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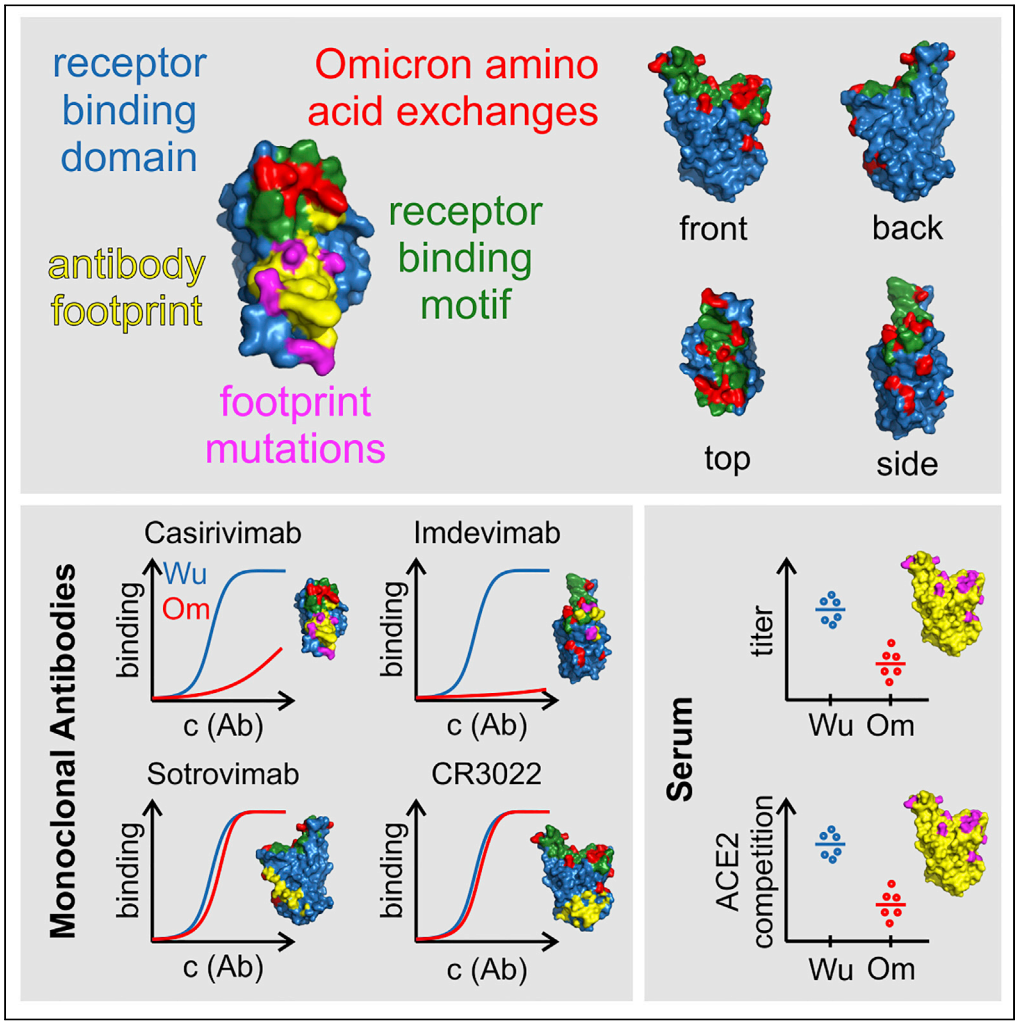


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## Highlights

Receptor binding motif antibodies Casirivimab and Imdevimab lose binding to Omicron

Binding of Sotrovimab and CR3022 is less affected by Omicrons mutations

IgG serum reactivity and competitive surrogate neutralization of Omicron are reduced

Loss of serum binding is accompanied by a pronounced loss of ACE2 competition

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# Omicron's binding to sotrovimab, casirivimab, imdevimab, CR3022, and sera from previously infected or vaccinated individuals

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## SUMMARY

**SARS-CoV-2 Omicron is the first pandemic variant of concern exhibiting an abrupt accumulation of mutations particularly in the receptor-binding domain that is a critical target of vaccination induced and therapeutic antibodies. Omicron's mutations did only marginally affect the binding of ACE2, and the two antibodies Sotrovimab and CR3022 but strongly impaired the binding of Casirivimab and Imdevimab. Moreover, as compared with Wuhan, there is reduced serum reactivity and a pronounced loss of competitive surrogate virus neutralization (sVN) against Omicron in naïve vaccinees and in COVID-19 convalescents after infection and subsequent vaccination. Finally, although the booster vaccination response conferred higher titers and better sVN, the effect was nonetheless significantly lower compared with responses against Wuhan. Overall, our data suggest that the antigenicity of Omicrons receptor binding motive has largely changed but antibodies such as Sotrovimab targeting other conserved sites maintain binding and therefore hold potential in prophylaxis and treatment of Omicron-induced COVID-19.**

## INTRODUCTION

Two years after onset of the SARS-CoV-2 pandemic, its global course is currently being taken over by the fifth variant of concern (VOC) B.1.1.529, which has been denominated Omicron (WHO, 2021). Currently, great efforts are being made to fill the knowledge gaps about the risk potential of Omicron (ECDC, 2021). Omicron, which has been first detected in specimen from Botswana and South Africa, displays a considerably higher number of mutations in the viral spike protein compared with previous VOCs (Gu et al., 2022; Saxena et al., 2021). It harbors 29 single amino acid exchanges, six deletions, and one amino acid in the spike protein, which is the most prominent antigen in current vaccine approaches. Fifteen mutations are located in the receptor binding domain (RBD), of which ten are part of the receptor binding motif (RBM) that is the main target of neutralizing antibodies (Figure 1A) (Piccoli et al., 2020).

Here, we describe Omicrons antigenic profile in comparison to the other VOCs and SARS-CoV-1 against the monoclonal antibodies Sotrovimab, Casirivimab, Imdevimab, and CR3022. Furthermore, we describe Omicrons affinity to its cellular receptor ACE2 in comparison to the other VOCs and SARS-CoV-1. To assess the profile of serum antibody binding to Omicron, we investigated a cohort of convalescent and SARS-CoV-2 naïve individuals before and after vaccination. Antibody binding assays and a sVNT show decreased affinity and competitive neutralization, respectively, of the tested sera to Omicron.

