Supplemental material to:

Length and secondary structure of the 5′ non-coding regions of mouse p53 mRNA transcripts - mouse as a model organism for p53 gene expression studies

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Figure legends

Figure S1. Sequences of the transcripts identified by 5′RACE. The sequences originated from three different samples: embryo, liver and thymus. At least twenty clones of each sample were sequenced. Start codon is underlined, first nucleotide of the mRNA-122 is denoted by red. Nucleotides preceding adenosine labelled in red are artifacts resulting from the 5′RACE procedure. The sequences for two transcripts with 247 nucleotide-long 5′UTR, which were identified in embryos, are not shown in the figure.

Figure S2. The abundance of the p53 mRNA transcript containing 247-nucleotide-long 5′UTR. The transcript was observed in a majority of analyzed tissues and its highest level was detected in heart, embryo, brain and spinal cord (denoted on the gel by asterisks). Three control reactions were conducted: C1- negative control reaction for cDNA synthesis, C2- negative PCR control reaction, C3- plasmid containing the sequence of mRNA-247 was used as a positive PCR control. Additional bands observed in lanes for testis and thymus samples are non-specific PCR products. β-actin was used as a loading control.

Figure S3. Structural probing of the 5′-terminal region of mRNA-122. Autoradiograms show the products of Pb^{2+}-induced cleavage **(A)**, SHAPE **(B)** and chemical modification by DMS in *in vitro* conditions **(C)** and in the cell culture **(D)** identified by reverse transcription with 5′-end-^{32}P-labeled DNA primers. cDNA products were analyzed on 8% polyacrylamide gels. Hairpin motifs shown in the secondary structure model of mRNA-122 (Fig. 3) are indicated in panel A, on the right side of each autoradiogram. Lanes: (-), control reaction; C, G, T and A, sequencing lines. In panel A black triangle represents increased Pb^{2+} concentrations; in panel B '+' denotes SHAPE reaction; in panel C the reaction with DMS was performed for 3, 5 and 10 min; in panel D the reaction was conducted for 10 min and it is marked as 'R'. Selected cytosine residues are labeled on the left of each autoradiogram.

Figure S4. Probing of the structure of the 5′-terminal region of mRNA-247. Autoradiograms show the products of Pb^{2+}-induced cleavage **(A)** and SHAPE reaction **(B)** identified by primer extension with 5′-end-^{32}P-labeled DNA primers and analyzed using 8% polyacrylamide gels. Selected hairpin motifs present in the secondary structure model of mRNA-247 (Fig. 4) are indicated in panel A, on the right of each autoradiogram. Lanes: (-): control reaction; C, G, T, A: sequencing lines. Black triangles represent increased Pb^{2+} concentrations in panel A, '+' denotes SHAPE reaction with the use of NMIA in panel B. Selected cytosine residues are labeled on the left of each radiogram.

Figure S5. Secondary structure probing of three selected isolated hairpins from the secondary structure model of mRNA-122 and/or mRNA-247: hairpin C(-200):G(-102) (A), C(-51):G9 (B) and A89:U140 (C). Autoradiograms show products of Pb^{2+}-induced cleavage reactions analyzed using 12% polyacrylamide gels. The experiments were conducted using 5′-^{32}P-end-labelled RNAs. Lines: T1, limited hydrolysis by RNase T1 in denaturing conditions; L, formamide ladder; (-), control reaction. Black triangles above autoradiograms represent decreased Pb^{2+} concentrations and selected nucleotide residues are labeled on the left side of each autoradiogram. Nucleotides are numbered according to the model of mRNA-247.

