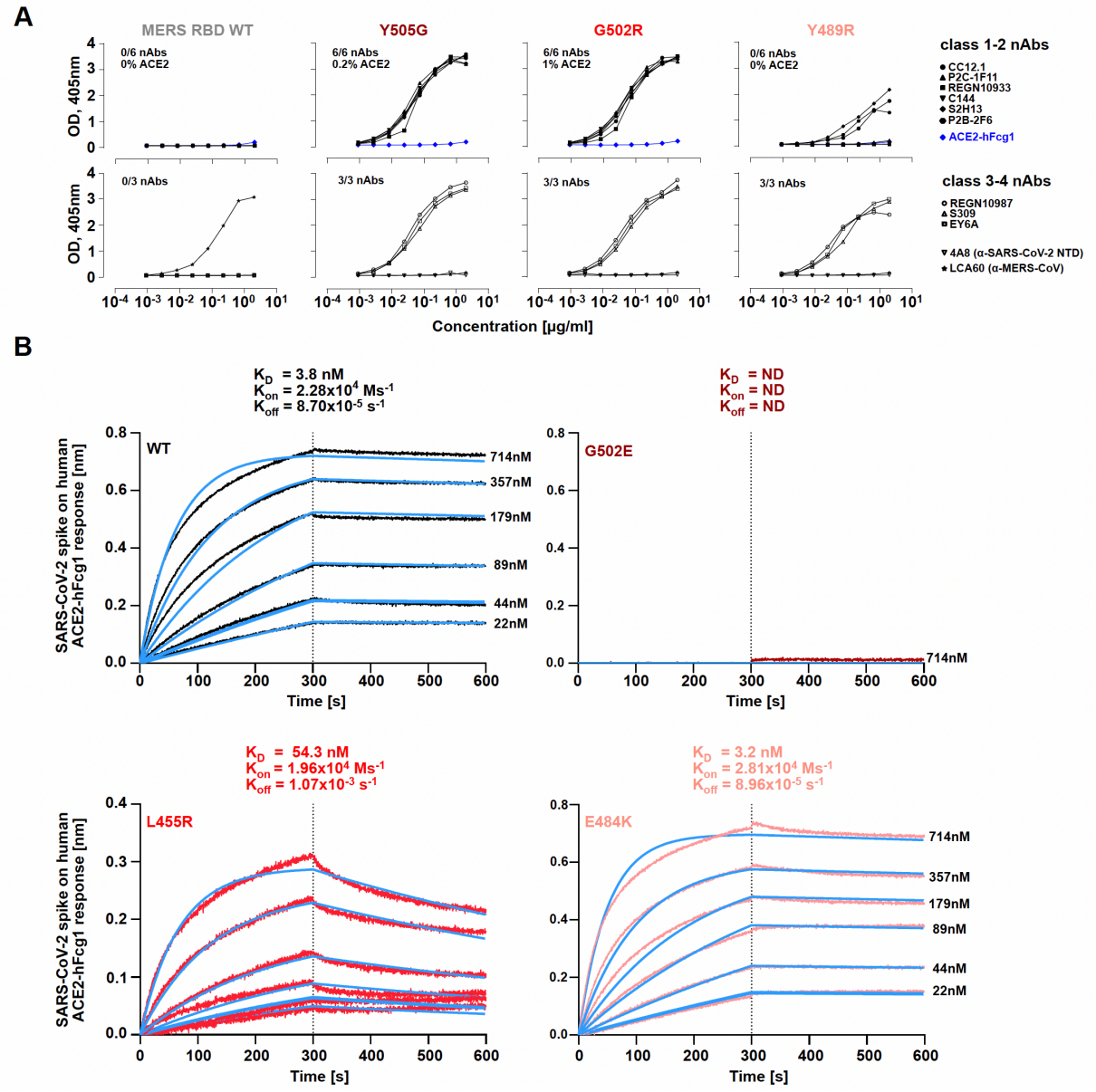
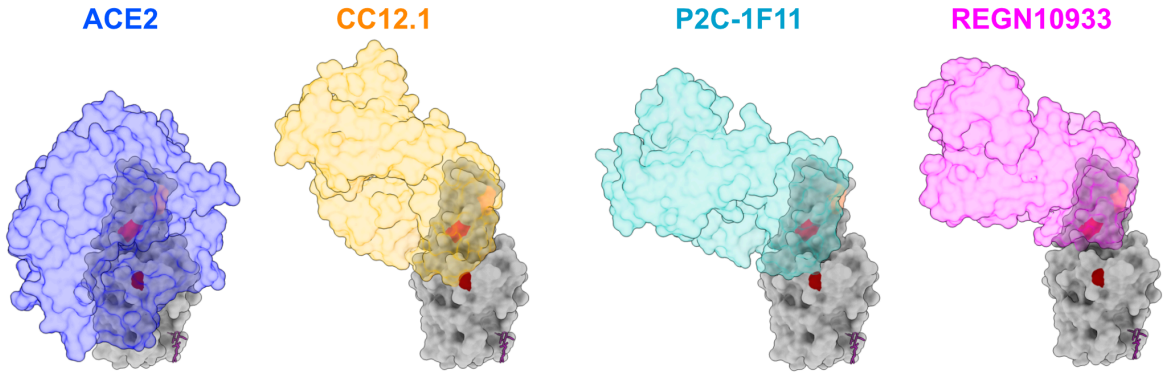
Score stratification of selected *in silico* candidates	RBD variant	Total score	ACE2 binding	Antibody escape	RBD expression
high	G502E	4.793	2.376	NA	2.418
high	G502D	4.708	1.899	NA	2.809
high	Y505G	4.110	2.357	NA	1.753
medium	G502R	3.195	2.497	NA	0.698
medium	L455R	3.047	-0.492	-0.824	3.199
medium	Y489S	3.039	2.497	NA	0.542
medium	Y505N	2.719	-0.119	0.341	2.496
low	F456Y	1.936	-0.586	0.339	2.183
low	Y505Q	1.586	0.152	0.305	1.128
low	L455E	1.592	-0.072	0.340	1.323
low	Y489R	1.470	1.554	NA	-0.083
low	F456A	0.297	0.040	-0.831	-0.083
low	E484Q	-2.847	-2.015	-0.826	-0.005
low	E484K	-2.933	-2.043	-0.806	-0.008

