

1 **Vaccination-infection interval determines cross-neutralization potency to SARS-CoV-2 Omicron**  
2 **after breakthrough infection by other variants**

3

4 **Authors:**

5 Sho Miyamoto<sup>1,8</sup>, Takeshi Arashiro<sup>1,2,8</sup>, Yu Adachi<sup>3,8</sup>, Saya Moriyama<sup>3,8</sup>, Hitomi Kinoshita<sup>4,8</sup>, Takayuki  
6 Kanno<sup>1</sup>, Shinji Saito<sup>1</sup>, Harutaka Katano<sup>1</sup>, Shun Iida<sup>1</sup>, Akira Ainai<sup>1</sup>, Ryutaro Kotaki<sup>3</sup>, Souichi Yamada<sup>4</sup>,  
7 Yudai Kuroda<sup>5</sup>, Tsukasa Yamamoto<sup>5</sup>, Keita Ishijima<sup>5</sup>, Eun-Sil Park<sup>5</sup>, Yusuke Inoue<sup>5</sup>, Yoshihiro Kaku<sup>5</sup>,  
8 Minoru Tobiume<sup>1</sup>, Naoko Iwata-Yoshikawa<sup>1</sup>, Nozomi Shiwa-Sudo<sup>1</sup>, Kenzo Tokunaga<sup>1</sup>, Seiya Ozono<sup>1</sup>,  
9 Takuya Hemmi<sup>1</sup>, Akira Ueno<sup>1</sup>, Noriko Kishida<sup>6</sup>, Shinji Watanabe<sup>6</sup>, Kiyoko Nojima<sup>7</sup>, Yohei Seki<sup>7</sup>, Takuo  
10 Mizukami<sup>7</sup>, Hideki Hasegawa<sup>6</sup>, Hideki Ebihara<sup>4</sup>, Ken Maeda<sup>5</sup>, Shuetsu Fukushi<sup>4\*</sup>, Yoshimasa Takahashi<sup>3\*</sup>,  
11 Tadaki Suzuki<sup>1,9\*</sup>

12

13 **Affiliations:**

14 <sup>1</sup>Department of Pathology, National Institute of Infectious Diseases; Tokyo, 162-8640, Japan.

15 <sup>2</sup>Center for Surveillance, Immunization, and Epidemiologic Research, National Institute of Infectious  
16 Diseases, Tokyo, Japan

17 <sup>3</sup>Research Center for Drug and Vaccine Development, National Institute of Infectious Diseases; Tokyo  
18 162-8640, Japan.

19 <sup>4</sup>Department of Virology I, National Institute of Infectious Diseases; Tokyo 162-8640, Japan.

20 <sup>5</sup>Department of Veterinary Science, National Institute of Infectious Diseases; Tokyo 162-8640, Japan.

21 <sup>6</sup>Center for Influenza and Respiratory Virus Research, National Institute of Infectious Diseases; Tokyo  
22 208-0011, Japan.

23 <sup>7</sup>Department of Safety Research on Blood and Biological Products, National Institute of Infectious

24 Diseases; 208-0011, Japan.

25 <sup>8</sup>These authors contributed equally

26 <sup>9</sup>Lead Contact

27

28 **\*Corresponding authors:**

29 fukushi@niid.go.jp (S.F.), ytakahas@niid.go.jp (Y.T.), and tksuzuki@nih.go.jp (T.S.)

30

31 **SUMMARY**

32 **Background**

33 The immune profile against SARS-CoV-2 has dramatically diversified due to a complex combination of  
34 exposure to vaccines and infection by various lineages/variants, likely generating a heterogeneity in  
35 protective immunity in a given population. To further complicate this, the Omicron variant, with numerous  
36 spike mutations, has emerged. These circumstances have created the need to assess the potential of  
37 immune evasion by the Omicron in individuals with various immune histories.

38 **Methods**

39 The neutralization susceptibility of the variants including the Omicron and their ancestor was comparably  
40 assessed using a panel of plasma/serum derived from individuals with divergent immune histories. Blood  
41 samples were collected from either mRNA vaccinees or from those who suffered from breakthrough  
42 infections by the Alpha/Delta with multiple time intervals following vaccination.

43 **Findings**

44 The Omicron was highly resistant to neutralization in fully vaccinated individuals without a history of  
45 breakthrough infections. In contrast, robust cross-neutralization against the Omicron were induced in  
46 vaccinees that experienced breakthrough infections. The time interval between vaccination and infection,  
47 rather than the variant types of infection, was significantly correlated with the magnitude and potency of  
48 Omicron-neutralizing antibodies.

49 **Conclusions**

50 Immune histories with breakthrough infections can overcome the resistance to infection by the Omicron,  
51 with the vaccination-infection interval being the key determinant of the magnitude and breadth of  
52 neutralization. The diverse exposure history in each individual warrants a tailored and cautious approach

53 to understanding population immunity against the Omicron and future variants.

54 **Funding**

55 This study was supported by grants from the Japan Agency for Medical Research and Development  
56 (AMED).

57

58 **KEYWORDS**

59 SARS-CoV-2; Omicron variant; COVID-19 vaccine; BNT162b2 mRNA vaccine; Breakthrough infection;

60 Neutralizing antibody

61

62

## 63 **Introduction**

64       Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2  
65 (SARS-CoV-2), continues to cause significant morbidity and mortality globally. Since the first detection  
66 of a new SARS-CoV-2 variant belonging to the Pango lineage B.1.1.529 in South Africa, it has spread  
67 rapidly worldwide, especially in African countries (Scott et al., 2021; The Lancet Infectious Diseases,  
68 2021). The World Health Organization (WHO) classified SARS-CoV-2 variant B.1.1.529 as a Variant of  
69 Concern (VOC), due to possible changes in viral characteristics, and designated it as “Omicron” (WHO,  
70 <https://www.who.int/>). The Omicron variant is characterized by approximately 30 amino acid mutations,  
71 three small deletions, and one insertion in the spike protein compared to the vaccine strain. Among these,  
72 15 mutations are located in the receptor-binding domain (RBD), which induces antibody evasion.  
73 Compared to previous VOCs, the Omicron variant contains a larger number of mutations in its spikes,  
74 which can dramatically alter its infectivity and immune evasion capabilities compared to any other variant  
75 to date, raising a serious global public health concern (Dejnirattisai et al., 2021; Leung and Wu, 2021; Lu  
76 et al., 2021).

77       A retrospective study using routine epidemiological surveillance data taken from several countries  
78 demonstrated that the Omicron variant was associated with a substantial ability to evade immunity from  
79 prior infection (Eggink et al., 2021; Goga et al., 2021; Pulliam et al., 2021). In addition, a number of  
80 studies have reported that the Omicron variant evades neutralization by sera collected from vaccinated or  
81 convalescent patients (Carreno et al., 2021; Dejnirattisai et al., 2021; Liu et al., 2021; Lu et al., 2021). In  
82 contrast, it has also been reported that booster immunization with an mRNA vaccine significantly increases  
83 the serum neutralizing potency against the Omicron variant (Doria-Rose et al., 2021; Edara et al., 2021;  
84 Gruell et al., 2021) and also provides a significant increase in protection against mild and severe disease

85 (Andrews et al., 2021; Hansen et al., 2021), indicating that an enhancement of pre-existing immunity  
86 induced by the ancestral virus antigens could overcome the antigenic shift of the Omicron variant and  
87 confer cross-protection against it. Moreover, it has been reported that sera from individuals who  
88 experienced COVID-19 vaccine breakthrough infections have improved cross-neutralization potency  
89 against SARS-CoV-2 variants, especially Delta variant breakthrough infections (Bates et al., 2021).  
90 Currently, the cumulative number of COVID-19 cases, seroprevalence of SARS-CoV-2, types of vaccines  
91 available, primary vaccination coverage, booster vaccine availability, and number of COVID-19 vaccine  
92 breakthrough infections vary in different regions of the world, and the immunity status of populations in  
93 each region is also diverse. To properly assess the risk of the spread of the Omicron variant in each region,  
94 it is necessary to understand the ability of the variant to evade immunity induced in various settings.  
95 Therefore, there is a need to better understand the susceptibility of the Omicron variant to neutralizing  
96 antibodies elicited by other variant breakthrough infections, as well as sera from vaccine recipients, to  
97 thoroughly assess the risk of immune evasion by the Omicron variant in populations with diverse immune  
98 histories to SARS-CoV-2. Here, we determined the neutralization susceptibility of the Omicron variant to  
99 antibodies from COVID-19 mRNA vaccine recipients with or without breakthrough infections and  
100 compared it to the ancestral SARS-CoV-2 lineage A virus, D614G, Beta, and Delta variants.

