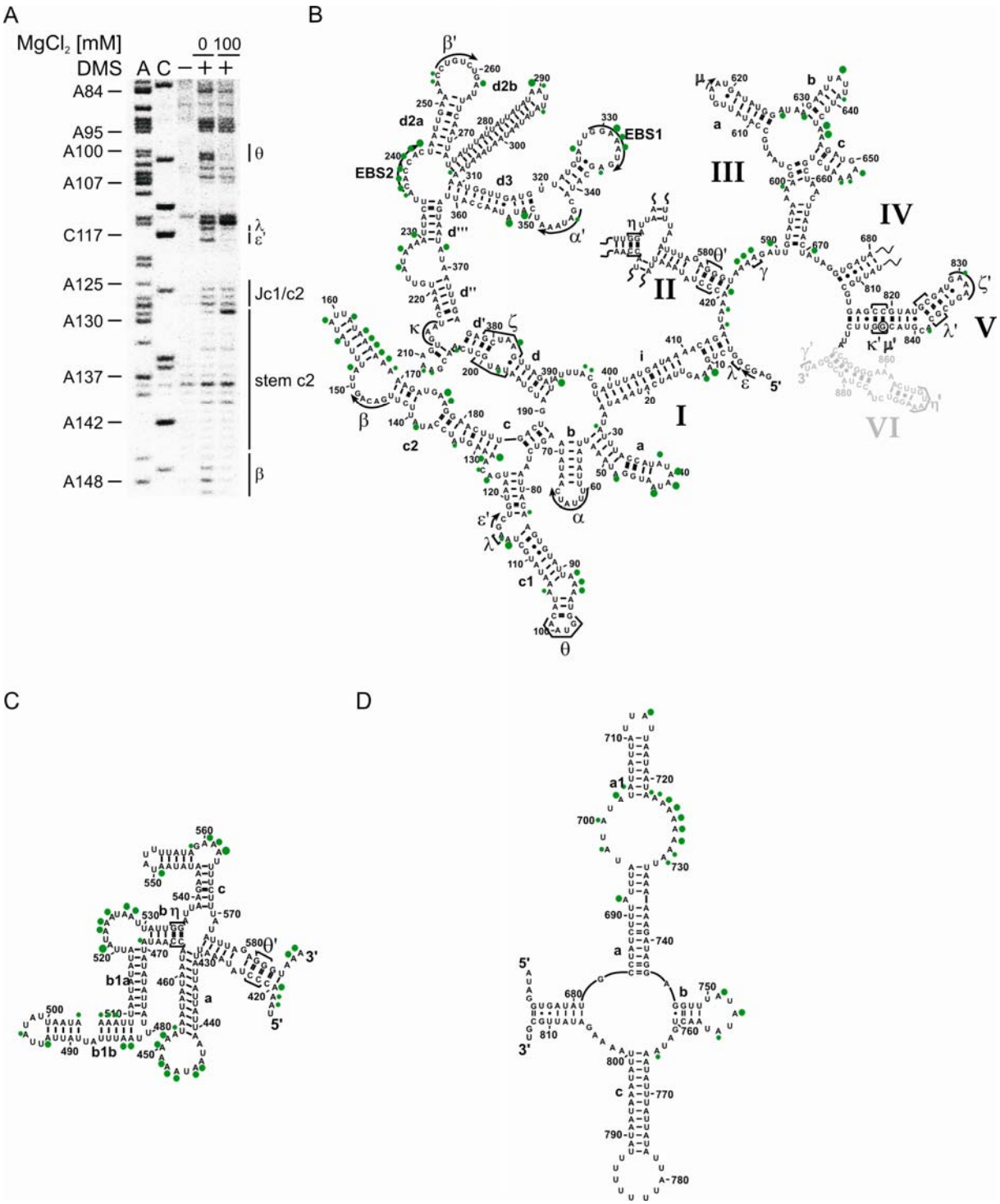
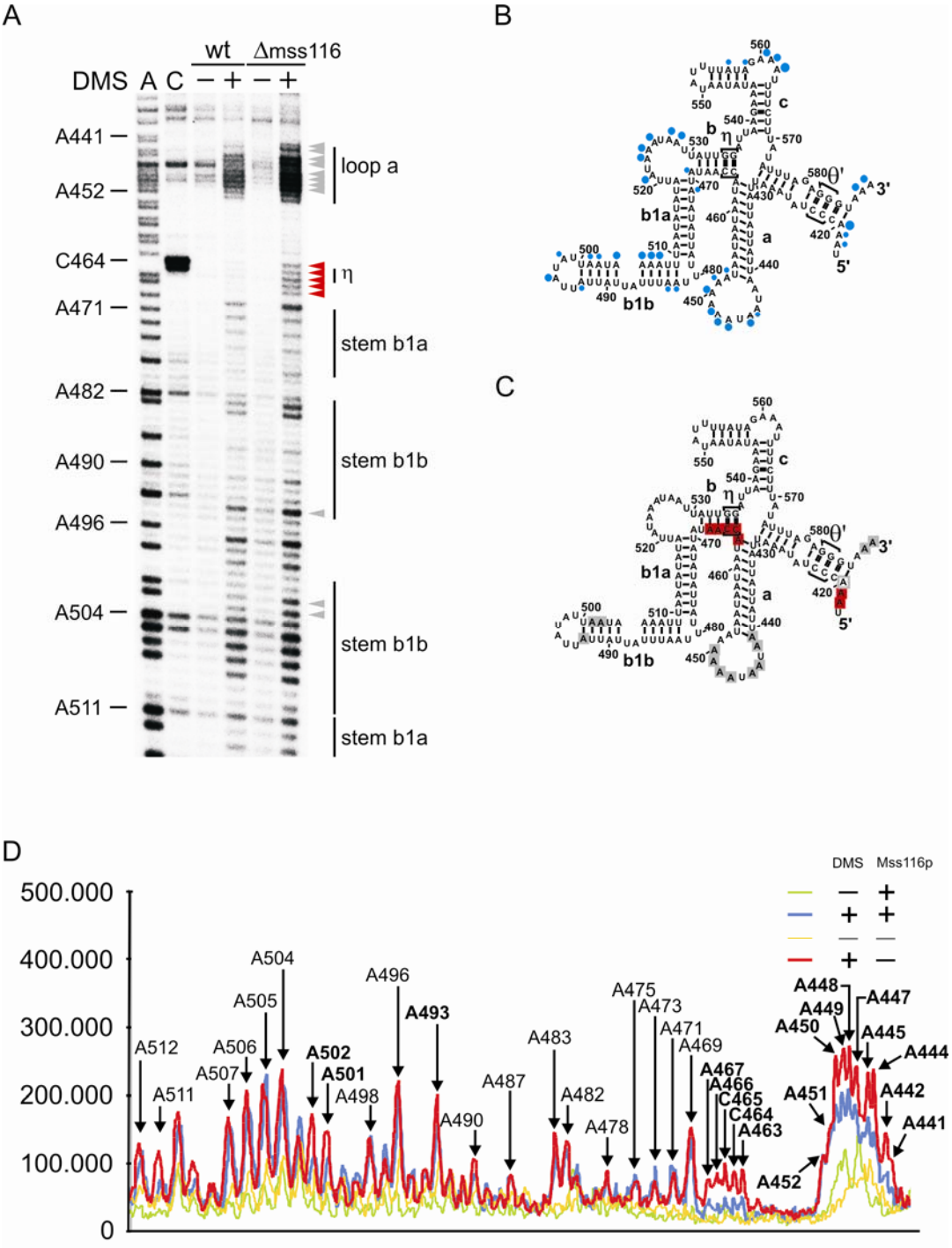
SUPPLEMENTARY FIGURES & TABLE



