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Supplementary Materials for

The SARS-CoV-2 spike protein binds and modulates estrogen receptors

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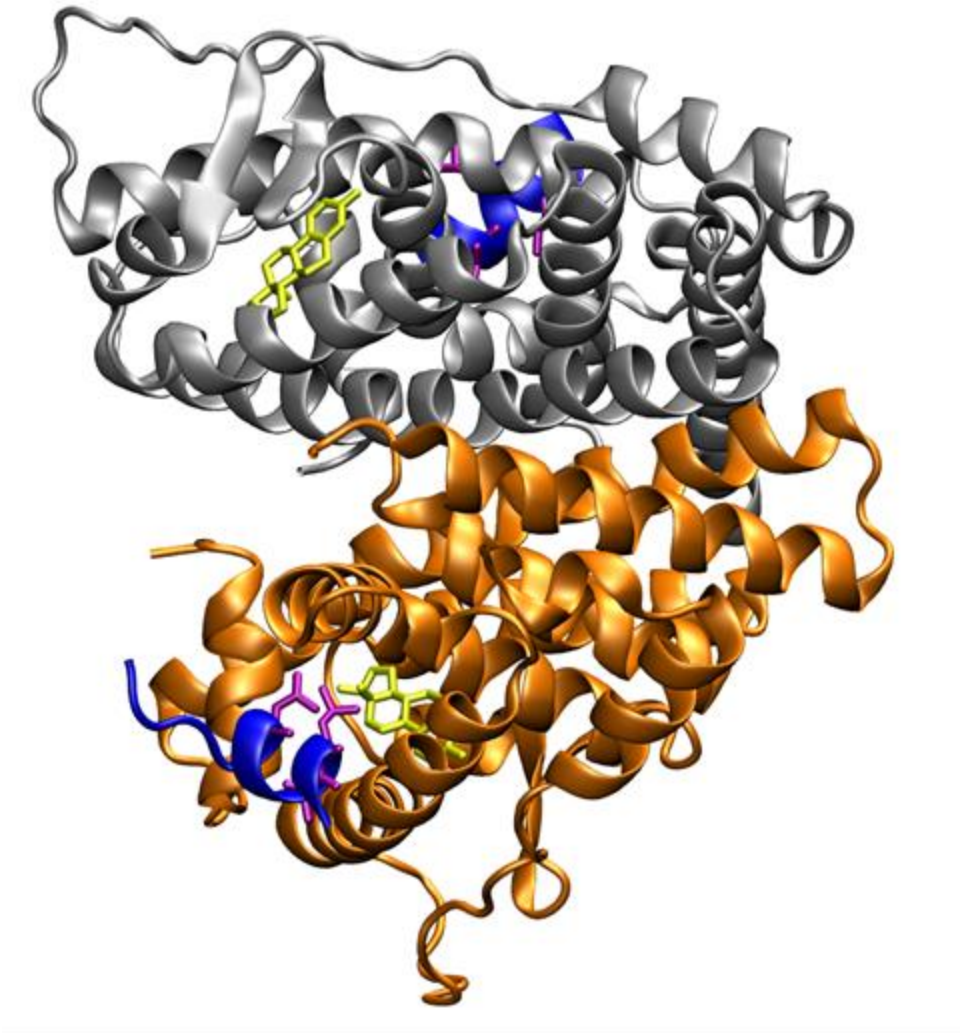

Fig. S1.

Crystal structure of ERα dimer in interaction with a NCOA1 fragment containing the LXD nuclear receptor coregulator (NRC) motif (pdb id 3UUD). The AF2 region in ERα (residues 302-552) is shown in silver and orange colors for the two monomers. The NCOA1 fragment containing the LHKLL (residues 690-694) is represented in blue color. Lateral chains of the three leucine residues are also represented in purple color. Estradiol is shown in yellow color.