101

102 **Results**

103 *High resistance of SARS-CoV-2 Omicron variant to neutralizing antibodies from mRNA vaccinees*

104 First, we tested the *in vitro* neutralization activity of plasma samples obtained from individuals  
105 vaccinated twice with BNT162b2 (Pfizer/BioNTech mRNA vaccine) against SARS-CoV-2 Pango lineage  
106 A (as a reference for ancestral strains), D614G (B.1), Beta variant (B.1.351), Delta variant (B.1.617.2),  
107 and Omicron variant (B.1.1.529) by vesicular stomatitis virus (VSV) pseudovirus-based and live-virus  
108 neutralization assays. Twenty health care workers who received two doses of mRNA vaccine were enrolled  
109 for blood donation at the early (median = 52.5 days) and late (median = 171.5 days) time points after the  
110 second dose (Supplemental Table 1). All volunteers were confirmed to be negative for anti-nucleocapsid  
111 antibodies in the pre-vaccinated plasma. Both VSV pseudovirus-based and live-virus neutralization assays  
112 with plasma samples from fully vaccinated individuals demonstrated a significant reduction in  
113 neutralization activity against the Omicron variant at early and late time points compared to the ancestral  
114 virus (Figure 1). Almost all samples lost all neutralizing activity against the Omicron variant in both VSV  
115 pseudovirus-based and live-virus neutralization assays, and the reduction in neutralization for the Omicron  
116 variant was greater than 18.5, or approximately 8-fold, at the early time point and 5.4, or 3.0-fold, at the  
117 late time point by VSV pseudovirus or live-virus neutralization assay, respectively, which was greater than  
118 that for the Beta variant, which has the most pronounced *in vitro* escape phenotype to date, or for the Delta  
119 variant (Figure 1).

120

121 *Cross-neutralization of the Omicron variant by sera from individuals with mRNA vaccine breakthrough*  
122 *Alpha or Delta variant infections*

123 While sera from individuals completing two doses of mRNA vaccination showed only limited

124 neutralizing activity, breakthrough infections after full vaccination induced elevated immune responses  
125 and cross-neutralization potency against SARS-CoV-2 variants (Bates et al., 2021). To investigate the  
126 neutralizing activity against the Omicron variant using sera from individuals who had mRNA vaccine  
127 breakthrough infections due to non-Omicron variants, convalescent sera (obtained 10-22 days after  
128 infection with Alpha or Delta variant; Table 1) were examined in VSV pseudovirus-based and live-virus  
129 neutralization assays. In the pseudovirus-based neutralization assay, neutralizing activity against the Delta  
130 variant was elevated compared to that against the ancestral virus and was particularly pronounced in those  
131 with breakthrough infection with the Delta variant, suggesting that breakthrough infection preferentially  
132 induces antibodies with high neutralizing activity against the infected variant (Figure 2A, 2B, 2C, and 2D).  
133 In contrast, the neutralizing activity against Beta or Omicron variants was markedly lower than that against  
134 the ancestral virus in the VSV pseudovirus-based neutralization assay, with a 3.8-fold or 9.7-fold decrease  
135 for Beta or Omicron variants, respectively (Figure 2A). In the live-virus neutralization assay, the  
136 neutralizing activity of the two isolates of the Omicron variant was also reduced by approximately 10-fold  
137 compared to that against the ancestral virus (Figure 2B). In contrast to the sera of mRNA vaccinees without  
138 breakthrough infections, we detected neutralizing activity against the Omicron variant in most sera of  
139 individuals with breakthrough infections in both assays, and there were large individual variations in the  
140 degree of reduction in neutralizing activity compared to the ancestral virus. Notably, some sera from  
141 individuals with breakthrough infections showed high cross-neutralizing activity and neutralized the  
142 Omicron variant to a level comparable to that of the other variants. In addition, the cross-neutralizing  
143 activity against the Omicron variant tended to be higher in sera from individuals with breakthrough  
144 infections than that against the Delta variant (Figure 2C, 2D). In the breakthrough cases included in this  
145 study, there was variation in the interval between vaccination and breakthrough infection, as epidemics



146 caused by the Alpha variant and those caused by the Delta variant occurred during different periods.  
147 Specifically, half of the breakthrough infections due to the Delta variant, which occurred later than the  
148 Alpha epidemic, occurred >60 days after vaccination (Figure 2E).

149

150 *Positive correlation between cross-neutralization activity against SARS-CoV-2 variants and interval*  
151 *between vaccination and breakthrough infection*

152 To understand the factors contributing to the high heterogeneity of cross-neutralizing activity against  
153 variants in sera from breakthrough cases, we evaluated the relationship between neutralizing activity  
154 against each variant and time between vaccine completion and breakthrough infection in each case.  
155 Intriguingly, in both pseudovirus-based and live-virus assays, the neutralizing activity against the ancestral  
156 virus and each variant, including Omicron, increased as the time between vaccination and breakthrough  
157 infection increased (Figure 3A–3K). Notably, the degree of correlation between neutralizing activity and  
158 the vaccination-to-infection interval tended to be stronger for Beta and Omicron variants, which were  
159 antigenically shifted from the ancestral virus (Figure 3C, 3E, 3H, 3J, and 3K). These trends did not differ  
160 significantly between sera after breakthrough infection with Alpha and Delta variants, except for  
161 neutralizing activity against the Delta variant. The neutralizing activity against the Delta variant by the  
162 sera obtained after Delta variant breakthrough infection showed a completely different trend from that of  
163 other serum-virus combinations (Figure 3D and 3I). Considering the effect of age on the cross-neutralizing  
164 activity against SARS-CoV-2, we excluded elderly patients whose sera were obtained only with a short  
165 interval between vaccination and breakthrough infection, and evaluated only sera obtained from patients  
166 younger than 60 years. We found a positive correlation between cross-neutralization activity against  
167 SARS-CoV-2 variants and the interval between vaccination and breakthrough infection, similar to the

168 results described above (Figure S1A–S1G). In addition, to evaluate the effect of the presence or absence  
169 of symptoms on cross-neutralizing activity, we compared the neutralizing activity between subjects with  
170 and without symptoms, but there was no significant difference in the neutralizing activity for each variant  
171 between subjects with and without symptoms (Figure S1H and S1I).

172

173 *Enhanced neutralizing potency index of anti-Omicron variant antibody in sera from individuals with*  
174 *breakthrough infections*

175 To assess the quality of neutralizing activity by distinguishing the antibodies with a high neutralizing  
176 potency from those with a lower potency which might be present in abundance, the neutralization potency  
177 index (NPI), which represents the average of the neutralization potencies of individual antibodies  
178 (Moriyama et al., 2021), was estimated in the sera from individuals who had breakthrough infections. The  
179 IgG titer of antibody against the RBD of the Omicron variant was greatly reduced by more than 40-fold  
180 compared to that of the ancestral virus (Figure 4A). Notably, as the interval between vaccination and  
181 infection increased, the IgG titer of antibody recognizing the RBD of the ancestral virus did not change  
182 whereas the IgG titer of antibodies against the Omicron variant RBD increased, suggesting that IgG  
183 antibodies recognizing the Omicron RBD were readily induced as this period extended (Figure 4B). The  
184 NPI against the ancestral virus, Beta, Delta, and Omicron variants was then calculated. The NPI was  
185 calculated using the neutralizing activity determined in pseudovirus-based and live-virus assays, and the  
186 NPI calculated in both assays demonstrated a significant increase in the Omicron variant compared with  
187 the ancestral virus (Figure 4C and 4D). Furthermore, evaluation of the relationship between NPI against  
188 the Omicron variant and vaccination-to-infection interval showed that, as with neutralizing activity, NPI  
189 was positively correlated with vaccination-to-infection interval: the longer the interval, the higher the NPI

190 and the higher the quality of antibodies produced (Figure 4E, 4F, and 4G). These results suggest that high-  
191 quality antibodies play a role in cross-neutralization against the Omicron variant in sera from individuals  
192 with breakthrough infections.

193

## 194 **Discussion**

195 The Omicron variant is a highly divergent SARS-CoV-2 variant with approximately 30 amino acid  
196 mutations in the spike protein, causing a severe evasion of humoral immunity induced by vaccines and  
197 previous infections (Carreno et al., 2021; Cele et al., 2021; Dejnirattisai et al., 2021; Lu et al., 2021). In  
198 this study, we demonstrated that sera from individuals with BNT162b2 mRNA vaccine breakthrough  
199 infection cases by SARS-CoV-2 Alpha or Delta variants showed robust cross-neutralizing potency against  
200 the Omicron variant, even though sera from vaccinees without breakthrough infection had greatly reduced  
201 neutralizing potency against the Omicron variant. Furthermore, a longer interval between vaccination and  
202 breakthrough infection was favorable for better antibody responses against the Omicron variant in serum  
203 from individuals who had non-Omicron variant breakthrough infections.

204 The Omicron variant is spreading rapidly in regions where the population has already been immunized  
205 by vaccines or previous infections. Elucidating the underlying factors that have led to marked immune  
206 evasion in immunized populations is urgently needed to mitigate the rapid global spread of the Omicron  
207 variant. It remains unclear whether the observed rapid growth rate of the Omicron variant can be attributed  
208 to immune evasion, increased intrinsic transmissibility, or a combination of the two. A recent analysis of  
209 data estimated that the risk of reinfection with the Omicron variant was significantly higher than that with  
210 the Delta variant (Pulliam et al., 2021). In addition, several reports have shown that after the primary series  
211 (two doses) of COVID-19 vaccines, the vaccine effectiveness against infection with the Omicron variant

212 was significantly reduced compared to that with the Delta variant (Andrews et al., 2021; Hansen et al.,  
213 2021). In contrast, studies using serum samples from individuals who had been previously infected and  
214 subsequently vaccinated with the mRNA vaccine and those who had received two doses of the mRNA  
215 vaccine followed by an mRNA vaccine booster found high levels of neutralization against the Omicron  
216 variant (Doria-Rose et al., 2021; Edara et al., 2021; Gruell et al., 2021). Furthermore, it was also reported  
217 that vaccine effectiveness against the Omicron variant after mRNA vaccine booster dramatically increased  
218 against SARS-CoV-2 infection (Andrews et al., 2021; Hansen et al., 2021). Taken together, these results  
219 suggest that part of the waning immunity to the Omicron variant in populations immunized with the  
220 primary vaccination series or previous infection due to other variants may be explained by a reduced serum  
221 neutralizing activity against the Omicron variant. Notably, enhanced neutralization of the Omicron variant  
222 by the ancestral virus-based booster vaccine indicated that the spike protein of the Omicron variant still  
223 shares some neutralizing epitopes with the ancient virus. Supporting this, multiple neutralizing monoclonal  
224 antibodies have been found to overcome the antigenic shift of the Omicron variant and cross-neutralize  
225 any SARS-CoV-2 variant, as well as their ancestral strain (Cameroni et al., 2021).

