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Supporting Information

Detection of Large Genomic RNA via DNAzyme-Mediated RNA Cleavage and Rolling Circle Amplification: SARS-CoV-2 as a Model

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Experimental Procedures

Chemicals

Materials used in this study were sourced as follows:

Electrophoresis reagents, buffers and salts – Bioshop Canada or Millipore Sigma
Polynucleotide Kinase – Thermo Scientific
T4 DNA Ligase – Thermo Scientific
Q5 DNA Polymerase – New England Biolabs
T7 RNA Polymerase – Invitrogen
DNase I – Thermo Scientific
FastAP – Thermo Scientific
 $\gamma^{32}\text{P}$ -ATP – Perkin Elmer
Pooled Human Saliva - Innovative Research
Proteinase K – Thermo Scientific
Pierce Detergent Removal Resin – Thermo Scientific
dNTPs & NTPs – Thermo Scientific
Phi29 DNA Polymerase – Thermo Scientific
RiboLock RNase Inhibitor – Thermo Scientific
SYBR Gold – Thermo Scientific
SYTO 9 – Thermo Scientific

Oligonucleotide Preparation

Oligonucleotides and PCR primers were obtained from Integrated DNA Technologies and purified by denaturing polyacrylamide gel electrophoresis (8 M urea, 1x TBE, 10% acrylamide) (dPAGE). Product bands were excised, crushed and eluted in elution buffer (10 mM Tris-HCl pH 7.5, 200 mM NaCl, 1 mM EDTA). Recovered elution buffer was ethanol precipitated with 2.5 volumes chilled absolute ethanol and pelleted by centrifugation at 20,000 $\times g$ for 20 min. Pellets were washed with 70% ethanol, dried and resuspended in nuclease-free water. All oligonucleotides were quantified by A_{260} measurements using a GE Nanovue spectrophotometer. Circular RCA templates were prepared by phosphorylation of 8 μM linear RCA templates with excess ATP using 0.02 U/ μl Polynucleotide Kinase (PNK) for 30 min at 37 °C. Phosphorylation reactions were then diluted to 500 nM RCA template concentration and circularly ligated with an equimolar amount of a ligation splint using 0.025 U/ μl T4 DNA Ligase and incubated at room temperature for 1 h. Products were “double purified” by dPAGE (run on dPAGE gel twice) and quantified as described above.

RNA Transcript Preparation

Vectors for production of SARS-CoV-2 transcripts were donated by Dr. Gerry Wright (McMaster University). Gene fragments were amplified from vectors by PCR, incorporating a T7 RNA polymerase promoter in the forward primer to facilitate *in vitro* transcription, using Q5 DNA polymerase. NSP12 and Spike (S) genes were split in to 2 and 5 fragments respectively, while Membrane (M), Nucleocapsid (N), NSP5, NSP1, NSP2, NSP3, NSP6, NSP8, NSP15, NSP16, NSP13 and NSP14 genes were produced as single transcripts. RNA transcription was performed at 37 °C for 2 h using 50 nM DNA template and 2 mM of each NTP in 1x transcription buffer containing 5 mM DTT, 0.005 U/ μl pyrophosphatase, 1 U/ μl RiboLock and 2.5 U/ μl T7 RNA polymerase. Following transcription, reactions were treated with 0.125 U/ μl DNase I at 37 °C for 20 min and purified on dPAGE. RNA radiolabeling was performed by dephosphorylation of 4 pmol RNA transcript with FastAP, followed by extraction with phenol-chloroform and chloroform, and by ethanol precipitation. Dephosphorylated RNA was then 5'-labeled with an excess of $\gamma^{32}\text{P}$ -ATP using PNK followed by dPAGE purification.

DNAzyme Kinetics

DNAzyme kinetic reactions were conducted in duplicate, where each reaction contained 500 nM DNAzyme and 2000 cpm (~400 pM) radiolabeled RNA transcript. Reactions were heated at 90 °C for 1 min, cooled to room temperature for 10 min to anneal, and initiated by addition of reaction buffer to 1x RB. Reactions were incubated at 23 °C and timepoints were taken after 0.5, 1, 2, 5, 10, 15, 30, 45, 60 and 90 min, and stopped by addition of QB to 1x and chilled on ice. An additional reaction without 1x RB was used as a $t = 0$ min timepoint reference. Reaction mixtures were resolved on 5% dPAGE, exposed on a storage phosphor screen and imaged using a Typhoon FLA 9500 Biomolecular Imager. Gel images were analysed using Image J and the observed rate constant (k_{obs}) was calculated by fitting the reaction curve to a one-phase or two-phase exponential association equation using GraphPad Prism.

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Correlation Analysis of DNAzyme Screening Activity

The analysis dataset consisted of 230 cleavage fraction datapoints from the screening study, DNAzyme sequences and RNA target (SRT) sequences. Nucleic acid structure prediction was performed using RNAsstructure version 6.4^[1]. DNAzyme and RNA folding was performed at 23 °C using the Fold program in the RNAsstructure package with DNA or RNA parameters, respectively. In all foldings, only the lowest energy structure was used for analysis. RNA sequences used for calculation of opening energy were prepared by masking DNAzyme binding regions to prevent folding during structure prediction. “RNA Opening” energy was calculated as (ΔG_{fold} RNA) – (ΔG_{fold} with DNAzyme binding site unbound). RNA sequences for calculation of proximity effects were prepared by extracting RNA sequences from SRTs centered on each DNAzyme binding site. Sites close to transcript ends were truncated at the beginning or end of the transcript if the flanking region extended beyond the ends of the transcript.

For correlation analysis, cleavage data was log10 transformed. Twenty-two DNAzymes with ≤ 0 cleavage activity were excluded from correlation analysis. Correlation analysis was performed in Python using the Pandas module corr function with the Pearson method. Calculation of the correlation p-values was performed using the stats module and pearsonr function.

Optimization of Saliva Treatment Method

Pooled human saliva (50 µl) was treated with one or more of the following as indicated in Figure S22, final volume 60 µl: 1) 2 µg/ml Proteinase K, 2) 0.1% SDS and 3) heating at 90 °C for 10 min. Reactions using SDS had SDS removed prior to downstream reactions by either 1) addition of 100 mM KCl, 10 min precipitation at room temperature, 5 min centrifugation, recovery of supernatant, or 2) washing with 30 µl pelleted Pierce Detergent Removal Resin, centrifugation and recovery of supernatant.

A trace amount of 5'-³²P-labeled RNA transcript was used to evaluate RNA stability in treated saliva. Five µl of treated saliva was mixed with ~2000 CPM 5'-³²P labeled SRT12 RNA and either 1x RB or 1x Heating Buffer (HB; 50 mM Tris pH 8.0, 5 mM EDTA) was added, final volume 10 µl. Reactions were then incubated at 90 °C or room temperature for 10 min as indicated in Figure S22. Finally, reactions were diluted with QB to 1x and resolved on a 10% dPAGE as previously described. Radiolabeled RNA products were visualized by storage phosphor imaging using a Typhoon FLA 9500 Biomolecular Imager.

Coupled DNAzyme-RCA Reactions

Human pooled donor saliva was treated by addition of 2.5 mg/ml Proteinase K, 10 mM Tris pH 8.0, 1 mM EDTA. Samples were incubated at 23°C for 2 min and then heated at 90°C for 10 min.

Coupled DNAzyme-RCA reactions are performed sequentially by first performing RNA cleavage, followed by addition of reagents for linear RCA to the same reaction mixture for detection. Cleavage reactions containing 50% (v/v) heat-inactivated saliva, 50 nM DNAzyme and 10 nM RNA transcript were initiated by addition of RB to 1x and incubated at 23 °C for 1 h. A control cleavage reaction excluding DNAzyme was set up identically and performed in parallel. Following DNAzyme cleavage, half the reaction was taken for RCA and set up as follows: 50% (v/v) DNAzyme reaction mixture, 10 nM CDT, 250 µM of each dNTP, 1x SYBR Gold, 0.25 U/µl PNK, 0.25 U/µl phi29 DNA Polymerase, phi29 reaction buffer (PB) containing 50 mM Tris pH 8.0, 10 mM MgCl₂, 66 mM KCl, 4 mM DTT, 0.1% Tween 20. Three clean RCA control reactions containing no saliva were set up: 1) 10 nM CDT only, 2) 10 nM CDT and 10 nM DNA primer, or 3) 10 nM CDT and 10 nM DNAzyme. Final reaction volumes were 20 µL. All reactions conducted in triplicate and incubated at 23 °C in a Bio-Rad CFX-96 real-time thermal cycler for 4 h while monitoring fluorescence using the FAM/SYBR filter.

Figure S24A shows linear RCA over a range of RNA concentrations. The DNAzyme cleavage reaction was performed in 1x RB containing 50% processed human saliva as well as 50 nM DNAzyme and 0.5-50 nM RNA transcript (23 °C for 1 h), followed by RCA in 1x RB containing 50% cleavage reaction mixture and 10 nM CDT. In addition, “CDT only”, “CDT + DNAzyme only” and “CDT + RNA only” were included as negative RCA controls. Each RCA reaction was monitored by following fluorescence in real time over 4 h (with the use of SYBR Gold). Figure S24B demonstrates that the normalized fluorescence exhibits a linear relationship with logarithmic concentration of the RNA transcript.

SUPPORTING INFORMATION

Results and Discussion

Table S1. RNA transcripts assessed as substrates of 10-23 DNAzymes.

| Transcript Identifier | Transcript Size (nt) | Gene Name | SARS-CoV-2 Genome Coverage (GenBank: MN908947.3) | Number of DNAzymes Screened | DNAzymes with FC > 0.2 |
|-----------------------|----------------------|--------------|--|-----------------------------|--|
| SRT1 | 543 | NSP1 | 265-805 | 11 | dZ 507a |
| SRT2 | 1916 | NSP2 | 805-2719 | 27 | dZ 1308a dZ 1940a dZ 2167a dZ 2426a |
| SRT3 | 1798 | NSP3 | 2997-4791 | 27 | dZ 3072a dZ 4076a |
| SRT4 | 921 | NSP5 | 10054-10972 | 10 | dZ 10325a dZ 10491a dZ 10800a |
| SRT5 | 844 | NSP6 | 10992-11832 | 12 | dZ 11567a |
| SRT6 | 584 | NSP8 | 12098-12679 | 11 | dZ 12262a |
| SRT7 | 1208 | NSP12 | 13469-14676 | 5 | dZ 13726a dZ 14172a |
| SRT8 | 1433 | NSP12 | 14767-16197 | 10 | dZ 15703a dZ 15921a |
| SRT9 | 1805 | NSP13 | 16236-18039 | 20 | dZ 16912a |
| SRT10 | 1583 | NSP14 | 18040-19620 | 19 | dZ 18583a |
| SRT11 | 1042 | NSP15 | 19620-20659 | 16 | dZ 19743a dZ 20134a dZ 20412a |
| SRT12 | 890 | NSP16 | 20659-21545 | 19 | dZ 21086a dZ 21338a |
| SRT13 | 769 | Spike | 21655-22420 | 4 | |
| SRT14 | 704 | Spike | 22421-23122 | 1 | |
| SRT15 | 478 | Spike | 23437-23911 | 1 | |
| SRT16 | 566 | Spike | 24109-24665 | 4 | |
| SRT17 | 682 | Spike | 24669-25343 | 3 | |
| SRT18 | 831 | ORF3a | 25393-26220 | 16 | dZ 25826a |
| SRT19 | 673 | Membrane | 26523-27192 | 4 | dZ 27137a |
| SRT20 | 1263 | Nucleocapsid | 28273-29533 | 10 | dZ 28350a dZ 28851a dZ 28704a |

FC = Fraction Cleavage

SUPPORTING INFORMATION

Table S2. Sequences of all 10-23 DNAzyme variants tested in this work.