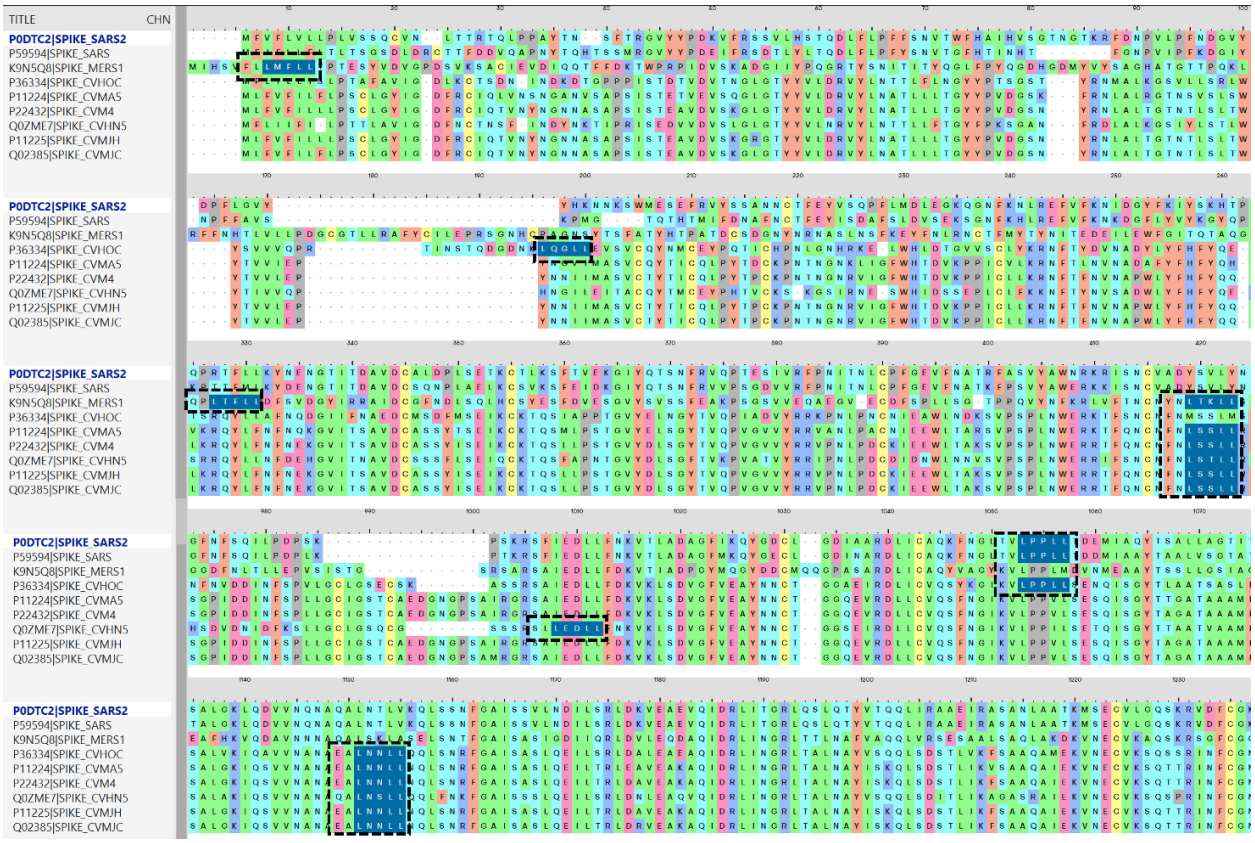

Fig. S2.

S protein sequence alignment and LDX motif identification. Amino acid patterns related to the LDX-like motif, conserved among different species, are highlighted in blue, and surrounded by black dotted boxes.