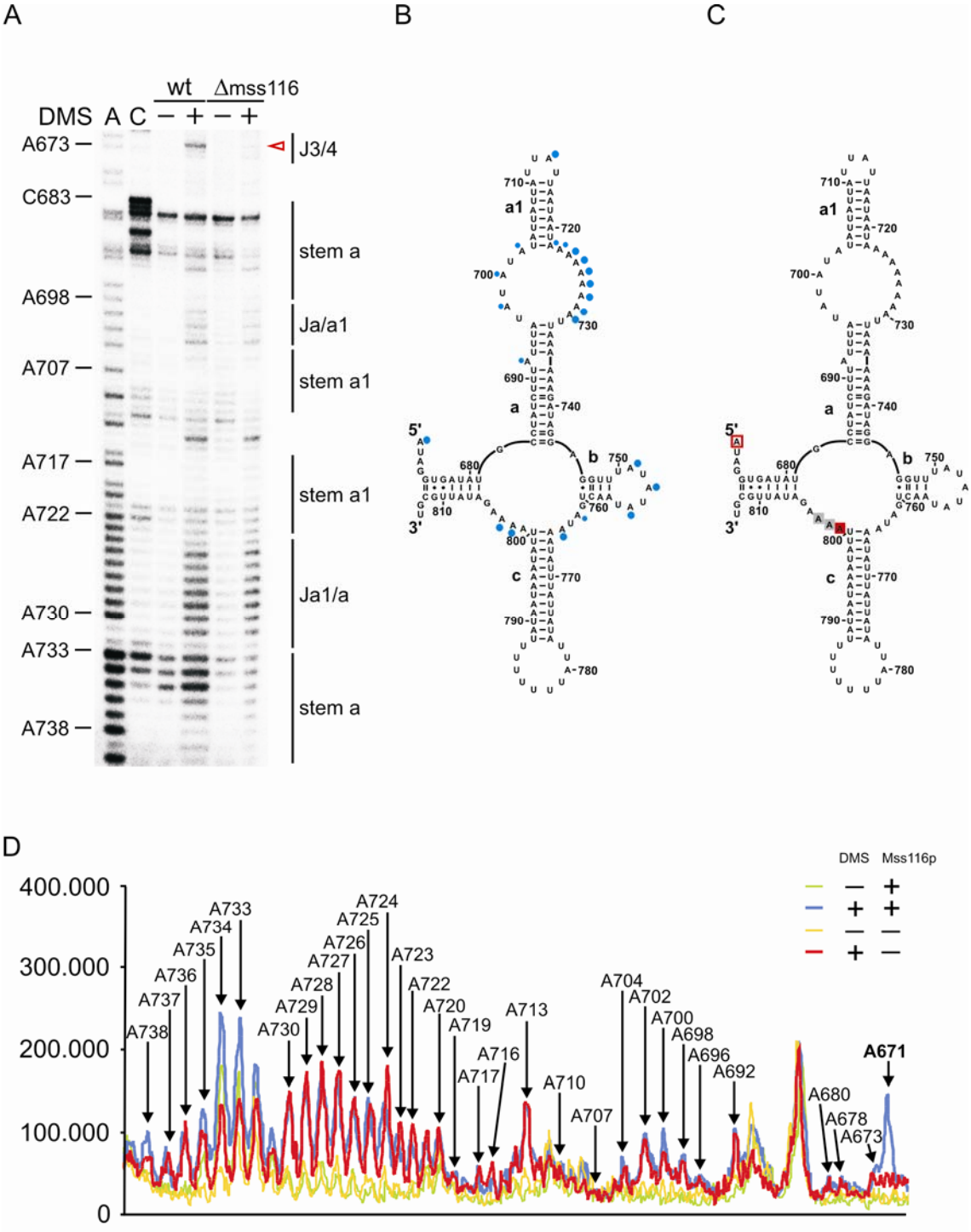
Supplementary Figure 1. Structural probing of the D123456 ai5γ ribozyme *in vitro*. (A) Representative primer extension gel showing the *in vitro* DMS modification pattern of the

