Supplementary Information - Antibody binding and ACE2 binding inhibition is significantly reduced for both the BA1 and BA2 omicron variants

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Supplementary Methods

Mass Spectrometry of omicron receptor binding domain (RBD)

The RBD omicron protein samples (5 µg) were N-deglycosylated using a PNGaseF reducing kit (Rapid PNGaseF reducing kit, New England Biolabs, Frankfurt am Main, Germany) by adding ¼ of the volume of reducing buffer included in the kit and denaturation and reduction for 5 minutes at 80 °C. Subsequently, 0.125 µL PNGaseF enzyme preparation, included in the kit, were added and deglycosylation was performed for 10 minutes at 50 °C. Prior to Liquid chromatography-mass spectrometry (LC-MS) analysis, the samples were diluted 1:3 with HisNaCl buffer (20 mmM His 140 mM NaCl, pH 6.0) and analyzed by liquid chromatography (HPLC) coupled to electrospray ionization (ESI) quadrupole time-of-flight (QTOF) MS. Samples (0.4 µg per injection) was desalted using reversed phase chromatography on a Dionex U3000 RSLC system (Thermo Scientific, Dreieich, Germany) using a Acquity BEH300 C4 column (1mm x 50mm, Waters, Eschborn, Germany) at 75°C and 150 µl/min flow rate applying a 11-min linear gradient with varying slopes. In detail, the gradient steps were applied as follows (min/% Eluent B): 0/5, 0.4/5, 2.55/30, 7/50, 7.5/99, 8/5, 8.75/99, 9.5/5, 10/99, 10.25/5 and 11/5. Eluent B was acetonitrile with 0.1% formic acid, and solvent A was water with 0.1% formic acid. To avoid contamination of the mass spectrometer with buffer salts, the HPLC eluate was directed into waste for the first 2 min. Continuous MS analysis was performed using a QTOF mass spectrometer (Maxis UHR-TOF; Bruker, Bremen, Germany) with an ESI source operating in positive ion mode. Spectra were taken in the mass range of 600–2000 m/z. External calibration was applied by infusion of tune mix via a syringe pump during a short time segment at the beginning of the run. Raw MS data were lock-mass corrected (at m/z 1221.9906) and further processed using Data Analysis 5.3 and MaxEnt Deconvolution software tools (Bruker).

Antigen Immobilisation on beads

SARS-CoV-2 wild-type Spike, RBD, S1 domain, S2 domain and Nucleocapsid were immobilised on magnetic MagPlex beads (Luminex) by EDC-sNHS coupling as previously described(1).RBDs from variants of concern and the Omicron Spike protein were immobilised on magnetic MagPlex beads (Luminex) by Anteo coupling (AMG Activation Kit for Multiplex Microspheres, #A-LMPAKMM-400, Anteo Technologies) as previously described(2). Following coupling, beads were stored at 4°C. Prior to experimentation, beads were then combined into a 25x Bead Mix and stored at 4°C until used. The antigens used in these experiments can be found in Table 2.

MULTICOV-AB

MULTICOV-AB, a previously published multiplex immunoassay was performed as described(1). A full list of antigens included within the assay are listed in Table 2. Samples were randomly allocated to plates to ensure that at least three sample of every sample group was included on each plate. Briefly, samples were thawed at room temperature, vortexed and then diluted 1:200 in assay buffer before being mixed 1:1 with 1x Bead Mix in a 96-well plate (final dilution 1:400). Samples were then incubated in darkness on a Thermomixer (20°C, 750 rpm, 2 hours) before being washed three times to remove unbound antibodies. To ensure retention of beads, a magnetic plate washer was used. To detect bound IgG, 3 µg/mL RPE-goat anti-human IgG was added to each well and then incubated for a further 45 mins on a Thermomixer. After another washing step, beads were resuspended in 100 µL of wash buffer, shaken for 3 mins at 1000 rpm, and then measured on a FLEXMAP3D instrument (No Timeout, Gate 7500-15000, Reporter Gain Standard PMT, 50 events). 3 Quality control samples were included in duplicate on each plate. All

samples were measured twice in two independent experiments. No sample failed QC. Raw median fluorescence intensity (MFI) values were normalized to a QC sample for all antigens as per(3) .