| DNAzyme | Transcript | Target Site (MN908947.3) | CDT | Sequence (5'→3') |
|------------|------------|-----------------------------|--------|---|
| 10-23 Core | | | | -----GGCTAGCTACAAACGA----- |
| dZ 341a | SRT1 | 341 | | AAGCCACGTACGAGCAGGCTAGCTACAAACGAGTCGCGAA |
| dZ 355a | SRT1 | 355 | | CGTGCCTCTGATAAGAGGCTAGCTACAAACGACTCCTCCA |
| dZ 426a | SRT1 | 426 | | CCTTTTCAACTCTAGGCTAGCTACAAACGATAAGGCCAC |
| dZ 468a | SRT1 | 468 | | ACGTTGATGAACACAGGCTAGCTACAAACGAGGGCTGT |
| dZ 483a | SRT1 | 483 | | AGTCGAGCATCCGAAGGCTAGCTACAAACGAGTTGATG |
| dZ 507a | SRT1 | 507 | RCA24b | AACCATAACATGACCAGGCTAGCTACAAACGAGAGGTGCA |
| dZ 558a | SRT1 | 558 | | TGTCTCACCACTACAGGGCTAGCTACAAACGACGTACTGA |
| dZ 578a | SRT1 | 578 | | ATGAGGGACAAGGACAGGCTAGCTACAAACGACAAGTGTC |
| dZ 648a | SRT1 | 648 | | GCCACCAGCTCCTTAGGCTAGCTACAAACGATACCGTTC |
| dZ 688a | SRT1 | 688 | | CGCCTAAGTCAAATGAGGCTAGCTACAAACGATTAGATC |
| dZ 765a | SRT1 | 765 | | TTCACGGTAACACCAAGGCTAGCTACAAACGATGCTATGT |
| dZ 846a | SRT2 | 846 | | CTCAAGAGGGTAGCCAGGCTAGCTACAAACGACAGGGCCA |
| dZ 866a | SRT2 | 866 | | GCTAGAAGGTCTTAAGGCTAGCTACAAACGAGCACTCAA |
| dZ 910a | SRT2 | 910 | | AGTCAGTTGTCGAGGCTAGCTACAAACGAAAAGTGCA |
| dZ 1015a | SRT2 | 1015 | | CAAAAGGTGTCTGAAGGCTAGCTACAAACGATCATAGCT |
| dZ 1051a | SRT2 | 1051 | | CATTGAAGGTGTCAAAGGCTAGCTACAAACGATTCTTGTC |
| dZ 1080a | SRT2 | 1080 | | TAAGGGAAATACAAAAGGCTAGCTACAAACGATTGGACAT |
| dZ 1168a | SRT2 | 1168 | | CAACTGGATAGACAGGGCTAGCTACAAACGACGAATTCT |
| dZ 1210a | SRT2 | 1210 | | TGAGAGTTGAAAGGCAGGCTAGCTACAAACGATTGGTT |
| dZ 1243a | SRT2 | 1243 | | CCATGAAGTTTACCAAGGCTAGCTACAAACGAAATGATCA |
| dZ 1308a | SRT2 | 1308 | RCA42b | ACCTTCTTAGTCAAAGGCTAGCTACAAACGATCTCAGTG |
| dZ 1338a | SRT2 | 1338 | | ATTTGGGTTAAGTAAAGGCTAGCTACAAACGACACAAGTA |
| dZ 1367a | SRT2 | 1367 | | CATGCTGGACAATAAAGGCTAGCTACAAACGATTAAACAA |
| dZ 1431a | SRT2 | 1431 | | TTTCAAGCCAGATTCAAGGCTAGCTACAAACGATATGGTAT |
| dZ 1475a | SRT2 | 1475 | | CAGCCTCCAAGGCAAGGCTAGCTACAAACGAACTGCGAC |
| dZ 1599a | SRT2 | 1599 | | AAGGTTGTCATTAAGGCTAGCTACAAACGACTTCGAA |
| dZ 1719a | SRT2 | 1719 | | AGTTTCCACAAAAGCAGGCTAGCTACAAACGATTGGAA |
| dZ 1759a | SRT2 | 1759 | | CAACAATTGTTGAAGGCTAGCTACAAACGAGCTTATA |
| dZ 1796a | SRT2 | 1796 | | GCTTTCTTTGTAAGGCTAGCTACAAACGATTAAAAT |
| dZ 1846a | SRT2 | 1846 | | GAGGACTCAGTATTGAGGCTAGCTACAAACGATTCTGTT |
| dZ 1940a | SRT2 | 1940 | RCA43b | TTCTGTAAAACACGAGGCTAGCTACAAACGAAATT |
| dZ 2020a | SRT2 | 2020 | | CCAATCAGATGTGAAGGCTAGCTACAAACGAAATCATAGC |
| dZ 2127a | SRT2 | 2127 | | GGGTTGAGTTTCAGGCTAGCTACAAACGAAAACAGTG |
| dZ 2167a | SRT2 | 2167 | RCA44b | CTACACCTCCCTAAAGGCTAGCTACAAACGATTCTTC |
| dZ 2244a | SRT2 | 2244 | | ACAATTGTCACCCAGGCTAGCTACAAACGAAATTTCAC |
| dZ 2276a | SRT2 | 2276 | | TGAACACTCTCTTAAGGCTAGCTACAAACGATTCTTG |
| dZ 2376a | SRT2 | 2376 | | AAATGTTCACCTAAAGGCTAGCTACAAACGATCAAGGCT |
| dZ 2426a | SRT2 | 2426 | RCA45b | TCTCTCTGGATTTAAGGCTAGCTACAAACGAACTTTC |
| dZ 3072a | SRT3 | 3072 | RCA46b | AAACTCTCTCTCAGGCTAGCTACAAACGAAATCACCT |
| dZ 3124a | SRT3 | 3124 | | TTTACCTGGTAATCAGGCTAGCTACAAACGACTTCAGTA |
| dZ 3207a | SRT3 | 3207 | | TTGTTGACTATCATCAGGCTAGCTACAAACGACTAACCAA |
| dZ 3377a | SRT3 | 3377 | | GCATTTTAATGATAGGCTAGCTACAAACGAAATTGTCAG |
| dZ 3419a | SRT3 | 3419 | | ACCACTGTTGGTTAGGCTAGCTACAAACGACTTTTAG |
| dZ 3512a | SRT3 | 3512 | | TCAGATTCAACTTGCAGGCTAGCTACAAACGAGGATTGT |
| dZ 3531a | SRT3 | 3531 | | ATTAGTAGCTATGTAAGGCTAGCTACAAACGACATCAGAT |
| dZ 3647a | SRT3 | 3647 | | CTCTTAAGAAGTTGAAGGCTAGCTACAAACGAGTCAC |
| dZ 3681a | SRT3 | 3681 | | TAGAACTTCGTGCTGAGGCTAGCTACAAACGATAAAATT |
| dZ 3706a | SRT3 | 3706 | RCA47b | TACCAAGCTGATAATAAGGCTAGCTACAAACGAGGTGCAAG |
| dZ 3755a | SRT3 | 3755 | | ACAGTATCTACACAAAGGCTAGCTACAAACGATCTTAAAG |
| dZ 3782a | SRT3 | 3782 | | AAGACAGCTAAGTAGAGGCTAGCTACAAACGATTTGTGC |
| dZ 3813a | SRT3 | 3813 | | TGAAAACAAGTTGTCAGGCTAGCTACAAACGAAAGAGATT |
| dZ 3908a | SRT3 | 3908 | | GGTTTACTTCAGTTAGGCTAGCTACAAACGAAAATGGCT |
| dZ 3960a | SRT3 | 3960 | | TCAACACAAGCTTTGAGGCTAGCTACAAACGATTCTTAT |
| dZ 4044a | SRT3 | 4044 | | TGGATGAAGATTGCCAGGCTAGCTACAAACGATAATGTCA |
| dZ 4076a | SRT3 | 4076 | RCA48b | ATGTCATGTCACTAAGGCTAGCTACAAACGAAAGAGTGG |
| dZ 4118a | SRT3 | 4118 | RCA49b | CATCACCCACTATATAGGCTAGCTACAAACGAGGAGCATC |
| dZ 4148a | SRT3 | 4148 | RCA50b | ACCACAGCAGTTAAAGGCTAGCTACAAACGAAACCTCTT |

CDT = Circular DNA Template

SUPPORTING INFORMATION

Table S2. Continued. Sequences of all 10-23 DNAzyme variants tested in this work.

| DNAzyme | Transcript | Target Site (MN908947.3) | CDT | Sequence (5'→3') |
|------------|------------|-----------------------------|--------|--|
| 10-23 Core | | | | -----GGCTAGCTACAAACGA----- |
| dZ 4239a | SRT3 | 4239 | | CGGGTAAGTGGTTATAGGCTAGCTACAAACGAAATTGTCT |
| dZ 4269a | SRT3 | 4269 | | CTCTACAGTGTAAACCAGGCTAGCTACAAACGATTAACCC |
| dZ 4298a | SRT3 | 4298 | | TTACACTTTAAGCAGGCTAGCTACAAACGATGTCTTG |
| dZ 4317a | SRT3 | 4317 | | TAGAATGTAAAAGGCAGGCTAGCTACAAACGATTTACAC |
| dZ 4343a | SRT3 | 4343 | | TGCTTCTCATTAGAGGCTAGCTACAAACGAAATAGATG |
| dZ 4386a | SRT3 | 4386 | | AAGCATTTCTCGCAAAGGCTAGCTACAAACGATCCAAGAA |
| dZ 4528a | SRT3 | 4528 | | TGGTGTAAAAGTAAAAGGCTAGCTACAAACGACTAGCAC |
| dZ 4731a | SRT3 | 4731 | | AGAAGAAGAAGTAAGGCTAGCTACAAACGAAACCATTA |
| dZ 10098a | SRT4 | 10098 | | TACTTGTACCATACAAGGCTAGCTACAAACGACCTCAACT |
| dZ 10140a | SRT4 | 10140 | | GTCATCAAGCCAAAGGCTAGCTACAAACGACGTAAAGT |
| dZ 10176a | SRT4 | 10176 | | AGAGGTGCAGATCACAGGCTAGCTACAAACGAGTCTGGA |
| dZ 10256a | SRT4 | 10256 | | ACATTACCGCCTGTAGGCTAGCTACAAACGACAAGAAAT |
| dZ 10325a | SRT4 | 10325 | | GGATTGGCTGTATCAAGGCTAGCTACAAACGACTTAAGCT |
| dZ 10338a | SRT4 | 10338 | | CTTAGGTGCTTAGGAGGCTAGCTACAAACGATGGCTGTA |
| dZ 10442a | SRT4 | 10442 | | GTGAAATTGGGCCTCAGGCTAGCTACAAACGAGCACATT |
| dZ 10491a | SRT4 | 10491 | RCA23b | GTTAAAACCAACACTAGGCTAGCTACAAACGACACATGAA |
| dZ 10599a | SRT4 | 10599 | | GTCAACAAAAGGTCCAGGCTAGCTACAAACGAAAAAGTTA |
| dZ 10800a | SRT4 | 10800 | | GAAAGAGGTCTCTAGTAGGCTAGCTACAAACGAGTCACAT |
| dZ 11062a | SRT5 | 11062 | | AAAAGAACAAAGACCAAGGCTAGCTACAAACGATGAGTACT |
| dZ 11085a | SRT5 | 11085 | | TAAAAAGGCATTTTCAGGCTAGCTACAAACGAAACAAAAAA |
| dZ 11111a | SRT5 | 11111 | | ATAGCAATAATACCCAGGCTAGCTACAAACGAAAGCAAAG |
| dZ 11217a | SRT5 | 11217 | | AGGCATATAGACCATAGGCTAGCTACAAACGATAAAATAA |
| dZ 11270a | SRT5 | 11270 | | AAACTAGTATCAACCAAGGCTAGCTACAAACGATTCACACC |
| dZ 11342a | SRT5 | 11342 | | CTTGTCTGTCTAAAGGAGGCTAGCTACAAACGATAGTAACA |
| dZ 11502a | SRT5 | 11502 | | GACAGTTGTAACACTACAGGCTAGCTACAAACGACTGAGTAG |
| dZ 11521a | SRT5 | 11521 | | TACCTCTGCCAAAAGGCTAGCTACAAACGATGACAGT |
| dZ 11567a | SRT5 | 11567 | | CCAGTTATGAAGAAAAGGCTAGCTACAAACGAGGGCAAT |
| dZ 11616a | SRT5 | 11616 | | AAAATAGCTAAGAAAAGGCTAGCTACAAACGAAATAAACT |
| dZ 11697a | SRT5 | 11697 | RCA25b | AGAAAACTAAGTAATCAGGCTAGCTACAAACGAAAACACCA |
| dZ 11730a | SRT5 | 11730 | | TCCCTGTGAATTCTAGGCTAGCTACAAACGAAATCTAAC |
| dZ 12156a | SRT6 | 12156 | | AGCACACGCCCTGCTCAGGCTAGCTACAAACGAAAGCTTCT |
| dZ 12174a | SRT6 | 12174 | | AACTTCAGAATCACCAAGGCTAGCTACAAACGATAGCAACA |
| dZ 12202a | SRT6 | 12202 | RCA26b | TCAAAAGACTCTTCAAGGCTAGCTACAAACGATTTTAAG |
| dZ 12262a | SRT6 | 12262 | | CATCTTTCCAACCTAGGCTAGCTACAAACGAGTTGCATG |
| dZ 12290a | SRT6 | 12290 | RCA27b | TTATACATTGGGTAGGCTAGCTACAAACGAAAGCTTGAT |
| dZ 12299a | SRT6 | 12299 | | CTAGCCTGTTTATACAGGCTAGCTACAAACGATTGGTCA |
| dZ 12350a | SRT6 | 12350 | RCA28b | AAAAGCATTGCTCGAGGCTAGCTACAAACGAAAGCACTAG |
| dZ 12359a | SRT6 | 12359 | | AGCATAGTAAAAGCAGGCTAGCTACAAACGATGTCGCA |
| dZ 12495a | SRT6 | 12495 | RCA29b | ATTTTATATGTGTTAGGCTAGCTACAAACGAAAGTCGTT |
| dZ 12557a | SRT6 | 12557 | | TCTACACCTGTTGGAGGCTAGCTACAAACGATTCCCACA |
| dZ 12618a | SRT6 | 12618 | RCA30b | TGCTAAATTAGGTGAAGGCTAGCTACAAACGATGTCCTATA |
| dZ 13533a | SRT7 | 13533 | | TGTAAAAGCCCTGAGGCTAGCTACAAACGAAACGACATC |
| dZ 13625a | SRT7 | 13625 | | ATCAATTAAATTGTCAGGCTAGCTACAAACGACTTCGTC |
| dZ 13726a | SRT7 | 13726 | | AAAGTCATGTTAGCAAGGCTAGCTACAAACGAAAGCTGGAC |
| dZ 14172a | SRT7 | 14172 | RCA18b | CCCTGGTCAAGGTTAAGGCTAGCTACAAACGAAATGGCAT |
| dZ 14578a | SRT7 | 14578 | | CCAGAACGACGCGTGCAGGCTAGCTACAAACGAAAGCAGGGT |
| dZ 14829a | SRT8 | 14829 | | GTTGCTGTATCACAGGCTAGCTACAAACGAAATTGTTGG |
| dZ 14984a | SRT8 | 14984 | | ACTCATTGAATCATAAAGGCTAGCTACAAACGAAAAGCTA |
| dZ 15029a | SRT8 | 15029 | | GACATTACGTTAGGCTAGCTACAAACGAAATGGAAA |
| dZ 15165a | SRT8 | 15165 | | CGGCTATTGATTCAAGGCTAGCTACAAACGAAATTTTTG |
| dZ 15202a | SRT8 | 15202 | RCA20b | TTGCTTGTCCAATTAGGCTAGCTACAAACGATAAGCTAG |
| dZ 15282a | SRT8 | 15282 | RCA21b | GGATAATCCCAACCCAGGCTAGCTACAAACGAAAGGTGAG |
| dZ 15439a | SRT8 | 15439 | RCA22b | GAACGCCACACATGAGGCTAGCTACAAACGACATTTCAC |
| dZ 15506a | SRT8 | 15506 | | AAAAACACTATTAGCAGGCTAGCTACAAACGAAAGCAGTT |
| dZ 15703a | SRT8 | 15703 | | TCAGAGAGTATCATCAGGCTAGCTACAAACGATGAGAAAAT |

CDT = Circular DNA Template

SUPPORTING INFORMATION

Table S2. Continued. Sequences of all 10-23 DNAzyme variants tested in this work.

