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Supporting Information

Characterization of Highly Efficient RNA-Cleaving DNAzymes that Function at Acidic pH with No Divalent Metal-Ion Cofactors

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M1	GTGCCAAGCTTACCGTCAC**N23**GAGATGTCGCCATCTCTTCCTATAGTGAGTCGTATTAG
P1	GTGCCAAGCTTACCG
P2	CTGCAGAATTCTAATACGACTCACTATAGGAAGAGATGGCGAC
P3	GGGCAGAATTCTAATACGACTCACTAT**rA**
P3.1	GGGCAGAATTCTAATACGACTCACTATA
P4	CAACAACAACAA**(Spacer18)**GTGCCAAGCTTACCG
Dz15WS	CTCACTAT**rA**GGAAGAGATGGCGACATCTCTTACAAACCCCAAACCTTCTCTT
Dz27WS	CTCACTAT**rA**GGAAGAGATGGCGACATCTCCTACCCTCAAGCGACTTCTCTCG
Dz15WS_E	GACATCTCTTACAAACCCCAAACCTTCTCTT
Dz27WS_E	GACATCTCCTACCCTCAAGCGACTTCTCTCG
Dz15WS_E C52T	GACATCTCTTATAAACCCCAAACCTTCTCTT
Dz15WS_E A53C	GACATCTCTTACCAACCCCAAACCTTCTCTT
Dz15WS_EA53G	GACATCTCTTACGAACCCCAAACCTTCTCTT
Dz15WS_E C56T	GACATCTCTTACAAATCCCAAACCTTCTCTT
Dz15WS_E A62G	GACATCTCTTACAAACCCCAAGCCTTCTCTT
Sub1	CTCACTAT**rA**GGAAGAGATGGC
Sub2	CTCACTA**rUrA**GGAAGAGATGGC
Sub3	CTCACTAT**rArG**GAAGAGATGGC
Sub4	CTCACTA**rUrArG**GAAGAGATGGC
Sub5	CTCACTAT**rG**GGAAGAGATGGC
Sub6	CTCACTAT**rC**GGAAGAGATGGC
Sub7	CTCACTAT**rU**GGAAGAGATGGC

Figure S1. Sequences of oligonucleotides used in this study.

Figure S2. Nucleotide sequences of the pH-dependent DNAzymes obtained by the *in vitro* selection. Regions that were randomized in the initial DNAzyme library are marked in green and single nucleotide changes within each selection group in that region are marked by blue fonts. In the constant regions all nucleotides that differ from those found at the corresponding position in the initial library are also denoted in blue. The catalytic cleavage site is marked as rA in red. In parentheses, the number of clones with identical sequences are shown.

