Electronic Supplementary Material (ESI) for Analyst.

Localized Surface Plasmon Resonance Aptasensor for Selective Detection of SARS-CoV-2 S1 Protein

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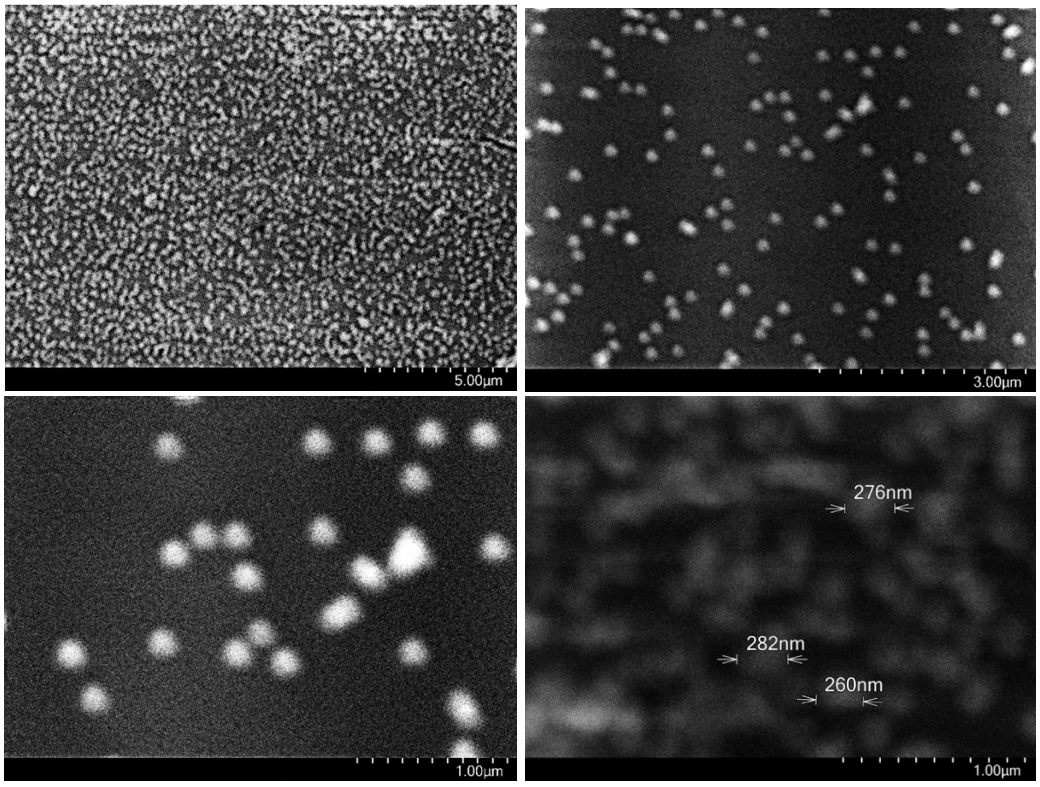

Fig. S1. SEM images of LSPR chip at various magnifications. The chip surface contains biotin film on gold nanoparticles.

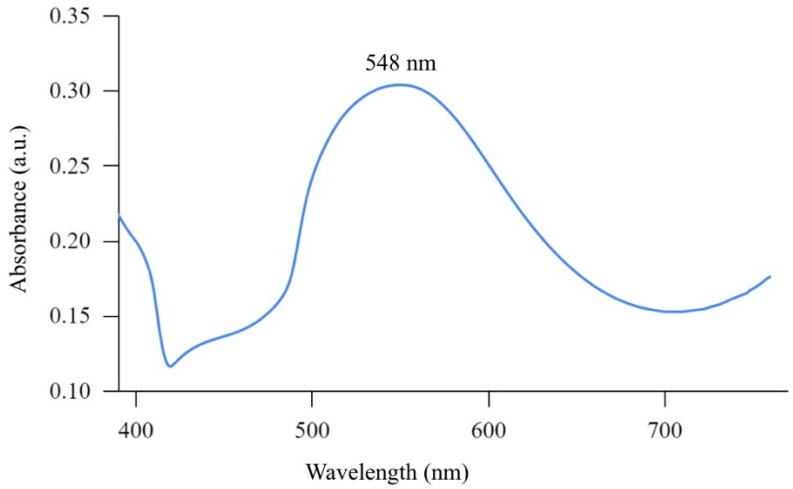

Fig. S2. Absorbance spectrum of LSPR Biotin-Au chip.

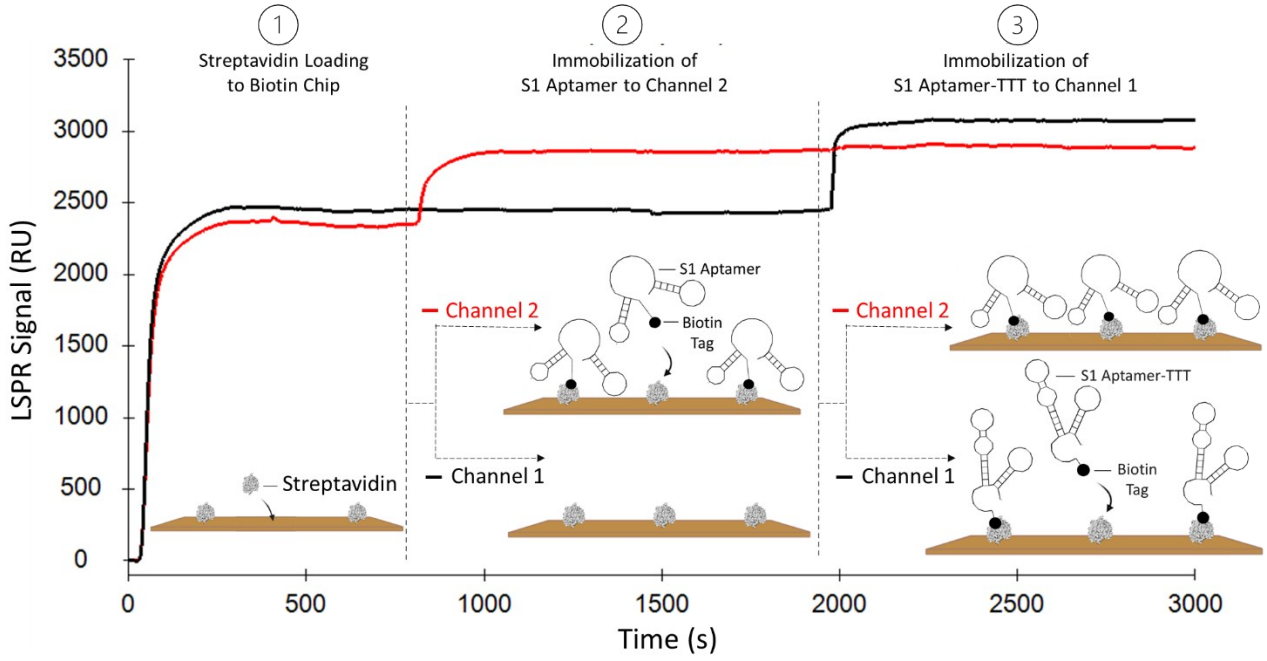

Fig. S3. Sensorgram showing fabrication process involving streptavidin protein loading to the biotin-gold chip followed by immobilization of S1 and S1-T aptamers on two different channels ([streptavidin] = 0.5 μM; [S1 aptamer] = 50 μg/mL; [S1 aptamer-T] = 50 μg/mL; flow rate = 20 μL/min).