## RESULTS

### SARS binding profiles of monoclonal antibodies and soluble ACE2

We first tested the binding affinities of soluble ACE2, the monoclonal antibodies Sotrovimab (S309), CR3022, Casirivimab (REGN-10933), and Imdevimab (REGN-10987), and a single convalescent plasma from early 2020 to the RBDs of ten different SARS-CoV strains (Figure 1B–1H). Sotrovimab is an RBD class 3 antibody (according to the nomenclature proposed by Barnes et al.) that recognizes a non-RBM-overlapping epitope containing a glycan (N343) that is conserved within sarbecoviruses and does not compete binding of ACE2

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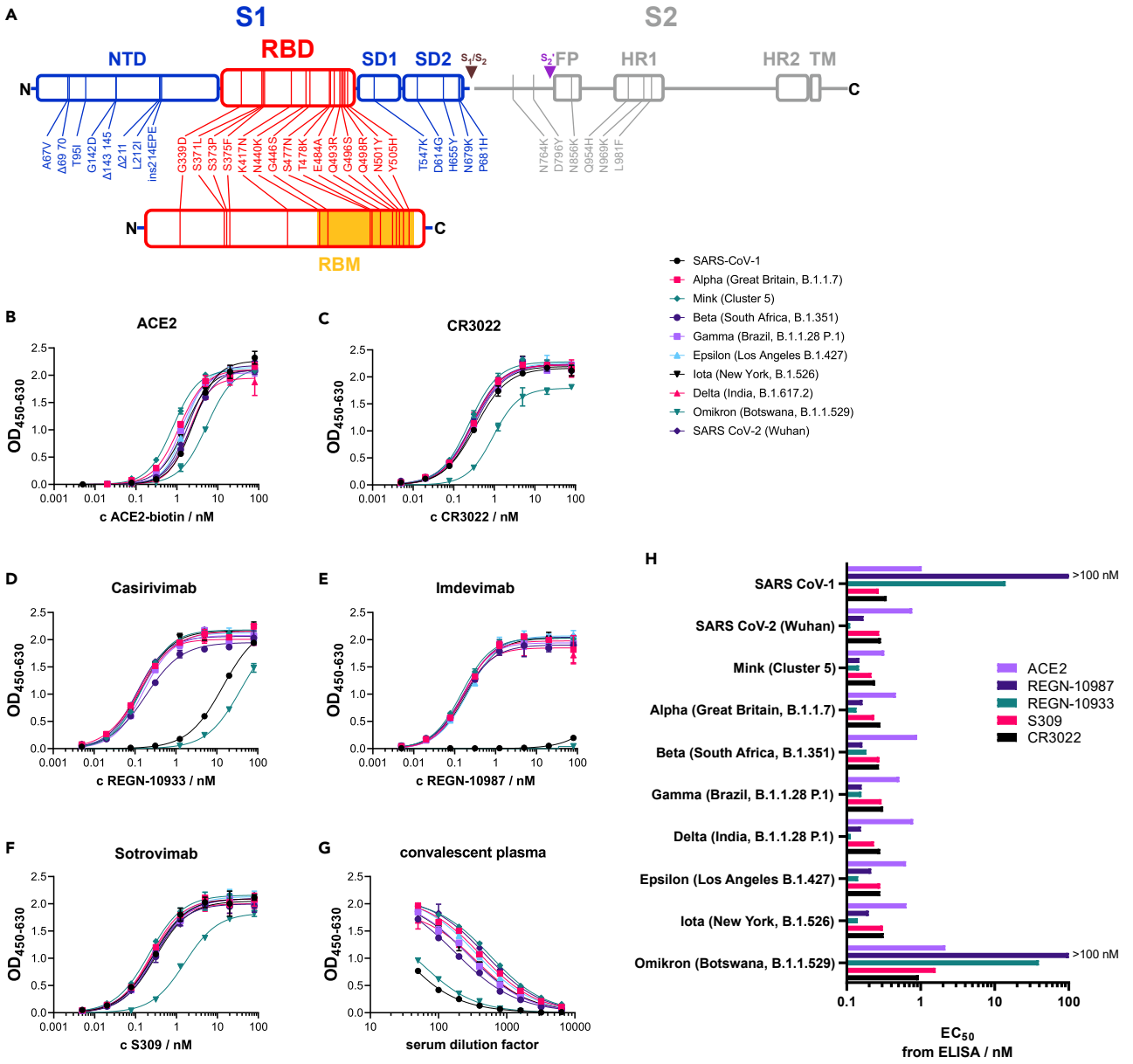


**Figure 1. ELISA binding profiles of 10 different RBDs of SARS-CoV-2 VOCs and VUIs and SARS-CoV-1 against a panel of monoclonal antibodies, soluble ACE2, and a representative convalescent plasma**

(A) Topological map displaying Omicron's amino acid exchanges within a spike protein's protomer and its RBD, respectively. RBM: receptor binding motif; triangles: Furin [black] and TMPRSS2 [gray] cleavage site; NTD: N-terminal domain; SD1: subdomain 1: SD2, subdomain 2; FP: fusion peptide; HR1: heptad repeat 1; HR2: heptad repeat 2; TM: transmembrane region. Binding profiles from ELISA experiments using (B) soluble ACE2, (C) CR3022, which was isolated based on its binding against SARS-CoV-1, (D) Casirivimab (REGN10933), (E) Imdevimab (REGN10987), (F) Sotrovimab (S309), and (G) a representative plasma from a COVID-19 convalescent donor from the first wave of SARS-CoV-2 infections. Titrations of the monoclonal antibodies were started at a concentration of 80 nM and eight 4-fold serial dilutions were measured. Convalescent serum was diluted 2-fold starting from a 1:50 dilution. Mean and standard deviation of duplicate measurements are given for all titrations.

(H) Half maximal effective concentrations ($EC_{50}$) were calculated from the binding curves (a low $EC_{50}$ corresponds to a high affinity of the ligand).

