

1 **Three-dose vaccination-induced immune responses protect against SARS-CoV-2**
2 **Omicron BA.2**

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33

34 **Summary**

35 **Background**

36 The ongoing outbreak of SARS-CoV-2 Omicron BA.2 infections in Hong Kong, the
37 model city of universal masking of the world, has resulted in a major public health
38 crisis. Although the third vaccination resulted in strong boosting of neutralization
39 antibody, vaccine efficacy and correlates of immune protection against the major
40 circulating Omicron BA.2 remains to be investigated.

41 **Methods**

42 We investigated the vaccine efficacy against the Omicron BA.2 breakthrough
43 infection among 470 public servants who had received different SARS-CoV-2
44 vaccine regimens including two-dose BNT162b2 (2×BNT, n=169), three-dose
45 BNT162b2 (3×BNT, n=170), two-dose CoronaVac (2×CorV, n=34), three-dose
46 CoronaVac (3×CorV, n=67) and third-dose BNT162b2 following 2×CorV
47 (2×CorV+1BNT, n=32). Humoral and cellular immune responses after three-dose
48 vaccination were further characterized and correlated with clinical characteristics of
49 BA.2 infection.

50 **Findings**

51 During the BA.2 outbreak, 27.7% vaccinees were infected. The timely third-dose
52 vaccination provided significant protection with lower incidence rates of
53 breakthrough infections (2×BNT 49.2% vs 3×BNT 13.1%, $p<0.0001$; 2×CorV 44.1%
54 vs 3×CoV 19.4%, $p=0.003$). Investigation of immune response on blood samples
55 derived from 92 subjects in three-dose vaccination cohorts collected before the BA.2
56 outbreak revealed that the third-dose vaccination activated spike (S)-specific memory
57 B cells and Omicron cross-reactive T cell responses, which correlated with reduced
58 frequencies of breakthrough infections and disease severity rather than with types of
59 vaccines. Moreover, the frequency of S-specific activated memory B cells was
60 significantly lower in infected vaccinees than uninfected vaccinees before vaccine-
61 breakthrough infection whereas $\text{IFN-}\gamma^+$ CD4 T cells were negatively associated with
62 age and viral clearance time. Critically, BA.2 breakthrough infection boosted cross-
63 reactive memory B cells with enhanced cross-neutralizing antibodies to Omicron
64 sublineages, including BA.2.12.1 and BA.4/5, in all vaccinees tested.

65 **Interpretation**

66 Our results imply that the timely third vaccination and immune responses are likely
67 required for vaccine-mediated protection against Omicron BA.2 pandemic. Although

68 BA.2 conferred the highest neutralization resistance compared with variants of
69 concern tested before the emergence of BA.2.12.1 and BA.4/5, the third dose
70 vaccination-activated S-specific memory B cells and Omicron cross-reactive T cell
71 responses contributed to reduced frequencies of breakthrough infection and disease
72 severity. Neutralizing antibody potency enhanced by BA.2 breakthrough infection
73 with previous 3 doses of vaccines (CoronaVac or BNT162b2) may reduce the risk for
74 infection of ongoing BA.2.12.1 and BA.4/5.

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82

83 **Key words**

84 SARS-CoV-2, COVID-19, Omicron, BA.2, Breakthrough infection, Neutralizing
85 antibody, T cell response

86

87 **Introduction**

88 To fight the ongoing SARS-CoV-2 pandemic, over 10 billion doses of COVID-19
89 vaccines under emergency use authorization (EUA) have been administered globally,
90 which has significantly reduced the rates of hospitalization, disease severity and death
91 ¹⁻⁵. Unfortunately, the emergence of variants of concern (VOCs), especially the
92 Omicron variants, have substantially threatened the vaccine efficacy ⁶. We recently
93 reported that waning anti-Omicron neutralizing antibody and T cell responses
94 especially among CoronaVac-vaccinees might pose a risk to vaccine-breakthrough
95 infections in Hong Kong ⁷. Although the third heterologous BNT162b2 vaccination
96 after 2-dose CoronaVac generates high neutralizing antibody responses against
97 ancestral and Omicron BA.1 than the third homologous CoronaVac booster ^{8,9},
98 vaccine efficacy and its correlations with the immune protection against the major
99 circulating Omicron BA.2 remains to be investigated ¹⁰⁻¹². In addition, it remains
100 unclear if BA.2 breakthrough infection would reduce the risk against ongoing
101 BA.2.12.1 and BA.4/5 reinfection by enhancing cross-reactive neutralizing antibody
102 potency.

103

104 **Materials and methods**

105 **Human subjects**

106 This study was approved by the Institutional Review Board of the University of Hong
107 Kong/Hospital Authority Hong Kong West Cluster (Ref No. UW 21-452). A total of
108 481 participants were recruited in this study. Written informed consent and
109 questionnaire of vaccination and infection were obtained from these subjects. Patients
110 provided the information of symptom onset date, type of symptoms, hospitalization,
111 duration of illness and the date of viral negative conversion as summarized in Table 1.
112 The vaccination record was officially registered by professional medical staff in the
113 governmental system called “LeaveHomeSafe”. The diagnosis of SARS-CoV-2
114 infection was confirmed by results of rapid antigen test and PCR, as well as
115 quarantine records enforced strictly by law. Peripheral blood mononuclear cells
116 (PBMCs) from 92 randomly selected-participants who had the third vaccination were
117 isolated from fresh blood samples before SARS-CoV-2 infection using Ficoll-Paque
118 density gradient centrifugation in our BSL-3 laboratory at the same day of blood
119 collection. The majority of purified PBMCs were used for immune cell phenotyping
120 whereas plasma samples were subjected to antibody testing. The rest of the cells were
121 cryopreserved in freezing medium (Synth-a-Freeze Cryopreservation Medium,
122 ThermoFisher Scientific) at 5×10^6 cells/mL at -150°C . Subjects included in the study
123 were required to complete vaccination (all dose) for at least 7 days, to allow the
124 manifestation of the delayed immune response to vaccination.

125

126 **Enzyme-linked immunosorbent assays (ELISA)**

127 Serum IgG binding antibodies to Spike were quantitated by ELISA using WHO
128 International Standard as standard. Briefly, different recombinant trimeric Spike
129 proteins derived from SARS-CoV-2 VOCs (Sino Biological) were diluted to final
130 concentrations of 1 $\mu\text{g/mL}$, then coated onto 96- well plates (Corning 3690) and
131 incubated at 4°C overnight. Plates were washed with PBST (PBS containing 0.05%
132 Tween-20) and blocked with blocking buffer (PBS containing 5% skim milk or 1%
133 BSA) at 37°C for 1 h. Two-fold serial dilution of WHO international standard (from
134 20 BAU/mL to 0.15625 BAU/mL) and plasma samples (400-fold diluted) were added
135 to the plates and incubated at 37°C for 1 h. Wells were then incubated with a
136 secondary goat anti-human IgG labeled with horseradish peroxidase (HRP) (1:5000
137 Invitrogen) TMB substrate (SIGMA). Optical density (OD) at 450 nm was measured
138 by SkanIt RE6.1 with VARIOSKAN Lux (Thermo Scientific).

139

140 **Pseudotyped viral neutralization assay**

141 To determine the neutralizing activity of subject’s plasma, the plasma was inactivated
142 at 56°C for 30 min prior to a pseudotyped viral entry assay. In brief, different SARS-

143 CoV-2 pseudotyped viruses were generated through co-transfection of 293T cells with
144 2 plasmids, pSARS-CoV-2 S and pNL4-3Luc_Env_Vpr, carrying the optimized
145 SARS-CoV-2 S gene and a human immunodeficiency virus type 1 backbone,
146 respectively. At 48 h post-transfection, viral supernatant was collected and frozen at
147 -150°C . Serially diluted plasma samples (from 1:20 to 1:14580) were incubated with
148 200 TCID₅₀ of pseudovirus at 37°C for 1 h. The plasma-virus mixtures were then
149 added into pre-seeded HEK293T-hACE2 cells. After 48 h, infected cells were lysed,
150 and luciferase activity was measured using Luciferase Assay System kits (Promega)
151 in a Victor3-1420 Multilabel Counter (PerkinElmer). The 50% inhibitory
152 concentrations (IC₅₀) of each plasma specimen were calculated to reflect anti-SARS-
153 CoV-2 potency.