226 In this study, we demonstrated that longer intervals between vaccination and breakthrough infection  
227 improved cross-neutralizing potency against the Omicron variant. COVID-19 vaccine breakthrough  
228 infections elicited robust cross-neutralizing antibody responses against several SARS-CoV-2 variants,  
229 which were largely recalled from memory B cells induced by previous vaccinations (Bates et al., 2021).  
230 Longer prime-boost intervals have been reported to result in higher antibody responses in both adenoviral  
231 vectors and mRNA vaccines (Grunau et al., 2021; Payne et al., 2021; Voysey et al., 2021), and mRNA  
232 vaccines induce a persistent germinal center B cell response in the draining lymph nodes at least 12 weeks  
233 after the second dose, which enables the generation of robust humoral immunity (Turner et al., 2021).

234 Notably, affinity maturation of IgG antibodies to the spike protein-conserved region, which is highly  
235 correlated with serum neutralization potency against antigenically drifted variants, persists for more than  
236 3 months after SARS-CoV-2 infection, presumably owing to the supply of long-lived plasma cells  
237 (Moriyama et al., 2021). These observations in vaccinees and convalescents suggest that longer intervals  
238 between priming and boosting might be favorable for improving the potency and breadth of neutralizing  
239 antibodies against SARS-CoV-2 through continued affinity maturation of variant-resistant IgG antibodies  
240 expressed on memory B cells and plasma cells. Furthermore, our observations also imply that the primary  
241 vaccination series with mRNA vaccines (2 doses) induces memory B cells to produce cross-neutralizing  
242 antibodies against Omicron variants, despite limited induction of serum anti-Omicron neutralizing  
243 antibodies.

244 SARS-CoV-2 variants that emerge repeatedly with no clear seasonality expand globally and replace  
245 existing variants in a few months, making it very difficult to develop a vaccine that is fully antigenically  
246 matched to the upcoming variants and has greatly impeded the employment of strategies similar to those  
247 used for seasonal influenza. Therefore, it is important to encourage research on vaccine development with  
248 enhanced cross-protection against not only the Omicron variant but also unidentified variants that are  
249 expected to appear in the future. This can be achieved by designing vaccine antigens with high cross-  
250 neutralizing antibody induction capacity against various variants, optimizing the vaccination interval,  
251 modifying the vaccination route, and elaborating adjuvants. Furthermore, it has been reported that booster  
252 immunization with an mRNA vaccine provides a significant increase in protection against mild and severe  
253 disease but is still less effective than immunization for other variants, highlighting the need to develop  
254 next-generation vaccines.

255 This study had several limitations. First, the number of samples evaluated was small. Second, since

256 sera from vaccine recipients without breakthrough infection at 6 months after vaccination had a low  
257 neutralization titer even against the ancestral virus in our assay, an accurate fold-reduction in neutralizing  
258 antibody titer could not be determined. Third, we did not include serum samples collected immediately  
259 (<10 days) after breakthrough infection. It is expected that the longer the interval between the vaccine and  
260 infection, the lower the antibody titer at the time of breakthrough infection. The possibility that reduced  
261 neutralizing activity at the time of breakthrough infection results in efficient viral replication in the upper  
262 respiratory tract, which may contribute to a better antibody response, was not evaluated in the present  
263 study. Fourth, we did not assess T cell immunity against SARS-CoV-2, including the Omicron variant,  
264 which contributes to protection when antibody titers are low in non-human primate models (McMahan et  
265 al., 2021) and may correlate with protection against severe disease. Finally, our investigation did not  
266 evaluate the actual risk of reinfection by the Omicron variant in individuals with a history of breakthrough  
267 infection.

268 In conclusion, breakthrough sera demonstrated improved cross-neutralization against the Omicron  
269 variant, and the time from vaccination to breakthrough infection was a key determinant of the magnitude  
270 and breadth of neutralizing activity against variants after breakthrough infection. These results suggest  
271 that population immunity is becoming increasingly diverse against Omicron and future variants depending  
272 on different settings, including varying degrees of exposure to existing variants, types of vaccines available,  
273 primary vaccination coverage, availability of booster vaccines, and the magnitude of COVID-19 vaccine  
274 breakthrough infections. Therefore, a tailored and cautious approach is warranted to understand the  
275 population immunity against Omicron and future variants.

276

277 **Acknowledgment**

278 This study was supported by grants from the Japan Agency for Medical Research and Development  
279 (AMED) (Grant Numbers by JP21fk0108104, JP20fk0108534, and JP21fk0108615). We thank Miki  
280 Akimoto, Aya Sato, Dai Izawa, Yusuke Sakai, Noriyo Nagata, Akiko Sataka, Asato Kojima, Izumi  
281 Kobayashi, Yuki Iwamoto, Yuko Sato, Milagros Virhuez Mendoza, Noriko Nakajima, Kenta Takahashi,  
282 Yuichiro Hirata, Masataka Tokita, Masanori Isogawa, Kazutaka Terahara, Takayuki Matsumura, Tomohiro  
283 Takano, Taishi Onodera, Eriko Izumiyama, Akira Dosaka, Kazuko Isoyama, Naoka Yoshida, Rieko Iwaki,  
284 and Emi Koda at NIID for their technical support, and Fukumi Nakamura-Uchiyama at Tokyo  
285 Metropolitan Bokutoh Hospital and Hidefumi Shimizu at JCHO Tokyo Shinjuku Medical Center for  
286 collection of blood samples from vaccinees. We also thank the following healthcare facilities, local health  
287 centers, and public health institutes for their contribution in providing us with valuable patient information  
288 and samples on breakthrough cases: Aki Health Center, Akiru Municipal Medical Center, Atsugi City  
289 Hospital, Akita Research Center for Public Health and Environment, Aomori Jikeikai Hospital, Aso Onsen  
290 Hospital, Chiba City Institute of Health and Environment, Chiba Prefectural Institute of Public Health,  
291 Chigasaki City Public Health Center, Chuhoku Branch Office for Public Health and Welfare, Daiwa  
292 Hospital (Osaka), Fukui Prefectural Institute of Public Health and Environmental Science, Fujimino  
293 Emergency Hospital, Fukuoka City Hospital, Gifu City Public Health Center, Gifu Prefectural General  
294 Medical Center, Gifu Prefectural Research Institute for Health and Environmental Sciences, Gunma  
295 Prefectural Institute of Public Health and Environmental Sciences, Gunma Saiseikai Maebashi Hospital,  
296 Hachioji City Public Health Center, Hakodate City Institute of Public Health, Hakodate Public Health  
297 Center, Harada Hospital (Hiroshima), Hyogo Prefectural Institute of Public Health Science, Ikegami  
298 General Hospital, IMS Fujimi General Hospital, IMS Sapporo Digestive Disease Center General Hospital,  
299 Inba Health and Welfare Center, International Goodwill Hospital, International University of Health and

300 Welfare Hospital, International University of Health and Welfare Mita Hospital, Ishikawa Prefectural  
301 Central Hospital, Ishikawa Prefecture Health and Welfare Department, Ishikawa Prefectural Institute of  
302 Public Health and Environmental Science, Itami City Hospital, Japanese Red Cross Gifu Hospital,  
303 Japanese Red Cross Kanazawa Hospital, Japanese Red Cross Kumamoto Hospital, Japanese Red Cross  
304 Narita Hospital, JCHO Kanazawa Hospital, JCHO Nankai Medical Center, JCHO Takanawa Hospital,  
305 Juntendo University Hospital, Kaga Medical Center, Kameda Medical Center, Kasai Clinic (Osaka),  
306 Kashiwa Public Health Center, Kawachi General Hospital, Kawaguchi Seiwa Hospital, Keio University  
307 Hospital, Keiwakai Ebetsu Hospital, Kitakyushu Public Health Center, Kitakyushu Public Health Institute,  
308 Kitasato University Kitasato Institute Hospital, Kobe City Nishi-Kobe Medical Center, Kobe Ekisaikai  
309 Hospital, Komatsu Municipal Hospital, Koriyama City Public Health Center, Kumamoto City Hospital,  
310 Kumamoto City Public Health Center, Kumamoto Prefectural Institute of Public-Health and  
311 Environmental Science, Kurume-shi Public Health Center, Kyoritsu Narashinodai Hospital, Kyoto City  
312 Institute of Health and Environmental Sciences, Kyoto Kujo Hospital, Kyoto Prefectural Institute of Public  
313 Health and Environment, Kyowakai Kyoritsu Hospital, Maebashi-shi Public Health Center, Makita  
314 General Hospital, Matsui Hospital (Tokyo), Matsumoto City Public Health Center, Mie Prefectural  
315 Institute of Public Health and Environmental Sciences, Minami Kaga Health and Welfare Center, Minoh  
316 City Hospital, Misato Kenwa Hospital, Mitsui Memorial Hospital, Mizushima Kyodo Hospital, Nadogaya  
317 Hospital, Nagano City Public Health Center, Nagano City Public Health Institute, Nagano Environmental  
318 Conservation Research Institute, Nagasaki Prefecture Iki Hospital, Nagayama Hospital (Osaka), Nanbu  
319 Tokushukai Hospital, Nanshu Orthopedics Hospital, Narita Tomisato Tokushukai Hospital, National  
320 Center for Global Health and Medicine, National Hospital Organization Kyoto Medical Center, National  
321 Hospital Organization Osaka National Hospital, National Hospital Organization Nagoya Medical Center,