ai5γ D123456 ribozyme – region at the c-c1-c2 three-way junction. This ribozyme construct resembles the spliced intron, as it contains all intron domains in their full length, but the exon sequences were deleted. A and C denote sequencing lanes. In the – lane natural stops of the Reverse Transcriptase are seen. In the + lanes the *in vitro* DMS pattern of the unfolded ribozyme (in the absence of Mg^{2+}) and of the folded RNA (in 100mM Mg^{2+}) is shown. (B) Summary map for the folded RNA (in 100mM Mg^{2+}): residues methylated by DMS *in vitro* are indicated with green filled circles. The size of the filled circles correlates with the relative modification intensity of individual bases. Notably, in the D123456 ribozyme domain 6 was not mapped, as it served as primer binding site to map the very 3' part of the intron. (C) Summary map of D2: residues methylated by DMS *in vitro* are indicated with green filled circles. The size of the filled circles correlates with the relative modification intensity of individual bases. (D) Summary map of D4: residues methylated by DMS *in vitro* are indicated with green filled circles. The size of the filled circles correlates with the relative modification intensity of individual bases.



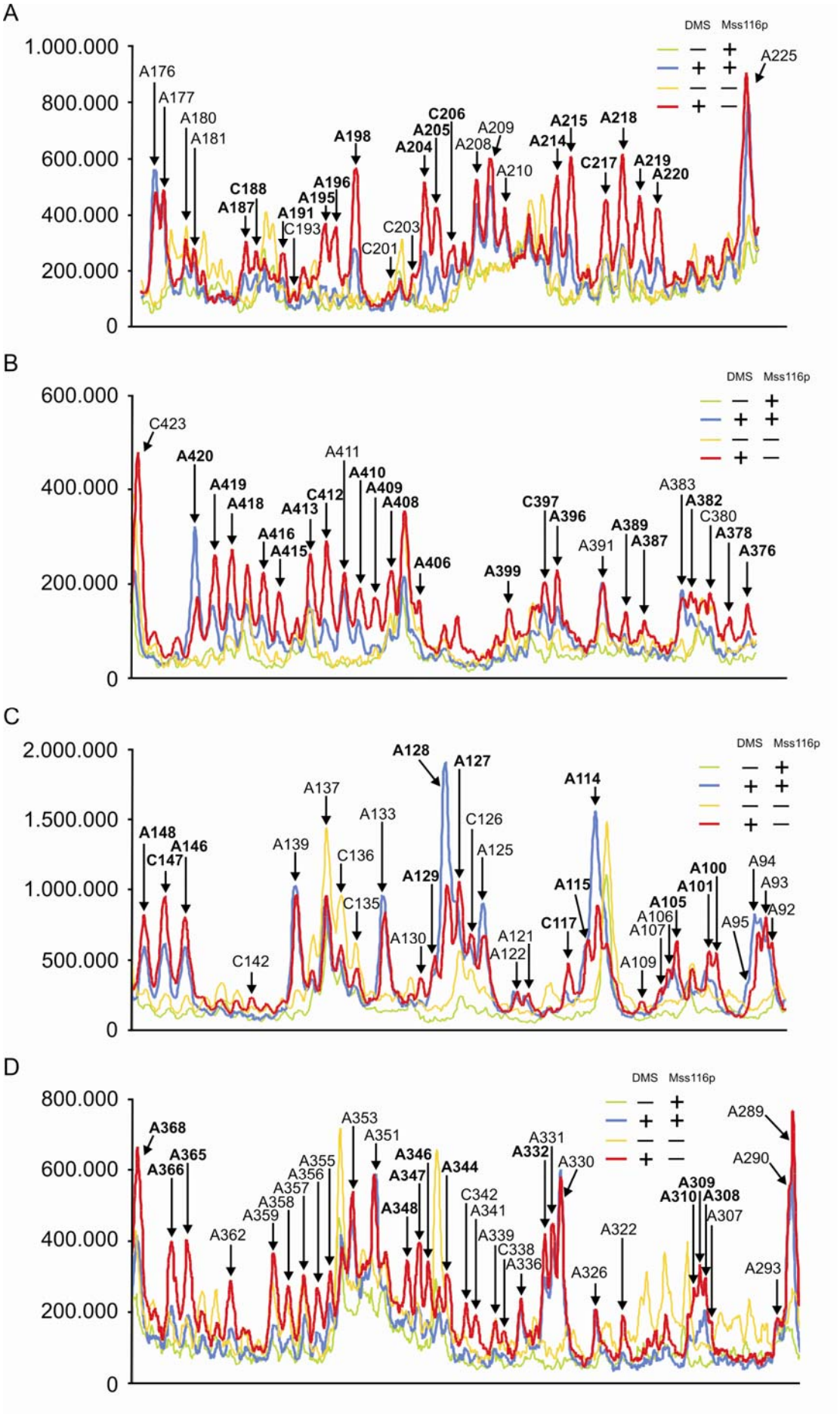
Supplementary Figure 2. Domain 2. (A) Representative gel showing the accessibility of residues in domain 2 in the wt and *mss116*-knockout strain. D2 harbors the tetraloop-receptors θ' and η forming long-range tertiary contacts with stem c1 in D1 and D6, respectively. Lanes are designated as in Fig. 1; symbol code as in Fig. 3. (B) Summary map of D2 in which residues methylated by DMS in the wt strain are indicated with blue filled circles. The size of

the filled circles correlates with the relative modification intensity of individual bases. (C) Differential summary map of D2: bases, the modification intensity of which changes in the absence of Mss116p, are highlighted. The closed squares indicate an increase in accessibility, while open squares represent bases with reduced accessibility in the absence of Mss116p. Strong changes in accessibility are displayed in red (>2-fold); while smaller changes are shown in gray (1.5 to 2-fold). (D) The normalized plot displays the modification intensity of nucleotides shown in the gel in panel (A) in the wt strain (blue) and *mss116*-knockout strain (red). The respective RT stop controls are shown in green and yellow, respectively. Nucleotides whose modification intensity changes in the absence of Mss116p are labeled in bold. In contrast, A469, A483, A496, A498 and A504-A507 remain equally modified in both strains and were therefore used for normalization.

