

1 **Broad neutralization of SARS-CoV-2 variants, including omicron, following**  
2 **breakthrough infection with delta in COVID-19 vaccinated individuals**

3

4 Thomas Lechmere<sup>1</sup>#, Luke B. Snell<sup>2</sup>#, Carl Graham<sup>1</sup>, Jeffrey Seow<sup>1</sup>, Zayed A. Shalim<sup>1</sup>,  
5 Themoula Charalampous,<sup>2</sup> Adela Alcolea-Medina,<sup>2</sup> Rahul Batra,<sup>2</sup> Gaia Nebbia,<sup>2</sup> Jonathan D.  
6 Edgeworth<sup>2</sup>, Michael H. Malim<sup>1</sup>, Katie J. Doores<sup>1</sup> \*

7

8 <sup>1</sup>Department of Infectious Diseases, School of Immunology & Microbial Sciences, King's  
9 College London, London, UK.

10 <sup>2</sup>Centre for Clinical Infection and Diagnostics Research, Department of Infectious Diseases,  
11 Guy's and St Thomas' NHS Foundation Trust, London, UK.

12

13 # These authors contributed equally

14 \* To whom correspondence should be addressed: [katie.doores@kcl.ac.uk](mailto:katie.doores@kcl.ac.uk)

15

16 **Abstract:**

17 Numerous studies have shown that a prior SARS-CoV-2 infection can greatly enhance  
18 the antibody response to COVID-19 vaccination, with this so called “hybrid immunity” leading  
19 to greater neutralization breadth against SARS-CoV-2 variants of concern. However, little is  
20 known about how breakthrough infection (BTI) in COVID-19 vaccinated individuals will impact  
21 the magnitude and breadth of the neutralizing antibody response. Here, we compared  
22 neutralizing antibody responses between unvaccinated and COVID-19 double vaccinated  
23 individuals (including both AZD1222 and BNT162b2 vaccinees) who have been infected with  
24 the delta (B.1.617.2) variant. Rapid production of Spike-reactive IgG was observed in the  
25 vaccinated group providing evidence of effective vaccine priming. Overall, potent cross-  
26 neutralizing activity against current SARS-CoV-2 variants of concern was observed in the BTI  
27 group compared to the infection group, including neutralization of the omicron (B.1.1.529)

**NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.**

28 variant. This study provides important insights into population immunity where transmission  
29 levels remain high and in the context of new or emerging variants of concern.

30

### 31 **Introduction:**

32 COVID-19 vaccines have proven to be critical in controlling SARS-CoV-2 infections  
33 worldwide. Vaccines based on the SARS-CoV-2 Wuhan-1 Spike protein generate neutralizing  
34 antibodies which constitute an important component of the protective capacity of COVID-19  
35 vaccines. Since the beginning of the global pandemic, variants of SARS-CoV-2 have arisen  
36 which encode mutations in the Spike protein. Until November 2021, the dominant circulating  
37 variant was B.1.617.2 (delta), but B.1.1.529 (omicron) is rapidly increasing globally  
38 ([https://www.nicd.ac.za/wpcontent/uploads/2021/11/Update-of-SA-sequencing-data-from-](https://www.nicd.ac.za/wpcontent/uploads/2021/11/Update-of-SA-sequencing-data-from-GISAID-26-Nov_Final.pdf)  
39 [GISAID-26-Nov\\_Final.pdf](https://www.nicd.ac.za/wpcontent/uploads/2021/11/Update-of-SA-sequencing-data-from-GISAID-26-Nov_Final.pdf)). There is concern that SARS-CoV-2 variants of concern (VOCs)  
40 might lead to a reduction in vaccine efficacy, in particular against omicron which encodes 31  
41 amino acid changes in the Spike protein.

42 To generate high titres of Spike reactive IgG with potent neutralization, double  
43 vaccination is required for both the BNT162b2 (based on mRNA encoding a stabilized Spike)  
44 and AZD1222 (based on a chimp adenovirus encoded Spike) vaccines (Ramasamy et al.,  
45 2021; Walsh et al., 2020). Importantly, several studies have shown that SARS-CoV-2 infection  
46 prior to vaccination can boost antibody titres and neutralizing activity, with this so called “hybrid  
47 immunity” leading to greater neutralization breadth against SARS-CoV-2 VOCs (Goel et al.,  
48 2021; Manisty et al., 2021; Reynolds et al., 2021; Saadat et al., 2021; Stamatatos et al., 2021).  
49 However, little is known about how breakthrough infection (BTI) in COVID-19 double  
50 vaccinated individuals will impact the magnitude and breadth of the neutralizing antibody  
51 response (Collier et al., 2021; Hacısuleyman et al., 2021; Kitchin, 2021), particularly in the  
52 face of the omicron variant where preliminary data shows that a 3<sup>rd</sup> vaccine dose is required  
53 for robust neutralization activity (Cameroni, 2021; Doria-Rose, 2021; Garcia-Beltran, 2021;  
54 Gruell, 2021; Schmidt, 2021) and predicted for high vaccine efficacy (Khoury, 2021). This  
55 information would provide important insights into population immunity in areas where

56 transmission levels remain high and where omicron is rapidly becoming the dominant strain.  
57 Here, we compared the magnitude and breadth of the antibody response in individuals  
58 infected with the SARS-CoV-2 delta VOC (vaccine naïve) to the antibody response in  
59 individuals who were double vaccinated prior to delta infection (breakthrough infection, BTI).

60

## 61 **Results**

### 62 **Cohort description**

63 We identified 42 individuals admitted to St Thomas' hospital who had previously  
64 received two COVID-19 vaccinations and subsequently tested positive for COVID-19. We note  
65 that at the time of writing, from the patients admitted to St Thomas' Hospital with COVID-19  
66 since the emergence of delta (n=635), 260 cases out of 332 (78%) where vaccination was  
67 known were either unvaccinated or partially vaccinated (one inoculation). In this study, 30/42  
68 (71%) of patients in the BTI group were admitted to hospital due to COVID-19, of which 11/30  
69 (37%) patients experienced severe disease (severity 4-5). 29/30 (97%) patients had  
70 underlying health conditions that predispose to severe disease and aged between 20-103  
71 years (median age 77 years, IQR 59-86) (**Table S1**). The remaining 12 participants in the BTI  
72 group (29%) were asymptomatic and admitted for reasons other than COVID-19. Patients  
73 were aged between 24-96 years (median age 62 years, IQR 37-72). Overall, the BTI group  
74 included individuals receiving both the AZD1222 vaccine (n = 23) and the BNT162b2 vaccine  
75 (n = 19). Discarded serum samples were collected between 0-53 days post onset of symptoms  
76 (POS) and longitudinal serum samples were collected where possible. The number of days  
77 post second vaccine ranged from 29-179 days (median 109 days).