(Barnes et al., 2020a; 2020b). CR3022 targets a highly conserved cryptic epitope distal from the receptor binding site and is a RBD class 4 antibody. Casirivimab is a RBD class 1 antibody whose epitope largely overlaps with the RBM (Wang et al., 2021). Imdevimab is a class 3 antibody that binds distal to the RBM but has some overlap with the ACE2-binding site and does sterically hinder ACE2 interaction (Barnes et al., 2020b; Wang et al., 2021). Of note, Casirivimab, Imdevimab, and Sotrovimab are approved for clinical use.

**Table 1. Characteristics of the investigated cohort**

| Study arm | Sex[a] | Age (median) | Vaccine[b] |
|---|---|---|---|
| COVID-19 convalescent | Female (11/24) | 53 | BioNTech: 5/11; Moderna: 3/11; AstraZeneca: 3/11 |
| | Male (13/24) | 37 | BioNTech: 6/13; Moderna: 4/13; AstraZeneca: 3/13 |
| | All (24/24) | 45 | BioNTech: 11/24; Moderna: 7/24; AstraZeneca: 6/24 |
| SARS-CoV-2 naïve | Female (17/24) | 41 | BioNTech: 17/17 |
| | Male (7/24) | 50 | BioNTech: 7/7 |
| | All (24/24) | 42 | BioNTech: 24/24 |

[a]Number of participants with specified gender per total number of participants is given in parentheses.
[b]Number of participants with specified vaccine per total number of participants is given.

We found a slightly reduced binding to ACE2 in our ELISA (Figure 1B). Strongest differences in the monoclonal antibodies binding profile and half maximal effective concentrations ($EC_{50}$) in comparison to the Wuhan RBD appear for SARS-CoV-1 and Omicron (Figure 1C–H). In fact, differences for these two variants regarding the low affinity against Casirivimab ($EC_{50}$ of 13.99 nM (SARS-CoV-1) and 39.44 nM (Omicron)) and Imdevimab ($EC_{50}$ of >100 nM for both variants) are in a comparable lower range, thereby reflecting their genetic distance to the Wuhan strain ($EC_{50}$ of 0.11 nM for Casirivimab and 0.17 nM for Imdevimab). This is also apparent when comparing the binding profile of the representative convalescent serum (Figure 1G). Of utmost importance, however, binding of Sotrovimab was largely preserved.

### Binding and neutralizing activity of SARS-CoV-2 naïve and COVID-19 convalescent sera

Next, we analyzed the IgG binding capacity of sera of 48 subjects after infection and/or vaccination to Wuhan RBD and Omicron RBD. The associated study cohort of COVID-19 convalescent and SARS-CoV-2 naïve control subjects has been described earlier (Glück et al., 2021). In this experiment, we used a subset of 24 samples from the postinfection arm and 24 samples from the SARS-CoV-2 naïve arm for our analyses. Characteristics of the subgroups are given in Table 1.

Anti-Wuhan RBD IgG serum titers (here expressed as $EC_{50}$ values) wane after infection as well as after vaccination (Figure 2). Titers are higher after vaccination of convalescents as compared with complete vaccination of SARS-CoV-2 naïve subjects, and booster vaccination after 6 months does not significantly increase the RBD serum titers as compared with after the initial full vaccination regimen. Waning of the antibody titers after vaccination postinfection is not as pronounced as in SARS-CoV-2 naive vaccinees. The time course of Omicron-RBD titers is comparable to Wuhan-RBD but the values are generally significantly lower. The median remaining titers against Omicrons RBD in comparison to Wuhans RBD are 41.5% postvaccination and 37% 6 months postvaccination in COVID-19 convalescents. In SARS-CoV-2 naïve subjects, the titers drop to median 25% post 2 × vaccination and are raised to median 46% of the Wuhan titers by a booster vaccination after 6 months.

To study differences in neutralizing capacity of the sera, we set up a surrogate virus neutralization assay that uses solid-phase-bound RBD and soluble ACE2 fused to a highly active and small luciferase (NanoLuc (Hall et al., 2012)). The assay measures the residual binding of ACE2-NanoLuc to RBD antigen bound to a solid phase in presence of competing serum-antibodies as compared with a control without serum. Expectedly, the findings from the serum titers against the Wuhan and Omicron RBD are reflected by the results from the sVNT (Figure 3). Competitive neutralizing antibody responses are low after infection, wane rapidly, are strongly boosted after vaccination, and are significantly lower against Omicron as compared with Wuhan strain.

## DISCUSSION

Our data show in accordance with others (Cameroni et al., 2021; Planas et al., 2021) that binding of RBM-targeting monoclonal antibodies is largely impaired, whereas in contrast antibodies that bind to other RBD epitopes still display high affinity to Omicron. When analyzing the sera from vaccinated or convalescent patients, we found that antibody responses against Omicron RBD were significantly lower compared with Wuhan RBD.

Our findings are in line with initial reports on the neutralization capacity of sera from convalescent and vaccinated subjects against Omicron, which showed a more than 10-fold drop in effective titers (Ai et al., 2021; Wang et al., 2022; Schubert et al., 2021; Wilhelm et al., 2021; Carreno et al., 2021; Hoffmann

