Electronic Supplementary Material (ESI) for RSC Chemical Biology.

Supporting Information

Targeting a Conserved Structural Element from the SARS-CoV-2 Genome using L-DNA Aptamers

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S1. Supplementary Text.

Materials and Methods.

General. Oligonucleotide synthesis reagents and D-nucleoside phosphoramidites were purchased from Glen Research (Sterling, VA). L-nucleoside phosphoramidites were purchased from ChemGenes (Wilmington, MA). Oligonucleotides were either purchased from Integrated DNA Technologies (Coralville, IA), or prepared in house by an Expedite 8909 DNA/RNA synthesizer. All oligonucleotides were purified by denaturing polyacrylamide gel electrophoresis (PAGE, 20%, 19:1 acrylamide: bisacrylamide) and desalted by ethanol precipitation before further use. The sequences of the oligonucleotides used in this study are listed in supplementary tables. Taq polymerase and reverse transcriptase were expressed and purified in house. MyOne^{TM} Streptavidin C1 magnetic Dynabeads and high-capacity streptavidin agarose beads (50% slurry) were purchased from Thermo Fisher Scientific (Waltham, MA). [γ-^{32}P]ATP and [α-^{32}P]dATP were purchased from Perkin Elmer (Waltham, MA). TOPO-TA cloning kit was obtained from Thermo Fischer Scientific. NTPs and dNTPs were purchased from Sigma Aldrich (St Louis, MO). Mass spectrometry analysis was performed by Novatia, LLC (Newtown, PA). Buffers were prepared by Milli-Q water (18.2 MΩ) and filtered with 0.22 μm membrane prior to use. All the other chemicals were purchased from either Sigma-Aldrich or Alfa Aesar and used as received.

In vitro selection

Preparation of ssDNA libraries. A 86 nt single stranded DNA (ssDNA) library containing a 45 nt random region flanked by two primer binding regions was amplified by polymerase chain reaction (PCR), containing 0.05 U/μL taq polymerase, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris (pH 9.0), 0.1% TRITON-X, 0.5 μM of forward and reverse primers, and 0.5 mM each of the four dNTPs (Table S1). All amplifications were conducted with the following program: i) 95°C for 3 min, (ii) 95°C for 30 s, (iii) 46°C for 30 s, (iv) 72°C for 1 min, and (v) 72°C for 5 min, with repeating steps (ii) to (iv) for N cycles as needed. The resulting dsDNA products were precipitated by ethanol, redispersed in 100 μL TE buffer (10 mM Tris, pH 7.6, and 1mM ethylenediaminetetraacetic acid (EDTA)), followed by the addition to 500 μL of high-capacity streptavidin coated agarose beads in WB buffer (50 mM NaCl, 10 mM Tris, pH 7.6) for immobilization. After incubating at room temperature for 30 min, the immobilized dsDNA library was washed twice with 500 μL WB buffer and once with water, followed by mixing with 300 μL ice cold EB buffer (50 mM NaOH, 1 mM EDTA) to release the nonbiotinylated strands. The eluent was immediately neutralized by 30 μL of NB buffer (1 M Tris, pH 7.6 and 3 M NaOAc)

and precipitated by ethanol. The obtained ssDNA library was further purified by denaturing PAGE (10%, 19:1 acrylamide:bis-acrylamide) before use.

Negative selection. This step was carried out before positive selection to remove any bead-binding and other non-specific oligonucleotides. Briefly, the ssDNA pool in 500 µL SB buffer (5 mM $MgCl_2$, 50 mM KCl, 20 mM NaCl, 25 mM Tris, pH 7.6) was heated at 90 °C for 3 mins and slowly cooled to 23 °C. The reaction mixture was then added to 1 mg streptavidin coated magnetic Dynabeads (washed and pre-blocked following a previously established protocol[1] and incubated at 23 °C for 2 h. After incubation, supernatant was collected for the positive selection and the beads were discarded. The concentration of ssDNA library was maintained as described in the Table S2 throughout each selection rounds. To further improve the specificity, 100 pmol of L-SARS-Cov-1 s2m RNA (s2m-Cov1), as negative counter competitor, was incubated with ssDNA pool at 23°C for 30 mins prior to bead binding starting at the round 6 selection.

Positive selection. 5' biotinylated L-SARS-Cov-2 s2m RNA target (L-s2m-Cov2) was heated at 70 °C for 1 min and slowly cooled to 23 °C, followed by addition to the retained supernatant from previous negative selection step. The reaction mixture was then incubated at 23 °C for 30 min before mixed with 1 mg tRNA pre-blocked streptavidin coated magnetic Dynabeads. After another 15 mins incubation, the beads were washed five times with 1 mL SB buffer under shaking to remove weakly bound ssDNA. Selection pressure was gradually increased throughout the successive rounds by lengthening the time of the washing steps (Table S2). Finally, the bound ssDNA was released with 300 µL EB buffer, immediately neutralized with NB buffer containing 4 µL glycogen (1 mg/mL), and ethanol precipitated. The pelleted ssDNA pool was redissolved in 20 µL TE buffer and directly amplified by PCR. The dsDNA product was ethanol precipitated and used to generate a new ssDNA pool for the next round selection.

TA cloning and sequencing. The enriched ssDNA from last SELEX round (Round 8) was amplified through PCR as before and purified by 2% agarose gel for cloning. In this case, the reverse primer (Rev. primer; Table S1) did not contain a 5′-biotin-TEG moiety. In brief, 4 µL of dsDNA template (3 ng/µL) was ligated with TOPO vector at 23 °C for 15 mins using TOPO-TA Cloning Kit. Afterwards, the ligated products were cloned into chemically competent *Escherichia coli* Top 10 cells. The bacteria were then plated on LB agar plate containing 50 mg/mL kanamycin and incubated at 37 °C for 16 hours. Twenty individual colonies were picked and

sequenced by Eton Biosciences Inc. (San Diego, CA). The sequencing data was analyzed with SnapGene software and sequences are listed in Table S3.

In Vitro Transcription of RNA

DNA templates used to prepare D-s2m-Cov2, D-s2m-Cov1, D-pre-miR-155, D-pre-miR-10b and D-pre-miR-21 were assembled from two overlapping synthetic oligonucleotides by cross extension reaction Table S7. The oligonucleotides (200 pmol of each) were annealed by heating at 90 °C for 1 min and slowly cool to room temperature in 100 µL mixture containing 6 mM $MgCl_2$, 150 mM KCl, 20 mM DTT, 100 mM Tris (pH 8.3) and 0.25 mM of each of the four dNTPs. Reverse Transcriptase (16 U/µL) was then added, and the extension reaction was carried out at 42 °C for 45 mins. The cross-extension products were ethanol precipitated and re-dispersed in water for in vitro transcription of RNA. Transcription mixture contains 10 U/µL T7 RNA polymerase, 25 mM $MgCl_2$, 10 mM DTT, 0.001 U/µL inorganic pyrophosphatase (IPP), 2 mM spermidine, 40 mM Tris (pH 7.9) and 5 mM of each the four NTPs. RNAs were ethanol precipitated and purified by denaturing PAGE (10%, 19:1 acrylamide:bis-acrylamide) after incubation at 37°C for 2 h.

Electrophoretic mobility shift assay (EMSA).

The dissociation constants K_d of aptamer&s2m-Cov2 complex were determined by EMSA. Briefly, 20 µL reaction mixture consisting of 1 nM of 5'-[^{32}P]-labeled s2m-Cov2, 5 mM $MgCl_2$, 50 mM KCl (except for Li^+-based experiment where 50 mM LiCl was used), 20 mM NaCl, 0.01 mg/mL tRNA and 25 mM Tris (pH 7.6) with various concentrations (0-2000 nM) of aptamers was incubated at 23 °C for 30 min. The complex samples were analyzed by native gel (8%, 19:1 acrylamide:bis-acrylamide) containing 5 mM $MgCl_2$, 50 mM KOAc, 20 mM NaOAc and 25 mM Tris (pH 7.6). Electrophoresis was carried out at 30 mA for 4 h and the images were collected on Typhoon FLA-9500 Molecular Imager (General Electric Co., Boston, MA). The binding specificity was characterized similarly with 2 µM aptamer and trace amount (1 nM) of 5'-[^{32}P]-labeled substrates including D-s2m-Cov2, D-s2m-Cov1, D-pre-miR-155, D-pre-miR-10b and D-pre-miR-21. As for the dimer experiment (Figure 5c), 1 µM aptamer, 1 nM D-s2m-B and 1 nM of 5'-[^{32}P]-labeled D-s2m-A were used in the reaction.

In-line probing analysis of RNA structure

A 30 µL reaction mixture containing 100 nM of 5′-[^{32}P]-labeled RNA (L-s2m-Cov2), either none or 20 µM D-aptamer (D-C1t or D-C3t), 10 mM $MgCl_2$, 50 mM KCl, 20 mM NaCl and 25 mM Tris

(pH 7.6) was incubated at 23 °C for either 24h or 48 h. The products were resolved by denaturing PAGE (15%, 19:1 acrylamide:bis-acrylamide) and analyzed by a Typhoon Molecular Imager (Figure S7).

Circular dichroism (CD) spectroscopy.

CD experiments were carried out as previously reported.[1] Briefly, DNA aptamers at a concentration of 9 μM were prepared in 25 mM Tris (pH 7.6) and 20 mM NaCl buffer supplement with either 50 mM KCl or LiCl. Samples were heated at 95°C for 3 mins and slowly cooled to room temperature to allow the proper structure formation. The spectra were obtained on an Applied Photophysics Chirascan spectrophotometer (Leatherhead) with a 1 mm path length quartz cuvette. All data were scanned with bandwidth of 1.0 nm at time interval per point of 1.5 s in the wavelength range of 220 to 310 nm at 23 °C.

S2. Supplementary Figures.

Figure S1

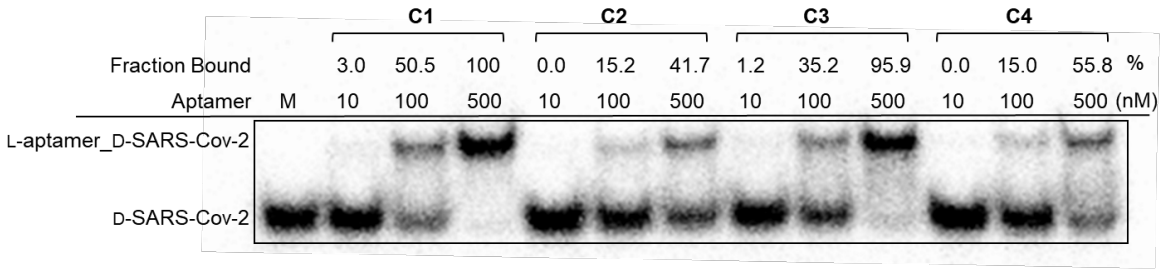

Figure S1. Screening of round 8 clones for binding L-s2m-Cov2 by EMSA (8% native PAGE; 19:1 acrylamide:bisacrylamide). Each clone (e.g. C1, C2, C3, and C4) represents a unique aptamer sequence from in vitro selection. All binding reactions contained 1 nM of 5'-[^{32}P]-labeled L-s2m-Cov2, the indicated aptamer concentration (nM), 5 mM $MgCl_2$, 50 mM KCl, 20 mM NaCl, 0.01 mg/mL of tRNA, and 25 mM Tris (pH 7.6). M represents unbound 5'-[^{32}P]-labeled L-s2m-Cov2 as a marker.

