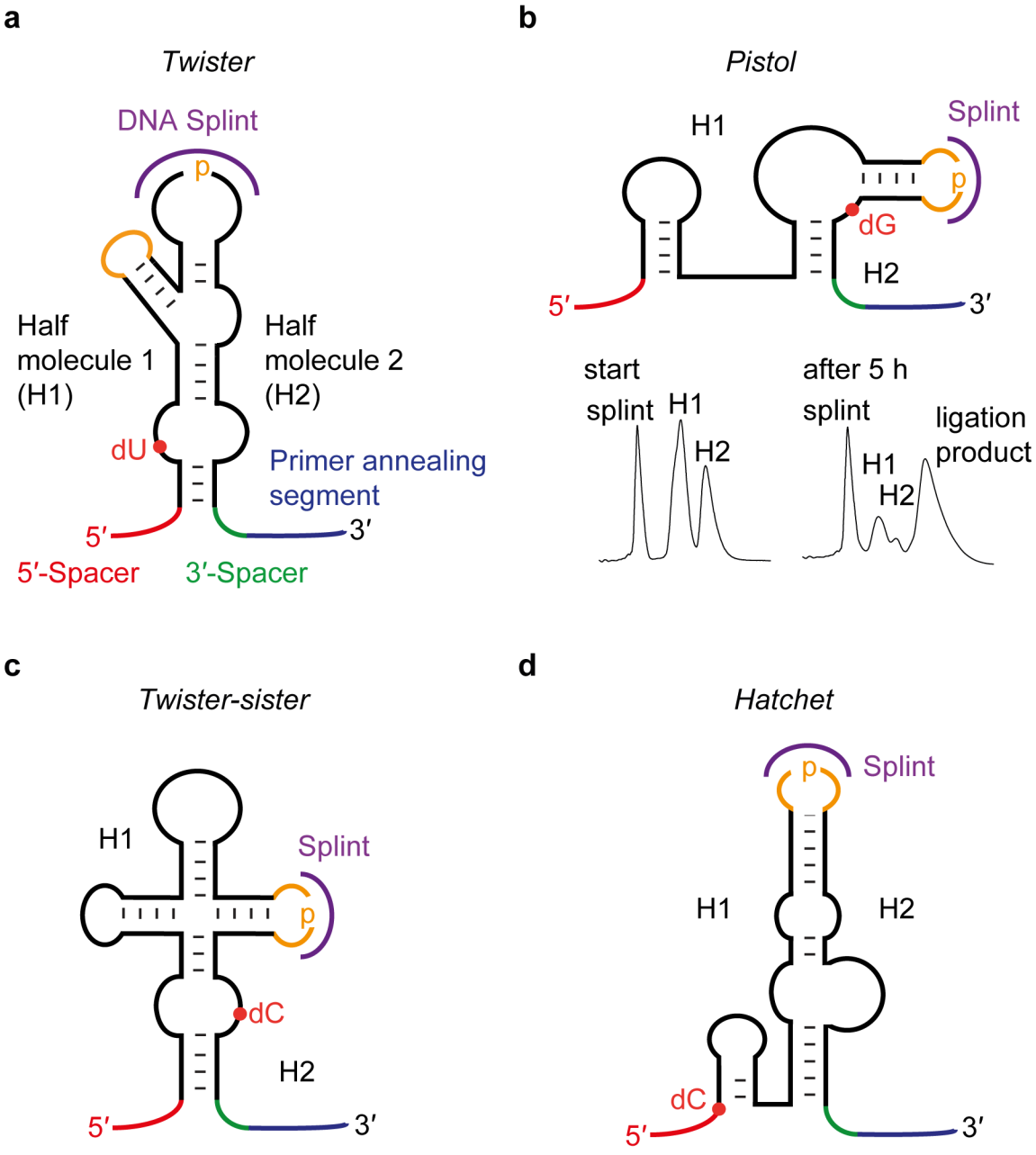
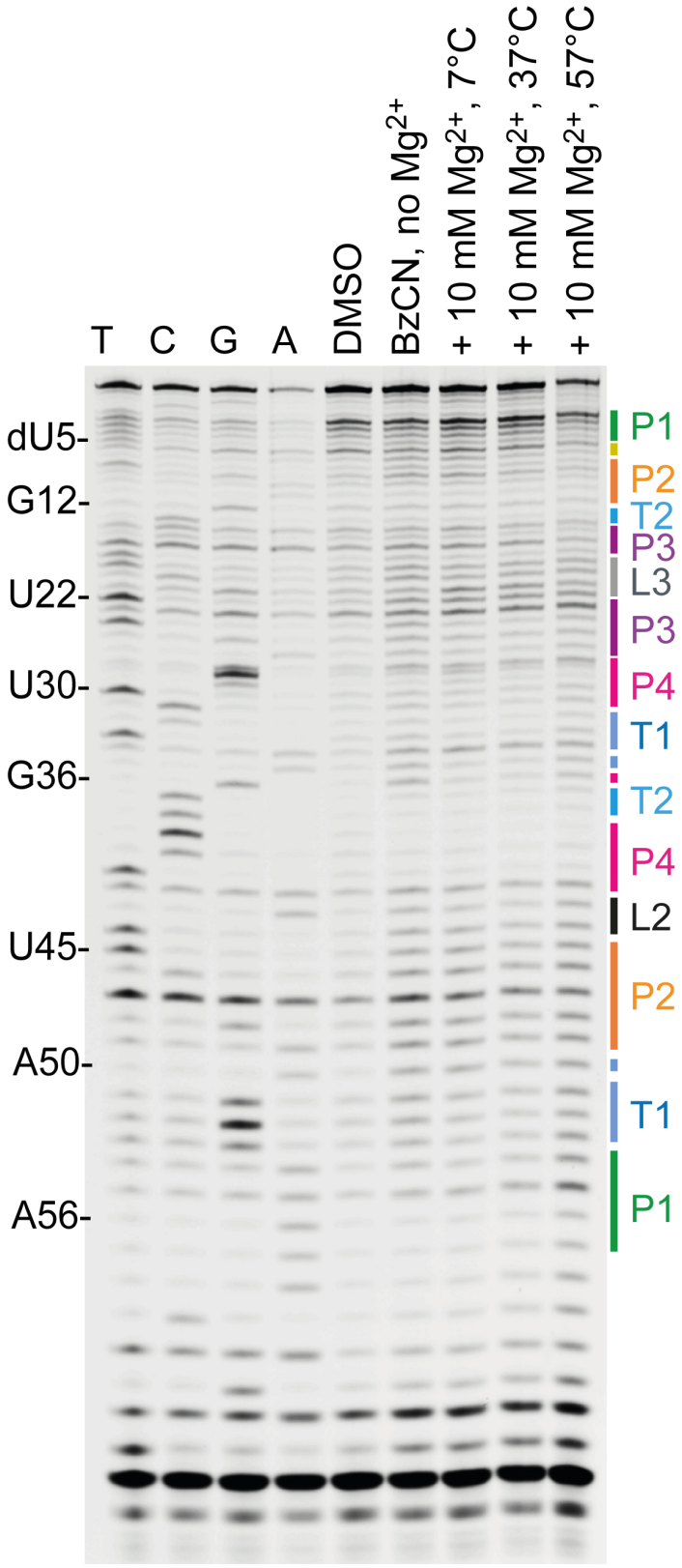
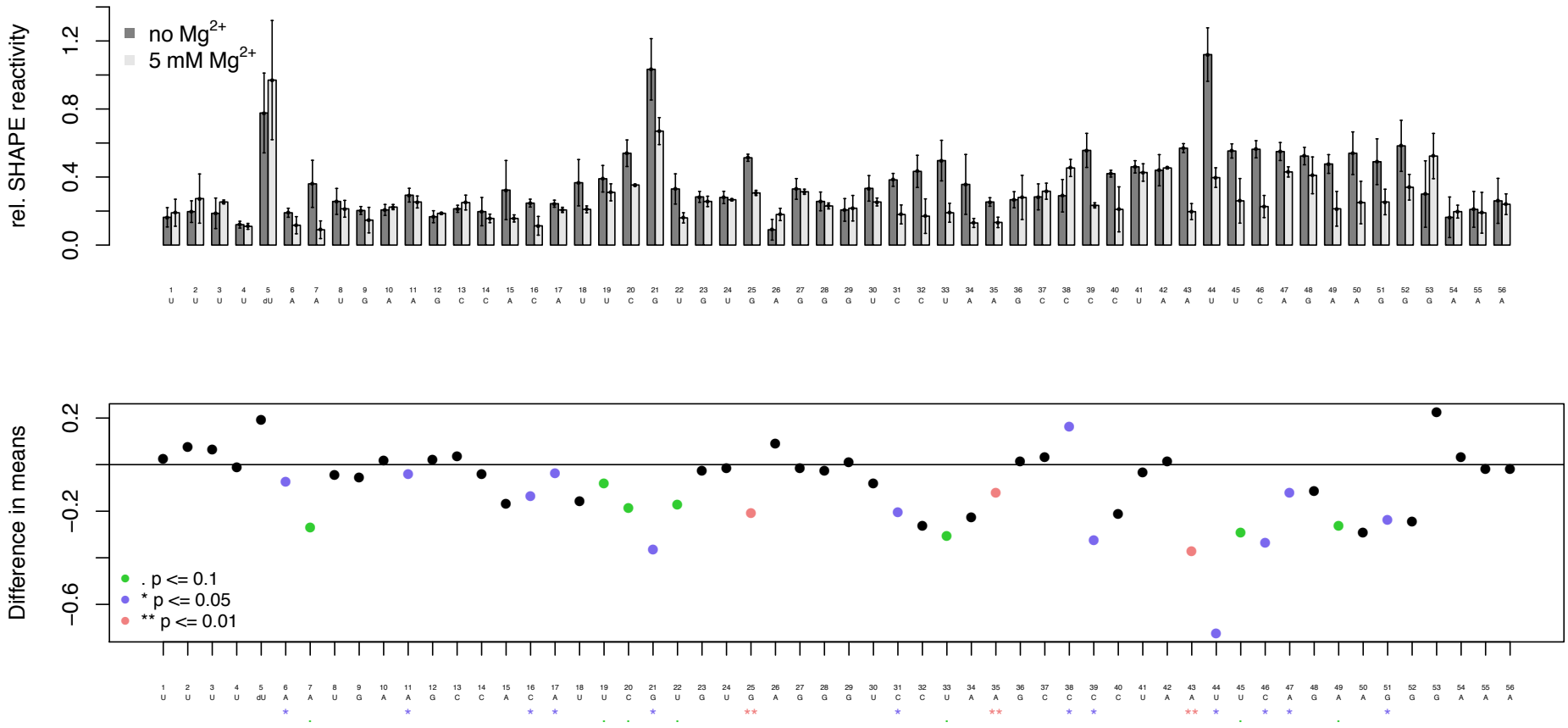


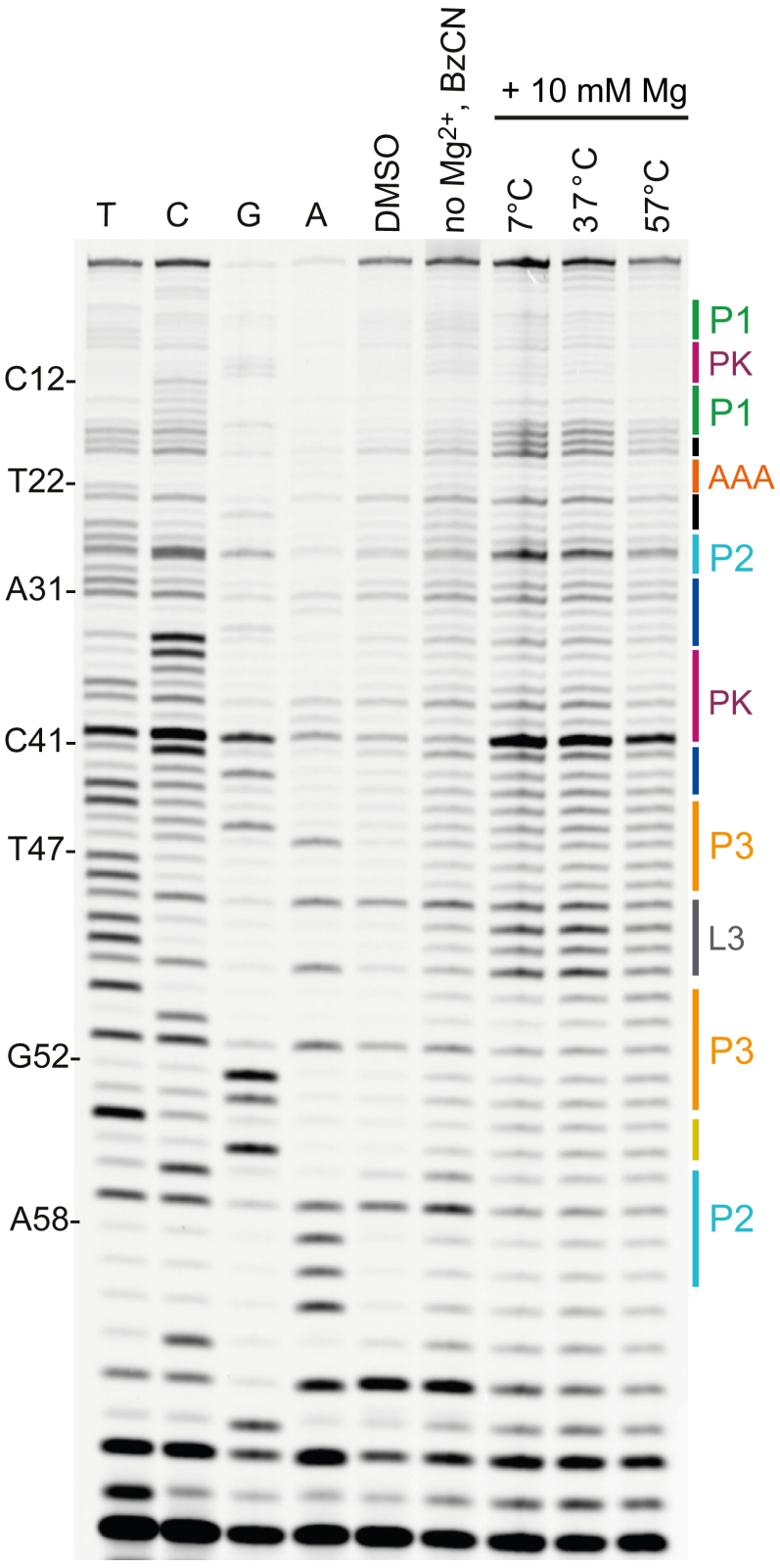
Supporting Figure S1. Preparation of ribozyme constructs for SHAPE probing. (**a**) Oligonucleotide setup used for enzymatic ligation of the twister ribozyme. (**b**) Same as (a) but for pistol ribozyme (upper panel); anion exchange (AE) HPLC analysis of