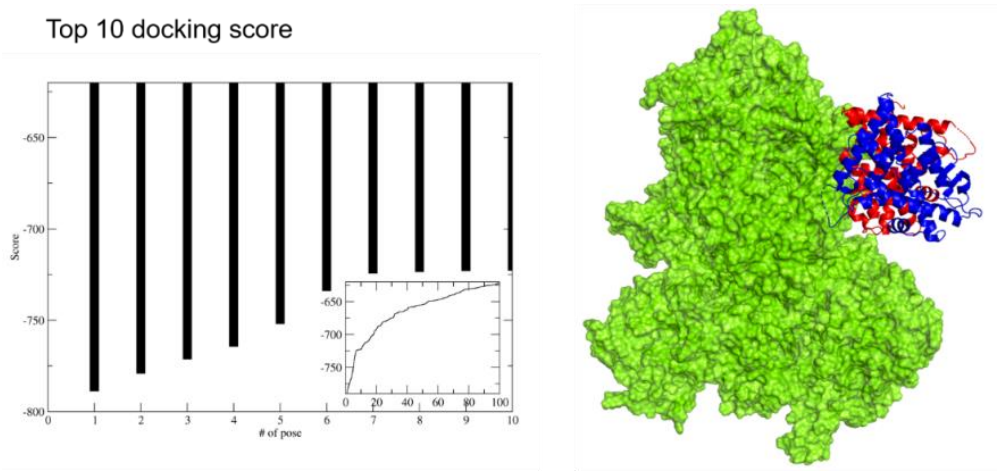

Fig. S3.

Binding hypothesis of ER to S by guided docking study. On the left side the plot reporting the top 10 docking scores and the score distribution along the 100 structures generated by the docking software (the smaller plot below). On the right side, the best 3D docking hypothesis. The ER dimer is in blue and red cartoon, S surface is shown in green.

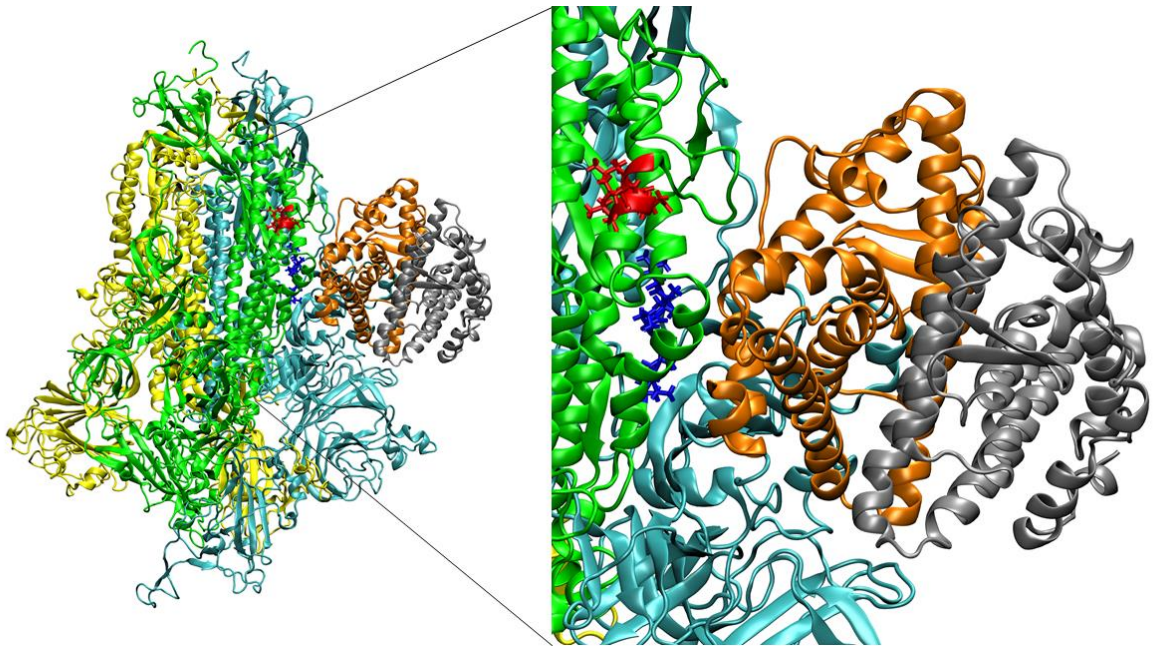

Fig. S4.

Snapshot of molecular dynamics frame for the S-ERα complex. The simulation shows the formation of a strong interaction surface between ER and S, in the region of the two conserved LXD motifs. The average number of protein-protein direct hydrogen bonds per timeframe is more than 14 after only few hundred nanoseconds.