Figure S1

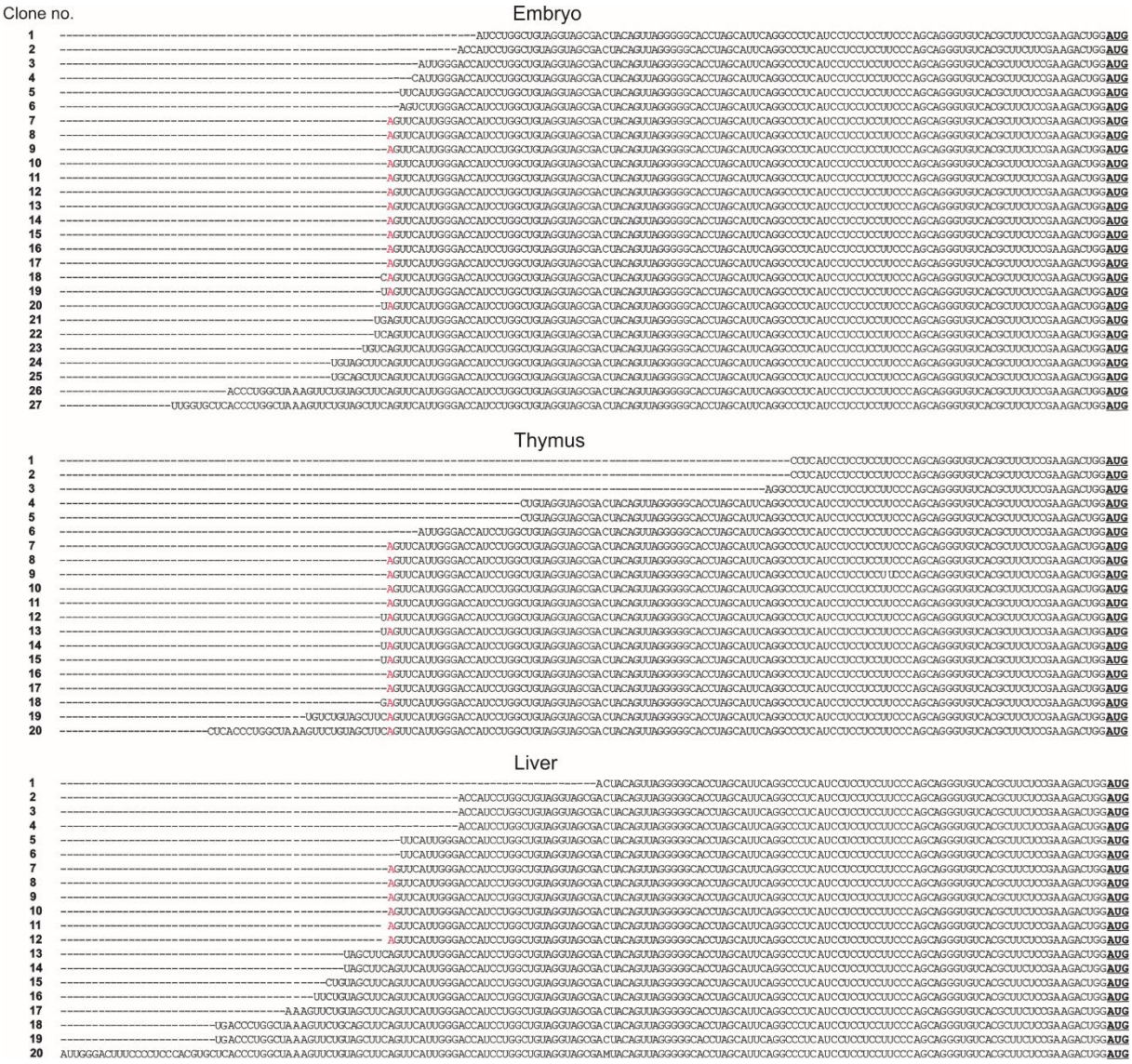


Figure S2