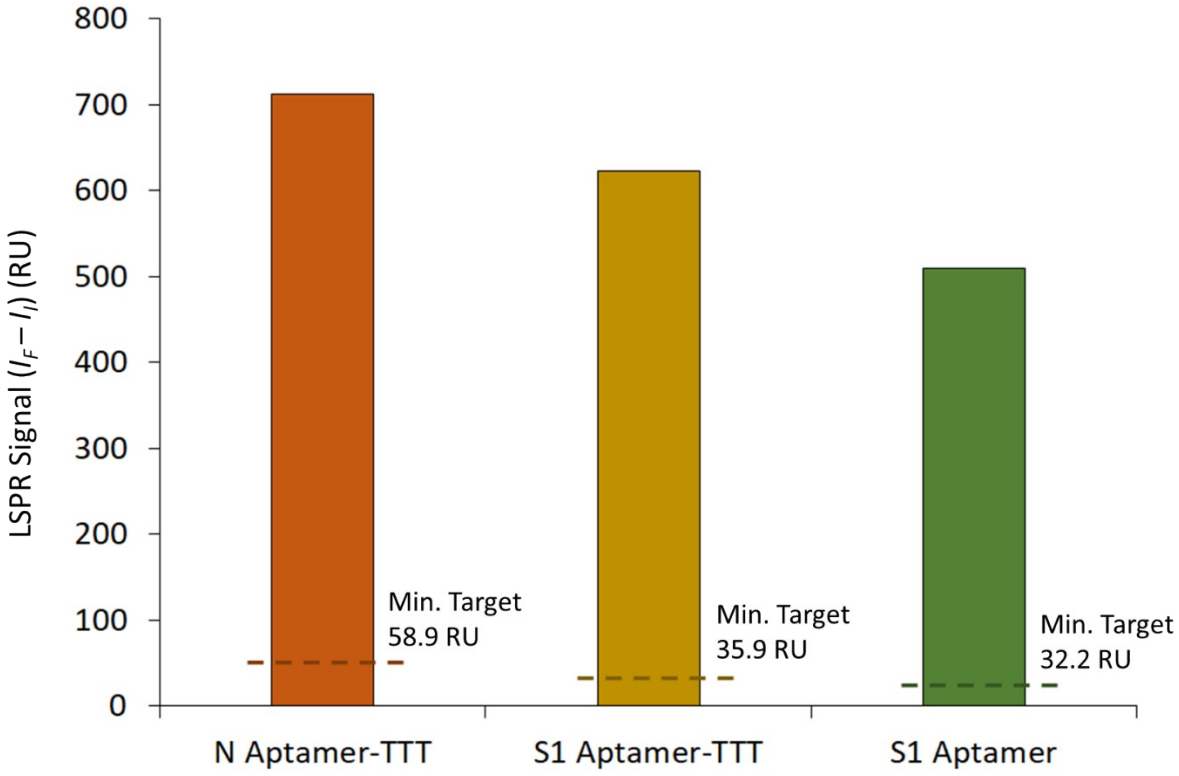

Fig. S4. Signal response after immobilization of N, S1- and S1-T aptamer on individual channels with minimum target values provided for each ([Aptamer] = 50 µg/mL).

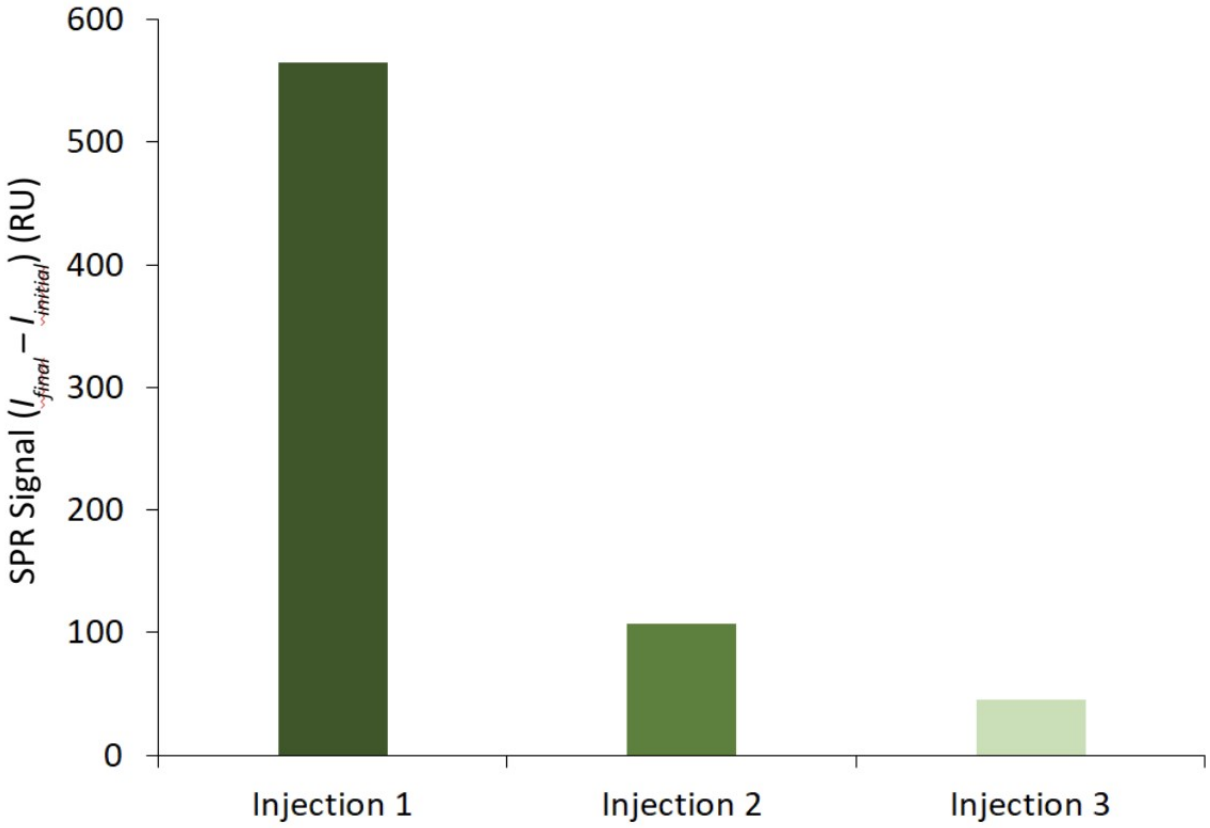

Fig. S5. Signal response after immobilization of S1 aptamer with 3 subsequent aptamer injections ([Aptamer] = 16 μg/mL).