Figure S2

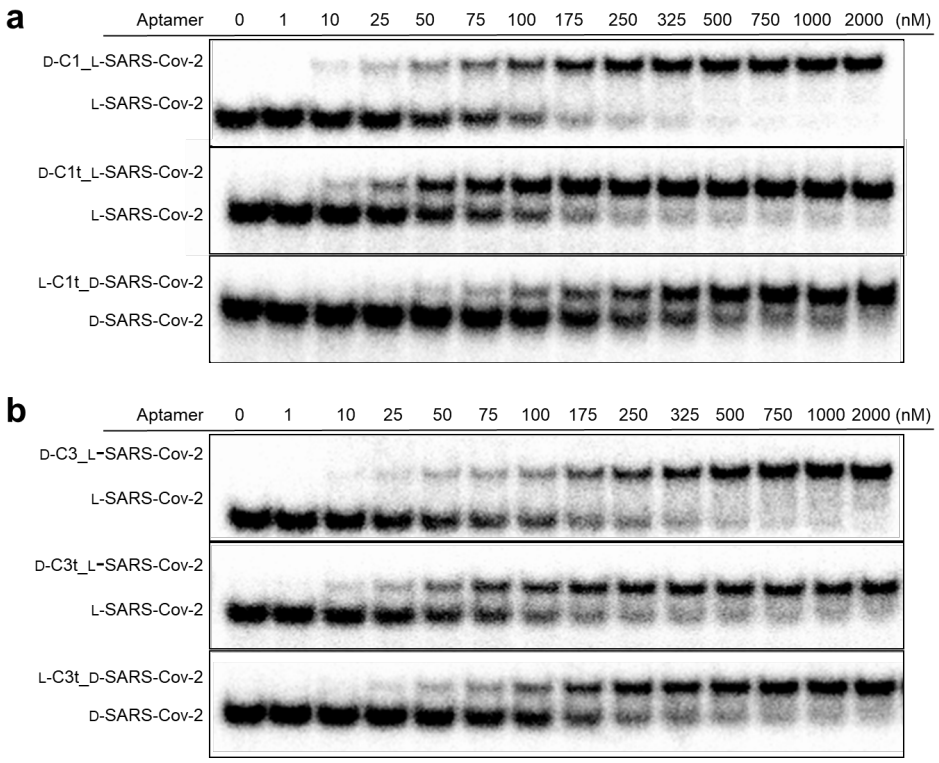

Figure S2. Representative EMSA gels (8% native PAGE; 19:1 acrylamide:bis-acrylamide) for the binding data depicted in Figure 2b,c. Full-length aptamers C1 (a) and C3 (b) or their truncated variants C1t and C3t (D and L) were incubated with s2m-CoV2 of the opposite stereochemistry. All binding reactions contained 1 nM of 5'-[^{32}P]-labeled s2m-Cov2, increasing concentration of aptamer (0-2000 nM), 5 mM $MgCl_2$, 50 mM KCl, 20 mM NaCl, 0.01 mg/mL of tRNA, and 25 mM Tris (pH 7.6).

Figure S3

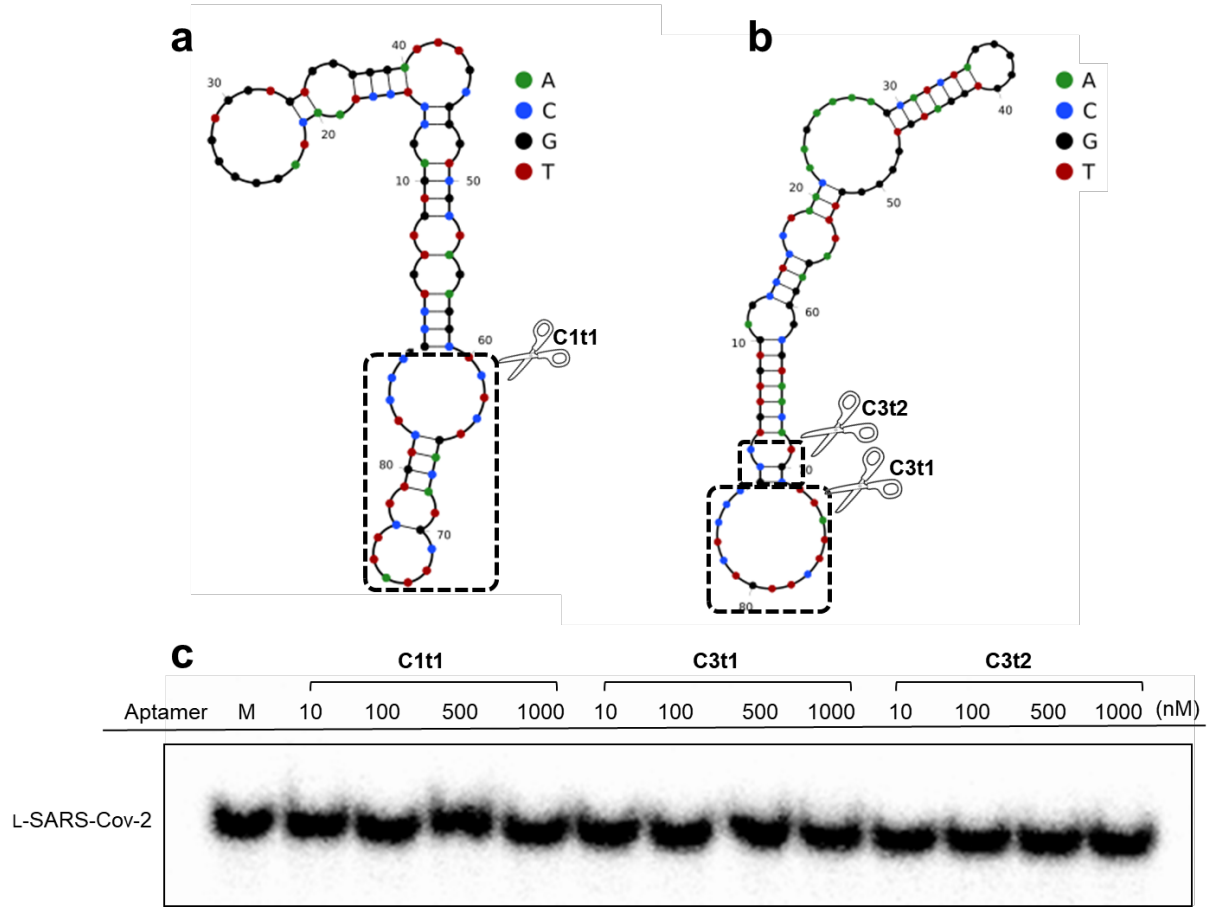

Figure S3. Truncation of D-C1 (a) and D-C3 (b) aptamers based on secondary structure predictions (NUPACK).[2] Boxes indicate truncated residues. (c) EMSA analysis of truncated variants D-C1t1, D-C3t1 and D-C3t2 binding to L-s2m-Cov2. All binding reactions contained 1 nM of 5'-[^{32}P]-labeled D-/L-s2m-Cov2, 10-2000 nM aptamer, 5 mM $MgCl_2$, 50 mM KCl, 20 mM NaCl, 0.01 mg/mL of tRNA, and 25 mM Tris (pH 7.6). M represents unbound 5'-[^{32}P]-labeled L-s2m-Cov2 as a marker. Secondary structure predictions made using NUPACK software.[2]

Figure S4

a

D-C1t_S AAGTGTGAGTTGTCAGTGTGTGCTGTGAGAGCGTCGTCGCGCTGCGCTGTGCGAGCGTGAT
D-C3t_S GAGCTGTGCTATGATAACGCTAAGCTGTATAGTGAGTGTGTCGCGTGAGATGAGAGATGTG

b

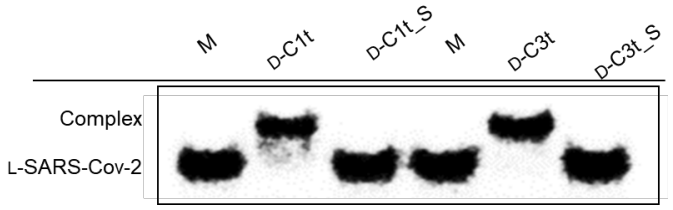

Figure S4 (a) Sequences of scrambled aptamers D-C1t_S and D-C3t_S. (b) EMSA analysis (8% native PAGE; 19:1 acrylamide:bis-acrylamide) of D-C1t, D-C3t, and their scrambled variants (D-C1t_S and D-C3t_S) binding to L-s2m-Cov2. All binding reactions contained 1 nM of 5'-[^{32}P]-labeled D-/L-s2m-Cov2, 2 μM aptamer, 5 mM $MgCl_2$, 50 mM KCl, 20 mM NaCl, 0.01 mg/mL of tRNA, and 25 mM Tris (pH 7.6). M represents unbound 5'-[^{32}P]-labeled L-s2m-Cov2 as a marker.

Figure S5

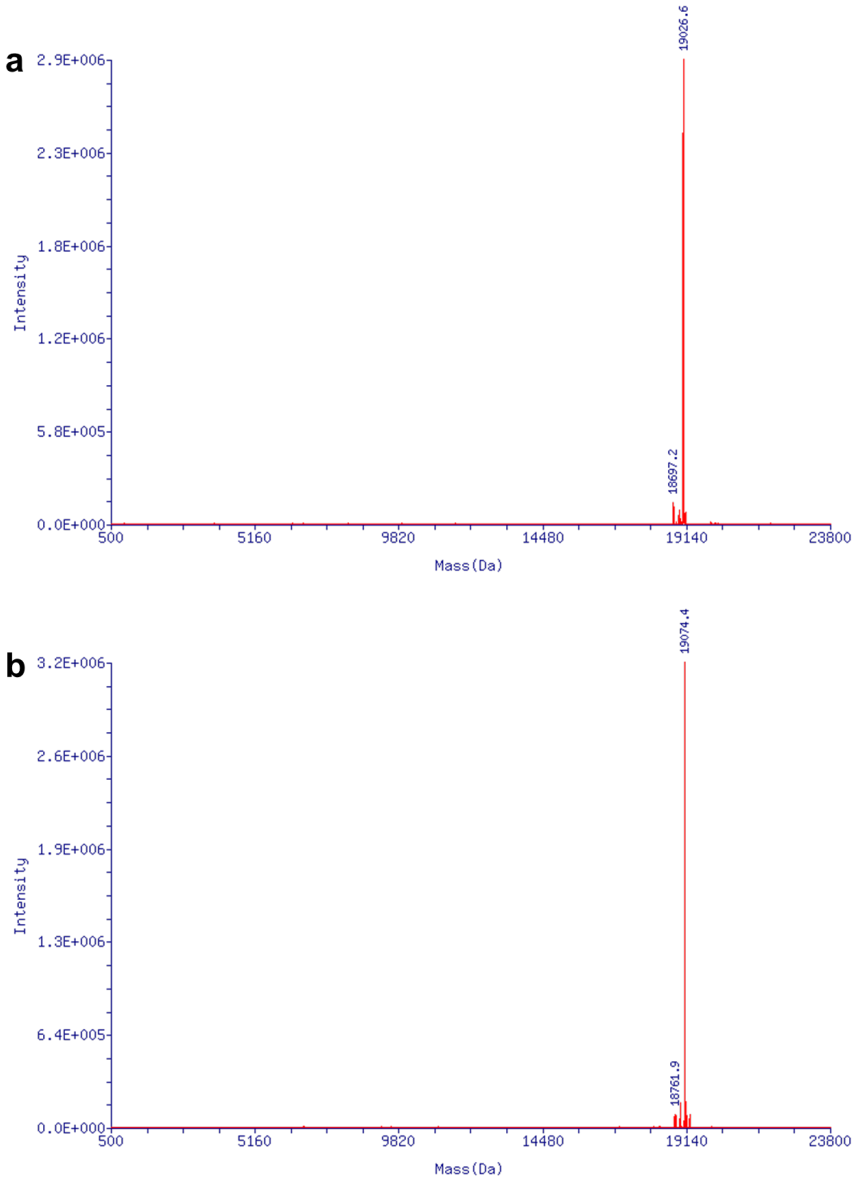

Figure S5. ESI-MS spectra of L-aptamers. (a) ESI-MS of L-C1t prepared by solid-phase synthesis. Mass calculated: 19026.3; Mass found: 19026.6. (b) ESI-MS of L-C3t prepared by solid-phase synthesis. Mass calculated: 19074.4; Mass found: 19074.4.