322 Niigata City Health Center, Niigata Prefectural Institute of Public Health and Environmental Sciences,  
323 Nippon Medical School Chiba Hokusoh Hospital, Nishinomiya City Public Health Center, Nitta ENT  
324 clinic, Obihiro Dai-ichi Hospital, Obihiro Health Center, Oita City Public Health Center, Oita Kouseiren  
325 Tsurumi Hospital, Oita Prefectural Institute of Health and Environment, Okayama Kyoritsu Hospital,  
326 Osaka Medical and Pharmaceutical University Hospital, Saiseikai Kanazawa Hospital, Saiseikai  
327 Moriyama Municipal Hospital, Saiseikai Yamaguchi Hospital, Saitama City Hospital, Saitama Nishi  
328 Kyodo Hospital, Sakura General Hospital (Aichi), Sakura Hospital (Kumamoto), Sapporo Public Health  
329 Office, Sasebo City General Hospital, Shibuya Clinic (Ishikawa), Shimane Prefectural Institute of Public  
330 Health and Environmental Science, Shimonoseki City Hospital, Shimonoseki Public Health Center, Shin  
331 Komonji Hospital, Shin-Yamanote Hospital, Shonan Daiichi Hospital, Suginami Public Health Center,  
332 Sumida Ward Public Health Center, Takasaki General Public Health Center, Tama Nambu Chiiki Hospital,  
333 Tamashima Central Hospital, Tochigi Prefectural Institute of Public Health and Environmental Science,  
334 Tokyo Medical and Dental University Medical Hospital, Tokyo Metropolitan Geriatric Hospital and  
335 Institute of Gerontology, Tokyo Metropolitan Institute of Public Health, Tokyo Women's Medical  
336 University Adachi Medical Center, Tonan Hospital, Tsuchiya General Hospital, Tsukuba Central Hospital,  
337 Tsurukawa Sanatorium Hospital, Ueda Health and Welfare Office, Utsunomiya City Institute of Public  
338 Health and Environment, and Yamanashi Prefectural Institute for Public Health and Environment. We also  
339 thank GISAID for the platform to share and compare our data with data submitted globally.

340

#### 341 **Author contributions**

342 Conceptualization, SF, YT, TS; Methodology, SMi, TA, SF, YA, SMo, HKi, KM, HKa, SI; Investigation,  
343 SMi, TA, YA, SMo, HKi, TK, SS, AA, RK, SY, YKu, TY, KI, EP, YI, YKa, MT, HKa, SI, NI, NS, KT,

344 SO, TH, AU, NK, SW, KN, YS, TM, KM; Writing – Original Draft, SMi, TA, YA, SMo, HKi, SF, YT,  
345 TS; Writing – Review & Editing, SMi, TA, YA, SMo, HKi, TK, SS, HKa, SI, AA, RK, SY, YKu, TY, KI,  
346 EP, YI, YKa, MT, NI, NS, KT, SO, TH, AU, NK, SW, KN, YS, TM, HH, HE, KM, SF, YT, TS;  
347 Visualization, SMi; Supervision, HH, HE, KM, SF, YT, TS; Project Administration, SF, YT, TS; Funding  
348 Acquisition, KM, YT, TS. SMi performed statistical analyses. SF, YT and TS had unrestricted access to  
349 all data. SMi, TA, YA, SMo, HKi, SF, YT, and TS prepared the first draft of the manuscript, which was  
350 reviewed and edited by all other authors. All authors agreed to submit the manuscript, read and approved  
351 the final draft, and take full responsibility of its content including the accuracy of the data and statistical  
352 analysis.

353

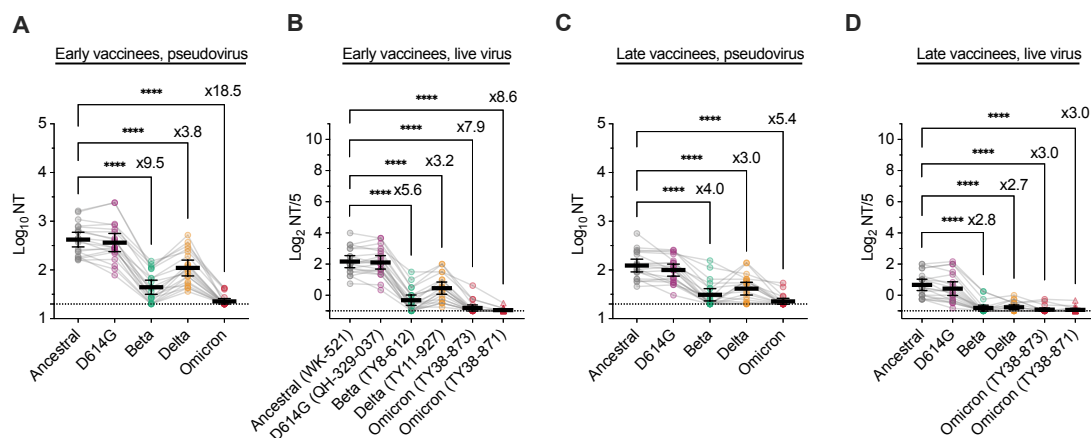
#### 354 **Declaration of interests**

355 The authors declare no competing interests.

356

357

358 **Figures**



359

360 **Figure 1.** High resistance of SARS-CoV-2 Omicron variant to neutralizing antibodies from mRNA

361 vaccinees (A, B) Neutralization titers (NTs) of early vaccinee serums against variants of SARS-CoV-2

362 pseudoviruses (A) and live viruses (B). (C, D) Neutralization titers of late vaccinee serums against variants

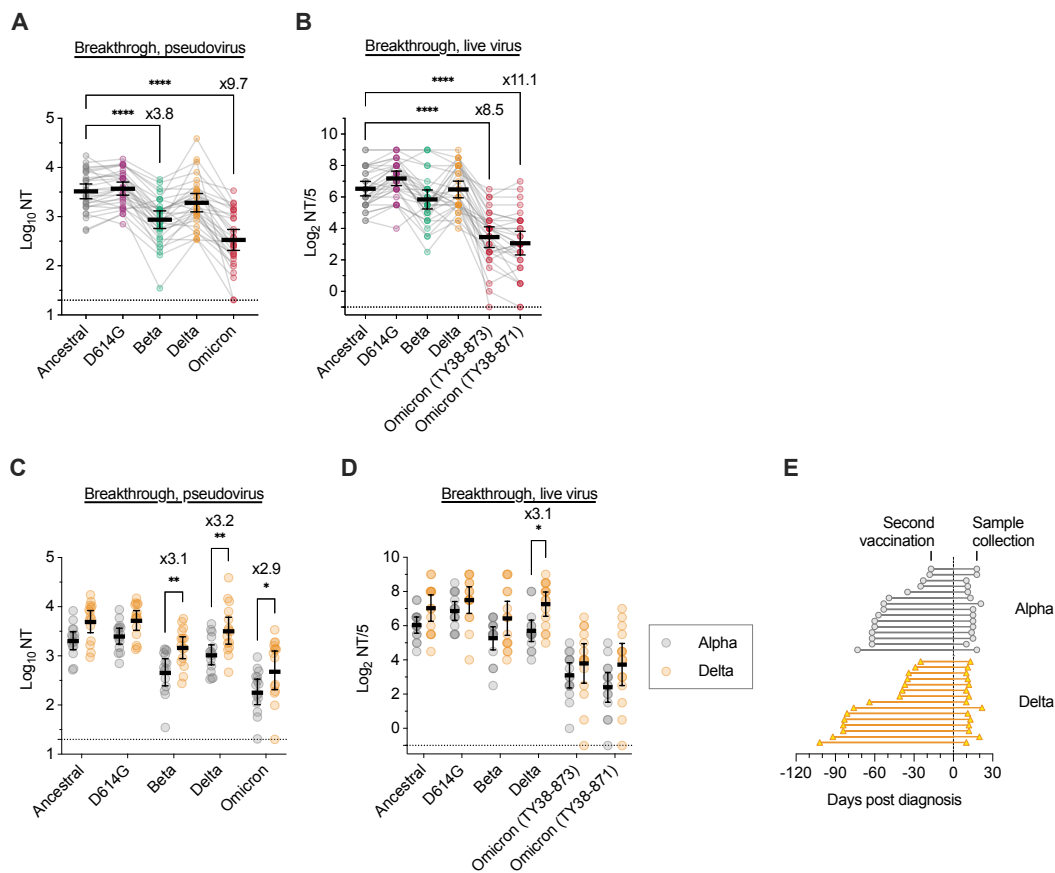
363 of SARS-CoV-2 pseudoviruses (C) and live viruses (D). Data from the same serum are connected with

364 lines, and the mean ± 95% CI of each serum titer is presented. The titers between the ancestral and variants

365 were compared using the one-way ANOVA with Dunnett's test; \*\*\*\* $p < 0.0001$ . Fold-reductions are

366 indicated above columns in statistically significant cases.

