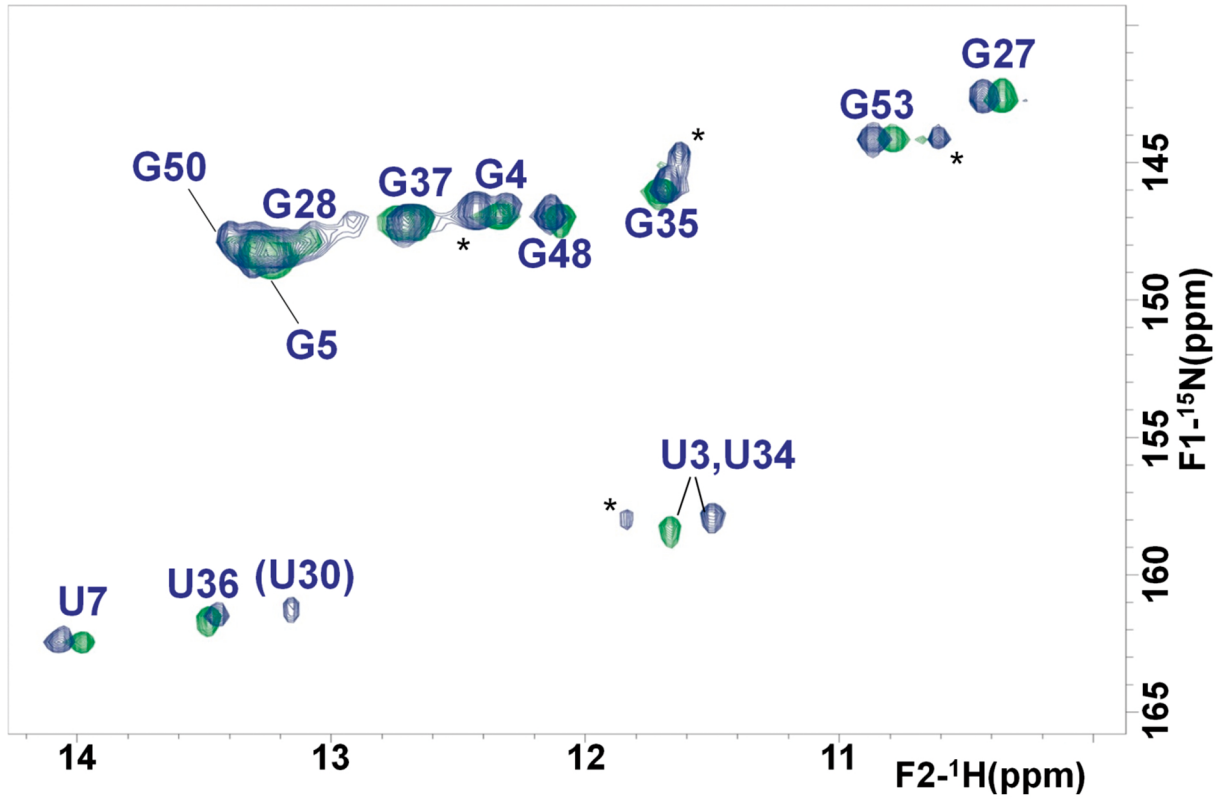

G79
G27
G64
G48
G57
G70
G35
G80
G37
G53
G68
G66
G4
(G32)
G5,G28,G50
G61,G88
U72
U3,U34
U7
U36
(U30)
U56
U85
U83
U60
U87
145
150
155
160
F1-15N(ppm)
14
13
12
11
F2-1H(ppm)

A

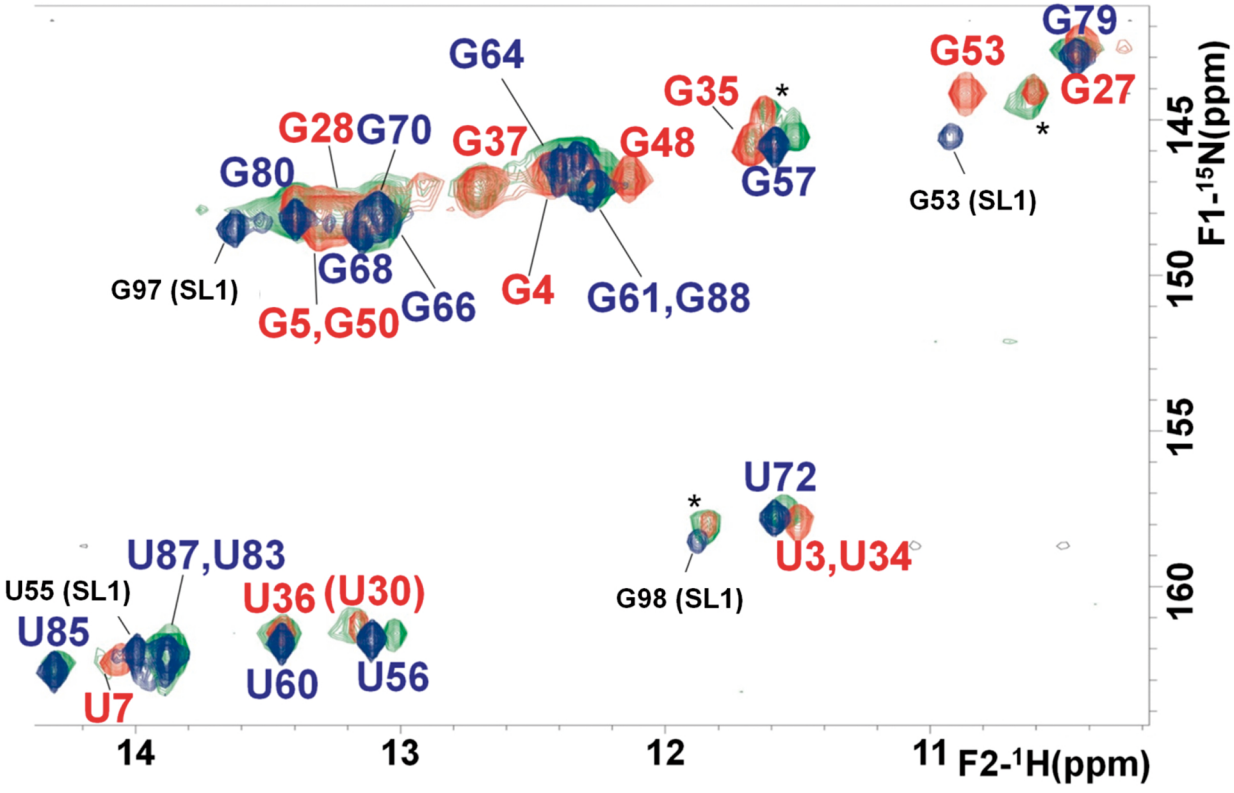


B

Fig. S6


G27
G53
G50
G28
G37
G4
G35
G48
G5
U3,U34
U7
U36
(U30)
14
13
12
11
F2-1H(ppm)
F1-15N(ppm)
145
150
155
160
165

A


G79
G64
G53
G27
G35
G28
G70
G37
G48
G80
G57
G53 (SL1)
G68
G97 (SL1)
G4
G61,G88
G5,G50
G66
U72
U87,U83
U3,U34
U55 (SL1)
G98 (SL1)
U36
(U30)
U85
U60
U56
U7
14
13
12
11
F2-1H(ppm)
F1-15N(ppm)
145
150
155
160

B

Fig. S7