Supporting Information

A SARS-CoV-2 Spike Binding DNA Aptamer that Inhibits Pseudovirus Infection by an RBD-Independent Mechanism**

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Author Contributions

A.S. developed the cellular binding and partly executed the cellular binding and infection assays, constructed the pseudotype viruses and wrote the manuscript. A.W. performed sequence analysis of the enriched libraries and conducted interaction analysis of the DNA libraries, aptamers and their variants. M.B. conducted infection and binding experiments. S.B. performed the automated selection experiments. V.F. expressed and purified proteins and performed the pulldown experiments. M.F. supervised experiments, discussed results and wrote the manuscript. G.M. conceived and designed the study, supervised and discussed results and wrote the manuscript. All authors have read and commented on the manuscript.
Supporting Information

Material and Methods

Coupling of SARS-CoV-proteins to Dynabeads His-Tag isolation & pulldown

For immobilization of SARS-CoV-2 proteins, Dynabeads His-Tag Isolation & Pulldown (ThermoFisher) were used. For this purpose, 9.6 nmol of SARS-CoV-2 proteins, prepared in 1 mL wash/binding buffer (50 mM Sodium-Phosphate, pH 8.0, 300 mM NaCl, 0.01% Tween®-20) were coupled to 100 µL of bead solution (40 mg beads/mL), according to the manufacturer’s protocol. The provided buffer, by the manufacturer, of the bead solution was discarded by separation before coupling, using a DynaMag™-2 Magnet (ThermoFisher). For the coupling reaction, the solution was incubated for 30 min on 4°C, using a Tube Revolver Model D-6050 (neoLab) rotating at a speed of 20 rpm. According to the manufacturer’s protocol, three washing steps with 1 mL wash/binding buffer were carried out, followed by one additional washing step with storing buffer (ssDNA selection = 1.25× PBS; 171.25 mM NaCl (Fisher Scientific), 3.38 mM KCl (Roth), 12.5 mM Na₂HPO₄ (Roth), 2.2 mM KH₂PO₄ (Roth), pH 7.4; 1 mg/mL Albumin (BSA) Fraction V (pH 7.0) (AppliChem); 3.25 mM MgCl₂ // 2’fRNA selection = 1.25× PBS; 171.25 mM NaCl (Fisher Scientific), 3.38 mM KCl (Roth), 12.5 mM Na₂HPO₄ (Roth), 2.2 mM KH₂PO₄ (Roth), pH 7.4; 1 mg/mL Albumin (BSA) Fraction V (pH 7.0) (AppliChem), before resuspending SARS-CoV-2-protein beads in 1 mL of storing buffer. In the particular case of competitor, 0.125 mg/mL Heparin was added to the storing buffer.

Automated selection of ssDNA aptamers

For the selection of ssDNA aptamers, the D2 DNA library (5′ – GGG AGA GGA GGG AGA TAG ATA TCA A – N40 – T TTC GTG GAT GCC ACA GGA C – 3′) was used (Ella Biotech GmbH, Martinsried, Germany). For amplifying the library, the following primers were used: forward primer (Cy5-D2 fw) 5′ Cy5 –GGG AGA GGA GGG AGA TAG ATA TCA A – 3′ and reverse primer (Phos-D2 rv) 5′ P – GTC CTG TGG CAT CCA CGA AA – 3′. PCR master mix for amplification reaction (colorless GoTaq® Flexi Buffer (Promega), 2 mM MgCl₂ (Roth), 0.2 mM dNTP (Genaxxon)). The PCR reaction was performed by using GoTaq® G2 Flexi DNA Polymerase (Promega), including 1 µM of Cy5-D2 fw and Phos-D2 rv primers, in a total reaction volume of 100 µL and the cycling program 30 s 95 °C, 30 s 62 °C, and 30 s 72 °C in a TRobot thermal cycler (Biometra). In the first four selection cycles 18 PCR cycles were used and in all following selection cycles 16. For all steps performed on the TRobot, an arched auto-sealing lid (Bio-Rad) was used to seal the reaction plate. 1 µL GoTaq® G2 Flexi DNA Polymerase (5u/µL, Promega) was added to start the PCR reaction. The automated pipetting steps were performed by a Biomek NX® (Beckman Coulter). The automated selection was started by 0.5 nmol of D2 ssDNA library pipetted to the SARS-CoV-proteins immobilized on Dynabeads His-Tag Isolation & Pulldown (ThermoFisher) and an incubation for 30 min at 37 °C while shaking at a speed of 700 rpm; pipetting up and down every 5 min during incubation. Selection buffer was PBS/3 mM MgCl₂/0.8 mg/mL BSA (PBS: 137 mM NaCl (Fisher Scientific), 2.7 mM KCl (Roth), 10 mM Na₂HPO₄ (Roth), 1.76 mM KH₂PO₄ (Roth), pH 7.4). After incubation, the samples were washed two times with 100µL wash buffer (PBS/3 mM MgCl₂). Washing steps were increased every selection cycle by two more washes until a total of eight washes per selection cycle was reached. Prior to PCR, the bound ssDNA molecules were recovered by incubation in ddH₂O (TKA Wasseraufbereitungssysteme GmbH) for 5 min at
80 °C. After PCR, a lambda exonuclease (final 20u, ThermoFisher) digestion was performed for 60 min at 37°C to generate ssDNA for the next selection cycle.