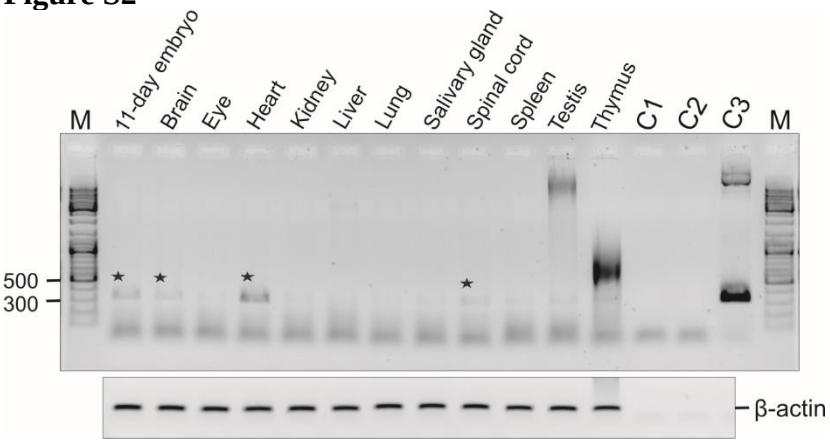
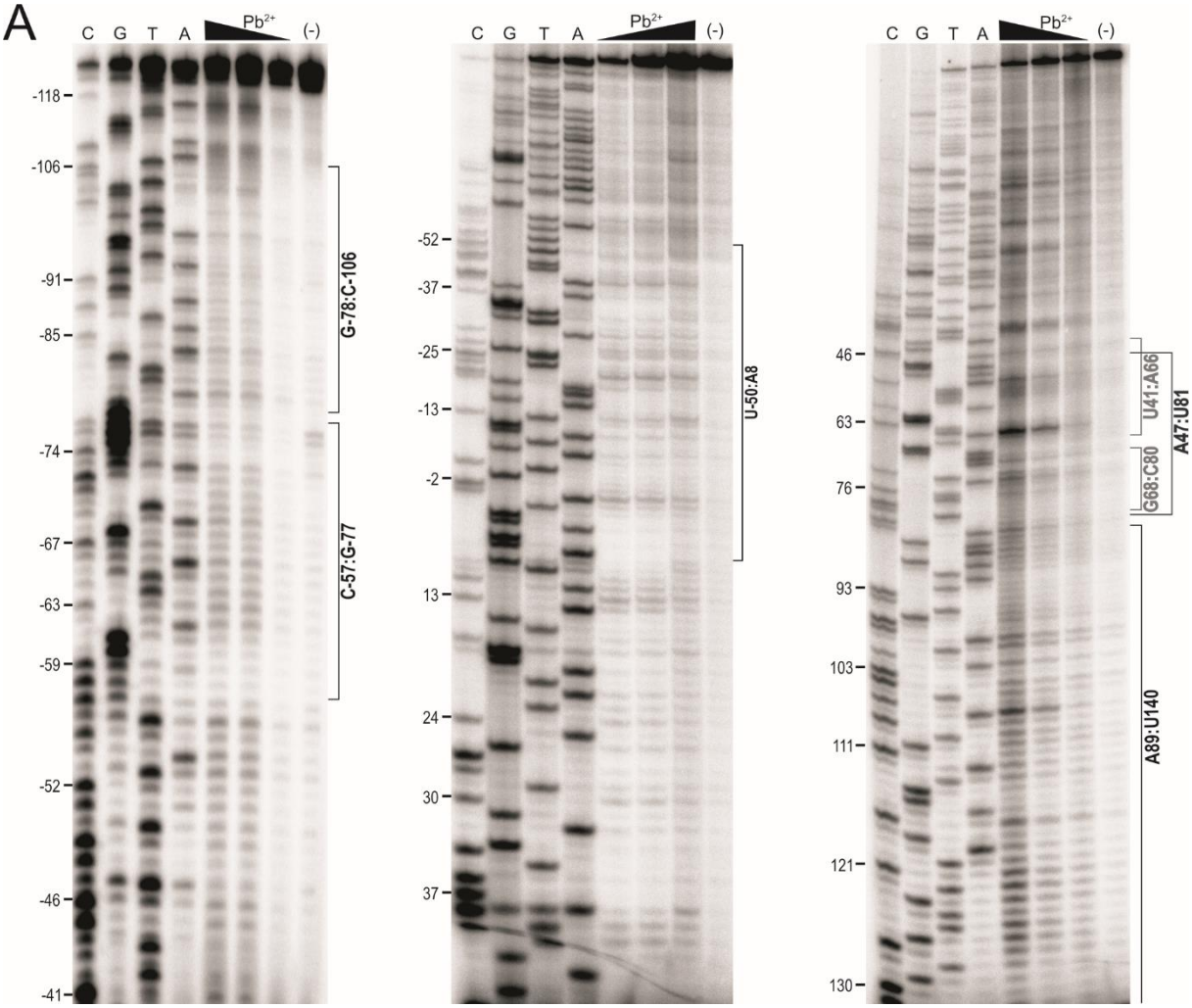