| DNAzyme | Transcript | Target Site (MN908947.3) | CDT | Sequence (5'→3') |
|------------|------------|-----------------------------|--------|---|
| 10-23 Core | | | | -----GGCTAGCTACAAACGA----- |
| dZ 15921a | SRT8 | 15921 | | CTGGGTAAGGAAGGTAGGCTAGCTACAAACGAACATAATC |
| dZ 16334a | SRT9 | 16334 | | TGATGTTGATATGACAGGCTAGCTACAAACGAGGTCTAA |
| dZ 16485a | SRT9 | 16485 | | CTTGTCCATTAGCACAGGCTAGCTACAAACGAAATGAAA |
| dZ 16501a | SRT9 | 16501 | | TTATATAAACCAAAAGGCTAGCTACAAACGATTGTCAT |
| dZ 16583a | SRT9 | 16583 | RCA33b | AATGTAATCACCAGCAGGCTAGCTACAAACGATTGTCAG |
| dZ 16727a | SRT9 | 16727 | RCA34b | AACTTCCCATTGAAAGGGCTAGCTACAAACGAGTAATTCT |
| dZ 16890a | SRT9 | 16890 | | AATCACCAACATTAAAGGCTAGCTACAAACGATTGTAAGT |
| dZ 16912a | SRT9 | 16912 | RCA35b | GTATGTTGATGTCAGCAGGCTAGCTACAAACGAAAAATAAT |
| dZ 16925a | SRT9 | 16925 | | TAATGGCATTAAGTGTAGGCTAGCTACAAACGAGTGATGTC |
| dZ 16981a | SRT9 | 16981 | | GGGTATAAGCCAGTAAGGCTAGCTACAAACGATCTAACAT |
| dZ 17207a | SRT9 | 17207 | | TTTATCTATAGGCAAAAGGCTAGCTACAAACGATTTTAAT |
| dZ 17344a | SRT9 | 17344 | | TCATCAAAGACAACTAGGCTAGCTACAAACGATCTGCTG |
| dZ 17378a | SRT9 | 17378 | | AAACACTCAAATCATAAAGGCTAGCTACAAACGATTGGCC |
| dZ 17406a | SRT9 | 17406 | | AGTGCTTAGCACGTAAGGCTAGCTACAAACGACTGGCATT |
| dZ 17498a | SRT9 | 17498 | | ACACACTGAATTGAAAGGCTAGCTACAAACGATTCTGGT |
| dZ 17522a | SRT9 | 17522 | RCA36b | GGACCTATAAGTTTCAGGCTAGCTACAAACGAAAGTCTAC |
| dZ 17567a | SRT9 | 17567 | | AACAAATTTCAGCAGGAGGCTAGCTACAAACGAAACGCCGA |
| dZ 17658a | SRT9 | 17658 | | TAACACCCTATAAAAGGCTAGCTACAAACGATTTTAAA |
| dZ 17713a | SRT9 | 17713 | | TCTCTTACACAGCCTAGGCTAGCTACAAACGATTGTCGCC |
| dZ 17730a | SRT9 | 17730 | | GGTTACGTGTAAGGAAGGCTAGCTACAAACGATCTCTTAC |
| dZ 17780a | SRT9 | 17780 | | AGCATTCTGTGAAATTAGGCTAGCTACAAACGAAAGGTGAA |
| dZ 18135a | SRT10 | 18135 | | AACCTCAGTTTGAAAGGCTAGCTACAAACGATTGTGTC |
| dZ 18153a | SRT10 | 18153 | | CAGGTATGTCACACAGGCTAGCTACAAACGAAAACCTC |
| dZ 18235a | SRT10 | 18235 | | TTAGGGTAACCATTAAAGGCTAGCTACAAACGATTGATAAT |
| dZ 18259a | SRT10 | 18259 | | GCTCTTCGCGGGTGAGGCTAGCTACAAACGAAAACATGT |
| dZ 18391a | SRT10 | 18391 | | CCTGTAGGTACAGCAAGGCTAGCTACAAACGATAGTTAA |
| dZ 18470a | SRT10 | 18470 | RCA37b | GAGGTGTTAAATTGAGGCTAGCTACAAACGACTCCAGGC |
| dZ 18535a | SRT10 | 18535 | | CTTAACATTGTACAAGGCTAGCTACAAACGACTTTATAC |
| dZ 18583a | SRT10 | 18583 | RCA38b | GCCCATAAAGACAATAGGCTAGCTACAAACGAGACTCTGT |
| dZ 18640a | SRT10 | 18640 | | GTGCGCTCAGGTCTAGGCTAGCTACAAACGATTTCACAA |
| dZ 18791a | SRT10 | 18791 | | GTTCCTTGTAGGTAGGCTAGCTACAAACGACTGTAAAA |
| dZ 18818a | SRT10 | 18818 | | ACCATGGACTTGTACAAGGCTAGCTACAAACGACAGATCA |
| dZ 18919a | SRT10 | 18919 | | ATTATAGGATATTCAAGGCTAGCTACAAACGAACTCCAGT |
| dZ 18941a | SRT10 | 18941 | | ATTAATCTTCAGTTCAAGGCTAGCTACAAACGACACCAATT |
| dZ 18973a | SRT10 | 18973 | RCA39b | ACAACCATGTGTTAAAGGCTAGCTACAAACGACTTTCTAC |
| dZ 19033a | SRT10 | 19033 | RCA40b | GCTTGTAGGTTACCAAGGCTAGCTACAAACGAGTCGTGAA |
| dZ 19182a | SRT10 | 19182 | | AATTCCAAAATAGGCAGGCTAGCTACAAACGAAACACCATC |
| dZ 19334a | SRT10 | 19334 | | AAACAAAGCACTTTAGGCTAGCTACAAACGACAAAAGCT |
| dZ 19376a | SRT10 | 19376 | | TGGACTGTAGAGTAAGGCTAGCTACAAACGAAAGAAAAT |
| dZ 19398a | SRT10 | 19398 | RCA41b | CTTGTTCATGAGAGGCTAGCTACAAACGATCACATGG |
| dZ 19699a | SRT11 | 19699 | | TAAACAGTGTATTAAAGGCTAGCTACAAACGAGATAAGAAA |
| dZ 19743a | SRT11 | 19743 | | TTTATTTCAAAACAGGCTAGCTACAAACGATCTACATC |
| dZ 19825a | SRT11 | 19825 | | TTATTGAGTATTTCAGGCTAGCTACAAACGACTCTGGTA |
| dZ 19892a | SRT11 | 19892 | | TATATGTCGGAGCAGGCTAGCTACAAACGACTCTTTG |
| dZ 19915a | SRT11 | 19915 | | ATAGAACAAACACCAAGGCTAGCTACAAACGAGTAGATA |
| dZ 19963a | SRT11 | 19963 | | GTGAGTGGTGCACAAAGGCTAGCTACAAACGACGTTTCAG |
| dZ 20103a | SRT11 | 20103 | | TGTGACTCCATTAAAGGCTAGCTACAAACGATAGTTGT |
| dZ 20134a | SRT11 | 20134 | RCA31b | TTGAACTGTGTTTTAGGCTAGCTACAAACGAGGCTTC |
| dZ 20156a | SRT11 | 20156 | | ACCATCAACTTCTAGGCTAGCTACAAACGAAATAATTG |
| dZ 20184a | SRT11 | 20184 | | AGTAAGTTTCAGGTAAAGGCTAGCTACAAACGATGTTGGAC |
| dZ 20216a | SRT11 | 20216 | | TTTAAATTCTGTAAAGGCTAGCTACAAACGATTCTACTC |
| dZ 20251a | SRT11 | 20251 | | AATTCTAAGAAATCAAGGCTAGCTACAAACGATTCCATT |
| dZ 20276a | SRT11 | 20276 | | CCGTCATGAAATTCAAGGCTAGCTACAAACGACCATAGCT |
| dZ 20412a | SRT11 | 20412 | RCA32b | GAATAAAATCTCTAAGGCTAGCTACAAACGATCAAAAGG |
| dZ 20426a | SRT11 | 20426 | | GTACTGTCCATTAGGAGGCTAGCTACAAACGAAAATCTT |

CDT = Circular DNA Template

SUPPORTING INFORMATION

Table S2. Continued. Sequences of all 10-23 DNAzyme variants tested in this work.

| DNAzyme | Transcript | Target Site (MN908947.3) | CDT | Sequence (5'→3') |
|------------|------------|-----------------------------|--------|---|
| 10-23 Core | | | | -----GGCTAGCTACACGA----- |
| dZ 20511a | SRT11 | 20511 | | CAAAATCATCAAGTAAGGCTAGCTACACGAAAATCAAT |
| dZ 20716a | SRT12 | 20716 | | CACTTTCTAATAGCAGGCTAGCTACACGATCTTGCA |
| dZ 20730a | SRT12 | 20730 | | AATTTGAAGGTACAGGCTAGCTACACGATTCTAA |
| dZ 20756a | SRT12 | 20756 | | TTTAGGTAATGTTGCAGGCTAGCTACACGATATCACCA |
| dZ 20788a | SRT12 | 20788 | | TGAGTATATTTCGCGAGGCTAGCTACACGATTATCA |
| dZ 20817a | SRT12 | 20817 | | TGTTAATGTGTTAACAGGCTAGCTACACGATTGACAC |
| dZ 20851a | SRT12 | 20851 | | AAATGTATAACTCTCAGGCTAGCTACACGATTATAGG |
| dZ 20882a | SRT12 | 20882 | | TGGTGCACACTCCTTAGGCTAGCTACACGACAGAACCA |
| dZ 20954a | SRT12 | 20954 | | GACAAAGTCATTAAGGAGCTAGCTACACGACTGAATCG |
| dZ 20992a | SRT12 | 20992 | | GTTGCACAATCACCAAGGCTAGCTACACGACAAAGTTG |
| dZ 21086a | SRT12 | 21086 | RCA51b | ACCCCTTTAGAGTCAGGCTAGCTACACGATTCTTT |
| dZ 21115a | SRT12 | 21115 | | TGTATAAACCCACAAAGGCTAGCTACACGAGTAAGTGA |
| dZ 21127a | SRT12 | 21127 | | GCTAGCTTTGTTGAGGCTAGCTACACGAAAACCCAC |
| dZ 21238a | SRT12 | 21238 | | GCATTACACATTAGTAAGGCTAGCTACACGAAAAGGCTG |
| dZ 21290a | SRT12 | 21290 | | GCCTGGTTGCCAACAGGCTAGCTACACGAAATTACAT |
| dZ 21313a | SRT12 | 21313 | | ATGACATAACCATCTAGGCTAGCTACACGATTGTCGC |
| dZ 21338a | SRT12 | 21338 | RCA52b | CCTCCAAAATATGTAAGGCTAGCTACACGATTGCATGC |
| dZ 21345a | SRT12 | 21345 | | TTGTATTCCCTCCAAAGGCTAGCTACACGAAATGTAATT |
| dZ 21390a | SRT12 | 21390 | | ATTTACTCATGTCAAGGCTAGCTACACGAAAAGATA |
| dZ 21467a | SRT12 | 21467 | | AGAAGAGATAAAATCAGGCTAGCTACACGAAATATTGA |
| dZ 21744a | SRT13 | 21744 | | ATGGAACCAAGTAACAGGCTAGCTACACGATGGAAAAG |
| dZ 21768a | SRT13 | 21768 | | ATTGGTCCCAGAGCACGGCTAGCTACACGAGTATAGCA |
| dZ 21969a | SRT13 | 21969 | | CAAAATGGATCATTAGGCTAGCTACACGAAAATTGA |
| dZ 22161a | SRT13 | 22161 | | AGAATATATTAAAGGCTAGCTACACGAAACCATCA |
| dZ 22614a | SRT14 | 22614 | | CTTCTGTTCCAAGCAGGCTAGCTACACGAAAACAGAT |
| dZ 23847a | SRT15 | 23847 | | TTAAAGCACCGGTTAACGGCTAGCTACACGATGTGACA |
| dZ 24178a | SRT16 | 24178 | | ACAGTGCAGAAGTGTAGGCTAGCTACACGATGAGCAAT |
| dZ 24390a | SRT16 | 24390 | | AAAGTTTCCAAGTGCAGGCTAGCTACACGATTGCTGTG |
| dZ 24468a | SRT16 | 24468 | | TGAAATTGCAACAAAGGCTAGCTACACGATGGAGCTA |
| dZ 24551a | SRT16 | 24551 | | TGAAGTCTGCCGTGAGGCTAGCTACACGACAACCTAT |
| dZ 24710a | SRT17 | 24710 | | GACTGAGGGAAAGGACAGGCTAGCTACACGAAAGATGAT |
| dZ 25097a | SRT17 | 25097 | | TCAATTCTTTGAAGGCTAGCTACACGAGTTACAA |
| dZ 25271a | SRT17 | 25271 | | CTACAGCAACTGGTCAGGCTAGCTACACGAAACAGAAA |
| dZ 25501a | SRT18 | 25501 | | GGGAGTGAGGCTTGTAGGCTAGCTACACGACGGTATCG |
| dZ 25524a | SRT18 | 25524 | | CGCCAACAATAAGCAGGCTAGCTACACGACCGAAAGG |
| dZ 25540a | SRT18 | 25540 | | ACAGCAAGAAGTGCAGGCTAGCTACACGAGCCAACAA |
| dZ 25556a | SRT18 | 25556 | | GAAGCGCTCTGAAAAAGGCTAGCTACACGAGCAAGAA |
| dZ 25596a | SRT18 | 25596 | | AGAGTCTAGTTGCCAGGCTAGCTACACGACTTTTT |
| dZ 25621a | SRT18 | 25621 | | TTGCAACAAAGTGAAGGCTAGCTACACGAAACCTTGG |
| dZ 25647a | SRT18 | 25647 | | AAACTGTTACAAACAAAGGCTAGCTACACGAAACAGCAA |
| dZ 25660a | SRT18 | 25660 | | AAAAGGTGTGAGTAAGGCTAGCTACACGATGTTACAA |
| dZ 25765a | SRT18 | 25765 | | CAAAGCCAAGCCTCAGGCTAGCTACACGATATTATTC |
| dZ 25806a | SRT18 | 25806 | | TGGCATCATAAAAGTAAAGGCTAGCTACACGAGGGTTTT |
| dZ 25826a | SRT18 | 25826 | | ATGCCAGCAAAGAAAAGGCTAGCTACACGAGTTGGCA |
| dZ 25847a | SRT18 | 25847 | | ACAATAGTCGTAACAAAGGCTAGCTACACGATAGTATGC |
| dZ 25937a | SRT18 | 25937 | | ACCAATCTGGTAGTCAGGCTAGCTACACGAGTTCAGAA |
| dZ 25967a | SRT18 | 25967 | | TTACTCCAGATTCCCAGGCTAGCTACACGATTTTCAGT |
| dZ 26072a | SRT18 | 26072 | | GATGAAGAAGGTAACAGGCTAGCTACACGAGTTCAACA |
| dZ 26155a | SRT18 | 26155 | | ATTACTGGATTAACAAAGGCTAGCTACACGATCCGGATG |
| dZ 26666a | SRT19 | 26666 | | AGGAAAATTAACCTAACGGCTAGCTACACGATATATACA |
| dZ 26718a | SRT19 | 26718 | | TAAACAGCAGCAAGCAGGCTAGCTACACGAAAAACAAG |
| dZ 26874a | SRT19 | 26874 | | GGCACGTTGAGAAGAAGGCTAGCTACACGAGTTAGTT |
| dZ 27137a | SRT19 | 27137 | | AATGGTCTGTGTTAACGGCTAGCTACACGATTATAGTT |
| dZ 28321a | SRT20 | 28321 | | CTGAGGGTCCACCAAAAGGCTAGCTACACGAGTAATGCG |
| dZ 28350a | SRT20 | 28350 | | TTCTCCATTCTGGTAGGCTAGCTACACGATGCCAGTT |
| dZ 28692a | SRT20 | 28692 | | GTGATCTTGGTAGGCTAGCTACACGATCAAGGCT |
| dZ 28704a | SRT20 | 28704 | | GCGGGTGCCAATGTGAGGCTAGCTACACGACTTTGGT |
| dZ 28722a | SRT20 | 28722 | | AGCATTGTTAGCAGGAGGCTAGCTACACGATGCCAGTG |

CDT = Circular DNA Template

SUPPORTING INFORMATION

Table S2. Continued. Sequences of all 10-23 DNAzyme variants tested in this work.

| DNAzyme | Transcript | Target Site (MN908947.3) | CDT | Sequence (5'→3') |
|------------|------------|-----------------------------|-----|--|
| 10-23 Core | | | | -----GGCTAGCTACAAACGA----- |
| dZ 28734a | SRT20 | 28734 | | TAGCACGATTGCAGCAGGCTAGCTACAAACGATGTTAGCA |
| dZ 28771a | SRT20 | 28771 | | AGAACGCCCTTGCAAGGCTAGCTACAAACGAGTTGTC |
| dZ 28851a | SRT20 | 28851 | | AGTTGAATTCTTGAAGGCTAGCTACAAACGATGTTGCGA |
| dZ 29172a | SRT20 | 29172 | | TGCAATTGCGGCCAAGGCTAGCTACAAACGAGTTGTAA |
| dZ 29212a | SRT20 | 29212 | | GCGACATTCCGAAGAAGGCTAGCTACAAACGAGCTGAAGC |

CDT = Circular DNA Template

Table S3. Pearson correlation coefficient and p-values comparing log(Cleavage) versus RNAstructure predicted lowest energy ΔG values for 1) free DNAzyme, 2) RNA opening energy (full length RNA target ΔG – binding site masked full length RNA target ΔG), 3) an RNA sequence flanking 25 nt upstream & downstream of the DNAzyme binding site, and 4) an RNA sequence flanking 50 nt upstream & downstream of the DNAzyme binding site.

| Pearson Correlation of Log(Cleavage) vs. Predicted ΔG Values @ 23°C | Correlation Coefficient | p-Value |
|---|-------------------------|-----------|
| Free DNAzyme | 0.343 | 0.000004 |
| RNA Opening | 0.230 | 0.0005517 |
| RNA Target Site ± 25nt | 0.192 | 0.0054549 |
| RNA Target Site ± 50nt | 0.211 | 0.0021680 |