pistol ribozyme ligation using T4 DNA ligase and a DNA splint (start and after 5 h) (lower panel). The ligation product was isolated by AE-HPLC. (**c**) Oligonucleotide setup used for enzymatic ligation of the twister-sister ribozyme. (**d**) Oligonucleotide setup used for enzymatic ligation of the hatchet ribozyme.



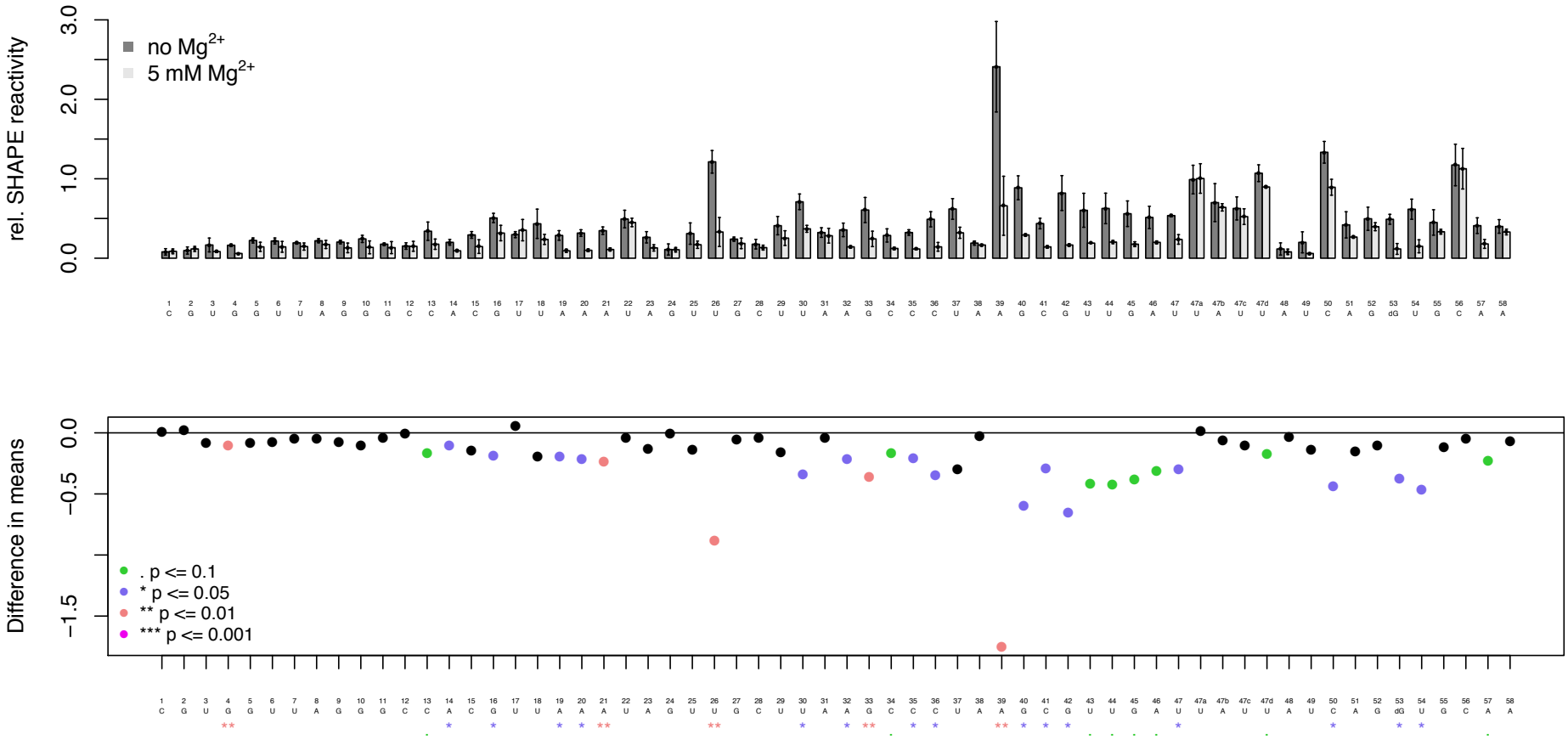
Supporting Figure S2. SHAPE probing of the *env22* twister ribozyme. Second example of a typical gel (see also Figure 1b). Lanes from left to right: T, C, G and A ladders, control in the absence of probing reagent, probing with BzCN, probing with BzCN in the presence of 10 mM $MgCl_2$ at 7, 37, and 57°C. Note that the color-coded stem-loop assignments at the right side of the gel corresponds to the actual residue that is affected (shifted +1 to nucleoside numbering on the left side).