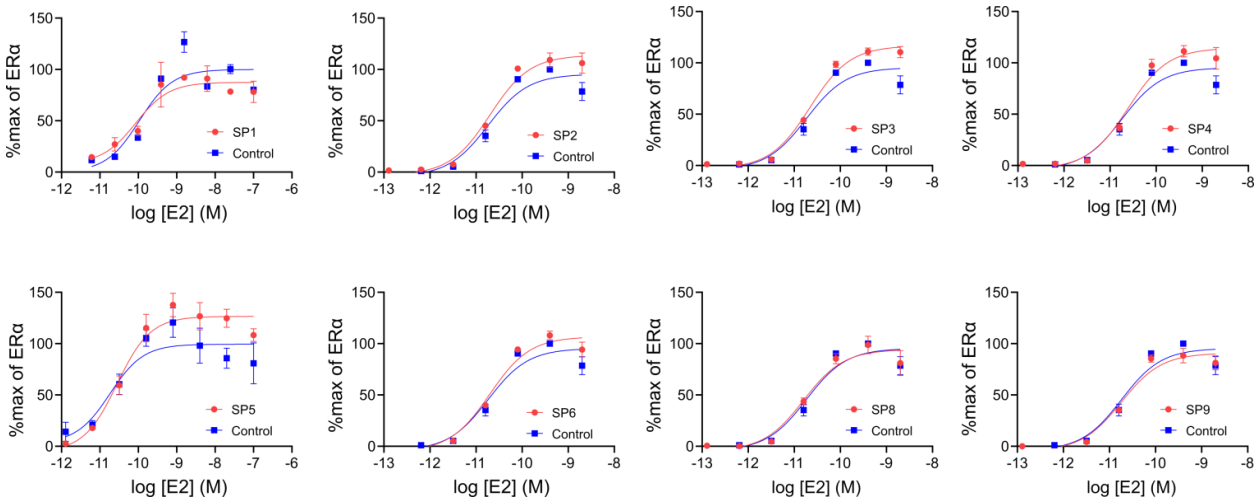

Fig. S5.

Effect of S peptides on ERα-mediated transcriptional activation. Data are shown as mean ± SEM.

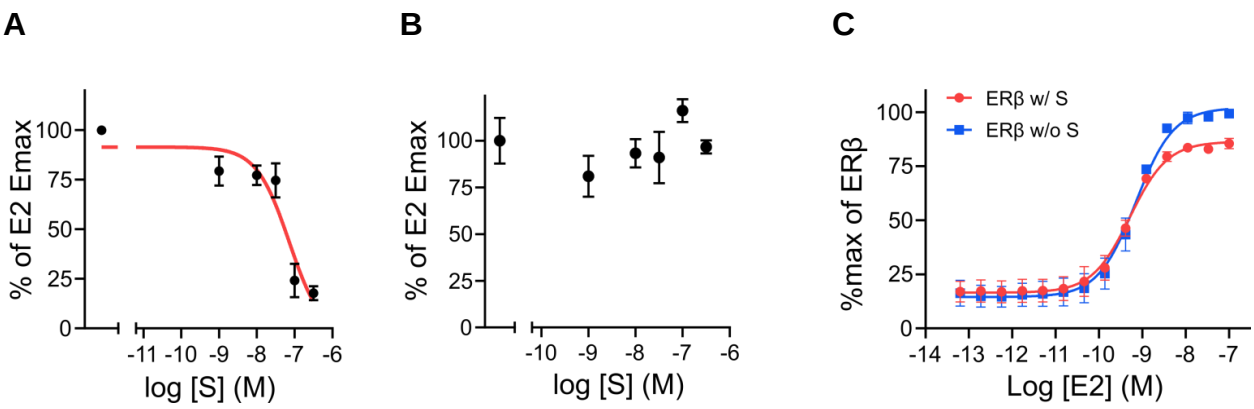

Fig. S6.