367



368

369 **Figure 2.** Cross-neutralization to the Omicron variant by sera from individuals who experienced mRNA

370 vaccine breakthrough Alpha or Delta variant infections (A, B) Neutralization titers of vaccine-

371 breakthrough case serums against variants of SARS-CoV-2 pseudoviruses (A) and live viruses (B). Data

372 from the same serum are connected with lines, and the mean  $\pm$  95% CI of each serum titer is presented.

373 The titers between the ancestral and variants were compared using the one-way ANOVA with Dunnett's

374 test; \*\*\*\* $p < 0.0001$ . Fold-reductions are indicated above columns in statistically significant cases. (C, D)

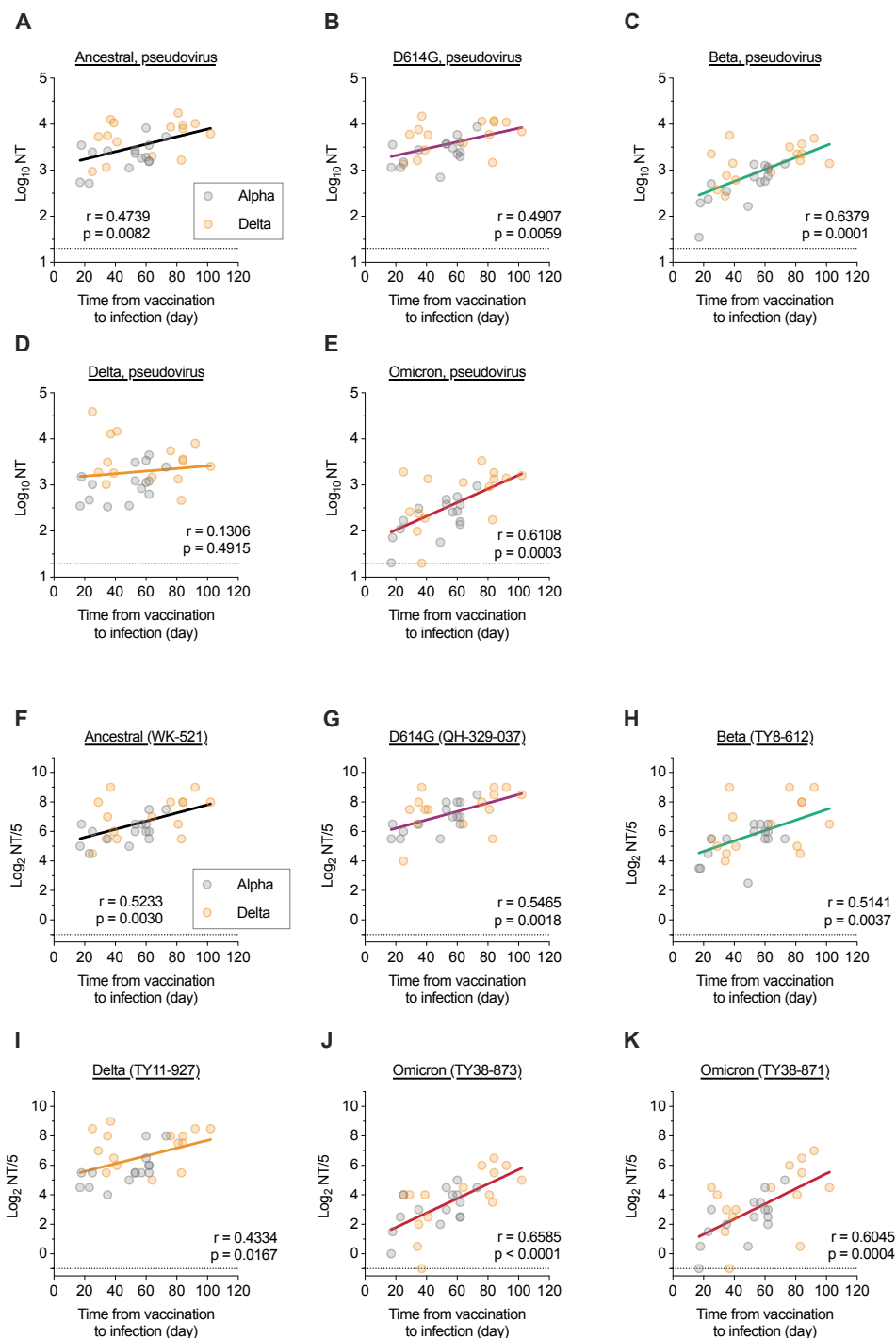
375 Comparison of the neutralization titers between background variant types of breakthrough infection

376 against variants of SARS-CoV-2 pseudo-viruses (C) and live viruses (D). The titers between the

377 background variants types were compared using the two-way ANOVA with Sidak's test; \* $p < 0.05$ , \*\* $p$

378  $< 0.01$ . Fold-increases are indicated above columns in statistically significant cases. (E) Timeline of sample

379 collection in vaccine breakthrough individuals: Alpha (n=15) and Delta (n=15). Timing from second  
380 vaccination to sample collection are indicated as line-connected circles. Day 0 (dotted line) indicates the  
381 day of COVID-19 diagnosis.  
382

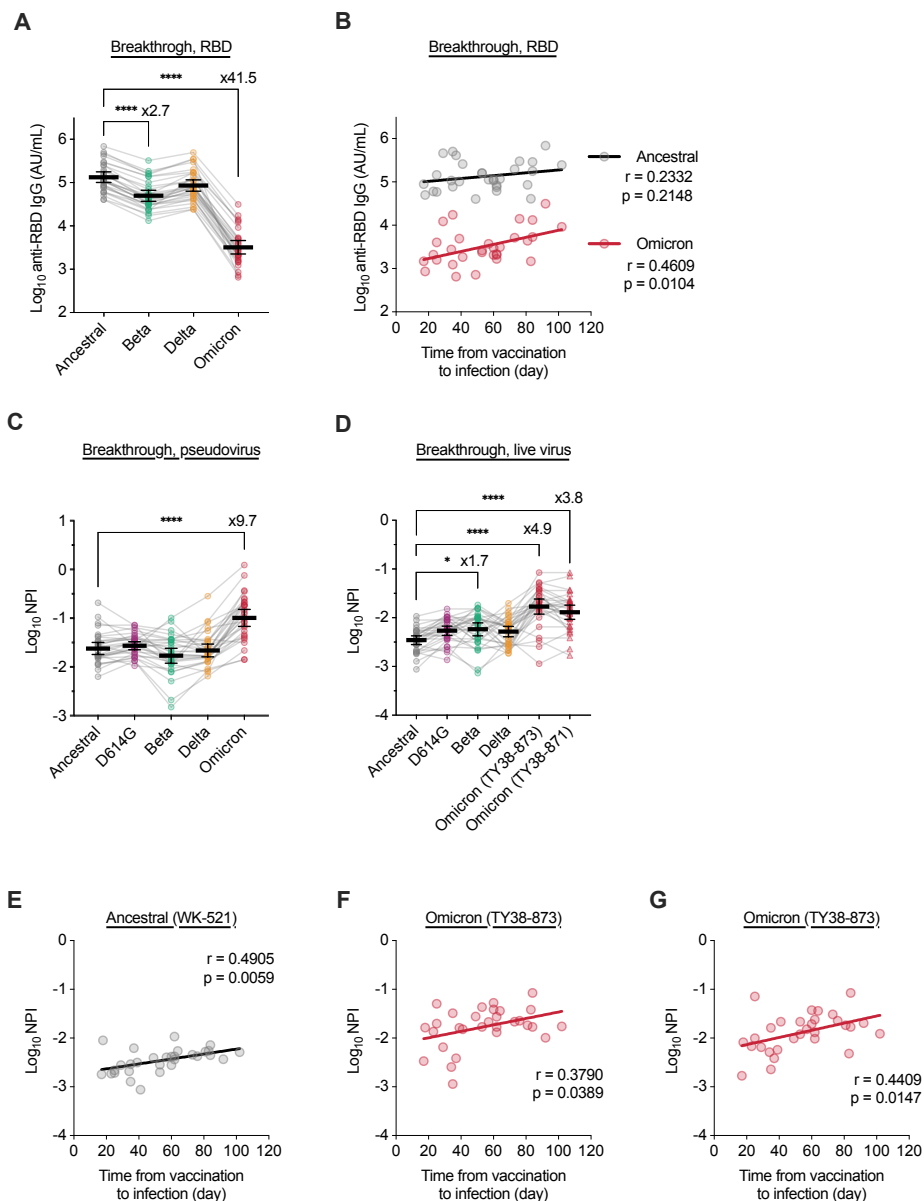


383

384 **Figure 3.** Positive correlation between cross-neutralization activity against SARS-CoV-2 variants and

385 interval between vaccination and breakthrough infection (A-K) Correlation between interval from