154

155 **Antigen-specific B cells**

156 To characterize the SARS-CoV-2 Spike-specific B cells, PBMCs from each vaccinee
157 were first stained with an antibody cocktail contained dead cell dye (Zombie aquae),
158 CD3-Pacific Blue, CD14-Pacific Blue, CD56-Pacific Blue, CD19-BV785, IgD-
159 BV605, IgG-PE, CD27-BV711, CD21-PE/Cy7, CD38-Percp/Cy5.5, CD11C-
160 APC/Fire750 and His-tag Spike protein. Cells were then washed with FACS buffer
161 (PBS with 2% FBS) and further stained with the secondary antibodies including APC
162 anti-His and DyLight 488 anti-his antibodies. Stained cells were acquired by
163 FACS Aria III Flow Cytometer (BD Biosciences) and analyzed with FlowJo software
164 (v10.6) (BD Bioscience).

165

166 **Intracellular cytokine staining (ICS)**

167 To measure antigen-specific T cell responses, PBMCs were stimulated with 2 $\mu\text{g}/\text{mL}$
168 Spike peptide pool (15-mer overlapping by 11) from SARS-CoV-2 ancestral or
169 Omicron variant, or 2 $\mu\text{g}/\text{mL}$ nucleocapsid protein (NP) peptide pool in the presence
170 of 0.5 $\mu\text{g}/\text{mL}$ anti-CD28 and anti-CD49d mAbs (Biolegend). Cells were incubated at
171 37°C for 9 hours and BFA was added at 3 h post incubation, as previously described
172 ¹¹. PMA/ionomycin stimulation was included as positive control. Cells were then
173 washed with staining buffer (PBS containing 2% FBS) and stained with mAbs against
174 surface markers, including dead cell dye (Zombie aqua), CD3-Pacific Blue, CD4-
175 Percp/Cy5.5, CD8-APC/Fire750, CD45RA-BV711, CCR7-BV785, CXCR5-APC,
176 CCR6-BV605. For intracellular staining, cells were fixed and permeabilized with BD
177 Cytofix/Cytoperm (BD Biosciences) prior to staining with the mAbs against IFN- γ -
178 PE, TNF- α -AF488 and IL-2-PE-Cy7. Stained cells were acquired by FACS Aria III
179 Flow Cytometer (BD Biosciences) and analyzed with FlowJo software (v10.6) (BD
180 Bioscience). Results were subtracted from percentage of unstimulated control.

181

182 **Correlation plots and heatmap visualizations**

183 Correlograms plotting the Spearman rank correlation coefficient (r), between all
184 parameter pairs were generated with the corrplot package (v0.84)¹³ running under R
185 (v3.6.1) in RStudio (1.1.456). Spearman rank two-tailed P values were calculated
186 using corr.test (psych v1.8.12) and graphed (ggplot2 v3.1.1) based on * $p < 0.05$,
187 ** $p < 0.01$, *** $p < 0.001$.

188

189 **Statistical analysis**

190 Statistical analysis was performed using PRISM 8.0. For between-group or multiple-
191 group categorical values comparison, two-sided chi-square tests or fisher's exact tests
192 were used. Unpaired Student's t tests were used to compare group means of GMT and
193 cell frequencies between two groups. The statistic details are depicted in the
194 respective legends. A P value < 0.05 was considered significant.

195

196 **Results**

197 **Demographic characteristics of breakthrough infection among 481 vaccinees**

198 Considering sociodemographic characteristics and exposure risk may also affect
199 vaccine efficacy. In this study, therefore, we only focus on 7247 subjects who are
200 public servants working for Hong Kong Government with comparable exposure risks.
201 During the time from January to March 2022 (Omicron BA.2 was first found in mid-
202 January 2022 and reached the peak in the early March as dominant strain in Hong
203 Kong^{10,14}), 5995 (82.7%) and 1012 (14%) study subjects had received two and three
204 doses of vaccinations, respectively, resulting in an overall vaccination rate of 96.7%.
205 During the recent fifth wave of COVID-19 in Hong Kong since the end of January
206 2022¹⁰, 470 (6.5%) subjects joined our follow-up study. These subjects had received
207 2-dose BNT162b2 (2×BNT, $n=169$), 3-dose BNT162b2 (3×BNT, $n=168$), 2-dose
208 CoronaVac (2×CorV, $n=34$), 3-dose CoronaVac (3×CorV, $n=67$) or a heterologous
209 booster dose of BNT162b2 after two prior doses of CoronaVac (2×CorV+1×BNT,
210 $n=32$) (Table 1). Among these 470 subjects, a total of 141 (128/470, 27.2%) infections
211 were confirmed by governmental reverse transcriptase-polymerase chain reaction
212 (RT-PCR) or lateral flow-based rapid antigen test (RAT) during the study period.
213 Gender difference in infection was not observed. Patients in 2×BNT were relatively
214 younger than 3×BNT (2×BNT vs 3×BNT: median 32 years vs median 40 years,
215 $p < 0.0001$), likely indicating the hesitation for taking the third dose BNT162b2 among
216 younger people. Patients who received two dose BNT162b2 were significantly
217 younger than patients who received two dose CoronaVac (2×CorV vs 2×BNT: median
218 41 years vs median 32 years, $p=0.0006$ (Table 1 and Supplementary Table 1), in line

219 with elderly people's preference of taking CoronaVac with less side effects. Moreover,
220 a shorter median interval between latest vaccination and symptom onset was noticed
221 for 3×BNT compared to 2×BNT (2×BNT vs 3×BNT: median 227 days vs median
222 48.5 days, $p<0.0001$) and for 3×CorV compared to 2×CorV (2×CorV vs 3×CorV:
223 median 237 days vs median 56 days, $p<0.0001$), respectively ([Table 1 and](#)
224 [Supplementary Table 1](#)).

225 Infections were found in both 2×BNT and 2×CorV groups with comparable incidence
226 rates of 49.2% (78/169) and 44.1% (15/34) ($p=0.828$), respectively. For the third dose
227 vaccination groups, however, both third homologous BNT162b2 (3×BNT: 22/168,
228 13.1%, $p<0.0001$) and CoronaVac vaccination (3×CorV: 13/67, 19.4%, $p=0.009$)
229 showed significantly reduced infection rate compared to 2×BNT and 2×CorV,
230 respectively. The third heterologous BNT162b2 vaccination group (2×CorV+1×BNT)
231 exhibited the lowest incident rate of 6.3% compared to the 2×CorV group ($p<0.0001$).
232 No statistical significance was found in the infection rates between any 3 dose groups,
233 although 3×BNT and 2×CorV+1×BNT showed lower infection rates than 3×CorV
234 ([Table 1 and Supplementary Table 1](#)). Notably, most infected subjects developed mild
235 disease, presenting three major symptoms including fever, cough and/or sore throat.
236 Asymptomatic infections were only found in 2×BNT groups with a low frequencies of
237 3.8% (3/78) ([Table 1](#)). The hospitalization rate was lower for 3×BNT (4.5%) than that
238 of 3×CorV (15.4%) patients. Comparable illness duration was observed in 2×BNT
239 (median 7 days) and 3×BNT (median 7.5 days) than those of 2×CorV (median 8 days)
240 and 3×CorV (median 8 days). There was no significant difference in terms of duration
241 time for viral antigen conversion to negativity between any groups ([Table 1 and](#)
242 [Supplementary Table 1](#)). These results suggested that the third dose vaccination by
243 both BNT162b2 and CoronaVac reduced the incident rate of BA.2 infection and the
244 third dose of BNT162b2 vaccination achieved a slightly lower hospitalization rate
245 compared with the third CoronaVac.