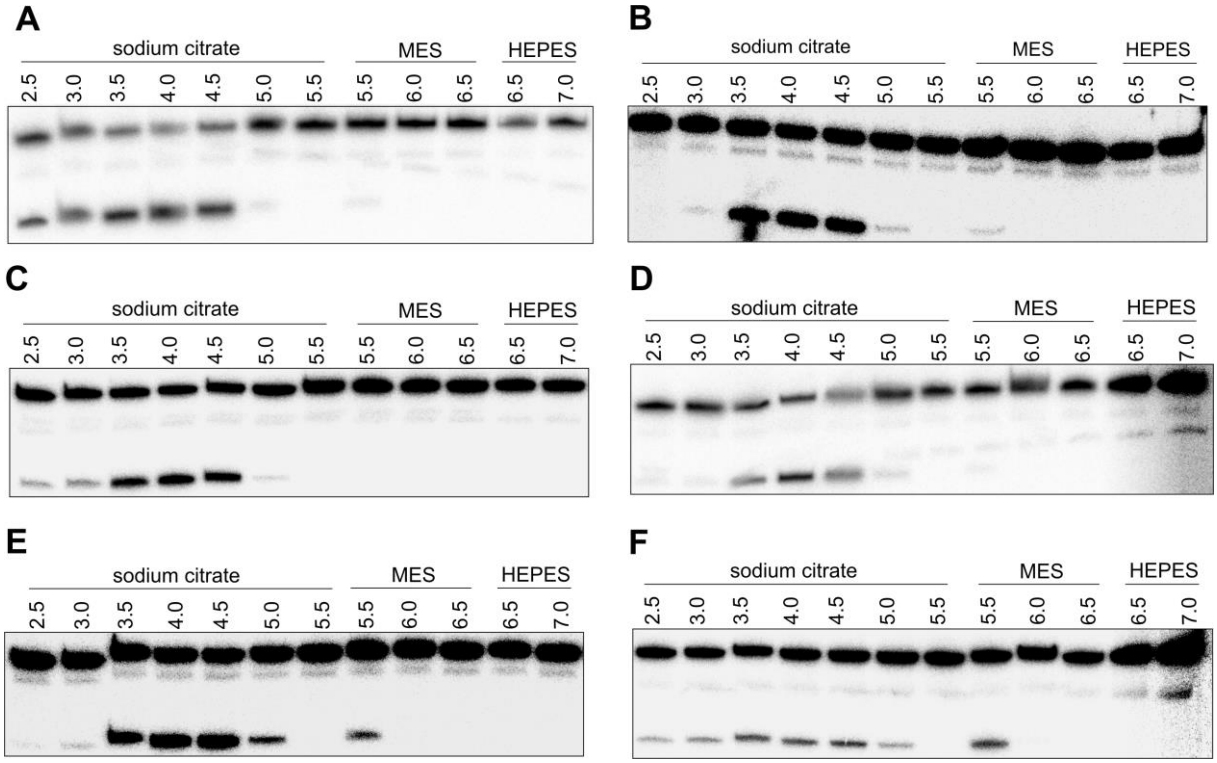

Figure S3. Autoradiograms showing pH specificity of *cis*-acting DNAzymes: (A) Dz27 (B) Dz22 (C) Dz24 (D) Dz40 (E) Dz15 (F) Dz42. The cleavage reactions were carried out at 25 °C for 60 min.