Figure S6

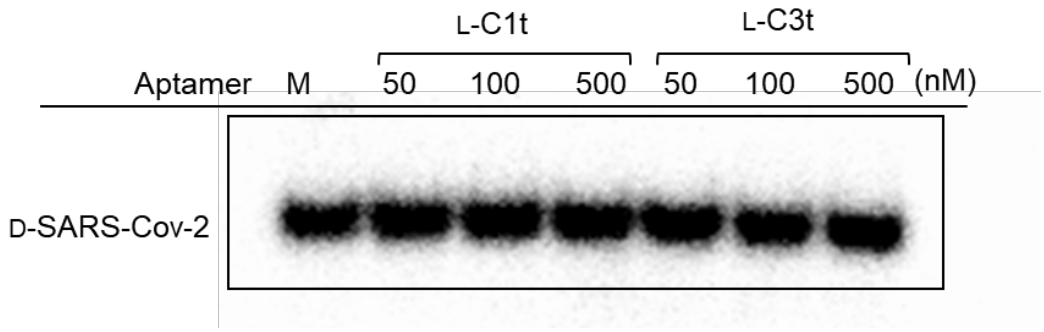

Figure S6. EMSA analysis (8% native PAGE; 19:1 acrylamide:bis-acrylamide) of L-C1t and L-C3t binding to D-s2m-Cov2 in the absence of K^+. All binding reactions contained 1 nM 5'-[^{32}P] labeled D-s2m-Cov2, 50-500 nM aptamer, 5 mM $MgCl_2$, 50 mM LiCl, 20 mM NaCl, 0.01 mg/mL of tRNA, and 25 mM TRIS (pH 7.6). M represents unbound 5'-[^{32}P]-labeled L-s2m-Cov2 as a marker.

Figure S7

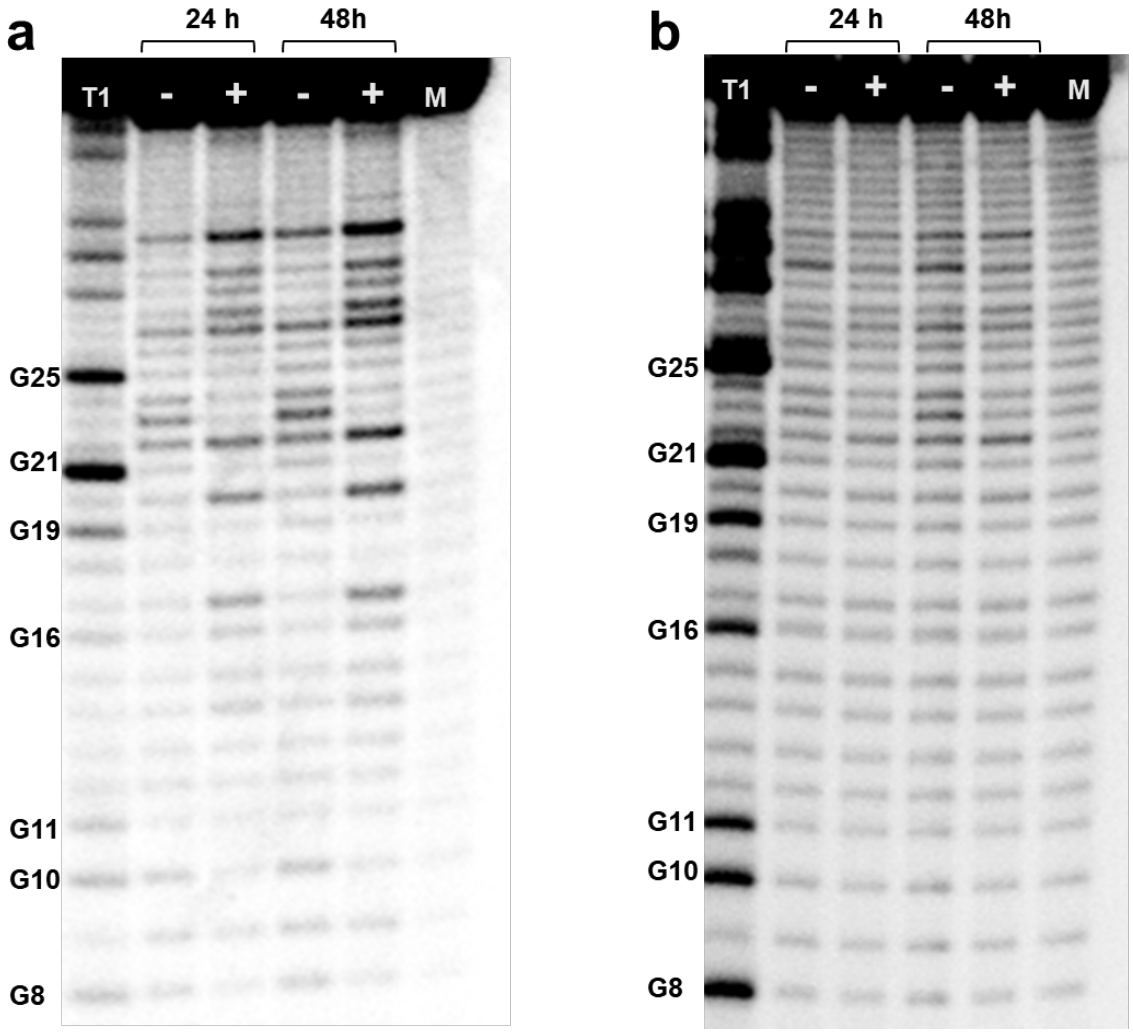

Figure S7. In-line probing analysis of D-s2m-Cov2 in the presence and absence of L-C1t (a) or L-C3t (b). All reactions contained 100 nM 5'-[^{32}P] labeled D-s2m-Cov2, either none (-) or 20 μM (+) of L-aptamer, 10 mM $MgCl_2$, 50 mM KCl, 20 mM NaCl, and 25 mM Tris (pH 7.6). Unreacted D-s2m-Cov2 (M) and material that had been partially digested with ribonuclease T1 (T1; cleaves after G residues) are shown in the far most right and left lanes, respectively.

Figure S8

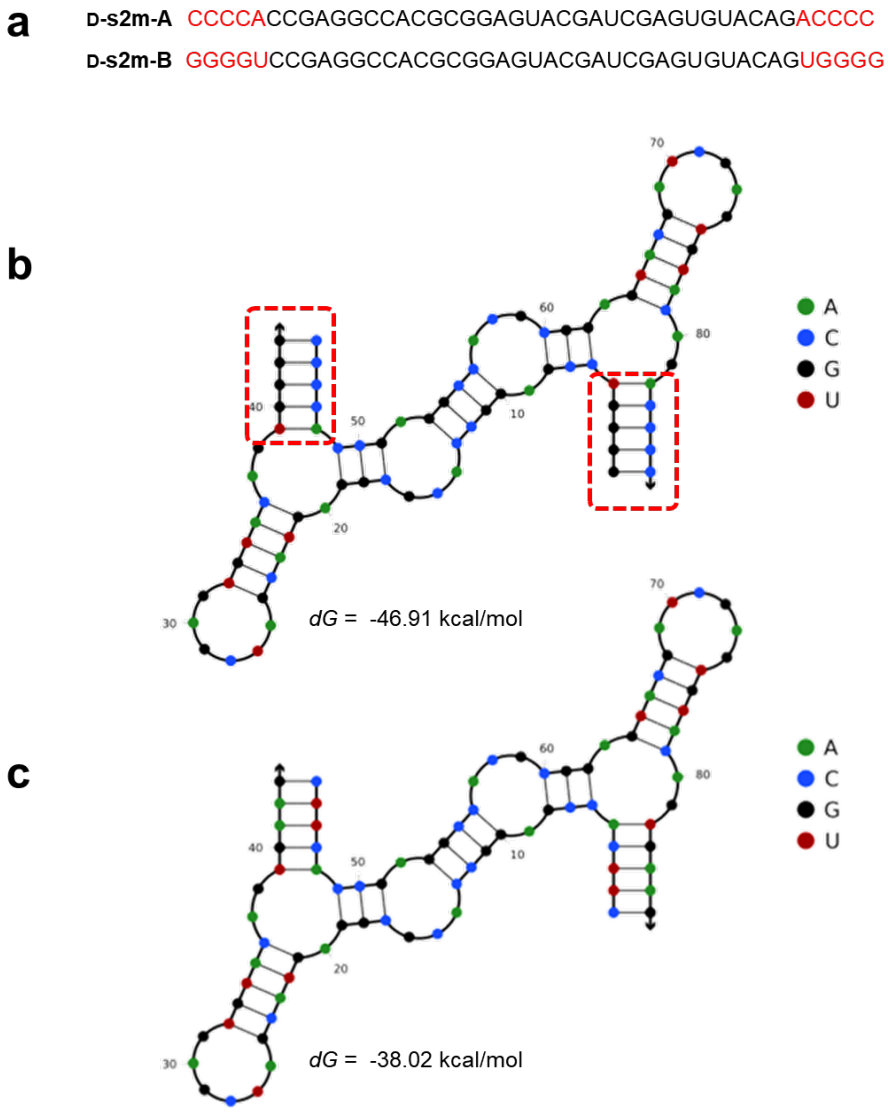

Figure S8. (a) Sequences of D-s2m-CoV2 hairpin variants (D-s2m-A and D-s2m-B) with cross-complementary lower stems (red highlights). (b) Predicted secondary structure of the heterodimer formed between D-s2m-A and D-s2m-B. (red highlights indicates the cross-complementary stems). (c) Predicted secondary structure of the homodimer formed by D-s2m-CoV2. Secondary structure predictions made using NUPACK software.[2]