246

247 **Activation of Spike-specific memory B cells by the third vaccination**

248 To characterize the third dose vaccination-induced immune responses, we were able
249 to obtain 92 blood samples donated by subjects in the same cohort including 41 from
250 3×BNT, 28 from 3×CorV and 21 from 2×CorV+1×BNT at median 23, 56 and 47 days
251 after the last vaccination, respectively, on January 27, 2022, right before BA.2
252 outbreak in Hong Kong^{10,14} ([Supplementary Table 2](#)). Considering that memory B cell
253 responses contribute to long-term immunological protection against COVID-19, we
254 measured the frequency of Spike (S)-specific B cells (gated on CD19⁺ IgG⁺ IgD⁻ cells)
255 after the third dose vaccination ([Figure 1A](#)). We found that the third dose of
256 BNT162b2, either 3×BNT (mean 2.83%) or 2×CorV+1×BNT (mean 1.33%), induced

257 significant higher frequency of S-specific B cells than 3×CorV (mean 0.35%) (Figure
258 1B). The significant boost effect of S-specific B cells was not observed by the third
259 dose of CoronaVac (Figure 1C). Moreover, S-specific B cells elicited by the third dose
260 of BNT162b2 reached the peak around 4-6 weeks and lasted for 3 months with a
261 higher mean frequency than that of 3×CorV (Figure 1D). Further phenotypical
262 analysis (Figure 1E) showed that the third dose of BNT162b2 resulted in elevated
263 frequency of activated memory B cells (AM, CD21⁻CD27⁺) compared with 2×BNT or
264 2×CorV whereas the third dose of CoronaVac enhanced the frequency of resting
265 memory (RM) B cells (Figure 1F). The frequency of AM reached the peak at 4 weeks
266 after the third booster and subsequently declined, accompanied by proportional
267 increase of RM, in both 3×BNT and 2×CorV+1×BNT groups whereas AM remained
268 unchanged in the 3×CorV group around two months (Figure 1G). These results
269 demonstrated that S-specific memory B cells were predominantly activated by the
270 third dose of BNT162b2 but insignificantly by the third dose of CoronaVac. However,
271 the third BNT162b2 vaccination following 2 doses of CoronaVac-boosted S-specific
272 B cells was comparable to those induced by three doses of BNT162b2, indicating that
273 BNT162b2 can recall and augment CoronaVac-induced memory B cells.

274

275 **The titer and breadth of neutralizing antibodies (NAbs) against a full panel of** 276 **current SARS-CoV-2 VOCs**

277 We then measured the titer and breadth of neutralizing antibodies (NAbs) against a
278 full panel of current SARS-CoV-2 VOCs including D614G, Alpha, Beta, Delta and
279 five Omicron variants (BA.1, BA.1.1, BA.2, BA.2.12.1 and BA.4/5) using the
280 pseudovirus assay as we previously described⁷. We included data from subjects who
281 previously received 2×BNT or 2×CorV at the activation (0-4 weeks) and memory (4-
282 15 weeks) periods were used for comparison⁷ (Supplementary Table 2). In line with
283 significantly higher frequencies of S-specific B cells, both 3×BNT- and
284 2×CorV+1×BNT-vaccinees displayed significantly stronger geometric mean 50%
285 neutralizing titers (GMT) than 3×CorV against all variants tested (Figure 2A). The
286 overall fold of neutralization resistance followed the order of Alpha < Beta < Delta <
287 Omicron lineages in all three vaccine groups. Interestingly, Omicron BA.2 and
288 BA.4/5 were more resistant to other VOCs with comparable reduction fold of GMT
289 while BA.2.12.1 showed a downward resistance compared to BA.2 among all
290 vaccinees (Figure 2B). According to the criteria that convalescent plasma antibody
291 titer >1:320 were eligible initially for SARS-CoV-2 therapy¹⁵ and considering that
292 the prophylactic administration of convalescent plasma at 1:320 dilution hardly
293 prevents SARS-CoV-2 infection in the hamster model¹⁶, we used 1:320 as the
294 threshold to define NAb titer: less than 1:320 as “Low”, 1:320-1:1280 as “Medium”

295 and above 1: 1280 as “High” for proportion analysis (Figure 2C). We found that 61%
296 of 3×BNT and 48% of 2×CorV+1×BNT vaccinees had high neutralization activity
297 (>1280) against D614G whereas none of 3×CorV vaccinees showed similar activities
298 (Figure 2C). For BA.2, neither 3×BNT nor 2×CorV+1×BNT vaccinees had high
299 neutralization activity, but 41% of 3×BNT and 29% of 2×CorV+1×BNT vaccinees
300 still had medium neutralization activity (321-1280). Strikingly, 68% of 3×CorV
301 vaccinees showed undetectable neutralization antibodies against BA.2. Similar
302 proportion of GMT magnitude was observed in all vaccine groups against BA.4/5
303 (Figure 2C). We also compared the binding antibody titers using different VOC spike
304 protein as the coating antigen. Since spike-specific IgG titers were correlated
305 positively with the neutralizing potency^{7,11}, we found that sera binding titers of
306 various VOCs in 3×BNT and 2×CorV+1×BNT groups were dramatically higher than
307 those in 3×CorV group (Figure 2D). However, as vaccine-induced NAb wane over
308 time⁷, we further compared the NAb titer between 2-dose and 3-dose vaccinees at the
309 similar time post-vaccination (without significant difference) (Supplementary Table 3).
310 The third dose of BNT162b2 induced significant higher NAb titers against all VOCs
311 in 3×BNT and 2×CorV+1×BNT groups compared to the 2-dose groups at both 0-4
312 weeks (activation) and >4 weeks (memory) after vaccination (Supplementary Table 3).
313 In contrast to weak boost effects by the third dose of CoronaVac in the 3×CorV group,
314 10.1-26.1-fold and 9.7-27.5-fold enhancements against Omicron variants at activation
315 and memory phases were observed after the third heterologous BNT162b2
316 (2×CorV+1×BNT), similar to the boost effects in the 3×BNT group (Supplementary
317 Table 3). Apart from the significantly increased NAb titers, the responder rates of
318 anti-BA.2 raised from 33% to 100%, from 0% to 38% and from 0% to 100% at 0-4
319 weeks; from 39% to 100%, from 0% to 35% and from 0% to 100% at >4 weeks in
320 3×BNT, 3×CorV and 2×CorV+1×BNT groups, respectively, post the last vaccination.
321 Consistently, BA.2 exhibited the most resistant profile to the boost effect, especially
322 in 3×CorV (Supplementary Table 4). These results demonstrated that the third
323 heterologous BNT162b2 vaccination in 2×CorV+1×BNT made significant
324 improvement on not only bringing the anti-Omicron responder rate to 100% but also
325 enhancing NAb titers close to 3×BNT at both 0-4 and >4 weeks (Supplementary Table
326 3 and Supplementary Table 4).

327

328 **Spike-specific CD4 and CD8 T cell responses**

329 T cell responses may play an important role in control of SARS-CoV-2 infection
330^{11,12,17}, CD4 and CD8 T cell responses to viral Spike (S) and nucleocapsid protein (NP)
331 were determined by measuring intracellular IFN- γ , TNF- α and IL-2 (Figure 3A and
332 3E). Since many amino acid mutations were found in Omicron Spike protein, we