78 Sera (n = 19) were also collected from unvaccinated individuals admitted to St Thomas'  
79 hospital due to COVID-19 who had a confirmed infection with the SARS-CoV-2 delta variant  
80 and experienced a range of disease severities with 9/19 (47%) patients experiencing severe  
81 disease (severity 4-5). Patients were aged between 25-82 years (median age 39 years, IQR  
82 30-51) and 9/19 (47%) had underlying health conditions (**Table S2**). Sera were collected  
83 between 12-22 days POS.

84

## 85 **IgG and IgM to Spike in breakthrough infection**

86 First, we measured the IgG and IgM responses to recombinant Spike (both WT and  
87 delta) in the two groups by ELISA. Sera from unvaccinated individuals infected with the delta  
88 variant at 12-22 days POS had higher delta Spike IgM levels than delta Spike IgG (**Figure 1A**)  
89 indicative of a primary immune response. Slightly higher IgG and IgM titres were observed  
90 against the delta recombinant Spike compared to WT Spike (**Figure 1B**).

91 For sera collected 12-22 days POS in the BTI group, delta Spike IgM levels in the BTI  
92 group were lower than the delta Spike IgG level (**Figure 1C**) indicative of a recall response. A  
93 similar trend was observed for both AZD1222 and BNT162b2 vaccinated individuals (**Figure**  
94 **S1A**). Where sequential serum samples were collected, nine individuals had undetectable or  
95 a very low Spike IgG response at the earliest timepoint POS (**Figure 1D and Figure S1B**).  
96 However, high titres of Spike specific IgG were detected several days later with only modest  
97 increases in IgM titres (**Figure 1E-F**). Six donors had IgG against Spike at early time points  
98 but lacked IgG to the SARS-CoV-2 Nucleoprotein (**Figure S1C-D**). Although this may provide  
99 insight into Spike IgG levels prior to infection, it is more likely due to a rapid Spike IgG recall  
100 response compared to a de novo IgG response to Nucleoprotein (**Figure 1E-F**). One  
101 participant (a renal transplant patient) had a high IgM response and low IgG response, similar  
102 to the vaccine naïve group, which suggests failed vaccine priming (**Figure 1C**). Interestingly,  
103 unlike the vaccine naïve group, the IgG and IgM titres against the WT and delta Spikes were  
104 comparable in the BTI group (**Figures 1D**).

105 Overall, these results indicate a rapid recall response due to prior vaccination in the  
106 BTI group and a primary immune response in the vaccine naïve group.

107

## 108 **Neutralization activity following breakthrough infection**

109 Next, we measured neutralization breadth and potency in the two groups using HIV-1  
110 (human immunodeficiency virus type-1) based virus particles, pseudotyped with SARS-CoV-  
111 2 Spike from different VOCs (wild-type (Wuhan), alpha (B.1.1.7), delta (B.1.617.2), mu

112 (B.1.621) and beta (B.1.351)) and a HeLa cell-line stably expressing the ACE2 receptor (Seow  
113 et al., 2020). The majority (17/19, 89%) of the vaccine naïve group produced a robust  
114 homologous neutralizing response against the delta VOC (**Figure 2A**). Cross-neutralization  
115 of the parental strain and other VOCs was detected for most individuals, albeit at a reduced  
116 potency. As we have reported previously (Dupont et al., 2021), the greatest reduction was  
117 observed against beta with a 9.4-fold reduction in the GMT, reflecting greater antigenic  
118 distance.

119 Sera collected between 12-22 days POS from individuals in the BTI group showed a  
120 robust homologous neutralizing response as well as strong cross-neutralization of the parental  
121 variant and VOCs (**Figure 2B**). Only a 1.2-fold reduction in GMT was observed against the  
122 more neutralization resistant beta VOC. Several individuals in the BTI group with sera  
123 collected soon after onset of symptoms showed no or very low neutralization against both WT  
124 and delta variants, however, potent neutralizing activity was detected several days later  
125 (**Figure 2C&D and S2A**). Geometric mean titres against the five variants were very similar  
126 between AZD1222 and BNT162b2 vaccinated individuals (**Figure S2B**). Three participants in  
127 the BTI group either failed to produce neutralizing antibodies or had titres close to baseline  
128 despite vaccination and SARS-CoV-2 infection. These individuals had underlying health  
129 conditions including cancer (one participant was undergoing rituximab treatment) and type-2  
130 diabetes.

131 As would have been anticipated, IgG ED<sub>50</sub> values correlated best with ID<sub>50</sub> values for  
132 the BTI group whereas IgM ED<sub>50</sub> values correlated best with ID<sub>50</sub> values for the unvaccinated  
133 group (**Figure 2E&F**) further highlighting the priming capacity of both the AZ and BNT162b2  
134 vaccines.

135

### 136 **BTI generates neutralizing activity against omicron**

137 In November 2021, omicron (B.1.1.529) was identified that encoded 31 amino acid  
138 mutations in the Spike protein (**Figure 3A**). Initial studies suggest that these mutations lead  
139 to large reductions in neutralization of sera from double vaccinated individuals. However,

140 administration of a third vaccine dose greatly enhances neutralization titres against omicron  
141 suggesting incomplete neutralization escape (Cameroni, 2021; Cele et al., 2021; Doria-Rose,  
142 2021; Garcia-Beltran, 2021; Gruell, 2021; Schmidt, 2021). Neutralization activity of a subset  
143 of 14 sera from the vaccine naïve group and 15 sera from the BTI group were measured  
144 against WT, delta and omicron variants (**Figure 3B&D**). In delta infected individuals, a 28.9-  
145 fold reduction in GMT against omicron compared to delta was measured compared to a 6.9-  
146 fold reduction in GMT for WT. Sera from two participants did not neutralize the omicron variant  
147 at the lowest dilution point (1:25). In contrast, all 15 sera from the BTI group neutralized the  
148 omicron variant with only a 4.5-fold reduction in GMT against omicron compared to delta GMT  
149 (**Figure 3C&E**). Three individuals showed a 21- to 81-fold reduction in ID<sub>50</sub> against omicron  
150 compared to ID<sub>50</sub> against delta, all of which were receiving treatment for underlying health  
151 conditions. These results further highlight the breadth of the neutralizing antibody response  
152 following BTI with the delta variant.