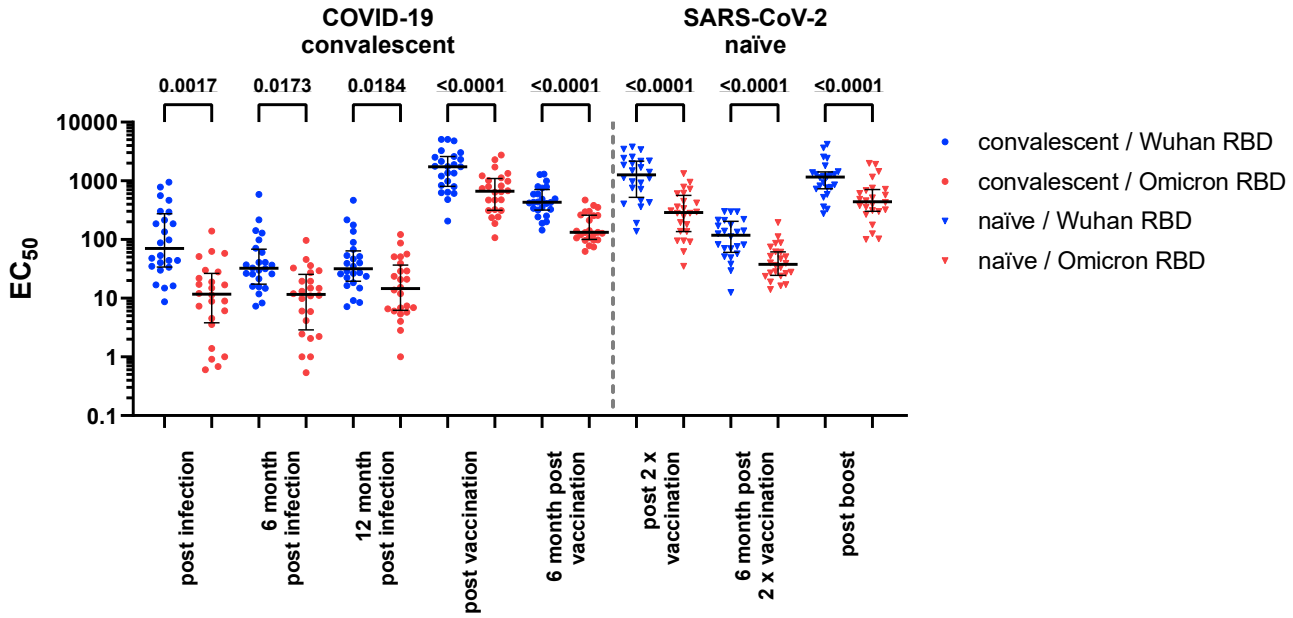


**Figure 2. Time course of serum binding titers against Wuhan and Omicron RBD**

Time course of binding titers (expressed as $EC_{50}$ values) in COVID-19 convalescent (left side of the vertical dashed line, circles) and SARS-CoV-2 naïve subjects (right side of the vertical dashed line, triangles) against Wuhan (blue) and Omicron (red) RBD. Both groups include an equal number of subjects ($n$ = 24), which were monitored over time. Median and interquartile ranges are given for every group. Significance was calculated using one-way analysis of variance (ANOVA) with Geisser-Greenhouse correction, and the respective p-values are given.

et al., 2021; Rössler et al., 2022). The IgG titers determined in this study, however, showed a considerably lower loss of binding capacity of serum antibodies compared with the reported loss of serum neutralizing capacity of Omicron (Carreno et al., 2021; Garcia-Beltran et al., 2022; Rössler et al., 2022; Schubert et al., 2021; Wilhelm et al., 2021). Interestingly, the loss of binding in our study is accompanied by a pronounced loss of ACE2 competition of the sera as detected in our sVNT. This is in line with the reported generally reduced serum-neutralization capacity against Omicron but also conforms to findings from Cao et al. and Cameroni et al. who described a sustained loss of affinity of a large number of RBM-directed monoclonal antibodies for Omicron (Cameroni et al., 2021; Cao et al., 2021). Nonetheless, Cao et al. reported that there are still a few, individual RBM-targeting monoclonal antibodies with retained binding to Omicron (Cao et al., 2021). This suggests that the polyclonal serum response in vaccinated naïve and convalescent subjects may reflect the presumed aggregated response of multiple, individual monoclonal antibody responses in which single antibodies with preserved binding and neutralizing ability may still be present. Ultimately, this might explain why the competitive neutralizing capacity is not completely lost in the sera of vaccinated convalescent and naïve subjects. Following this reasoning, we found in line with others that booster immunization is able to increase antibody responses to Omicron. Nonetheless, this response did not match the levels reached against Wuhan RBD in neither antibody titers nor sVNT, again confirming the results of other studies (Carreño et al., 2021; Garcia-Beltran et al., 2022; Gruell et al., 2022; Hoffmann et al., 2021; Muik et al., 2022; Schmidt et al., 2021).

At the same time, more and more data are emerging on either maintained protection after recovery from COVID-19 or vaccination or a generally lower severity of Omicron-induced COVID-19 in convalescent and vaccinated subjects (Andrews et al., 2021; Ferguson, 2021a, 2021b; Goga et al., 2021; Kuhlmann et al., 2021; Wolter et al., 2021). Therefore, other factors than neutralization seem to convey protection from severe COVID-19 in these patients. For instance, other antibody effector functions such as nonneutralizing effector functions of antibodies may be involved, which have also been previously reported in the development of HIV and influenza vaccines (Asthagiri Arunkumar et al., 2019; Bonsignori et al., 2012; Corey et al., 2015; Haynes et al., 2012; Ng et al., 2019).

Very recently BA.1, BA.2, and BA.3 sublineages evolved, and further lineages will follow, with novel serotypes. In this respect, it is very encouraging that Sotrovimab, which is known to bind to various