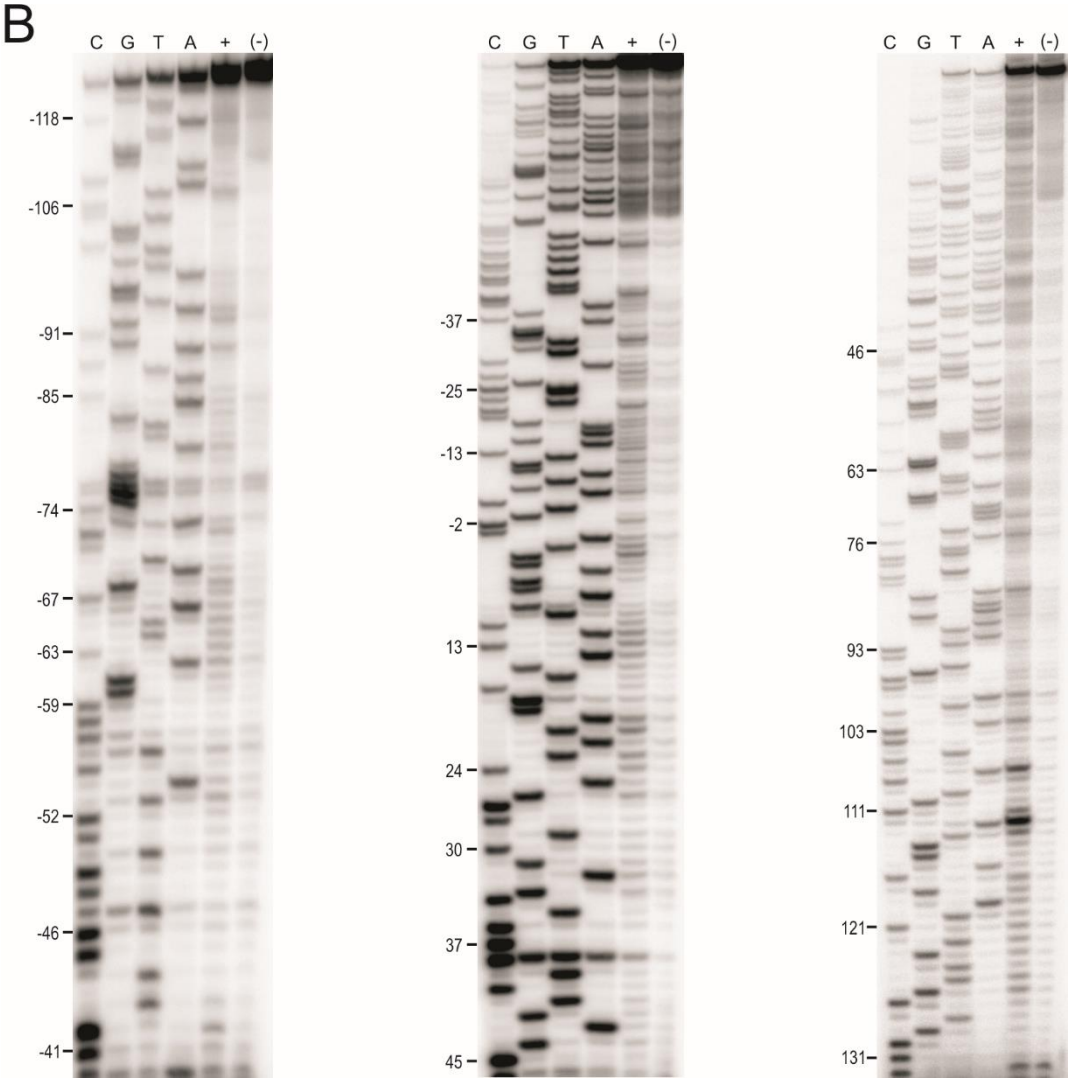