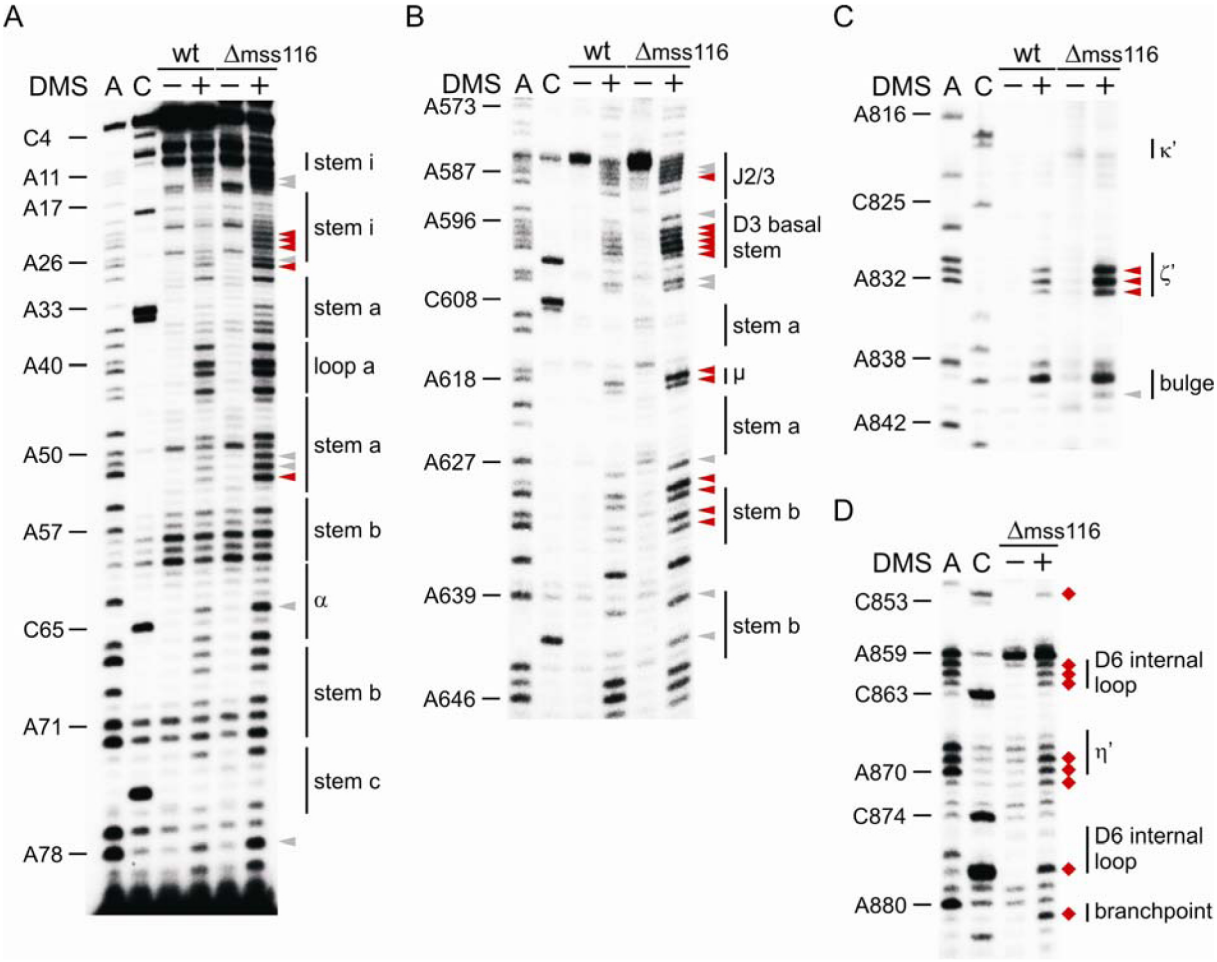


Supplementary Figure 3. Domain 4. (A) Representative gel showing the accessibility of residues in domain 4 in the wt and *mss116*-knockout strain. Lanes are designated as in Fig. 1; symbol code as in Fig. 3. (B) Summary map of D4 in which residues methylated by DMS in the wt strain are indicated with blue filled circles. The size of the filled circles correlates with