153

## 154 **Discussion**

155 These data demonstrate that whilst 2-doses of COVID-19 vaccine (both BNT162b2 or  
156 AZD1222) was not sufficient to provide sterilizing immunity against SARS-CoV-2 infection in  
157 these particular individuals, breakthrough infection generated a strong anamnestic response.  
158 Although this study cannot provide information on the titre of neutralizing antibody required for  
159 protection against infection with the delta VOC, longitudinal sampling revealed that six  
160 participants who had undetectable neutralization or ID<sub>50</sub> ~25 against delta VOC at the earliest  
161 timepoint sampled rapidly developed IgG to Spike and serum neutralizing activity upon  
162 infection showing both AZD1222 and BNT162b2 vaccination primed their immune system to  
163 respond rapidly upon SARS-CoV-2 infection. 30/42 (71%) of the BTI group were admitted to  
164 hospital due to COVID-19 after BTI, the median age was 77 years and only 1/30 (3%) had no  
165 comorbidities that would predispose to severe disease. This suggests the group admitted with  
166 BTI were at particular risk of severe disease due to advancing age and/or co-morbidities.  
167 Indeed, advancing age was the main criterion with which vaccination schedule was based in

168 the UK, meaning that those over 70 years were amongst the first to be offered vaccination in  
169 January 2021. As such, vaccine-induced immunity may have waned in this group due to the  
170 longer interval between vaccination and exposure, facilitating subsequent BTI (Levin et al.,  
171 2021; Mizrahi et al., 2021; Shrotri et al., 2021; Tartof et al., 2021). Indeed, the median time  
172 elapsed since last vaccination in the BTI group was 109 days with 24/30 (80%) being  
173 vaccinated over 10 weeks prior to symptom onset. Others have described waning of vaccine-  
174 induced immunity against delta after 10 weeks, especially in older age groups (Andrews, 2021;  
175 Israel et al., 2021). Notably, the unvaccinated group were much younger and a large  
176 proportion had no co-morbidities.

177         When comparing the immune response of the BTI group and the vaccine naïve group,  
178 we observe that prior vaccination led to a more potent and broader neutralizing antibody  
179 response during the acute phase of infection, including against the highly mutated omicron  
180 variant. As we do not have matched sera collected prior to breakthrough infection we cannot  
181 comment on the breadth of the nAb response prior to infection. However, in this vaccinated  
182 cohort, boosting is occurring with a heterologous Spike which may contribute to the  
183 broadening of the serum neutralizing activity. In addition, all individuals in this study received  
184 an extended booster regime (8-12 weeks post prime) which has been suggested to generate  
185 a broader response than the short (3-4 week) boost regime (Payne et al., 2021; Tauzin, 2021).  
186 Further studies examining the antibody response at the monoclonal level is needed to  
187 determine if the broader serum activity is due to individual antibodies or a de novo response  
188 directed against the delta Spike. Broadening of the neutralizing antibody response has been  
189 reported at later timepoints following natural infection (~6-10 months) (Dupont et al., 2021;  
190 Gaebler et al., 2021) and therefore, despite narrow serum neutralization breadth in the vaccine  
191 naïve group, convalescent sera collected at later timepoints would be expected to have  
192 broader neutralizing activity. The large decrease in neutralization of viral particles  
193 pseudotyped with omicron Spike by sera from delta infected individuals highlights the large  
194 antigenic distance between the delta and omicron Spike glycoproteins (Dupont et al., 2021;  
195 Liu et al., 2021).

196           Although the omicron VOC is more neutralization resistant, several studies have  
197 reported smaller fold-reductions in serum neutralization potency for omicron following 3-doses  
198 of COVID-19 vaccination (range 4 - 7 fold) compared to those who had received only 2 vaccine  
199 doses (range 20-fold to >40-fold) (Cameroni, 2021; Doria-Rose, 2021; Garcia-Beltran, 2021;  
200 Gruell, 2021; Liu, 2021; Schmidt, 2021). Overall, the data presented here suggest that a  
201 breakthrough SARS-CoV-2 delta infection is also acting as an effective booster which could  
202 provide broad protection against current VOCs, including omicron. As new VOCs arise with  
203 new/unique combinations of mutations, our data suggests a broad neutralizing antibody  
204 response generated by a combination of vaccination and infection may provide immunity  
205 against other/emerging VOCs. This study provides important insights into population immunity  
206 and can inform public health measures where SARS-CoV-2 transmission levels remain high.  
207  
208



209 **Methods**

210 **Ethics**

211 Collection of surplus serum samples was approved by South Central – Hampshire B  
212 REC (20/SC/0310). SARS-CoV-2 cases were diagnosed by RT–PCR of respiratory samples  
213 at St Thomas’ Hospital, London. Sera were selected on the availability of longitudinal samples  
214 and knowledge of timing and type of COVID-19 vaccination.

215

216 **COVID-19 severity classification.**

217 Disease severity was determined as previously described (Dupont et al., 2021; Seow et al.,  
218 2020). Patients diagnosed with COVID-19 were classified as follows: (0) Asymptomatic or no  
219 requirement for supplemental oxygen; (1) Requirement for supplemental oxygen (fraction of  
220 inspired oxygen ( $F_{iO_2}$ ) < 0.4) for at least 12 h; (2) Requirement for supplemental oxygen ( $F_{iO_2}$   
221  $\geq$  0.4) for at least 12 h; (3) Requirement for non-invasive ventilation/continuous positive airway  
222 not a candidate for escalation above level one (ward-based) care; (4) Requirement for  
223 intubation and mechanical ventilation or supplemental oxygen ( $F_{iO_2}$  > 0.8) and peripheral  
224 oxygen saturations <90% (with no history of type 2 respiratory failure (T2RF)) or <85% (with  
225 known T2RF) for at least 12 h; (5) Requirement for ECMO.

226

227 **Virus sequencing**

228 Delta variant infection was confirmed using whole genome sequencing as previously  
229 described (Dupont et al., 2021) or using MT-PCR (Hale et al., 2021).

230

231 **Plasmids.**

232 WT, B.1.1.7, B.1.351, B.1.621, B.1.617.2 and B.1.1.529 codon optimized Spike  
233 plasmids were obtained from Wendy Barclay (Imperial College London). The final 19 amino  
234 acids were removed using an K1255\* mutation. B.1.1.7 mutations introduced were  $\Delta$ H69/V70,  
235  $\Delta$ Y144, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H. B.1.351 mutations

236 introduced were D80A, D215G, Delta242-244, R246I, K417N, E484K, N501Y, D614G,  
237 A701V. B.1.617.2 mutations introduced were: T19R, G142D,  $\Delta$ 156-157, R158G, L452R,  
238 T478R, D614G, P681R, D950N. B.1.621 mutations introduced were: T95I,  
239 Y144T/144insS/Y145N, R346K, E484K, N501Y, D614G, P681H, D950N. B.1.1.529 mutations  
240 introduced were: A67V,  $\Delta$ 69-70, T95I, G142D/ $\Delta$ 143-145,  $\Delta$ 211/L212I, ins214EPE, G339D,  
241 S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493K, G496S,  
242 Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K,  
243 Q954H, N969K, L981F.