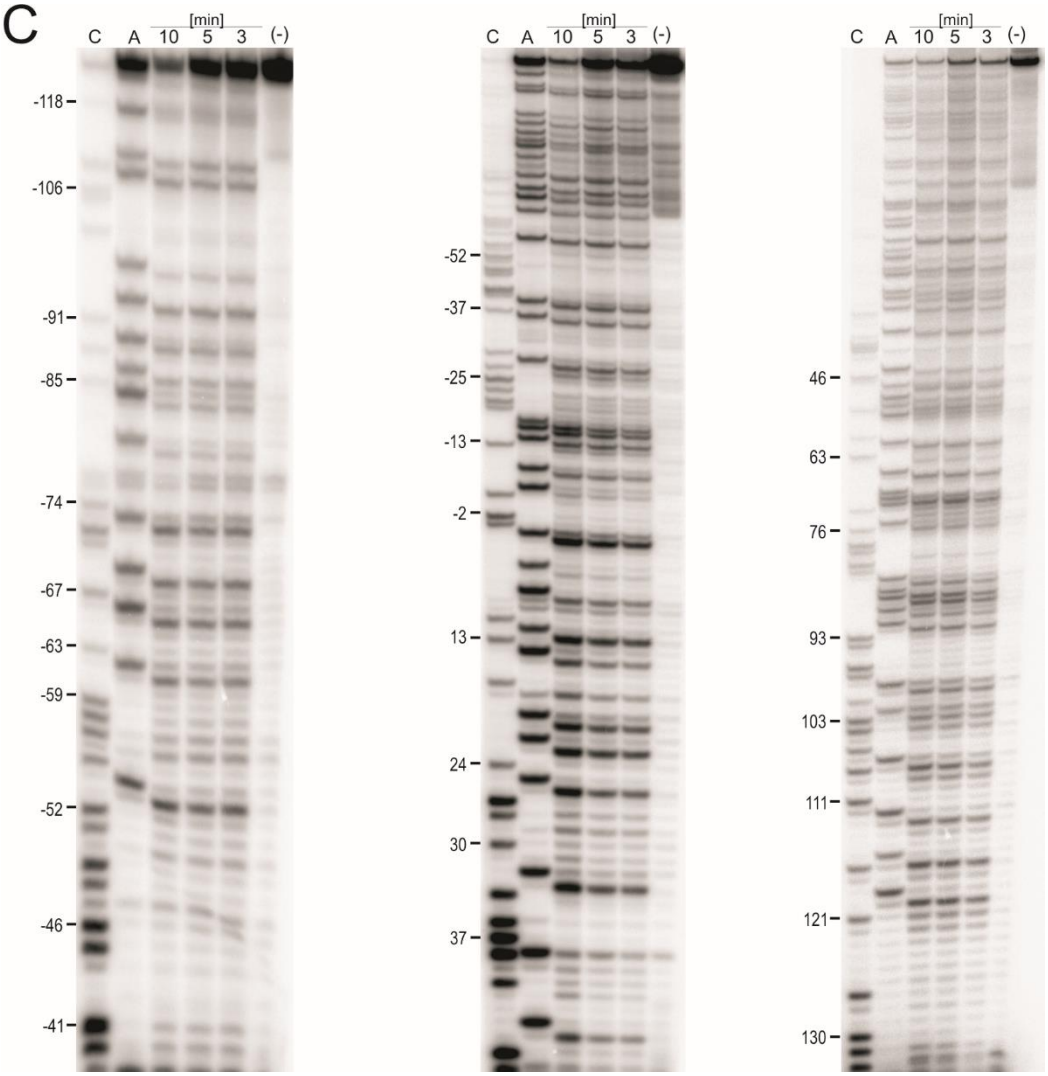
Figure S3


A
C G T A Pb2+ (-)
-118
-106
-91
-85
-74
-67
-63
-59
-52
-46
-41
G-78:C-106
C-57:G-77
C G T A Pb2+ (-)
-52
-37
-25
-13
-2
13
24
30
37
U-50:A8
C G T A Pb2+ (-)
46
63
76
93
103
111
121
130
U41:A66
G68:C80
A47:U81
A89:U140