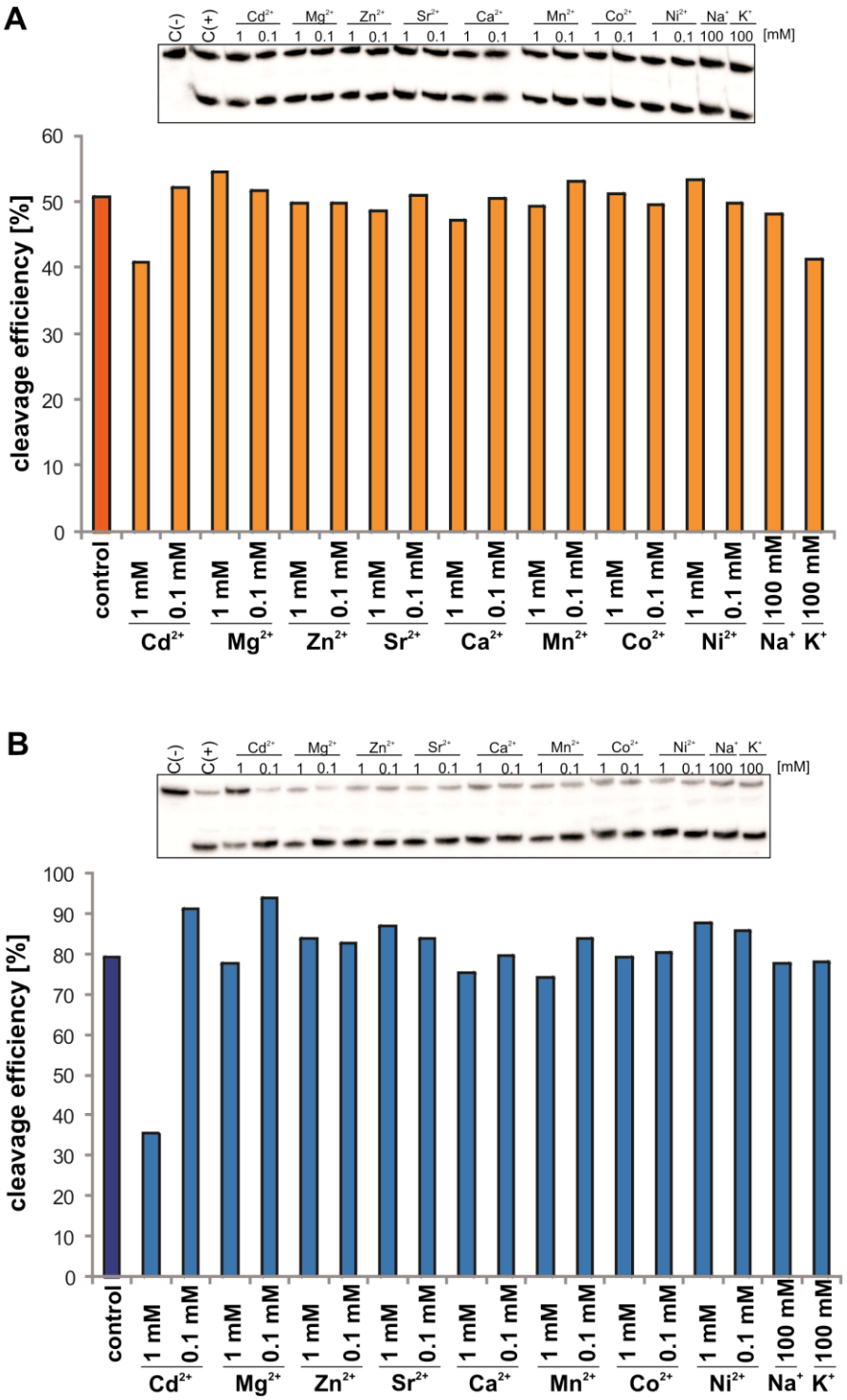

Figure S4. The impact of metal ions on pH-dependent DNAzymes (A) Dz15 (B) Dz27. The cleavage efficiency was determined after 60 min in the presence of different metal ions. The assays were carried out in sodium citrate buffer pH 4.0 for Dz15 and pH 4.5 for Dz27 . The cleavage extents were measured after 60 min incubation at 25°C. Lanes: C(-), control reaction without metal ions at pH 7.0; C(+), control reaction without metal ions at pH 4.0 and 4.5.