Table S4. Kinetic parameters of 10-23 DNAzymes that cleaved long SARS-CoV-2 RNA transcripts and corresponding 25 nt short transcripts.

| DNAzyme | RNA Target | k_{obs} (min ⁻¹) | γ_{max} (%) |
|-----------|-----------------------------|--------------------------------|--------------------|
| dZ 25524a | SRT18 (831 nt) | 0.00023 | 74 |
| | Complementary Oligo (25 nt) | 0.045 | 87 |
| dZ 12618a | SRT6 (584 nt) | 0.027 | 78 |
| | Complementary Oligo (25 nt) | 0.058 | 84 |
| dZ 25806a | SRT18 (831 nt) | 0.090 | 86 |
| | Complementary Oligo (25 nt) | 0.16 | 84 |
| dZ 13726a | SRT7 (1208 nt) | 0.094 | 80 |
| | Complementary Oligo (25 nt) | 0.54 | 84 |
| dZ 10325a | SRT4 (921 nt) | 0.066 | 71 |
| | Complementary Oligo (25 nt) | 0.090 | 85 |

Table S5. Sequence of Primers and CDT used in the quasi-exponential RCA system.

| Oligo | Sequence (5'→3') |
|-----------------------------|--|
| Circular DNA Template (CDT) | TCCCCATTTATCCTTCTACAAGCCGCATTAGTTAGAGTTTTCTATTAGGA |
| RNA Primer | UAAUGC GGCUUGUAGAAAGG |
| Primer 2 (P2) | TAGTTAGAGTTTTCTATTAGGATCCCCATT |

SUPPORTING INFORMATION

Table S6. Clinical patient sample SARS-CoV-2 test results for nasopharyngeal samples (NPS) and saliva samples. NPS samples were tested at Hamilton Regional Medical Laboratory Program. In NPS samples tested using a target panel including S Gene, "STF" refers to S Gene Target Failure and is suggestive of the B.1.1.529 variant in the context of circulating variants at the time of sample collection. Samples were assigned a presumed variant based on epidemiological context at the time of sample collection, indicative RT-PCR results, or if confirmed by sequencing. Coupled DNAzyme-RCA results were performed in triplicate using saliva, the mean RFU values are reported at 1 h reaction time, and the mean RFU rates, as determined between 20 - 60 min reaction time, are reported.

| ID | SARS-CoV-2 Status | NPS Ct Value | | | | | Presumed Variant (PANGO Lineage) | Saliva Ct Value | DNAzyme-RCA Saliva | | |
|------|-------------------|--------------|--------|----------|--------|--------|----------------------------------|-----------------|--------------------|---------------|-----|
| | | UTR | E Gene | ORF Gene | N Gene | S Gene | | N1 Gene Target | Mean RFU | Mean RFU Rate | |
| PS1 | POSITIVE | 21.32 | 18.85 | | | | B.1.1.7* | 27.82 | 30974 | 513 | |
| PS2 | POSITIVE | 25.1 | 24.1 | | | | B.1.1.7* | 34.03 | 25150 | 424 | |
| PS3 | POSITIVE | 16.26 | 14.72 | | | | B.1.1.7* | 30.63 | 33012 | 576 | |
| PS4 | POSITIVE | 19.61 | 17.41 | | | | B.1.1.7* | 34.32 | 33945 | 544 | |
| PS5 | POSITIVE | 18.6 | 16.46 | | | | B.1.1.7* | 28.04 | 28970 | 504 | |
| PS6 | POSITIVE | 18.9 | 14.62 | | | | B.1.1.7* | 34.39 | 19395 | 301 | |
| PS7 | POSITIVE | 13.48 | 1.83 | | | | P.1* | 32.09 | 27424 | 454 | |
| PS8 | POSITIVE | 24.95 | 23.12 | | | | WT or B.1.617.2‡ | 31.79 | 24983 | 404 | |
| PS9 | POSITIVE | 26.14 | 24.15 | | | | WT or B.1.617.2‡ | 26.6 | 22641 | 353 | |
| PS10 | POSITIVE | 3.8 | 28.6 | | | | WT or B.1.617.2‡ | 30.4 | 36130 | 586 | |
| PS11 | POSITIVE | 16.48 | 14.48 | | | | B.1.617.2* | 34.3 | 27627 | 459 | |
| PS12 | POSITIVE | 17.9 | 16.1 | 12.92 | 12.79 | 13.86 | B.1.617.2‡ | 30.75 | 13690 | 181 | |
| PS13 | POSITIVE | | | 9.68 | 7.16 | STF | B.1.1.529* | 31.83 | 23708 | 378 | |
| PS14 | POSITIVE | | | | 12.89 | 1.32 | STF | B.1.1.529* | 33.37 | 31623 | 486 |
| NS1 | NEGATIVE | | | | | | | | 18767 | 275 | |
| NS2 | NEGATIVE | | | | | | | | 11571 | 136 | |
| NS3 | NEGATIVE | | | | | | | | 16745 | 253 | |
| NS4 | NEGATIVE | | | | | | | | 16835 | 247 | |
| NS5 | NEGATIVE | | | | | | | | 18356 | 273 | |
| NS6 | NEGATIVE | | | | | | | | 19830 | 306 | |
| NS7 | NEGATIVE | | | | | | | | 9811 | 85 | |
| NS8 | NEGATIVE | | | | | | | | 20509 | 318 | |
| NS9 | NEGATIVE | | | | | | | | 13909 | 185 | |
| NS10 | NEGATIVE | | | | | | | | 20144 | 315 | |
| NS11 | NEGATIVE | | | | | | | | 14703 | 246 | |
| NS12 | NEGATIVE | | | | | | | | 13758 | 180 | |
| NS13 | NEGATIVE | | | | | | | | 16593 | 240 | |
| NS14 | NEGATIVE | | | | | | | | 13824 | 183 | |
| NS15 | NEGATIVE | | | | | | | | 21110 | 337 | |

STF = S Gene Target Failure, *N501 mutation detected, ^Whole genome sequencing, ‡Inferred variant based on circulating variants at the time of sample collection.

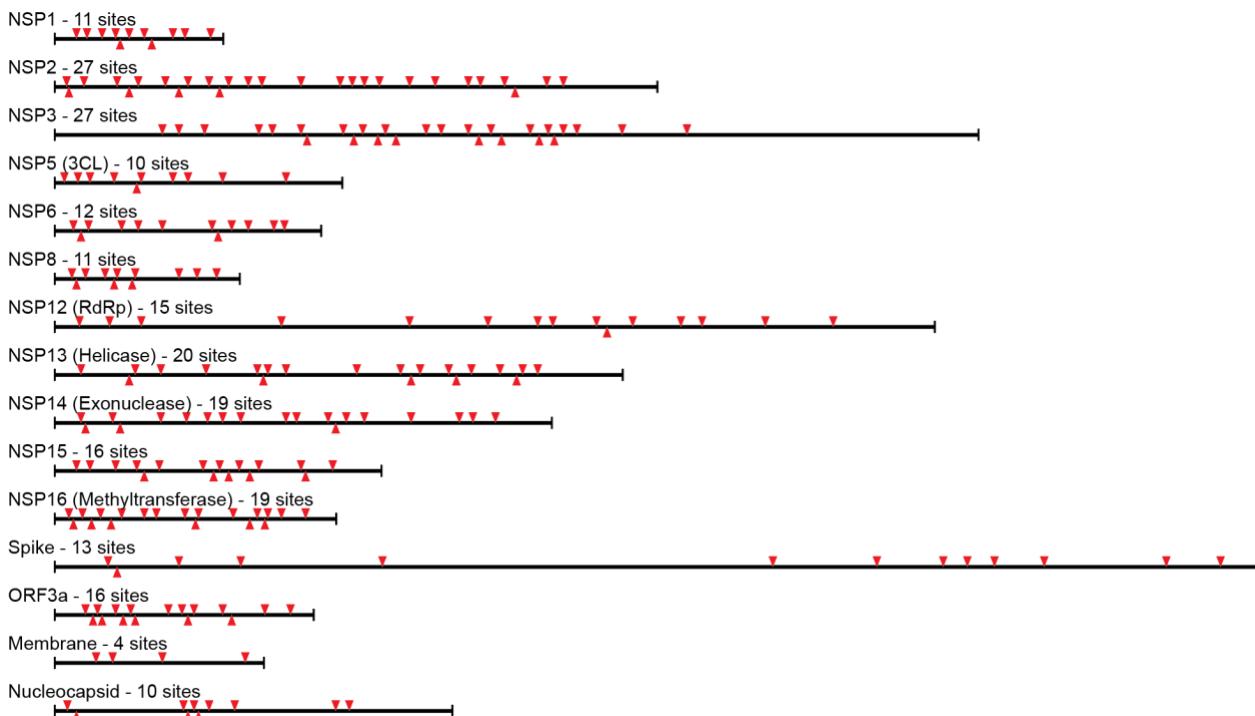
SUPPORTING INFORMATION

Table S7. A list of saliva-based SARS-CoV-2 molecular tests. Test times are approximate and encompass saliva sample preparation, test apparatus preparation and reaction time. The targeted SARS-CoV-2 genes are also listed.

| Technology | Technical Requirements | Target | Test time | # of clinical samples | LOD | Sensitivity | Specificity | Ref. |
|---|------------------------|----------------------------------|-------------|-----------------------|---------------|-------------|-------------|-----------|
| DNAzyme-Rolling Circle Amplification | 30 °C | NSP14 | 2 hr 15 min | 29 | 196 copies/µl | 86% | 100% | This test |
| RT-qPCR | Thermal cycler | ORF1ab / ORF8 / RdRp / E / N / S | 2-24 hr | 76-1939 | - | 50%-96% | 70.4%-100% | [2] |
| Strand displacement and amplification SDA (Saliva)* | 42 °C | S | 1 hr 15 min | 5 | 10 copies/µl | 40% | 100% | [3] |
| RT-Loop-mediated Isothermal Amplification (LAMP) | 65 °C | ORF1ab/ORF7a/S | 1 hr | 44 | 25 copies/µl | 83% | 100% | [4] |
| CRISPR/ Specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) (Saliva)* | 60 °C | N | 2 hr | 12 | 10 copies/µl | 93.1% | 98.5% | [5] |
| Recombinase Polymerase Amplification (RPA)* | 42 °C | S | 2 hr | 18 | 0.8 copies/µl | 100% | 100% | [6] |

NSP = non structured protein, ORF = open reading frame, RdRp = RNA-dependant RNA polymerase, E = envelope, N = nucleocapsid, S = spike, *Indicates the use of spiked saliva samples instead of patient samples.

Figure S1. Overview of SARS-CoV-2 RNA target sites across studied genes. Sites selected for DNAzyme cleavage screening indicated in red.



SUPPORTING INFORMATION

Figure S2. Titration of MgCl₂ in 10-23 DNAzyme-mediated cleavage of 25 nt SARS CoV-2 RNA substrates to evaluate optimal MgCl₂ concentration.

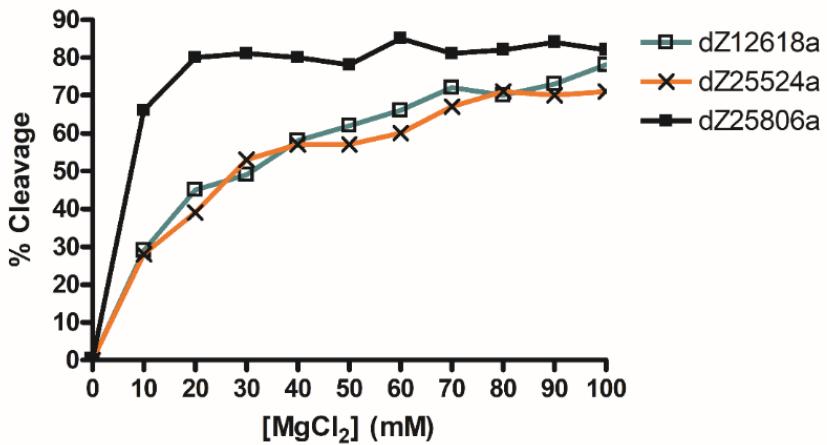
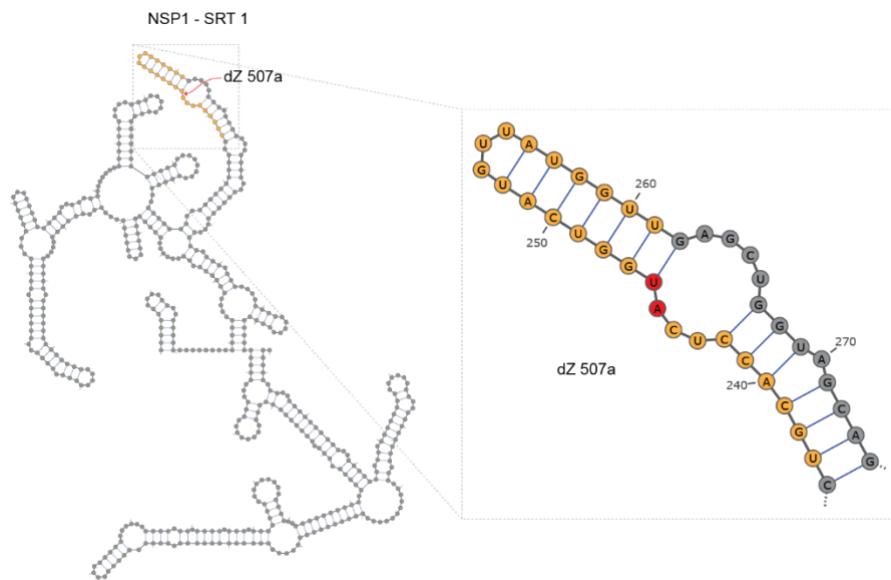
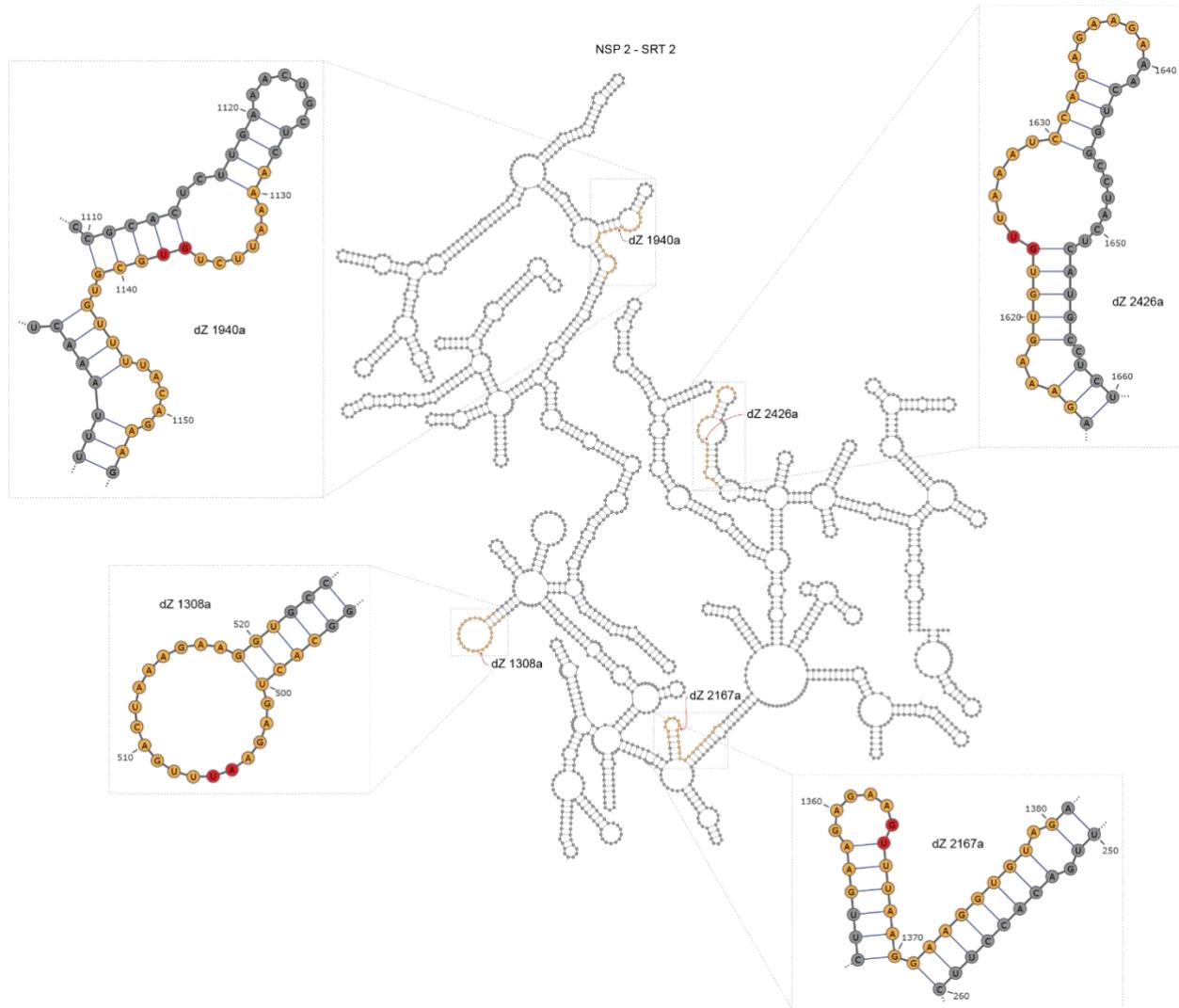


Figure S3. NSP1 RNA transcript secondary structure predicted using RNAfold webserver. Binding region of DNAzymes with >=20% FC (dZ 507a) indicated in orange. The enlargement shows sequence, local structure and cleavage junction (red).