Figure S9

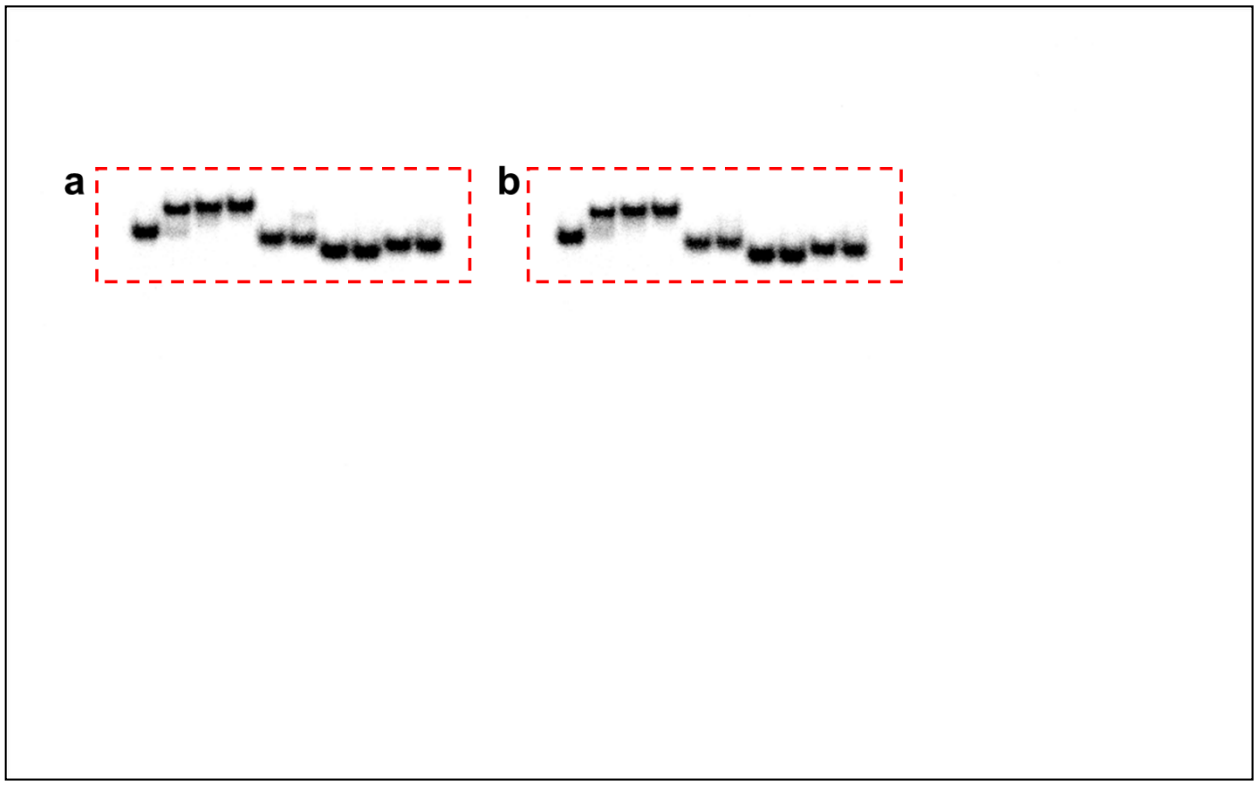

Figure S9. Uncropped gel image for Figure 5a top panel (a) and 5a bottom panel (b). Red frame indicates the cropped region shown in Figure 5a.

Figure S10

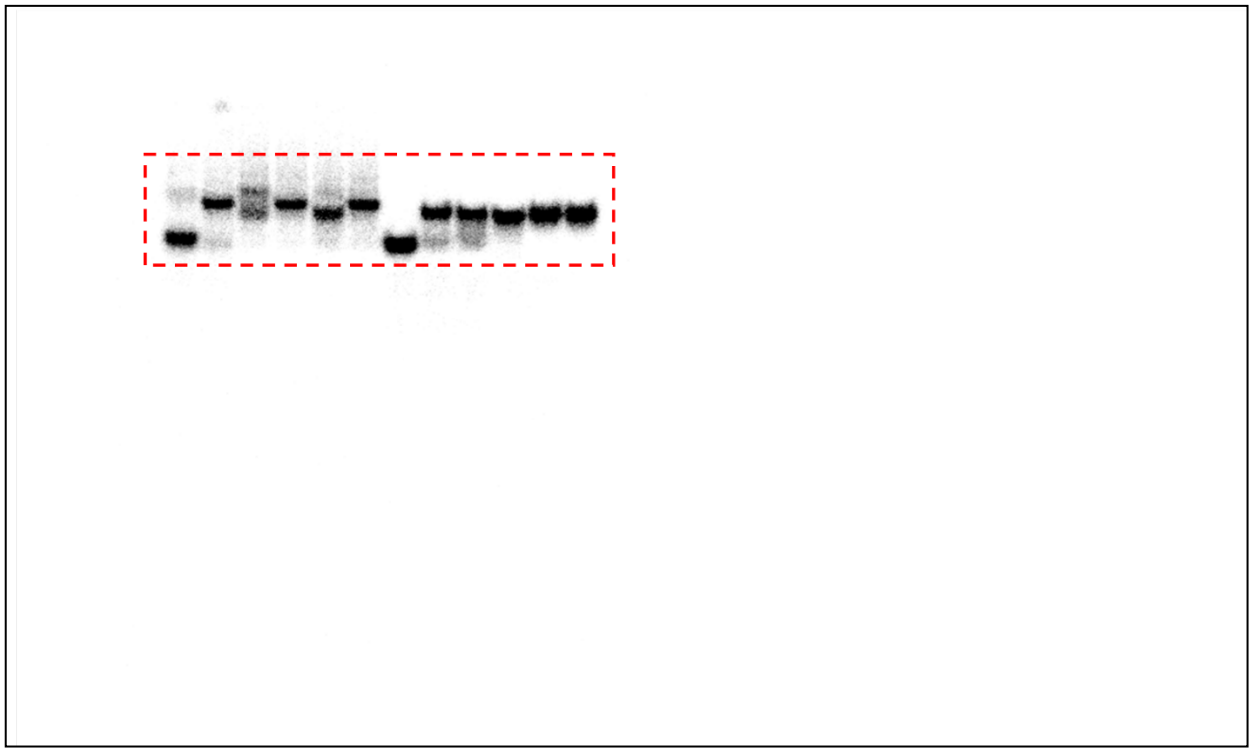

Figure S10. Uncropped gel images for Figure 5c. Red frame indicates the cropped region shown in Figure 5c.

Figure S11

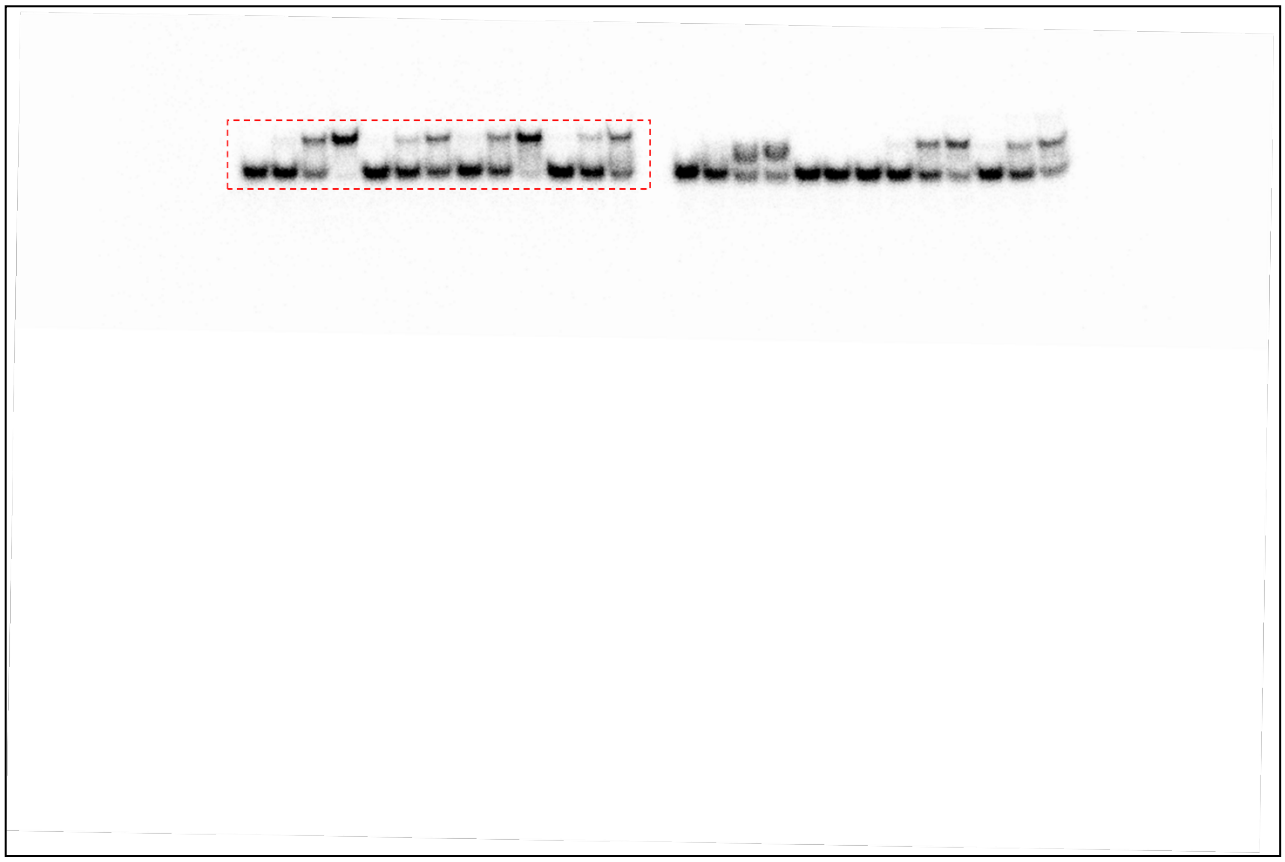

Figure S11. Uncropped gel images for Figure S1. Red frame indicates the cropped region shown in Figure S1.

Figure S12

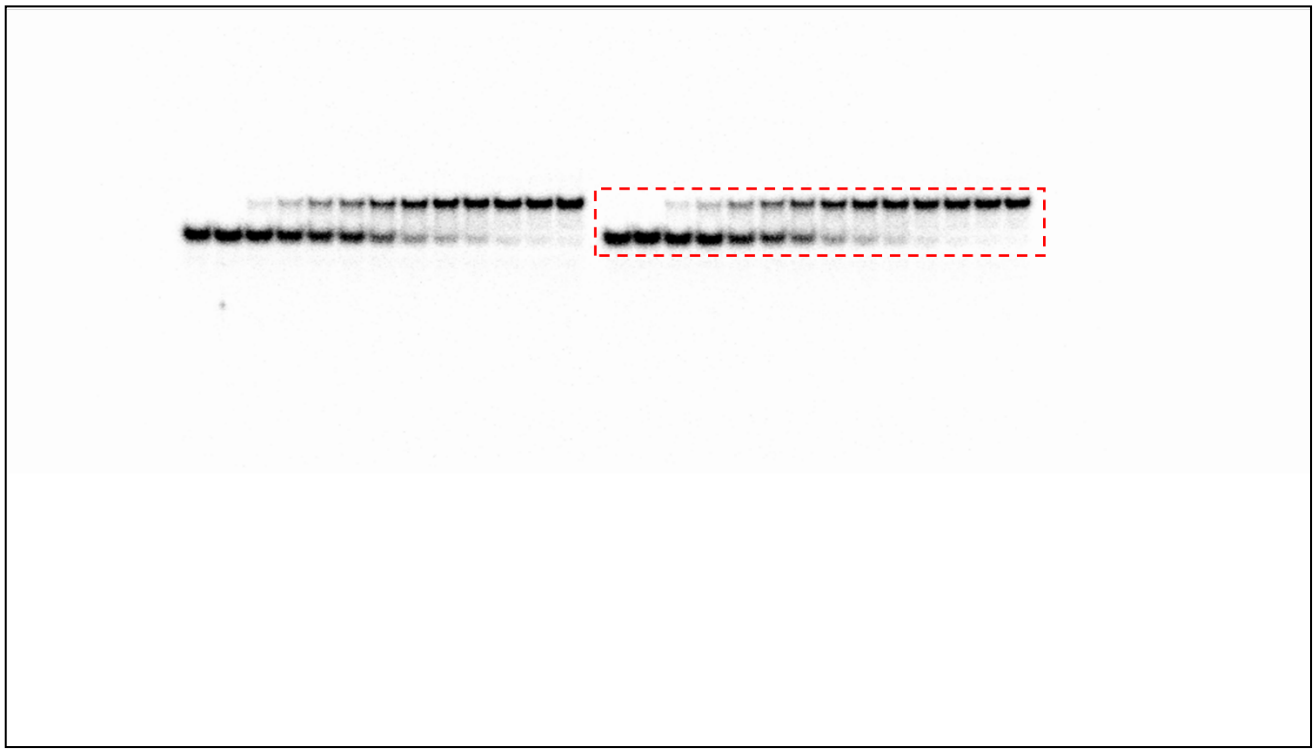

Figure S12. Uncropped gel images for Figure S2a (top panel). Red frame indicates the cropped region shown in Figure S2a (top panel).