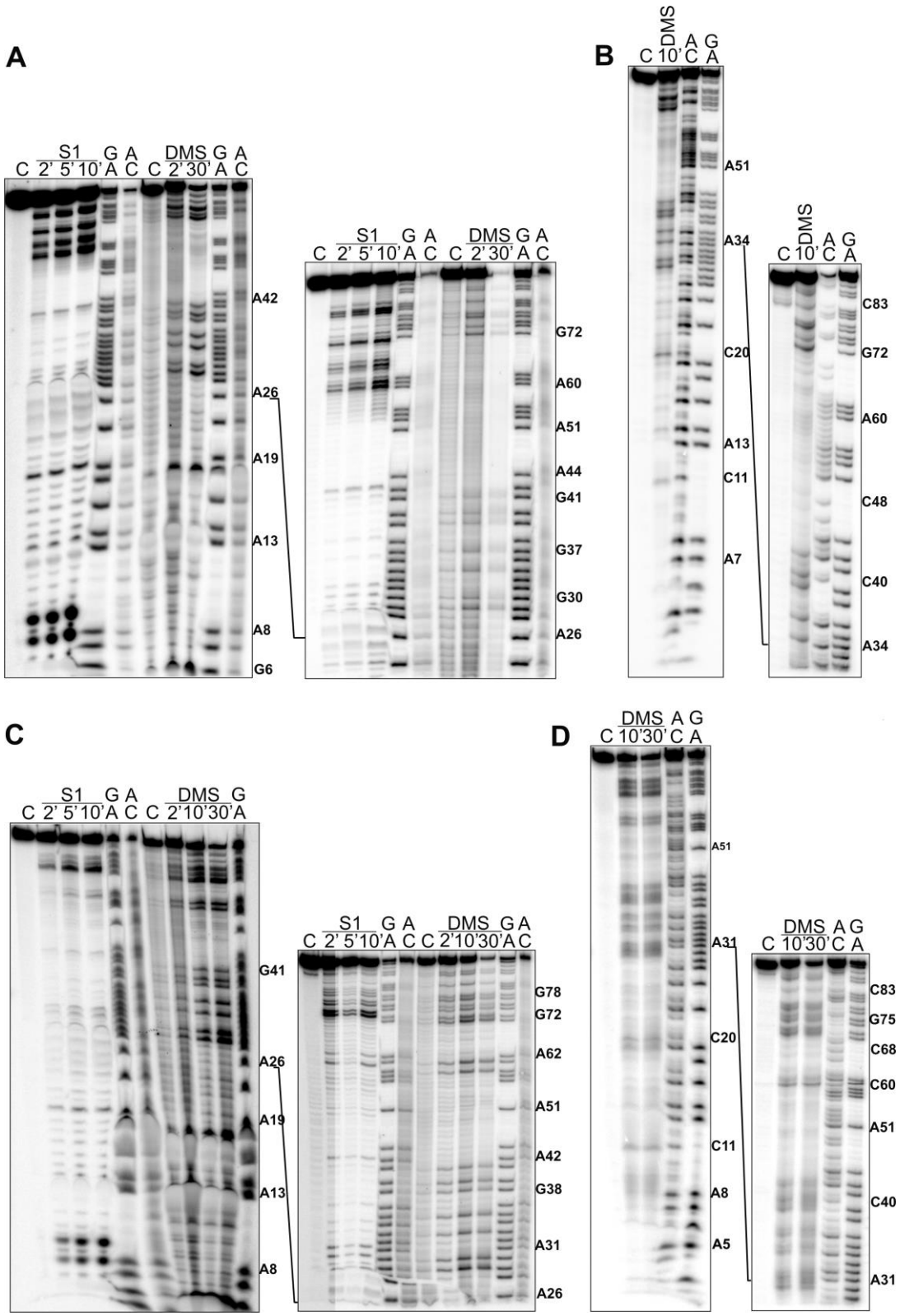

Figure S5. Probing of the structure of *cis*-acting DNAzymes (A-B) Dz15 and (C-D) Dz27. Autoradiograms of S1 nuclease digestion and the probing of guanosine residues with 0.2% DMS (A and C). Autoradiograms showing the probing of cytosine residues with 0.4% DMS (B and D). All reactions were performed with 5'-^{32}P-end-labeled DNAzymes at 25 °C. Lanes: C, control reaction; AC and GA, sequencing lanes. Selected nucleotide residues are numbered in the autoradiograms on the right.