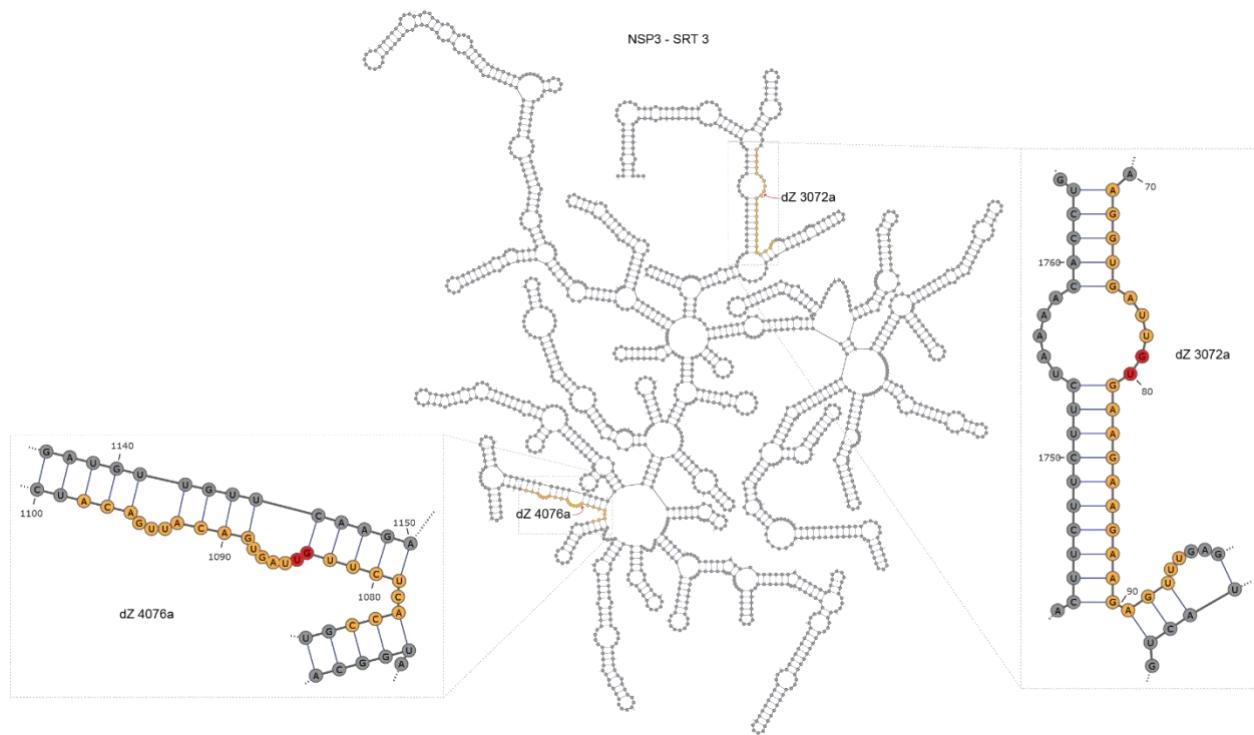
SUPPORTING INFORMATION

Figure S4. NSP2 RNA transcript secondary structure predicted using RNAfold webserver. Binding regions of DNAzymes with >=20% FC (dZ 1940a, dZ 2426a, dZ 1308a and dZ 2167a) indicated in orange. The enlargements show sequence, local structure and cleavage junctions (red).



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Figure S5. NSP3 RNA transcript secondary structure predicted using RNAfold webserver. Binding regions of DNAzymes with >=20% FC (dZ 4076a, and dZ 3072a) indicated in orange. The enlargements show sequence, local structure and cleavage junctions (red).



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Figure S6. NSP5 RNA transcript secondary structure predicted using RNAfold webserver. Binding regions of DNAzymes with $\geq 20\%$ FC (dZ 10325a, dZ 10491a and dZ 10800a) indicated in orange. The enlargements show sequence, local structure and cleavage junctions (red).

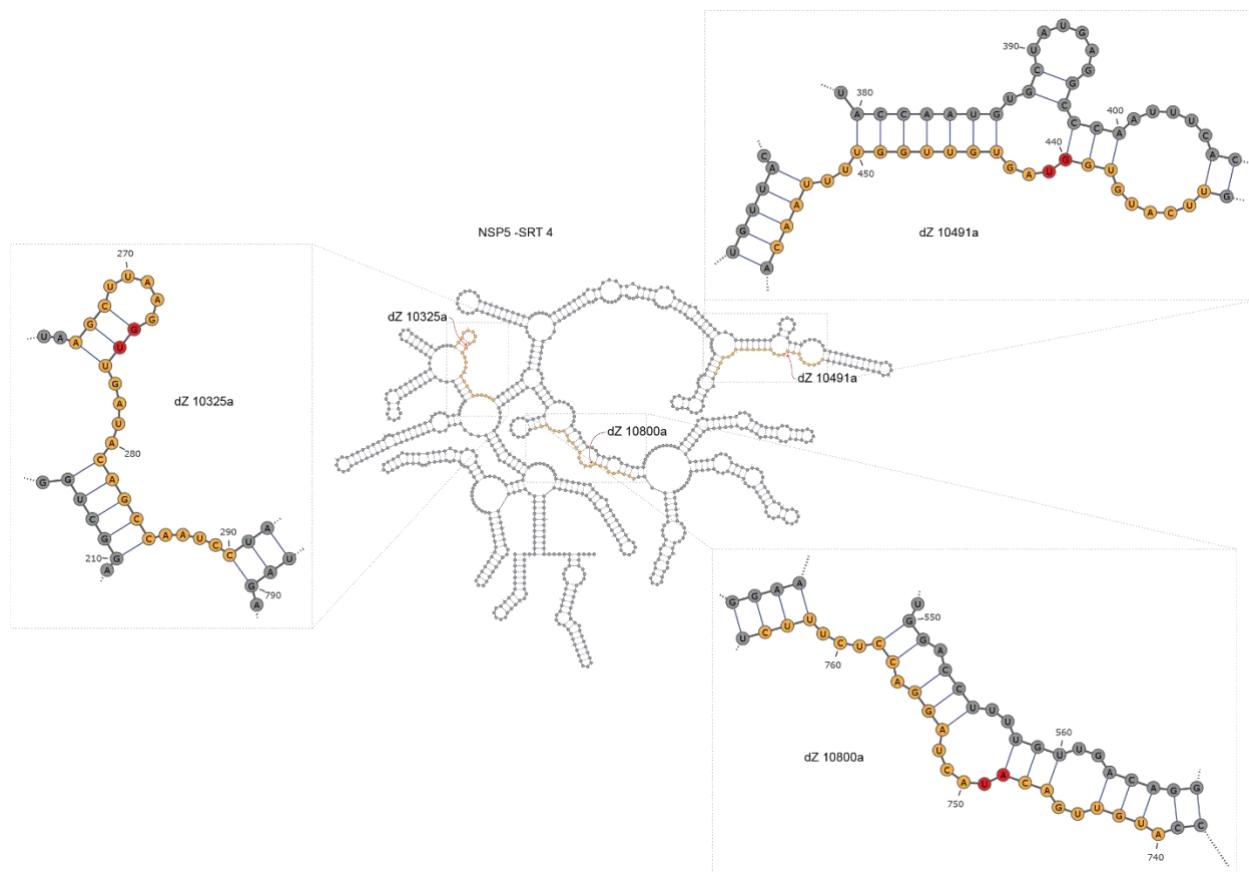
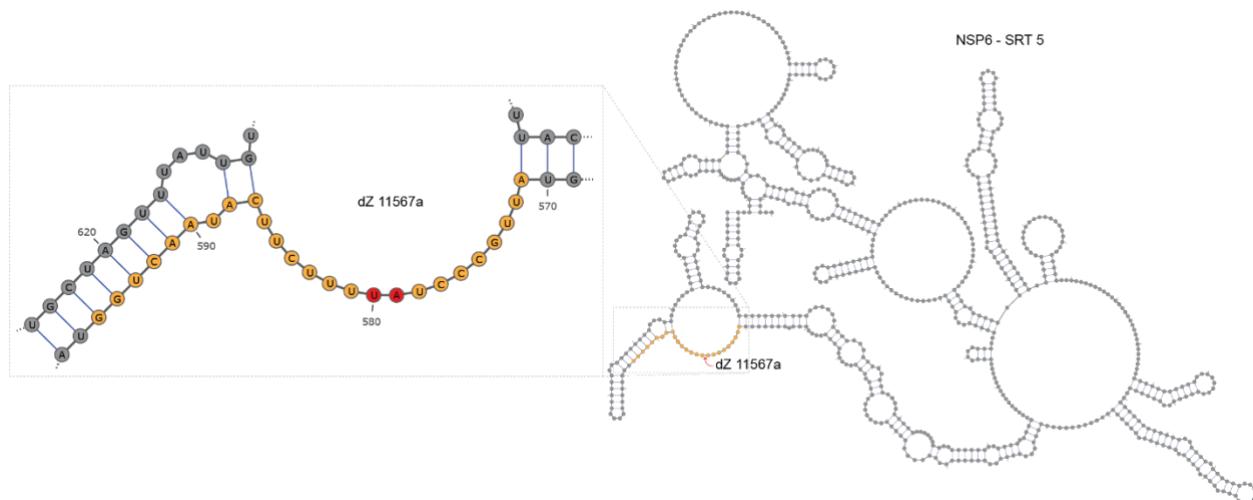


Figure S7. NSP6 RNA transcript secondary structure predicted using RNAfold webserver. Binding regions of DNAzymes with $\geq 20\%$ FC (dZ 11567a) indicated in orange. The enlargement shows sequence, local structure and cleavage junctions (red).



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Figure S8. NSP8 RNA transcript secondary structure predicted using RNAfold webserver. Binding regions of DNAzymes with >=20% FC (dZ 12262a) indicated in orange. The enlargement shows sequence, local structure and cleavage junctions (red).