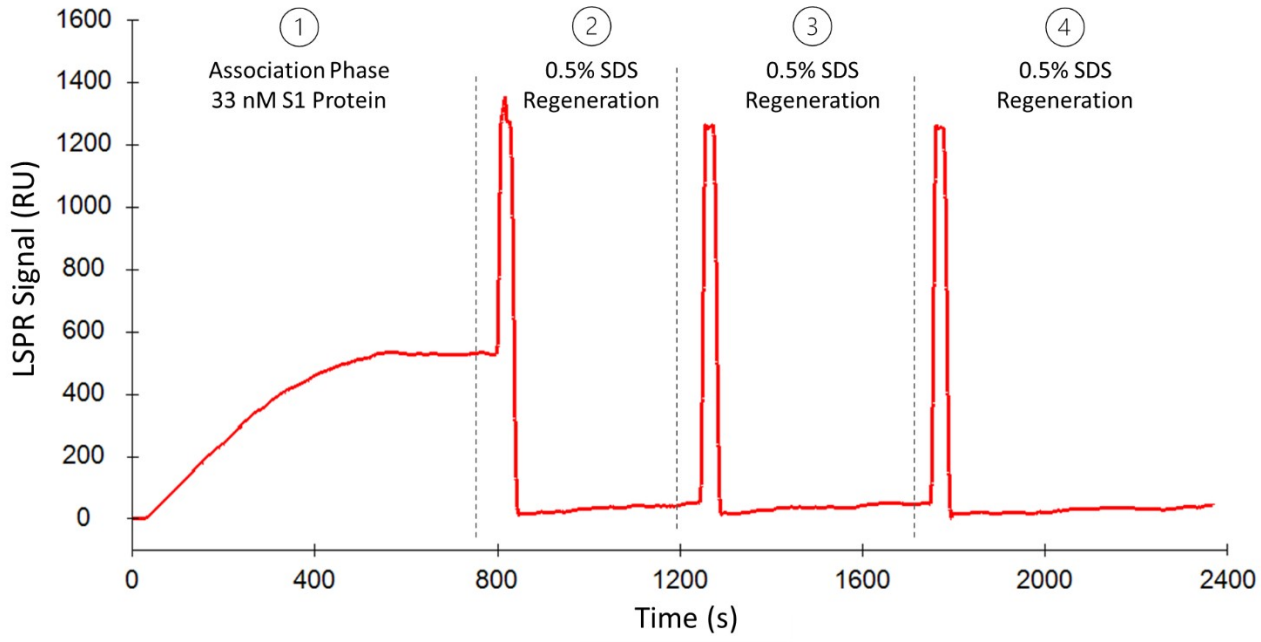

Fig. S6. Representative sensorgram of the S1 protein binding (1) followed by the regeneration optimization with subsequent injections of 0.5% SDS buffer (2-3) ([S1 Protein] = 33 nM (2.5 µg/mL), regeneration flow rate = 150 µL/min).

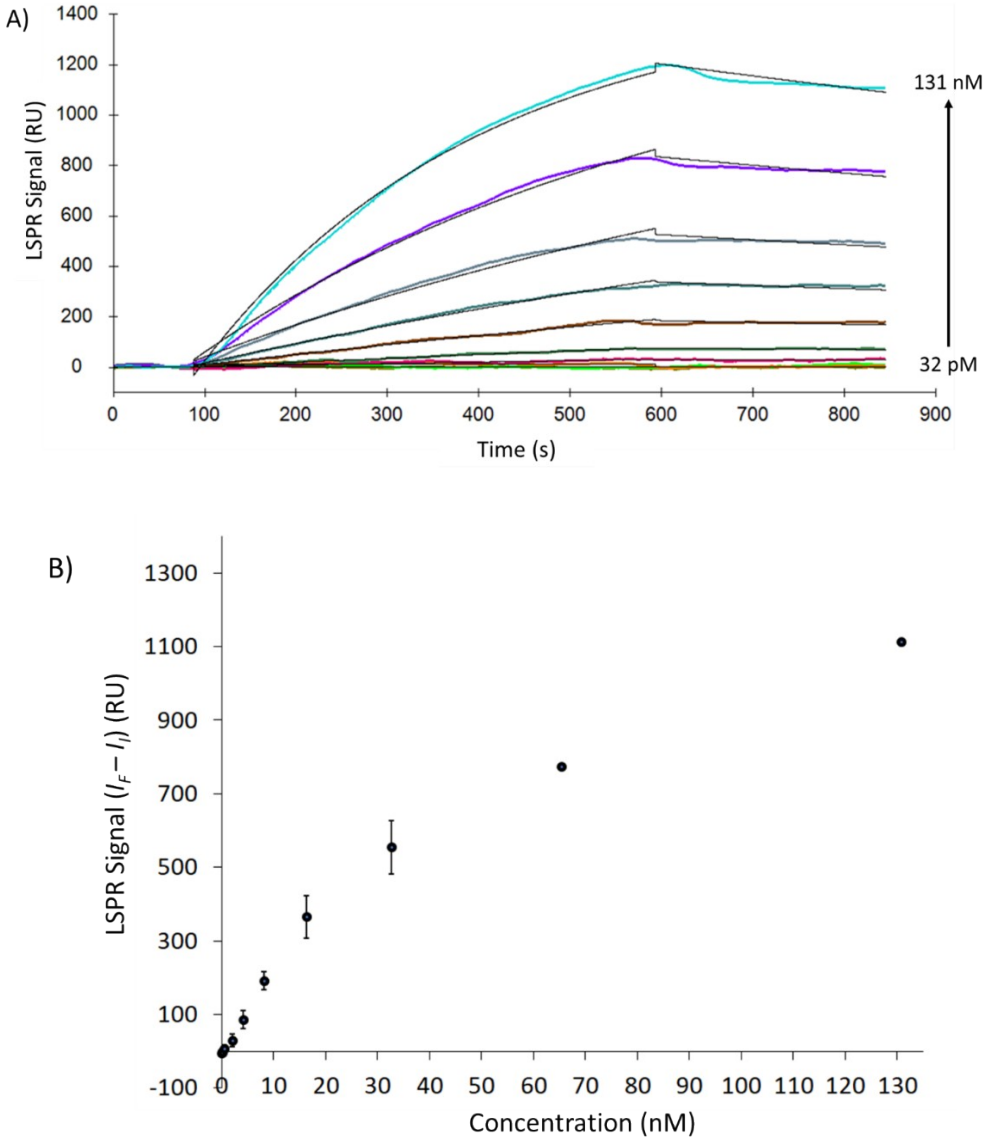

Fig. S7. (A) Representative sensorgram of fitted data (black lines) for varying concentrations of S1 protein (colored lines) using the S1 aptasensor ([S1 protein] = 32 pM to 131 nM). (B) LSPR signal change as a function of S1 protein concentration determined from sensorgram (A) ([S1 protein] = 0 to 131 nM).