Supplementary Figure 1. Stratification of *in silico* determined SARS-CoV-2 RBD candidates into three categories. RBD candidates were categorized into high (dark red), medium (red) and low (light red) score groups. Shown are the total scores as well as individual values for ACE2 binding, antibody escape, and RBD expression for candidates tested *in vitro*. NA, not applicable.

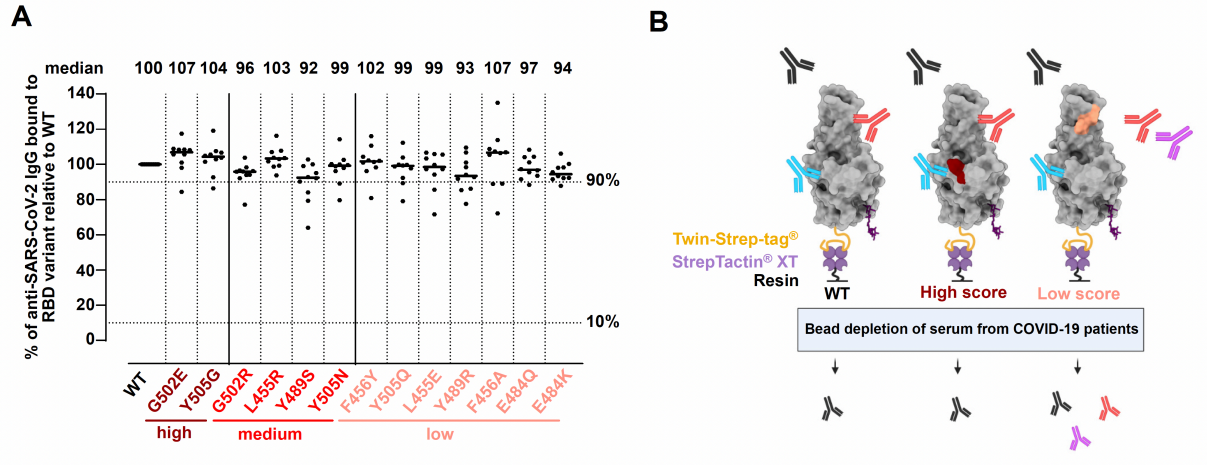


Supplementary Figure 2. Binding analysis of RBD candidates to ACE2 and selected nAbs. (A) Different anti-SARS-CoV-2 neutralizing antibodies (nAbs) of classes 1-2 (upper panels) and 3-4 (lower panels) were tested in ELISA for binding to indicated SARS-CoV-2 RBD variants with different *in silico* scores. Anti-spike NTD 4A8 antibody as well as anti-MERS-CoV antibody LCA60 served as negative controls. OD curves at 405 nm, the number of remaining nAbs bound as well as % of ACE2 binding compared to RBD WT are displayed. OD, 405nm, optical density for wavelength at 405 nm. (B) BLI sensorgrams showing the binding affinity via dissociation constant (K_D), on-rate (K_{on}) and off-rate (K_{off}) of human ACE2-hFcg1 affinity to SARS-CoV-2 spike WT and three variants, representative for each *in silico* score group. K_D