S trimer (A), but not S-RBD (B), inhibits E2-induced ERα DNA binding in MCF-7 nuclear extracts. Transfected S inhibits E2-stimulated ERβ transcriptional activity (C). Data are shown as mean ± SEM.

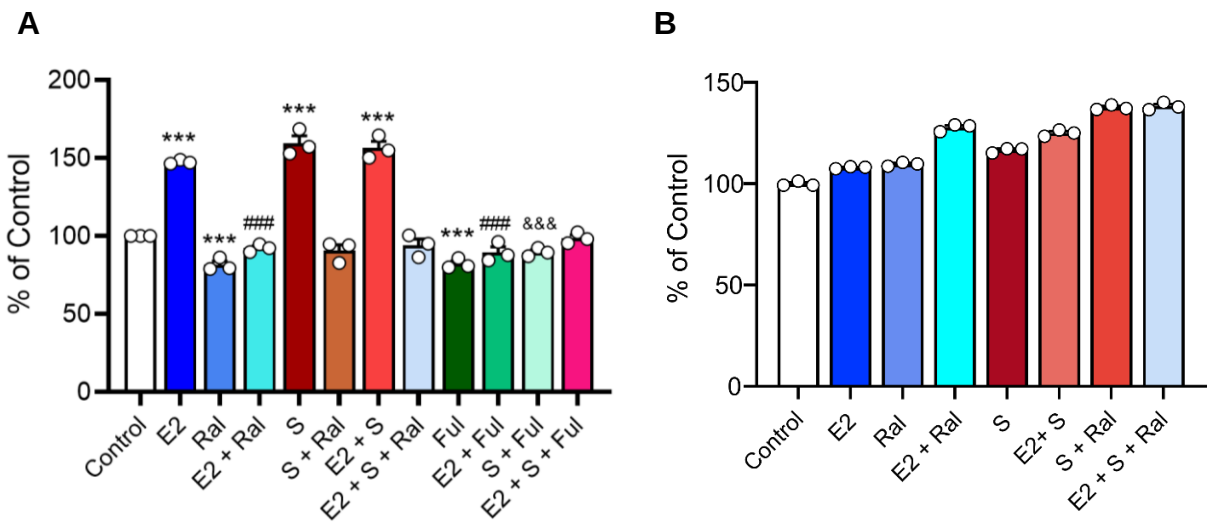

Fig. S7.

(A) Fulvestrant (ICI 182,780) (1 µM) and raloxifene (2 µM) blocked MCF-7 proliferation induced by S (10 ng/mL or E2 (1 nM), (***P*<0.01, ****P*<0.001 versus control; $^{\#\#\#}P$<0.001 versus E2; $^{\&\&\&}P$<0.001 versus S; one-way ANOVA with post hoc Tukey test). Results are mean ± SD of 3 independent experiments. (B) BrdU Proliferation assay for MDA-MB-231 cell line. All the treatments alone or in combination do not significantly affect cell proliferation. The assay was performed in triplicate. Results are shown as mean ± SD of one representative experiment.