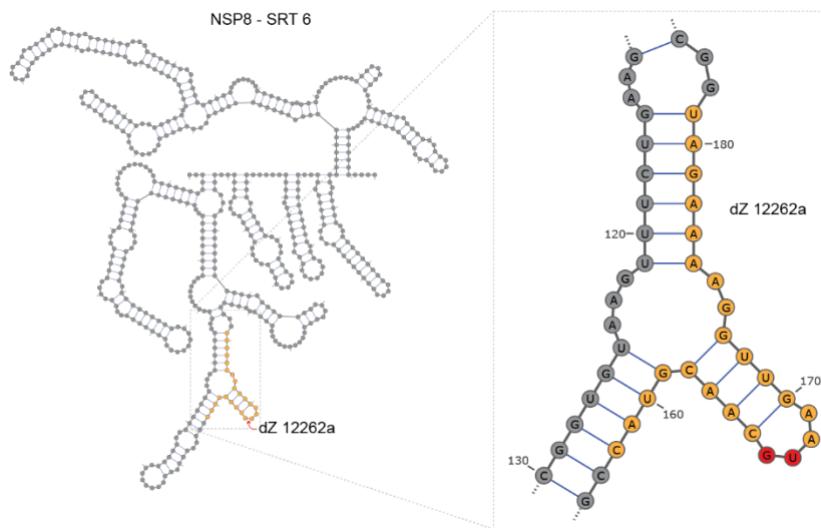
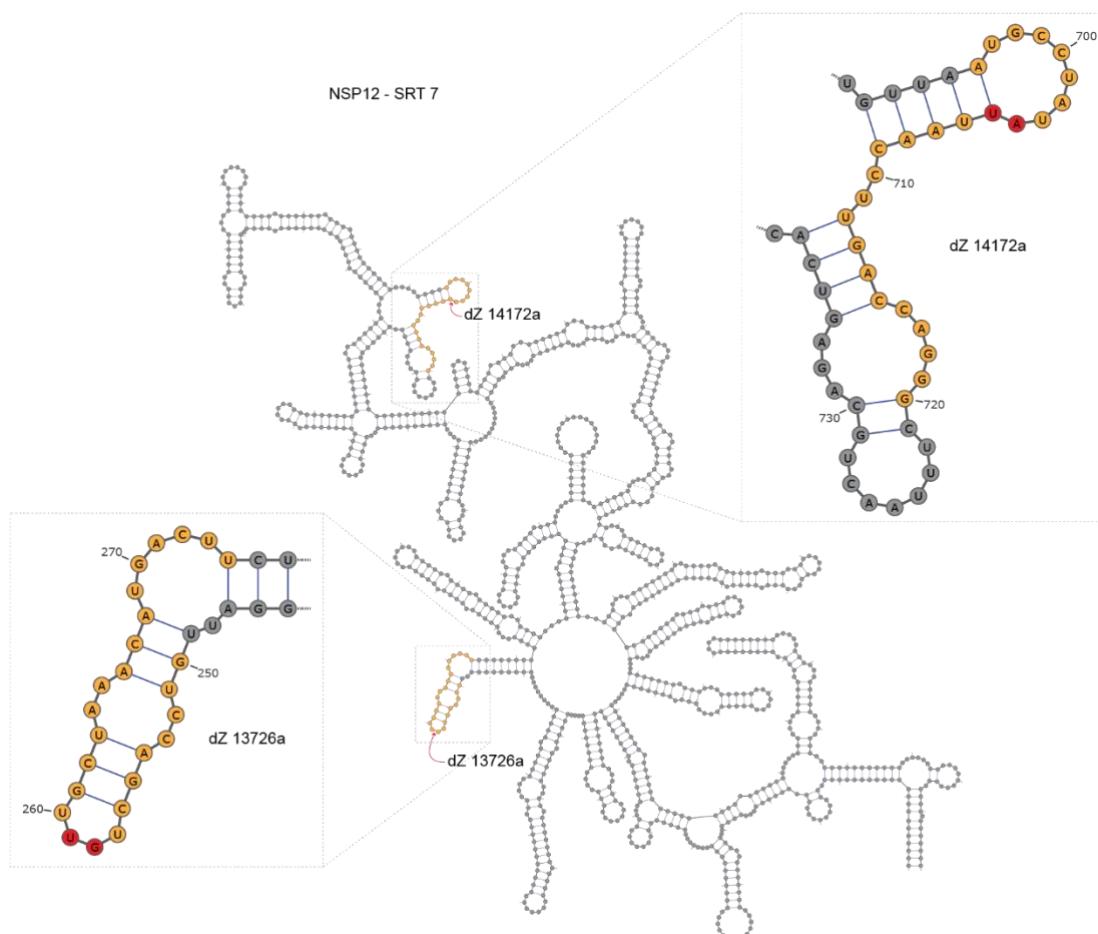
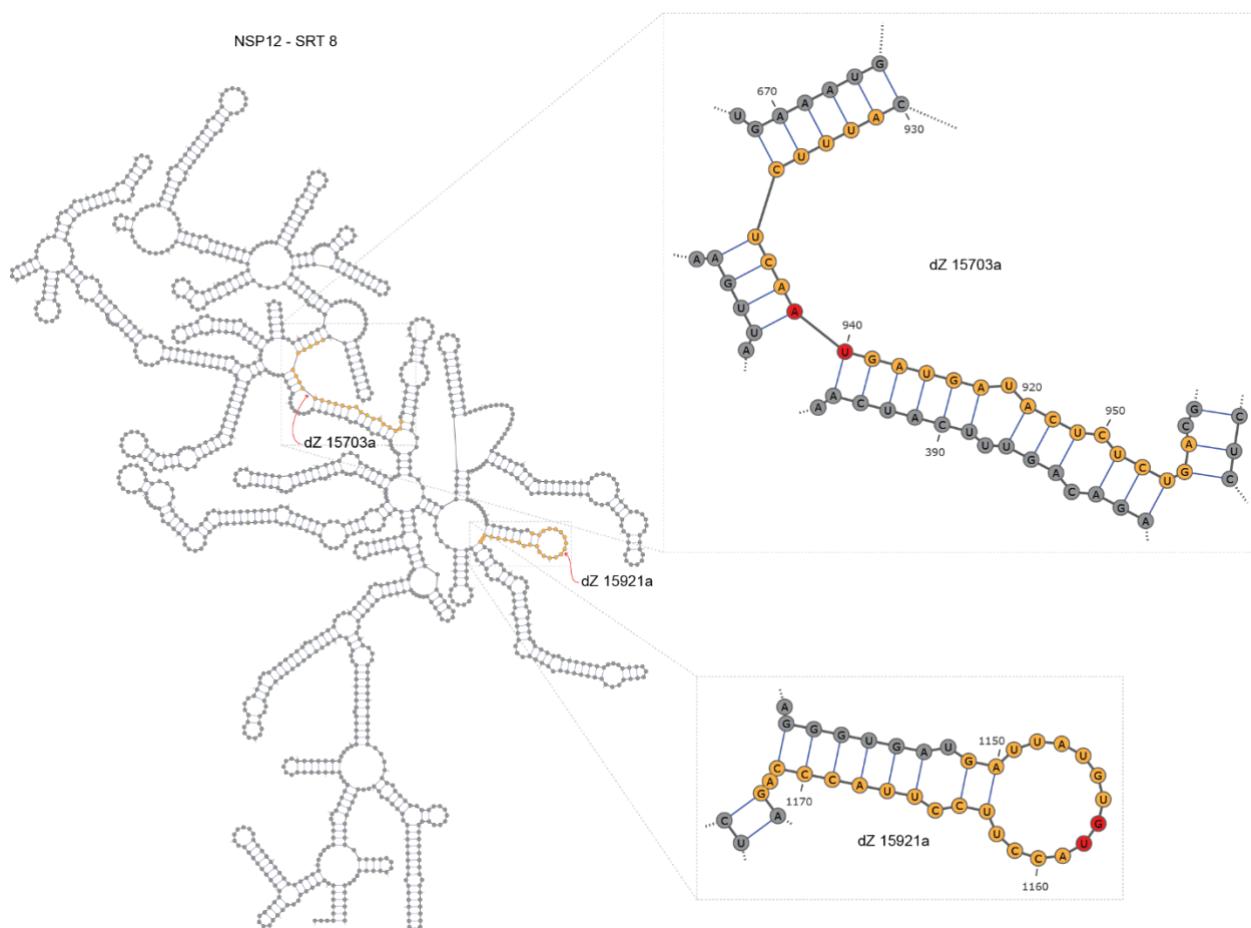


Figure S9. NSP12(SRT 7) RNA transcript secondary structure predicted using RNAfold webserver. Binding regions of DNAzymes with >=20% FC (dZ 13726a and dZ 14172a) indicated in orange. The enlargements show sequence, local structure and cleavage junctions (red).



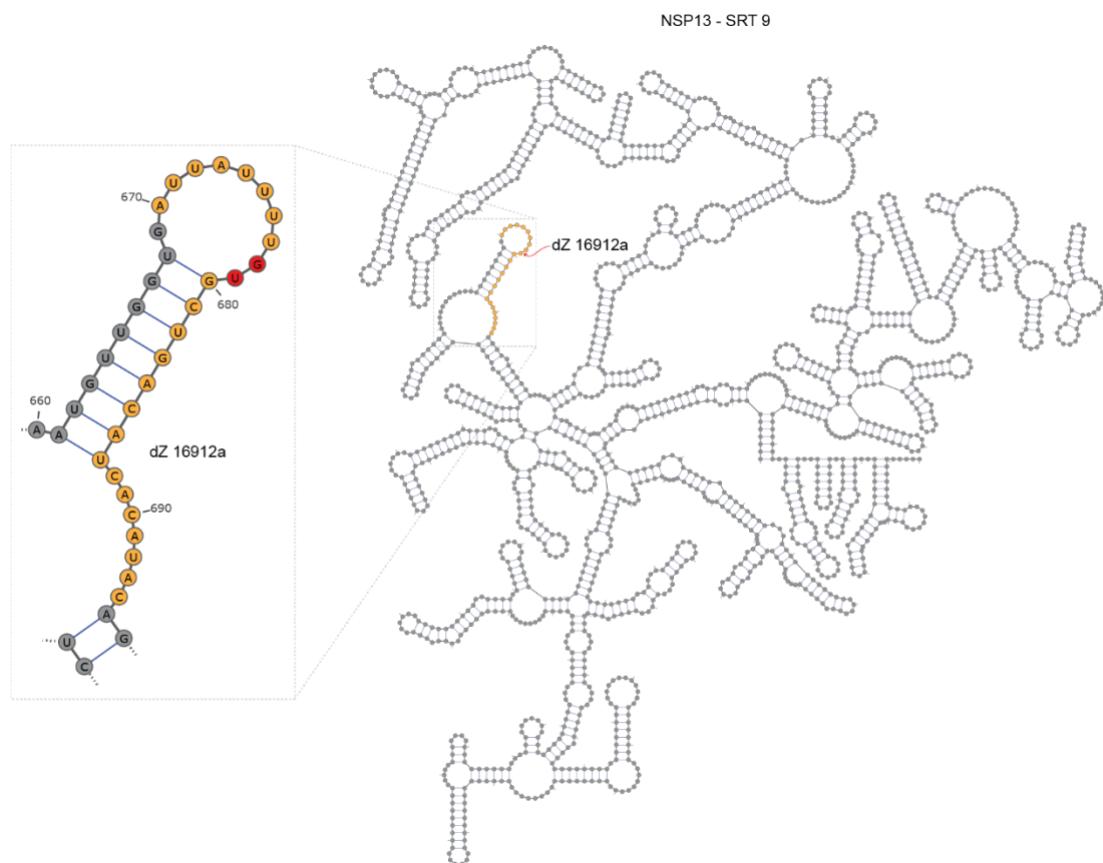
SUPPORTING INFORMATION

Figure S10. NSP12(SRT 8) RNA transcript secondary structure predicted using RNAfold webserver. Binding regions of DNAzymes with >=20% FC (dZ 15703a and dZ 15921a) indicated in orange. The enlargements show sequence, local structure and cleavage junctions (red).



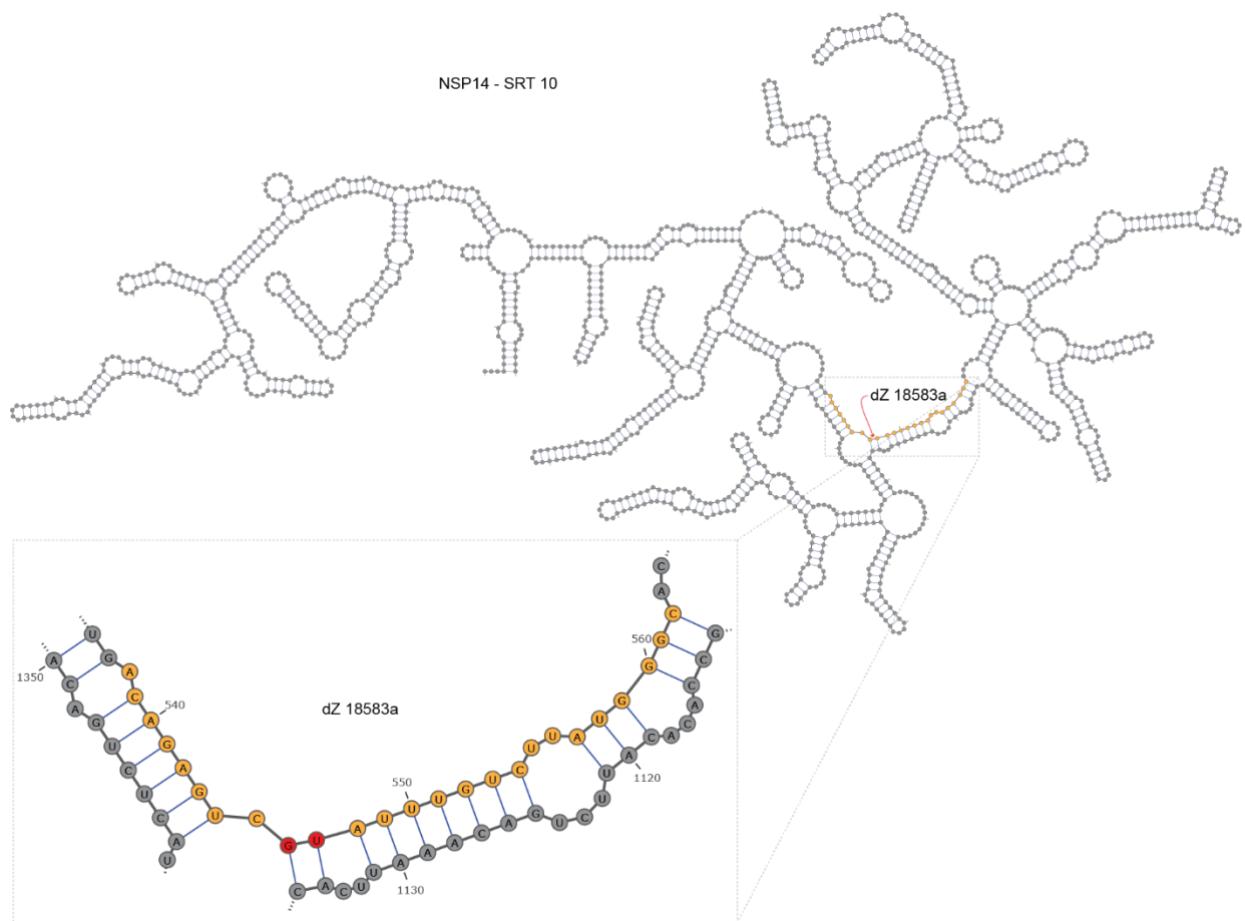
SUPPORTING INFORMATION

Figure S11. NSP13 RNA transcript secondary structure predicted using RNAfold webserver. Binding regions of DNAzymes with >=20% FC (dZ 16912a) indicated in orange. The enlargement shows sequence, local structure and cleavage junctions (red).



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Figure S12. NSP14 RNA transcript secondary structure predicted using RNAfold webserver. Binding regions of DNAzymes with >=20% FC (dZ 18583a) indicated in orange. The enlargement shows sequence, local structure and cleavage junctions (red).



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Figure S13. NSP15 RNA transcript secondary structure predicted using RNAfold webserver. Binding regions of DNAzymes with $\geq 20\%$ FC (dZ 20134a, dZ 20412a and dZ 19743a) indicated in orange. The enlargements show sequence, local structure and cleavage junctions (red).