**Agarose gel analysis**

Agarose LE (Genaxxon) was used to prepare 4% agarose gels pre-stained with ethidium bromide (Roth). 1 µL 6x DNA Loading Dye (Thermo Scientific) was mixed with 5 µL of dsDNA products and loaded on the gel. As reference, 4 µL of GeneRuler Ultra Low Range DNA Ladder (Thermo Scientific) were loaded. A Genoplex system (VWR) was used for visualization of the stained dsDNA.

**DNA interaction analysis**

For interaction studies of the selected libraries and individual sequences CoV2-S, RBD, CoV-S or ACE2 were immobilized on magnetic particles (ThermoFisher) via a His-tag according to manufacturer’s instructions and stored in 1.25× PBS with 1 mg/ml BSA at 4°C. 4 µl of the bead suspension were incubated with 500 nM DNA in a final volume of 10 µl in PBS with 3 mM MgCl₂ and 0.8 mg/ml BSA at 37°C in a thermomixer (Eppendorf) and 650 rpm. After incubation, the beads were washed three times with 100 µl PBS/3 mM MgCl₂. Bound Cy5-labeled sequences were analyzed by flow cytometry counting approximately 20,000 events. Unmodified sequences were analyzed via OliGreen (ThermoFisher) fluorescence. Therefore, bound DNA sequences were recovered in 100 µl ddH₂O for 5 min at 95°C, cooled down to 4°C and incubated in 200 µl TE buffer (10 mM Tris/HCl pH 7.5, 1 mM ethylenediaminetetraacetic acid) with a 1:500 dilution of OliGreen. Fluorescence intensity was measured on a Tecan Ultra plate reader (Tecan) at excitation and emission wavelengths of λ = 500 nm and λ = 525 nm, respectively.

**NGS**

The starting library and all enriched libraries were analyzed by NGS using the Illumina HiSeq1500 platform. Samples were prepared according to. Briefly, 12 different index primers were attached to the different libraries via PCR, allowing the analysis of 12 samples in parallel on one lane. PCR products were purified using the NucleoSpin Clean-Up kit (Macherey Nagel), and equal amounts of PCR product of each library were mixed to a final amount of 2 µg DNA. For the hybridization to the flow cell a subsequent adapter ligation step was performed according to the manufacturer’s instructions (TruSeq DNA PCR-Free Sample Preparation Kit LT, Illumina). Samples were purified by agarose gel purification. The NGS data was analyzed using the inhouse AptaNext software and MEME suite. Five families from cycle 12 were identified and the most abundant sequences were tested in a FACS binding assay. Secondary structures of the aptamers were predicted with Mfold.

**SPR**

Binding affinities of the full-length sequences were assessed by surface plasmon resonance on a BiAcore 3000 instrument (GE Healthcare Europe). All buffers were filtered and degassed prior to use. 50 nM of biotinylated aptamers SP5, SP6 and SP7 (flow cells 2-4) and the control SP5sc (flow cell 1) were immobilized on XanTec SAD chips (XanTec Bioanalytics) with a flow rate of 10 µl/min at 25 °C in 0.5 M NaCl until values of ~200 response units were reached. The CoV2-S protein (PBS/3 mM MgCl₂ and 1 mg/ml BSA) was injected at different concentrations of 1, 3.2, 10, 32, 100, 200, 316, 700 and 1000 nM for 180 s at 25 °C and 37°C. The dissociation
time was 400 s, followed by a regeneration step (0.5% sodium dodecyl sulfate). Data was evaluated using the BIAevaluation 4.1 (Biacore) software: 1:1 binding with drifting baseline.