Figure S13

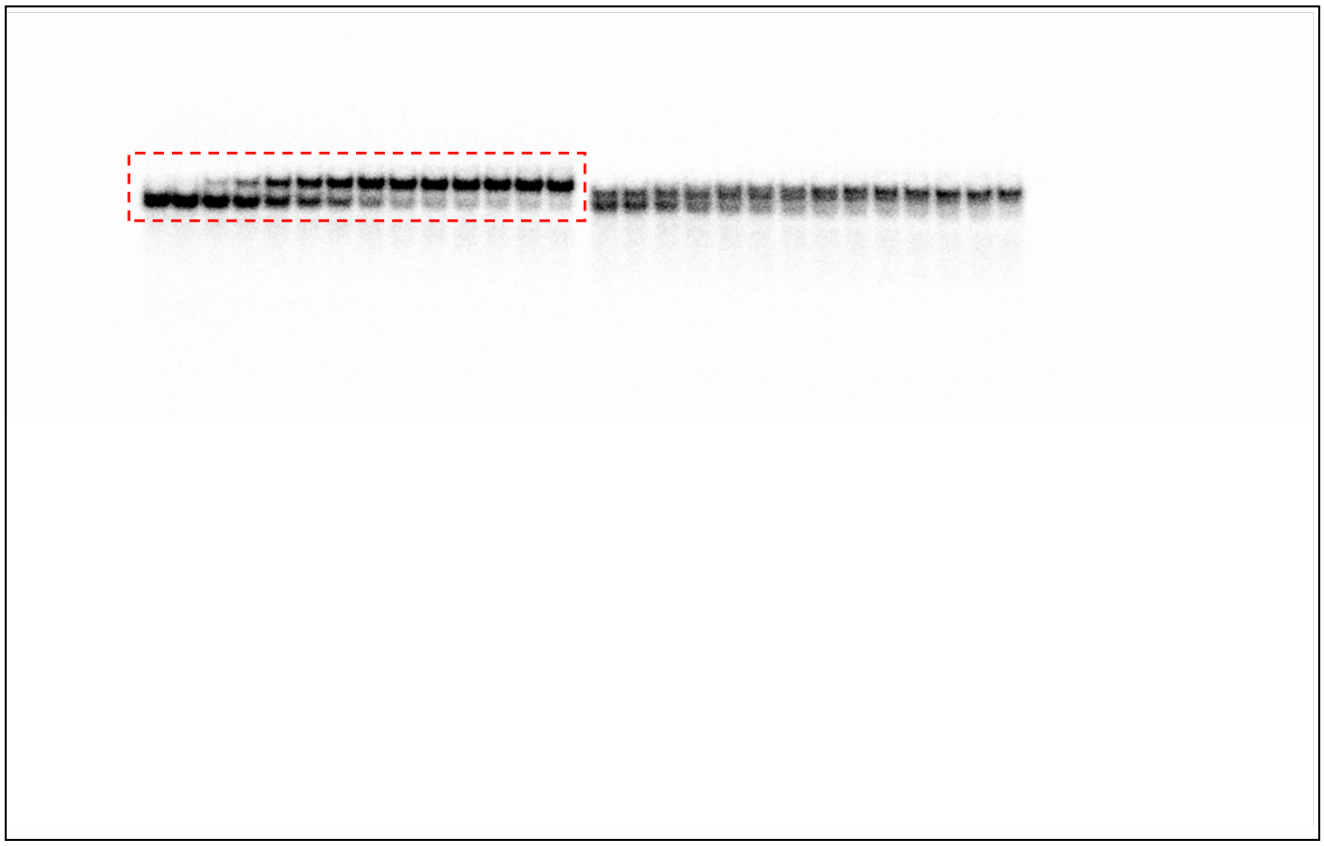

Figure S13. Uncropped gel images for Figure S2a (middle panel). Red frame indicates the cropped region shown in Figure S2a (middle panel).

Figure S14

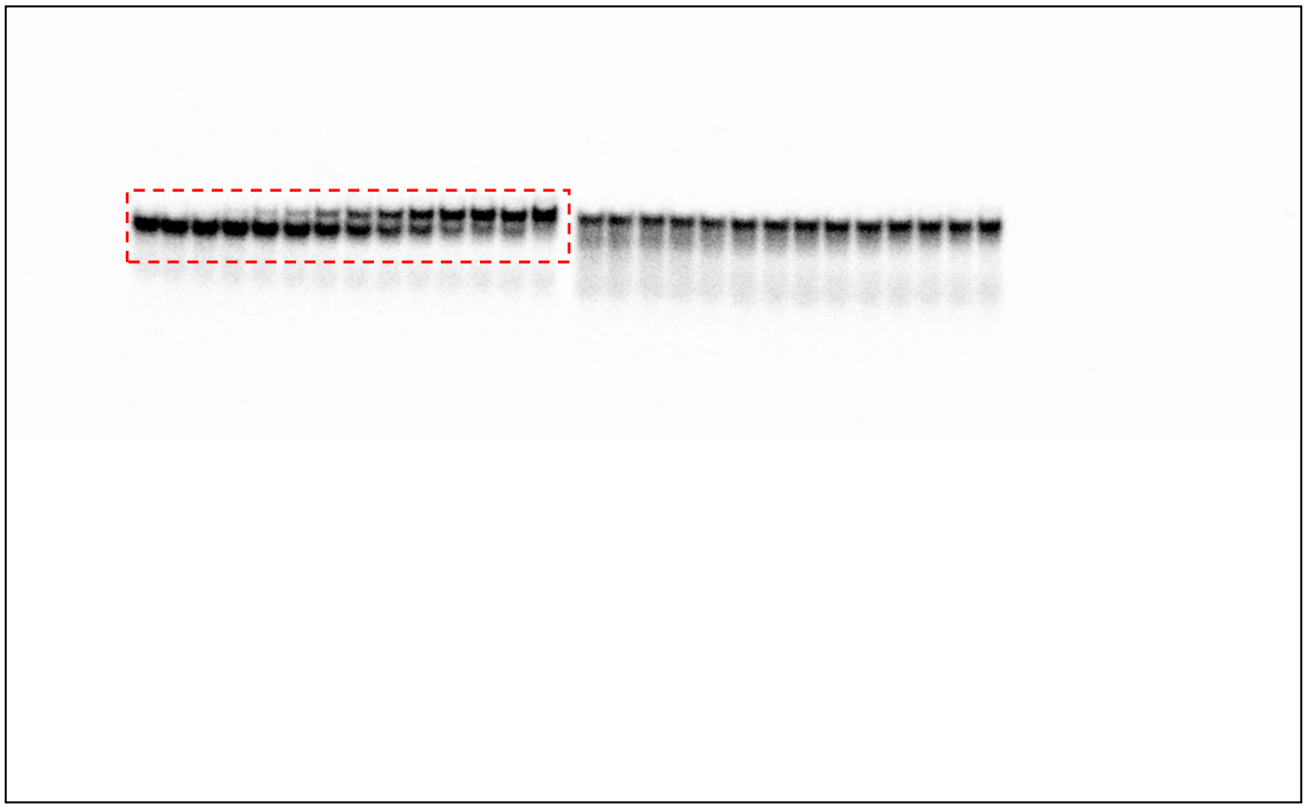

Figure S14. Uncropped gel images for Figure S2a (bottom panel). Red frame indicates the cropped region shown in Figure S2a (bottom panel).

Figure S15

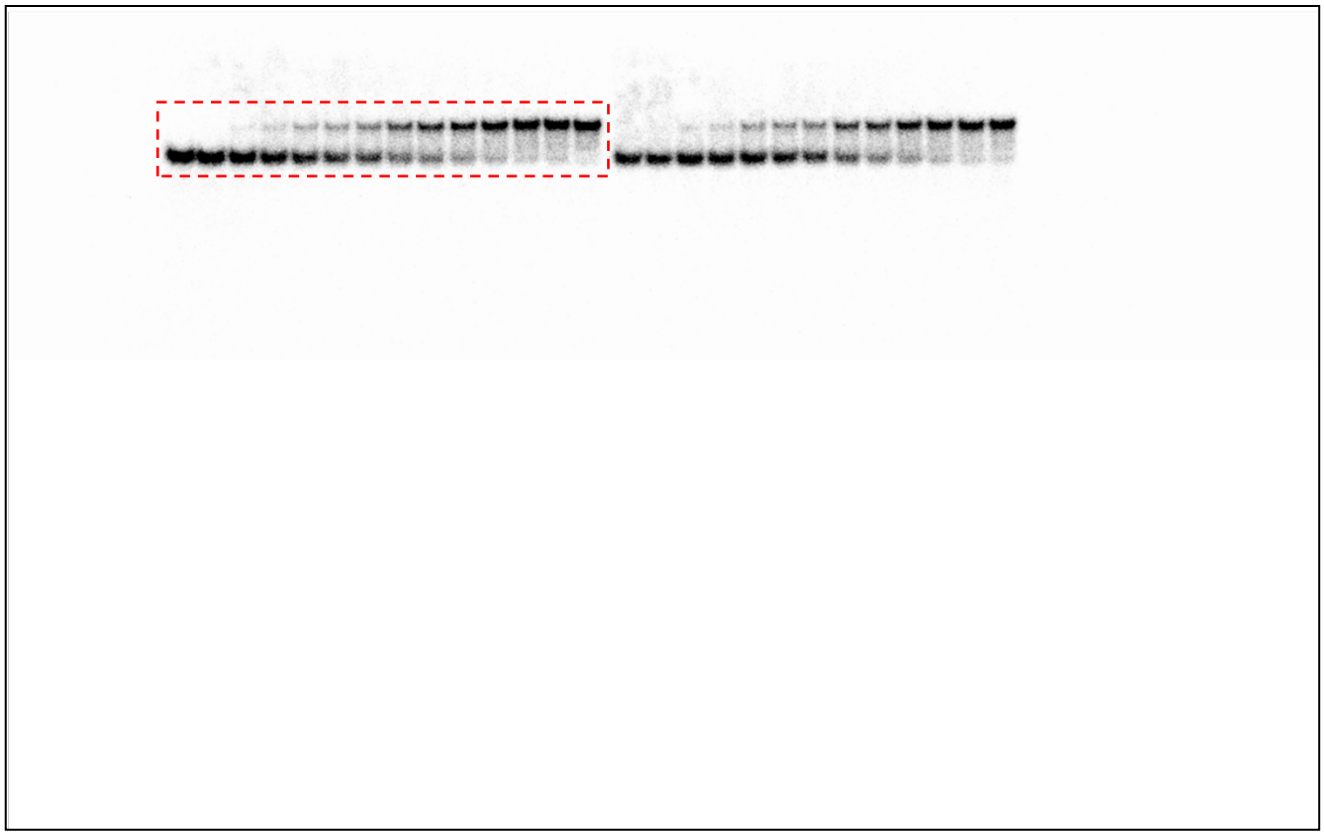

Figure S15. Uncropped gel images for Figure S2b (top panel). Red frame indicates the cropped region shown in Figure S2b (top panel).