B


C G T A + (-)
-118
-106
-91
-85
-74
-67
-63
-59
-52
-46
-41
C G T A + (-)
-37
-25
-13
-2
13
24
30
37
45
C G T A + (-)
46
63
76
93
103
111
121
131

C


C A [min] 10 5 3 (-)
-118
-106
-91
-85
-74
-67
-63
-59
-52
-46
-41
C A [min] 10 5 3 (-)
-52
-37
-25
-13
-2
13
24
30
37
C A [min] 10 5 3 (-)
46
63
76
93
103
111
121
130

D

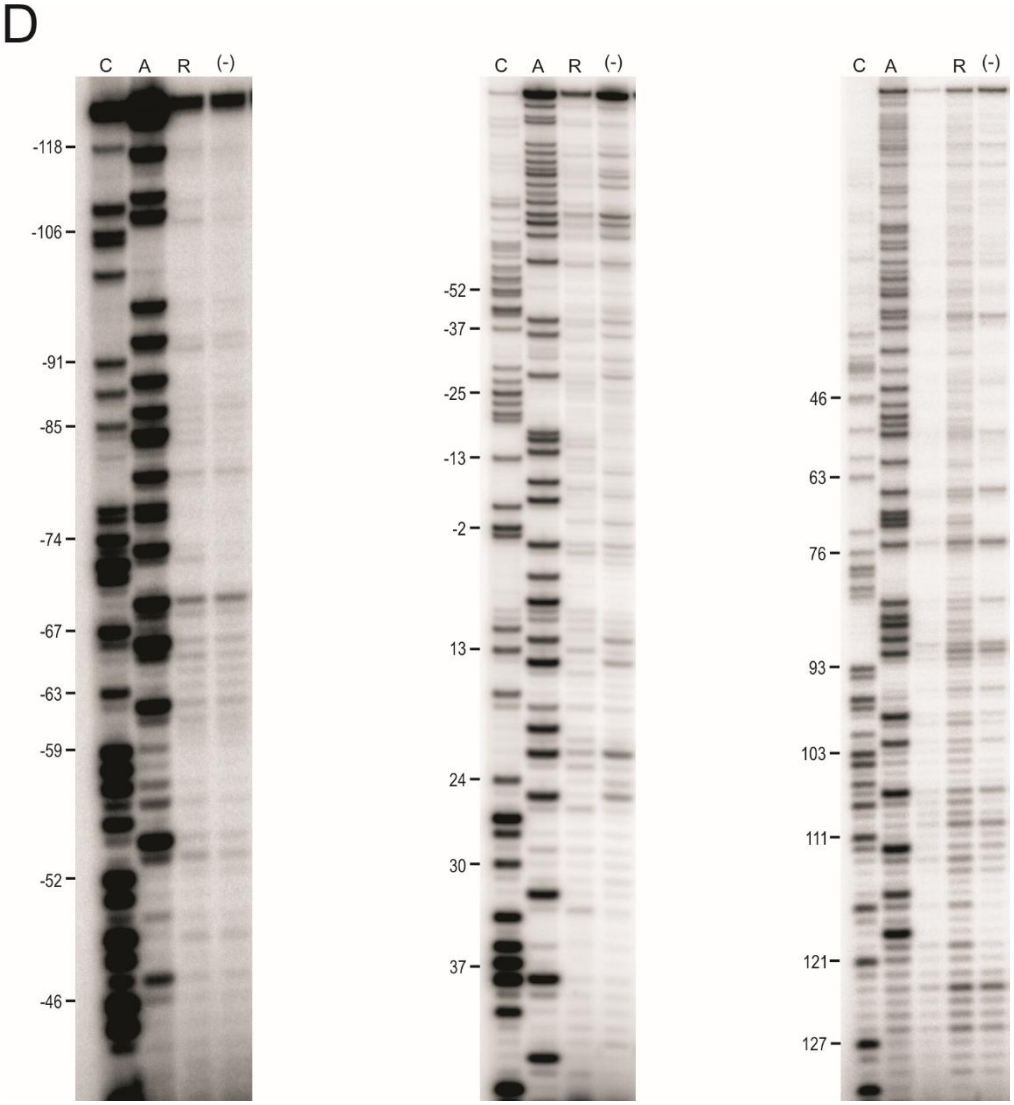

C A R (-)
-118
-106
-91
-85
-74
-67
-63
-59
-52
-46
C A R (-)
-52
-37
-25
-13
-2
13
24
30
37
C A R (-)
46
63
76
93
103
111
121
127