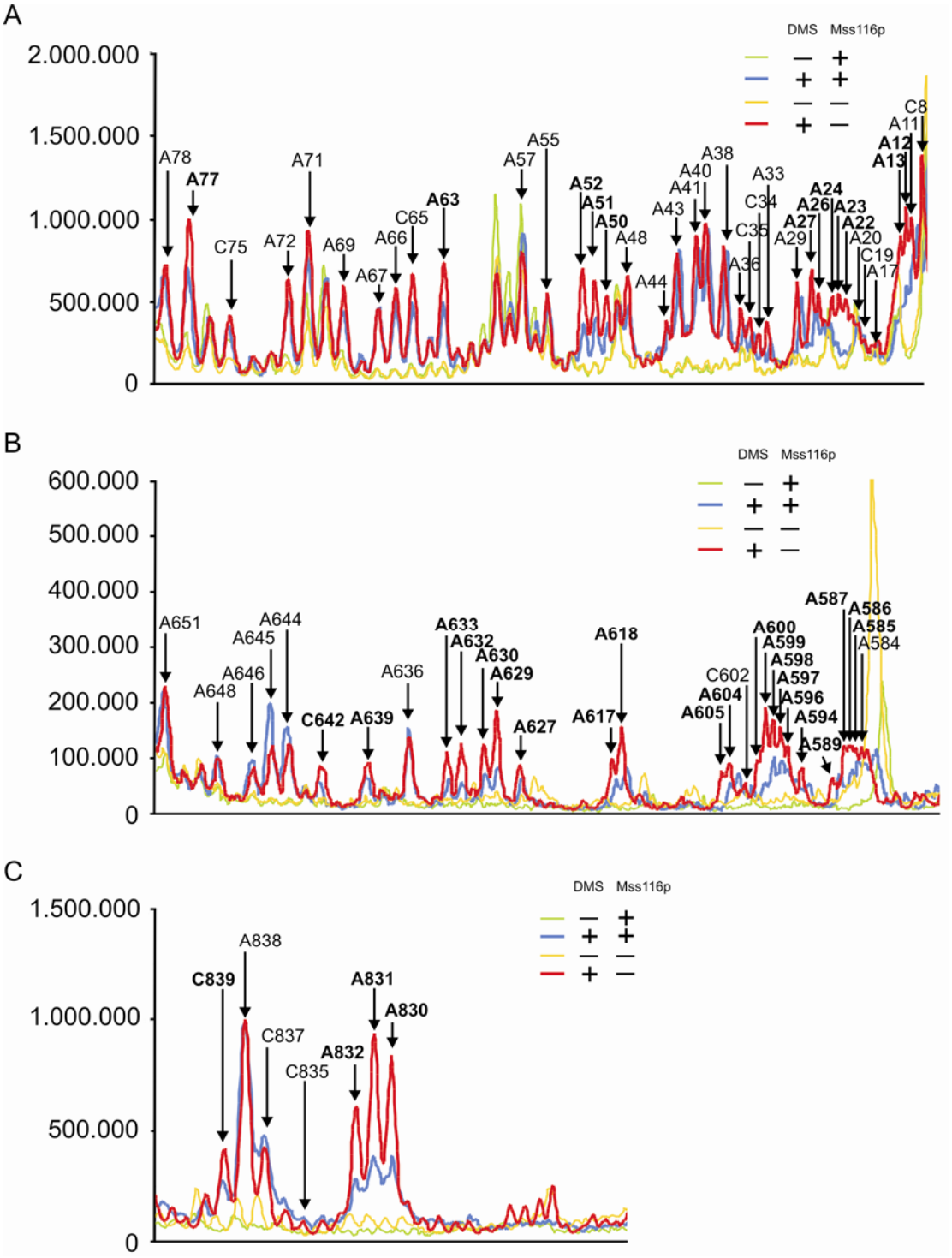
the relative modification intensity of individual bases. (C) Differential summary map of D4: bases, the modification intensity of which changes in the absence of Mss116p, are highlighted. The closed squares indicate an increase in accessibility, while open squares represent bases with reduced accessibility in the absence of Mss116p. Strong changes in accessibility are displayed in red (>2-fold); while smaller changes are shown in gray (1.5 to 2-fold). (D) The normalized plot displays the modification intensity of nucleotides shown in the gel in panel (A) in the wt strain (blue) and *mss116*-knockout strain (red). The respective RT stop controls are shown in green and yellow, respectively. Nucleotides whose modification intensity changes in the absence of Mss116p are labeled in bold. In contrast, A692, A702, A713, A725-A730, and A736-A737 remain equally modified in both strains and were therefore used for normalization.


A
DMS Mss116p
1.000.000
800.000
600.000
400.000
200.000
0
A176
A177
A180
A181
C188
A187
A191
C193
A195
A196
A198
C201
C203
A204
A205
C206
A208
A209
A210
A214
A215
C217
A218
A219
A220
A225
B
DMS Mss116p
600.000
400.000
200.000
0
C423
A420
A419
A418
A416
A415
A413
C412
A411
A410
A409
A408
A406
A399
C397
A396
A391
A389
A387
A383
A382
C380
A378
A376
C
DMS Mss116p
2.000.000
1.500.000
1.000.000
500.000
0
A148
C147
A146
C142
A139
A137
C136
A133
C135
A130
A128
A127
C126
A125
A129
A121
A122
A114
A115
C117
A109
A106
A107
A105
A100
A101
A95
A94
A93
A92
D
DMS Mss116p
800.000
600.000
400.000
200.000
0
A368
A365
A366
A362
A359
A358
A357
A356
A355
A353
A351
A348
A347
A346
A344
C342
A341
A339
C338
A336
A332
A331
A330
A326
A322
A309
A310
A308
A307
A289
A290
A293