333 measured ancestral and Omicron S-specific T cell responses in parallel. Significantly
334 higher mean frequencies of S-specific IFN- γ^+ CD4 T cells were found in 3×BNT
335 (ancestral: 0.070% and Omicron: 0.080%) than those in 3×CorV (ancestral: 0.025%
336 and Omicron: 0.023%) and in 2×CorV+1×BNT (ancestral: 0.034% and Omicron:
337 0.030%) (Figure 3B). No significant differences of S-specific IFN- γ^+ and
338 polyfunctional CD4 T cells were found between ancestral and Omicron (Figure 3B
339 and 3C). There were also no significant differences between 2×BNT and 3×BNT, and
340 between 2×CorV and 3×CorV at activation period (Figure 3D, left). However, the
341 third BNT162b2 vaccination in the 2×CorV+1×BNT group recalled significant higher
342 frequency of S-specific IFN- γ^+ cells and responder rate than those in the 3×CorV
343 group at the memory phase (Figure 3D, right). In addition, significantly higher mean
344 frequencies of S-specific IFN- γ^+ CD8 T cells were found in 3×BNT (ancestral: 0.084%
345 and Omicron: 0.098%) than those in 3×CorV (ancestral: 0.017% and Omicron:
346 0.015%) and in 2×CorV+1×BNT (ancestral: 0.021% and Omicron: 0.013%) (Figure
347 3F). The frequency of S-specific polyfunctional CD8 T cells were relatively higher in
348 3×BNT than those in 3×CorV and 2×CorV+1×BNT (Figure 3G). Significant increase
349 of S-specific IFN- γ^+ CD8 T cells was not observed in 3×BNT compared to that in
350 2×BNT at acute (Figure 3H, left) but observed at the memory period (Figure 3H,
351 right). CoronaVac, however, did not show similar activities. Besides the Spike, weak
352 nucleocapsid protein (NP)-specific IFN- γ^+ CD4 and CD8 T cells were observed in 3
353 groups although more CD4 T cell responders (67%) were found in 3×CorV
354 (Supplementary Figure 1), indicating the pre-existing of cross-reactive NP-specific T
355 cell responses in unexposed donors¹⁸. Considering that S-specific circulating T
356 follicular helper cells (cTFH, CD45RA⁻CXCR5⁺CD4⁺) are associated with potent
357 NAb responses¹⁹, we found that the frequency of IFN- γ^+ cTFH cells were low with
358 mean 0.033-0.048%, 0.01-0.023% and 0.017-0.059% in 3×BNT, 3×CorV and
359 2×BNT+1×CorV groups, respectively (Supplementary Figure 2A-2B). However, the
360 responder rate was higher in 3×BNT (20-22%) and 2×BNT+1×CorV (14-24%) than
361 that of 3×CorV (7-10%) (Supplementary Figure 2B). These results indicated that the
362 third dose of BNT162b2 vaccination significantly improved S-specific IFN- γ^+ ,
363 polyfunctional and memory T cells in 3×BNT but not in 2×CorV+1×BNT and
364 3×CorV.

365

366 **Associations among humoral, cellular immune response and breakthrough** 367 **infection features**

368 Immune correlation analysis was subsequently conducted for 23 antigen-specific
369 measurements together with gender, age, time interval between second and third
370 vaccinations, sampling time after third dose of vaccination and infection. Consistent

371 with the kinetics of AM proportion, S-specific AM correlated negatively with time
372 after the third dose of vaccination in the 2×CorV+1×BNT group (Figure 4C). Positive
373 correlations between S-specific B cells and NAbs were observed in both 3×BNT and
374 2×CorV+1×BNT groups while the RM was positively associated with NAbs in the
375 3×CorV group (Figure 4A-C, green rectangle). Consistently, significant positive
376 correlations were found in NAbs titers against all 7 viral variants (Figure 4A-C,
377 purple triangles). Since the third dose vaccination by BNT162b2 or CoronaVac did
378 not improve S-specific CD4 T cell responses among 2×CorV vaccinees, positive
379 correlations among S-specific CD4 T cells, S-specific B cells and NAbs were limited
380 to the 3×BNT group (Figure 4A, red rectangle). However, positive correlations
381 between S-specific cTFH cells and NAbs were observed in 3×BNT and
382 2×CorV+1×BNT, but not in 3×CorV (Figure 4A-C, yellow rectangles). Interestingly,
383 in the 3×BNT group, Omicron S-specific CD4 T cell and cTFH responses exhibited
384 stronger correlation with S-specific B cell and the broadly NAbs than those with
385 ancestral S-specific CD4 T cell and cTFH responses (Figure 4A, yellow rectangle).
386 We then combined all three groups for overall analysis (Figure 4D). Strong positive
387 correlations were consistently found in NAbs titers against all 7 viral variants (Figure
388 4D, purple triangle). Both age and S-specific RM B cells were negatively correlated
389 with NAb activity (Figure 4D, purple rectangle) whereas S-specific AM B cells were
390 positively correlated with neutralizing activity (Figure 4D, green rectangle). Moreover,
391 the frequency of S-specific AM B cells was significantly lower in infected vaccinees
392 than uninfected vaccinees before vaccine-breakthrough infection (Figure 4E) whereas
393 the anti-BA.2 NAb titer did not achieve statistical significance (Figure 4F). Notably,
394 few vaccinees (2/12, 16.7%) with NAb titer higher than 1:320 became infected. We
395 further analyzed the relationships between immune responses and clinical
396 characteristics among our study subjects who were subsequently infected by BA.2
397 (Figure 4G). NAb titer was negatively correlated with hospitalization rate (Figure 4G,
398 purple rectangle), indicating the importance of NAb in reducing COVID-19 severity.
399 Age was positively correlated with viral negative conversion time, suggesting a longer
400 viral clearance time among older patients (Figure 4G, black square). Notably, IFN- γ ⁺
401 CD4 T cells were negatively associated with age and viral negative conversion time
402 (Figure 4G, red squares). In addition, hospitalization was negatively correlated with
403 the interval between second and third dose of vaccinations and with the interval
404 between third dose of vaccination and symptom onset, likely suggesting the
405 importance of the optimal timing for the third dose vaccination (Figure 4G, black
406 rectangle). These results demonstrated that the third dose vaccination-induced NAbs
407 and T cell response contributed to reducing risk of severe clinical outcomes after
408 infection.

409

410 **Immune responses after Omicron BA.2 breakthrough infection and the fourth**
411 **vaccination**

412 Rapidly recalled antibody and T cell responses were observed in vaccine
413 breakthrough infections by SARS-CoV-2 variants^{17,20-22}. At median 137 (range 122-
414 164) days post symptom onset ([Supplementary Table 5](#)), we able to harvest the blood
415 sample from five 3×BNT, three 3×CorV and one 2×CorV+1×BNT subject who had a
416 BA.2 breakthrough infections. Six 3×BNT, seven 3×CorV and ten 2×CorV+1×BNT
417 subjects who never had infection were also included. For comparison, we also
418 included three subjects who received the fourth vaccination with BNT162b2
419 following three-dose CoronaVac (3×CorV+1×BNT) ([Supplementary Table 5](#)). We
420 first measured the frequency of S-specific B cells and found that BA.2 S-specific B
421 cells were consistently lower than ancestral S-specific B cells among all vaccinees no
422 matter with or without BA.2 infection (2.2-3.1-fold and 1.1-2.3-fold difference among
423 uninfected and infected vaccinees, respectively) ([Figure 5A-C](#)). Among uninfected
424 vaccinees, the frequency of BA.2 S-specific B cells in 3×CorV group (mean 0.05%)
425 was significantly lower than those in 3×BNT (mean 0.38%) and 2×CorV+1×BNT
426 (mean 0.17%) groups ([Figure 5B](#)). Although BA.2 infection increased BA.2 S-
427 specific B cells in 3×CorV (mean 0.18%), it was still significantly lower than those in
428 3×BNT group (mean 0.53%) and lower than 3×CorV+1×BNT group (mean 0.48%)
429 without significance ([Figure 5C](#)). In contrast to B cell response, all vaccinees showed
430 similar CD4 and CD8 T cell responses to ancestral and Omicron Spike, and BA.2
431 infection did not boost a higher T cell response than uninfected vaccinees ([Figure 5D-](#)
432 [I](#)). Moreover, uninfected and infected 3×CorV showed lower T cell responses than
433 those in 3×BNT and 3×CorV+1×BNT without significance ([Figure 5F and 5I](#)).
434 Particularly, markedly higher CD8 T cells were found in 3×BNT uninfected vaccinees
435 than those in 3×CorV and 2×CorV+1×BNT uninfected vaccinees even at a long term
436 after vaccination (>4 months) ([Figure 5H](#)). These results indicated that BA.2 infection
437 boosted cross-reactive B cells rather than T cells to ancestral and Omicron Spike.

438

439 **Neutralizing antibody titer after BA.2 breakthrough infection and the fourth**
440 **vaccination**

441 Since broadly neutralizing activity would be boosted by an increased number of
442 exposures to SARS-CoV-2 antigens (vaccination or infection) among vaccinees
443^{17,21,23,24}, pairwise comparison of neutralizing activity was analyzed using the plasma
444 sample collected before (1st) and after (2nd) BA.2 breakthrough infection. Three-dose
445 and 4-dose uninfected vaccinees were also included ([Supplementary Table 5](#)).