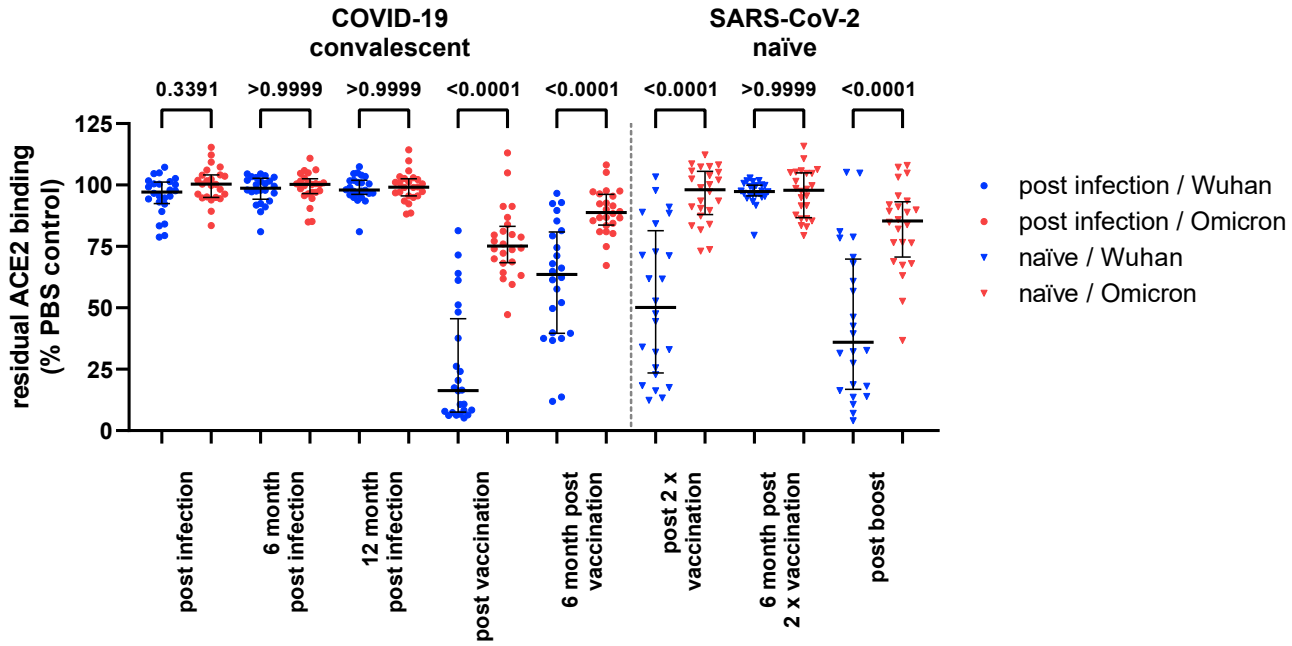


**Figure 3. Time course of serum neutralizing reactivity against Wuhan and Omicron in a sVNT**

Time course of neutralizing antibodies against Wuhan and Omicron as measured by a surrogate virus neutralization test. The assay measures the residual binding of ACE2 to the respective RBD variant after incubation with an analyzed serum at 1:50 dilution. Thus, values at 100% reflect absence of antibodies that neutralize by competition with the receptor, and values at 0% represent a very strong neutralization with no remaining binding signal from the soluble receptor. The two analyzed groups are COVID-19 convalescent (left side of the vertical dashed line, circles, $n = 24$) and SARS-CoV-2 naïve subjects (right side of the vertical dashed line, triangles, $n = 24$). The antigens were Wuhan (blue) and Omicron (red) RBD. Median and interquartile ranges are given for every group. Significance was calculated using one-way ANOVA with Geisser-Greenhouse correction and the respective p-values are given.

sarbecoviruses including SARS-CoV-1 and SARS-CoV-2, binds to Omicron as well (Cameroni et al., 2021; Cao et al., 2021; Gupta et al., 2021; Liu et al., 2021; Planas et al., 2021). Nevertheless, the clinical development of existing and additional broadly neutralizing antibodies as well as further antiviral agents against coronaviruses and the rapid adaptation of vaccines are urgently needed (Cameroni et al., 2021; Cao et al., 2021; Planas et al., 2021).

Based on the finding of a retained affinity of Sotrovimab to Omicron, it will be interesting to investigate structural conditions and constrains of this interaction. Determination of the high-resolution structure of the complex of Sotrovimab and stabilized Omicron spike trimers of the novel lineages and their respective RBDs will help to understand the molecular conditions of Sotrovimab's breadth. As Sotrovimab partially involves interactions with the conserved glycan at position N343, differences in glycan processing, e.g. as a result from a changed cell tropism, may further alter Sotrovimab's binding properties. This is likely to be an important feature of future escape variants and can already be investigated by mutational studies and glyco-engineering. Along these lines, forced *in vitro* viral selection studies could provide further information on the structural plasticity of Sotrovimabs epitope. Finally, comparative analysis of the characteristics of nonvaccinated Omicron-convalescent sera with convalescent sera from the pre-Omicron era and Omicron-breakthrough-infection sera will be of utmost interest. It will be interesting to see if the drop of neutralization will be compensated by newly induced RBM-reactivity or if other RBD-core or non-RBD epitopes are preferentially boosted and if this ultimately broadens protection against circulating and future emerging coronaviruses.

## Limitations of the study

As a limitation of this study, only RBD-directed antibody binding and serum titers were measured. Differences may appear when measuring against whole spike protein or against other antigens, although RBD titers have been shown to correlate well with spike titers in case of Wuhan and may thus be predictive (Peterhoff et al., 2021a). Along these lines, we found using our ELISA setup that ACE2 affinity to Omicron is reduced. This was described by Schubert et al. as well where the authors also used an ELISA to characterize the receptor

interaction (Schubert et al., 2021). In contrast, however, Cameroni et al. performed surface plasmon resonance measurements and found slightly enhanced binding affinity (Cameroni et al., 2021). This may reflect different assay-specific test characteristics. More sensitive and differentiate methods for binding affinity analysis as well as investigation of the molecular structure of the antibody-antigen-complex might help to clarify this. Finally, our sVNT is likely not reflecting the complete serum neutralization capacity, as it mainly detects neutralization by ACE2 receptor competition and may miss noncompetitive neutralization or competitive neutralization of potential alternative ligand structures (Clausen et al., 2020; Lempp et al., 2021). Such data would be available from comparative pseudotype or real-virus neutralization assays.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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## AUTHOR CONTRIBUTIONS