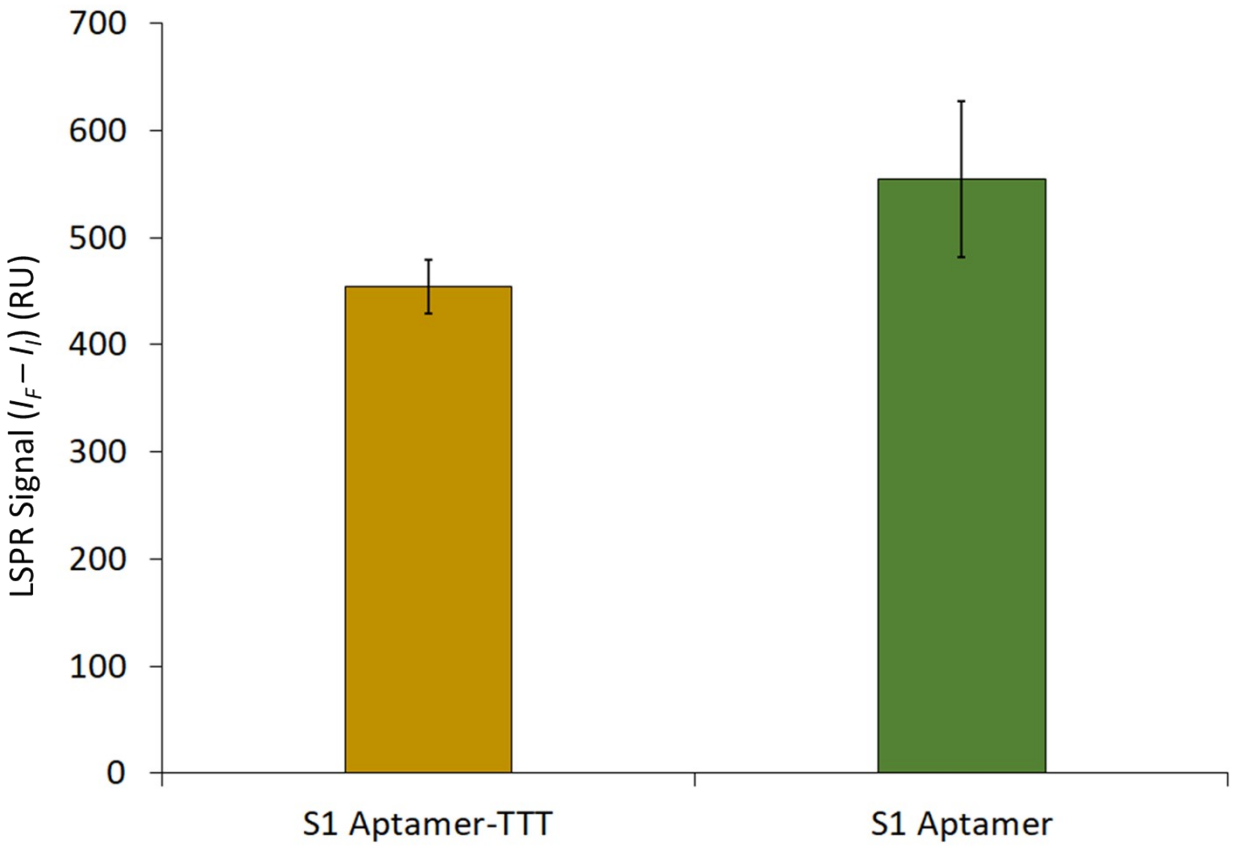

Fig. S8. Comparison of LSPR signal responses for S1 protein using the S1 Aptamer-T or the S1 Aptamer ([Aptamer] = 50 µg/mL, data represent average and error bars of triplicate measurements).

Table S1. Experimental fitting parameters for various SARS-CoV-2 proteins using S1 aptamer

Protein	k_a (1/M*s)	k_d (1/s)	K_D (nM)
SARS-CoV-2 S1	$9.26 \times 10^4 \pm 1.05 \times 10^4$	$3.72 \times 10^{-5} \pm 1.66 \times 10^{-5}$	0.41 ± 0.23
SARS-CoV-2 S2	$2.37 \times 10^5 \pm 3.82 \times 10^4$	$1.51 \times 10^{-3} \pm 4.03 \times 10^{-4}$	6.29 ± 0.69
SARS-CoV-2 RBD	$4.10 \times 10^4 \pm 1.13 \times 10^3$	$6.98 \times 10^{-4} \pm 5.40 \times 10^{-4}$	17.28 ± 13.75

Table S2. Experimental fitting parameters for various SARS-CoV-2 proteins using S1-T aptamer

Protein	k_a (1/M*s)	k_d (1/s)	K_D (nM)
SARS-CoV-2 S1	$6.65 \times 10^4 \pm 1.22 \times 10^4$	$1.01 \times 10^{-4} \pm 5.19 \times 10^{-5}$	1.48 ± 0.7
SARS-CoV-2 S2	$1.86 \times 10^5 \pm 9.39 \times 10^4$	$1.84 \times 10^{-2} \pm 2.91 \times 10^{-2}$	104.13 ± 160.2
SARS-CoV-2 RBD	$8.25 \times 10^4 \pm 3.36 \times 10^3$	$5.02 \times 10^{-4} \pm 8.21 \times 10^{-4}$	5.94 ± 9.7

Table S3. Experimental fitting parameters for various SARS-CoV-2 proteins using N-T aptamer

Protein	k_a (1/M*s)	k_d (1/s)	K_D (nM)
SARS-CoV-2 S1	$2.22 \times 10^5 \pm 9.05 \times 10^4$	$8.44 \times 10^{-4} \pm 3.62 \times 10^{-4}$	3.78 ± 0.07
SARS-CoV-2 S2	$3.92 \times 10^4 \pm 2.55 \times 10^4$	$4.55 \times 10^{-2} \pm 1.08 \times 10^{-2}$	2389.67 ± 2867.2
SARS-CoV-2 RBD	$1.52 \times 10^5 \pm 1.83 \times 10^5$	$7.87 \times 10^{-3} \pm 8.78 \times 10^{-3}$	152.69 ± 167.1

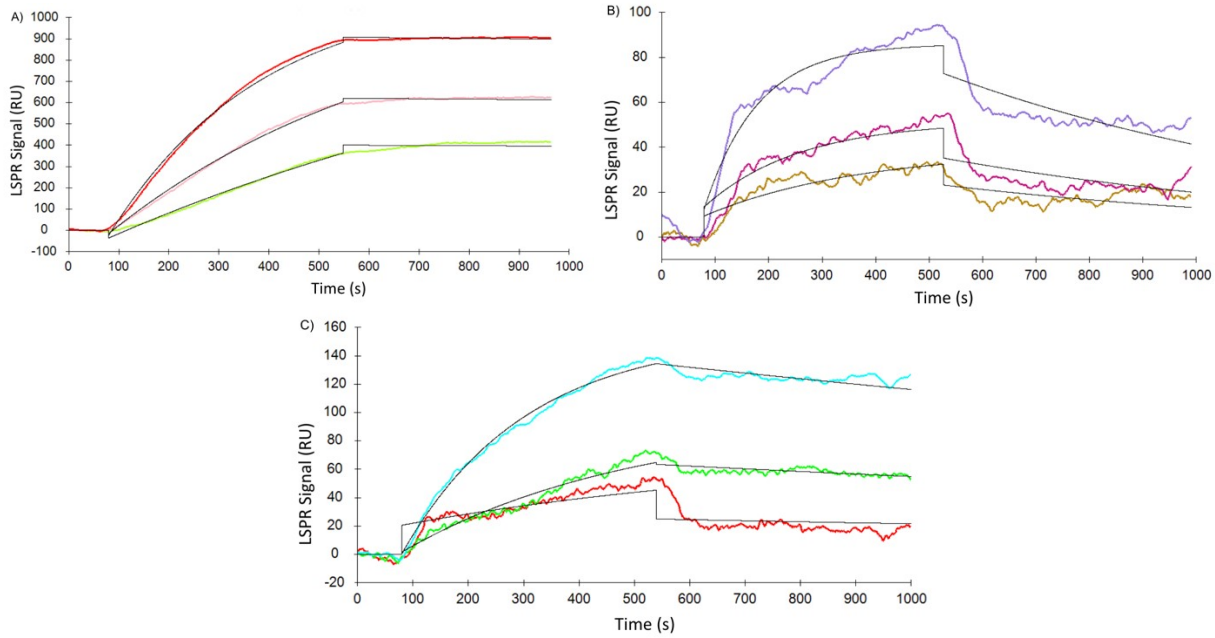

Fig. S9. Representative sensorgrams of fitted data (black lines) for varying concentrations of A) S1 protein (colored lines), B) S2 protein, C) RBD protein using the S1 aptasensor ([protein] = 0 - 2.5 $\mu g/mL$).