244

#### 245 **Glycoprotein expression and purification.**

246 The recombinant wild-type (Wuhan-1 strain) and delta (B.1.617.2) consist of a pre-  
247 fusion S ectodomain residues 1–1138 with proline substitutions at amino acid positions 986  
248 and 987, a GGGG substitution at the furin cleavage site (amino acids 682–685) and an N  
249 terminal T4 trimerisation domain followed by a Strep-tag II (Brouwer et al., 2020). Spike was  
250 expressed in HEK 293 Freestyle cells and purified using StrepTactinXT Superflow high  
251 capacity 50% suspension according to the manufacturer’s protocol by gravity flow (IBA Life  
252 Sciences).

253 N protein was obtained from the James lab at LMB, Cambridge. The N protein is a  
254 truncated construct of the SARS-CoV-2 N protein comprising residues 48–365 with an N  
255 terminal uncleavable hexahistidine tag. N was expressed in *E. Coli* using autoinducing media  
256 for 7h at 37°C and purified using immobilised metal affinity chromatography (IMAC), size  
257 exclusion and heparin chromatography.

258

#### 259 **Spike IgG titres by ELISA**

260 ELISA was carried out as previously described (Seow et al., 2020). All sera were heat-  
261 inactivated at 56°C for 30 mins before use in the in-house ELISA. High-binding ELISA plates  
262 (Corning, 3690) were coated with antigen (N or Spike (WT or delta)) at 3  $\mu$ g/mL (25  $\mu$ L per  
263 well) in PBS overnight at 4°C. Wells were washed with PBS-T (PBS with 0.05% Tween-20)

264 and then blocked with 100  $\mu$ L 5% milk in PBS-T for 1 hr at room temperature. Wells were  
265 emptied and a titration of serum starting at 1:50 and using a 6-fold dilution series in milk was  
266 added and incubated for 2 hr at room temperature. Control reagents included CR3009 (2  
267  $\mu$ g/mL), CR3022 (0.2  $\mu$ g/mL), negative control plasma (1:25 dilution), positive control plasma  
268 (1:50) and blank wells. Wells were washed with PBS-T. Secondary antibody was added and  
269 incubated for 1 hr at room temperature. IgM was detected using Goat-anti-human-IgM-HRP  
270 (horseradish peroxidase) (1:1,000) (Sigma: A6907) and IgG was detected using Goat-anti-  
271 human-Fc-AP (alkaline phosphatase) (1:1,000) (Jackson: 109-055-098). Wells were washed  
272 with PBS-T and either AP substrate (Sigma) was added and read at 405 nm (AP) or 1-step  
273 TMB (3,3',5,5'-Tetramethylbenzidine) substrate (Thermo Scientific) was added and quenched  
274 with 0.5 M H<sub>2</sub>SO<sub>4</sub> before reading at 450 nm (HRP). Half-maximal binding (EC<sub>50</sub>) was calculated  
275 using GraphPad Prism. Measurements were carried out in duplicate.

276

#### 277 **SARS-CoV-2 pseudotyped virus particle preparation.**

278 Pseudotyped HIV-1 virus incorporating the SARS-CoV-2 Spike protein (either wild-  
279 type, B.1.1.7, B.1.351, B.1.621, B.1.617.2 or B.1.1.529) were prepared as previously  
280 described (Dupont et al., 2021). Viral particles were produced in a 10 cm dish seeded the day  
281 prior with 5x10<sup>6</sup> HEK293T/17 cells in 10 ml of complete Dulbecco's Modified Eagle's Medium  
282 (DMEM-C, 10% FBS and 1% Pen/Strep) containing 10% (vol/vol) foetal bovine serum (FBS),  
283 100 IU/ml penicillin and 100  $\mu$ g/ml streptomycin. Cells were transfected using 90  $\mu$ g of PEI-  
284 Max (1 mg/mL, Polysciences) with: 15 $\mu$ g of HIV-luciferase plasmid, 10  $\mu$ g of HIV 8.91 gag/pol  
285 plasmid and 5  $\mu$ g of SARS-CoV-2 spike protein plasmid.(Grehan et al., 2015; Thompson et  
286 al., 2020) The supernatant was harvested 72 hours post-transfection. Pseudotyped virus  
287 particles was filtered through a 0.45 $\mu$ m filter, and stored at -80°C until required.

288

#### 289 **Neutralization assay with SARS-CoV-2 pseudotyped virus.**

290 Serial dilutions of serum samples (heat inactivated at 56°C for 30mins) were prepared  
291 with DMEM media (25µL) (10% FBS and 1% Pen/Strep) and incubated with pseudotyped  
292 virus (25µL) for 1-hour at 37°C in half-area 96-well plates. Next, Hela cells stably expressing  
293 the ACE2 receptor were added (10,000 cells/25µL per well) and the plates were left for 72  
294 hours. Infection levels were assessed in lysed cells with the Bright-Glo luciferase kit  
295 (Promega), using a Victor™ X3 multilabel reader (Perkin Elmer). Each serum sample was run  
296 in duplicate and was measured against the five SARS-CoV-2 variants within the same  
297 experiment using the same dilution series.