Conceptualization, methodology, validation, and formal analysis of the study were accounted by DP. Investigation was conducted by AM, LT, VG, and DP. Supervision and project administration were done by DP and AG. Resources were provided by AG and TG. Funding Acquisition was done by DP, TG, JK, BSc, and AG. Writing of the manuscript and visualization of the data were done by DP. Review and editing of the manuscript was done by AP, JK, BSa, JJ, RW, BSc, AG, and DP. All authors approved the submitted version.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Antibodies | | |
| Polyclonal rabbit anti-human-IgG, HRP-conjugated | Agilent | Cat# P021402-2; RRID: AB_2893418 |
| CR3022 | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | NCBI accession numbers: DQ168569, DQ168570 |
| REGN10933 (Casirivimab) | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | PDB code 6XDG |
| REGN-10987 (Imdevimab) | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | PDB code 6XDG |
| S309 (Sotrovimab) | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | PDB code 6WPT |
| Biological samples | | |
| Human serum samples | Kliniken Südostbayern Hospital Network, Germany; Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| Chemicals, peptides, and recombinant proteins | | |
| SARS CoV-1 RBD | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | NCBI accession number: AY274119 |
| SARS CoV-2 (Wuhan) RBD | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | GISAID accession number: EPI_ISL_ 402119 |
| Alpha (Great Britain, B.1.1.7) RBD [N501Y] | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| Beta (South Africa, B.1.351) RBD [K417N, E484K, N501Y] | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| Gamma (Brazil, B.1.1.28 P.1) RBD [K417T, E484K, N501Y] | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| Delta (India, B.1.617.2) RBD [L452R, T478K] | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| Epsilon (Los Angeles, B.1.427) RBD [L452R] | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| Iota (New York, B.1.526) RBD [S477N, E484K] | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| Omikron (Botswana, B.1.1.529) RBD [G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H] | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Mink (Cluster 5) RBD [Y453F] | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| soluble biotinylated ACE2 | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | ACE2: Uniprot ID Q9BYF1 |
| NanoLuc-ACE2 | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | ACE2: Uniprot ID Q9BYF1<br>NanoLuc: Uniprot ID Q9GV45 |
| ExpiFectamine™ 293 Transfection Kit | Thermo Fisher Scientific | Cat# A14524 |
| Expi293™ Expression Medium | Thermo Fisher Scientific | Cat# A1435101 |
| Opti-MEM™ I Reduced Serum Medium | Thermo Fisher Scientific | Cat# 31985062 |
| HisTrap Excel 5 mL | Cytiva | Cat# 17371206 |
| PD-10 Desalting Columns (Sephadex G-25) | Cytiva | Cat# 17085101 |
| Amicon Ultra-15, 10 kDa | Merck Millipore | Cat# UFC901024 |
| HiTrap MabSelect SuRe 1 mL | Cytiva | Cat# 11003493 |
| HiTrap DEAE FF | Cytiva | Cat# 17515401 |
| Mikrogen TMB Substrate Solution | Mikrogen | Cat# 12003 |
| Dulbecco's Phosphate Buffered Saline | Gibco | Cat# 14190-094 |
| Tween 20 | Caelo | Cat# 3472 |
| Fat free milk powder | Heirler | www.heirler.de |
| Imidazole | Sigma | Cat# 56749 |
| Glycine | Sigma | Cat# 50046 |
| HEPES | Sigma | Cat# 54457 |
| Sulfuric acid | Supelco | Cat# 1.09072 |
| **Critical commercial assays** | | |
| Streptavidin-POD Conjugate | Merck Millipore | Cat# 11089153001 |
| BirA biotin-protein ligase standard reaction kit | Avidity | Cat# BirA500 |
| Nano-Glo Luciferase Assay Reagent | Promega | Cat# N1120 |
| **Oligonucleotides** | | |
| Mutagenesis primer N439K_fw: GCCTGGAACAGCAAgAACCTGGACTCCAAAG | Eurofins Genomics | N/A |
| Mutagenesis primer N439K_rev: CTTTGGAGTCCAGGTTcTTGCTGTTCCAGGC | Eurofins Genomics | N/A |
| Mutagenesis primer Y453F_fw: ACTACAATTACCTGTtCCGGCTGTTCCGGAAG | Eurofins Genomics | N/A |
| Mutagenesis primer Y453F_rev: CTTCCGGAACAGCCGGaACAGGTAATTGTAGT | Eurofins Genomics | N/A |
| Mutagenesis primer S477N_fw: TCTATCAGGCCGGCAatACCCCTTGCAACGGC | Eurofins Genomics | N/A |
| Mutagenesis primer S477N_rev: GCCGTTGCAAGGGGTatTGCCGGCCTGATAGA | Eurofins Genomics | N/A |
| Mutagenesis primer E484K_fw: CCTTGCAACGGCGTGaagGGCTTCAACTGCTAC | Eurofins Genomics | N/A |
| Mutagenesis primer E484K_rev: GTAGCAGTTGAAGCCcttCACGCCGTTGCAAGG | Eurofins Genomics | N/A |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Mutagenesis primer N501Y_fw: GGCTTTCAGCCCACAtATGGCGTGGGCTACC | Eurofins Genomics | N/A |
| Mutagenesis primer N501Y_rev: GGTAGCCCACGCCATaTGTGGGCTGAAAGCC | Eurofins Genomics | N/A |
| Mutagenesis primer L452R_fw: GGCAACTACAATTACAGATACCGGCTGTTCCGG | Eurofins Genomics | N/A |
| Mutagenesis primer L452R_rev: CCGGAACAGCCGGTATCTGTAATTGTAGTTGCC | Eurofins Genomics | N/A |
| Mutagenesis primer K417T_fw: CCTGGACAGACAGGCACAATCGCCGACTACAAC | Eurofins Genomics | N/A |
| Mutagenesis primer K417T_rev: GTTGTAGTCGGCGATTGTGCCTGTCTGTCCAGG | Eurofins Genomics | N/A |
| Mutagenesis primer K417N_fw: CCTGGACAGACAGGCAACATCGCCGACTACAAC | Eurofins Genomics | N/A |
| Mutagenesis primer K417N_rev: GTTGTAGTCGGCGATGTTGCCTGTCTGTCCAGG | Eurofins Genomics | N/A |
| Mutagenesis primer P337L_fw: ATCACCAATCTGTGCctgTTCGGCGAGGTGTTC | Eurofins Genomics | N/A |
| Mutagenesis primer P337L_rev: GAACACCTCGCCGAAcagGCACAGATTGGTGAT | Eurofins Genomics | N/A |
| Mutagenesis primer R346S_fw: GTGTTCAATGCCACCtctTTCGCCTCTGTGTAC | Eurofins Genomics | N/A |
| Mutagenesis primer R346S_rev: GTACACAGAGGCGAAagaGGTGGCATTGAACAC | Eurofins Genomics | N/A |
| Mutagenesis primer T478K_fw: TCAGGCCGGCAGCAAGCCTTGCAACGGCG | Eurofins Genomics | N/A |
| Mutagenesis primer T478K_rev: CGCCGTTGCAAGGCTTGCTGCCGGCCTGA | Eurofins Genomics | N/A |
| **Recombinant DNA** | | |
| pcDNA5/FRT/TO_SARS-CoV-1_RBD | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pcDNA5/FRT/TO_SARS-CoV-2_RBD | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pcDNA5/FRT/TO_SARS-CoV-2_RBD_Wuhan | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pcDNA5/FRT/TO_SARS-CoV-2_RBD_Alpha | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pcDNA5/FRT/TO_SARS-CoV-2_RBD_Beta | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pcDNA5/FRT/TO_SARS-CoV-2_RBD_Gamma | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pcDNA5/FRT/TO_SARS-CoV-2_RBD_Delta | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| pcDNA5/FRT/TO_SARS-CoV-2_RBD_Epsilon | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pcDNA5/FRT/TO_SARS-CoV-2_RBD_Iota | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pcDNA5/FRT/TO_SARS-CoV-2_RBD_Omicron | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pcDNA5/FRT/TO_SARS-CoV-2_RBD_Mink | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pAb_LC_lambda_empty (pcDNA5/FRT/TO derivate providing a murine IgG1 signal peptide and the constant regions of lambda light chain) | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pAb_LC_kappa_empty (pcDNA5/FRT/TO derivate providing a murine IgG1 signal peptide and the constant regions of kappa light chain) | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pAb_HC_empty (pcDNA5/FRT/TO derivate providing a murine IgG1 signal peptide and the constant regions of IgG1 heavy chain) | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pAb_HC_CR3022 | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pAb_LC_kappa_CR3022 | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pAb_HC_REGN-10933 | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pAb_LC_kappa_REGN-10933 | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pAb_HC_REGN-10987 | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pAb_LC_lambda_REGN-10987 | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pAb_HC_S309 | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| pAb_LC_kappa_S309 | David Peterhoff, Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany | N/A |
| **Software and algorithms** | | |
| GraphPad Prism 9.2.0 | GraphPad Software | www.graphpad.com |
| SPSS Statistics 26 | IBM | www.ibm.com |
| CorelDRAW 2018 | Corel Corporation | www.coreldraw.com |
| HydroControl | Tecan Group | www.tecan.com |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Microplate Manager Ver. 5.2.1 | Bio-Rad Laboratories | www.bio-rad.com |
| Other | | |
| S-Monovette | Sarstedt | Cat# 01.1601.014 |
| Nunc Maxisorp Plates | Thermo Fisher Scientific | Cat# 446469 |
| LumiNunc 96-well plate | Thermo Fisher Scientific | Cat# 437796 |
| HydroFlex microplate washer | Tecan | N/A |
| Microplate Reader Model 680 | Bio-Rad | N/A |
| VICTOR 3 1420 Multilabel Counter | PerkinElmer | N/A |