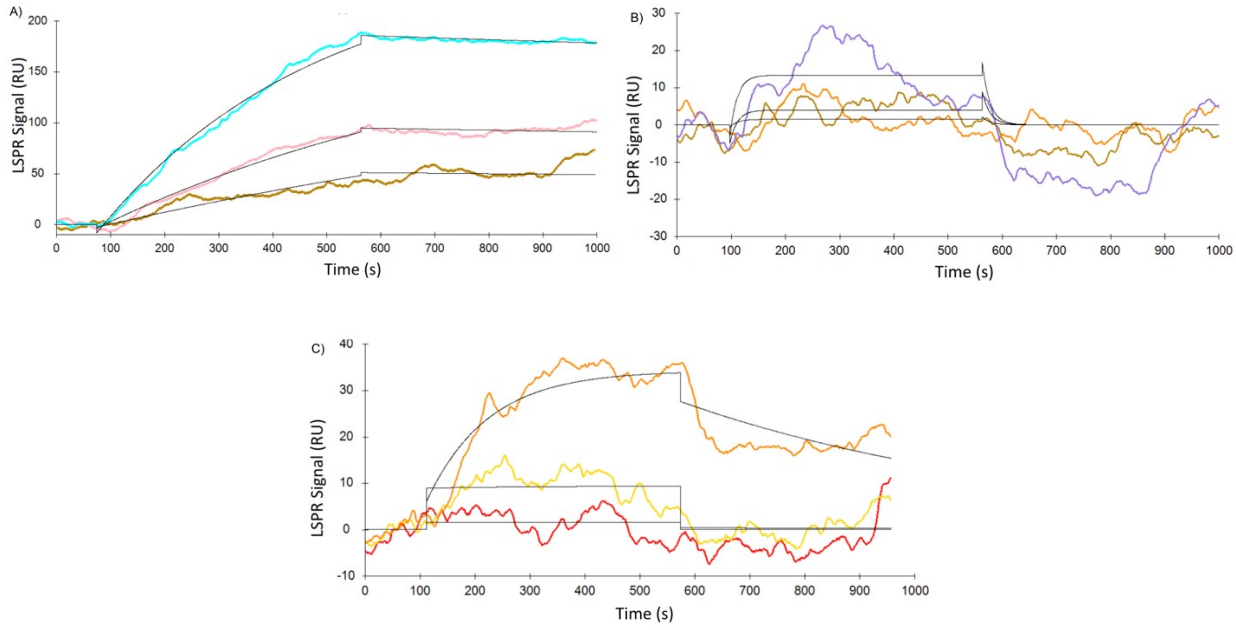

Fig. S10. Representative sensorgrams of fitted data (black lines) for varying concentrations of A) S1 protein (colored lines), B) S2 protein, C) RBD protein using the S1-T aptasensor ([protein] = 0 - 2.5 $\mu g/mL$).

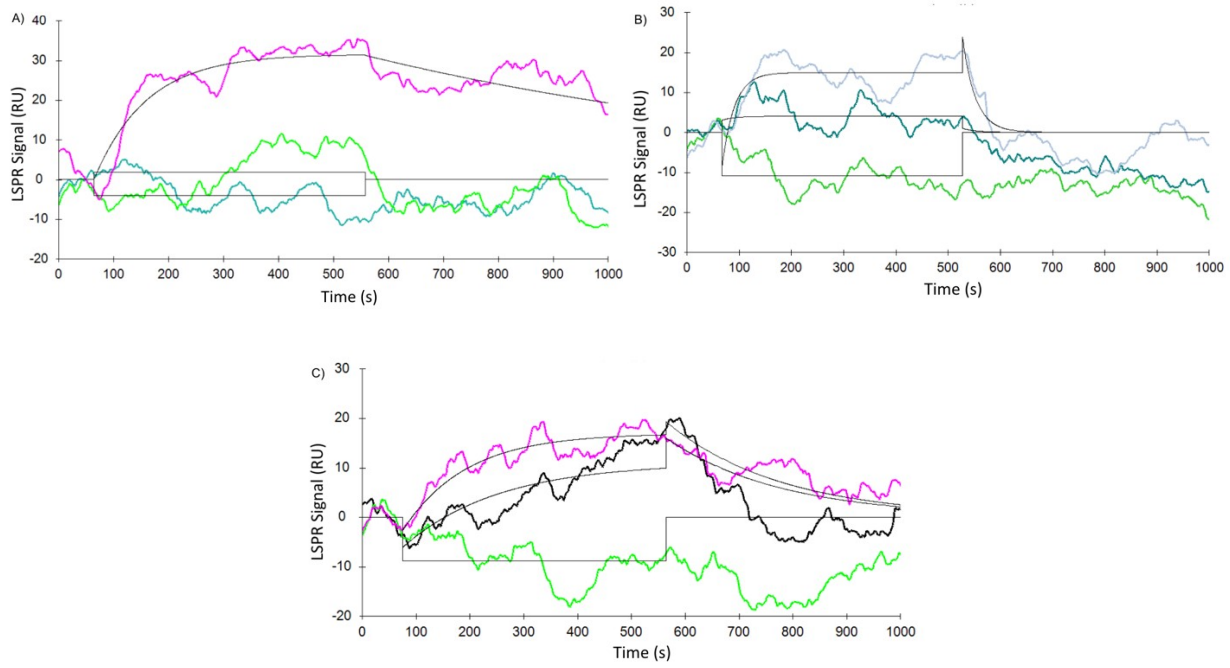

Fig. S11. Representative sensorgrams of fitted data (black lines) for varying concentrations of A) S1 protein (colored lines), B) S2 protein, C) RBD protein using the N-T aptasensor ([protein] = 0 - 2.5 $\mu g/mL$).