298

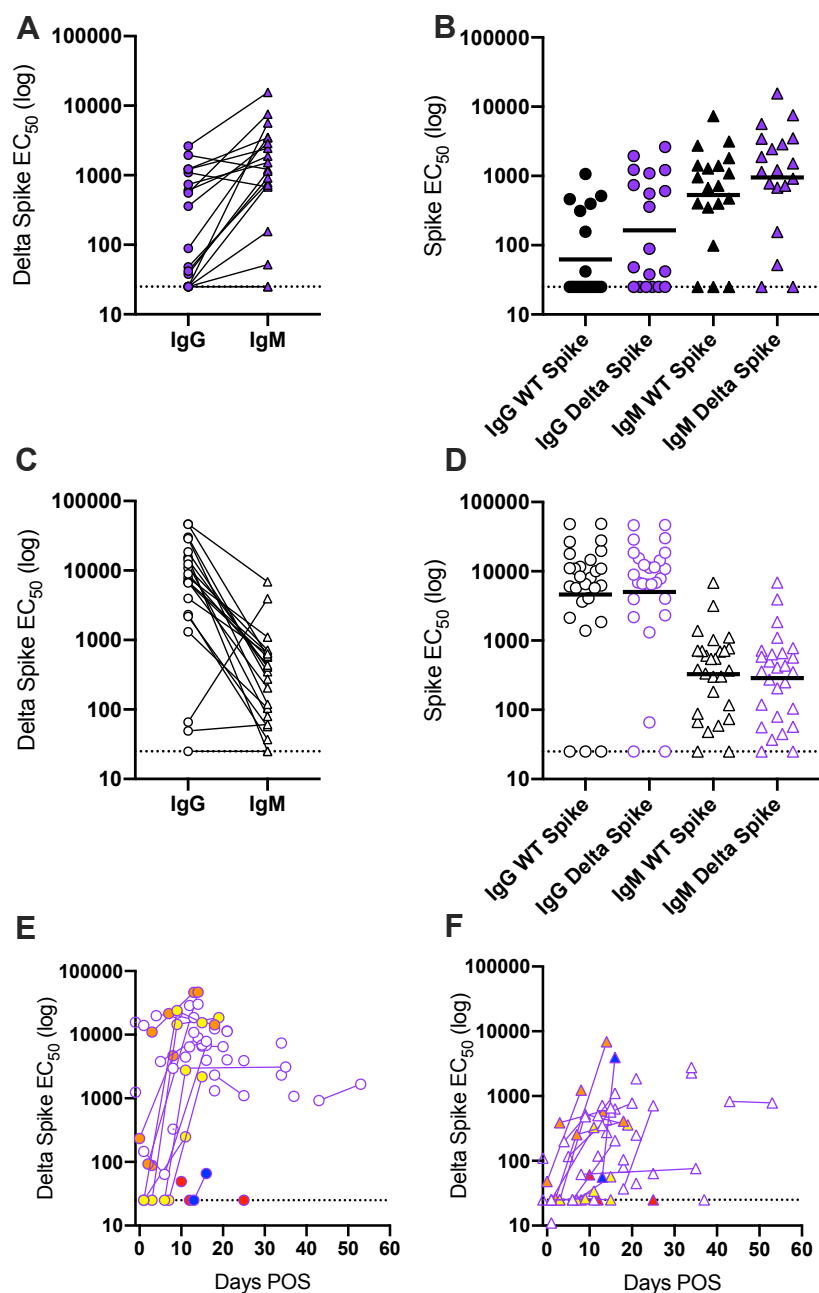
299 **Statistical analysis.**

300 Analyses were performed using GraphPad Prism v.8.3.1.

301

302

303 **Figures**  
304



305

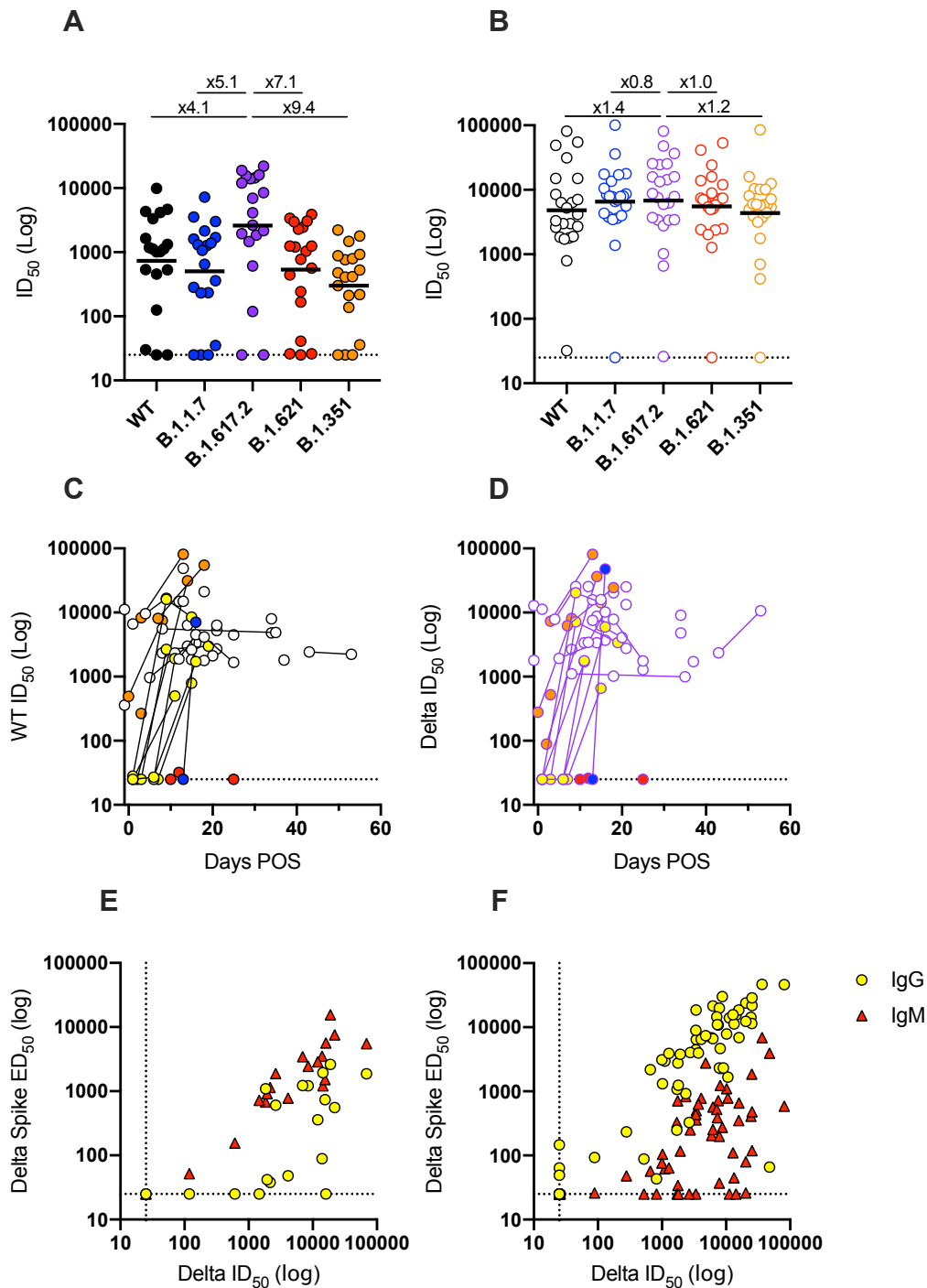
306

307 **Figure 1: Differences in antibody binding between delta infected individuals and**  
308 **COVID-19 vaccinated individuals experiencing delta breakthrough infection. A)**

309 Difference in IgG and IgM titres for sera collected 12-22 days POS for the delta infection  
310 (vaccine naïve) group. **B)** Comparison of the IgG and IgM ED<sub>50</sub> values against recombinant

311 WT and delta Spikes for the vaccine naïve group. Black horizontal lines show the geometric  
312 mean titres. **C)** Difference in IgG and IgM titres for sera for the BTI group. **D)** Comparison of

313 the IgG and IgM ED<sub>50</sub> values against recombinant WT and delta Spikes for the BTI group.  
314 Black horizontal lines show the geometric mean titres. **E)** Longitudinal IgG ED<sub>50</sub> against  
315 recombinant delta Spike in the BTI group. **F)** Longitudinal IgM ED<sub>50</sub> against recombinant delta  
316 Spike in the BTI group. Donors with IgM>IgG are shown in blue, donors who do not  
317 seroconvert are shown in red, donors with high Spike IgG but no N IgG at <7days POS are  
318 shown in orange, and donors with low Spike IgG at <7 days POS that rapidly increases are  
319 shown in yellow.