386 vaccination to breakthrough infection and the neutralization titers of the serums against variants of SARS-  
387 CoV-2 pseudoviruses (A-E) and live viruses (F-K). The correlation plots represent against ancestral (A,  
388 F), D614G (B, G), Beta (C, H), Delta (E, I), and Omicron (F, J, K) pseudo and live viruses, respectively.  
389 The regression line, Pearson correlation  $r$  value, and  $p$  value are shown.  
390



391

392 **Figure 4.** *Enhanced neutralizing potency index of anti-Omicron variant antibody in breakthrough*

393 *infection sera (A) IgG titers of vaccine-breakthrough case serums against ancestral RBD and RBD mutants*

394 *of SARS-CoV-2 variants. Data from the same serum are connected with lines, and the mean ± 95% CI of*

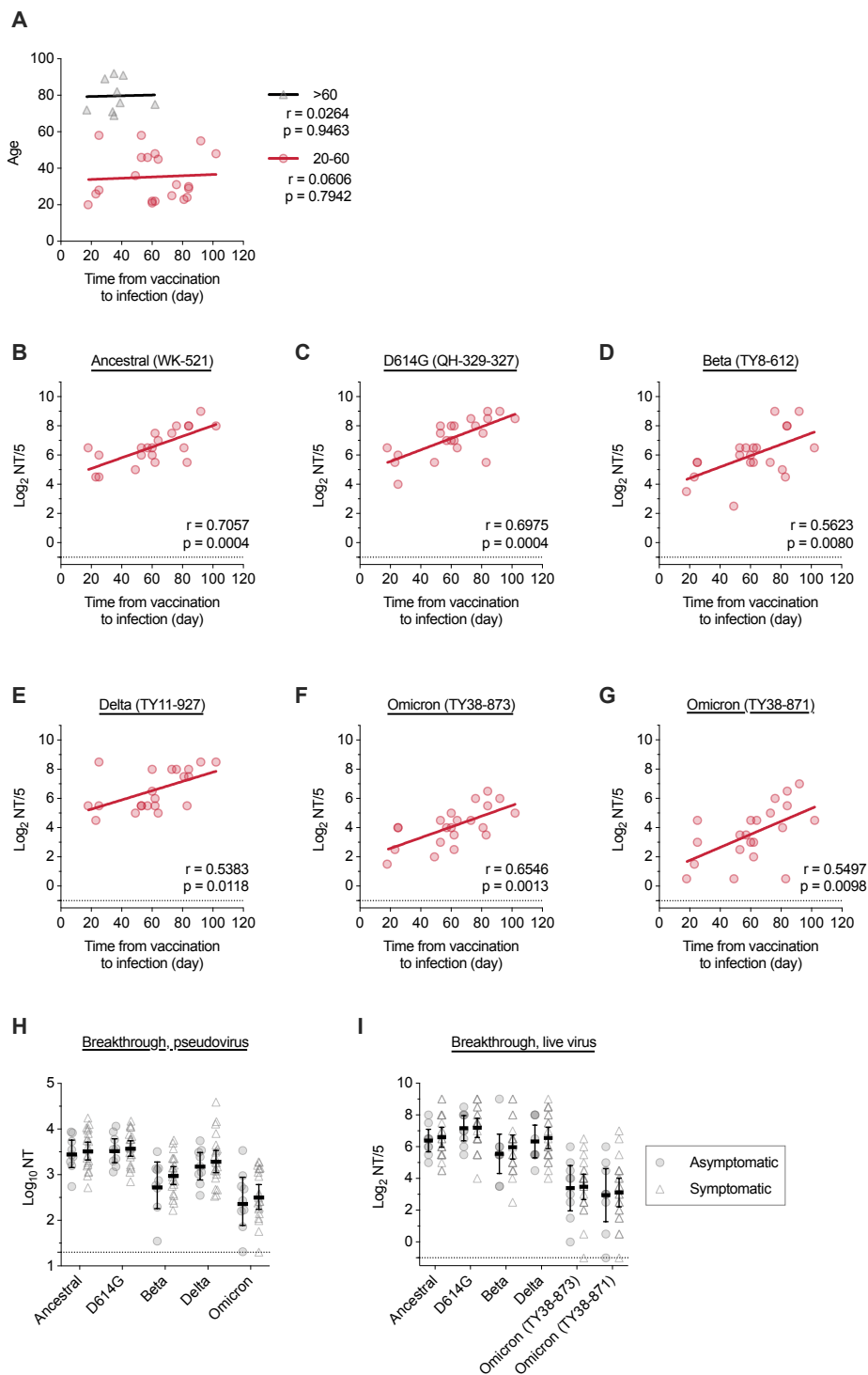
395 *each serum titer is presented. The titers between the ancestral and variants were compared using the one-*

396 *way ANOVA with Dunnett's test; \*\*\*\*p < 0.0001. Fold-reductions are indicated above columns in*



397 statistically significant cases. (B) Correlation between interval from vaccination to breakthrough infection  
398 and the IgG titers of the serums against the ancestral (gray) and Omicron (red) RBDs. The regression line,  
399 Pearson correlation  $r$  value, and  $p$  value are shown. (C, D) Neutralization potency index (NPI) of vaccine-  
400 breakthrough case serums against variants of SARS-CoV-2 pseudoviruses (C) and live viruses (D). Data  
401 from the same serum are connected with lines, and the mean  $\pm$  95% CI of each serum titer is presented.  
402 The titers between the ancestral and variants were compared using the one-way ANOVA with Dunnett's  
403 test; \* $p < 0.05$ , \*\*\*\* $p < 0.0001$ . Fold-increases are indicated above columns in statistically significant cases.  
404 (E-G) Correlation between interval from vaccination to breakthrough infection and the NPI of the serums  
405 against ancestral and Omicron variants of SARS-CoV-2 live viruses. The correlation plots represent  
406 against ancestral (E) and Omicron (F, G) live viruses, respectively. The regression line, Pearson correlation  
407  $r$  value, and  $p$  value are shown.  
408

409 Supplemental information



411 **Figure S1.** *Effect of age and symptoms in vaccine breakthrough individuals on the neutralization titers,*  
412 *Related to Figure 3 (A) Correlation of age of the individuals with time interval from vaccination to*  
413 *breakthrough infection. Data were stratified by age (20-60 or >60 years). (B-F) Correlation between*  
414 *interval from vaccination to breakthrough infection and the neutralization titers of the serums against*  
415 *variants of SARS-CoV-2 live viruses in the 20-60 years individuals. The correlation plots represent against*  
416 *ancestral (B), D614G (C), Beta (D), Delta (E), and Omicron (F, G) live viruses, respectively. The*  
417 *regression line, Pearson correlation  $r$  value, and  $p$  value are shown. (H, I) Comparison of the neutralization*  
418 *titers between the asymptomatic and symptomatic cases against variants of SARS-CoV-2 pseudoviruses*  
419 *(H) and live viruses (I). The titers between the asymptomatic and symptomatic cases were compared using*  
420 *the two-way ANOVA;  $p = 0.1598$  (H),  $p = 0.4710$  (I).*

421

422

423 **Supplemental Table 1. Characteristics of participants in this study**

	<b>Vaccinated only</b>	<b>Breakthrough infection (All)</b>	<b>Breakthrough infection (Alpha variant infection)</b>	<b>Breakthrough infection (Delta variant infection)</b>
Number of subjects	20	30	15	15
Age, y	42 (33-48)	46 (26-71)	36 (22-58)	55 (30-76)
Male sex	4 (20.0)	10 (33.3)	1 (6.7)	9 (60.0)
<b>Vaccine</b>	BNT162b2 (2 doses)	BNT162b2 (2 doses)	BNT162b2 (2 doses)	BNT162b2 (2 doses)
<b>Severity</b>				
Asymptomatic	N/A	9 (30.0)	8 (53.3)	1 (6.7)
Mild	N/A	17 (56.7)	7 (46.7)	10 (66.7)
Moderate*	N/A	4 (13.3)	0 (0.0)	4 (26.7)
Dose interval (days)	21 (21-21)	21 (21-21)	21 (20-21)	21 (21-21)
Infection to sera collection (days)	N/A	13 (11-15)	15 (11-18)	12 (10-13)
Dose 2 to infection (days)	N/A	55 (35-74)	53 (25-62)	64 (35-84)
Dose 2 to sera collection (days)	Early: 31.5 (29-34) Late: 150.5 (148.25-152.75)	72 (46-91)	72 (36-75)	74 (46-96)
<b>Period of infection</b>	N/A	April 30-August 31, 2021	April 30-August 14, 2021	June 18-August 31, 2021

424 Median (interquartile range) or n (%)

425 \*Moderate includes individuals who required oxygen

426

427

428 **Methods**

429 *Human subjects and sampling*

430 Human plasma samples obtained from vaccinated health care workers without infection, who received  
431 two doses of BNT162b2 (Pfizer/BioNTech) mRNA vaccine, were collected on approximately day 51 and  
432 day 161 after the first vaccination, with written informed consent prior to enrollment and ethics approval  
433 by the medical research ethics committee of the National Institute of Infectious Diseases (NIID) for the  
434 use of human subjects (#1321). Blood was collected in Vacutainer CPT tubes (BD Biosciences) and  
435 centrifuged at  $1800 \times g$  for 20 min. Peripheral blood mononuclear cells (PBMCs) were suspended in  
436 plasma and harvested into conical tubes, followed by centrifugation at  $300 \times g$  for 15 min. The plasma  
437 was transferred into another conical tube, centrifuged at  $800 \times g$  for 15 min, and the supernatant transferred  
438 into another tube to completely remove PBMCs. Human serum samples obtained from breakthrough cases  
439 were also included in this study. Demographic information, vaccination status, and respiratory samples to  
440 determine the type of variant infected among breakthrough cases in this report were collected as part of  
441 the public health activity led by NIID under the Infectious Diseases Control Law and were published on  
442 the NIID website to meet statutory requirements. Sera were collected concurrently for clinical testing  
443 provided by NIID with patient consent, and neutralization assays for this report were performed using  
444 residual samples as a research activity with ethics approval by ethics approval from the medical research  
445 ethics committee of NIID for the use of human subjects (#1275) and informed consent. To examine  
446 neutralization, plasma and serum samples were heat-inactivated at  $56^{\circ}\text{C}$  for 30 min before use.