RBDCoV-ACE2

RBDCoV-ACE2, a previously published multiplex ACE2 inhibition assay(2), analyzes neutralizing antibody activity through ACE2 binding inhibition. A full list of antigens included in this assay can be found as Table 2. Briefly, 1:25 diluted samples from MULTICOV-AB, were further diluted to 1:200 in ACE2 buffer(2), which contains 300 ng/mL biotinylated ACE2. Samples were then mixed 1:1 with 1x VOC bead mix in 96 well plates and incubated for 2 hours in darkness on a thermomixer (750 rpm, 20°C). Following this initial incubation, samples were washed to remove unbound ACE2 using an automated magnetic plate washer. Bound ACE2 was detected by adding 2 µg/mL RPE-labelled streptavidin and incubating for a further 45 mins. After washing to remove unbound fluorophores, beads were resuspended in 100 µL washing buffer and shaken for 3 mins at 1000 rpm. Plates were measured once on a FLEXMAP3D instrument (No Timeout, Gate 7500-15000, Reporter Gain Standard PMT, 50 events). As controls, 3 wells with 150 ng/mL ACE2, 2 blank wells and 3 wells with a QC sample were included. ACE2 binding inhibition was calculated as a percentage, with 100% indicating maximum ACE2 binding inhibition and 0% indicating no ACE2 binding inhibition. Samples with an ACE2 binding inhibition less than 20% are classified as non-responders(2).

Biolayer Interferometry (BLI)

Purified RBD_{wt}, RBD_{δ}, RBD_{oBA1} and RBD_{oBA2} were biotinylated with Sulfo-NHS-LC-LC-Biotin (Thermo Fisher Scientific) in 5 molar excess at ambient temperature for 30 min. Excess of biotin was removed by size exclusion chromatography using Zeba™

Spin Desalting Columns 7K MWCO 0.5 ml (Thermo Fisher Scientific) according to the manufacturer's protocol. Analysis of binding kinetics of RBD specific antibodies in serum samples were performed using the Octet RED96e system (Sartorius) as per the manufacturer's recommendations. In brief, 5 µg/ ml of each biotinylated RBD diluted in Octet buffer (PBS, 0.1% BSA, 0.02% Tween20) was immobilized on streptavidin coated biosensor tips (SA, Sartorius) for 20 s. In the association step, serum samples at a 1:100 dilution were reacted for 720 s followed by dissociation in Octet buffer for 1200 s. Every run was normalized to a healthy control sample (pre-pandemic) lacking RBD specific antibodies and for each sample technical duplicates (n = 2) were performed. Data were analyzed using the Octet Data Analysis HT 12.0 software applying the 1:1 fitting model for the dissociation step. The binding profile response of each sample is illustrated as the mean wavelength shift in nm. Binding kinetics for ACE2 were performed by immobilizing 5 µg/ ml of each biotinylated RBD diluted in Octet buffer on streptavidin coated biosensor tips (SA, Sartorius) for 20 s. Dilution series ranging from 50 to 6.25 nM of ACE2 (Sino Biological) were applied for 300 s and one reference was included per run, followed by a dissociation step in Octet buffer (480s). For affinity determination, the 1:1 global fit of the Data Analysis HT 12.0 software was used.