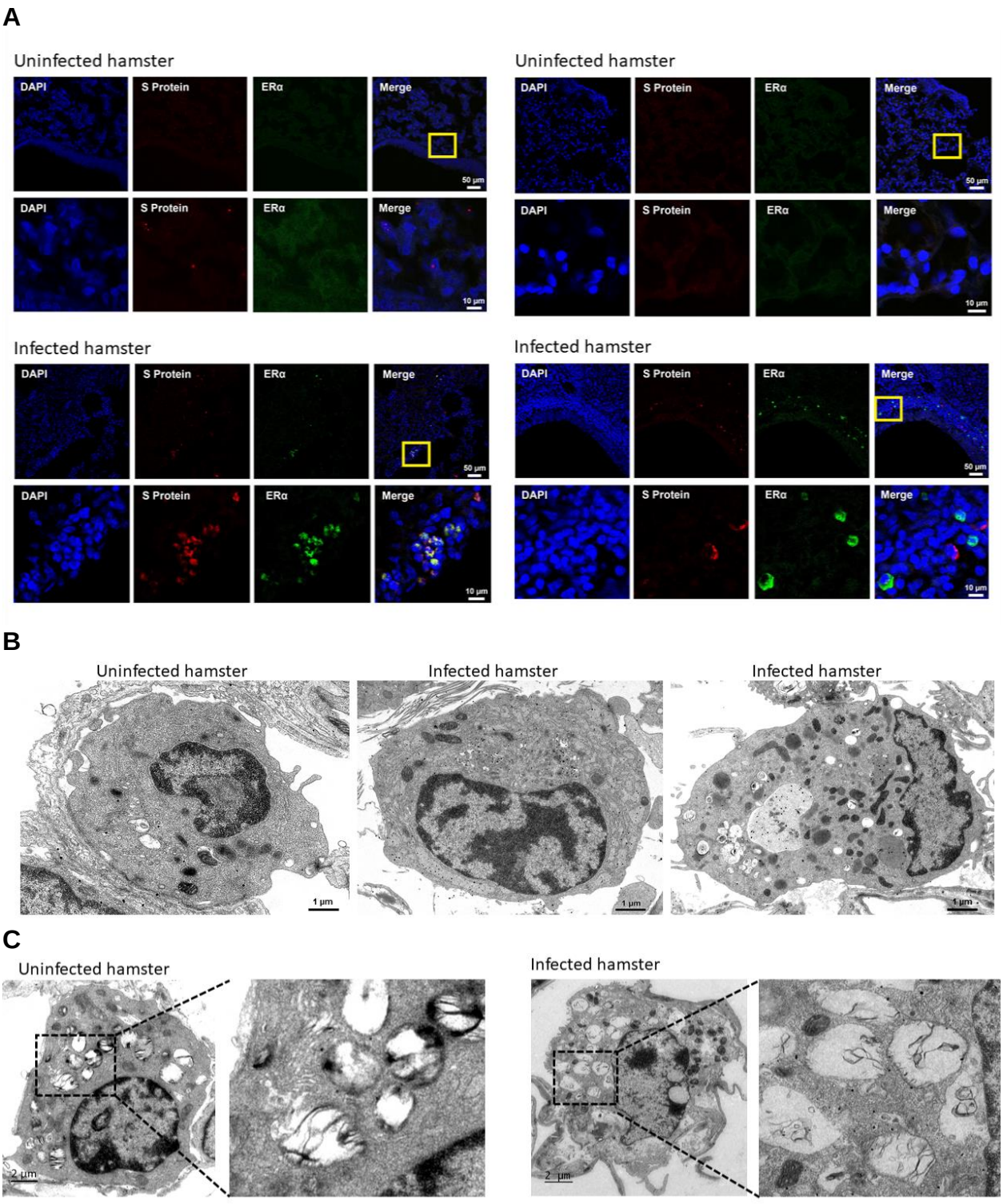

Fig. S8.
SARS-CoV-2 infection induces cytoplasmic expression of ERα protein in hamsters. Immunohistochemistry showing colocalization of S and ERα immunoreactivity in infected lung hamster (A). Immunogold EM showing ERα-bound gold nanoparticles in hamster alveolar macrophages (B) and pulmonary type II cells (C).