446 Consistent to our previous findings in two-dose vaccinees⁷, NAb titer of uninfected
447 vaccinees waned over time, especially when against BA.2.12.1 and BA.4/5 (Figure
448 6A-E), but the waning effect was not observed in NAbs against D614G (Figure 6A).
449 However, 100% and 90% of the uninfected 3×BNT and 2×CorV+1×BNT vaccinees
450 were maintained measurable NAbs against all Omicron variants whereas more
451 uninfected 3×CorV vaccinees (4/7) loosed neutralizing capacity against Omicron
452 BA.4/5. Notably, the fourth vaccination can boost higher NAbs titers and responder
453 rates for 3×CorV vaccinees (Figure 6A-E). Moreover, different 3-dose vaccinees after
454 BA.2 breakthrough infection and 3×CorV+1×BNT vaccinees consistently exhibited a
455 stronger GMT against BA.1 (3×BNT: 3653, 3×CorV: 582 and 2×CorV+1×BNT: 221)
456 and BA.2 (3×BNT: 3005, 3×CorV: 742 and 2×CorV+1×BNT: 417) than those against
457 BA.2.12.1 (3×BNT: 1857, 3×CorV: 531 and 2×CorV+1×BNT: 135) and BA.4/5
458 (3×BNT: 957, 3×CorV: 200 and 2×CorV+1×BNT: 94) (Figure 6A-E). This boost
459 effect by BA.2 breakthrough infection was more profound in 3×CorV vaccinees with
460 the highest fold-change (up to 21.2-fold increased for BA.2) in GMT against Omicron
461 sublineages (Figure 6A-E). The results indicated that BA.2 breakthrough infection
462 and the fourth vaccination enhanced cross-neutralizing antibodies to Omicron
463 sublineages in all vaccinees.

464

465 Discussion

466 Clinical trials have demonstrated that a third heterologous booster vaccination by
467 EUA SARS-CoV-2 mRNA vaccines (BNT162b2 and mRNA-1273) increased
468 neutralizing antibody titer accompanied by better prevention and lower disease
469 severity than the initial two doses with BBIP-CorV or CoronaVac during the Gamma
470 and Delta epidemics²⁵⁻²⁹. After the emergence of the Omicron variants, some cohort
471 studies reported that Omicron BA.1 infection was associated with milder disease and
472 shorter duration of clinical symptoms than Delta infection³⁰⁻³⁵.

473

474 The third vaccination was helpful in reducing the infection and hospitalization rates
475 during the Delta and Omicron BA.1 prevalence in other countries^{25,36,37}. Till now, the
476 association between immune responses induced by the third vaccination and Omicron
477 BA.2 breakthrough infection remains unknown. In this study, we investigated the
478 immune responses of vaccinees after they received the third vaccination right before
479 the explosive fifth wave of SARS-CoV-2 epidemic caused by Omicron BA.2 in Hong
480 Kong^{10,14}. We also followed up the infection status and clinical outcomes of our study
481 subjects during the wave period. We found that the third dose of either BNT162b2 or
482 CoronaVac led to significantly lower infection rates than those who received the
483 standard 2-dose vaccination regimen, particularly in the heterologous

484 2×CorV+1×BNT group. Furthermore, the third BNT162b2 resulted in significantly
485 higher rates of asymptomatic and lower rates of hospitalization than 3×CorV group.
486 Our findings, therefore, provided critical knowledge on understanding the role of third
487 vaccination-induced immune responses in protection against the globally spreading
488 Omicron BA.2 infections.

489

490 Omicron BA.2 has higher transmissibility and immune evasion than Omicron BA.1
491 ^{38,39}, explaining its rapid spread in Hong Kong and other places ^{40,41}. Since the end of
492 January 2022, BA.2 has quickly dominated the fifth wave of SARS-CoV-2 epidemic
493 in Hong Kong, where the universal masking policy remains unchanged, with a shorter
494 doubling time of 1.28 days than 1.6-2.8 days of BA.1 ¹⁰. BA.2 shares 21 mutations in
495 the Spike with BA.1. Although Q496S and N501Y mutations are missing in the BA.2
496 S-BRD domain, unique S371F, T376A, D405N and R408S mutations have been
497 found ³⁹. Due to these mutations, we and others ^{39,42} demonstrated that NAb titers
498 against BA.2 showed 0.97-1.18 and 1.14-1.42 time lower than those against BA.1 at
499 0-4 weeks and >4 weeks after third vaccination by BNT162b2 or CoronaVac. Also,
500 we consistently found that BA.2 confers the highest NAb resistance compared with
501 other VOCs including BA.1 and BA.1.1 before emergence of BA.4/5. While 59-71%
502 and 29-41% BNT162b2 booster recipients had low (IC₅₀: 20-320) and median (IC₅₀:
503 321-1280) NAbs against BA.2, 66% CoronaVac booster recipients had undetectable
504 (IC₅₀<20) NAbs. Surprisingly, although the third BNT162b2 vaccination boosted
505 higher anti-BA.2 NAb titer and responder rate as well as a more S-specific T cell
506 responses than the third CoronaVac, there was no significant difference in incidence
507 of breakthrough infections between 3×BNT and 3×CorV. Firstly, the majority of our
508 vaccinees, including 3×BNT and 3×CorV, have a low neutralizing antibody titer at
509 the time of exposure, rendering them susceptible to BA.2 breakthrough infection. Ten
510 of twelve vaccinees who had IC₅₀<320 NAb against BA.2 became infected, which is
511 consistent to the animal study that the prophylactic administration of convalescent
512 plasma at 1:320 dilution hardly prevents SARS-CoV-2 infection in hamster model ¹⁶.
513 Secondly, both CoronaVac and BNT162b2 hardly induce enough mucosal
514 neutralizing antibody or T cell responses for prevention ⁴³, as Omicron replicates
515 faster and stronger than wild type and Delta variant in the nasal and bronchial
516 compartments but less efficiently in the lung parenchyma ⁴⁴⁻⁴⁶. Critically, although
517 CoronaVac displays lower immunogenicity than BNT162b2, it still induced memory
518 B cell and T cell responses that can be recalled for protection as demonstrated in the
519 3×CorV vaccinees with BA.2 breakthrough infection. Therefore, the recalled immune
520 response, especially the comparable T cell responses, which are invoked by the BA.2
521 breakthrough infection in participants who received different vaccine regimens.

522 In addition, three doses of either CoronaVac or BNT162b2 vaccines provided similar
523 and high protection against Omicron infection-induced severe outcomes^{47,48}. Such
524 BA.2 infection-mediated immune activation might be even more profound among
525 3×CorV vaccinees, resulting in significantly reduced infection and hospitalization
526 rates compared with 2×CorV vaccinees. Therefore, when all vaccinees were analyzed
527 together, we found that S-specific activated memory B cell subset was a significant
528 factor in preventing BA.2 infection because these AM B cells could differentiate into
529 long-lived plasma cells⁴⁹ and are associated with expansion of memory B cells, and
530 the re-establishment of B cell memory after the third vaccination^{23,50}. Moreover, T
531 cell responses could be another protective factor because they may recognize mutated
532 viral variants without significantly reducing the potency⁵¹. We found that both
533 BNT162b2 and CoronaVac-induced T cell responses cross-reacted to Omicron S
534 peptides with comparable activities to ancestral S^{52,53}. Since S-specific T cells are
535 associated with the control and clearance of the ongoing infection¹², potent T cell
536 responses correlated with fewer hospitalization among patients who received the third
537 vaccination.

538 While we studied the BA.2 variant, the BA.2.12.1, BA.4, and BA.5 have raised and
539 increased resistance compared to previous VOCs to vaccine-induced NAb through
540 the L452R/Q and F486V mutations in the Spike⁵⁴⁻⁵⁶. We confirmed that BA.2
541 breakthrough infection and the fourth vaccination effectively boosts neutralizing
542 antibody against BA.2.12.1 and BA.4/5. This can explain why BA.1/BA.2 infection in
543 vaccinated persons were less at risk of BA.4/5 infection than individuals infected with
544 a pre-Omicron VOCs⁵⁷. However, BA.2 breakthrough infections mainly recalled
545 vaccine-induced ancestral Spike-specific memory B cells, which may drive further
546 mutation of virus and variant-associated reinfection^{55,58,59,60}.