Supplementary Figure 4. Mss116p promotes formation of the κ–ζ element *in vivo*. Normalized plots are shown for the representative gels shown in Figs. 3 and 4. Comparable plots were prepared for all gels (and for at least three independent experiments) and the data were summarized in the differential maps shown in Fig. 5 and Supplementary Figs. 2C and 3C. (A) The plot shows the modification intensity of nucleotides in the 5' part the κ–ζ element in the wt strain (blue) and *mss116*-knockout strain (red). The respective RT stop controls are shown in green and yellow, respectively. Nucleotides whose modification intensity changes in the absence of Mss116p are labeled in bold. In contrast, A176-A177, A208-A210 and A225 remain equally modified in both strains and were therefore used for normalization. (B) The plot shows the modification intensity of nucleotides in the 3' part the κ–ζ element in both genetic backgrounds. Color code as in (A). Again, nucleotides whose modification intensity changes in the absence of Mss116p are labeled in bold. In contrast, A383, A391 and A411 remain equally modified in both strains and were therefore used for normalization. (C) The plot shows the modification intensity of nucleotides in the c-c1-c2 three-way junction, which harbors the θ, λ, ε' and β elements forming long-range tertiary contacts with distinct regions of the intron, in both genetic backgrounds. Color code as in (A). Again, nucleotides whose modification intensity changes in the absence of Mss116p are labeled in bold. In contrast, A92-A94, A133 and A139 remain equally modified in both strains and were therefore used for normalization. (D) The plot shows the modification intensity of nucleotides in the in stem d3 including the exon binding site 1 (EBS1) and the α' element, which forms an intra-domain contact with the closing loop of stem b, in both genetic backgrounds. Color code as in (A). Again, nucleotides whose modification intensity changes in the absence of Mss116p are labeled in bold. In contrast, A289-A290, A330-A331, A351 and A353 remain equally modified in both strains and were therefore used for normalization.