ELONA
33 ng/ml of the RBD-nanobody VHH E (kindly provided by Dr. F.-I. Schmidt and Dr. P.-A. König (Core Facility Nanobodies, Medical Faculty, University of Bonn) was immobilized in 20 µl bicarbonate/carbonate buffer (pH 9.6) on 96 half-well microtiter plates (MICROLON® 600, VWR) at 4°C overnight followed by two washing steps with 100 µl PBS with 0.05% Tween 20. Wells were blocked with PBS including 5% BSA for 1 hour at RT, followed by two washing steps with 100 µl PBS/3 mM MgCl₂. Afterwards the CoV2-S protein or the pseudovirus (~15,000 particles) were added in PBS with 3 mM MgCl₂ and 0.8 mg/ml BSA and incubated in a final volume of 20 µl at RT, followed by two washing steps with PBS/3 mM MgCl₂. Next, biotinylated DNA aptamers [500 nM] and controls in PBS/3 mM MgCl₂ and 0.8 mg/ml BSA were added in a final volume of 20 µl at RT, followed by two washing steps with PBS/3 mM MgCl₂. Streptavidin-HRP (GE Healthcare) in a 1:1000 dilution in PBS/3 mM MgCl₂ was added (20 µl) at RT, followed by two washing steps with PBS/3 mM MgCl₂. Finally, 100 µl ABTS (ThermoFisher) were added per well, incubated at RT for 40 min and the absorbance at λ = 405 nm was measured on a Tecan Nanoquant (Tecan).

Constructs and plasmids
Plasmids for SARS-CoV-2-Se, SARS-CoV-2-S-HexaPro and SARS-CoV-2-Se (kindly provided by Jason McLellan, The University of Texas at Austin, USA) code for the prefusion-stabilized ectodomains of the S proteins and carry on the C-terminus a trimerization motif, a HRV 3C cleavage site, 8xHis and TwinStrep tags. SARS-CoV-2-S(D614G)-∆19 codes for the S protein (GenBank NC_045512) containing the D614G mutation and lacking the C-terminal 19 amino acids thus deleting the ER-retention motif and enhancing transport to the plasma membrane. SARS-CoV-2-S(D614G)-∆19-HiBiT contains the HiBiT tag (Promega) at the very C-terminus. SARS-CoV-2-S-RBD codes for amino acids 319 - 591 of the S protein and contains the signal peptide of the S protein on the N-terminus to allow secretion and an HRV 3C cleavage site, 8xHis and TwinStrep tags on the C-terminus. Proteins denoted by ∆His or ∆ST do not contain the respective tag but are otherwise identical to their tag-containing counterparts. ACE2e contains, after cleavage of the signal peptide, amino acids 19 - 615 of human ACE2 (UniProt Q9BYF1), an N-terminal myc tag and a C-terminal HRV 3C cleavage site following by an 8xHis tag. The myc and ACE2 coding sequence was amplified form pCEP4-myc-ACE2 (addgene #141185). With the exception of SARS-CoV-2-S(D614G)-∆19-HiBiT which was cloned into pmCherry-N1 (Takara) replacing mCherry all these proteins were cloned into pCAG which is based on pCAGGS only lacking the SV40 ori of the latter. pET-Sumo-Nek7 contains the full-length human Nek7 (UniProt Q8TDX7) cloned into pET-Sumo (Thermo). All constructs were assembled from PCR-amplified fragments using Q5 DNA Polymerase (NEB) or synthetic genes (Eurofins) except for the pCAG backbone which was linearized by restriction digestion. For assembly the NEBuilder HiFi DNA Assembly Master Mix was used (NEB). Coding sequences of all constructs were verified by Sanger sequencing (Eurofins).