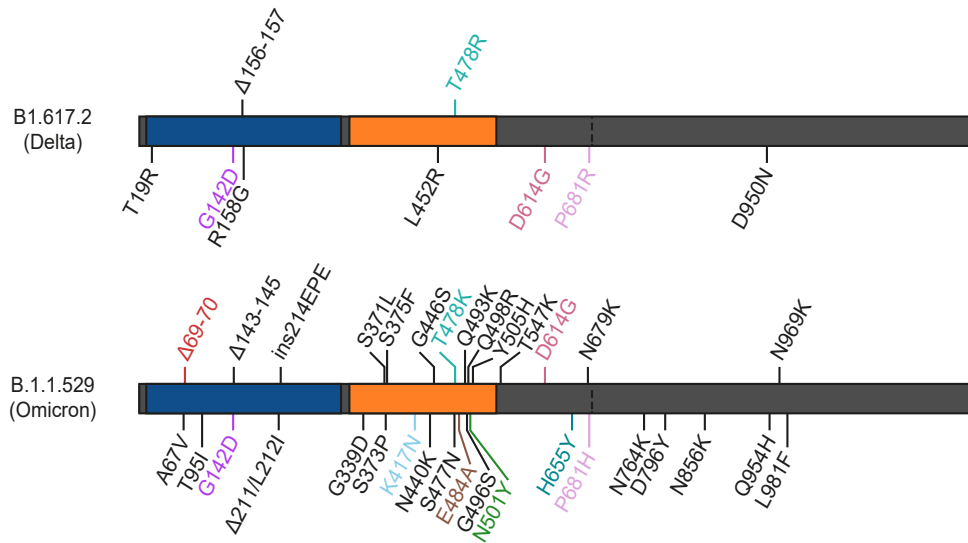
320

321 **Figure 2: Differences in neutralizing antibody response between delta infected**  
 322 **individuals and COVID-19 vaccinated individuals experiencing delta breakthrough**  
 323 **infection.** ID<sub>50</sub> of neutralization against WT (black) and VOCs alpha (blue), delta (purple), mu  
 324 (red) and beta (orange) for sera from **A)** SARS-CoV-2 delta infected individuals and **B)** BTI  
 325 individuals. Black line shows the geometric mean titre. Fold decrease in GMT compared to  
 326 delta are shown above. Longitudinal neutralization potency of sera in BTI individuals against

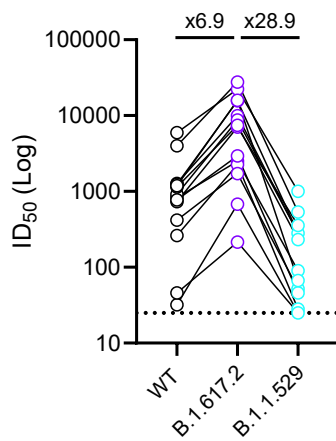
327 **C)** WT pseudovirus particles and **D)** delta pseudovirus particles. Donors with IgM>IgG are  
328 shown in blue, donors who do not seroconvert are shown in red, donors with high Spike IgG  
329 but no N IgG at <7days POS are shown in orange, and donors with low Spike IgG at <7 days  
330 POS that rapidly increases are shown in yellow. Data for the alpha, beta and mu VOCs is  
331 shown in **Figure S2A**. Correlation (Spearman,  $r$ ) between ID<sub>50</sub> of neutralization and IgM or  
332 IgG ED<sub>50</sub> for delta Spike binding for **E)** delta infected individuals (IgM:  $r = 0.92$ ,  $r^2 = 0.90$ ,  $p$   
333  $<0.0001$  and IgG:  $r = 0.66$ ,  $r^2 = 0.43$ ,  $p = 0.001$ ) and **F)** COVID-19 vaccinated individuals  
334 experiencing breakthrough infection (IgM:  $r = 0.61$ ,  $r^2 = 0.38$ ,  $p <0.0001$  and IgG:  $r = 0.83$ ,  $r^2$   
335  $= 0.75$ ,  $p <0.0001$ ). A linear regression was used to calculate the goodness of fit ( $r^2$ ). The  
336 dotted lines represent the lowest serum dilution used in each assay. IgG is shown with yellow  
337 circles and IgM shown with red circles.



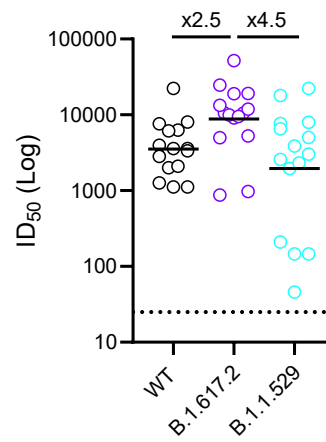
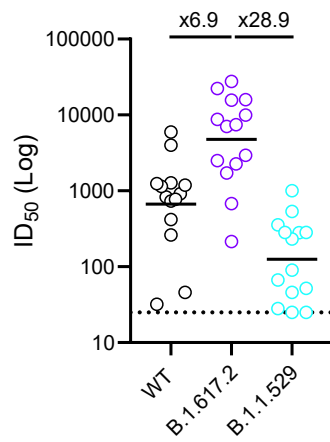
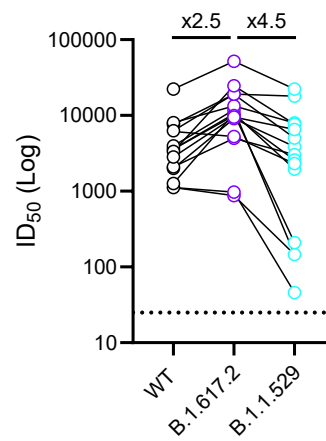
**A**



**B**



**C**



339 **Figure 3: Neutralization of omicron in BTI and delta infected individuals. A)** Schematic  
340 showing mutations in the delta (B.1.617.2) and omicron (B.1.1.529) Spikes. Select sera from  
341 **B)** SARS-CoV-2 delta infected (vaccine naïve) individuals (13-22 days POS) and **C)** BTI  
342 individuals (12-21 days POS) was tested against WT, delta and omicron VOCs. ID<sub>50</sub> of  
343 neutralization against WT (black) and VOCs delta (purple), and omicron (turquoise) for each  
344 participant are linked. Geometric mean titres against WT, delta and omicron VOCs for **D)**  
345 SARS-CoV-2 delta infected individuals (13-22 days POS) and **E)** BTI individuals (12-21 days  
346 POS). Black line shows the geometric mean titre. Fold decrease in GMT against omicron  
347 compared to WT and delta are shown above.