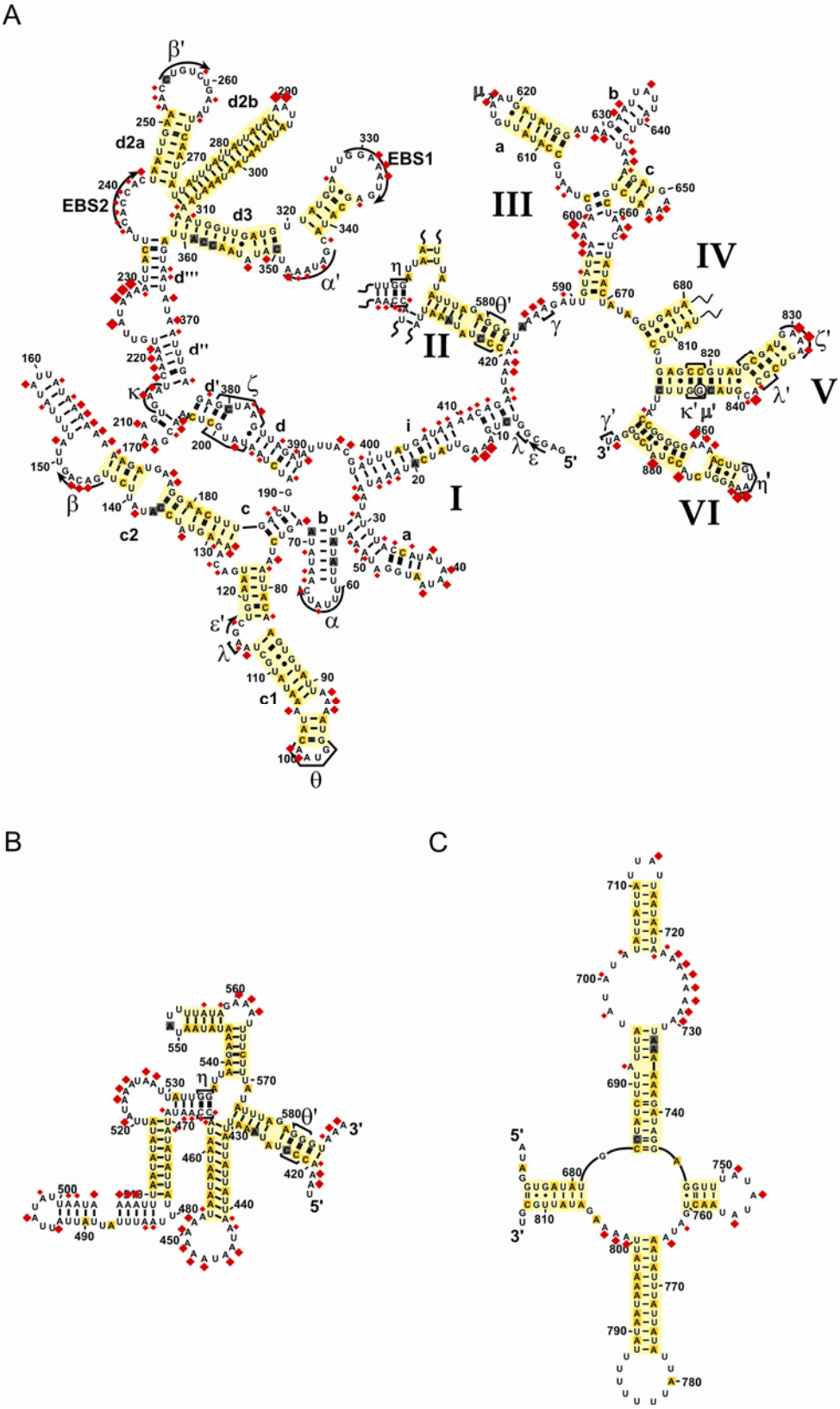


Supplementary Figure 5. Mss116p-induced conformational changes within the ai5γ intron *in vivo.* (A) Representative gel showing the accessibility of residues in stems a, b, c and i of D1 in the wt and *mss116*-knockout strain. Lanes are designated as in Fig. 1; symbol and color code as in Fig. 3. (B) Representative gel showing the accessibility of residues in J2/3 and domain 3 in the wt and *mss116*-knockout strain. Lanes are designated as in Fig. 1; symbol and color code as in Fig. 3. (C) Representative gel showing the accessibility of residues in domain 5 in the wt and *mss116*-knockout strain. Of particular interest are the dinucleotide bulge and the tetraloop ζ'. Lanes are designated as in Fig. 1; symbol and color code as in Fig. 3. (D) Representative gel showing the *in vivo* DMS modification pattern of D6 of the ai5γ intron in the *mss116*-knockout strain. Sites of modification are indicated with a red diamond. The normalized plots derived from the gels of panel (A)-(C) are shown in Supplementary Fig. 6.



Supplementary Figure 6. Mss116p promotes formation of tertiary interactions *in vivo*. Normalized plots are shown for the representative gels shown in Supplementary Fig. 5. Comparable plots were prepared for all gels (and for at least three independent experiments) and the data were summarized in the differential maps shown in Fig. 5 and Supplementary Figs. 2C and 3C. (A) The plot shows the modification intensity of residues in stems a, b, c and

i of D1 in the wt strain (blue) and *mss116*-knockout strain (red). The respective RT stop controls are shown in green and yellow, respectively. Nucleotides whose modification intensity changes in the absence of Mss116p are labeled in bold. In contrast, A29, A38, A40-A41, A43, A55, A67, C75, and A78 remain equally modified in both strains and were therefore used for normalization. (B) The plot shows the modification intensity of