Protein expression and purification
With the exception of Nek7 which was expressed in E. coli BL21(DE3) proteins were expressed in FreeStyle 293F cells (Thermo). 293F cells at 1x10⁶ cells / ml in FreeStyle 293 Expression Medium (Thermo) were transfected with 1 mg plasmid and 2 mg PEI max (Polysciences) per
liter of cells. 3 - 5 days after transfection proteins were purified from the culture medium after removing cells and debris by centrifugation (10 min, 800 g, rt, followed by 30 min, 10000 g, 4 °C). The cleared medium was adjusted to 50 mM HEPES/KOH, pH 7.8 / 300 mM NaCl / 25 mM imidazole and loaded overnight onto a column containing 2 ml Protino Ni-NTA Agarose (Macherey-Nagel) per liter of medium. After washing with 50 mM HEPES/KOH, pH 7.8 / 300 mM NaCl / 25 mM imidazole proteins were eluted in the same buffer containing 1 M imidazole. Eluted proteins were concentrated using Vivaspin Turbo concentrators (Sartorius) and loaded on a Superose 6 column (Cytiva) equilibrated in 20 mM HEPES/KOH, pH 7.8 / 150 mM NaCl to remove aggregated material. Peak fractions were pooled, concentrated, flash-frozen in liquid nitrogen and stored at -80 °C. Purification of proteins lacking the 8xHis tag was done by replacing the Ni-NTA column by a StrepTactin column (IBA). Elution was achieved by 30 mM desthiobiotin. To remove the 8xHis TwinStrep tags from SARS-CoV-2-S-RBD and SARS-CoV-Se the proteins were incubated overnight with HRV 3C protease (Thermo) and passed over a Ni-NTA agarose column. The flow-through was concentrated and further purified by size exclusion chromatography as above.

**Pulldown assays**

For aptamer pulldowns 1 µM 5´-biotinylated SP6 or SP6C, respectively, were incubated with 0.5 µM of the indicated proteins (without TwinStrep tag) for 30 min at rt in buffer PB (PBS / 4 mM MgCl₂ / 2.5 µM BSA). For the RBD competition, 0.5 µM S protein together with 2.5 µM RBD (without 8xHis and TwinStrep tags) were used. An aliquot was removed (input) and 100 µl pre-equilibrated Hydrophilic Streptavidin Magnetic Beads (NEB) were added. After 30 min incubation at rt on an overhead rotator beads were collected on a magnet and an aliquot was removed from the supernatant (unbound). After washing two times with 500 µl PBS / 3 mM MgCl₂ proteins were eluted by boiling in Lämmli sample buffer (eluate). For spike-ACE2 complex pulldowns 1 µM SARS-CoV2-S-HexaPro and ACE2 (without 8xHis tag) were incubated in the presence of 3 µM SP6 or VHH E, as indicated, in buffer PB. An aliquot was removed (input) and 20 µl pre-equilibrated HisPur Ni-NTA Magnetic Beads (Thermo) were added. After 30 min incubation at rt on an overhead rotator beads were collected on a magnet and an aliquot was removed from the supernatant (unbound). After washing two times with 250 µl buffer PW proteins were eluted with 25 mM HEPES/KOH, pH 7.8 / 150 mM NaCl / 1 M imidazole (eluate). Samples were separated by SDS-PAGE. Coomassie-stained gels were scanned on an Odyssey Sa (Licor).

**Analytical size exclusion chromatography**

5 µM SARS-CoV-2-S-RBD labeled with Dye488 (Serva) (kindly provided by Olaf Stemmann, University of Bayreuth, Germany) was incubated with 10 µM ACE2(19-615) for 5 min on ice in the presence or absence of 10 µM SP6 or VHH E, respectively, in SEC buffer (20 mM Hepes/NaOH / 150 mM NaCl / 3 mM KCl / 3 mM MgCl₂, pH 7.8). After centrifugation (20000 g, 5 min, 4 °C) the samples (25 µl) were analyzed on a Superose 6 10/300 GL column (Cytiva), equilibrated in SEC buffer, at a flow rate of 0.5 ml/min using a 1260 Infinity II LC System (Agilent). Absorbance was recorded at 488 nm to specifically monitor RBD, at 280 nm for total protein and at 260 nm for DNA.