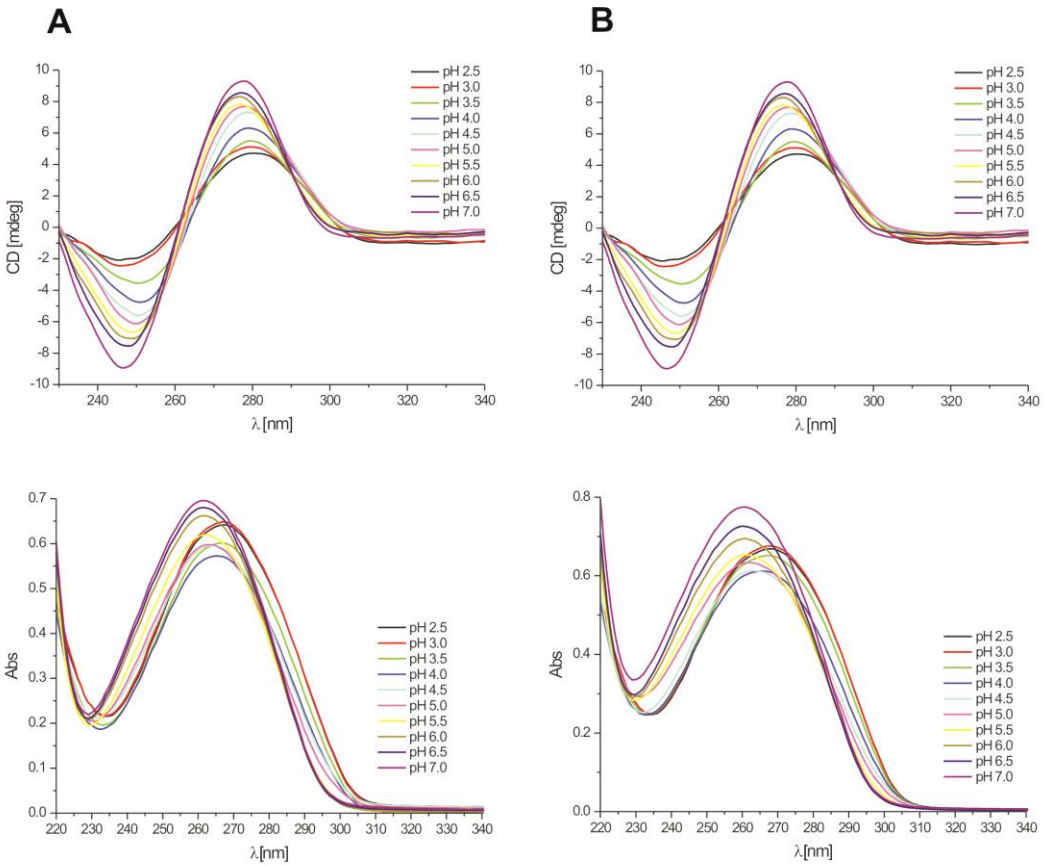

Figure S6. UV and CD spectra of DNAzymes (A) Dz15WS and (B) Dz27WS at different pH values. A non-cleavable versions of DNAzymes were applied in which adenosine at the cleavage site was replaced by its deoxy analogue. The spectra were recorded in 50 mM sodium citrate.