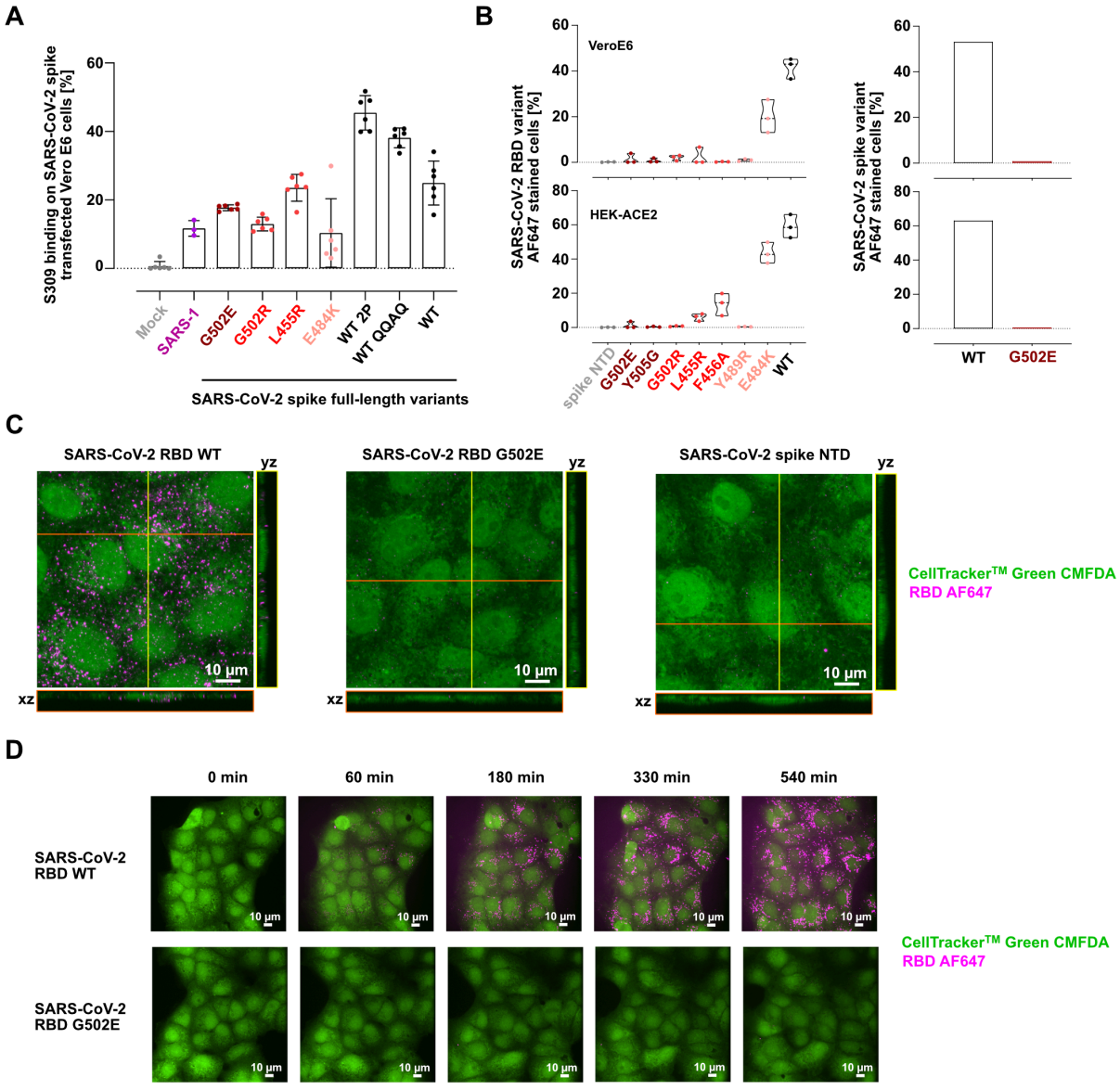
values were calculated using a 1:1 global fitting model and only R^2 values above 0.98 were considered. ND, not detected.