**Pseudovirus generation**

VSV pseudotypes were generated as published.32 Briefly, Hek293T cells transfected with pCAG-SARS-CoV-2-S(D614G)-Δ19 or pcDNA3.1-VSV-G, respectively, were inoculated with
VSV-ΔG* (kindly provided by Gert Zimmer, Institute of Virology and Immunology, Mittelhäusern, Switzerland). In VSV-ΔG* the VSV-G open reading frame is replaced by an expression cassette for GFP and firefly luciferase allowing infected cells to be detected by GFP fluorescence or luciferase activity. After 1 h incubation at 37 °C the inoculum was removed, the cells were washed with DMEM and cultivated for 16 - 18 h in DMEM / 2 % FBS / 30 mM Hepes at 34 °C. The culture medium containing the pseudotyped particles was clarified from cellular debris by centrifugation (800 g, 5 min, rt). Aliquots were flash-frozen in liquid nitrogen and stored at -80 °C.

Infection
Vero E6 cells were cultivated in DMEM (Thermo) / 10 % FBS (PAN) at 37 °C and 8 % CO₂. The day before infection 5x10⁴ cells were plated per well of a 24well plate. Virus particles pseudotyped with SARS-CoV-2-S(D614G)-Δ19 or VSV-G were pre-incubated for 20 min at rt with the indicated agent in DMEM / 3 mM MgCl₂. The culture medium was removed from the cells and replaced by 150 µl pre-incubated virus (MOI ≈ 0.1). After incubation for 1 h at 37 °C 0.5 ml DMEM / 10 % FBS / 3 mM MgCl₂ was added and the cells were cultivated for 16 - 18 h. Cells were detached with trypsin, fixed for 20 min at rt with 4 % formaldehyde, pelleted (800 g, 5 min, rt) and resuspended in PBS. Infection rate was determined as percentage of GFP-positive cells by flow cytometry (BectonDickinson). Data analysis was done with FlowJow 10.7.1 (BectonDickinson). Doublets were excluded (for gating see Supporting Fig. 4c). For statistical analysis the non-parametric Kruskal-Wallis test and Dunn’s multiple comparison post-test was used because due to the small sample size of n=5 Gaussian distribution for the values could not be tested. Analysis was performed with Prism 5.0f (GraphPad).

Binding
1x10⁴ Vero E6 cells were plated per well of two 96well plates the day before. Virus particles pseudotyped with SARS-CoV-2-S(D614G)-Δ19-HiBiT were pre-incubated for 20 min at rt with the indicated agent in DMEM / 3 mM MgCl₂. The culture medium was removed from the cells, replaced by 100 µl pre-incubated virus, and the cells were incubated for 1 h at 37 °C. The inoculum was completely removed and 50 µl of Nano-Glo HiBiT Lytic Reagent (Promega), beforehand diluted with an equal volume of PBS, was added. After 15 min incubation at rt luminescence was measured with an Infinite M1000 Pro (Tecan). Statistical analysis was done as above, n=8, each with 3 technical replicates.
Overview on sequences of aptamers used in this study (point mutations are depicted in red bold letters)