Figure S16

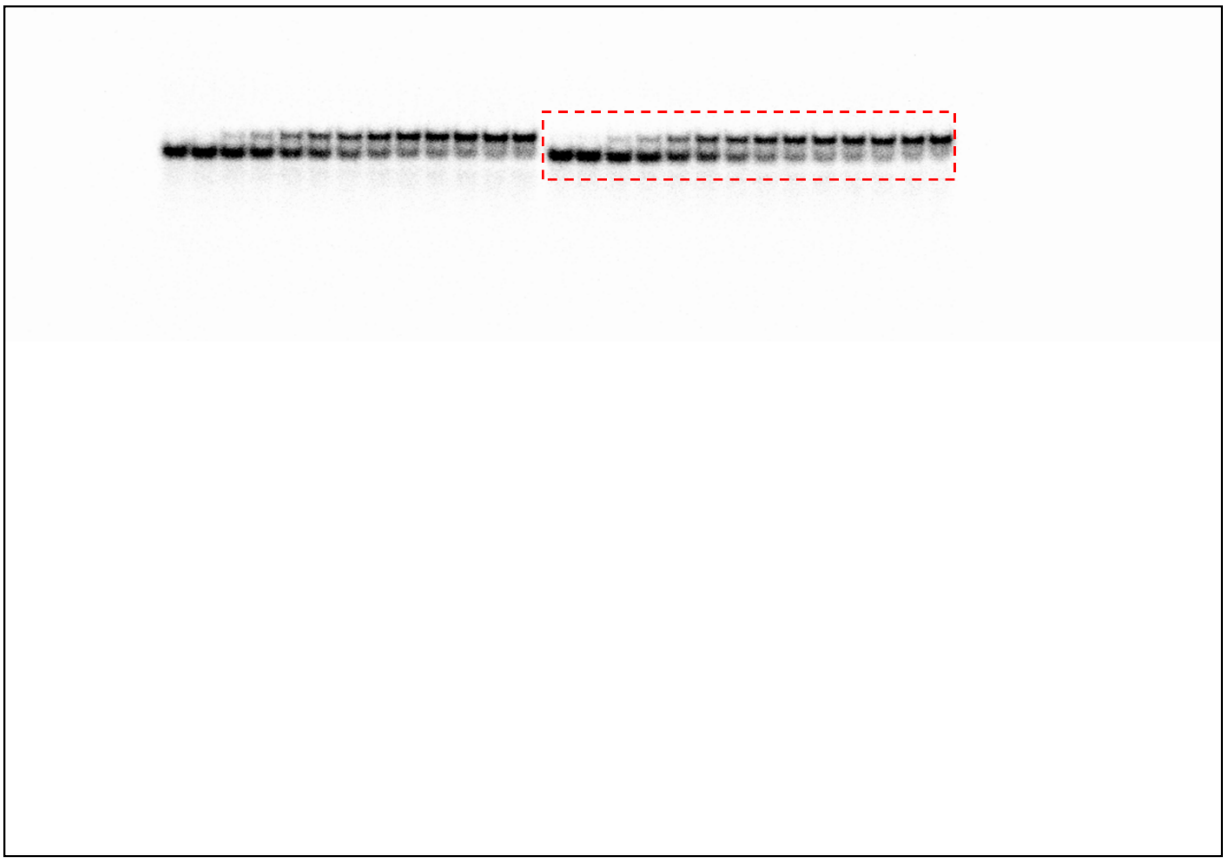

Figure S16. Uncropped gel images for Figure S2b (middle panel). Red frame indicates the cropped region shown in Figure S2b (middle panel).

Figure S17

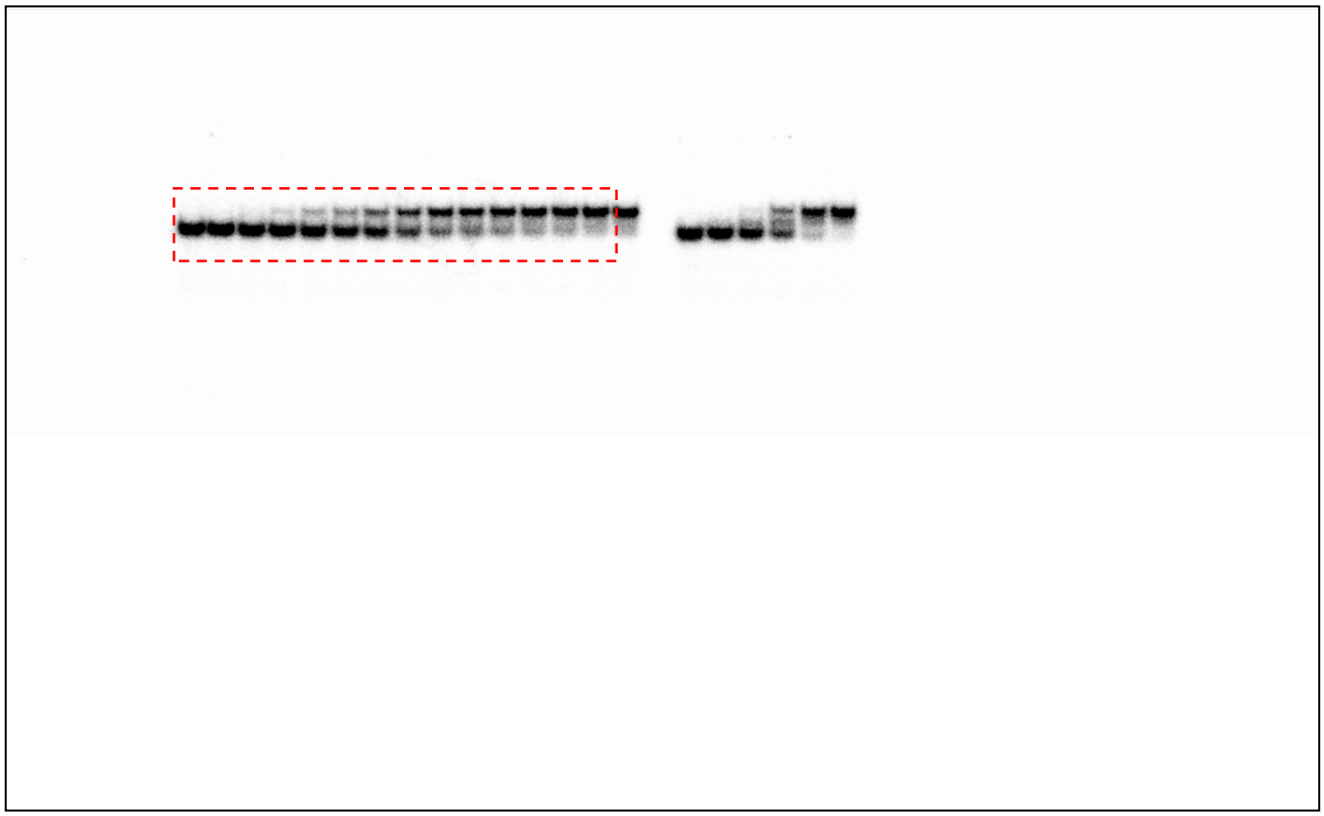

Figure S17. Uncropped gel images for Figure S2b (bottom panel). Red frame indicates the cropped region shown in Figure S2b (bottom panel).

Figure S18

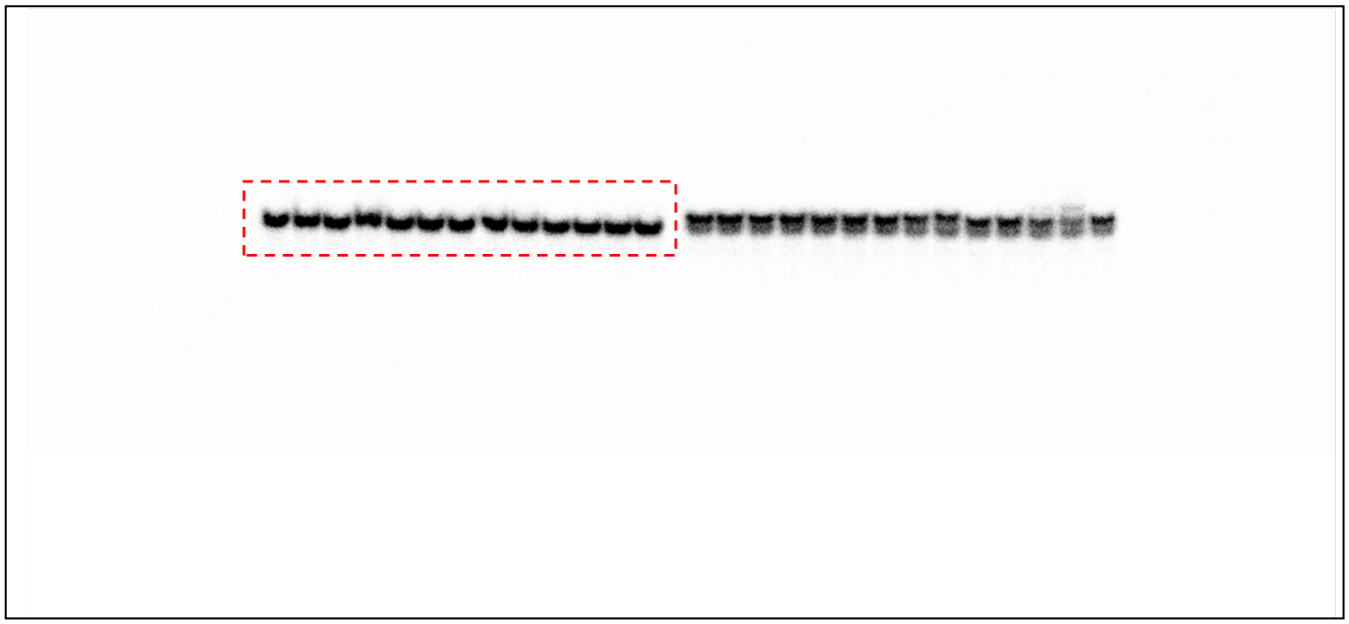

Figure S18. Uncropped gel images for Figure S3c. Red frame indicates the cropped region shown in Figure S3c.