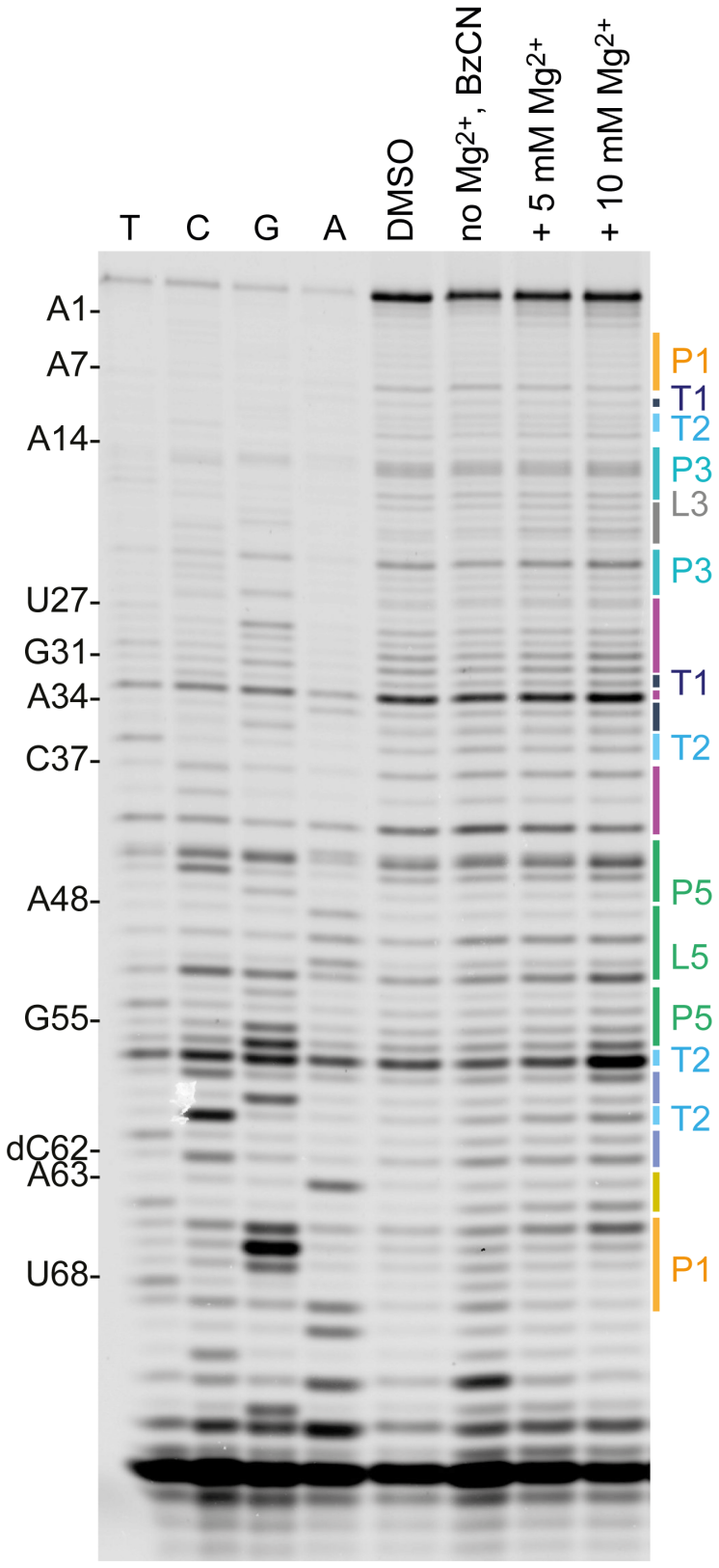
Supporting Figure 3. Quantification of the SHAPE probing data for the complete *env22* twister RNA sequence. The normalized reactivity data of each nucleotide with the mean of three independent experiments are shown (top). The Student's t-test (2-sided paired) for assessing the statistical significance was applied and the corresponding plot is show (bottom).