447

448 *SARS-CoV-2 virus*

449 The SARS-CoV-2 ancestral strain WK-521 (lineage A, GISAID ID: EPI\_ISL\_408667), D614G strain QH-

450 329-037 (lineage B.1, GISAID: EPI\_ISL\_529135), Beta variant TY8-612 (lineage B.1.351, GISAID:  
451 EPI\_ISL\_1123289), Delta variant TY11-927 (lineage B.1.617.2, GISAID:EPI\_ISL\_2158617), and  
452 Omicron variant TY38-873 (lineage BA.1, GISAID: EPI\_ISL\_7418017) and TY38-871 (lineage BA.1,  
453 GISAID: EPI\_ISL\_7571618) were used in this study, which were isolated using VeroE6/TMPRSS2 cells  
454 at NIID with ethics approval by the medical research ethics committee of NIID for the use of human  
455 subjects (#1178). To isolate viruses belonging to the Omicron variant (TY38-873; GISAID ID:  
456 EPI\_ISL\_7418017, and TY38-871; GISAID: EPI\_ISL\_7571618), respiratory specimens, which were  
457 collected from individuals being screened at airport quarantine stations in Japan and then transferred to  
458 NIID for whole genome sequencing, were subjected to viral isolation using VeroE6/TMPRSS2 cells at  
459 NIID. The TY38-871 virus harbors an additional R346K mutation.

460

#### 461 *Cells*

462 293T cells, obtained from American Type Culture Collection, were cultured in Dulbecco's modified Eagle  
463 medium (DMEM; FUJIFILM Wako Chemical, Kanagawa, Japan) supplemented with 10% fetal bovine  
464 serum at 37°C and 5% CO<sub>2</sub>. Expi293F cells were maintained in the Expi293 expression medium (Thermo  
465 Fisher Scientific). VeroE6/TMPRSS2 cells (JCRB1819, Japanese Collection of Research Bioresources  
466 Cell Bank) were maintained in low glucose DMEM (Fujifilm) containing 10% heat-inactivated fetal  
467 bovine serum (Biowest), 1 mg/mL geneticin (Thermo Fisher Scientific), and 100 U/mL  
468 penicillin/streptomycin (Thermo Fisher Scientific) at 37°C supplied with 5% CO<sub>2</sub>.

469

#### 470 *Recombinant RBD antigens*

471 Human codon-optimized sequences coding the SARS-CoV-2 RBD (amino acids: 331-529) with the

472 following mutations were cloned into the mammalian expression vector pCAGGS: Beta, K417N / E484K  
473 / N501Y; Delta, L452R / T478K; Omicron, G339D / S371L / S373P / S375F / K417N / N440K / G446S /  
474 S477N / T478K / E484A / Q493R / G496S / Q498R / N501Y / Y505H. Recombinant Avi-tagged His-  
475 tagged proteins were produced using Expi293F cells, according to the manufacturer's instructions  
476 (Thermo Fisher Scientific), in the presence of the secreted BirA-Flag plasmid (Addgene) and biotin.  
477 Recombinant proteins were purified from the culture supernatant using Ni-NTA agarose (Qiagen).

478

#### 479 *Electrochemiluminescence immunoassay (ECLIA)*

480 IgG titers for variant RBDs were measured using multiplex kits (Meso Scale Discovery) according to the  
481 manufacturer's instructions. Briefly, plates were coated with biotinylated RBDs premixed with linkers at  
482 4°C overnight. The plates were washed with phosphate-buffered saline supplemented with 0.05% Tween-  
483 20 and incubated with the MSD Blocker A reagent (Meso Scale Discovery) at room temperature for 1 h  
484 with shaking. Plasma samples diluted with MSD Diluent 100 (Meso Scale Discovery) were added to the  
485 plates after washing, and the plates were incubated at room temperature for 2 h with shaking. The plates  
486 were washed and incubated with sulfo-tag-conjugated anti-human IgG (Meso Scale Discovery) at room  
487 temperature for 1 h with shaking. Finally, the plates were measured for electrochemiluminescence using  
488 MESOQuickPlex SQ 120 (Meso Scale Discovery) after washing and adding MSD Gold read buffer B  
489 (Meso Scale Discovery).

490

#### 491 *VSV pseudovirus production*

492 The VSV pseudovirus bearing SARS-CoV-2 spike proteins was generated as previously described (Tani  
493 et al., 2021). Briefly, the spike genes of the SARS-CoV-2 ancestral strain, D614G, Beta, and Delta variants

494 were obtained from viral RNA extracted from SARS-CoV-2 strain WK-521 for ancestral strain, TY4-920  
495 for D614G strain (lineage B.1.1, GISAID ID: EPI\_ISL\_2931303), TY8-612 for Beta, and TY11-927 for  
496 Delta, respectively, by RT-PCR using PrimeScript II High Fidelity One Step RT-PCR kit (Takara-Bio,  
497 Shiga, Japan). The spike gene of the SARS-CoV-2 Omicron variant was obtained from RNA extracted  
498 from nasopharyngeal swab specimens of patients infected with the SARS-CoV-2 Omicron variant (TY38-  
499 873) by RT-PCR, as described above. The cytoplasmic 19 aa-deleted SARS-CoV-2 spike genes were  
500 cloned into the CAGGS/MCS expression vector. The spike sequence in the expression plasmids was  
501 confirmed by Sanger sequencing. 293T cells transfected with expression plasmids encoding the spike  
502 genes of SARS-CoV-2 ancestral strain, D614G, Beta, Delta, and Omicron variants were infected with G-  
503 complemented VSV $\Delta$ G/Luc. After 24 h, the culture supernatants containing VSV pseudoviruses were  
504 collected and stored at -80°C until use.

505

#### 506 *VSV pseudovirus-based neutralization assay*

507 Neutralization of SARS-CoV-2 pseudovirus was performed as previously described (Moriyama et al.,  
508 2021). Briefly, serially diluted plasma (five-fold serial dilution of the plasma from vaccinated participants,  
509 or eight-fold serial dilution of the plasma from breakthrough infected patients, starting at 1:10 dilution)  
510 was mixed with equal volume of the VSV pseudoviruses bearing SARS-CoV-2 spike protein and incubated  
511 at 37°C for 1 h. The mixture was then inoculated into VeroE6/TMPRSS2 cells seeded on 96 well solid  
512 white flat-bottom plates (Corning). At 24 h post-infection, the infectivity of VSV pseudovirus was assessed  
513 by measuring luciferase activity using the Bright-Glo Luciferase Assay System (Promega, Madison, WI,  
514 USA) and GloMax Navigator Microplate Luminometer (Promega). The half-maximal inhibitory  
515 concentration (IC<sub>50</sub>) is presented as the neutralization titer.



516

517 *Live virus neutralization assay*

518 Live virus neutralization assays were performed as previously described (Moriyama et al., 2021; Onodera  
519 et al., 2021). Briefly, plasma or serum samples were serially diluted (2-fold dilutions starting from 1:5) in  
520 high-glucose DMEM supplemented with 2% fetal bovine serum and 100 U/mL penicillin/streptomycin  
521 and were mixed with 100 TCID<sub>50</sub> SARS-CoV-2 viruses, WK-521 (ancestral strain), QH-329-037 (D614G  
522 strain), TY8-612 (Beta variant), TY11-927 (Delta variant), TY38-873 (Omicron variant), and TY38-871  
523 (Omicron variant), followed by incubation at 37°C for 1 h. The virus-plasma mixtures were placed on  
524 VeroE6/TMPRSS2 cells (JCRB1819) seeded in 96-well plates and cultured at 37°C with 5% CO<sub>2</sub> for 5  
525 days. After culturing, the cells were fixed with 20% formalin (Fujifilm Wako Pure Chemicals) and stained  
526 with crystal violet solution (Sigma-Aldrich). The mean cut-off dilution index with > 50% cytopathic effect  
527 from two to four multiplicate series is presented as the neutralizing titer. Assays on sera from vaccinated  
528 individuals without infection were performed in multiple independent laboratories within NIID to confirm  
529 the findings, with adjustments made using rabbit sera immunized with RBD (ancestral strain). Since sera  
530 from individuals who suffered from breakthrough infections were limited in quantity, the assay was  
531 performed once. All experiments using live viruses were performed in a biosafety level 3 laboratory at  
532 NIID, Japan.

533

534 *Ethical statement approval*

535 All samples, protocols, and procedures were approved by the Medical Research Ethics Committee of NIID  
536 for the use of human subjects (Approval numbers 1178, 1275, 1316, and 1321).