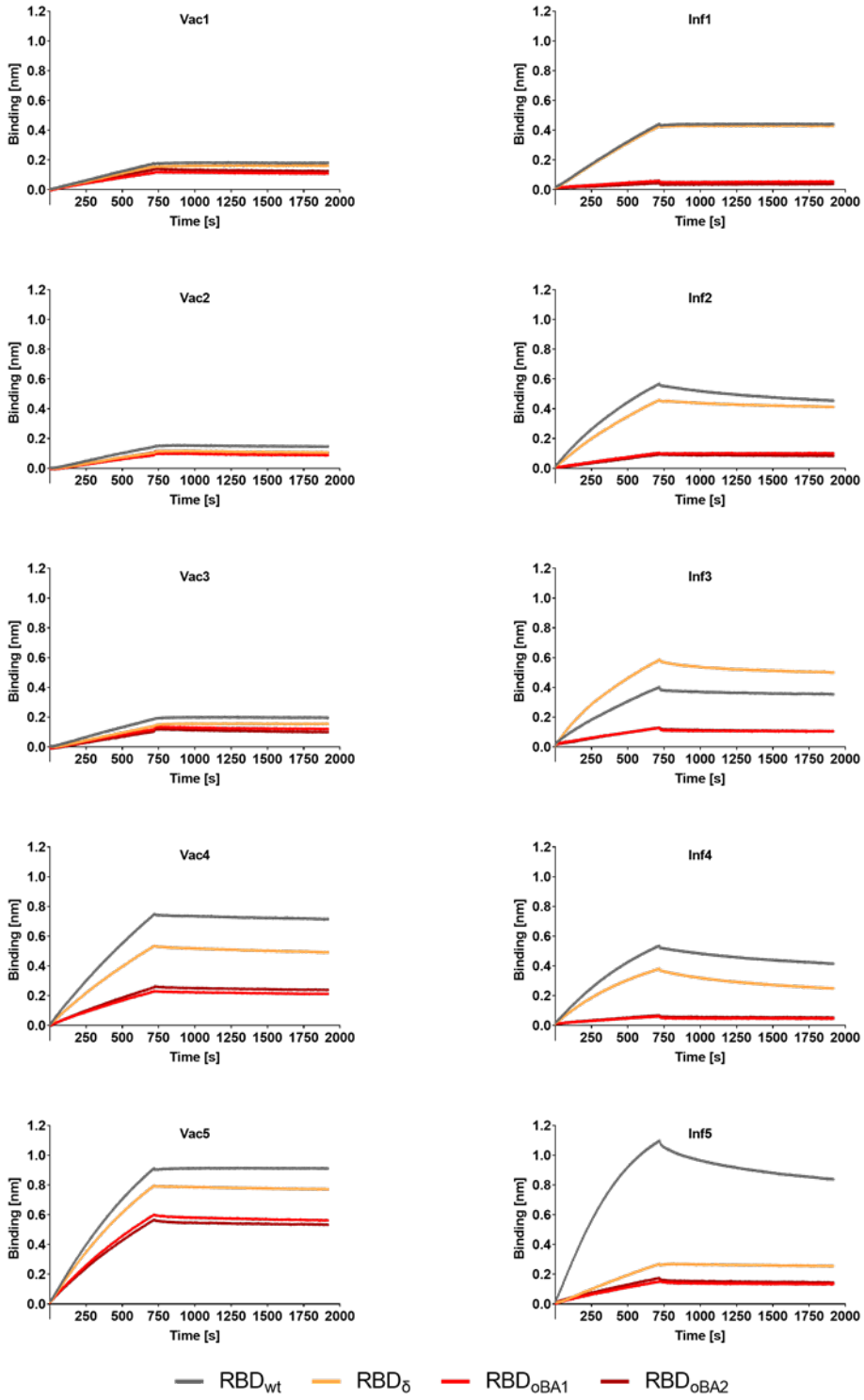
Data Analysis

Data was collated and matched to metadata in Excel 2016. Data visualisation was done in RStudio (Version 1.2.5001 running R version 3.6.1). Additional packages "gplots" and "beeswarm" were used for specific displays. The "lm" function of R's "stats" library was used for linear regression analyzes. Correlation analyzes were performed using the "cor" function of R's "stats" library. The "wilcox.test" function from R's "stats" library was used to perform both Mann-Whitney-U Tests (two-sided) in order to estimate significance of observed differences between different groups, and

Wilcoxon Signed Rank Analysis (two-sided) to estimate the significance of observed differences between antigens for the same samples. Graphs were exported from RStudio and further edited in Inkscape (Version 0.92.4) to generate final figures. Biolayer inferometry graphical representation was prepared using GraphPad Prism Software (Version 9.0.0).