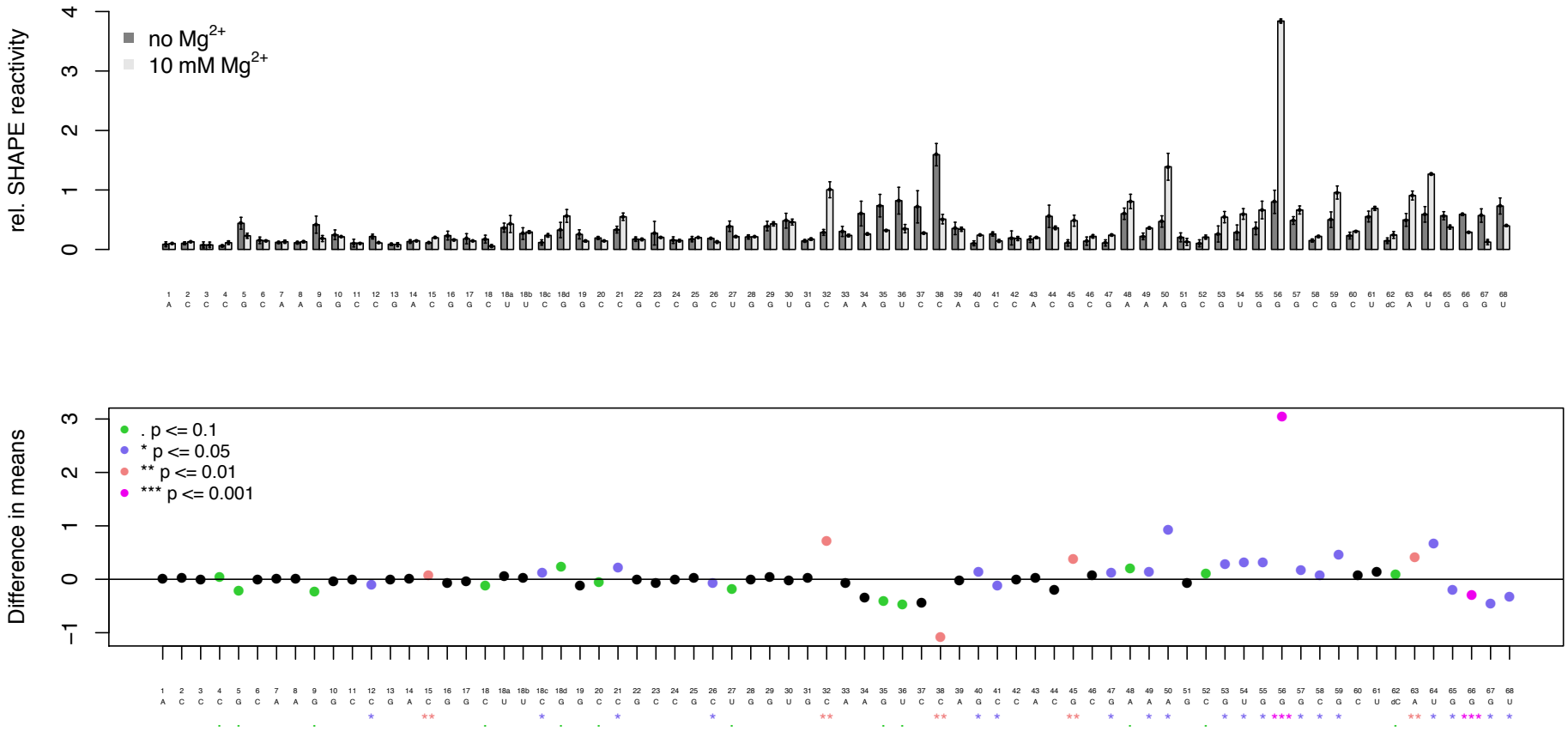
Supporting Figure S4. SHAPE probing of the *env25* pistol ribozyme. Second example of a typical gel (see also Figure 2b). Lanes from left to right: T, C, G and A ladders, control in the absence of probing reagent, probing with BzCN, probing in the presence of 10 mM $MgCl_2$ at 7, 37, and 57°C. Note that the color-coded stem-loop assignments at the right side of the gel corresponds to the actual residue that is affected (shifted +1 to nucleoside numbering on the left side).



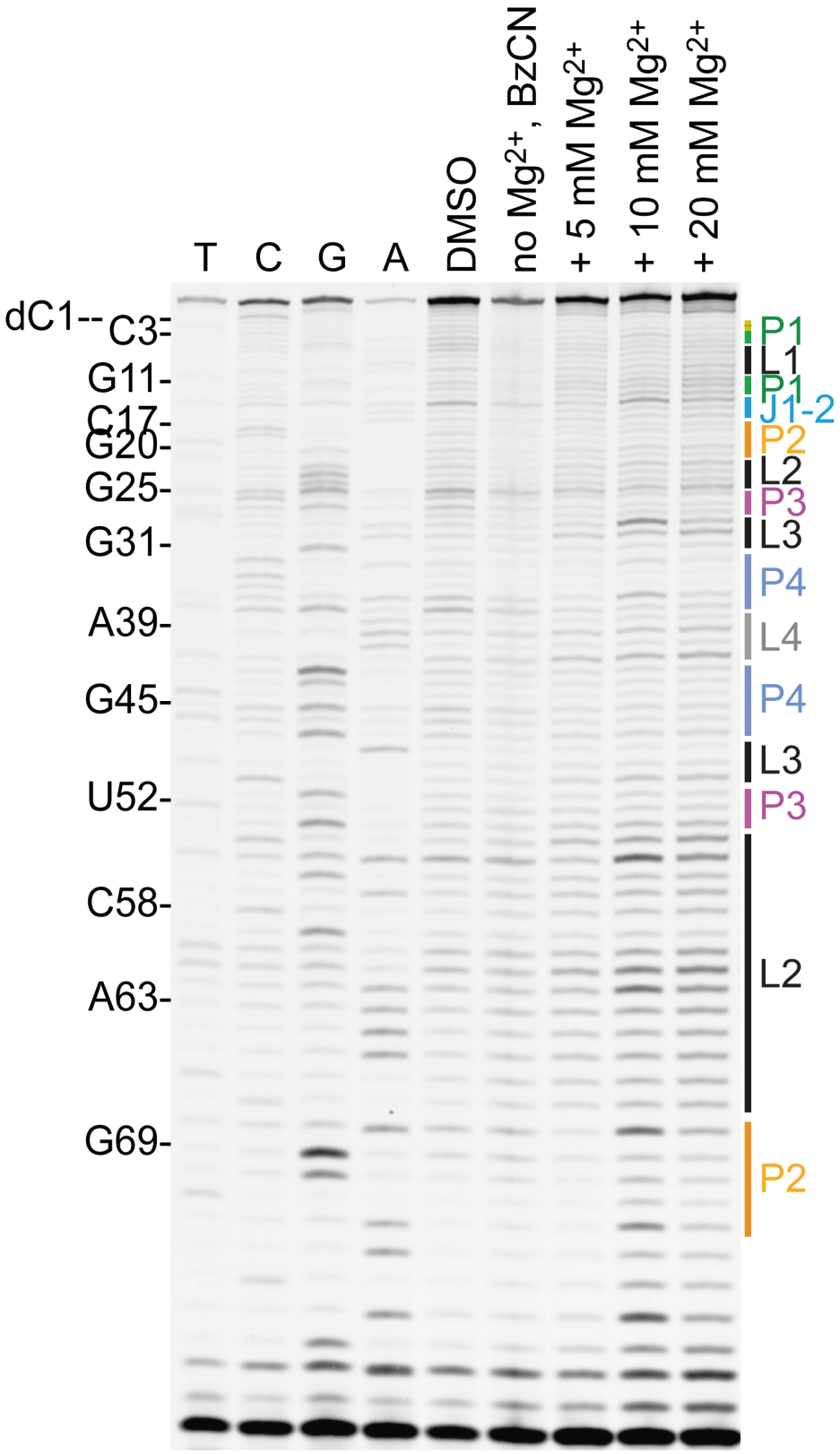
Supporting Figure 5. Quantification of the SHAPE probing data for the complete *env25* pistol RNA sequence. The normalized reactivity data of each nucleotide with the mean of three independent experiments are shown (top). The Student's t-test (2-sided paired) for assessing the statistical significance was applied and the corresponding plot is show (bottom).