Figure S4

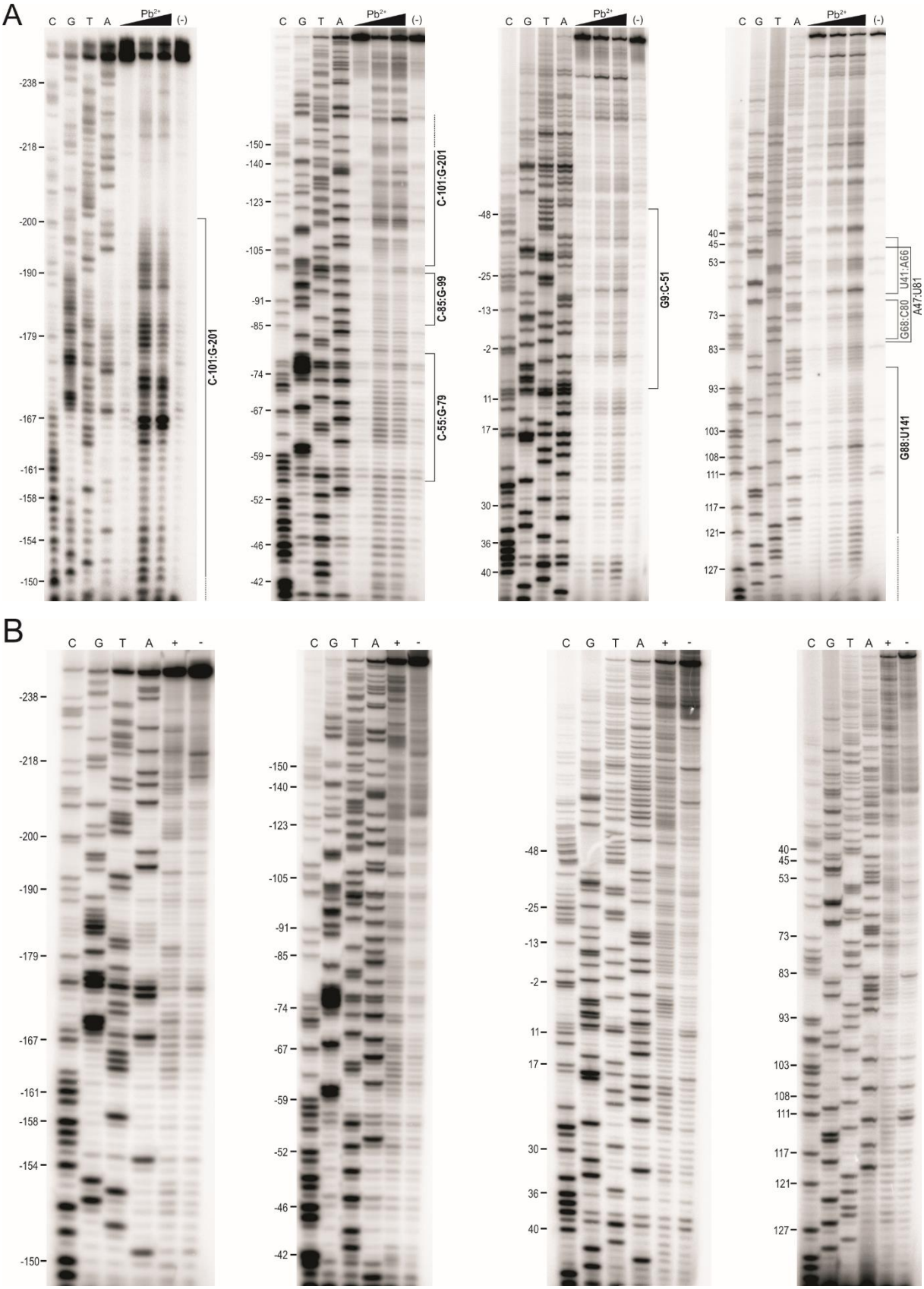
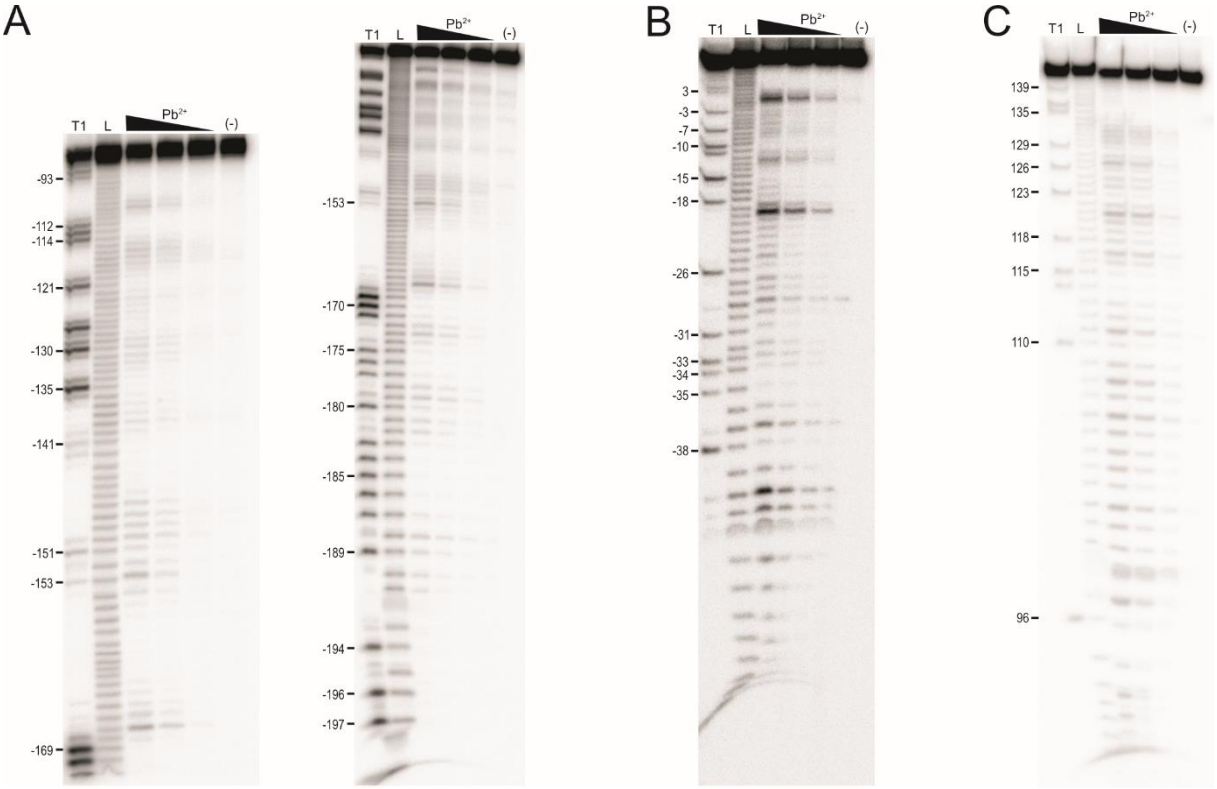

A
C G T A Pb2+ (-)
C-101:G-201
C-101:G-201
C-85:G-99
C-55:G-79
G9:C-51
G68:C80
U41:A66
A47:U81
G88:U141
B
C G T A + -

Figure S5


A
T1
L
Pb2+
(-)
-93
-112
-114
-121
-130
-135
-141
-151
-153
-169
-153
-170
-175
-180
-185
-189
-194
-196
-197
B
3
-3
-7
-10
-15
-18
-26
-31
-33
-34
-35
-38
C
139
135
129
126
123
118
115
110
96