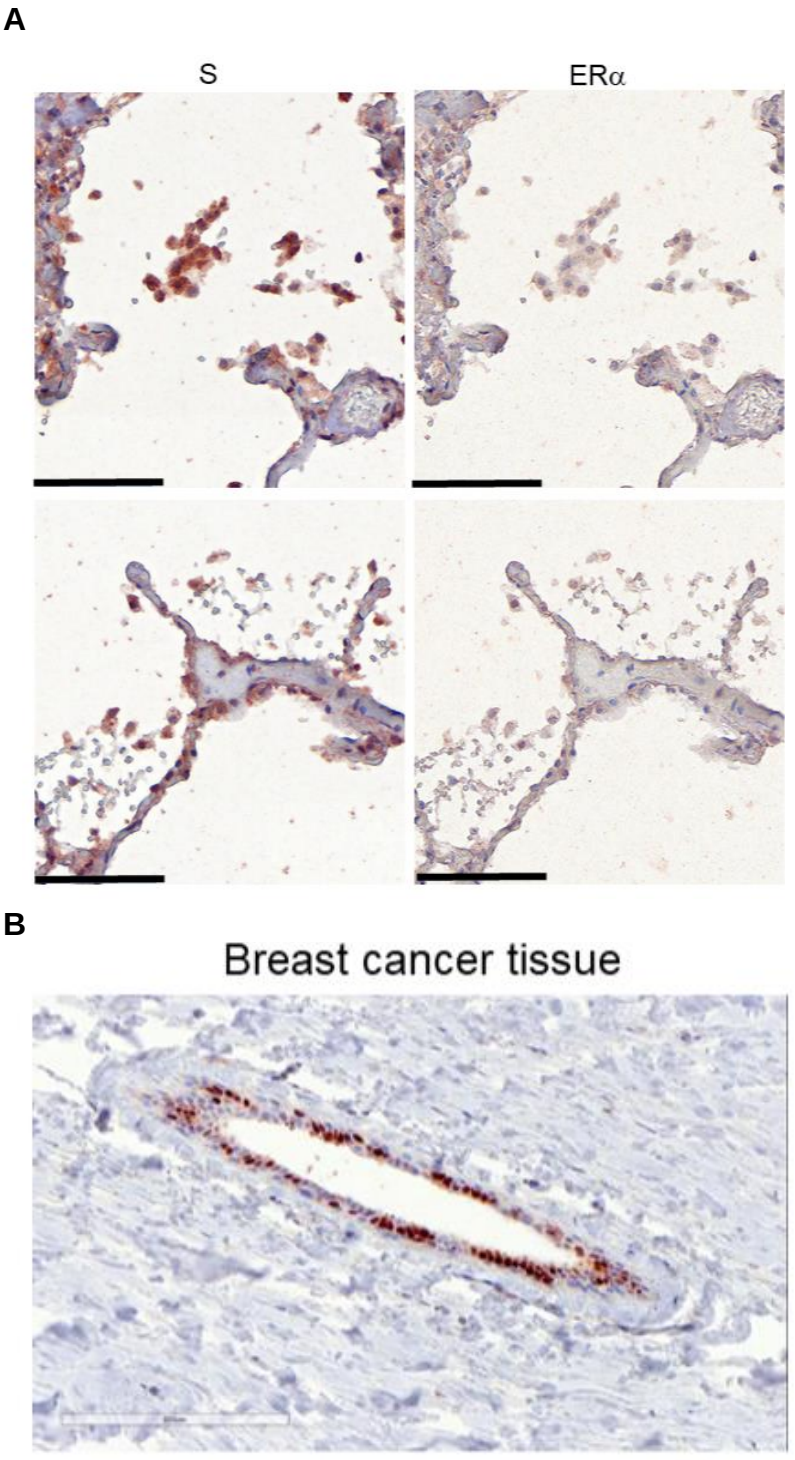

Fig. S9.

S and ERα immunostaining in SARS-CoV-2-infected human lung showing S-ERα colocalization. Scale bar 100 nm (A). Immunohistochemistry showing ERα immunoreactivity in breast cancer tissue. Scale bar 200 nm (B).