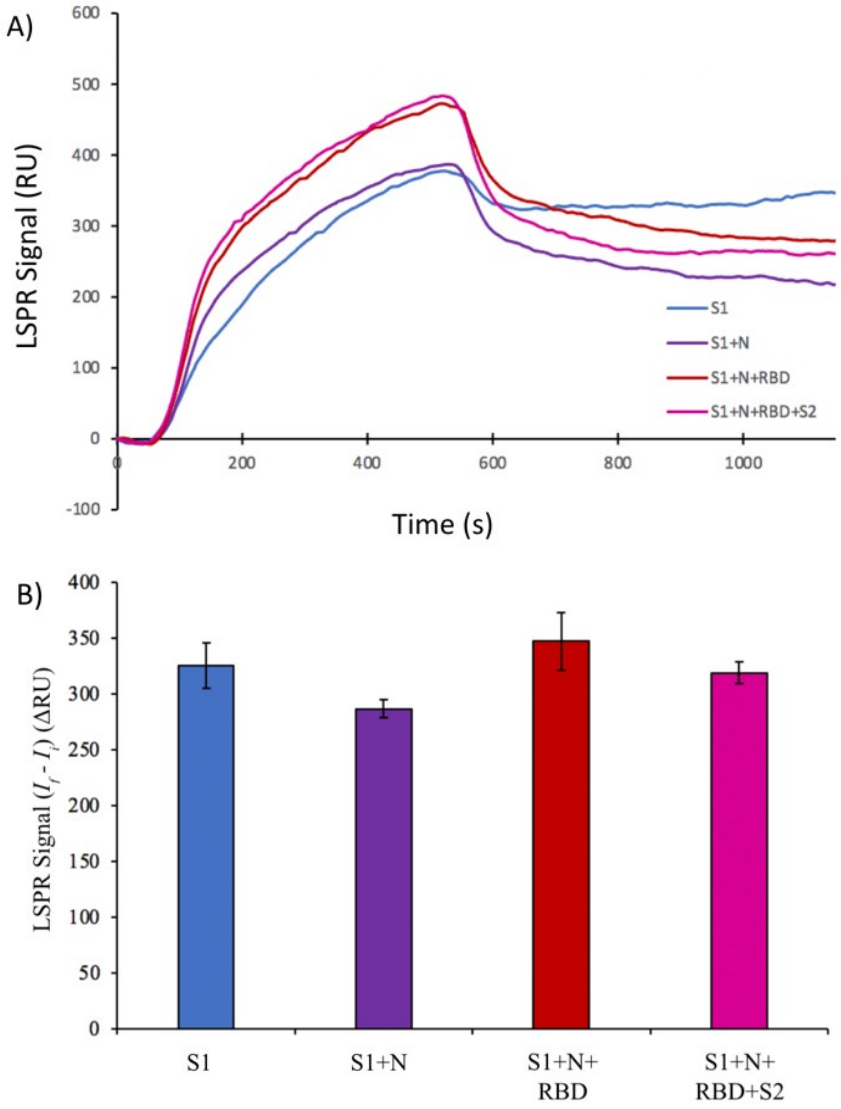

Fig. S12. A) Representative sensorgram and B) plot of LSPR signals at t=610 s, of SARS-CoV-2 S1, and various mixtures; S1+N, S1+N+RBD, S1+N+RBD+S2 ([each protein] = 2.5 $\mu g/mL$).

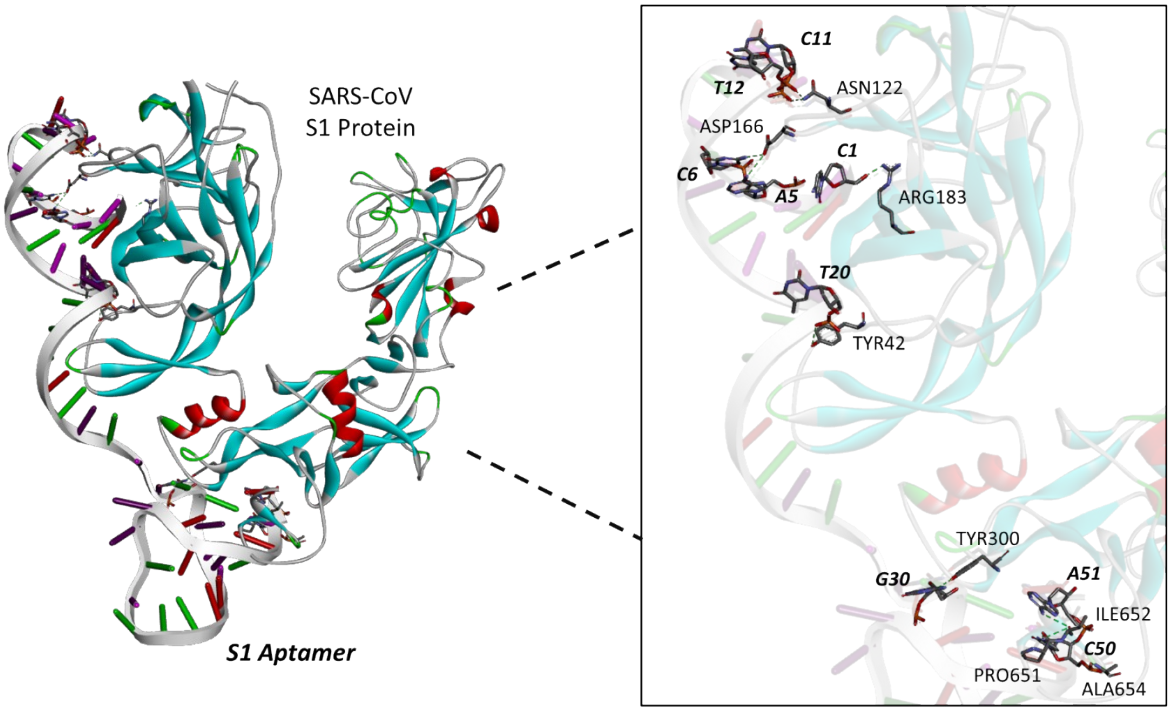

Fig. S13. Molecular docking modelling of SARS-CoV S1 protein (PDB ID: 6CRZ) and S1 aptamer using PatchDock WebServer and visualized with Discovery Studio Visualizer Software.

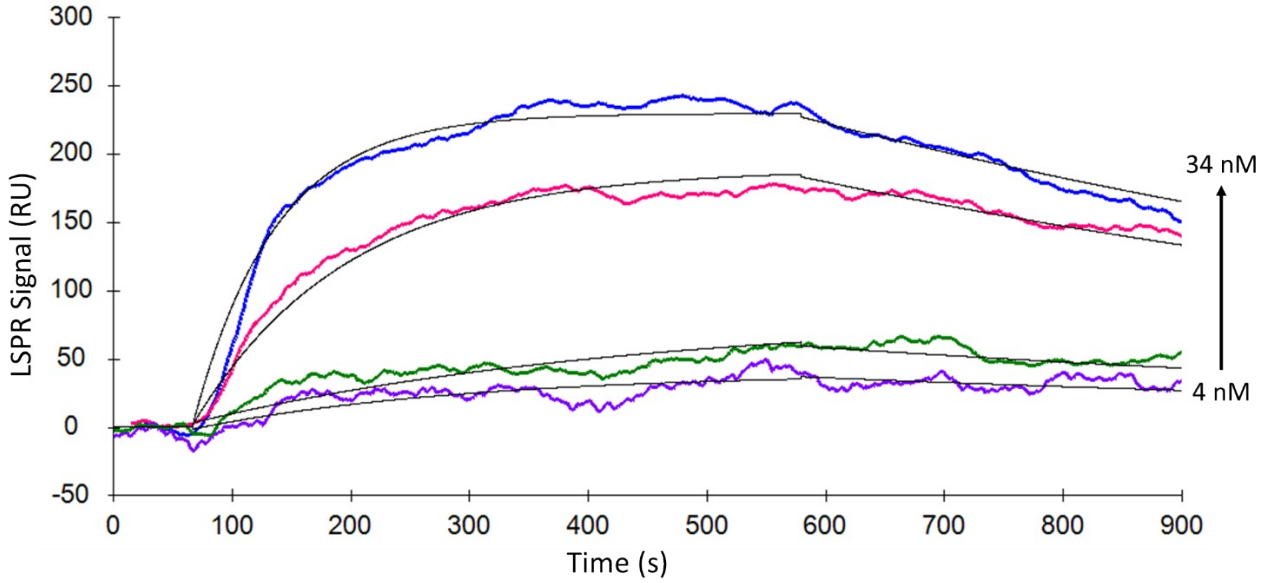

Fig. S14. Representative sensorgram of fitted data (black lines) for various concentrations of SARS-CoV S1 protein (colored lines) using the S1 aptasensor ([SARS CoV S1] = 4.2, 8.4, 16.8 and 33.6 nM (0.3 to 2.5 μg/mL)).