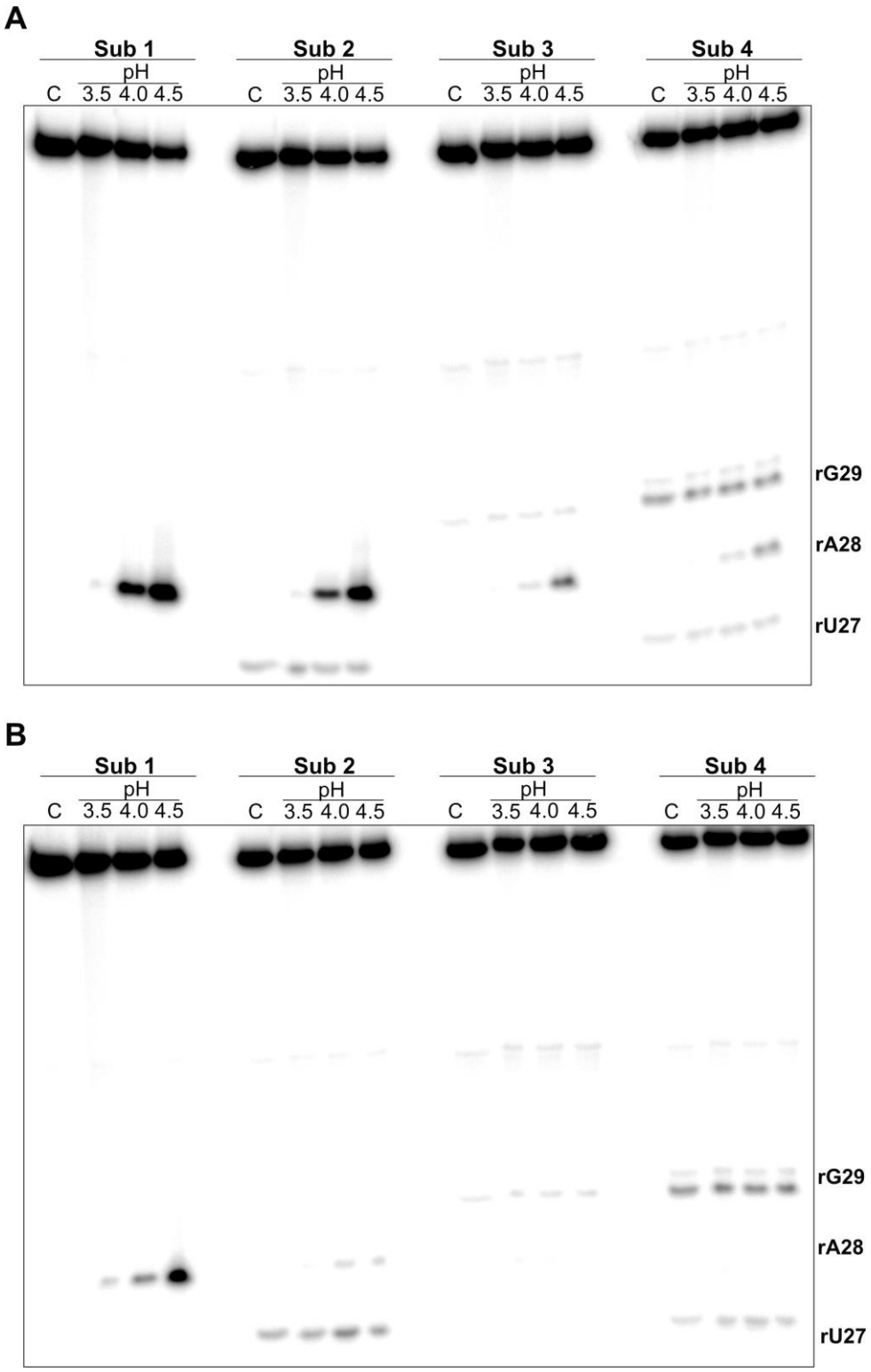

Figure S7. Autoradiograms of cleavage of *trans*-acting DNAzymes (A) Dz15WS_E (B) Dz27WS_E in the presence of substrates with additional ribonucleotides within the catalytic cleavage site (Sub1 with 27-TrAG-29, Sub2 with 27-rUrAG-29, Sub3 with 27-TrArG-29 and Sub4 with 27-rUrArG-29). The catalytic activities were assessed after 3 hours incubation at 37°C. Lane: C, control reaction.