residues in J2/3 and domain 3 in both genetic backgrounds. Color code as in (A). Again, nucleotides whose modification intensity changes in the absence of Mss116p are labeled in bold. In contrast, A636, A646, A648 and A651 remain equally modified in both strains and were therefore used for normalization. (C) The plot shows the modification intensity of residues in domain 5 in both genetic backgrounds. Color code as in (A). Again, nucleotides whose modification intensity changes in the absence of Mss116p are labeled in bold. In contrast, C837 and A838 remain equally modified in both strains and were therefore used for normalization.



Supplementary Figure 7. In the absence of Mss116p the ai5γ intron does form most of the secondary structure but lacks its tertiary fold. (A) Summary map of residues methylated by DMS in the *mss116*-knockout strain (red filled diamonds), whereby the summary map of

Domain 2 is shown in (B) and that of Domain 4 in (C). The size of the filled diamonds correlates with the relative modification intensity of individual bases. Helical structures formed in the absence of Mss116p are highlighted in yellow (darker yellow boxes indicate the A and C residues that were not methylated). Gray shaded boxes indicate RT stops precluding obtaining information of these A and C residues.

Supplementary Table 1

Oligos used as primers in the reverse transcription reaction.

Oligo [a]	Sequence (5' to 3')
Sc 82	TGTTACCATTTTAATACACTTG
Sc 160	GTTCCTTCATCTTTTTTTTATAA
Sc 234	GTTTTCAATTAGTGGTGTAAG
Sc 312	TTCCAATACATAACATCAACC
Sc 375	GTAAATATCTAACTTAGCTCTC
Sc 440	TTGGTATTATTATTATTTTTTATTATTA
Sc 520	TATTTCTTAATCCAATAATTATTTATA
Sc 588	GCATTAGCTTTTTATACAATC
Sc 656	CACCTATAGTATAAGTTAGCAG
Sc 682	ATAATAATAATATATATATAAATAAAGATAGGC
Sc 754	ATTTATCAGTTATATATAAACCTCC
Sc 805	CGGCTCACGCAATATC
Sc 866	CCCGATAGGTAGACCTTTAC
Sc -6	GAAAATAGCACCCATTGATAATAC

[a] Except for Sc -6, which binds to the downstream exon, all oligos anneal to the intron.