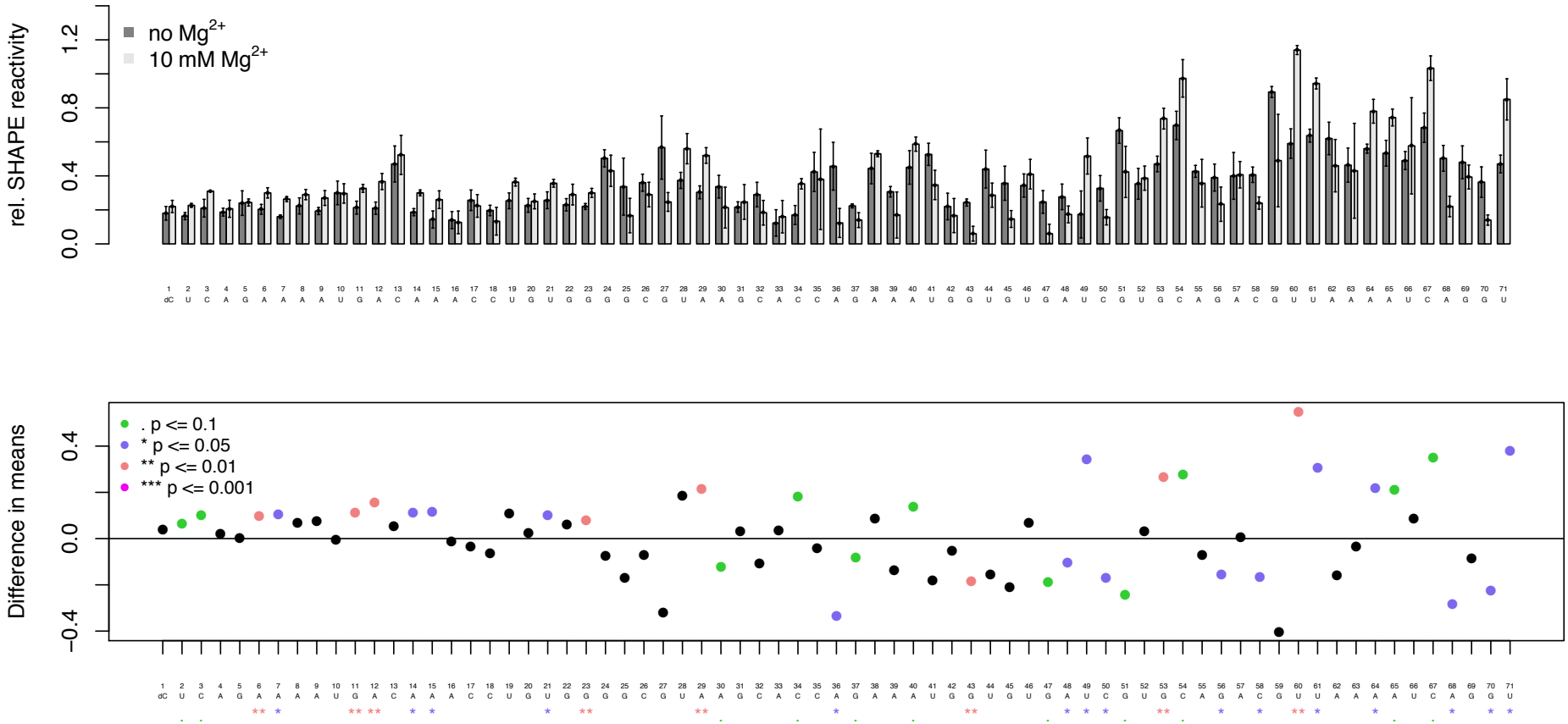
Supporting Figure 6. SHAPE probing of the twister-sister ribozyme. Second example of a typical gel (see also Figure 3b). Lanes from left to right: T, C, G and A ladders, control in the absence of probing reagent, probing with BzCN, probing in the presence of 5 and 10 mM $MgCl_2$ at 37° C. Note that the color-coded stem-loop assignments at the right side of the gel corresponds to the actual residue that is affected (shifted +1 to nucleoside numbering on the left side).