| Name | Sequence |
|------|----------|
| SP1  | GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP2  | GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP3  | GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP4  | GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP5  | GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP5sc| GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP6  | GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP6C | GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP6.51| GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP6.45| GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP6.41| GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP6.34| GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP6.34A| GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP6.34G| GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP6.34C| GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP6.30| GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP6.19| GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| SP7  | GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| RBD1 | GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| RBD2 | GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| RBD3 | GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
| RBD4 | GGGAGAGGGAGGAGATAGATATCAGAGGCAGGAGGAGGGGCCCACCCAAACACGTTACACTTTCTGCGATGCACAGGAC |
Supporting Figure 1: a) Agarose gel analysis of the PCR products obtained from the selection cycles 1-12 of the automated selection targeting CoV2-S. ULR: GeneRuler Ultra Low Range DNA Ladder; 1-12 indicate the selection cycle. b) Number of analyzed sequences per selection cycle by NGS. c) Nucleotide distribution of the random region at the different positions in the
starting library and selection cycles 2 to 12. d) The predicted families 30, 29, 13, and 22 obtained by NGS analysis based on motif search by MEME.
Supporting Figure 2: a,b) Sensograms of the SPR analysis of biotinylated aptamers SP5, SP6 and SP7 immobilized on a streptavidin chip and the CoV2-S at concentrations between 1-1000 nM at 37°C (a) (N = 4) and 25°C (b) (N = 2). c) OligoGreen-based assay to evaluate the interaction of the aptamers SP5, SP6 and SP7 having different modifications (Cy5, biotin or unmodified) at the 5’-end with CoV2-S. N = 2, mean +/- SD d) secondary structure prediction by Mfold of the aptamer SP6. Nucleotides 26 to 59 of the truncated aptamer SP6.34 are highlighted. SP6C depicts the single point mutant G50C.
Supporting Figure 3: a-c) Agarose gel analysis of the PCR products obtained from the selection cycles 1-12 of the automated selections targeting RBD and using conventional selection conditions (a), 10% of target (b) or heparin as competitor (c) during the selection. ULR: GeneRuler Ultra Low Range DNA Ladder; 1-12 indicate the selection cycle. d) Interaction analysis of the enriched libraries (a)-(e) binding to RBD and CoV2-S. SL: Starting library, R12CoV2-S (library enriched for CoV2-S as in Fig. 1a). e) Agarose gel analysis of the PCR products obtained from the re-selection cycles 13-115 and 13*-115* of the automated selections targeting RBD and starting from the library of selection cycle 12 enriched for CoV2-S (Fig. 1a). ULR: GeneRuler Ultra Low Range DNA Ladder; 13-15 indicate the selection cycles
using conventional selection conditions; 13*-15* indicate selection cycles using 10% of the target during the selection. f) Interaction analysis of the enriched libraries from (e) binding to RBD and CoV2-S. SL: Starting library, R12CoV2-S (library enriched for CoV2-S as in Fig. 1a). g,h) Nucleotide distribution at the different positions of the random region of the libraries obtained from the re-selection experiments targeting RBD shown in (e,f). i) The predicted families 1 and 2 obtained by NGS analysis based on motif search by MEME from libraries 15* and 15. Interaction of aptamers RBD1-4 with RBD (j) and CoV2-S (k). SL: Starting library; R15: as R15 in f. Enrichment profiles of RBD1-4 during the selection targeting CoV2-S (selection cycles 1-12, l,m) and the two different re-selection conditions, conventional conditions (cycles 13-15, l) and with less target protein (cycles 13-15, m). d, f, j, k: N = 2, mean +/- SD.
Supporting Figure 4: Top panel: Interaction analysis of the RBD-binding aptamers RBD 1C and RBD 4C\(^{[17]}\) using flow cytometry. Shown is the Mean fluorescence of CoV-2S- bound DNA in comparison to the aptamers SP3, SP4, and SP5 (Fig. 1), non-binding controls (SP5sc, SP1sc), the starting library (SL), and the enriched library R12 selected for CoV-2S binding (Fig. 1). bottom panel: Similar analysis but instead of the CoV-2S the isolated RBD domain was used for the interaction study, along with the CoV-2S binding aptamer SP5 (Fig. 1), non-binding control SP5sc, the starting library (SL) and the enriched library R9 1/10 selected for binding to RBD (Supporting Fig. 3d). This experiment was performed using 500 nM of the indicated ssDNA in PBS pH 7.4, 0.8mg/ml BSA and 4 mM MgCl\(_2\). N = 2, mean +/- SD.
Supporting Figure 5: The indicated combinations of proteins and aptamer were separated on a Sepharose 6 10/300 column. In the upper graph the Dye488-labeled RBD is selectively detected, while in the middle graph total protein and DNA and in the lower graph preferentially DNA are visualized. As SP6 has a very strong absorption the peak at 30 min in the 280 nm trace containing SP6 was cropped. The peak at 23 min corresponds to an oligomeric form of SP6. Note, that in the upper graph the curves for RBD / ACE2 and RBD / ACE2 / SP6 are virtually identical demonstrating that complex formation between RBD and ACE2 is not affected by SP6. Retention times of RBD, ACE2 or RBD/ACE2 complex are indicated by green, black or blue arrows, respectively.
Supporting Figure 6: a) ELONA assay in sandwich format using a RBD-binding nanobody to capture CoV2-S and the indicated biotinylated oligonucleotide sequence [500 nM] to detect bound CoV2-S. N = 3, error represents standard deviation b) SARS-CoV-2-S pseudovirus
infection. n=5, *** p<0.001, ** p<0.01. c) Gating strategy for quantifying GFP expressing cells by flow cytometry.

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