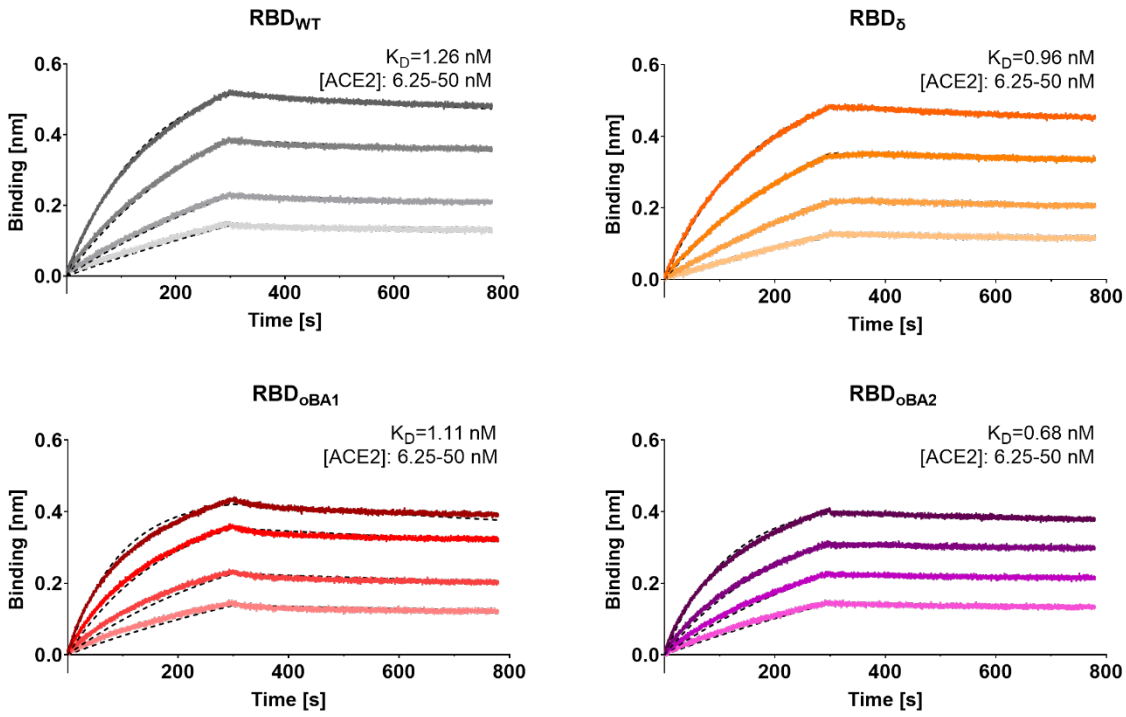
Supplementary Figures

Supplementary Figure 1: **Binding kinetics of RBD specific antibodies from serum samples of vaccinated and convalescent individuals.**



Biotinylated RBD_{wt}, RBD_{δ}, RBD_{oBA1}, RBD_{oBA2} were immobilized on streptavidin biosensor tips and binding kinetics of serum samples from vaccinated (n = 5, Vac) and convalescent (n =5, Inf) individuals were analyzed using BLI. All sensograms are illustrated as mean of technical duplicates (n = 2).

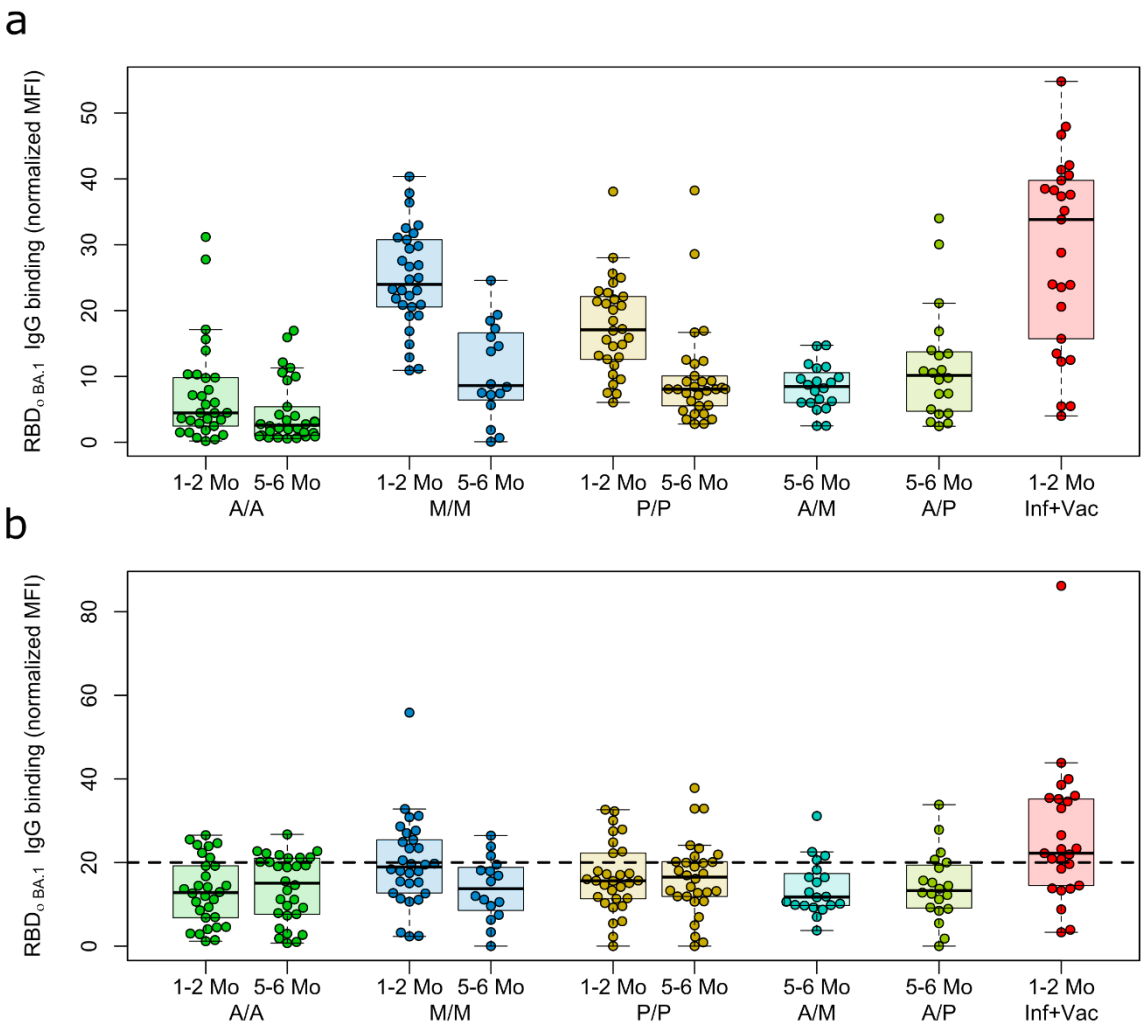
Supplementary Figure 2: **Binding kinetics of ACE2 to RBD_{wt}, RBD_{δ}, RBD_{oBA1} and RBD_{oBA2} using BLI.**