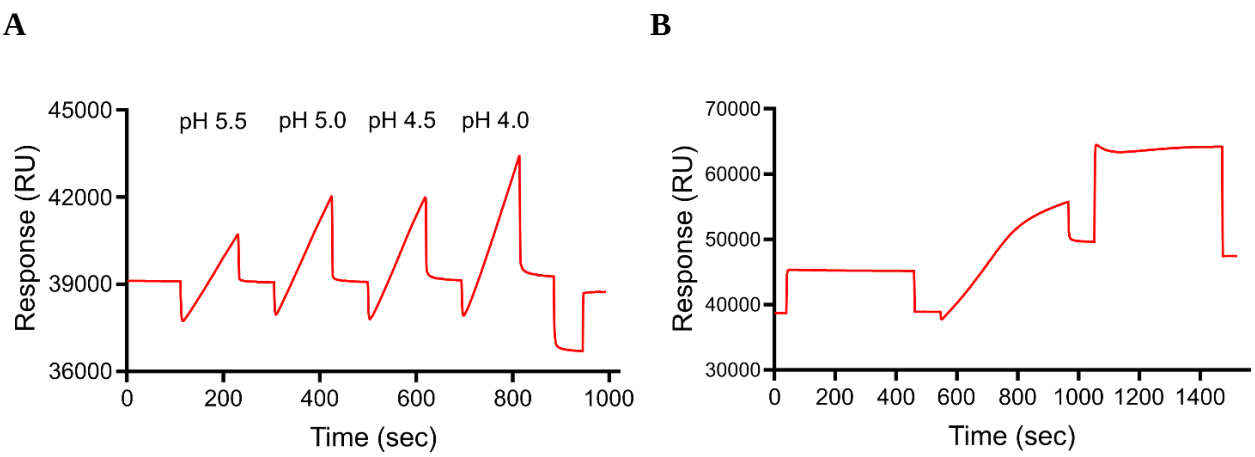

Fig. S10.

(A) The sensorgram shows the effect of buffer pH on immobilization of S to the chip surface. (B) Sensorgram illustrating the immobilized amount of S at pH 4.

Table S1

Database ID	Mean signal used	Mean Z-Factor	Mean Z-Score	Mean CI P-Value	Mean CV	Description
NP_001019799.1	2553.66	0.44708	0.829635	0.043687	0.04742	NRP1 / Neuropilin-1 Protein
BC020803.1	6186.558	0.57108	7.054942	0.002179	0.09359	Developmentally regulated GTP binding protein 1 (DRG1)
BC033792.1	2805.59	0.671277	2.627567	0.013592	0.04339	Tumor protein D52-like 3 (TPD52L3)
NM_016059.3	3130.755	0.641917	3.062002	0.010238	0.024877	Peptidylprolyl isomerase (cyclophilin)-like 1 (PPIL1)
NM_016508.2	5234.063	0.594467	5.65815	0.003209	0.081497	Cyclin-dependent kinase-like 3
NM_022140.2	2422.185	0.554883	1.70084	0.043508	0.058637	Band 4.1-like protein 4A
NM_145010.1	8754.735	0.656837	9.09587	0.001407	0.074957	chromosome 10 open reading frame 63 (C10orf63)

The data was obtained from three independent Protoarrays

Table S1. Proteins identified by protein-protein interaction with [^{125}I]S using the Protoarray platform.

Table S2

INTERACTION PROTEINS	SCORE
ERα > NCOA1	0.997
ERα > NCOA2	0.995
ERβ > NCOA1	0.994
ERβ > NCOA2	0.971
ERβ > NCOA3	0.976

Table S2. Scores showing strong interaction between ERα/β and NCOAs.

Table S3.

Virus Species	UNIPROT CODE
Severe acute respiratory syndrome coronavirus 2 (2019-nCoV) (SARS-CoV-2)	SPIKE_SARS2
Severe acute respiratory syndrome coronavirus (SARS-CoV)	SPIKE_SARS
Middle East respiratory syndrome-related coronavirus (MERS-CoV)	SPIKE_MERS1
Human coronavirus HKU1 (isolate N5) (HCoV-HKU1)	SPIKE_CVHN5
Human coronavirus OC43 (HCoV-OC43)	SPIKE_CVHOC
Murine coronavirus (strain A59) (MHV-A59) (Murine hepatitis virus)	SPIKE_CVMA5
Murine coronavirus (strain 4) (MHV-4) (Murine hepatitis virus)	SPIKE_CVM4
Murine coronavirus (strain JHM) (MHV-JHM) (Murine hepatitis virus)	SPIKE_CVMJH
Murine coronavirus (strain JHMV / variant CL-2) (MHV) (Murine hepatitis virus)	SPIKE_CVMJC

Table S3. Coronavirus' S proteins used for sequence and amino acids pattern analysis.