547 There are some limitations in our study. Firstly, most of our infected vaccinees were
548 confirmed to have been infected by self-RAT, thus the effect of different vaccine
549 regimens on controlling viral loads could not be determined. It remains necessary to
550 compare the dynamics and magnitudes of the recalled immune responses among
551 vaccinees with BA.2 breakthrough infection patients in the future. Secondly, it should
552 be noted that the median interval time between the latest vaccination and symptom
553 onset for the 2×BNT (227 days) and 2×CorV (237 days) groups was significantly
554 longer than those for 3 dose vaccination groups, including 3×BNT (48.5 days),
555 3×CorV (56 days) and 2×CorV+1×BNT (25.5 days). Although NAb potency wanes
556 over time⁷, we and others consistently found that timely boost vaccination not only
557 restore waning NAb titers but also broaden the breadth of NAb, which is able to
558 cross-neutralize VOCs, including Omicron^{8,23,50,61}. Thirdly, only one sample can be
559 harvested from 2×CorV+1×BNT vaccinees with BA.2 infections. It's hard to

560 conclude the outcome of BNT162b2 booster for two-dose CoronaVac vaccinees
561 during BA.2 breakthrough infection.

562

563 In summary, we report that 3×BNT and 3×CorV provided better protection against
564 SARS-COV-2 BA.2 than 2×BNT and 2×CorV. High frequencies of S-specific
565 activated memory B cells and cross-reactive T cell responses induced by the third
566 vaccination are critical for protection and illness reduction during the Omicron BA.2
567 breakthrough infection. Enhanced neutralization induced by BA.2 breakthrough
568 infection and the fourth vaccination may help to reduce the risk for infection of
569 ongoing BA.2.12.1 and BA.4/5.

570

571 **Contributors**

572 Z.C. and R.Z. conceived and designed the study. R.Z. and Z.C. designed experiments,
573 analyzed data, and wrote the manuscript. Z.C., R.Z. X.L., Y.-C.K., H.-Y.K., I.F.-N.H.,
574 and K.-Y.Y coordinated donor recruitment and specimen collection. R.Z., N.L., H.H.,
575 D.Y., Q.P. prepared the clinical sample. R.Z., N.L. and H.H. performed the flow
576 cytometry analysis. R.Z., N.L. and D.Y. performed the pseudoviral neutralization
577 assay. Z.D. did the correlation analysis.

578

579 **Data sharing**

580 The authors declare that the data supporting the findings of this study are available
581 from the corresponding author upon request.

582

583 **Declaration of interests**

584 We declare no competing interests.

585

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601

602 **Figurer legends**

603 **Figure 1. Activation of Spike-specific memory B cells by the third dose**

604 **vaccination.** (A) Representative flow cytometry plots showing staining patterns of
605 SARS-CoV-2 Spike probes on memory B cells (IgD⁻ IgG⁺ CD19⁺). (B) Quantified
606 results depict the percentage of Spike⁺ B cells in 3×BNT (orange), 3×CorV (blue)
607 and 2×CorV+1×BNT (purple) groups at median 23, 55 and 47 days after the third
608 dose vaccination. (C) Comparisons of Spike⁺ B cell frequency between 2-dose
609 (sample collected at median 28 days after second vaccination for 2×BNT and 2×CorV
610 groups) and 3-dose (sample collected at median 16, 20 and 18.5 days after third
611 vaccination for 3×BNT, 3×CorV and 2×CorV+1×BNT groups, respectively) cohorts
612 within 4 weeks after the last vaccinations. (D) Cross-sectional analysis of Spike-
613 specific B cells by time after third dose vaccination. The connection lines indicate the
614 mean value. (E) Phenotypes of Spike-specific B cells were defined by using CD21
615 and CD27 markers. (F) Pie chart showed the proportion of activated (AM),
616 tissue-like (TLM) memory, intermediate memory (IM) and resting-memory (RM) B
617 cells. (G) Cross-sectional analysis of the percentage of AM (upper) and RM (bottom)
618 in the Spike-specific B cells by time after third vaccination. The connection lines
619 indicate the mean value.

620

621 **Figure 2. The titer and breadth of neutralizing antibodies (NAbs) against a full**

622 **panel of current SARS-CoV-2 VOCs.** (A) The geometric mean titers (GMT) of
623 neutralizing antibody (IC₅₀ represents serum dilution required to achieve 50% virus
624 neutralization) against nine SARS-CoV-2 strains were measured by pseudovirus-
625 based assay among 3×BNT (orange), 3×CorV (blue) and 2×CorV+1×BNT vaccinees
626 (purple) at median 23, 55 and 47 days after the third dose vaccination. Numbers under
627 the x-axis indicate the responder rates (IC₅₀>20 termed 'responder'). (B) GMT of
628 neutralizing antibody were depicted on the top of Figure. The green lines indicate the
629 change of GMT among variants. Numbers on the top of dots indicate the fold change
630 of different VOC relative to D614G. Each symbol represents an individual donor with
631 a line indicating the mean of each group. (C) Proportion of four neutralizing antibody
632 magnitudes among vaccinees. (D) Levels of anti-Spike IgG (BAU/mL) of all
633 vaccinated subjected are shown as mean ± SEM. Dotted line represents value of 64.5
634 BAU/mL used as the limit of detection (LOD). Statistics were generated by using 2-
635 tailed Student's t test. *p<0.05; **p<0.01; ***p<0.001.

636

637 **Figure 3. Spike-specific CD4 and CD8 T cell responses.** PBMCs were stimulated
638 with the Spike peptide pools from ancestral or Omicron SARS-CoV-2 prior to
639 intracellular cytokine staining assay. Representative flow cytometry plots showing
640 single positive of IFN- γ^+ or TNF- α^+ or IL-2 $^+$ as well as the polyfunctional cells with
641 ≥ 2 cytokines among CD4 $^+$ (A) and CD8 $^+$ (E) T cells. Paired analysis of the
642 frequencies of IFN- γ -producing CD4 $^+$ (B) and CD8 $^+$ (F) T cells as well as the
643 frequencies of polyfunctional CD4 $^+$ (C) and CD8 $^+$ (G) T cells to ancestral (open dots)
644 or Omicron (solid dots) Spike among the 3 \times BNT (orange), 3 \times CorV (blue) and
645 2 \times CorV+1 \times BNT (purple) vaccinees. The mean frequencies were depicted under the
646 x-axis. The frequencies of IFN- γ -producing CD4 $^+$ (D) and CD8 $^+$ (H) T cell to ancestral
647 Spike among 2 \times BNT, 3 \times BNT, 2 \times CorV, 3 \times CorV and 2 \times CorV+1 \times BNT vaccinees at 0-
648 4 weeks (left) and >4 weeks (right) periods after vaccinations. Undetected (UD): % of
649 IFN- γ^+ cells $<0.00781\%$. The green lines in B, C, F, G indicate the change of mean
650 responses to ancestral and Omicron Spike. The responses are depicted as the
651 background-subtracted percentage of S-specific T cells (Background subtraction
652 refers to the subtraction of the values of the negative control sample from the peptide-
653 stimulated sample). The responder rates were depicted on the top of dots (% of IFN- γ^+
654 cells $>0.00781\%$ termed ‘responder’ after subtracted from percentage of unstimulated
655 control). Each symbol represents an individual donor with a line indicating the mean
656 of each group. Statistics were generated by using 2-tailed Student’s t test. Ns: no
657 significance, * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

658

659 **Figure 4. Associations among humoral, cellular immune response and**
660 **breakthrough infection features.** Correlogram of immune responses among 3 \times BNT
661 (A), 3 \times CorV (B), 2 \times CorV+1 \times BNT (C) and overall (D) vaccinees. Comparison of
662 AM $^+$ B cell frequency on Spike-specific B cells (E) and neutralizing titer against
663 BA.2 (F) between uninfected and infected vaccinees. Uninfected vaccinees, infected
664 3 \times BNT vaccinees, infected 3 \times CorV vaccinees and infected 2 \times CorV+1 \times BNT
665 vaccinees were presented as grey, orange, blue and purple dots, respectively. Statistics
666 were generated by using 2-tailed Student’s t test. * $p<0.05$. (G) Correlogram of clinical
667 characteristics and immune responses among patients. Spearman rank order
668 correlation values (r) are shown from red (-1.0) to blue (1.0); r values are indicated by
669 color and square size. p values are indicated by white asterisks. The green rectangles
670 denote SARS-CoV-2 Spike-specific B cell features, the purple triangle and rectangles
671 denote anti-SARS-CoV-2 variants’ neutralizing antibody features, the red rectangles
672 denote the SARS-CoV-2 Spike-specific CD4 T cell features, the yellow rectangle
673 denotes the SARS-CoV-2 Spike-specific cTFH features and the black rectangles

674 denotes clinical characteristic features.