348

349 **Acknowledgements**

350           This work was funded by; Fondation Dormeur, Vaduz for funding equipment to KJD,  
351 Huo Family Foundation Award to MHM, KJD, MRC Genotype-to-Phenotype UK National  
352 Virology Consortium (MR/W005611/1 to MHM, KJD), and Wellcome Trust Investigator Award  
353 106223/Z/14/Z to MHM. CG is supported by the MRC-KCL Doctoral Training Partnership in  
354 Biomedical Sciences (MR/N013700/1). This work was supported by the Department of Health  
355 via a National Institute for Health Research comprehensive Biomedical Research Centre  
356 award to Guy's and St Thomas' NHS Foundation Trust in partnership with King's College  
357 London and King's College Hospital NHS Foundation Trust. This study is part of the EDCTP2  
358 programme supported by the European Union (grant number RIA2020EF-3008 COVAB). The  
359 views and opinions of authors expressed herein do not necessarily state or reflect those of  
360 EDCTP. This project is supported by a joint initiative between the Botnar Research Centre for  
361 Child Health and the European & Developing Countries Clinical Trials Partnership (KJD).

362           Thank you to Philip Brouwer, Marit van Gils and Rogier Sanders for the Spike protein  
363 construct, Leo James and Jakub Luptak for the N protein, Wendy Barclay and Thomas  
364 Peacock (Imperial) for providing the Spike plasmids and James Voss and Deli Huang  
365 (Scripps) for providing the Hela-ACE2 cells.

366

367

## 368 References

- 369 Andrews, N. (2021). Vaccine effectiveness and duration of protection of Comirnaty, Vaxzevria  
370 and Spikevax against mild and severe COVID-19 in the UK. medRxiv.
- 371 Brouwer, P.J.M., Caniels, T.G., van der Straten, K., Snitselaar, J.L., Aldon, Y., Bangaru, S.,  
372 Torres, J.L., Okba, N.M.A., Claireaux, M., Kerster, G., *et al.* (2020). Potent neutralizing  
373 antibodies from COVID-19 patients define multiple targets of vulnerability. *Science* 369, 643-  
374 650.
- 375 Cameroni, E. (2021). Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron  
376 antigenic shift. bioRxiv.
- 377 Cele, S., Jackson, L., Khan, K., Khoury, D.S., Moyo-Gwete, T., Tegally, H., Scheepers, C.,  
378 Amoako, D., Karim, F., Bernstein, M., *et al.* (2021). SARS-CoV-2 Omicron has extensive but  
379 incomplete escape of Pfizer BNT162b2 elicited neutralization and requires ACE2 for infection.  
380 medRxiv.
- 381 Collier, A.Y., Brown, C.M., McMahan, K., Yu, J., Liu, J., Jacob-Dolan, C., Chandrashekar, A.,  
382 Tierney, D., Ansel, J.L., Rowe, M., *et al.* (2021). Immune Responses in Fully Vaccinated  
383 Individuals Following Breakthrough Infection with the SARS-CoV-2 Delta Variant in  
384 Provincetown, Massachusetts. medRxiv.
- 385 Doria-Rose, N.A. (2021). Booster of mRNA-1273 Vaccine Reduces SARS-CoV-2 Omicron  
386 Escape from Neutralizing Antibodies. medRxiv.
- 387 Dupont, L., Snell, L.B., Graham, C., Seow, J., Merrick, B., Lechmere, T., Maguire, T.J.A.,  
388 Hallett, S.R., Pickering, S., Charalampous, T., *et al.* (2021). Neutralizing antibody activity in  
389 convalescent sera from infection in humans with SARS-CoV-2 and variants of concern. *Nat*  
390 *Microbiol* 6, 1433-1442.
- 391 Gaebler, C., Wang, Z., Lorenzi, J.C.C., Muecksch, F., Finkin, S., Tokuyama, M., Cho, A.,  
392 Jankovic, M., Schaefer-Babajew, D., Oliveira, T.Y., *et al.* (2021). Evolution of antibody  
393 immunity to SARS-CoV-2. *Nature* 591, 639-644.
- 394 Garcia-Beltran, W.F. (2021). mRNA-based COVID-19 vaccine boosters induce neutralizing  
395 immunity against SARSCoV-2 Omicron variant. medRxiv.
- 396 Goel, R.R., Apostolidis, S.A., Painter, M.M., Mathew, D., Pattekar, A., Kuthuru, O., Gouma,  
397 S., Hicks, P., Meng, W., Rosenfeld, A.M., *et al.* (2021). Distinct antibody and memory B cell  
398 responses in SARS-CoV-2 naive and recovered individuals following mRNA vaccination. *Sci*  
399 *Immunol* 6.
- 400 Grehan, K., Ferrara, F., and Temperton, N. (2015). An optimised method for the production of  
401 MERS-CoV spike expressing viral pseudotypes. *MethodsX* 2, 379-384.
- 402 Gruell, H. (2021). mRNA booster immunization elicits potent neutralizing serum activity  
403 against the SARS-CoV-2 Omicron variant. medRxiv.
- 404 Haciasuleyman, E., Hale, C., Saito, Y., Blachere, N.E., Bergh, M., Conlon, E.G., Schaefer-  
405 Babajew, D.J., DaSilva, J., Muecksch, F., Gaebler, C., *et al.* (2021). Vaccine Breakthrough  
406 Infections with SARS-CoV-2 Variants. *N Engl J Med* 384, 2212-2218.
- 407 Hale, R., Crowley, P., Dervisevic, S., Coupland, L., Cliff, P.R., Ebie, S., Snell, L.B., Paul, J.,  
408 Williams, C., Randell, P., *et al.* (2021). Development of a Multiplex Tandem PCR (MT-PCR)  
409 Assay for the Detection of Emerging SARS-CoV-2 Variants. *Viruses* 13.
- 410 Israel, A., Merzon, E., Schaffer, A.A., Shenhar, Y., Green, I., Golan-Cohen, A., Ruppin, E.,  
411 Magen, E., and Vinker, S. (2021). Elapsed time since BNT162b2 vaccine and risk of SARS-  
412 CoV-2 infection: test negative design study. *BMJ* 375, e067873.
- 413 Khoury, D.S. (2021). A meta-analysis of Early Results to predict Vaccine efficacy against  
414 Omicron. medRxiv.
- 415 Kitchin, D. (2021).  
416 Ad26.COVS.S breakthrough infections induce high titers of antibodies capable of neutralizing  
417 variants of concern. medRxiv.
- 418 Levin, E.G., Lustig, Y., Cohen, C., Fluss, R., Indenbaum, V., Amit, S., Doolman, R., Asraf, K.,  
419 Mendelson, E., Ziv, A., *et al.* (2021). Waning Immune Humoral Response to BNT162b2 Covid-  
420 19 Vaccine over 6 Months. *N Engl J Med*.