	K_D [nM]	k_{on} [10^5 M^{-1} s^{-1}]	k_{off} [10^{-4} s^{-1}]
RBD_{wt}	1.26 ± 0.01	1.37 ± 0.00	1.72 ± 0.01
RBD_{δ}	0.96 ± 0.01	1.24 ± 0.00	1.19 ± 0.01
RBD_{oBA1}	1.11 ± 0.01	2.10 ± 0.01	2.32 ± 0.02
RBD_{oBA2}	0.68 ± 0.01	1.75 ± 0.00	1.19 ± 0.02

For biolayer interferometry (BLI)-based affinity measurements, biotinylated RBD_{wt}, RBD_{δ}, RBD_{oBA1} and RBD_{oBA2} were immobilized on streptavidin biosensors. Kinetic measurements were performed using four concentrations of purified ACE2 ranging from 6.25 nM to 50 nM (illustrated with gradually lighter shades). The table summarizes affinities (K_D), association (k_{on}), and dissociation constants (k_{off}) of ACE2 determined for the different RBD variants.

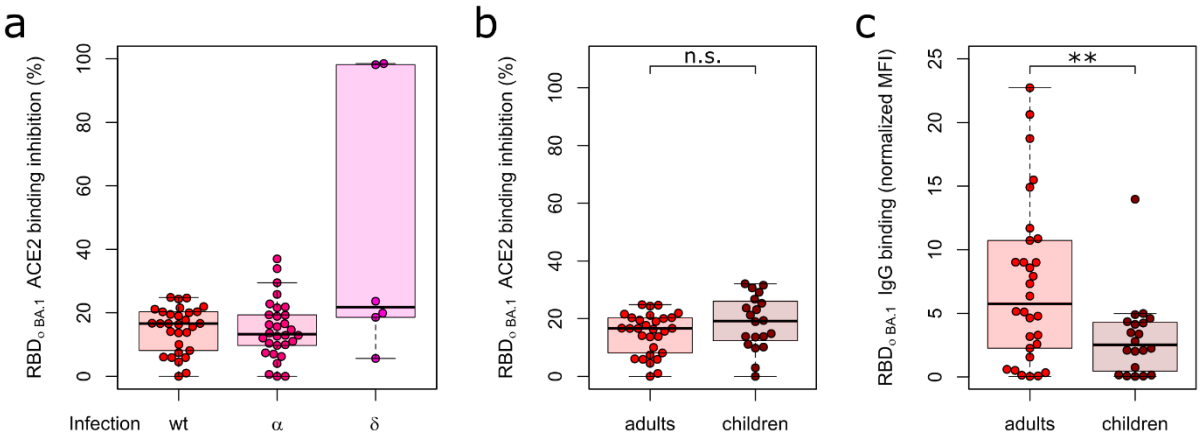
Supplementary Figure 3: **IgG binding capacity and ACE2 binding inhibition for vaccinated samples towards BA1**.



Binding response towards omicron was analyzed by either MULTICOV-AB (a) or RBDCoV-ACE2 (b) for samples from different vaccine schemes (n=30 for all sample groups, except for mRNA-1273 5-6 months (n=16), heterologous vaccine schemes (both n=20) and infected and vaccinated (Inf+vac, n=25). To determine the effect of time post-vaccination, samples from both 1-2 months and 5-6 months post-vaccination were included. A/A = AZD1222. M/M = mRNA-1273. P/P = BNT162b2. A/M = first dose AZD1222, second dose mRNA-1273. A/P = first dose AZD1222, second dose BNT162b2. Boxes represent the median, 25th and 75th percentiles, whiskers show the largest and smallest non-outlier values. Outliers were determined

by 1.5 IQR. The 20% cut off for non-responders is indicated by the dashed line on b. The equivalent data for BA2 is shown in the main manuscript file as Figure 3.

Supplementary Figure 4: **Differences in IgG binding response and ACE2 binding inhibition towards BA1 among different populations of convalescent samples.**



Comparative ACE2 binding inhibition (a and b) and IgG binding capacity (c) between convalescent samples from different pandemic waves (a) and adults and children (b and c) for Omicron BA1. (a) ACE2 binding inhibition towards BA1 for individuals infected with WT (n=30), alpha (n=30) or delta (n=6). (b) There is no significant difference (p=0.18) in ACE2 binding inhibition between adults (n=30) and children (n=20). (c) Adults have significantly higher IgG binding capacity (p=0.01) towards BA1 than children. Boxes represent the median, 25th and 75th percentiles, whiskers show the largest and smallest non-outlier values. Outliers were determined by 1.5 IQR. Statistical significance was calculated by two-sided Mann-Whitney U.

Supplementary Table 1: **Median fold reduction in IgG binding capacity from wild-type to respective variant**

Group	Vaccinated	Convalescent
WT	1	1
alpha	1.19 (1.06 – 1.32)	1.17 (0.95 – 1.39)
beta	2.84 (1.80 – 3.88)	3.46 (1.24 – 5.67)
gamma	1.65 (1.17 – 2.13)	1.96 (1.03 – 2.90)
delta	1.21 (1.03 – 1.38)	1.25 (0.95 – 1.55)
omicron BA1	2.32 (0.96 – 3.68)	4.09 (1.31 – 6.87)
omicron BA2	2.14 (0.96 – 3.32)	2.99 (0.93 – 5.05)
lambda	1.73 (1.24 – 2.20)	2.03 (1.29 – 2.76)
mu	3.01 (1.60 – 4.42)	3.62 (1.25 – 5.99)

Median fold change in reduction towards WT for all other variants included within the MULTICOV-AB assay. The IQR is shown in brackets. Values are shown for both vaccinated (n=226) and convalescent (n=86) samples. WT – wild-type (B.1. isolate).

Supplementary Table 2: **Median fold reduction in IgG binding capacity from omicron BA1 to respective variant**

Group	Vaccinated	Convalescent
WT	2.32 (0.96 – 3.68)	4.09 (1.31 – 6.87)
alpha	1.91 (0.85 – 2.96)	3.16 (0.40 – 5.91)
beta	0.83 (0.62 – 1.04)	0.99 (0.67 – 1.32)
gamma	1.39 (0.97 – 1.81)	1.76 (1.07 – 2.45)
delta	1.91 (1.03 – 2.80)	3.00 (1.27 – 4.73)
omicron BA1	1	1
omicron BA2	1.10 (1.03 – 1.17)	1.18 (1.02 – 1.34)
lambda	1.31 (0.79 – 1.83)	1.69 (0.73 – 2.66)
mu	0.78 (0.57 – 1.00)	0.94 (0.50 – 1.38)

Median fold change in reduction towards omicron BA1 for all other variants included within the MULTICOV-AB assay. The IQR is shown in brackets. Values are shown for both vaccinated (n=226) and convalescent (n=86) samples. WT – wild-type (B.1. isolate).

Supplementary Table 3: **Median fold reduction in IgG binding capacity from omicron BA2 to respective variant**

Group	Vaccinated	Convalescent
WT	2.13 (0.95 – 3.32)	2.99 (0.93 – 5.05)
alpha	1.72 (0.86 – 2.58)	2.47 (0.53 – 4.41)
beta	0.76 (0.58 – 0.93)	0.84 (0.63 – 1.04)
gamma	1.26 (0.91 – 1.61)	1.45 (0.90 – 2.00)
delta	1.71 (1.00 – 2.43)	2.30 (0.87 – 3.74)
omicron BA1	0.91 (0.85 – 0.97	0.85 (0.73 – 0.96)
omicron BA2	1	1
lambda	1.18 (0.74 – 1.62)	1.32 (0.62 – 2.01)
mu	0.72 (0.54 – 0.90)	0.80 (0.52 – 1.09)

Median fold change in reduction towards omicron BA2 for all other variants included within the MULTICOV-AB assay. The IQR is shown in brackets. Values are shown for both vaccinated (n=226) and convalescent (n=86) samples. WT – wild-type (B.1. isolate).

Supplementary Table 4: **Statistical significance between variants for IgG binding capacity**

	WT	alpha	beta	gamma	delta	omicron BA1	omicron BA2	lambda	mu
WT		2.37×10^{-50}	2.50×10^{-55}	4.75×10^{-54}	1.76×10^{-49}	4.72×10^{-55}	6.86×10^{-54}	3.06×10^{-55}	2.35×10^{-55}
alpha			7.46×10^{-54}	1.88×10^{-53}	7.87×10^{-4}	1.56×10^{-54}	1.05×10^{-52}	3.75×10^{-55}	2.35×10^{-55}
beta				6.05×10^{-54}	2.35×10^{-55}	8.75×10^{-21}	1.06×10^{-39}	9.28×10^{-53}	6.44×10^{-10}
gamma					2.74×10^{-49}	1.55×10^{-52}	7.59×10^{-45}	4.08×10^{-16}	3.34×10^{-54}
delta						4.46×10^{-55}	1.61×10^{-52}	2.35×10^{-55}	2.35×10^{-55}
omicron BA1							1.26×10^{-50}	6.04×10^{-44}	5.68×10^{-24}
omicron BA2								6.01×10^{-23}	4.47×10^{-39}
lambda									2.14×10^{-53}
mu									

Statistical analysis as determined by Wilcoxon Signed Rank between all variants for IgG binding capacity. WT – wild-type (B.1. isolate).

Supplementary Table 5: **Median ACE2 binding inhibition of the respective variant**

Group	Vaccinated	Convalescent
WT	26.11 (38.7)	27.25 (45.55)
alpha	20.63 (30.37)	30.23 (36.32)
beta	11.38 (18.48)	8.87 (14.52)
gamma	10.81 (17.34)	9.41 (17.19)
delta	16.67 (27.97)	15.72 (34.75)
omicron BA1	15.26 (11.26)	16.44 (11.54)
omicron BA2	8.03 (9.92)	10.88 (12.65)
lambda	18.79 (33.09)	16.13 (27.63)
mu	9.12 (15.08)	8.07 (12.16)

Median ACE2 binding inhibition for all RBD variants included within the RBDCoV-ACE2 assay. The IQR is shown in brackets. Values are shown for both vaccinated (n=226) and convalescent (n=86) samples. WT – wild-type (B.1. isolate).

Supplementary Table 6**: Responder rate for different variants**

	WT	alpha	beta	gamma	delta	BA1	BA2	lambda	mu
Infected	54 (63)	51 (59)	19 (22)	22 (26)	38 (44)	28 (33)	12 (14)	34 (40)	17 (20)
Vaccinated	142 (63)	116 (51)	70 (31)	68 (30)	98 (43)	68 (30)	19 (8)	107 (47)	51 (23)
Negative	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Samples were classified as responsive based on having an ACE2 binding inhibition greater than 20% within the RBDCoV-ACE2 assay. Number of samples classified as responsive is shown, with this displayed as a percentage of all samples of this type in brackets. Values are shown for both vaccinated (n=226), convalescent (n=86) and negative (n=15) samples. WT – wild-type (B.1. isolate).

Supplementary Table 7: **Statistical significance between variants for ACE2 binding inhibition**

	WT	alpha	beta	gamma	delta	omicron BA1	omicron BA2	lambda	mu
WT		0.04	8.40×10^{-17}	7.22×10^{-18}	2.12×10^{-6}	3.99×10^{-11}	5.99×10^{-29}	4.00×10^{-5}	2.76×10^{-23}
alpha			2.58×10^{-12}	3.44×10^{-13}	2.24×10^{-3}	6.88×10^{-6}	8.97×10^{-24}	0.02	8.16×10^{-19}
beta				0.77	2.77×10^{-4}	1.20×10^{-6}	0.02	1.70×10^{-5}	0.04
gamma					8.97×10^{-5}	5.61×10^{-7}	0.03	4.61×10^{-6}	0.09
delta						0.53	1.12×10^{-10}	0.50	5.10×10^{-8}
omicron BA1							1.46×10^{-20}	0.15	2.92×10^{-13}
omicron BA2								8.82×10^{-13}	0.95
lambda									1.12×10^{-9}
mu									

Statistical analysis as determined by Wilcoxon Signed Rank between all variants for ACE2 binding inhibition. WT – wild-type (B.1. isolate).