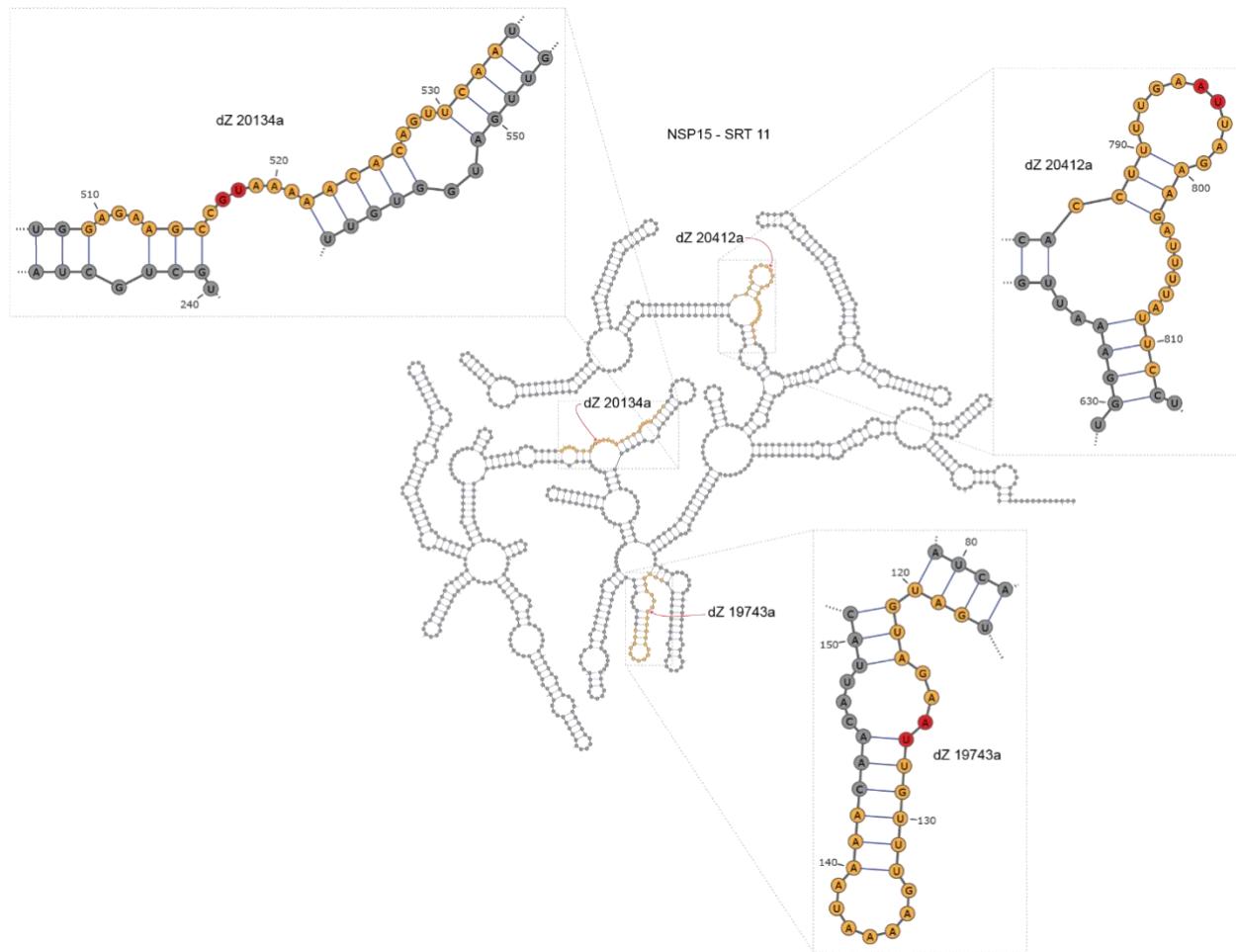
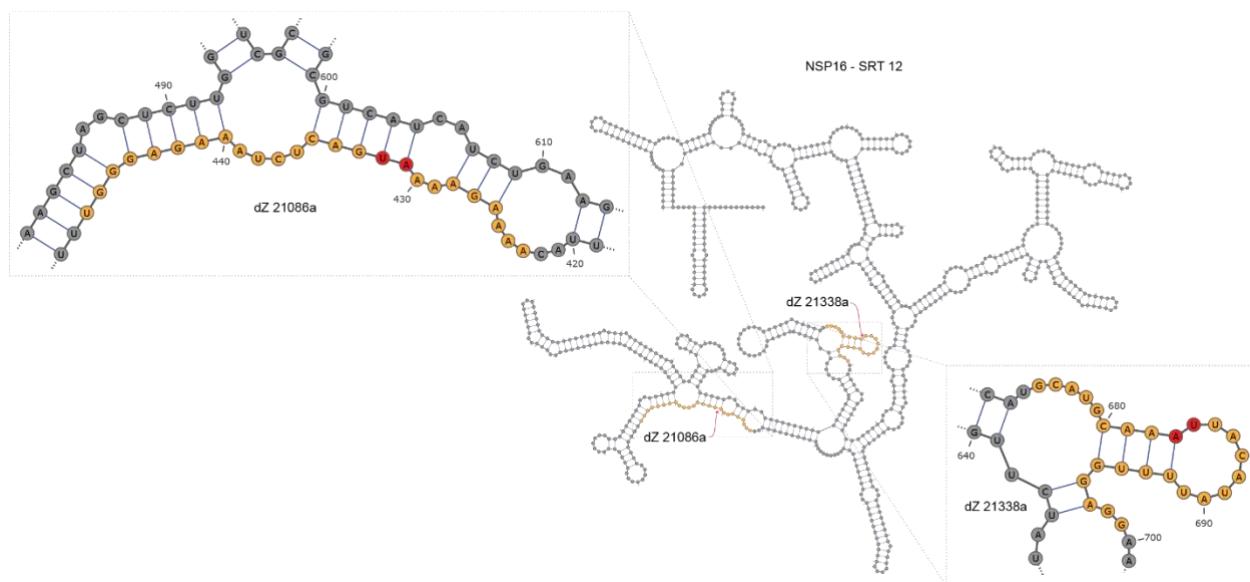


Figure S14. NSP16 RNA transcript secondary structure predicted using RNAfold webserver. Binding regions of DNAzymes with $\geq 20\%$ FC (dZ 21086a and dZ 21338a) indicated in orange. The enlargements show sequence, local structure and cleavage junctions (red).



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Figure S15. ORF3a RNA transcript secondary structure predicted using RNAfold webserver. Binding regions of DNAzymes with $\geq 20\%$ FC (dZ 25806a) indicated in orange. The enlargement shows sequence, local structure and cleavage junctions (red).

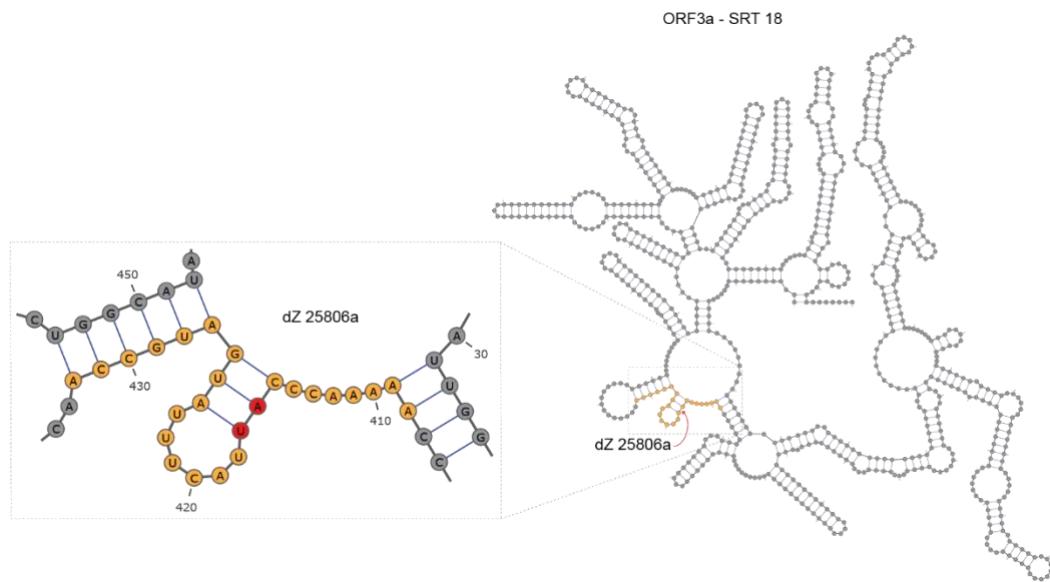
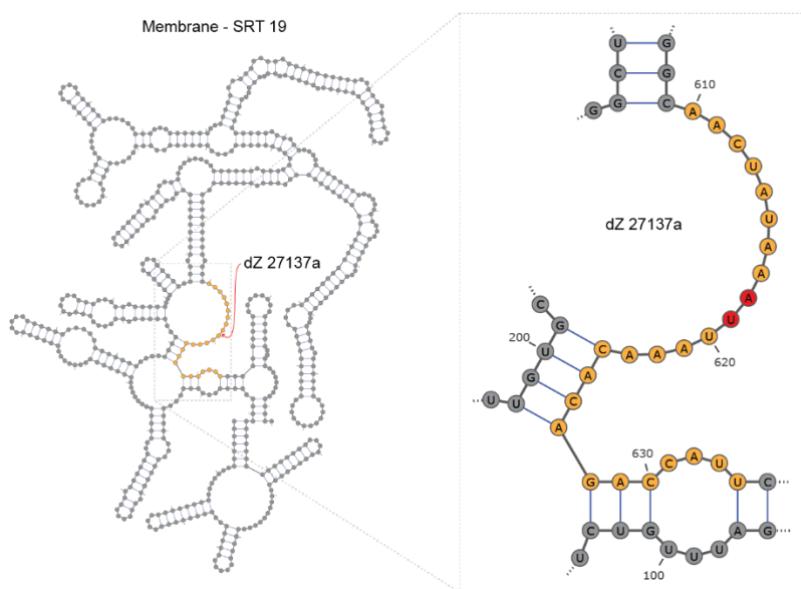


Figure S16. Membrane RNA transcript secondary structure predicted using RNAfold webserver. Binding regions of DNAzymes with $\geq 20\%$ FC (dZ 27137a) indicated in orange. The enlargement shows sequence, local structure and cleavage junctions (red).



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Figure S17. Nucleocapsid RNA transcript secondary structure predicted using RNAfold webserver. Binding regions of DNAzymes with >=20% FC (dZ 28851a, dZ 28704a and dZ 28350a) indicated in orange. The enlargements show sequence, local structure and cleavage junctions (red).

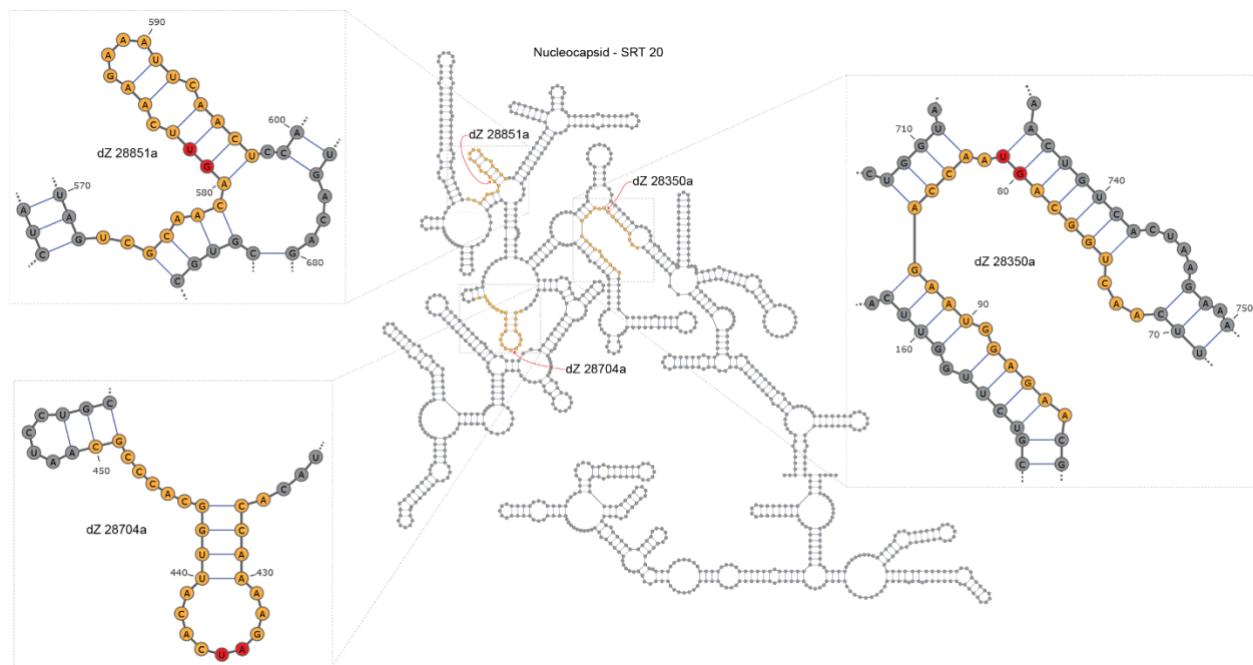
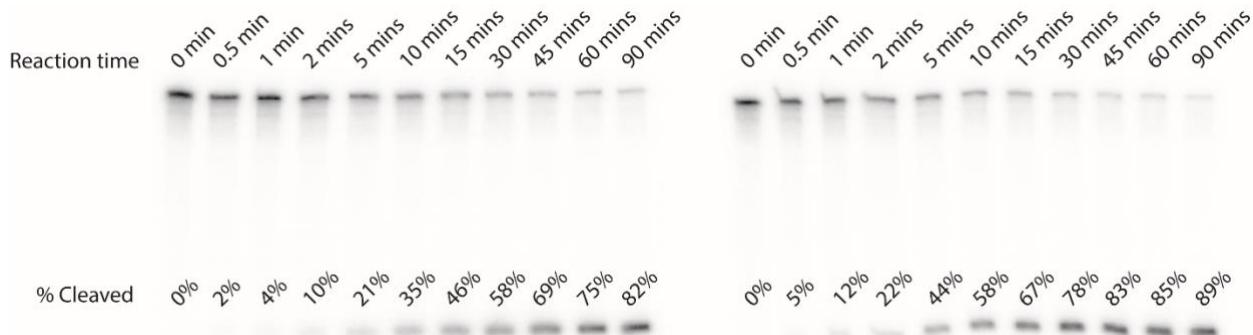
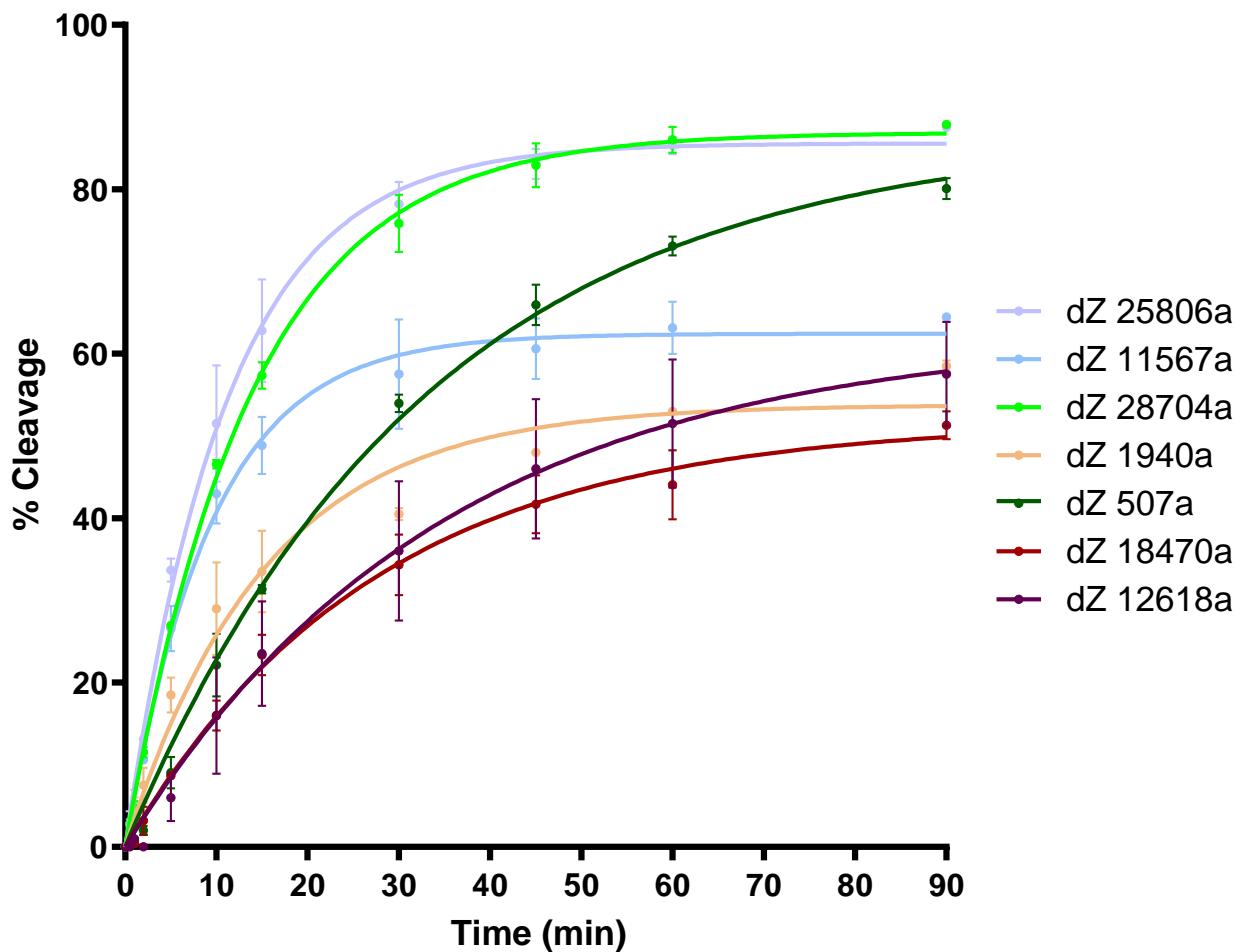


Figure S18. Time course of dZ 13726a cleavage of SRT7 (1208 nt) 5'-³²P-RNA in duplicate on dPAGE.