537

538 *Statistical analysis*

539 Data analysis and visualization were performed using GraphPad Prism software. For statistical analysis,  
540 one-way ANOVA with Dunnett's test and two-way ANOVA with Sidak's test were used to compare the  
541 titers. The Pearson correlation coefficient was used to assess correlations between titers and time intervals.  
542 Statistical significance was set at  $p < 0.05$ .

543

544

545 **References**

- 546 Andrews, N., Stowe, J., Kirsebom, F., Toffa, S., Rickeard, T., Gallagher, E., Gower, C., Kall, M., Groves,  
547 N., O'Connell, A.-M., et al. (2021). Effectiveness of COVID-19 vaccines against the Omicron (B.1.1.529)  
548 variant of concern.
- 549 Bates, T.A., McBride, S.K., Winders, B., Schoen, D., Trautmann, L., Curlin, M.E., and Tafesse, F.G. (2021).  
550 Antibody Response and Variant Cross-Neutralization After SARS-CoV-2 Breakthrough Infection. JAMA.
- 551 Cameroni, E., Bowen, J.E., Rosen, L.E., Saliba, C., Zepeda, S.K., Culap, K., Pinto, D., VanBlargan, L.A.,  
552 De Marco, A., di Iulio, J., et al. (2021). Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron  
553 antigenic shift. Nature.
- 554 Carreno, J.M., Alshammary, H., Tcheou, J., Singh, G., Raskin, A., Kawabata, H., Sominsky, L., Clark, J.,  
555 Adelsberg, D.C., Bielak, D., et al. (2021). Activity of convalescent and vaccine serum against a B.1.1.529  
556 variant SARS-CoV-2 isolate.
- 557 Cele, S., Jackson, L., Khoury, D.S., Khan, K., Moyo-Gwete, T., Tegally, H., San, J.E., Cromer, D.,  
558 Scheepers, C., Amoako, D., et al. (2021). Omicron extensively but incompletely escapes Pfizer BNT162b2  
559 neutralization. Nature.
- 560 Dejnirattisai, W., Shaw, R.H., Supasa, P., Liu, C., Stuart, A.S.V., Pollard, A.J., Liu, X., Lambe, T., Crook,  
561 D., Stuart, D.I., et al. (2021). Reduced neutralisation of SARS-CoV-2 omicron B.1.1.529 variant by post-  
562 immunisation serum. Lancet.
- 563 Doria-Rose, N.A., Shen, X., Schmidt, S.D., O'Dell, S., McDanal, C., Feng, W., Tong, J., Eaton, A.,  
564 Magliano, M., Tang, H., et al. (2021). Booster of mRNA-1273 Vaccine Reduces SARS-CoV-2 Omicron  
565 Escape from Neutralizing Antibodies. MedRxiv.
- 566 Edara, V.-V., Manning, K.E., Ellis, M., Lai, L., Moore, K.M., Foster, S.L., Floyd, K., Davis-Gardner, M.E.,  
567 Mantus, G., Nyhoff, L.E., et al. (2021). mRNA-1273 and BNT162b2 mRNA vaccines have reduced  
568 neutralizing activity against the SARS-CoV-2 Omicron variant.
- 569 Eggink, D., Andeweg, S.P., Vennema, H., van Maarseveen, N., Vermaas, K., Vlaemynck, B., Schepers, R.,  
570 van Gageldonk-Lafeber, A.B., van den Hof, S., Reusken, C.B.E.M., et al. (2021). Increased risk of  
571 infection with SARS-CoV-2 Omicron compared to Delta in vaccinated and previously infected individuals,  
572 the Netherlands, 22 November to 19 December 2021.

- 573 Goga, A.E., Bekker, L.-G., Garret, N., Reddy, T., Yende-Zuma, N., Fairall, L., Moultrie, H., Takalani, A.,  
574 Trivelli, V., Faesen, M., et al. (2021). Breakthrough Covid-19 infections during periods of circulating Beta,  
575 Delta and Omicron variants of concern, among health care workers in the Sisonke Ad26.COV2.S vaccine  
576 trial, South Africa.
- 577 Gruell, H., Vanshylla, K., Tober-Lau, P., Hillus, D., Schommers, P., Lehmann, C., Kurth, F., Sander, L.E.,  
578 and Klein, F. (2021). mRNA booster immunization elicits potent neutralizing serum activity against the  
579 SARS-CoV-2 Omicron variant.
- 580 Grunau, B., Goldfarb, D.M., Asamoah-Boaheng, M., Golding, L., Kirkham, T.L., Demers, P.A., and  
581 Lavoie, P.M. (2021). Immunogenicity of Extended mRNA SARS-CoV-2 Vaccine Dosing Intervals. *JAMA*.
- 582 Hansen, C.H., Schelde, A.B., Moustsen-Helms, I.R., Emborg, H.-D., Krause, T.G., Moelbak, K.,  
583 Valentiner-Branth, P., and The Infectious Disease Preparedness Group at Statens Serum Institut (2021).  
584 Vaccine effectiveness against SARS-CoV-2 infection with the Omicron or Delta variants following a two-  
585 dose or booster BNT162b2 or mRNA-1273 vaccination series: A Danish cohort study.
- 586 Leung, K., and Wu, J.T. (2021). Managing waning vaccine protection against SARS-CoV-2 variants.  
587 *Lancet*.
- 588 Liu, L., Iketani, S., Guo, Y., Chan, J.F.-W., Wang, M., Liu, L., Luo, Y., Chu, H., Huang, Y., Nair, M.S., et  
589 al. (2021). Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. *Nature*.
- 590 Lu, L., Mok, B.W.-Y., Chen, L.-L., Chan, J.M.-C., Tsang, O.T.-Y., Lam, B.H.-S., Chuang, V.W.-M., Chu,  
591 A.W.-H., Chan, W.-M., Ip, J.D., et al. (2021). Neutralization of SARS-CoV-2 Omicron variant by sera  
592 from BNT162b2 or Coronavac vaccine recipients. *Clin. Infect. Dis.*
- 593 McMahan, K., Yu, J., Mercado, N.B., Loos, C., Tostanoski, L.H., Chandrashekar, A., Liu, J., Peter, L.,  
594 Atyeo, C., Zhu, A., et al. (2021). Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nature*  
595 *590*, 630–634.
- 596 Moriyama, S., Adachi, Y., Sato, T., Tonouchi, K., Sun, L., Fukushi, S., Yamada, S., Kinoshita, H., Nojima,  
597 K., Kanno, T., et al. (2021). Temporal maturation of neutralizing antibodies in COVID-19 convalescent  
598 individuals improves potency and breadth to circulating SARS-CoV-2 variants. *Immunity* *54*, 1841-  
599 1852.e4.
- 600 Onodera, T., Kita, S., Adachi, Y., Moriyama, S., Sato, A., Nomura, T., Sakakibara, S., Inoue, T., Tadokoro,

- 601 T., Anraku, Y., et al. (2021). A SARS-CoV-2 antibody broadly neutralizes SARS-related coronaviruses and  
602 variants by coordinated recognition of a virus-vulnerable site. *Immunity* 54, 2385-2398.e10.
- 603 Payne, R.P., Longet, S., Austin, J.A., Skelly, D.T., Dejnirattisai, W., Adele, S., Meardon, N., Faustini, S.,  
604 Al-Taei, S., Moore, S.C., et al. (2021). Immunogenicity of standard and extended dosing intervals of  
605 BNT162b2 mRNA vaccine. *Cell* 184, 5699-5714.e11.
- 606 Pulliam, J.R.C., van Schalkwyk, C., Govender, N., von Gottberg, A., Cohen, C., Groome, M.J., Dushoff,  
607 J., Mlisana, K., and Moultrie, H. (2021). Increased risk of SARS-CoV-2 reinfection associated with  
608 emergence of the Omicron variant in South Africa.
- 609 Scott, L., Hsiao, N.-Y., Moyo, S., Singh, L., Tegally, H., Dor, G., Maes, P., Pybus, O.G., Kraemer, M.U.G.,  
610 Semenova, E., et al. (2021). Track Omicron's spread with molecular data. *Science* 374, 1454–1455.
- 611 Tani, H., Kimura, M., Tan, L., Yoshida, Y., Ozawa, T., Kishi, H., Fukushi, S., Saijo, M., Sano, K., Suzuki,  
612 T., et al. (2021). Evaluation of SARS-CoV-2 neutralizing antibodies using a vesicular stomatitis virus  
613 possessing SARS-CoV-2 spike protein. *Viol. J.* 18, 16.
- 614 The Lancet Infectious Diseases (2021). Emerging SARS-CoV-2 variants: shooting the messenger. *Lancet*  
615 *Infect. Dis.*
- 616 Turner, J.S., O'Halloran, J.A., Kalaidina, E., Kim, W., Schmitz, A.J., Zhou, J.Q., Lei, T., Thapa, M., Chen,  
617 R.E., Case, J.B., et al. (2021). SARS-CoV-2 mRNA vaccines induce persistent human germinal centre  
618 responses. *Nature* 596, 109–113.
- 619 Voysey, M., Costa Clemens, S.A., Madhi, S.A., Weckx, L.Y., Folegatti, P.M., Aley, P.K., Angus, B., Baillie,  
620 V.L., Barnabas, S.L., Bhorat, Q.E., et al. (2021). Single-dose administration and the influence of the timing  
621 of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled  
622 analysis of four randomised trials. *Lancet* 397, 881–891.