675

676 **Figure 5. Immune responses after Omicron BA.2 breakthrough infection and the**
677 **fourth vaccination**

678 (A) Representative flow cytometry plots showing staining patterns of SARS-CoV-2
679 ancestral or BA.2 Spike probes on memory B cells (IgD⁻ IgG⁺ CD19⁺). (B-C)
680 Quantified results depict the percentage of ancestral (empty) and BA.2 (solid) Spike⁺
681 B cells in uninfected (B) and infected (C) 3×BNT (orange), 3×CorV (blue),
682 2×CorV+1×BNT (purple) and 3×CorV+1×BNT (grey) groups. The numbers above
683 the x-axis indicate the fold-change in frequency of positive B cells to ancestral and
684 BA.2 Spike. The numbers under x-axis indicate the mean frequencies of ancestral or
685 BA.2-specific B cells. Undetected (UD): % of Spike⁺ cells<0.03125%. (D and G)
686 Representative flow cytometry plots showing the IFN-γ⁺ cells among CD4⁺ (D) and
687 CD8⁺ (G) T cells to negative control, ancestral Spike and Omicron Spike peptide
688 pools. Quantified results depict the percentage of ancestral (empty) and Omicron
689 (solid)-specific IFN-γ⁺ cells in uninfected (E and H) and infected (F and I) 3×BNT
690 (orange), 3×CorV (blue), 2×CorV+1×BNT (purple) and 3×CorV+1×BNT (grey)
691 groups. The numbers above the figures indicate the fold-change in frequency of
692 positive T cells to ancestral and BA.2 Spike. The numbers under x-axis indicate the
693 mean frequencies of ancestral or Omicron-specific IFN-γ⁺ cells T cells. Undetected
694 (UD): % of IFN-γ⁺ cells<0.00781%. Each symbol represents an individual donor.
695 Statistics were generated by using 2-tailed Student's t test. Ns: no significance, *
696 p<0.05; **p<0.01.

697

698 **Figure 6. Neutralizing antibody titer after BA.2 breakthrough infection and the**

699 **fourth vaccination. (A-E)** The neutralizing antibody (IC₅₀ represents serum dilution
700 required to achieve 50% virus neutralization) against five SARS-CoV-2 strains were
701 measured by pseudovirus-based assay among uninfected and infected 3×BNT
702 (orange), 3×CorV (blue), 2×CorV+1×BNT (purple) and 3×CorV+1×BNT (grey)
703 before (1st, empty dots) and after (2nd, solid dots) BA.2 infection or the fourth
704 vaccination. Black dots and lines represent the breakthrough infection sample in each
705 group. Numbers on the figure top indicate the fold-change in NAb titer between 1st
706 and 2nd sample. Numbers under the x-axis indicate the geometric mean titers (GMT).
707 Statistics were generated by using 2-tailed Student's t test. *p<0.05; ***p<0.001; ns:
708 not significant. (F-H) The ratio of SARS-CoV-2 VOC NAb IC₅₀ normalized against
709 the D614G NAb IC₅₀. Orange line, blue line and purple line represent uninfected
710 3×BNT, 3×CorV and 2×CorV+1×BNT vaccinees. Black lines represent the infected
711 vaccinees in each group. Numbers on the figure top indicate the ratio for

712 corresponding VOCs.

713

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883

884 **Supplementary materials**

885 **Supplementary Table 1. Significance in demographic characteristics among each**
886 **vaccine cohort.**

887

888 **Supplementary Table 2. Characteristics of the third doses of SARS-CoV-2**
889 **vaccine cohorts.**

890

891 **Supplementary Table 3. Comparison in neutralizing antibody titers between 2-**
892 **dose and 3-dose vaccinations.**

893

894 **Supplementary Table 4. Comparison in antibody responder rates between 2-dose**
895 **and 3-dose vaccination.**

896

897 **Supplementary Table 5. Characteristics of three doses and 4 doses of SARS-CoV-**
898 **2 vaccinees with or without BA.2 infection.**

899

900 **Supplementary Figure 1. SARS-CoV-2 NP-specific T cell responses. PBMCs from**
901 **vaccinees were subjected to the intracellular cytokine staining assay against NP**

902 peptide pool. IFN- γ^+ cells were gated on CD4 (**A**) and CD8 (**B**) T cells, respectively.
903 Quantified results depict the percentage of IFN- γ^+ cells as background subtracted
904 data from the same sample stimulated with negative control (anti-CD28/CD49d only).
905 Each symbol represents an individual donor with a line indicating the mean of each
906 group among the 3×BNT (orange), 3×CorV (blue) and 2×CorV+1×BNT (purple)
907 vaccinees. The mean frequency of IFN- γ^+ cells and responder rates were depicted
908 under x-axis (% of IFN- γ^+ cells>0.00781% termed ‘responder’ after subtracted from
909 percentage of unstimulated control). Undetected (UD): % of IFN- γ^+ cells<0.00781%.
910

911 **Supplementary Figure 2. SARS-CoV-2 Spike-specific cTFH responses.** PBMCs
912 from vaccinees were subjected to the intracellular cytokine staining assay against
913 Spike peptide pools from ancestral or Omicron SARS-CoV-2. (**A**) IFN- γ^+ cells were
914 gated on cTFHs. (**B**) Quantified results depict the percentage of IFN- γ^+ cells as
915 background subtracted data from the same sample stimulated with negative control
916 (anti-CD28/CD49d only). Each symbol represents an individual donor with a line
917 indicating the mean of each group to ancestral (open dots) or Omicron (solid dots)
918 Spike among the 3×BNT (orange), 3×CorV (blue) and 2×CorV+1×BNT (purple)
919 vaccinees. The mean frequency of IFN- γ^+ cells and responder rates were depicted
920 under x-axis. Undetected (UD): % of IFN- γ^+ cells<0.00781%. Statistics were
921 generated by using 2-tailed Student’s t test. Ns: no significanceNs: no significance.

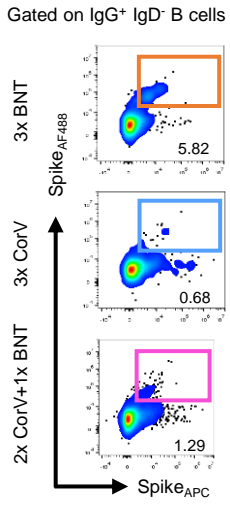
Table 1. Demographic characteristics of breakthrough infection among 470 vaccinees

Vaccinations	2xBNT (n=169)	3xBNT (n=168)	2xCorV (n=34)	3xCorV (n=67)	2xCorV+1xBNT (n=32)
Infection rate % (No. patient/Total No.)	49.2% (78/169)	13.1% (22/168)	44.1% (15/34)	19.4% (13/67)	6.3% (2/32)
Patients	(n=78)	(n=22)	(n=15)	(n=13)	(n=2)
Age, year (ranges in parentheses)	32 (24-58)	40 (27-60)	41 (24-64)	50 (20-62)	47.5 (37-58)
Gender					
Male (% of all participants)	60 (48.8%)	14 (12.3%)	9 (42.9%)	8 (18.2%)	2 (7.1%)
Female (% of all participants)	18 (39.1%)	8 (14.8%)	6 (46.2%)	5 (21.7%)	0 (0%)
Median interval days between latest vaccination and symptom onset (ranges in parentheses)	227 (140-332)	48.5 (10-111)	237 (52-341)	56 (7-109)	25.5 (10-41)
Asymptomatic rate % (No. Asymptomatic patient/No. total patient)	3.8% (3/78)	0% (0/22)	0 % (0/15)	0% (0/13)	0% (0/2)
Disease severity	Mild	Mild	Mild	Mild	Mild
Number of symptoms (ranges in parentheses)	4 (0-6)	3 (1-5)	3 (1-6)	2 (1-5)	3.5 (3-5)
Presentation to hospital % (No. patients presenting to hospital/No. total patient)	19.2% (15/78)	4.5% (1/22)	20% (3/15)	15.4% (2/13)	50% (1/2)
Duration of illness, days (ranges in parentheses)	7 (0-19)	7.5 (2-19)	8 (6-21)	8 (2-14)	9.5 (2-17)
The interval days between symptom onset and two negative RAT	8 (1-20)	9 (6-13)	8 (6-12)	9 (3-14)	8 (5-11)