Table S4. Curve fitting data for SARS-CoV S1 protein with the S1 aptasensor. The 1:1 binding model was used in the TraceDrawer Software to obtain the k_a, k_d, and K_D values. Values represent triplicate measurements.

	k_a (1/M*s)	k_d (1/s)	K_D (nM)
SARS-CoV	$3.99 \times 10^5 \pm 2.65 \times 10^4$	$1.17 \times 10^{-3} \pm 2.10 \times 10^{-4}$	2.9 ± 0.4

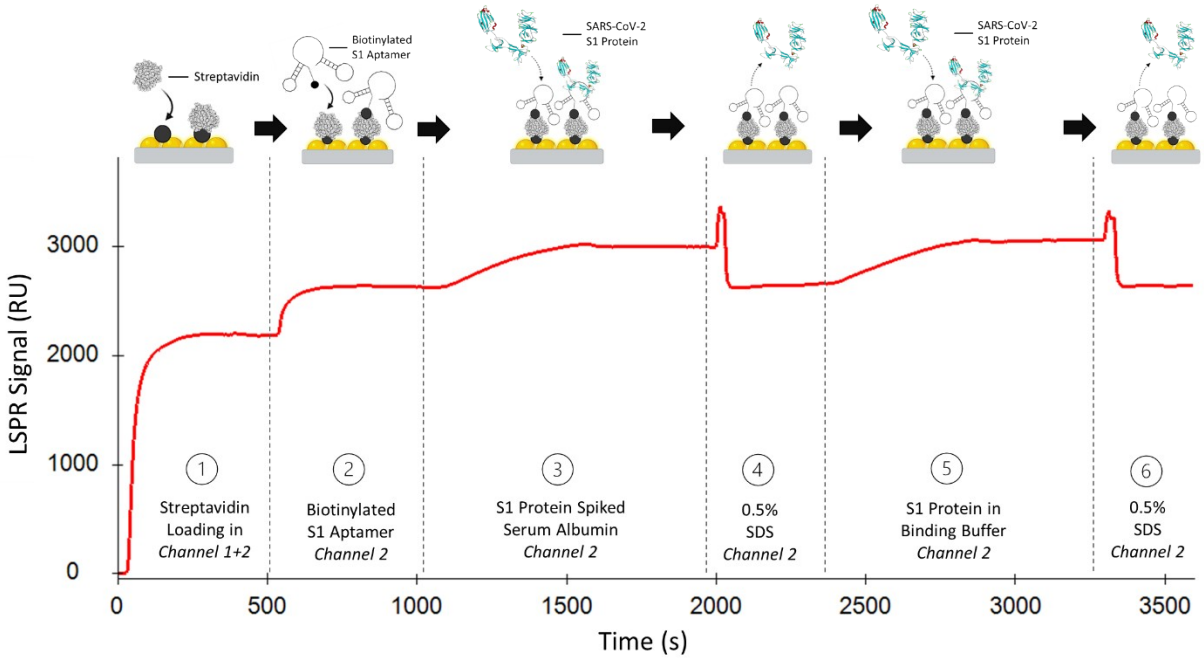

Fig. S15. Sensorgram showing sensor fabrication and performance: (1) streptavidin protein loading to the biotin-gold chip (flow rate = 20 µL/min); followed by (2) immobilization of S1 aptamer (flow rate = 20 µL/min); then (3) injection of 1000x diluted serum albumin spiked with S1 protein sample (flow rate = 10 µL/min); (4) surface regeneration with 0.5% SDS (flow rate = 150 µL/min); (5) injection of S1 protein in buffer (flow rate = 20 µL/min); (6) surface regeneration with 0.5% SDS (flow rate = 150 µL/min) ([streptavidin] = 0.5 µM; [S1 aptamer] = 50 µg/mL; [S1 protein] = 0.25 µg/mL).

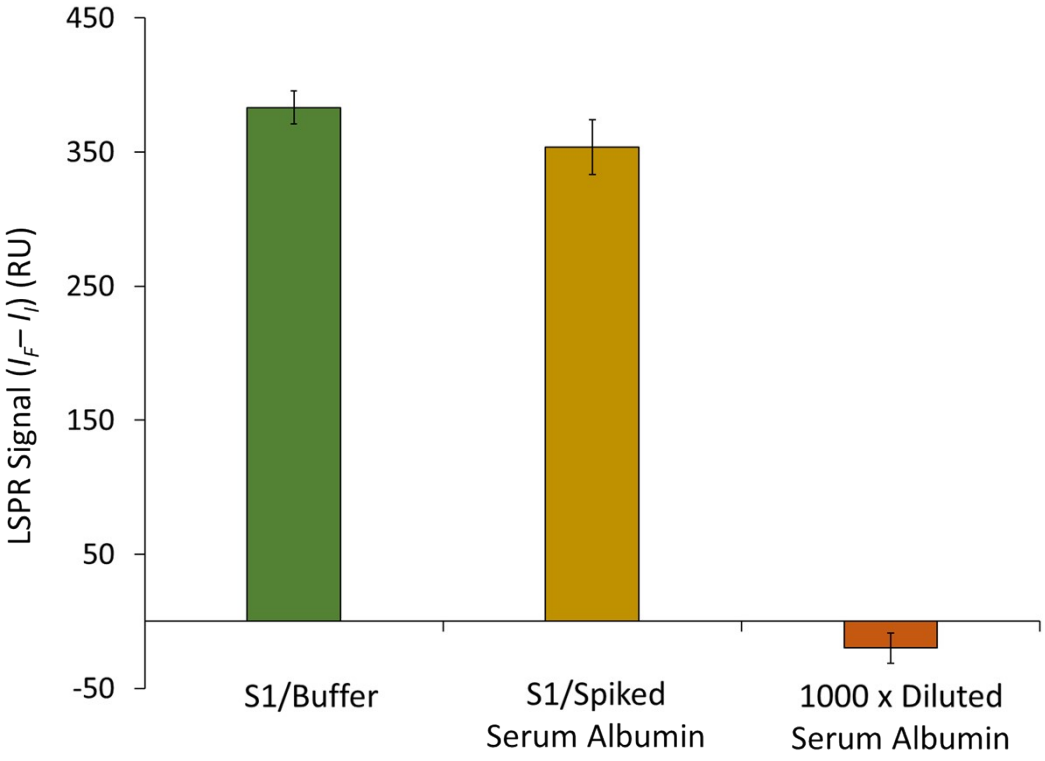

Fig. S16. Plot of LSPR signal change for SARS-CoV-2 S1 protein in spiked buffer solution compared to the 1000x diluted spiked serum albumin solution with or without S1 protein ([S1 Protein] = 2.5 μg/mL; signals represent average of triplicate measurements with standard deviations shown as error bars).