Figure S19

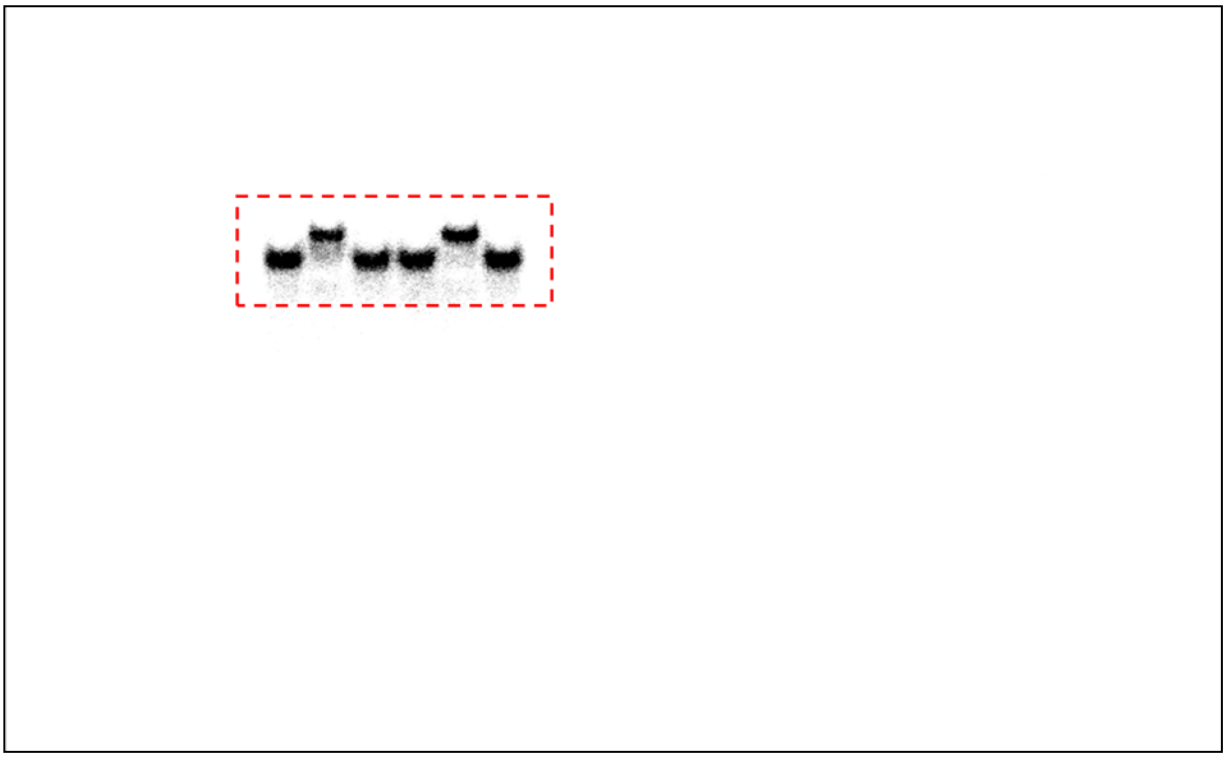

Figure S19. Uncropped gel images for Figure S4b. Red frame indicates the cropped region shown in Figure S4b.

Figure S20

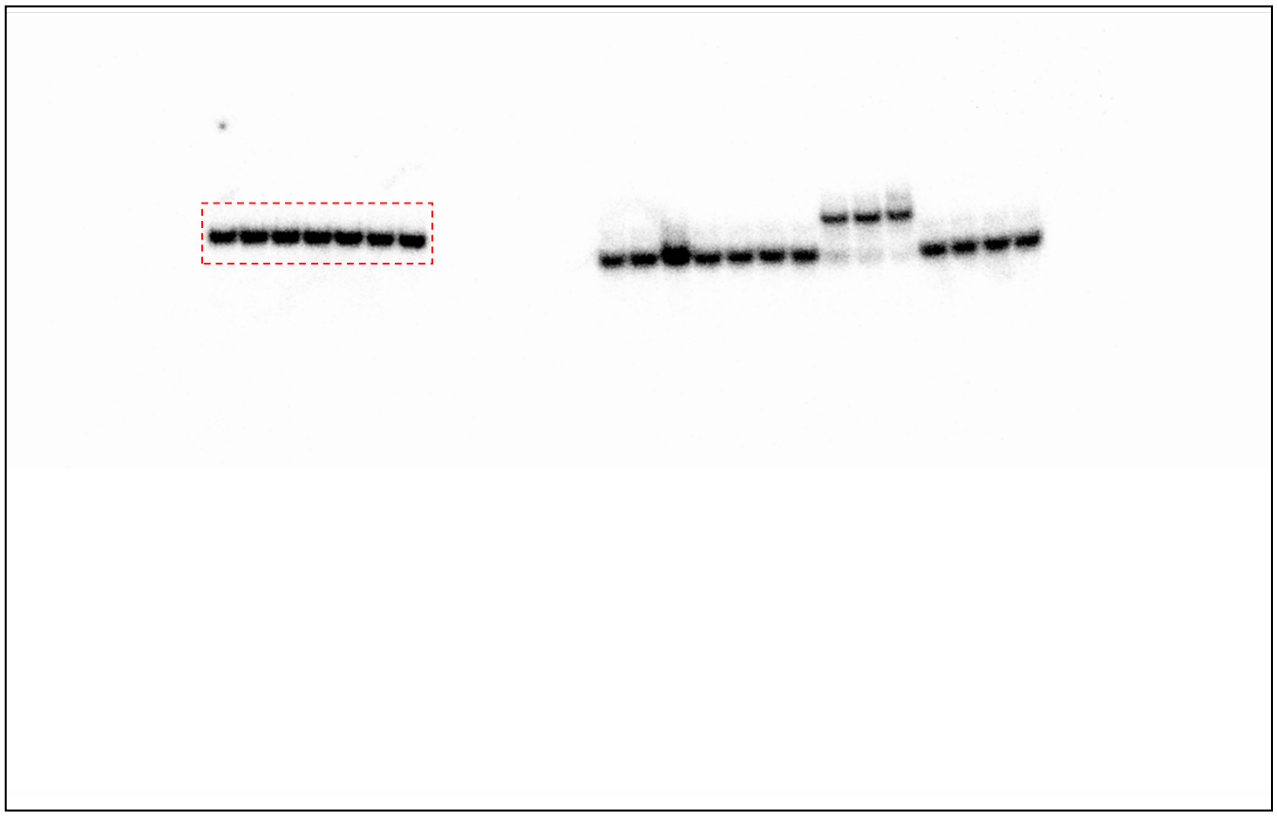

Figure S20. Uncropped gel images for Figure S6. Red frame indicates the cropped region shown in Figure S6.

Figure S21

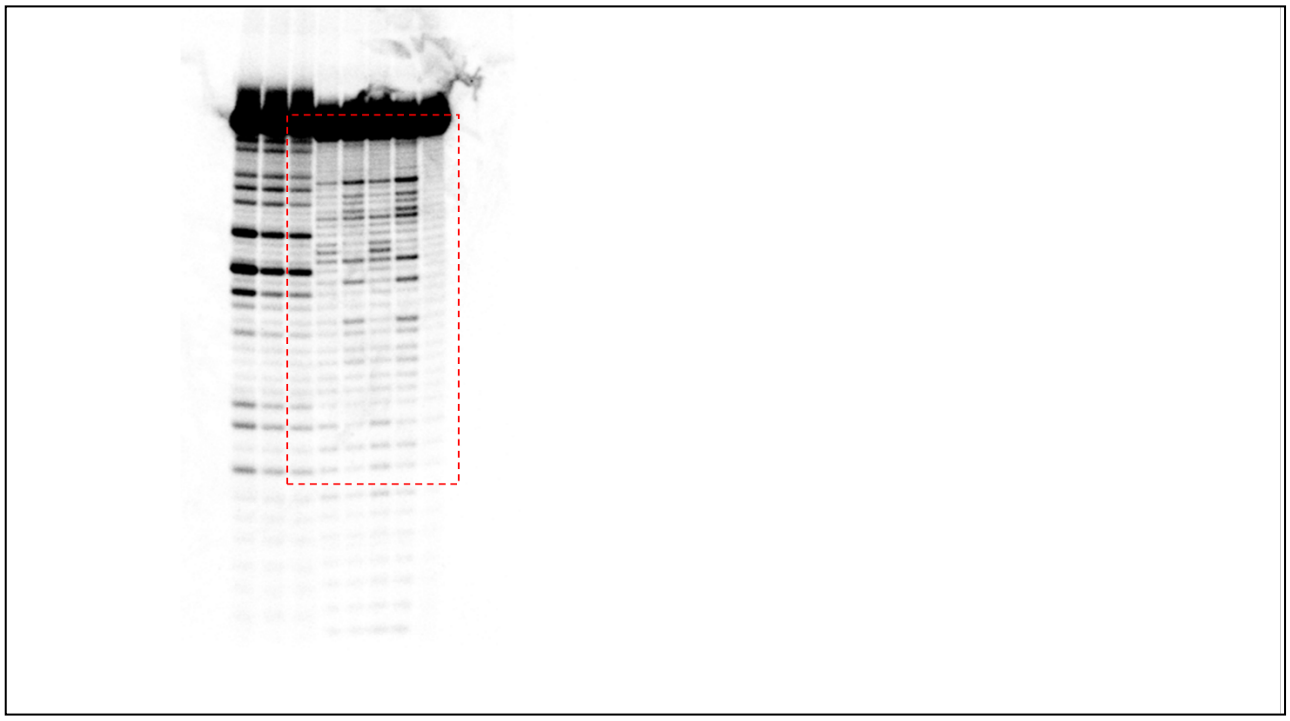

Figure S21. Uncropped gel images for Figure S7a. Red frame indicates the cropped region shown in Figure S7a.

Figure S22

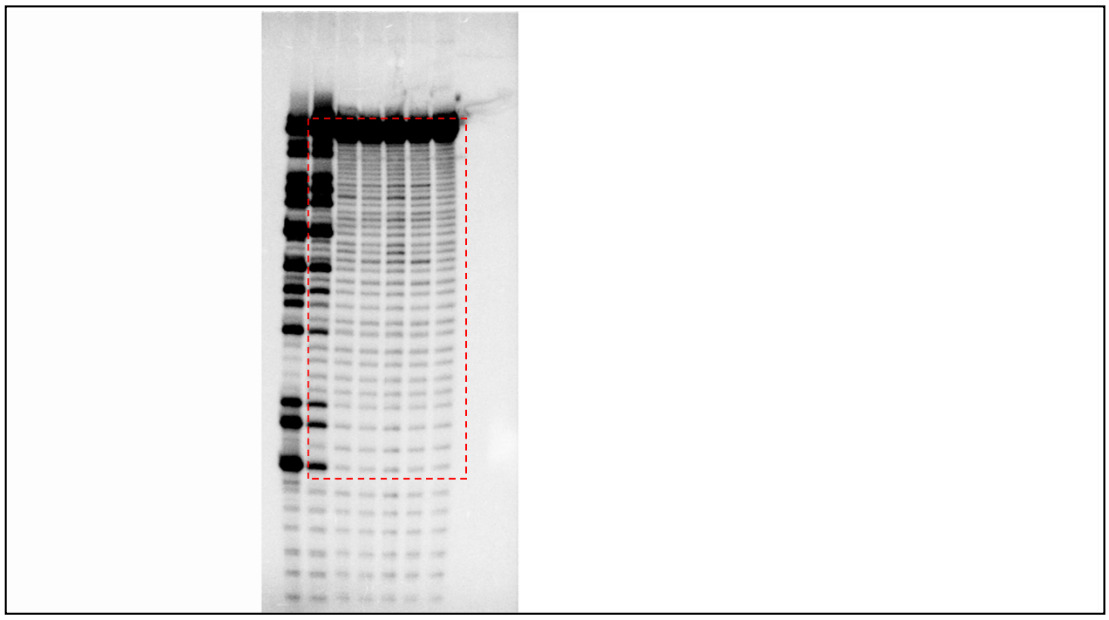

Figure S22. Uncropped gel images for Figure S7b. Red frame indicates the cropped region shown in Figure S7b.

S3. Supplementary Tables.

Table S1. Oligos associated with *in vitro* selection.

Oligos	Sequences (5' to 3')	Type
Lib. N45	GCCTGTTGTGAGCCTCCTAAC (N45) CATGCTTATTCTTGTCTCCC [a]	D-DNA
Fwd. primer	GCCTGTTGTGAGCCTCCTAAC	D-DNA
Rev. primer	5'-/Biotin-TEG/GGGAGACAAGAATAAGCATG	D-DNA
L-s2m-Cov2	/Biotin/CUUCACCGAGGCCACGCGGAGUACGAUCGAGUGUACAGUGAAG	L-RNA
L-s2m Cov1	/Biotin/CUUCACCGAGGCCACGCGGAGUACGAUCGAGGGUACAGUGAAG	L-RNA

Table S2. Detailed conditions used for *in vitro* selection. N.S. = negative selection; P.S. = positive selection.