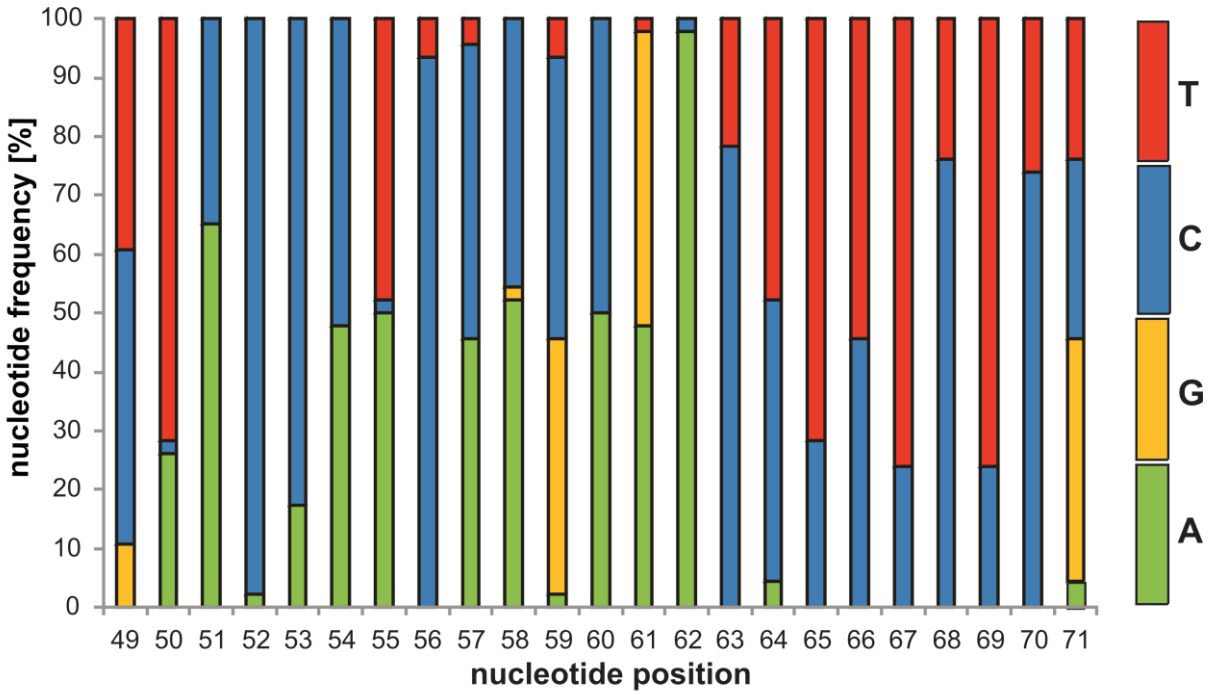

Figure S8. Statistical analysis of sequence variations of all obtained *cis*-acting DNAzymes. The variations were determined for each position of the sequence corresponding to 23-nucleotide random region of initial library.

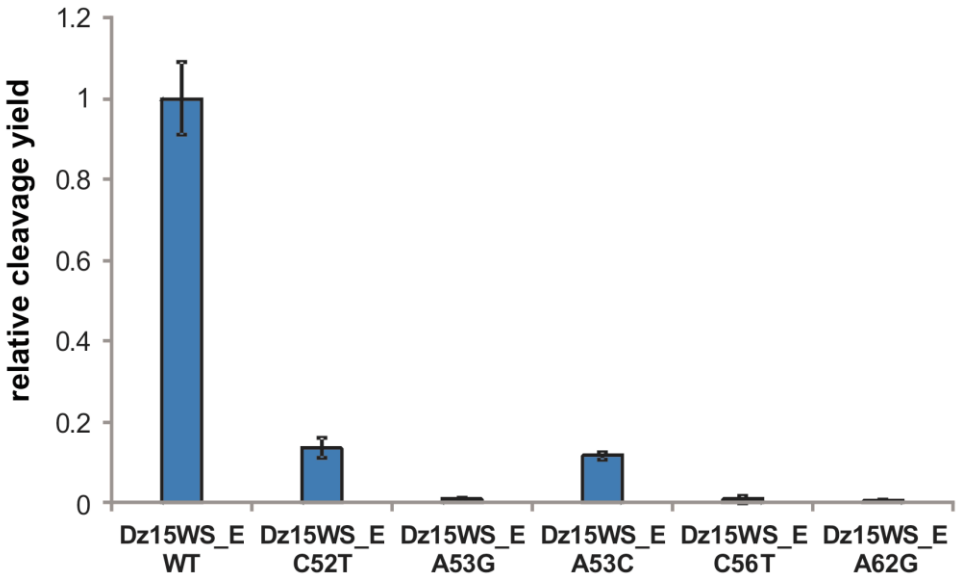

Figure S9. Effect of individual mutations at position 52,53,56 and 62 on the cleavage activity of *trans*-acting Dz15WS_E. Cleavage reactions were performed at pH 4.5 in the presence of oligonucleotide substrate containing single riboadenosine (rA) bond at the cleavage position. The cleavage extents were measured after 3 hours incubation at 37°C.