Supplementary Figure 3. Binding footprint of ACE2 and selected class 1 nAbs on the SARS-CoV-2 RBD WT. Molecular surface representation of ACE2 (blue) and class 1 antibodies CC12.1 (orange), P2C-1F11 (teal) and REGN10933 (magenta) binding to SARS-CoV-2 RBD WT (grey). A high (G502, dark red), medium (L455, red) and low (E484, light red) score exchange site of the RBD WT are highlighted.

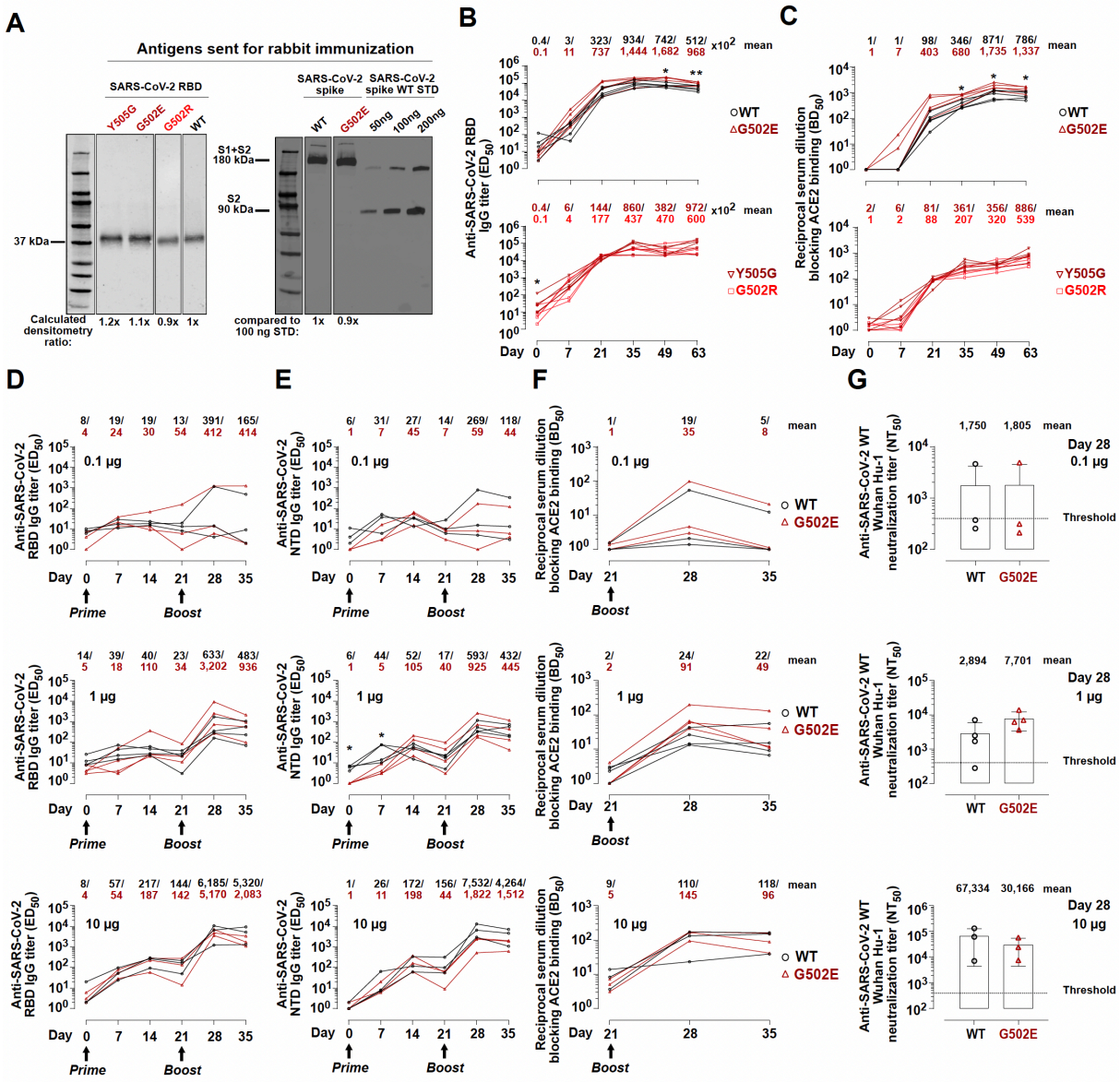


Supplementary Figure 4. Sera from COVID-19 patients were subjected to ELISA and a pull-down assay was performed with RBD variants of different score. (A) Binding of IgG serum antibodies from COVID-19 patients (n = 10) to indicated SARS-CoV-2 RBD mutants was determined by ELISA. Anti-RBD reactivity of antibodies to the respective variants is shown as % of binding compared to RBD WT. Median is shown. (B) Scheme depicting antibody pull-down from sera of COVID-19 patients using WT, high, or low score RBD candidates. High score candidates as well as WT show higher antibody capture from the serum than low score candidates.



Supplementary Figure 5. Surface staining of ACE2 and membrane-bound SARS-CoV-2 spike proteins as well as internalization of the soluble RBD WT by Vero E6 cells. (A) S309 antibody staining was performed on Vero E6 cells transfected with full-length transmembrane SARS-CoV-2 spike variants and respective binding determined via flow cytometry after 24 h. Mean +/- SD of % positive cells is shown for n = 3-6 technical replicates. (B) Surface staining of Vero E6 (top) and HEK Flp-In T-REx 293 pFRT/TO/FLAG/HA cells constitutively expressing ACE2 (HEK-ACE2; bottom) was conducted with fluorescently labelled RBD (left panel; n = 3 technical replicates) or spike proteins (right panel) with indicated mutations of high (dark red), medium (red) and low score (light red) and % of binding is shown. The SARS-CoV-2 spike NTD served as a negative control. (C) Representative confocal