421 Liu, C., Ginn, H.M., Dejnirattisai, W., Supasa, P., Wang, B., Tuekprakhon, A., Nutalai, R.,  
422 Zhou, D., Mentzer, A.J., Zhao, Y., *et al.* (2021). Reduced neutralization of SARS-CoV-2  
423 B.1.617 by vaccine and convalescent serum. *Cell*.  
424 Liu, L. (2021). Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2.  
425 *bioRxiv*.  
426 Manisty, C., Otter, A.D., Treibel, T.A., McKnight, A., Altmann, D.M., Brooks, T., Noursadeghi,  
427 M., Boyton, R.J., Semper, A., and Moon, J.C. (2021). Antibody response to first BNT162b2  
428 dose in previously SARS-CoV-2-infected individuals. *Lancet* 397, 1057-1058.  
429 Mizrahi, B., Lotan, R., Kalkstein, N., Peretz, A., Perez, G., Ben-Tov, A., Chodick, G., Gazit, S.,  
430 and Patalon, T. (2021). Correlation of SARS-CoV-2-breakthrough infections to time-from-  
431 vaccine. *Nat Commun* 12, 6379.  
432 Payne, R.P., Longet, S., Austin, J.A., Skelly, D.T., Dejnirattisai, W., Adele, S., Meardon, N.,  
433 Faustini, S., Al-Taei, S., Moore, S.C., *et al.* (2021). Immunogenicity of standard and extended  
434 dosing intervals of BNT162b2 mRNA vaccine. *Cell* 184, 5699-5714 e5611.  
435 Ramasamy, M.N., Minassian, A.M., Ewer, K.J., Flaxman, A.L., Folegatti, P.M., Owens, D.R.,  
436 Voysey, M., Aley, P.K., Angus, B., Babbage, G., *et al.* (2021). Safety and immunogenicity of  
437 ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults  
438 (COV002): a single-blind, randomised, controlled, phase 2/3 trial. *Lancet* 396, 1979-1993.  
439 Reynolds, C.J., Pade, C., Gibbons, J.M., Butler, D.K., Otter, A.D., Menacho, K., Fontana, M.,  
440 Smit, A., Sackville-West, J.E., Cutino-Moguel, T., *et al.* (2021). Prior SARS-CoV-2 infection  
441 rescues B and T cell responses to variants after first vaccine dose. *Science*.  
442 Saadat, S., Rikhtegaran Tehrani, Z., Logue, J., Newman, M., Frieman, M.B., Harris, A.D., and  
443 Sajadi, M.M. (2021). Binding and Neutralization Antibody Titers After a Single Vaccine Dose  
444 in Health Care Workers Previously Infected With SARS-CoV-2. *JAMA* 325, 1467-1469.  
445 Schmidt, F. (2021). Plasma neutralization properties of the SARS-CoV-2 Omicron variant.  
446 *medRxiv*.  
447 Seow, J., Graham, C., Merrick, B., Acors, S., Pickering, S., Steel, K.J.A., Hemmings, O.,  
448 O'Byrne, A., Kouphou, N., Galao, R.P., *et al.* (2020). Longitudinal observation and decline of  
449 neutralizing antibody responses in the three months following SARS-CoV-2 infection in  
450 humans. *Nat Microbiol* 5, 1598-1607.  
451 Shrotri, M., Navaratnam, A.M.D., Nguyen, V., Byrne, T., Geismar, C., Fragaszy, E., Beale, S.,  
452 Fong, W.L.E., Patel, P., Kovar, J., *et al.* (2021). Spike-antibody waning after second dose of  
453 BNT162b2 or ChAdOx1. *Lancet* 398, 385-387.  
454 Stamatatos, L., Czartoski, J., Wan, Y.H., Homad, L.J., Rubin, V., Glantz, H., Neradilek, M.,  
455 Seydoux, E., Jennewein, M.F., MacCamy, A.J., *et al.* (2021). mRNA vaccination boosts cross-  
456 variant neutralizing antibodies elicited by SARS-CoV-2 infection. *Science*.  
457 Tartof, S.Y., Slezak, J.M., Fischer, H., Hong, V., Ackerson, B.K., Ranasinghe, O.N.,  
458 Frankland, T.B., Ogun, O.A., Zamparo, J.M., Gray, S., *et al.* (2021). Effectiveness of mRNA  
459 BNT162b2 COVID-19 vaccine up to 6 months in a large integrated health system in the USA:  
460 a retrospective cohort study. *Lancet* 398, 1407-1416.  
461 Tauzin, A. (2021). Strong humoral immune responses against SARS-CoV-2 Spike after  
462 BNT162b2 mRNA vaccination with a 16-week interval between doses. *medRxiv*.  
463 Thompson, C.P., Grayson, N.E., Paton, R.S., Bolton, J.S., Lourenco, J., Penman, B.S., Lee,  
464 L.N., Odon, V., Mongkolsapaya, J., Chinnakannan, S., *et al.* (2020). Detection of neutralising  
465 antibodies to SARS-CoV-2 to determine population exposure in Scottish blood donors  
466 between March and May 2020. *Euro Surveill* 25.  
467 Walsh, E.E., Frenck, R.W., Jr., Falsey, A.R., Kitchin, N., Absalon, J., Gurtman, A., Lockhart,  
468 S., Neuzil, K., Mulligan, M.J., Bailey, R., *et al.* (2020). Safety and Immunogenicity of Two RNA-  
469 Based Covid-19 Vaccine Candidates. *N Engl J Med* 383, 2439-2450.  
470