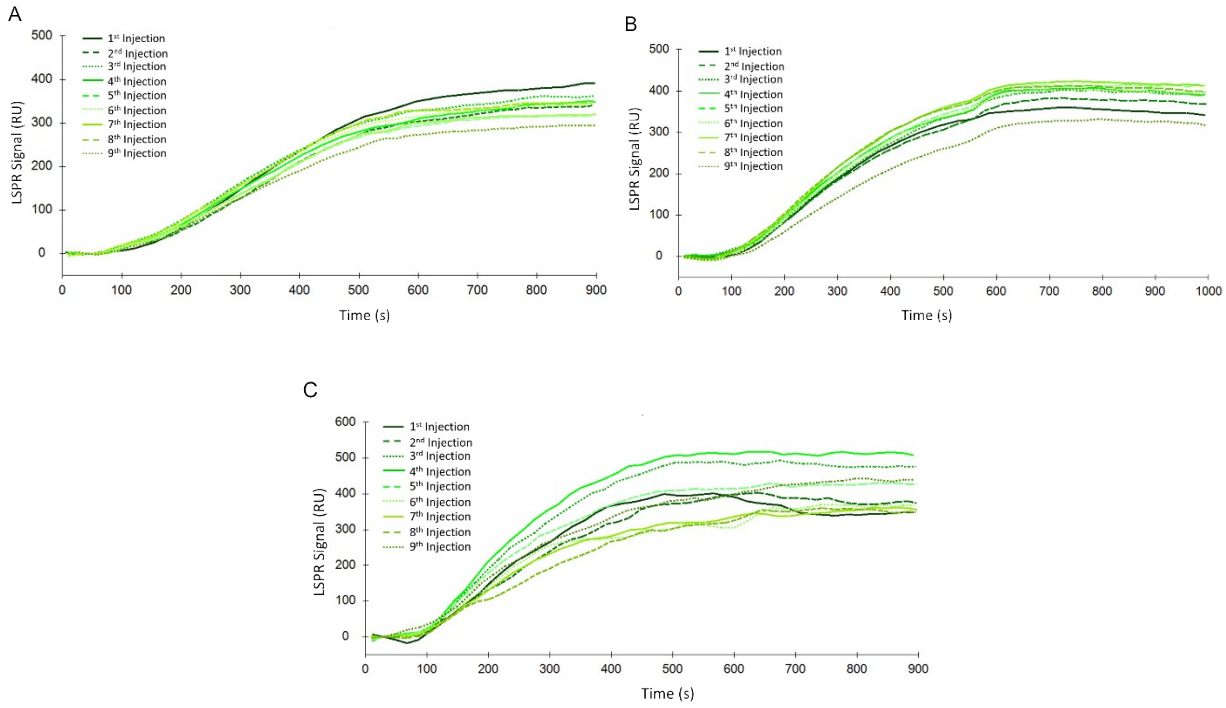

Fig. S17. Representative sensorgrams showing repeatability of the SARS-CoV-2 S1 protein injections using offline prepared S1 aptamer sensor after specific storage time: (A) at t = 0 days; (B) t = 10 days; (C) t = 24 days (all S1 protein injections were followed by regeneration using 0.5% SDS buffer; [S1 Protein] = 1 μg/mL).

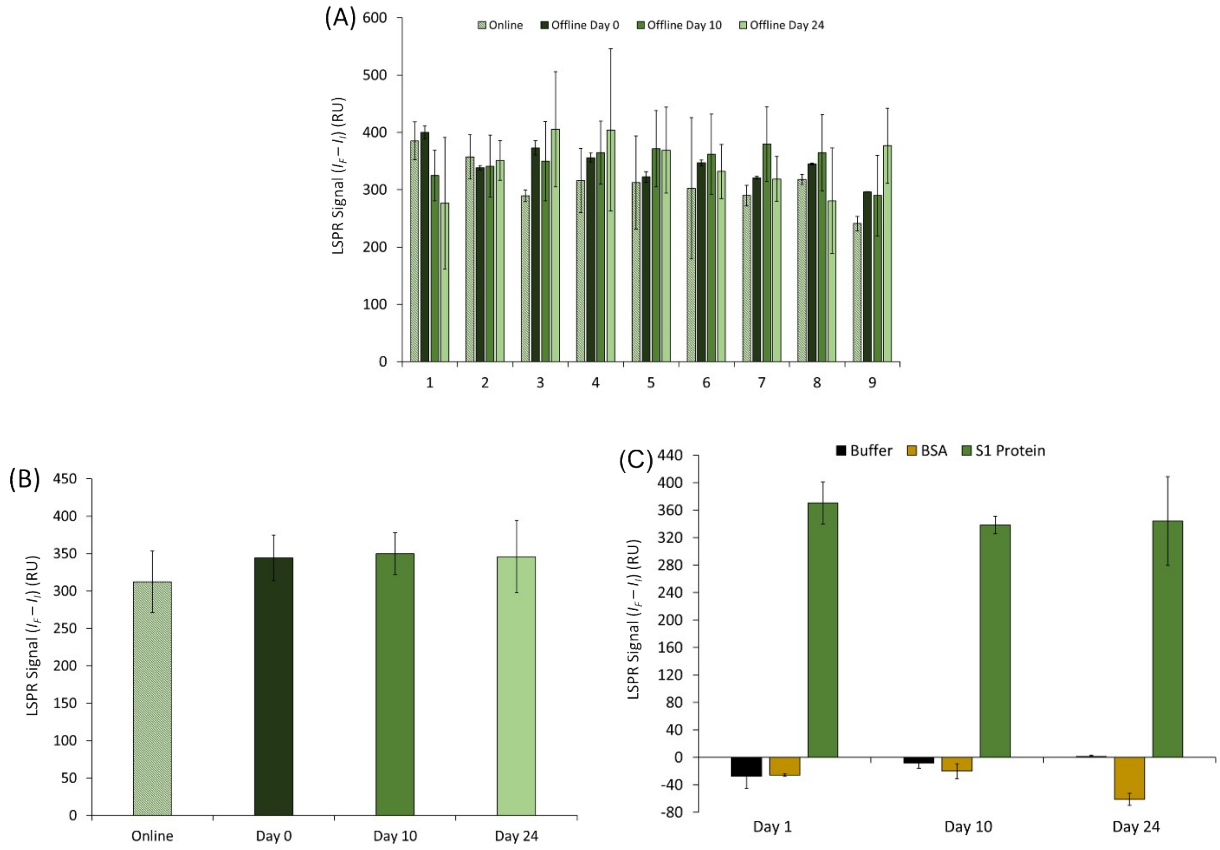

Fig. S18. (A) LSPR signals for 9 repeat injections of S1 protein using the online sensor or offline-prepared sensor with the shelf-life = 0, 10 or 24 days (data represent average of duplicate measurements with error bars showing standard deviation); (B) Average LSPR signals for the online or offline-prepared sensors used for 9 repeat injections of S1 protein on 0, 10 or 24 days; (C) Average LSPR signals for the offline-prepared sensor used for triplicate injections of blank buffer, BSA and S1 Protein ([S1 protein] = [BSA] = 1 μg/mL; regeneration conditions = 0.5% SDS).