Rounds	1	2	3	4	5	6	7	8
[Lib. N45] (μM)	2	0.2	0.2	0.2	0.2	0.2	0.2	0.02
[L-s2m-CoV2] (μM)	0.2	0.2	0.2	0.2	0.2	0.2	0.02	0.004
$[Mg^{2+}]$ (mM)	5	5	5	5	5	2	2	2
N.S. (mins) [a]	60	120	120	120	120	120	120	120
P.S. (mins) [b]	30	30	30	30	30	30	30	30
Temp. (^{o}C)	23	23	23	23	23	23	23	23
Wash time (mins) [c]	<1	1	5	10	10	1	10	10
Times Washed [d]	5	5	5	5	5	5	5	5
Wash volume (mL) [e]	1	1	1	1	1	1	1	1

[a] The time of each DNA library was incubated with streptavidin-coated beads (round 1 to 5) or counter targets (round 6 to 8) in negative selection (N.S.) process.

[b] The time of each DNA library was incubated with L-s2m-CoV2 in positive selection (P.S.) process. Following immobilization of L-s2m-Cov2 to streptavidin-coated beads, unbound sequences were washed away using the indicated volume of SB buffer.

[c] The bead washing time in minutes.

[d] The number of washing steps.

[e] The wash buffer volume.

Table S3. Sequences of individual clones (N45 region) isolated from Lib.N45 following 8 rounds of *in vitro* selection. The clones with the highest affinity for s2m-Cov2 is shown in red.

Aptamers	Sequences (5' to 3')	Occurrences
C1	TAG GGG GTG GTG TGG GGG ATT TGC GGG TCG CTA GAG GCT CTC TGA	8
C2	TAG TAT GGG GGT GGC GTG GGG GGA AGG GGG AAG GTG AGG TCA CGG	1
C3	AAG AAA AGC ATC TAG GGG GTG GAT GTG GGG GTT TAG AGG GCG TAA	1
C4	TGG GGG TGG CGC GGG GGG TTT GAG GGA GCT AGA AGG TCA CAA TGG	1
C5	CGG GGG TGG TCG CGG GGG CTT GGT GGG TCC TAA GCG GCG CAC AAA	1
C6	GTA GAT ATG GTG GGG GTG GAG GGG AGG GTG TTA GTG AGC GCG AGT	1
C7	TAG TAG GGG GTG GCG AGG GGG GAA GGA GGG AAG TTA GTG CGG CTA	1
C8	GGG GGT GGT GTG GGG GAT GTG AGG GCT ATA GAG GTT CAG CTA TGG	1
C9	GCT GAA TAG GGG GCG GCT GTG GGG GTT AAG TGG GTC GCG TTA GGA	1
C10	TAC GGG GGC GGC GTG GGG GTG TCA AGT GGG CGT GTC GGC TAG CAA	1
C11	GGG GGT GGT GCG GGG GTA CTG TGG GTA AGC AAT ACA GGG TCA TAA	1

Table S4. Sequences of truncation variants used to determine the minimal binding domain of aptamer D-C1. Residues from fixed primer binding sites are underlined. n.d. = no binding observed. The minimal binding domain is shown in red.

Aptamer	Truncations	nt	Sequences (5'-3')	Binding
D-C1t1	3'-27nt	59	GCC TGT TGT GAG CCT CCT AAC TAG GGG GTG GTG TGG GGG ATT TGC GGG TCG CTA GAG GC	n.d.
D-C1t2	5'-5nt	81	T TGT GAG CCT CCT AAC TAG GGG GTG GTG TGG GGG ATT TGC GGG TCG CTA GAG GCT CTC TGA CAT GCT TAT TCT TGT CTC CC	+++
D-C1t3	5'-10nt	76	AG CCT CCT AAC TAG GGG GTG GTG TGG GGG ATT TGC GGG TCG CTA GAG GCT CTC TGA CAT GCT TAT TCT TGT CTC CC	++
D- C1t4	3'-5nt	81	GCC TGT TGT GAG CCT CCT AAC TAG GGG GTG GTG TGG GGG ATT TGC GGG TCG CTA GAG GCT CTC TGA CAT GCT TAT TCT TGT	+++
D- C1t5	3'-10nt	76	GCC TGT TGT GAG CCT CCT AAC TAG GGG GTG GTG TGG GGG ATT TGC GGG TCG CTA GAG GCT CTC TGA CAT GCT TAT T	+++
D- C1t6	3'-15nt	71	GCC TGT TGT GAG CCT CCT AAC TAG GGG GTG GTG TGG GGG ATT TGC GGG TCG CTA GAG GCT CTC TGA CAT GC	+++
D- C1t7	3'-20nt	66	GCC TGT TGT GAG CCT CCT AAC TAG GGG GTG GTG TGG GGG ATT TGC GGG TCG CTA GAG GCT CTC TGA	+
D- C1t8	5'-5nt, 3'-10nt	71	T TGT GAG CCT CCT AAC TAG GGG GTG GTG TGG GGG ATT TGC GGG TCG CTA GAG GCT CTC TGA CAT GCT TAT T	+++
D- C1t9	5'-5nt, 3'-15nt	66	T TGT GAG CCT CCT AAC TAG GGG GTG GTG TGG GGG ATT TGC GGG TCG CTA GAG GCT CTC TGA CAT GC	++
D- C1t10 (D- C1t)	5'-5nt, 3'-20nt	61	T TGT GAG CCT CCT AAC TAG GGG GTG GTG TGG GGG ATT TGC GGG TCG CTA GAG GCT CTC TGA	+++

Table S5. Sequences of truncation variants used to determine the minimal binding domain of aptamer D-C3. Residues from fixed primer binding sites are underlined. n.d. = no binding observed. The minimal binding domain is shown in red.

Aptamer	Truncations	nt	Sequences (5'-3')	Binding
D-C3t1	3'-15nt	71	GCC TGT TGT GAG CCT CCT AAC AAG AAA AGC ATC TAG GGG GTG GAT GTG GGG GTT TAG AGG GCG TAA CAT GC	n.d.
D-C3t2	5'-3nt, 3'-18nt	65	TGT TGT GAG CCT CCT AAC AAG AAA AGC ATC TAG GGG GTG GAT GTG GGG GTT TAG AGG GCG TAA CA	n.d.
D-C3t3	5'-5nt	81	T TGT GAG CCT CCT AAC AAG AAA AGC ATC TAG GGG GTG GAT GTG GGG GTT TAG AGG GCG TAA CAT GCT TAT TCT TGT CTC CC	+++
D- C3t4	5'-10nt	76	AG CCT CCT AAC AAG AAA AGC ATC TAG GGG GTG GAT GTG GGG GTT TAG AGG GCG TAA CAT GCT TAT TCT TGT CTC CC	+++
D- C3t5	3'-5nt	81	GCC TGT TGT GAG CCT CCT AAC AAG AAA AGC ATC TAG GGG GTG GAT GTG GGG GTT TAG AGG GCG TAA CAT GCT TAT TCT TGT	++
D- C3t6	3'-10nt	76	GCC TGT TGT GAG CCT CCT AAC AAG AAA AGC ATC TAG GGG GTG GAT GTG GGG GTT TAG AGG GCG TAA CAT GCT TAT T	n.d.
D- C3t7	5'-15nt	71	CCT AAC AAG AAA AGC ATC TAG GGG GTG GAT GTG GGG GTT TAG AGG GCG TAA CAT GCT TAT TCT TGT CTC CC	+++
D- C3t8	5'-20nt,	66	C AAG AAA AGC ATC TAG GGG GTG GAT GTG GGG GTT TAG AGG GCG TAA CAT GCT TAT TCT TGT CTC CC	+++
D- C3t9	5'-25nt,	61	AA AGC ATC TAG GGG GTG GAT GTG GGG GTT TAG AGG GCG TAA CAT GCT TAT TCT TGT CTC CC	n.d.
D- C3t10 (D- C3t)	5'-20nt, 3'-5nt	61	C AAG AAA AGC ATC TAG GGG GTG GAT GTG GGG GTT TAG AGG GCG TAA CAT GCT TAT TCT TGT	++

Table S6. G-quadruplex analysis using QGRS Mapper software.[3] G-score comparison of L-C1t and L-C3t to previously reported L-DNA aptamers L-T9t and L-AdU5t[1], which have confirmed G-quadruplex structures. The predicted G-quadruplex motif is underlined and guanosines involved are colored red.

Name	Sequences (5' to 3')	G-score
C1t	TTGTGAGCCTCCTAACTAGGGGGTGGTGTGGGGGATTTGCGGGTCG CTAGAGGCTCTCTGA	36
C3t	CAAGAAAAGCATCTAGGGGGTGGATGTGGGGGTTTAGAGGGCGTAA CATGCTTATTCTTGT	33
T9t	CCTAACGGTTAAGAGTGTGGGGGGAGGGAGAGGTTGCTTGGGGCGCG GGATGCATGC	64
AdU5t	AGCCTCCTAACAGGCACGAACCGGGGTGGGGAAGGTTGGAGTCGAA GGAGTGGGGCATGC	35

Table S7. Sequences of various D-RNAs used in this work and the two DNA strands (sense and antisense) used to prepare them via cross-extension reaction.

Name	Sequences (5' to 3')	Type
D-s2m-Cov2	CUUCACCGAGGCCACGCGGAG UACGAUCGAGUGUACAGUGAAG	D-RNA
sense	TTCTAATACGACTCACTATAGGCTTCACCGAGGCCACGCGGAGTA	D-DNA
antisense	CTTCACTGTACACTCGATCGTACTCCGCGTGGCCTCG	D-DNA
D-s2m-Cov1	CUUCACCGAGGCCACGCGGAGUACGAUCGAGGGUACAGUGAAG	D-RNA
sense	TTCTAATACGACTCACTATAGGCTTCACCGAGGCCACGCGGAGTA	D-DNA
antisense	CTTCACTGTACCCTCGATCGTACTCCGCGTGGCCTCG	D-DNA
D-pre-miR-155	GGUGAUAGGGGUUUUUGCCUCCAACUGACUCCUACACC	D-RNA
sense	TTCTAATACGACTCACTATAGGTGATAGGGGTTTTTGCCTCCAACTGA CTCCTACACC	D-DNA
antisense	GGTGTAGGAGTCAGTTGGAGGCAAAAACCCCTATCACCTATAGTGAG TCGTATTAGAA	D-DNA
D-pre-miR-10b	GAAUUUGUGUGGUAUCCGUAUAGUCACAGAUUC	D-RNA
sense	TTCTAATACGACTCACTATAGAATTTGTGTGGTATC	D-DNA
antisense	GAATCTGTGACTATACGGATACCACACAAATTCTAT	D-DNA
D-pre-miR-21	GGUGUUGACUGUUGAAUCUCAUGGCAACACC	D-RNA
sense	TTCTAATACGACTCACTATAGGTGTTGACTGTTGA	D-DNA
antisense	GGTGTTGCCATGAGATTCAACAGTCAACACCTATA	D-DNA

S4. References.

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3. Kikin, O.; D'Antonio, L.; Bagga, P. S., QGRS Mapper: a web-based server for predicting G-quadruplexes in nucleotide sequences. *Nucleic Acids Res.* **2006,** *34*, W676-W682.