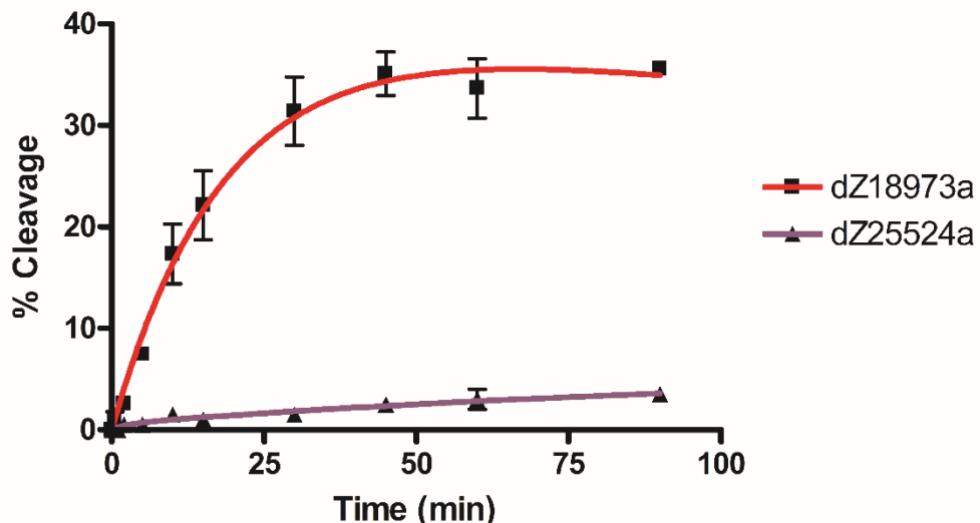
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Figure S19. Kinetic profile of DNAzyme candidates. Single-turnover cleavage reactions of DNAzymes against their matching RNA substrates. Mean data points, SD and best-fit curve ($Y = Y_{\max} (1 - e^{-kt})$) are plotted.



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Figure S20. Kinetic profile of DNAzyme candidates from Figure 4 that fit to the two-phase exponential association equation ($Y = Y_{\max 1} (1 - e^{-k_1 t}) + Y_{\max 2} (1 - e^{-k_2 t})$). dZ18973a: r^2 Single exponential = 0.9731; r^2 Double exponential = 0.9739. dZ25524a: r^2 Single exponential = 0.7818; r^2 Double exponential = 0.8006.



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Figure S21. Sequences of circular DNA templates and complementary RNA primers generated by corresponding DNAzymes.



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Figure S21. Continued. Sequences of circular DNA templates and complementary RNA primers generated by corresponding DNAzymes.



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Figure S21. Continued. Sequences of circular DNA templates and complementary RNA primers generated by corresponding DNAzymes.



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Figure S22. Comparison of various treatments and additives for stabilization of RNA in human saliva. Pooled human saliva was treated with heat, SDS, Proteinase K, RB (Reaction Buffer) or HB (Heating Buffer). KCl Precip and Resin refer to methods for removal of SDS. 5'-³²P labeled SRT12 RNA transcript was then spiked into pretreated saliva samples, heated again where indicated and incubated at room temperature for 10 min to evaluate the stability of unprotected RNA in the mixture. A saliva treatment using Proteinase K, HB and heat was most effective in preventing degradation of the RNA transcript.

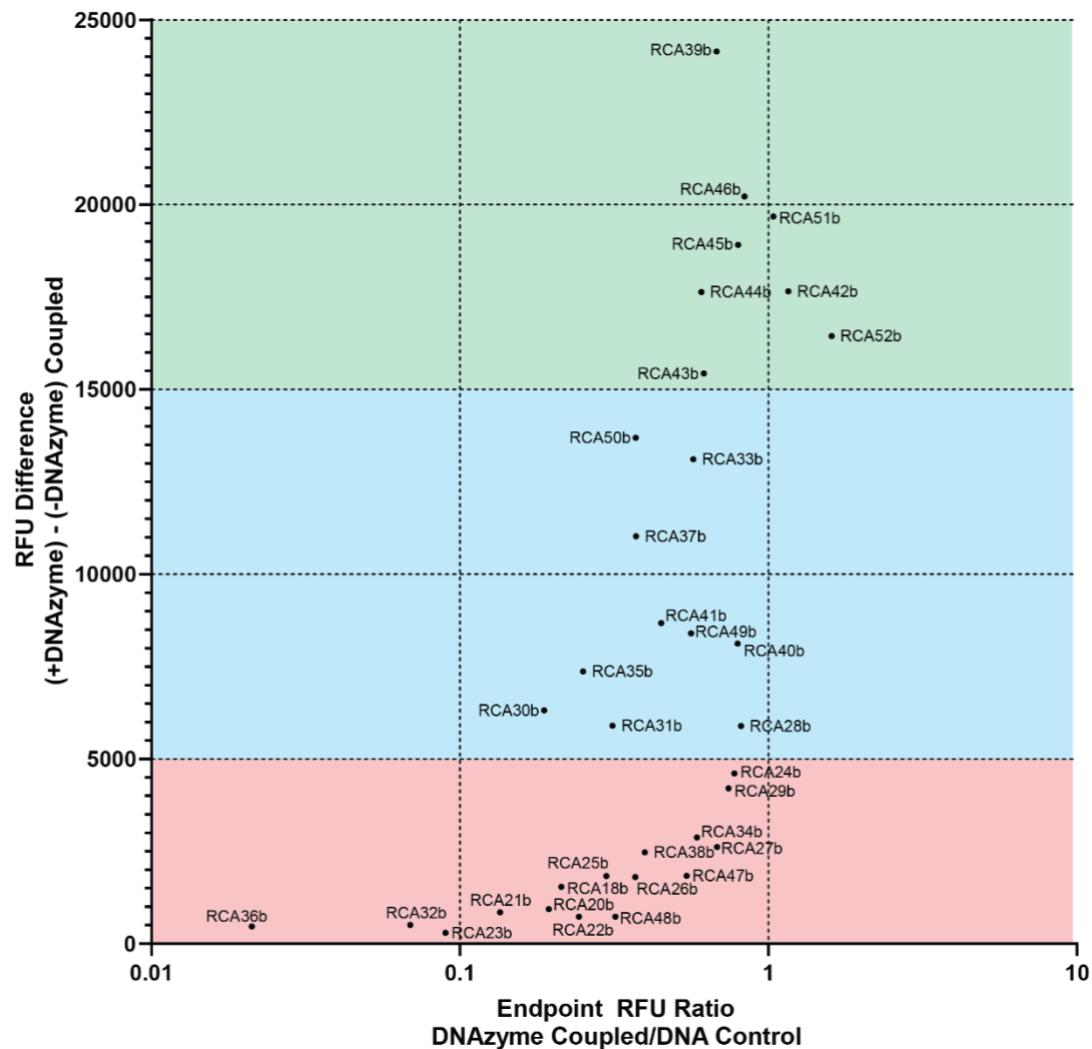
| Lane # | Heat-PK | | | | Heat+PK | | | SDS | | | |
|----------------|---------|---|---|---|---------|---|---|-----|---|----|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| RNA Transcript | + | + | + | + | + | + | + | + | + | + | + |
| ~50% Saliva | | + | + | + | + | + | + | + | + | + | + |
| Heated | | + | + | + | + | + | + | | | | |
| 0.1% SDS | | | | | | | + | + | + | | |
| KCl Precip | | | | | | | | + | | | |
| Resin | | | | | | | | | + | | |
| Proteinase K | | | | | + | + | + | | | | |
| RB | | | | + | | + | | + | + | + | + |
| HB | | | + | | | + | | | | | |



SDS = Sodium dodecyl sulfate, RB = Reaction Buffer, HB = Heating Buffer

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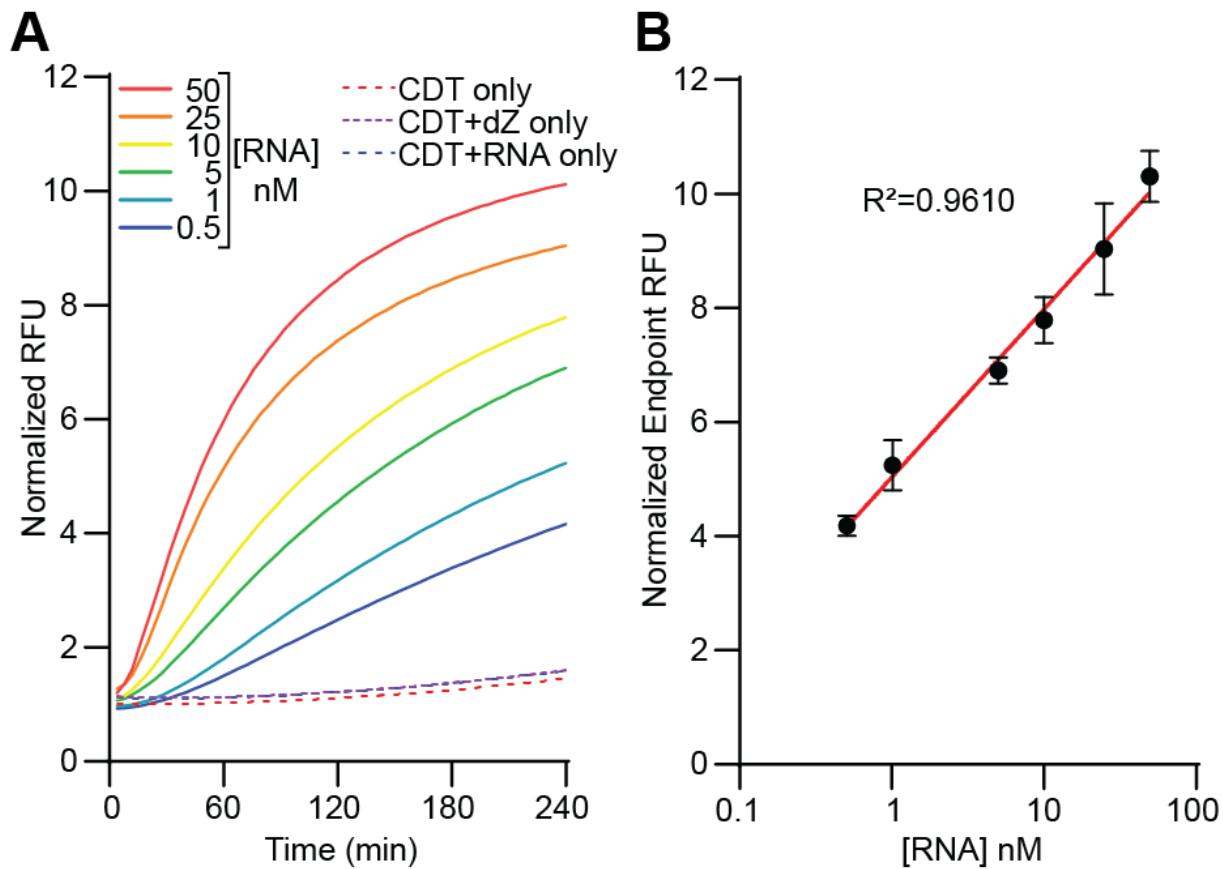
Figure S23. Complete version of coupled DNAzyme-mediated RNA cleavage and RNA primed RCA reaction from Figure 5D.



RFU = Relative Fluorescence Units

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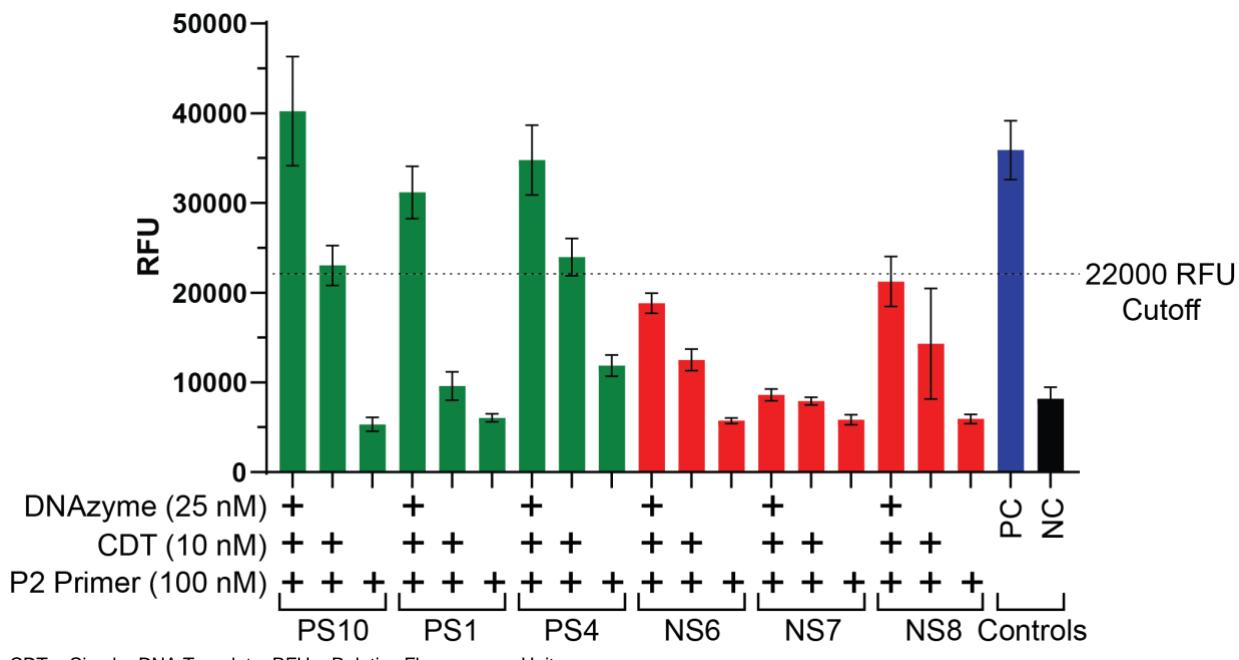
Figure S24. Dose responses of DNAzyme-coupled linear RCA reaction. (A) Normalized fluorescence vs. time for the listed RCA reactions. (B) Normalized fluorescence taken at 240 min vs [RNA]. DNAzyme was used at 50 nM, and NSP14 RNA was varied between 0.5-50 nM. “CDT only”, :CDT+RNA only” and “CDT+DNAzyme only” served as negative RCA controls.



CDT = Circular DNA Template, dZ = 10-23 DNAzyme, RFU = Relative Fluorescence Units

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Figure S25. Bar plot representing mean endpoint RFU for control reactions using 3 positive (P#) and negative (N#) patient saliva samples. Control reactions setup as +DNAzyme, -DNAzyme and +CDT. Dotted line indicates the cut-off point for the assay at 22,000 RFU. The error bars represent the standard deviation from the mean obtained using triplicate reactions.



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Author Contributions

YL, JDB, JG, CDMF, LS, KM and AC designed the experiments and interpreted the data. GP, BJS, CB, JG and DY oversaw collection of patient saliva samples. DW performed PCR analysis on patient saliva samples. JG, CN and AM performed all DNAzyme and RCA experiments and analyzed the data. YL, JDB, CF, AM, CN and JG wrote the manuscript. All authors edited the manuscript..