## RESOURCE AVAILABILITY

### Lead contact

Requests should be directed to the lead contact, David Peterhoff (david.peterhoff@ur.de).

### Materials and availability

Materials are available on personal request under a material transfer agreement.

### Data and code availability

- Datasets generated in this study are available on personal request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

## EXPERIMENTAL MODEL AND SUBJECT DETAILS

### Study cohort

Employees of the Kliniken Südostbayern Hospital Network (Germany) who recovered from COVID-19 between April and June 2020 were asked to participate in the prospective cohort study. Directly after recovery and after 30 weeks, participants were asked to provide a serum sample. Approximately one year after COVID-19, participants were offered a one dose booster vaccination against COVID-19. Those who agreed to be vaccinated were asked to provide another serum sample directly before the vaccination and at least 14 days thereafter.

SARS CoV-2 naïve donors, without evidence of prior COVID-19 according to symptoms, negative anti-SARS-CoV-2 antibodies and negative SARS-CoV-2 PCR-tests served as controls and underwent the standard two-dose vaccine schedule between February and April 2021. The participants were asked to provide a serum-sample immediately prior to vaccination and at least 14 days after the complete regimen.

Both groups were asked for a further serum sample 6 months after complete vaccination. SARS-CoV-2 naïve donors received their booster vaccination 8 months after the prime-regimen and were asked to donate a further serum sample 14 days after vaccination.

Information about sex and median age of the analyzed subjects and sample size of all experimental groups is specified in Table 1.

### Ethical issues

The study was approved by the ethical committee of the Faculty for Medicine, University of Regensburg, Regensburg, Germany (reference number 20-1896-101). The study complies with the 1964 Helsinki declaration and its later amendments. All participants provided written informed consent.

## METHOD DETAILS

### Serum preparation

Blood sampling was accomplished by venipuncture (S-Monovette, Sarstedt, Nürnbrecht, Germany). Serum was obtained by centrifugation 1–6 h after blood drawl and stored at – 20°C.

### Protein expression and purification

The SARS-CoV-1 and SARS-CoV-2 (Wuhan Hu-1) RBD-encoding sequences were codon usage-optimized and synthesized by GeneArt AG (Thermo Fisher Scientific). The RBD was cloned into a modified pcDNA5/FRT/TO vector encoding an N-terminal mini-tPA signal peptide (sequence: MDAMKRGLCCVLLLCGAVFVSPSAA, described in Peterhoff et al., 2021b) and a C-terminal avi-hexahistidine tag (sequence: GGS-GLNDIFEAQKIE WHE-GS-HHHHHH). The plasmids encoding the RBD of the SARS-CoV-2 VOCs were generated by introducing the corresponding mutations via overlap extension polymerase chain reaction into the Wuhan sequence (Higuchi et al., 1988). Due to the high number of amino acid exchanges, Omicron RBD (PANGO lineage B.1.1.529, GISAID accession ID EPI_ISL_6640917, containing amino acid exchanges G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H) was generated separately by gene synthesis.

Expression of the antigens was accomplished by transient transfection of Expi293F™ cells (Thermo Fisher Scientific; A14527) with plasmids encoding the SARS-CoV-2 RBD variants according to the manufacturer's recommendations. After 5 days of protein expression, supernatants were harvested by centrifugation, loaded onto an immobilized metal chelate affinity chromatography (IMAC) column (HisTrap Excel, Cytiva), washed with Dulbecco's Phosphate Buffered Saline (PBS, Sigma) containing 10 mM imidazole (Sigma) and eluted over a linear gradient of 10–500 mM imidazole in PBS. The protein was immediately buffer exchanged to PBS and concentrated to approximately 1–2 mg/mL by ultrafiltration (Amicon Ultra-15, 10 kDa, Merck Millipore).