images from 2 independent experiments of fixed samples display absorption of the RBD WT compared to the G502E variant by Vero E6 cells after 90 minutes of incubation. SARS-CoV-2 spike NTD served as a negative control. Shown are z projections with maximum intensity as well as orthogonal views. Scale bars, 10 µm. (D) Live-cell imaging of technical duplicates was conducted with a Nikon spinning disk confocal microscope monitoring SARS-CoV-2 RBD WT and G502E internalization kinetics by Vero E6 cells over the course of 9 h. Shown are representative images from different time points (0, 60, 180, 330 and 540 min) as z projections with maximum intensity. Scale bars, 10 µm. In (C-D), cytoplasm stain with CellTracker™ CMFDA is shown in green and AF647 labelled RBD in magenta.



Supplementary Figure 6. Rabbits generate high titers of spike-specific IgG following immunization with the SARS-CoV-2 spike variant G502E. (A) Indicated SARS-CoV-2 RBD and spike proteins quantified by Nanodrop measurement were analyzed by Western blot prior to rabbit immunization. Densitometry ratios (bottom) of Western blot bands were determined using the Gel Analyzer software setting 100 ng of RBD WT or commercial spike WT protein as standard (STD). (B-C) Rabbits were vaccinated in a 14-day booster interval with SARS-CoV-2 RBD WT, G502E, Y505G, and G502R (n = 5 rabbits per condition). Respective sera were analyzed at indicated time points over the course of 63 days. Shown are serum dilutions at which 50% of IgG is bound to the SARS-CoV-2 RBD WT (ED_{50}, (B)), and the serum dilutions at which 50% of ACE2 binding to RBD WT is blocked (BD_{50}, (C)). (D-G) Rabbits were primed

(Day 0) and boosted (Day 21) with 0.1 (n = 3 rabbits per condition), 1 (n = 4 rabbits per condition) or 10 µg (n = 3 rabbits per condition) SARS-CoV-2 spike WT (black) or G502E (dark red) protein. The ED_{50} of IgG reactive for SARS-CoV-2 spike RBD (D), and the spike NTD (E) for up to 35 days after prime for all three immunization groups are shown. Day 21, 28 and 35 bleeds were tested for their potential of blocking ACE2 binding to RBD WT (BD_{50}; (F)) and day 28 sera were subjected to SARS-CoV-2 WT Wuhan Hu-1 PsV neutralization assay (NT_{50}; (G)). Threshold was set by the average NT_{50} of day 35 sera from rabbits immunized with an irrelevant antigen. In (B-C), two-tailed unpaired *t*-tests or unpaired non-parametric Mann-Whitney tests were used. In (E), unpaired Mann-Whitney test was applied. In (B-F) mean as trendlines and in (G) mean +/-SD is displayed. *P* = *<0.05; *P* = **<0.01.

Raw_files_Western_blot: