bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

1 **Title:** SARS-CoV-2 evolution and patient immunological history shape the breadth and
2 potency of antibody-mediated immunity
3

4 **Authors:** Maria Manali[1*], Laura A. Bissett[1*], Julien A. R. Amat[1,2], Nicola Logan[1], Sam Scott[1],
5 Ellen C. Hughes[1], William T. Harvey[1], Richard Orton[1], Emma C. Thomson[1], Rory N.
6 Gunson[3], Mafalda Viana[4#], Brian Willett[1#] and Pablo R Murcia[1#].
7
8 **Affiliations:**
9
10 [1] MRC-University of Glasgow Centre for Virus Research. Glasgow, United Kingdom.
11 [2] School of Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University
12 of Glasgow, Glasgow, United Kingdom.
13 [3] West of Scotland Specialist Virology Centre, NHS Greater Glasgow and Clyde, Glasgow,
14 United Kingdom.
15 [4] Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow,
16 Glasgow, United Kingdom.
17
18
19 * Equal contribution
20 # Co-corresponding authors
21
22 **Short title:** SARS-CoV-2 exposure history shapes antibody-mediated immunity
23

24 **Keywords:** SARS-CoV-2; immunological history; antibody-mediated immunity; virus
25 neutralisation, virus evolution.
26

27 **Abstract**
28
29 Since the emergence of SARS-CoV-2, humans have been exposed to distinct SARS-CoV-2
30 antigens, either by infection with different variants, and/or vaccination. Population immunity is
31 thus highly heterogeneous, but the impact of such heterogeneity on the effectiveness and
32 breadth of the antibody-mediated response is unclear. We measured antibody-mediated
33 neutralisation responses against SARS-CoV-2_{Wuhan}, SARS-CoV-2α, SARS-CoV-2δ and
34 SARS-CoV-2o pseudoviruses using sera from patients with distinct immunological histories,
35 including naive, vaccinated, infected with SARS-CoV-2_{Wuhan}, SARS-CoV-2α or SARS-CoV-2δ,
36 and vaccinated/infected individuals. We show that the breadth and potency of the antibody-
37 mediated response is influenced by the number, the variant, and the nature (infection or
38 vaccination) of exposures, and that individuals with mixed immunity acquired by vaccination
39 and natural exposure exhibit the broadest and most potent responses. Our results suggest
40 that the interplay between host immunity and SARS-CoV-2 evolution will shape the
41 antigenicity and subsequent transmission dynamics of SARS-CoV-2, with important
42 implications for future vaccine design.
43

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

44 **Author Summary**

45 Neutralising antibodies provide protection against viruses and are generated because of
46 vaccination or prior infections. The main target of anti-SARS-CoV-2 neutralising antibodies is
47 a protein called Spike, which decorates the viral particle and mediates viral entry into cells. As
48 SARS-CoV-2 evolves, mutations accumulate in the spike protein, allowing the virus to escape
49 antibody-mediated immunity and decreasing vaccine effectiveness. Multiple SARS-CoV-2
50 variants have appeared since the start of the COVID-19 pandemic, causing various waves of
51 infection through the population and infecting -in some cases- people that had been previously
52 infected or vaccinated. Since the antibody response is highly specific, individuals infected with
53 different variants are likely to have different repertoires of neutralising antibodies. We studied
54 the breadth and potency of the antibody-mediated response against different SARS-CoV-2
55 variants using sera from vaccinated people as well as from people infected with different
56 variants. We show that potency of the antibody response against different SARS-CoV-2
57 variants depends on the particular variant that infected each person, the exposure type
58 (infection or vaccination) and the number and order of exposures. Our study provides insight
59 into the interplay between virus evolution and immunity, as well as important information for
60 the development of better vaccination strategies.

61 **Introduction**

62 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in December
63 2019[1] causing the largest pandemic of the XXI century. Since the start of the pandemic,
64 different viral lineages emerged, exhibiting highly dynamic transmission patterns[2-5]. This is
65 illustrated by the epidemiology of COVID-19 in the United Kingdom since the first introduction
66 of SARS-CoV-2 in late February 2020, when the Wuhan strain (SARS-CoV-2_W) was first
67 reported. A D614G variant of the Wuhan strain circulated almost exclusively until September
68 of the same year, when the alpha strain (SARS-CoV-2α) appeared, initially in the Southeast
69 of England[2]. By December 2020, SARS-CoV-2α was the dominating variant. At this point in
70 time, the UK started a COVID-19 vaccination program that took place with unprecedent pace:
71 by July 1st ,2021, ~47 million people (mainly adults) had received at least one vaccine dose[6].
72 However, during this period, a new variant (delta, SARS-CoV-2δ) emerged in India, reaching
73 the UK in April 2021, and quickly became the most prevalent lineage, until November 2021,
74 when the omicron variant (SARS-CoV-2o) was introduced and quickly replaced SARS-CoV-
75 2δ. Over a period of approximately 2 years (February 2020 to March 2022) the UK population
76 experienced four COVID-19 pandemic waves, each of them caused by a different SARS-CoV-
77 2 variant (Wuhan, alpha, delta and omicron). In addition, SARS-CoV-2δ and SARS-CoV-2o
78 infections have been reported in individuals that had been previously vaccinated or infected

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

79 by preceding variants[7, 8]. As a result, population immunity against SARS-CoV-2 is likely to
80 be highly heterogeneous. The impact of such immunological heterogeneity on SARS-CoV-2
81 fitness is far from clear. As antibody-mediated immunity is considered a correlate of
82 protection[9, 10], identifying the factors that affect the humoral immune response is key for
83 COVID-19 preparedness and to design more effective vaccines. Our overall objective was to
84 quantify the breadth and potency of antibodies elicited by different immune histories against
85 distinct SARS-CoV-2 variants. To this end, we measured antibody-mediated immunity against
86 SARS-CoV-2_W, SARS-CoV-2α, SARS-CoV-2δ, and SARS-CoV-2o using convalescent serum
87 samples from the Glasgow patient population collected between March 31st, 2020, and
88 September 22nd, 2021. Our sampling strategy captured the complex immunological landscape
89 described above and included sera from naive, vaccinated, infected, as well as vaccinated
90 and infected individuals. Importantly, by combining patient metadata (date of positive PCR)
91 with virus genomic epidemiology (prevalence of circulating lineages over time) we were able
92 to select confidently serum samples from patients exposed to three major variants that
93 circulated in the UK (Wuhan, alpha and delta).

94

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

95 **Results**

96 A schematic description of the study is shown in Fig 1. Serum samples (n=353) from
97 biobanked material that had been collected for serological surveillance studies[11] were
98 selected. The immunological history of each patient at the time of sampling was compiled
99 based on serological status using an ELISA assay that tested for SARS-CoV-2 spike 1 (S1)
100 and receptor binding domain (S-RBD)[11] together with metadata associated with each clinical
101 specimen. Associated metadata consisted of date of serum collection, SARS-CoV-2 PCR
102 status (including date and result of diagnosis) and vaccination status (including date of
103 vaccination and number of doses). Samples were initially classified in four broad groups: *naive*
104 (N, 30 samples), *vaccinated* (V, 55 samples) *infected* (I, 91 samples) and *infected and*
105 *vaccinated* (I_V, 177 samples). Further, sera from the I and I_V group were stratified based on
106 the infecting variant as *infected-Wuhan* (I_W, 37 samples), *infected-alpha* ($I\alpha$, 39 samples),
107 *infected-delta* ($I\delta$, 15 samples), *infected-Wuhan-vaccinated* (I_{WV}, 60 samples), *infected-alpha-*
108 *vaccinated* ($I\alpha_V$, 69 samples), and *infected-delta-vaccinated* ($I\delta_V$, 48 samples). The date of
109 PCR confirmation and the prevalence of each variant at the time of diagnosis was used to
110 infer the most likely infecting variant (see materials and methods and S1 Fig). All serum
111 samples were processed as follows: first they were tested using a multiplex
112 electrochemiluminescence assay against the spike (S), and nucleocapsid (N) proteins to
113 quantify antibody concentrations. Further, serum samples were subjected to virus
114 neutralisation assays (VNAs)[11] at a fixed dilution (1:50) using HIV (SARS-CoV-2)
115 pseudotypes carrying the S glycoprotein of either SARS-CoV-2_W, SARS-CoV-2α, SARS-CoV-
116 2δ, or SARS-CoV-2o. Every sample that displayed 50% neutralisation to at least one SARS-
117 CoV-2 variant was subject to antibody titration as previously described [12].

118 Quantification of S and N antibody levels for each group of patients is shown in Fig 2. As
119 expected, sera from patients in the naive group (neither vaccinated nor infected) exhibited the
120 lowest levels of anti-S antibodies because they had not been exposed to the Spike antigen of
121 SARS-CoV-2. Patients that had been infected displayed higher levels of anti-S antibodies than
122 vaccinated ones, whereas both were significantly lower to those observed in sera from patients
123 that had been infected and vaccinated. Sera from patients that had been infected possessed
124 higher levels of anti-N antibodies than those that had been infected and vaccinated (Fig 2). Of
125 note, vaccinated patients had lower levels of anti-N than naive individuals. Overall, these
126 results are consistent with previous reports suggesting that exposure to SARS-CoV-2 antigens
127 by vaccination and infection results in higher levels of anti-SARS-CoV-2 antibodies than
128 vaccination alone [13-15].

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

129 We next measured the neutralisation activity of each serum sample at a fixed dilution against
130 SARS-CoV-2_W, SARS-CoV-2α, SARS-CoV-2δ, and SARS-CoV-2o using virus pseudotypes.
131 The efficiency of neutralisation varied depending on the SARS-CoV-2 variant tested (Fig 3A),
132 and the immunological history of the patients (Fig 3B). When the chronological order of
133 appearance of each variant is considered, a pattern of neutralisation reduction consistent with
134 antigenic drift emerges. This is illustrated by the ladder-like distribution of the median
135 percentage neutralisation (Fig 3A) and becomes even more evident when neutralisation levels
136 are compared between SARS-CoV-2o and all the other variants, as the former, more evolved
137 S, is neutralised less effectively. A similar trend of neutralisation reduction is observed
138 between SARS-CoV-2_W and SARS-CoV-2δ in the V and I_V groups (Fig 3A). We also observed
139 that virus neutralisation efficiency increases depending on the number and type of exposures,
140 irrespective of the variant tested (Fig 3B). As a result, the I_V group exhibited the highest
141 neutralisation values against all variants (Fig 3B).

142 To quantify more accurately the neutralising potency of the antibody-mediated response
143 among the four broad groups (N, V, I, I_V), we titrated neutralising antibodies against each
144 variant. Consistent with our previous results, neutralising titres significantly decreased as
145 SARS-CoV-2 evolved (Fig 4A). Indeed, the aforementioned "ladder-like" effect was even more
146 evident. Also consistent with our previous results, the number and type of antigen exposure
147 events had a significant impact on virus neutralisation titres (Fig 4B). Patients derived from
148 the I_V group displayed significantly higher neutralising antibody titres compared to every other
149 group across all variants (Fig 4B). In turn, infected patients exhibited variable titres against
150 each variant when compared to vaccinated patients: for example, differences between these
151 two groups were non-significant when SARS-CoV-2_W, SARS-CoV-2α and SARS-CoV-2δ were
152 compared. However, patients from the V group displayed significantly higher antibody titres
153 against SARS-CoV-2o, albeit neutralisation efficiency was still very low. Collectively, these
154 results suggest that SARS-CoV-2 antigenic evolution is directional (SARS-CoV-2 evolved to
155 escape antibody-mediated immunity) and that the number and type of exposure events affect
156 the breadth and potency of the antibody-mediated response.

157 To understand better how humoral immunity is affected by the antigenicity of SARS-CoV-2
158 variants, we stratified the I and I_V groups according to the strains that had infected the patients.
159 This analysis revealed a trend consistent with homologous immunity as sera from patients that
160 had been infected with SARS-CoV-2_W, SARS-CoV-2α or SARS-CoV-2δ displayed highest
161 potency against their infecting variants, albeit differences were not always statistically
162 significant (Fig 5A). Notably, patients that had been vaccinated and infected with SARS-CoV-
163 2δ showed overall the highest neutralisation potency against all variants (Fig 5B). As this group
164 of patients had been exposed to the most phylogenetically distant antigens (SARS-CoV-2_W by

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

165 vaccination and SARS-CoV-2δ by infection), this result suggests not only that heterologous
166 exposure results in a broader and more effective humoral response but also that the level of
167 antigenic differences between variants affects the potency of the antibody-mediated response.
168 In addition, we observed some differences among patients that had been vaccinated and
169 infected with each SARS-CoV-2 variant: for example, patients that had been infected with
170 SARS-CoV-2α exhibited lower neutralisation efficiency against SARS-CoV-2δ than against
171 SARS-CoV-2_W or SARS-CoV-2α (Fig 5A). Titration of neutralising antibodies enabled us to
172 quantify neutralisation biases towards specific SARS-CoV-2 variants. Generally, patients
173 infected by specific variants exhibited significantly different neutralising titres against other
174 SARS-CoV-2 variants (Fig 6A and B) and this effect was also evident among vaccinated and
175 infected patients. For example, patients infected with SARS-CoV-2δ exhibited high levels of
176 neutralising antibodies against SARS-CoV-2δ but significantly lower titres against all other
177 variants (Fig 6A), whereas patients infected with SARS-CoV-2_W or SARS-CoV-2α displayed
178 similar levels of neutralising antibodies against SARS-CoV-2_W and SARS-CoV-2α but lower
179 levels against SARS-CoV-2δ and even lower against SARS-CoV-2o (Fig 6A). Overall, titres
180 in patients that had been infected only were lower to those measured in patients that had been
181 infected and vaccinated (Fig 6A and B). The only exception was observed in sera from patients
182 infected with SARS-CoV-2δ, whose neutralising antibody titres against the homologous
183 antigen was similar in vaccinated and infected patients (Fig 6B). Neutralising antibody
184 responses seemed to display immunological preferences: for example, patients that had been
185 vaccinated and infected with SARS-CoV-2δ exhibited significantly higher neutralisation levels
186 against SARS-CoV-2_W (the vaccine variant) than SARS-CoV-2δ (the infecting variant),
187 suggesting the stimulation of an anamnestic response. In contrast, patients that had been
188 vaccinated but infected with SARS-CoV-2α showed similar neutralising antibody titres against
189 SARS-CoV-2_W and SARS-CoV-2α. Of note, when neutralising antibody titres were compared
190 across all patient groups, those vaccinated and infected with SARS-CoV-2δ or infected with
191 SARS-CoV-2_W and then vaccinated, displayed the highest titres against all variants (Fig 6B),
192 consistent with the notion that immunity conferred via infection *and* vaccination results in
193 broader and more potent humoral responses.

194 As the I_V group exhibited the highest antibody titres against all variants, we wanted to
195 determine if the order in which patients were exposed to SARS-CoV-2 (either vaccination first
196 or infection first) played any role in the breadth and potency of antibody mediated
197 neutralisation. To test this, we focused on the $I\alpha_V$ group, which exhibited a similar number of
198 patients that had been either infected first (n=28) or vaccinated first (n=41). Sera from patients
199 that had been infected first and then vaccinated exhibited significantly higher neutralisation
200 efficiency against every variant (Fig 7A) and also higher titres of neutralising antibodies (Fig

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

201 7B). Overall, this result highlights that the type of exposure (vaccination or infection) and the
202 order in which different types of exposure occur have a significant impact on the breadth and
203 potency of humoral immunity against SARS-CoV-2.

204 **Discussion**

205 Our study shows that the immunological landscape of SARS-CoV-2 is highly heterogeneous
206 and has been shaped by the complex interplay between host immunity and virus evolution.
207 Infection by, or vaccination against, SARS-CoV-2 does not elicit lifelong immunity[16, 17] but
208 instead result in a variety of immune phenotypes, which are likely to influence both
209 transmission dynamics and disease outcomes. We demonstrate that multiple factors influence
210 the breadth and potency of the antibody-mediated response against SARS-CoV-2 and include
211 antigenicity of the exposing pathogen, number of exposures, and exposure type (infection
212 and/or vaccination). While T-cell responses play an important role in SARS-CoV-2
213 immunity[18], we could not evaluate the impact of cellular-mediated immunity due to the nature
214 of our samples (i.e., sera). In line with previous studies, we show that mutations that appeared
215 during SARS-CoV-2 evolution reduce antibody mediated neutralisation[19, 20], suggesting
216 that evolution of the spike gene of SARS-CoV-2 is directional and driven by immune selection.
217 This is consistent with reports of reinfections by novel variants[21, 22]. Our results showing
218 that all serum samples exhibited lowest neutralising activity against pseudoviruses carrying
219 the spike glycoprotein of SARS-CoV-2o (Figs 4A and 6A) support this view. As all currently
220 licenced vaccine preparations express the S glycoprotein of SARS-CoV-2_W, it is expected that
221 the risk of reinfections in vaccinated-only individuals increases as SARS-CoV-2 evolves.
222 Similarly, for infected-only individuals, the risk of reinfection will likely increase as the antigenic
223 distance between the viruses involved in primary and secondary infection increases, and thus
224 is a function of time. Our results also show that more exposure events result in broader and
225 more potent antibody-mediated responses (Figs 3B and 5B), and this protective effect is also
226 influenced by the antigenic nature of the viruses involved in the primary infection (or
227 vaccination) and subsequent infections. This finding suggests that updates of the vaccine
228 strains (or development of multivalent vaccines) will improve protection against evolving
229 variants, and also that increased transmission of antigenically divergent SARS-CoV-2 viruses
230 among previously exposed individuals will result in future higher levels of protection. We also
231 show that primary infection followed by vaccination results in more potent humoral responses
232 (Fig 7), which indicates that the type and order of exposure events have a significant impact
233 on the breadth and potency of the antibody mediated response. These findings are consistent
234 with recent reports [13, 14, 23]. While it is not advisable to promote the acquisition of SARS-
235 CoV-2 immunity by natural infection given the risk of severe disease and/or death due to

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

236 COVID-19 in naive individuals, our results suggest that vaccines based on live-attenuated
237 viruses might provide increased protection.

238 In sum, our work underscores the complexity of the immunological landscape of SARS-CoV-
239 2. While our results will inform the development of better epidemiological models to predict
240 the future transmission dynamics of SARS-COV-2[24], further clinical studies are needed to
241 determine the impact of exposure history on disease presentation to prepare better for the
242 future disease burden of COVID-19 as this disease becomes endemic.

243 **Materials and Methods**

244 **Ethics statement.** Ethical approval was provided by NHSGGC Biorepository (application
245 550).

246 **Serum samples**. Random residual biochemistry serum samples (~41,000) from primary
247 (general practices) and secondary (hospitals) healthcare settings were collected by the
248 NHSGGC Biorepository between the 31st of March 2020 and 22nd of September 2021.
249 Associated metadata included date of sample collection, date of positive PCR result, date of
250 first and second vaccination and vaccine manufacturer. Seronegative samples were selected
251 based on their ELISA results (SARS-CoV-2 S1 or SARS-CoV-2 RBD) and the absence of a
252 positive PCR test result or record of vaccination. Samples from vaccinated patients were
253 selected based on their ELISA result (SARS-CoV-2 S1 or SARS-CoV-2 RBD), record of
254 vaccination with 1 or 2 doses at least 14 days prior to blood collection and absence of a
255 positive PCR test result for at least 14 days after sampling. Samples from infected patients
256 had no record of vaccination, were ELISA positive (SARS-CoV-2 S1 or SARS-CoV-2 RBD)
257 and had a positive PCR test result at least 14 days before sample collection. Samples from
258 infected and vaccinated patients were identified by their positive ELISA result, the presence
259 of positive PCR test result and record of vaccination with 1 or 2 doses at least 14 days prior
260 to blood collection. Samples from infected and infected and vaccinated patients were further
261 stratified to infecting variants (based on the date of the positive PCR test), by identifying key
262 time periods during which each variant was most predominant. All serum samples were
263 inactivated at 56ºC for 30 minutes before being tested.

264 **Cells.** HEK293T and 293-ACE2 cells were maintained at 37°C, 5% CO_2, in Dulbecco's
265 modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum, 2mM L-
266 glutamine, 100µg/ml streptomycin and 100 IU/ml penicillin. HEK293 cells were used to
267 produce HEK293-ACE2 target cells by stable transduction with pSCRPSY-hACE2 and were
268 maintained in complete DMEM supplemented with 2µg/ml puromycin. HEK293T cells were
269 used for the generation of HIV(SARS-CoV-2) pseudotypes.

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

270 **IgG quantification.** IgG antibodies against SARS-CoV-2 the spike and nucleocapsid proteins
271 were measured using an MSD V-PLEX COVID-19 Coronavirus Panel 2 (K15369) kit. Multiplex
272 Meso Scale Discovery electrochemiluminescence (MSD-ECL) assays were performed
273 according to the manufacturer's instructions. Briefly, 96-well plates were blocked at room
274 temperature for at least 30 minutes. Plates were then washed; samples were diluted 1:5000
275 and added to the plates along with serially diluted reference standard and serology controls.
276 Plates were incubated for two hours and further washed. SULFO-TAG detection antibody was
277 added, and plates were incubated for one hour. After incubation, plates were washed and read
278 using a MESO Sector S 600 plate reader. Data were generated by Methodological Mind
279 software and analysed using MSD Discovery Workbench (v4.0). Results were normalised to
280 standard(s) and expressed as MSD arbitrary units per ml (AU/ml).

281 **Neutralisation assays.** Pseudotype-based neutralisation assays were carried out as
282 described previously[11]. HEK293T cells were transfected with the appropriate SARS-CoV-2
283 Spike gene expression vector (Wuhan, Alpha, Delta, or Omicron) together with p8.9171 and
284 pCSFLW72 using polyethylenimine (PEI, Polysciences, Warrington, USA). HIV (SARS-CoV-
285 2) pseudotype-containing supernatants were harvested 48 hours post-transfection, aliquoted
286 and frozen at -80°C prior to use. Gene constructs bearing the Wuhan (D614G), Alpha
287 (B.1.1.7), Delta (B.1.617.2) and Omicron (B.1.1.529) Spike genes were based on the codon-
288 optimised spike sequence of SARS-CoV-2 and generated by GenScript Biotech. Constructs
289 bore the following mutations relative to the Wuhan-Hu-1 sequence (GenBank: MN908947):
290 Wuhan(D614G) – D614G; Omicron (BA.1, B.1.1.529) - A67V, Δ69-70, T95I, G142D/Δ143-
291 145, Δ211/L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S,
292 S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y,
293 N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F; Alpha (B.1.1.7)- L18F, Δ69-
294 70, Δ144, N501Y, A570D, P681H, T716I, S982A, D1118H; Delta (B.1.617.2)- T19R, G142D,
295 Δ156-157, R158G, L452R, T478K, D614G, P681R, D950N.

296 Neutralisation efficiency was measured first using a fixed dilution of serum samples in
297 duplicates. Samples with neutralising activity ≥ 50% relative to the no serum control were then
298 titrated by serial dilutions. Each sample was serially diluted in triplicate from 1:50 to 1:36450
299 in complete DMEM, incubated for 1 hour with HIV (SARS-CoV-2) pseudotypes, and plated
300 onto HEK239-ACE2 target cells. After 48 hours, luciferase activity was measured by adding
301 Steadylite Plus chemiluminescence substrate and analysed using a Perkin Elmer EnSight
302 multimode plate reader. Antibody titres were estimated by interpolating the point at which
303 infectivity had been reduced to 50% of the value for the no serum control samples. Samples
304 that did not have an antibody titre were arbitrary assigned a value of 50 (the lowest dilution
305 available).

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

306 **Statistical analysis.** Shapiro-Wilk tests were performed to assess data homoscedasticity. As
307 data were found not to be normally distributed, non-parametric pairwise Wilcoxon Rank Sum
308 tests were carried out to assess statistically significant differences in antibody levels between
309 groups and viruses. Holm's method was used to adjust p-values to account for multiple
310 statistical comparisons. Separate tests were performed for each group when comparing
311 between viruses, and for each virus when comparing between groups. Pairwise comparisons
312 were presented as connected dotplots, highlighting the significance levels of each of the paired
313 comparisons. All analyses and data visualisations were executed using the stats[25] and
314 ggplot2[26] packages respectively, from R version 4.0.5.

315 **Data availability.** Data of each sample, including metadata and results of each assays, are
316 included in S1 Appendix.

317 **Acknowledgements.** We thank Massimo Palmarini for scientific advice and the NHS Greater
318 Glasgow and Clyde Biorepository for providing serum samples.

319
320 **References**
321

322 1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from
323 Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-33. Epub
324 2020/01/25. doi: 10.1056/NEJMoa2001017. PubMed PMID: 31978945; PubMed Central
325 PMCID: PMCPMC7092803.
326 2. Davies NG, Abbott S, Barnard RC, Jarvis CI, Kucharski AJ, Munday JD, et al.
327 Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science.
328 2021;372(6538). Epub 2021/03/05. doi: 10.1126/science.abg3055. PubMed PMID:
329 33658326; PubMed Central PMCID: PMCPMC8128288.
330 3. Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, et al.
331 Detection of a SARS-CoV-2 variant of concern in South Africa. Nature.
332 2021;592(7854):438-43. Epub 2021/03/11. doi: 10.1038/s41586-021-03402-9. PubMed
333 PMID: 33690265.
334 4. Faria NR, Mellan TA, Whittaker C, Claro IM, Candido DDS, Mishra S, et al.
335 Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. Science.
336 2021;372(6544):815-21. Epub 2021/04/16. doi: 10.1126/science.abh2644. PubMed PMID:
337 33853970; PubMed Central PMCID: PMCPMC8139423.
338 5. Viana R, Moyo S, Amoako DG, Tegally H, Scheepers C, Althaus CL, et al. Rapid
339 epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. Nature. 2022.
340 Epub 2022/01/19. doi: 10.1038/s41586-022-04411-y. PubMed PMID: 35042229.
341 6. GOV.UK. Coronavirus (COVID-19) in the UK-Vaccinations. Available from:
342 https://coronavirus.data.gov.uk/details/vaccinations.
343 7. Pulliam JRC, van Schalkwyk C, Govender N, von Gottberg A, Cohen C, Groome MJ,
344 et al. Increased risk of SARS-CoV-2 reinfection associated with emergence of Omicron in
345 South Africa. Science. 2022:eabn4947. Epub 2022/03/16. doi: 10.1126/science.abn4947.
346 PubMed PMID: 35289632.
347 8. Pouwels KB, Pritchard E, Matthews PC, Stoesser N, Eyre DW, Vihta KD, et al.
348 Effect of Delta variant on viral burden and vaccine effectiveness against new SARS-CoV-2
349 infections in the UK. Nat Med. 2021;27(12):2127-35. Epub 2021/10/16. doi:

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

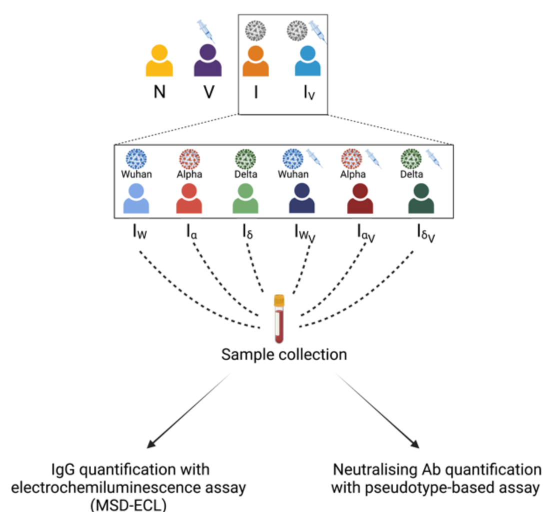
350 10.1038/s41591-021-01548-7. PubMed PMID: 34650248; PubMed Central PMCID:
351 PMCPMC8674129.
352 9. Earle KA, Ambrosino DM, Fiore-Gartland A, Goldblatt D, Gilbert PB, Siber GR, et
353 al. Evidence for antibody as a protective correlate for COVID-19 vaccines. Vaccine.
354 2021;39(32):4423-8. Epub 2021/07/03. doi: 10.1016/j.vaccine.2021.05.063. PubMed PMID:
355 34210573; PubMed Central PMCID: PMCPMC8142841.
356 10. Corbett KS, Nason MC, Flach B, Gagne M, O'Connell S, Johnston TS, et al. Immune
357 correlates of protection by mRNA-1273 vaccine against SARS-CoV-2 in nonhuman
358 primates. Science. 2021;373(6561):eabj0299. Epub 2021/09/17. doi:
359 10.1126/science.abj0299. PubMed PMID: 34529476; PubMed Central PMCID:
360 PMCPMC8449013.
361 11. Hughes EC, Amat JAR, Haney J, Parr YA, Logan N, Palmateer N, et al. Severe Acute
362 Respiratory Syndrome Coronavirus 2 Serosurveillance in a Patient Population Reveals
363 Differences in Virus Exposure and Antibody-Mediated Immunity According to Host
364 Demography and Healthcare Setting. J Infect Dis. 2021;223(6):971-80. Epub 2020/12/29.
365 doi: 10.1093/infdis/jiaa788. PubMed PMID: 33367847; PubMed Central PMCID:
366 PMCPMC7798933.
367 12. Davis C, Logan N, Tyson G, Orton R, Harvey WT, Perkins JS, et al. Reduced
368 neutralisation of the Delta (B.1.617.2) SARS-CoV-2 variant of concern following
369 vaccination. PLoS Pathog. 2021;17(12):e1010022. Epub 2021/12/03. doi:
370 10.1371/journal.ppat.1010022. PubMed PMID: 34855916; PubMed Central PMCID:
371 PMCPMC8639073.
372 13. Bates TA, McBride SK, Leier HC, Guzman G, Lyski ZL, Schoen D, et al.
373 Vaccination before or after SARS-CoV-2 infection leads to robust humoral response and
374 antibodies that effectively neutralize variants. Sci Immunol. 2022;7(68):eabn8014. Epub
375 2022/01/26. doi: 10.1126/sciimmunol.abn8014. PubMed PMID: 35076258.
376 14. Reynolds CJ, Gibbons JM, Pade C, Lin KM, Sandoval DM, Pieper F, et al.
377 Heterologous infection and vaccination shapes immunity against SARS-CoV-2 variants.
378 Science. 2022;375(6577):183-92. Epub 2021/12/03. doi: 10.1126/science.abm0811. PubMed
379 PMID: 34855510.
380 15. Walls AC, Sprouse KR, Bowen JE, Joshi A, Franko N, Navarro MJ, et al. SARS-
381 CoV-2 breakthrough infections elicit potent, broad, and durable neutralizing antibody
382 responses. Cell. 2022;185(5):872-80 e3. Epub 2022/02/07. doi: 10.1016/j.cell.2022.01.011.
383 PubMed PMID: 35123650; PubMed Central PMCID: PMCPMC8769922.
384 16. Chia WN, Zhu F, Ong SWX, Young BE, Fong SW, Le Bert N, et al. Dynamics of
385 SARS-CoV-2 neutralising antibody responses and duration of immunity: a longitudinal
386 study. Lancet Microbe. 2021;2(6):e240-e9. Epub 2021/03/30. doi: 10.1016/S2666-
387 5247(21)00025-2. PubMed PMID: 33778792; PubMed Central PMCID: PMCPMC7987301.
388 17. Thomas SJ, Moreira ED, Jr., Kitchin N, Absalon J, Gurtman A, Lockhart S, et al.
389 Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine through 6 Months. N Engl J
390 Med. 2021;385(19):1761-73. Epub 2021/09/16. doi: 10.1056/NEJMoa2110345. PubMed
391 PMID: 34525277; PubMed Central PMCID: PMCPMC8461570.
392 18. Moss P. The T cell immune response against SARS-CoV-2. Nat Immunol.
393 2022;23(2):186-93. Epub 2022/02/03. doi: 10.1038/s41590-021-01122-w. PubMed PMID:
394 35105982.
395 19. Ibarrondo FJ, Hofmann C, Ali A, Ayoub P, Kohn DB, Yang OO. Previous Infection
396 Combined with Vaccination Produces Neutralizing Antibodies with Potency against SARS-
397 CoV-2 Variants. mBio. 2021;12(6):e0265621. Epub 2021/12/08. doi: 10.1128/mBio.02656-
398 21. PubMed PMID: 34872349; PubMed Central PMCID: PMCPMC8649753.

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

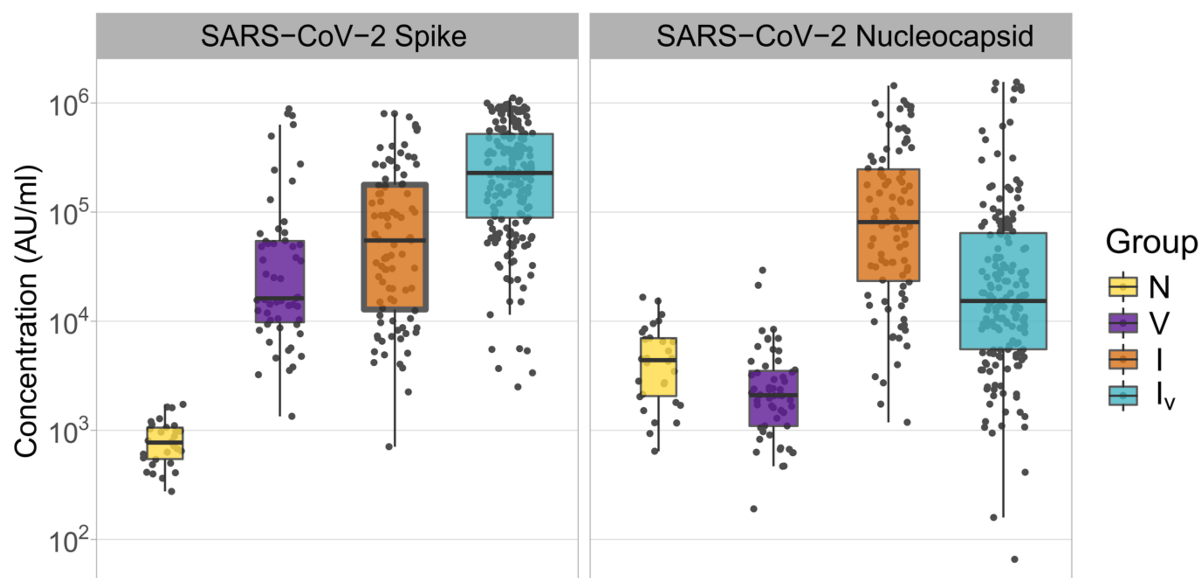
399 20. Cele S, Jackson L, Khoury DS, Khan K, Moyo-Gwete T, Tegally H, et al. Omicron
400 extensively but incompletely escapes Pfizer BNT162b2 neutralization. Nature.
401 2022;602(7898):654-6. Epub 2022/01/12. doi: 10.1038/s41586-021-04387-1. PubMed
402 PMID: 35016196; PubMed Central PMCID: PMCPMC8866126.
403 21. Levine-Tiefenbrun M, Yelin I, Alapi H, Katz R, Herzel E, Kuint J, et al. Viral loads
404 of Delta-variant SARS-CoV-2 breakthrough infections after vaccination and booster with
405 BNT162b2. Nat Med. 2021;27(12):2108-10. Epub 2021/11/04. doi: 10.1038/s41591-021-
406 01575-4. PubMed PMID: 34728830.
407 22. Mlcochova P, Kemp SA, Dhar MS, Papa G, Meng B, Ferreira I, et al. SARS-CoV-2
408 B.1.617.2 Delta variant replication and immune evasion. Nature. 2021;599(7883):114-9.
409 Epub 2021/09/07. doi: 10.1038/s41586-021-03944-y. PubMed PMID: 34488225; PubMed
410 Central PMCID: PMCPMC8566220.
411 23. Keeton R, Tincho MB, Ngomti A, Baguma R, Benede N, Suzuki A, et al. T cell
412 responses to SARS-CoV-2 spike cross-recognize Omicron. Nature. 2022. Epub 2022/02/02.
413 doi: 10.1038/s41586-022-04460-3. PubMed PMID: 35102311.
414 24. Saad-Roy CM, Wagner CE, Baker RE, Morris SE, Farrar J, Graham AL, et al.
415 Immune life history, vaccination, and the dynamics of SARS-CoV-2 over the next 5 years.
416 Science. 2020;370(6518):811-8. Epub 2020/09/23. doi: 10.1126/science.abd7343. PubMed
417 PMID: 32958581; PubMed Central PMCID: PMCPMC7857410.
418 25. Team RC. R: A language and environment for statistical computing. 2013.
419 26. Wickham H. ggplot2. Wiley Interdisciplinary Reviews: Computational Statistics.
420 2011;3(2):180-5.

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

422 **Figures**



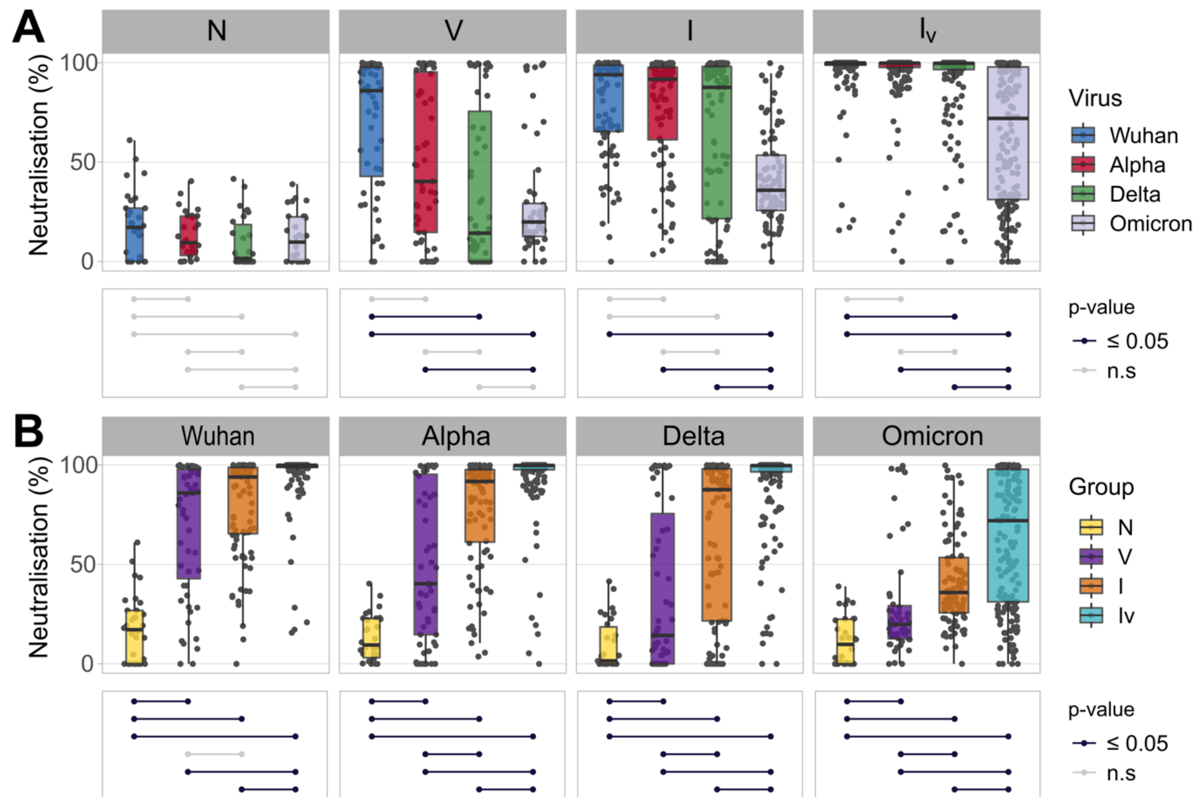
423 **Figure 1.** Schematic representation of the study design. Coloured silhouettes represent different patient
424 groups (N= naive, V= vaccinated, I= infected, I_V= infected and vaccinated). I and I_V were further stratified
425 based on the infecting strain (I_W= infected with SARS-CoV-2_W, $I\alpha$= infected with SARS-CoV-2α, $I\delta$=
426 infected with SARS-CoV-2δ, I_{WV}= vaccinated and infected with SARS-CoV-2_W, $I\alpha_V$= vaccinated and
427 infected with SARS-CoV-2α, $I\delta_V$= vaccinated and infected with SARS-CoV-2δ). Serum samples were
428 tested for the presence of IgG antibodies against SARS-CoV-2 S and N, and also tested in virus
429 neutralisation assays using pseudotyped viruses carrying the S glycoprotein of specific SARS-CoV-2
430 variants (see methods).
431



432

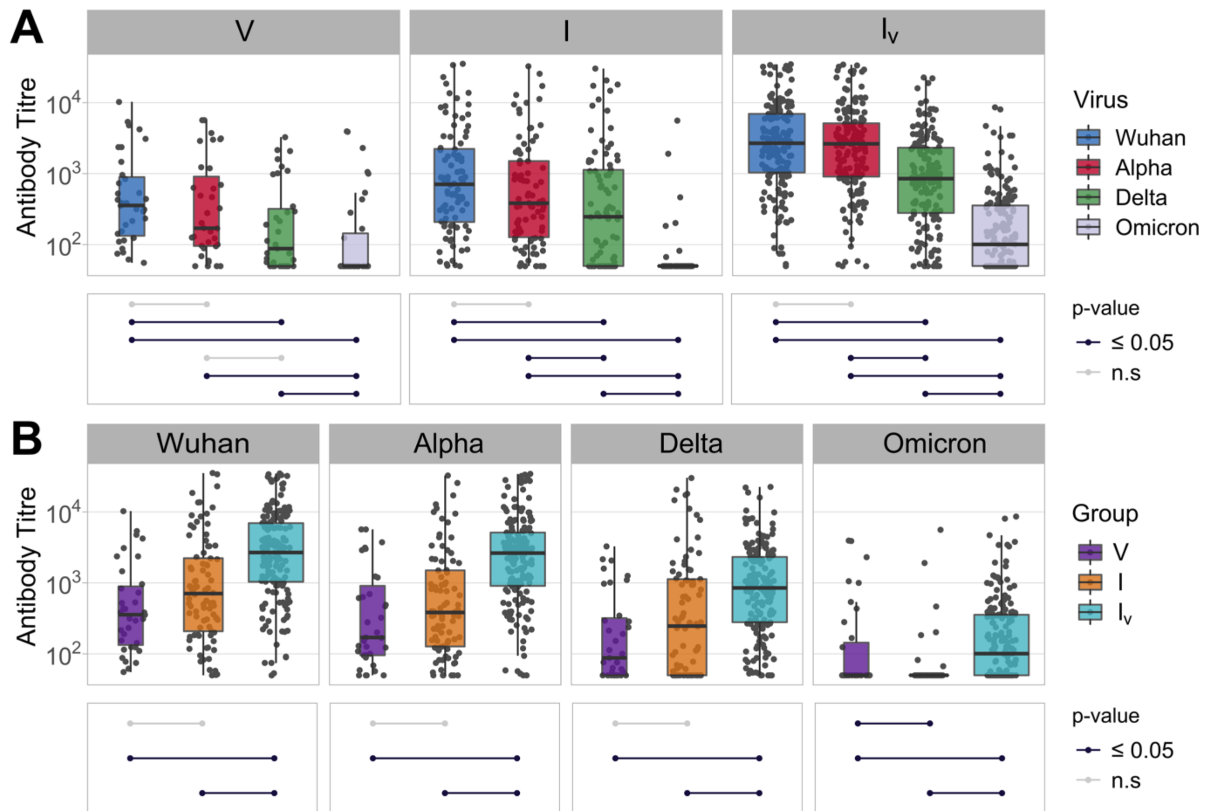
433 **Figure 2**. Concentrations of SARS-CoV-2 Spike and Nucleocapsid antibodies in samples derived from
434 patients with different histories of SARS-CoV-2 exposure. The name of the antigens is shown at the top
435 of each panel. Patient groups are defined as N: naïve (yellow); V: vaccinated (purple); I: infected
436 (orange); I_V: infected and vaccinated (cyan). Antibody concentrations are shown in MSD arbitrary
437 units/ml. Boxplots displayed the interquartile range and median values.
438

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.



439
440 **Figure 3**. Neutralising responses elicited against pseudotyped viruses carrying the S protein of different
441 SARS-CoV-2 variants according to patient exposure history to SARS-CoV-2. (A) Sera from patients
442 were grouped based on immunological histories (N: naive; V: vaccinated; I: infected; I_V: infected and
443 vaccinated). Neutralising activity was measured using Wuhan (blue), Alpha (red), Delta (green) and
444 Omicron (grey) spike glycoprotein bearing HIV(SARS-CoV-2) pseudotypes and plotted per patient
445 group (A) and per SARS-CoV-2 S variant (B). Neutralisation was measured at a fixed dilution (1:50).
446 Each point represents the mean of two replicates. Boxplots displayed the interquartile range and median
447 values. Significance levels between patient groups or pseudotyped viruses were tested using pairwise
448 Wilcoxon test, and are shown in bottom panels as connected dotplots.
449

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

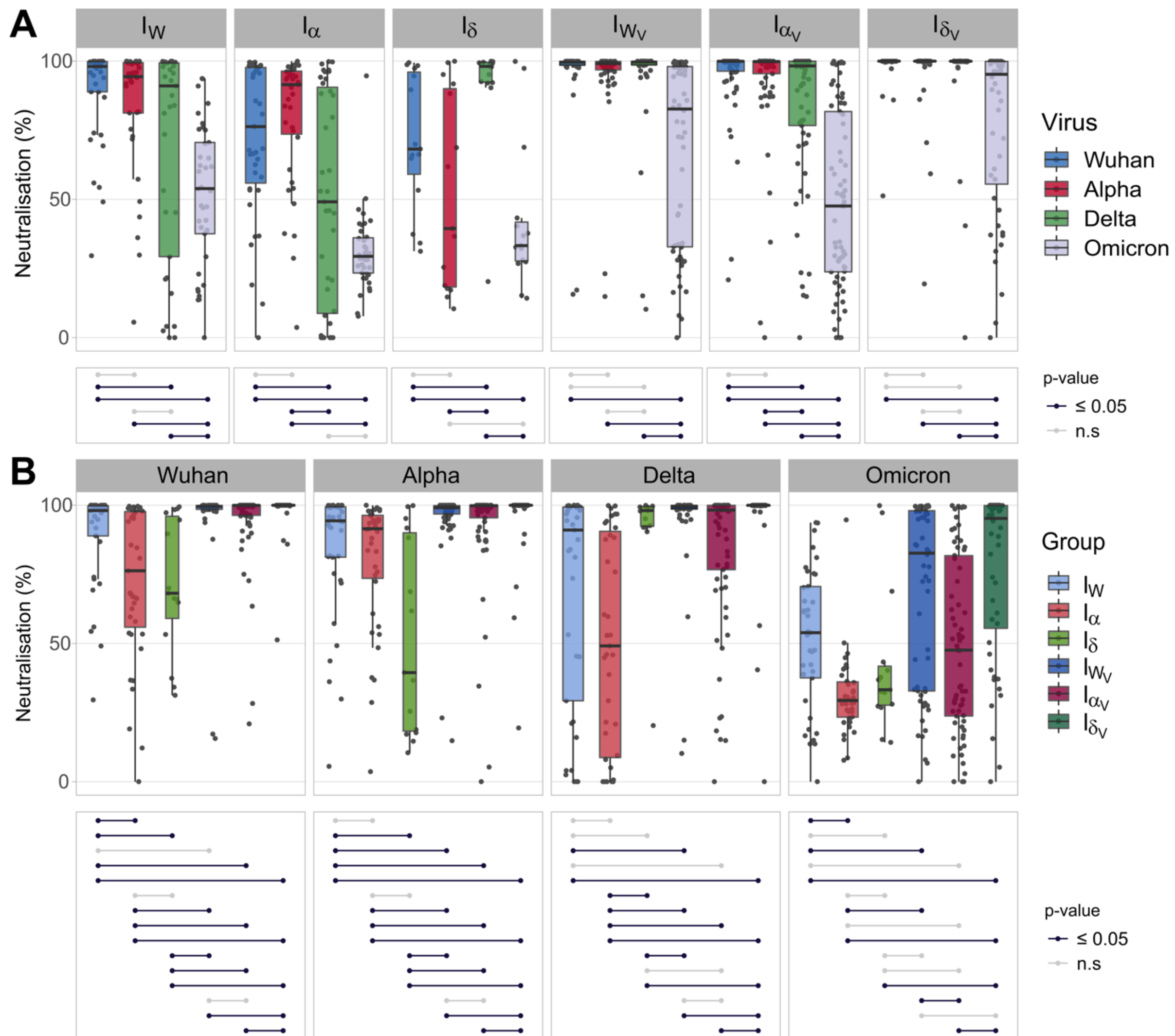


450

451 **Figure 4**. Neutralising antibody titres against SARS-CoV-2 variants in sera from patients with different
452 histories of SARS-CoV-2 exposure (V: vaccinated (purple); I: infected (orange); I_V: infected and
453 vaccinated (cyan)). Neutralising activity was measured using Wuhan (blue), Alpha (red), Delta (green)
454 and Omicron (grey) spike glycoprotein bearing HIV(SARS-CoV-2) pseudotypes and plotted per patient
455 group (A) and per SARS-CoV-2 S variant (B). Each point represents the mean of three replicates.
456 Boxplots displayed the interquartile range and median values. Significance levels between patient
457 groups or pseudotyped viruses were tested using pairwise Wilcoxon test, and are shown in bottom
458 panels as connected dotplots.

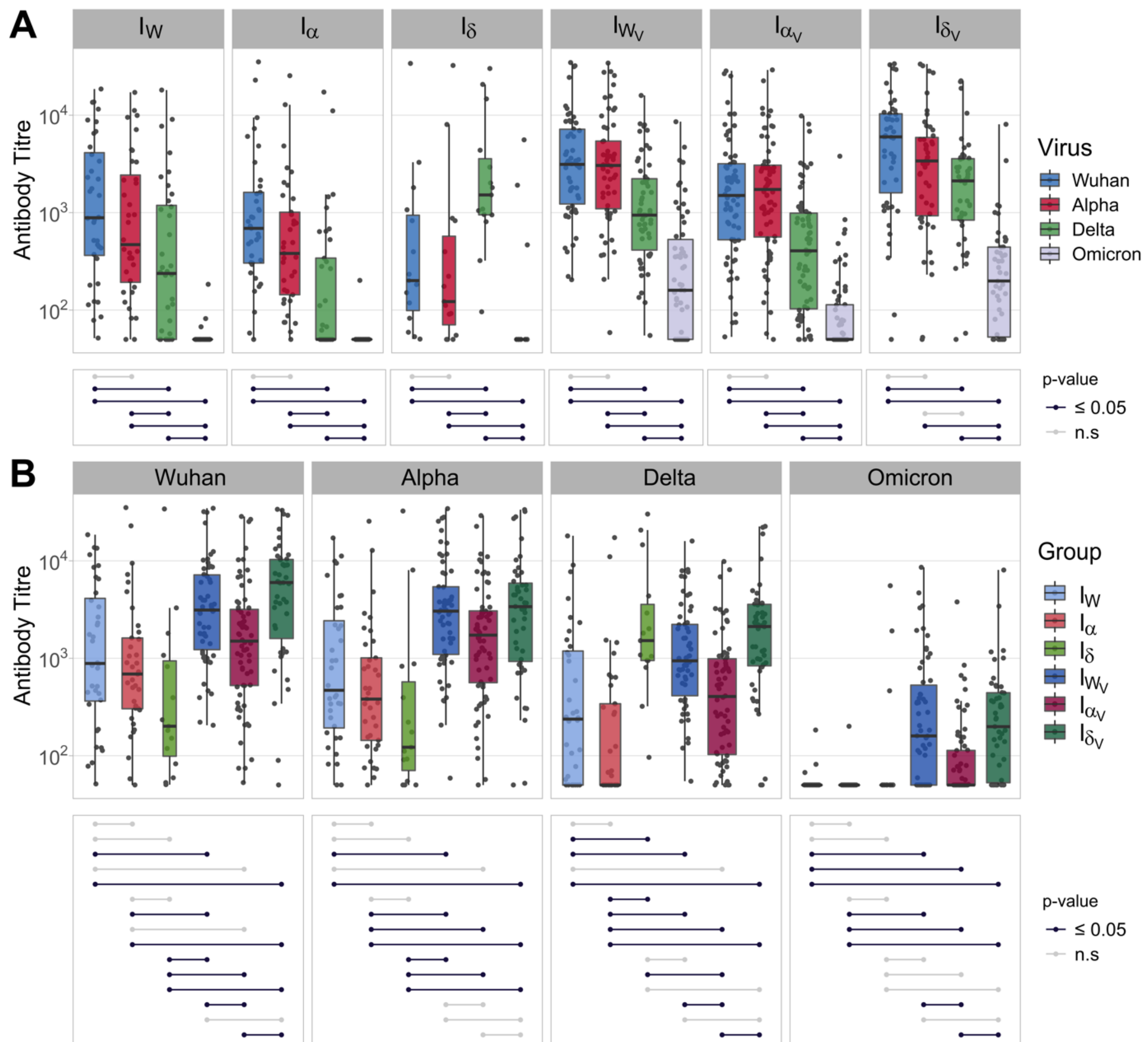
459

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.



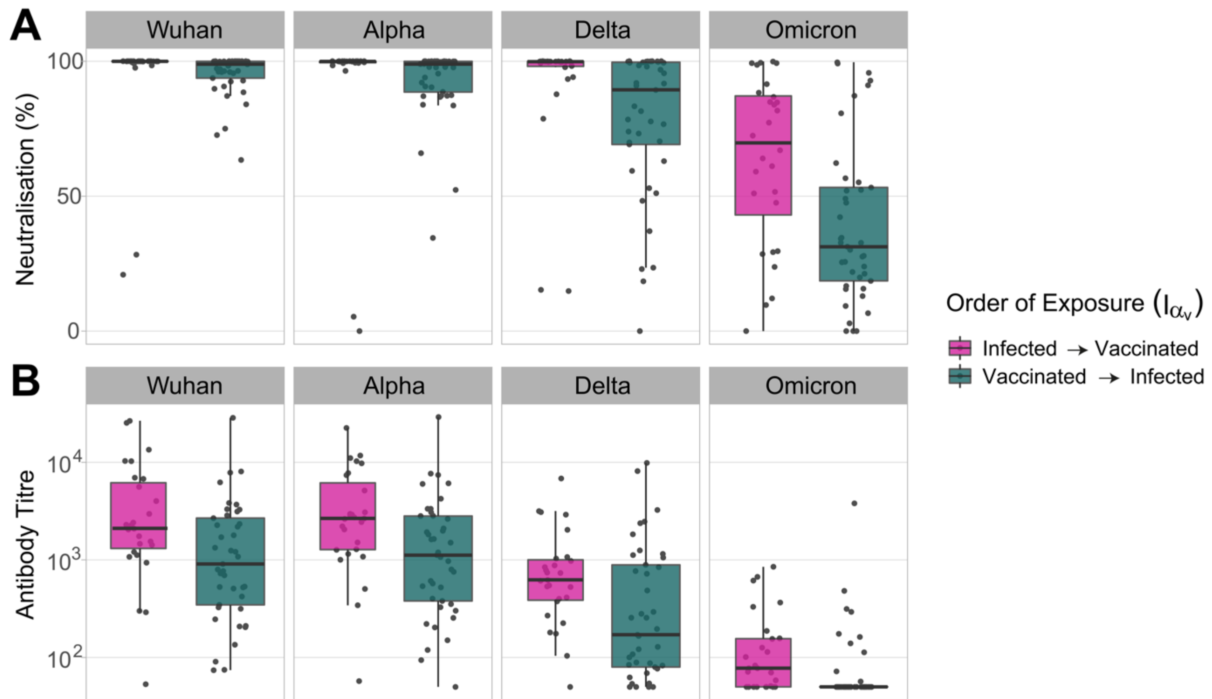
460
461 **Figure 5**. Neutralising responses elicited against pseudotyped viruses carrying the S protein of different
462 SARS-CoV-2 variants according to patient exposure history. (A) Sera from patients were grouped based
463 on immunological histories taking into account the infecting SARS-CoV-2 variant (I_W: infected with
464 SARS-CoV-2_W (light blue), $I\alpha$: infected with SARS-CoV-2α (light red), $I\delta$: infected with SARS-CoV-2δ
465 (light green), I_{WV} infected with SARS-CoV-2_W and vaccinated (dark blue), $I\alpha_V$: infected with SARS-CoV-
466 2α (dark red) and vaccinated, $I\delta_V$: infected with SARS-CoV-2δ and vaccinated, (dark green)).
467 Neutralising activity was measured using Wuhan (blue), Alpha (red), Delta (green) and Omicron (grey)
468 spike glycoprotein bearing HIV(SARS-CoV-2) pseudotypes and plotted per patient group (A) and per
469 SARS-CoV-2 S variant (B). Neutralisation was measured at a fixed dilution (1:50). Each point
470 represents the mean of two replicates. Boxplots displayed the interquartile range and median values.
471 Significance levels between patient groups or pseudotyped viruses were tested using pairwise Wilcoxon
472 test, and are shown in bottom panels as connected dotplots.
473

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

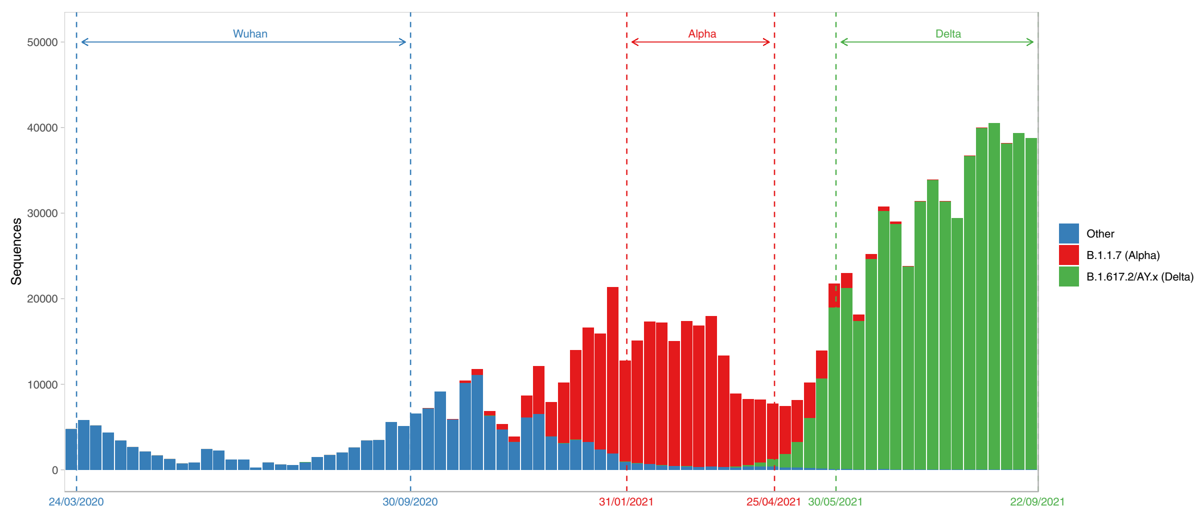


474
475
476 **Figure 6**. Neutralising antibody titres against SARS-CoV-2 variants in sera from patients with different
477 histories of SARS-CoV-2 exposure taking into account the infecting SARS-CoV-2 variant (I_W: infected
478 with SARS-CoV-2_W (light blue), $I\alpha$: infected with SARS-CoV-2α (light red), $I\delta$: infected with SARS-CoV-
479 2δ (light green), I_{WV} infected with SARS-CoV-2_W and vaccinated (dark blue), $I\alpha_V$: infected with SARS-
480 CoV-2α and vaccinated (dark red), $I\delta_V$: infected with SARS-CoV-2δ and vaccinated (dark green)).
481 Neutralising activity was measured using Wuhan (blue), Alpha (red), Delta (green) and Omicron (grey)
482 spike glycoprotein bearing HIV(SARS-CoV-2) pseudotypes and plotted per patient group (A) and per
483 SARS-CoV-2 S variant (B). Each point represents the mean of three replicates. Boxplots displayed the
484 interquartile range and median values. Significance levels between patient groups or pseudotyped
485 viruses were tested using pairwise Wilcoxon test, and are shown in bottom panels as connected
486 dotplots.

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.06.490867; this version posted May 6, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.



487
488
489 **Figure 7**. Neutralising responses against pseudotyped viruses carrying the S protein of different SARS-
490 CoV-2 variants were measured (A) and titrated (B) in patients that had been either infected and
491 vaccinated (pink) or vaccinated and infected (seagreen), with SARS-CoV-2α. Serum samples were
492 subject to neutralisation assays using lentiviruses pseudotyped with the S protein of SARS-CoV-2_W
493 (Wuhan) SARS-CoV-2α (Alpha), SARS-CoV-2δ (Delta) or SARS-CoV-2o (Omicron). (A) Neutralisation
494 was measured at a fixed dilution (1:50) and each data point is the mean of two replicates. (B) Antibody
495 titres were calculated by interpolating the point at which infectivity had been reduced to 50% of the
496 value for the non-serum control samples. Each point represents the mean of three replicates.
497
498 **Supporting Information**
499



500
501 **S1 Figure**. Number of SARS-CoV-2 variants sequenced in the United Kingdom between March 2020
502 and September 2021. Variants are colour-coded, and the key is shown on the right of the graph. Vertical
503 dashed lines show the time periods used to select samples as described in the main text and methods.
504 **S1 Appendix.** Assay results and metadata of each serum sample used in this study.