Monoclonal antibody light chain and heavy chain variable domain sequences were retrieved from NCBI GenBank or RSCB PDB (NCBI accession number DQ168569 and DQ168570 for CR3022; pdb code 6WPT for S309; pdb code 6XDG for REGN-10933 and REGN-10987). The sequences were codon optimized and synthesized by GeneArt AG (Thermo Fisher Scientific) and cloned into a pcDNA5/FRT/TO derivate providing a murine IgG1 signal peptide and the constant regions of lambda light chain (S309, REGN-10933) or kappa light chain (CR3022, REGN-10987) or human IgG1 heavy chain. The monoclonal antibodies were transiently expressed at a gene dosage ratio of 1 for light and heavy chain plasmids in Expi293F™ cells (Thermo Fisher Scientific; A14527) according to the manufacturer's recommendations. The antibodies were purified from the supernatants by protein A based affinity purification. Elution from the column (HiTrap MabSelect SuRe, Cytiva) was accomplished by a pH step using 100 mM glycine buffer pH 3.2 and the eluted antibodies were immediately buffer exchanged to PBS.

ACE2-NanoLuc was codon optimized and synthesized by GeneArt AG (Thermo Fisher Scientific). The variant is an N-terminal fusion of the 19 kDa catalytic domain of the shrimp *Oplophorus gracilirostris* luciferin 2-monooxygenase (NanoLuc, Uniprot ID Q9GV45) with the soluble ACE2 (amino acid 20–732) metalloprotease separated by a 12 amino acid linker (sequence: $GSG_4SG_4S$). The construct provides a C-terminal avi-octahistidine purification tag (sequence: GS-GLNDIFEAQKIEWHE-GS-HHHHHHHH). Expression was performed in Expi293F as described above and purification was accomplished by IMAC and subsequent anion exchange chromatography (HiTrap DEAE Sepharose, Cytiva) using a gradient from 10 mM to 1 M NaCl, in HEPES pH 6.8. The protein was buffer exchanged to PBS and stored at 4°C.

Soluble ACE2 (amino acid 20–732) was codon optimized and synthesized by GeneArt AG (Thermo Fisher Scientific) and cloned into a pcDNA5/FRT/TO derivate providing a mini-tPA-signal peptide and an avi-octahistidine tag (sequence GS-GLNDIFEAQKIEWHE-GS-HHHHHHHH). The protein was purified as described for ACE2-NanoLuc. Site specific biotinylation was performed using BirA (BirA biotin-protein ligase standard reaction kit, Avidity).

### ELISA using monoclonal antibodies and soluble ACE2

Antigens were coated at a concentration of 2 μg/ml in PBS to the plastic surface of Nunc Maxisorp 96-well plates (Thermo Fisher Scientific) over night at 4°C. Plates were blocked with 5% fat free milk powder in PBS containing 0.1% Tween 20 (Caelo) (PBS-T) and fourfold serial dilutions starting at 80 nM in 1% fat free milk powder in PBS-T

were applied and incubated for 1 h at room temperature. After washing with PBS-T, anti-human IgG horse raddish peroxidase (HRP) conjugate (Dako/Agilent) for monoclonal antibodies or Streptavidin-POD Conjugate (Roche) for biotinylated soluble ACE2 in 1% fat free milk powder in PBS-T was added for 1 h and the plates were subsequently developed with TMB substrate solution (Mikrogen) and stopped with 1.0 N sulfuric acid (Sigma Aldrich). Optical density was measured in a plate reader at 450 and 630 nm (Microplate Reader Model 680, Bio-Rad) and 630 nm values were subtracted from 450 nm values as background.

### Detection of RBD-specific serum antibodies by ELISA

Anti-RBD antibody levels in serum were detected by an ELISA utilizing the SARS-CoV-1- and SARS-CoV-2-RBDs described above. The validation of the ELISA was previously described (Peterhoff et al., 2021a). The assay detects RBD-specific antibodies responses with high specificity and sensitivity and the detected antibody levels were shown to correlate well with the virus neutralization capacity of the respective serum sample. ELISA-results were expressed as effective concentration 50 ($EC_{50}$) resulting from titrations in 2.5 fold serial serum dilutions starting at 1:40 serum dilution. IgA serum reactivities were determined at a single serum dilution of 1:100 and are expressed as optical densities of the sample/background ratios (signal/cut-off; S/CO) as determined for SARS CoV-2 Wuhan RBD.

### ACE2-NanoLuc surrogate virus neutralization assay

Conditions for optimal dynamic detection range of neutralizing antibodies in the surrogate virus neutralization test (sVNT) were determined by three-dimensional cross-titration of antigen, NanoLuc-ACE2 and neutralizing antibodies. For serum sVNTs, sera were diluted 1:50 in 1% fat free milk in PBS (Gibco) 0.1 % Tween 20 (Caelo) (PBS-T) and added to RBD-coated and pre-blocked ELISA plate. After 1 h incubation the plate was washed with PBS-T and 200 nM NanoLuc-ACE2 in PBS-T was added for 30 min. After washing with PBS-T, 50 µl Nano-Glo Luciferase Assay Reagent (Promega) was added to each well and the luminescence signal was detected within 20 minutes in a 96 well luminescence reader (VICTOR Plate Reader, PerkinElmer).

The luminescence counts per second were normalized to the signal of a control well without serum competition and to the median signal from all SARS CoV-2 naïve sera.

## QUANTIFICATION AND STATISTICAL ANALYSIS

Descriptive statistics were calculated from raw data using SPSS (SPSS Statistics 26, IBM, New York/USA) or GraphPad Prism (GraphPad Prism for Windows 9.0; GraphPad, San Diego/USA). For multiple comparisons one-way ANOVA was performed with Geisser-Greenhouse correction and p-values > 0.0500 were not considered significant. Graphs were generated with Graphpad Prism.