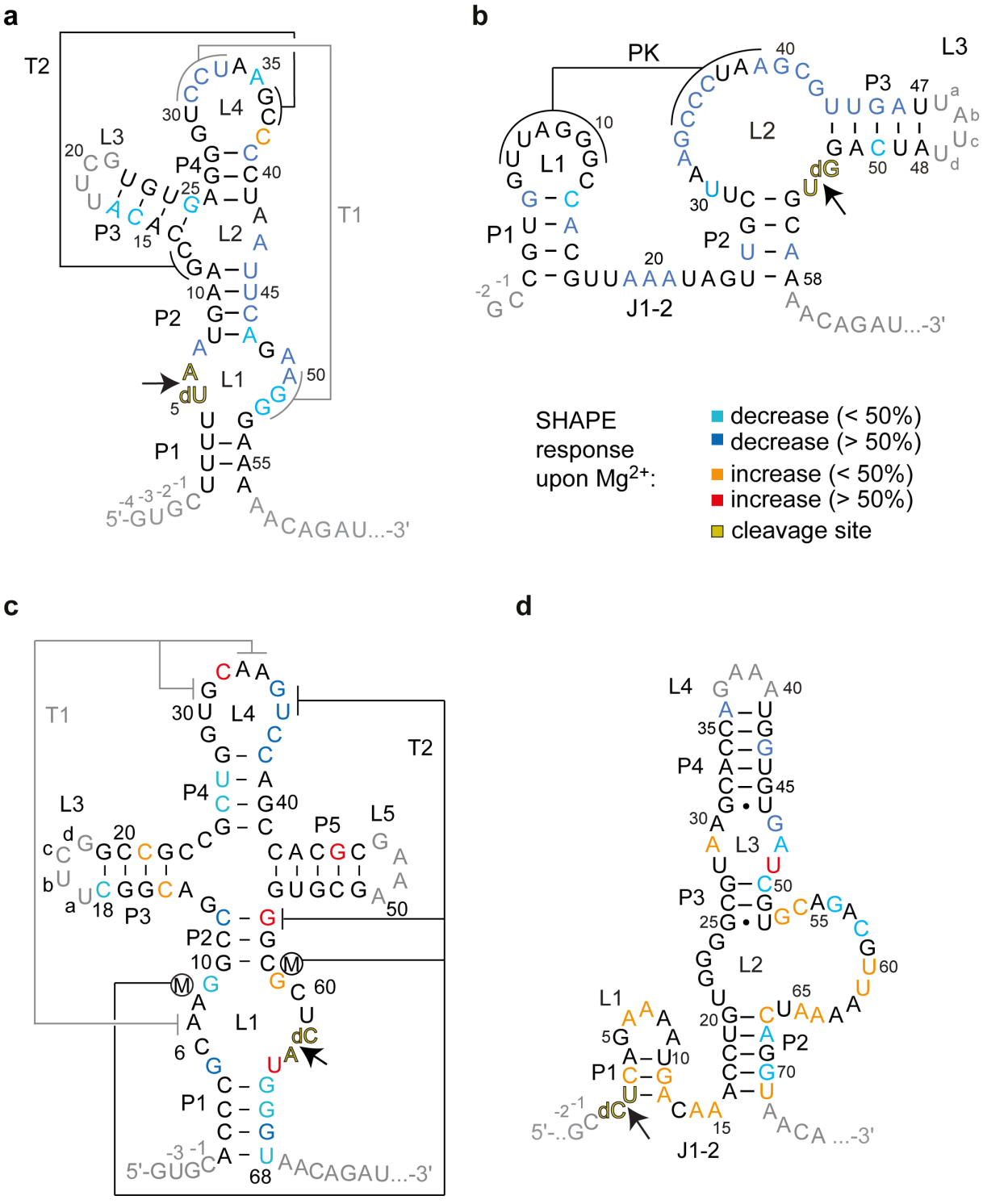
Supporting Figure 7. Quantification of the SHAPE probing data for the complete twister-sister RNA sequence. The normalized reactivity data of each nucleotide with the mean of three independent experiments are shown (top). The Student's t-test (2-sided paired) for assessing the statistical significance was applied and the corresponding plot is show (bottom).



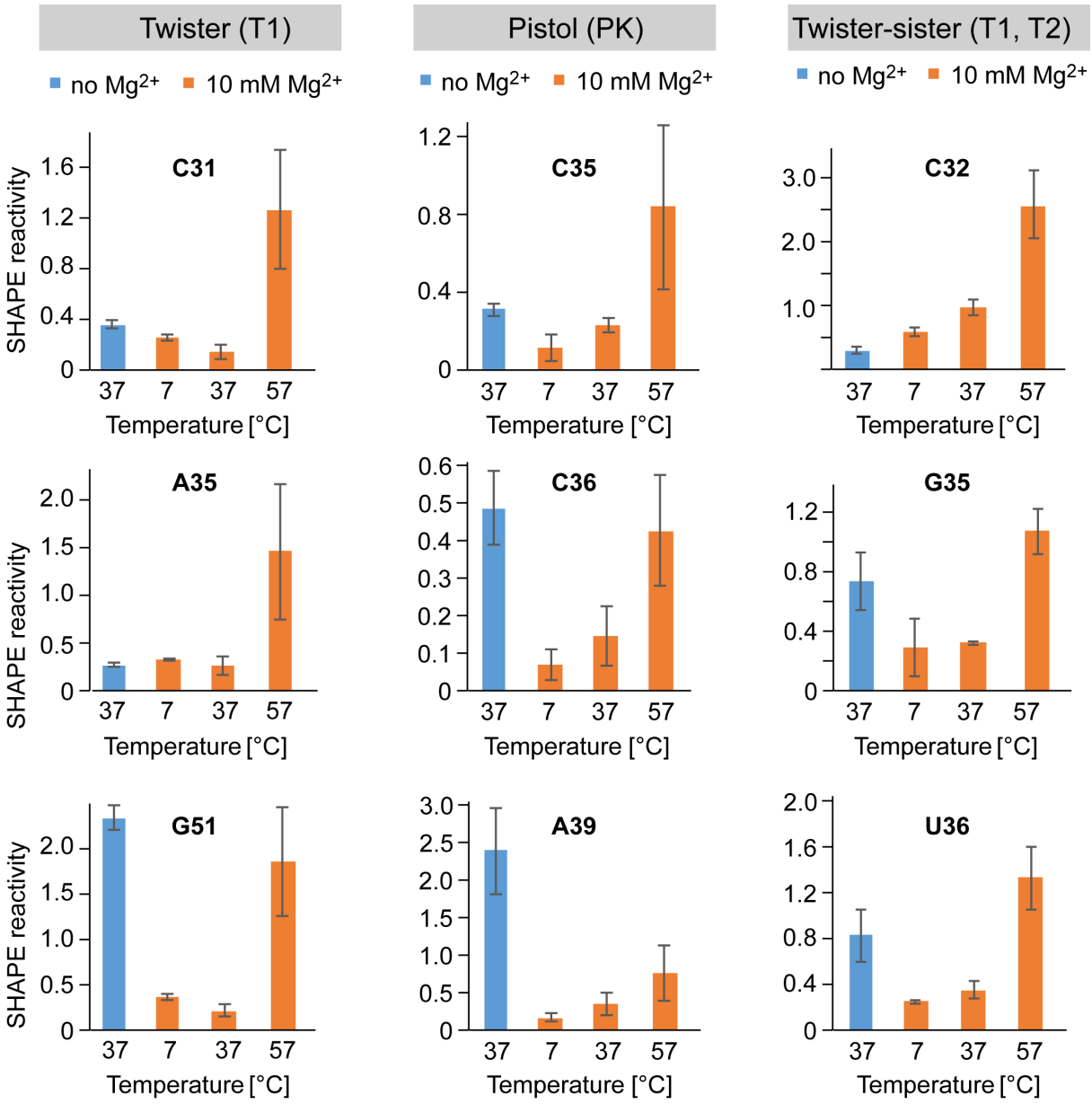
Supporting Figure 8. SHAPE probing of the hatchet ribozyme. Second example of a typical gel (see also Figure 4b). Lanes from left to right: T, C, G and A ladders, control in the absence of probing reagent, probing with BzCN, probing in the presence of 5, 10, and 20 mM $MgCl_2$ at room temperature. Note that the color-coded stem-loop assignments at the right side of the gel corresponds to the actual residue that is affected (shifted +1 to nucleoside numbering on the left side).



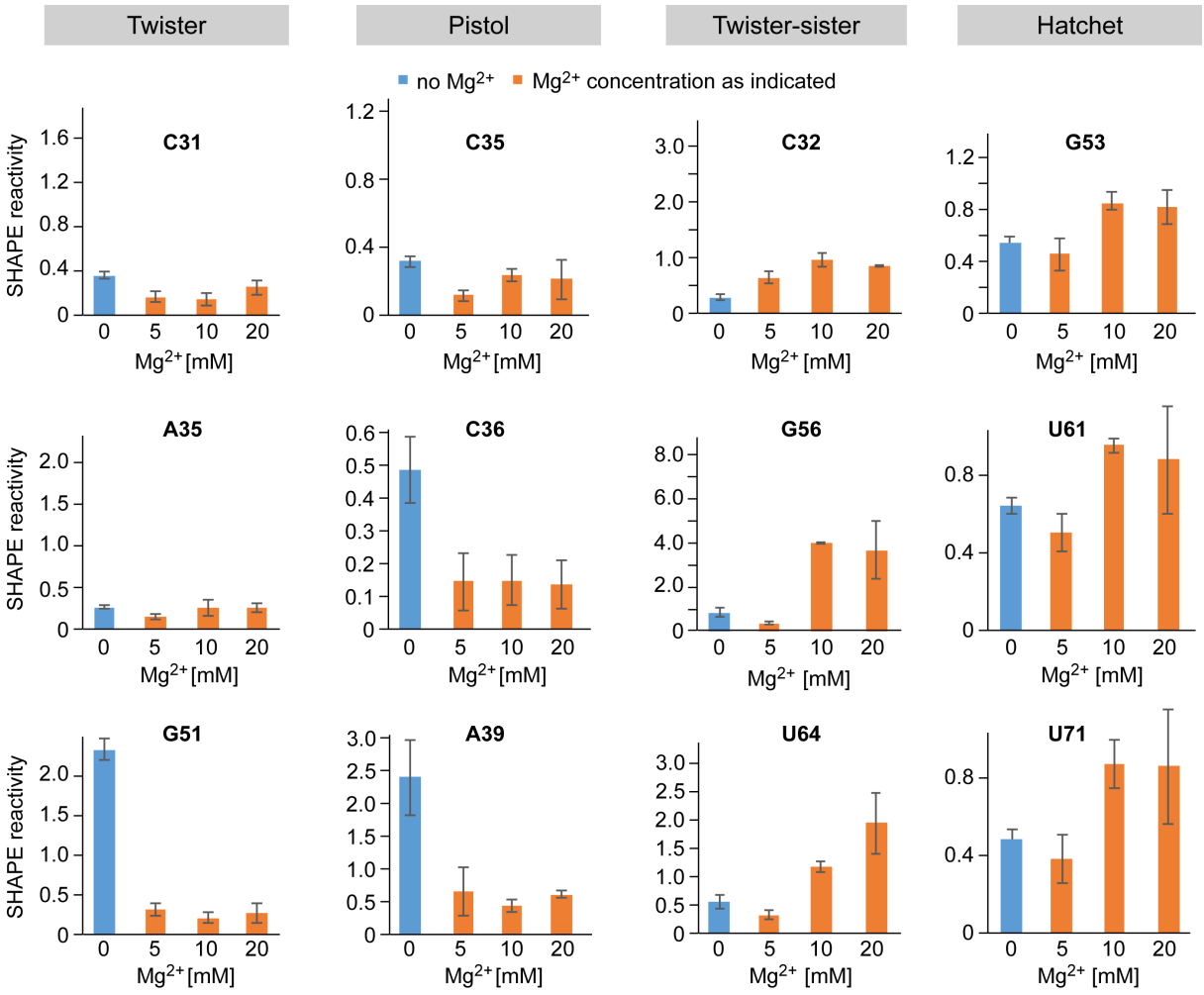
Supporting Figure 9. Quantification of the SHAPE probing data for the complete hatchet RNA sequence. The normalized reactivity data of each nucleotide with the mean of three independent experiments are shown (top). The Student's t-test (2-sided paired) for assessing the statistical significance was applied and the corresponding plot is show (bottom).



Supporting Figure S10. Comparison of SHAPE reactivities in response to Mg^{2+} projected onto the secondary structure models of the for ribozymes. (**a**) Twister ribozyme (no versus 5 mM Mg^{2+}). (**b**) Pistol ribozyme (no versus 5 mM Mg^{2+}) (**c**) Twister-sister (no versus 10 mM Mg^{2+}). (d) Hatchet ribozyme (no versus 10 mM Mg^{2+}). The color code used for increases versus decreases in SHAPE reactivities is shown in panel b; black nucleosides did not show pronounced SHAPE reactivity changes in response to Mg^{2+}.



Supporting Figure S11. Comparison of SHAPE reactivities of selected nucleotides (as indicated) in response to temperature with respect to pseudoknot formations of twister, pistol and twister-sister ribozymes.



Supporting Figure S12. Comparison of SHAPE reactivities of selected nucleotides of the four ribozymes (as indicated) in response to varying concentrations of Mg^{2+} at 37°C. The nucleotides were selected according to their structural importance as discussed in the main text: Twister C31, A35, G51: pseudoknot formation; pistol C35, C36, A39: pseudoknot formation; twister-sister C32: pseudoknot formation; twister-sister G56: 4-way junction; twister-sister U64: cleavage site/pocket; hatchet G53, U61, U71: currently no 3D structure available, depicted nucleotides were selected to most pronounced changes in SHAPE reactivity; G53 of hatchet is highly conserved.