Supplementary Table 8: **Summary of the binding response and dissociation constant (k_{off}) of the individual serum samples.**

	Response [nm]				**Dissociation constant k_{off} [10^{-5} s^{-1}]**			
	RBD_{wt}	**RBD_{δ}**	**RBD_{oBA1}**	**RBD_{oBA2}**	**RBD_{wt}**	**RBD_{δ}**	**RBD_{oBA1}**	**RBD_{oBA2}**
Vac1	0.175 ± 0.008	0.154 ± 0.018	0.113 ± 0.002	0.134 ± 0.007	0.010 ± 0.000	0.010 ± 0.000	6.565 ± 1.280	8.205 ± 2.326
Vac2	0.143 ± 0.005	0.104 ± 0.005	0.089 ± 0.004	0.105 ± 0.008	3.895 ± 0.148	7.880 ± 1.824	10.405 ± 0.841	9.965 ± 1.605
Vac3	0.186 ± 0.011	0.139 ± 0.002	0.117 ± 0.000	0.101 ± 0.004	0.010 ± 0.000	0.010 ± 0.000	8.660 ± 0.014	14.950 ± 2.192
Vac4	0.741 ± 0.034	0.531 ± 0.020	0.227 ± 0.003	0.254 ± 0.003	3.080 ± 0.170	6.050 ± 0.269	6.325 ± 0.375	6.420 ± 0.141
Vac5	0.908 ± 0.019	0.791 ± 0.045	0.596 ± 0.066	0.562 ± 0.038	0.010 ± 0.000	2.200 ± 0.099	4.165 ± 0.120	3.110 ± 0.863
Inf1	0.439 ± 0.009	0.425 ± 0.072	0.059 ± 0.004	0.044 ± 0.003	0.010 ± 0.000	0.181 ± 0.242	0.010 ± 0.000	0.010 ± 0.000
Inf2	0.565 ± 0.015	0.456 ± 0.011	0.103 ± 0.006	0.092 ± 0.007	16.700 ± 0.141	7.630 ± 0.184	0.010 ± 0.000	7.125 ± 1.167
Inf3	0.399 ± 0.072	0.581 ± 0.099	0.126 ± 0.025	0.128 ± 0.014	5.635 ± 0.502	10.050 ± 0.071	4.105 ± 1.082	9.115 ± 0.092
Inf4	0.534 ± 0.019	0.380 ± 0.039	0.060 ± 0.002	0.067 ± 0.001	18.750 ± 0.212	32.350 ± 0.354	5.185 ± 3.882	7.245 ± 0.926
Inf5	1.094 ± 0.025	0.267 ± 0.010	0.149 ± 0.014	0.174 ± 0.000	18.800 ± 0.283	4.315 ± 0.516	6.240 ± 1.541	8.025 ± 2.157

Binding kinetics of serum samples from (n = 5, Vac) and convalescent (n =5, Inf) individuals to the different RBD variants were analyzed using BLI. Binding response and dissociation constant (k_{off}) determined by the 1:1 fitting model of the individual serum samples were summarized as mean ± SD.

Supplementary Table 9: **Differences in response rate towards the Spike and RBD for BA1 and WT**

	Spike WT	Spike BA1	RBD WT	RBD BA1
Convalescent	37 (43)	3 (3)	54 (63)	28 (33)
Vaccinated	78 (35)	11 (5)	142 (63)	68 (30)
Negative	0 (0)	0 (0)	0 (0)	0 (0)

Samples were classified as responsive based on having an ACE2 binding inhibition greater than 20% within the RBDCoV-ACE2 assay. Number of samples classified as responsive is shown, with this displayed as a percentage of all samples of this type in brackets. Values are shown for both vaccinated (n=226), convalescent (n=86) and negative (n=15) samples. WT – wild-type (B.1. isolate).

References

1. Becker M, Strengert M, Junker D, Kaiser PD, Kerrinnes T, Traenkle B, et al. Exploring beyond clinical routine SARS-CoV-2 serology using MultiCoV-Ab to evaluate endemic coronavirus cross-reactivity. Nature Communications. 2021;12(1):1152.

2. Junker D, Dulovic A, Becker M, Wagner TR, Kaiser PD, Traenkle B, et al. COVID-19 patient serum less potently inhibits ACE2-RBD binding for various SARS-CoV-2 RBD mutants. medRxiv. 2021:2021.08.20.21262328.

3. Becker M, Dulovic A, Junker D, Ruetalo N, Kaiser PD, Pinilla YT, et al. Immune response to SARS-CoV-2 variants of concern in vaccinated individuals. Nature Communications. 2021;12(1):3109.