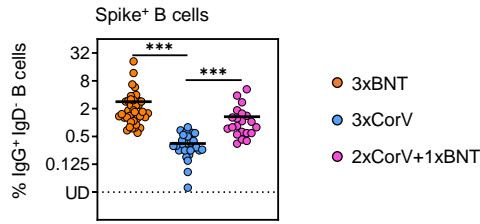
Values displayed are medians, with ranges in parentheses

Figure 1

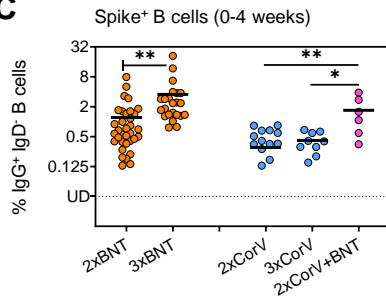
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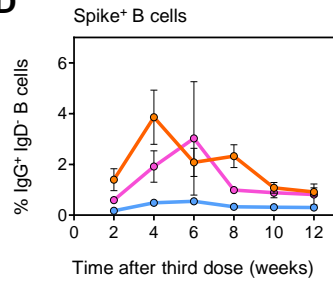
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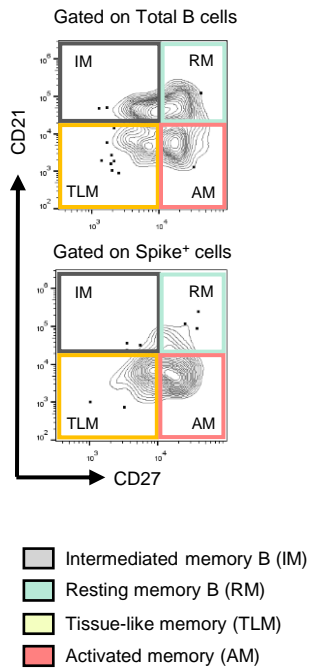
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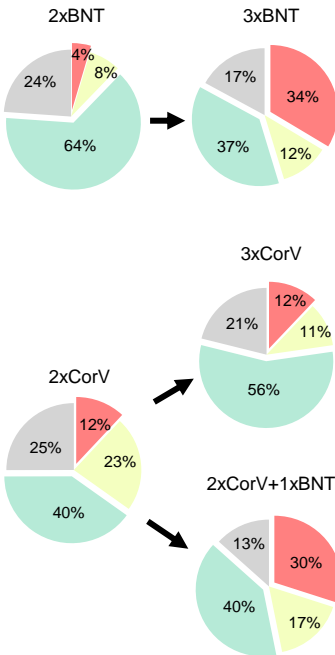
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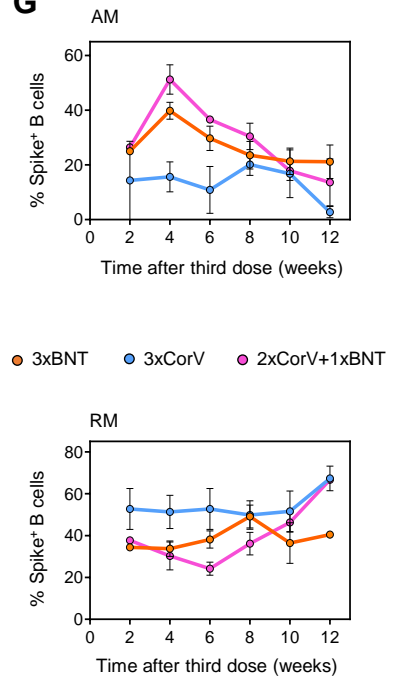


Figure 2

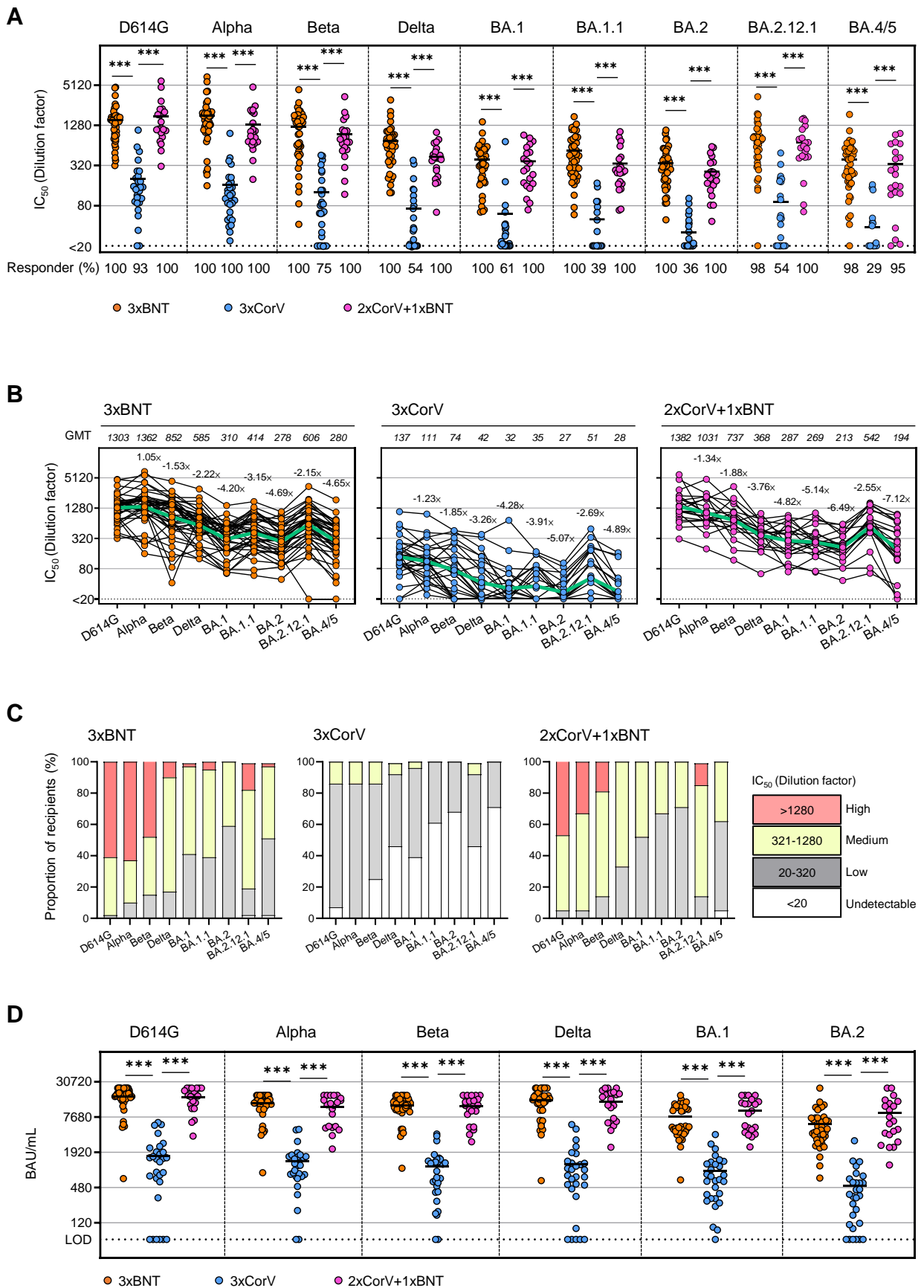


Figure 3

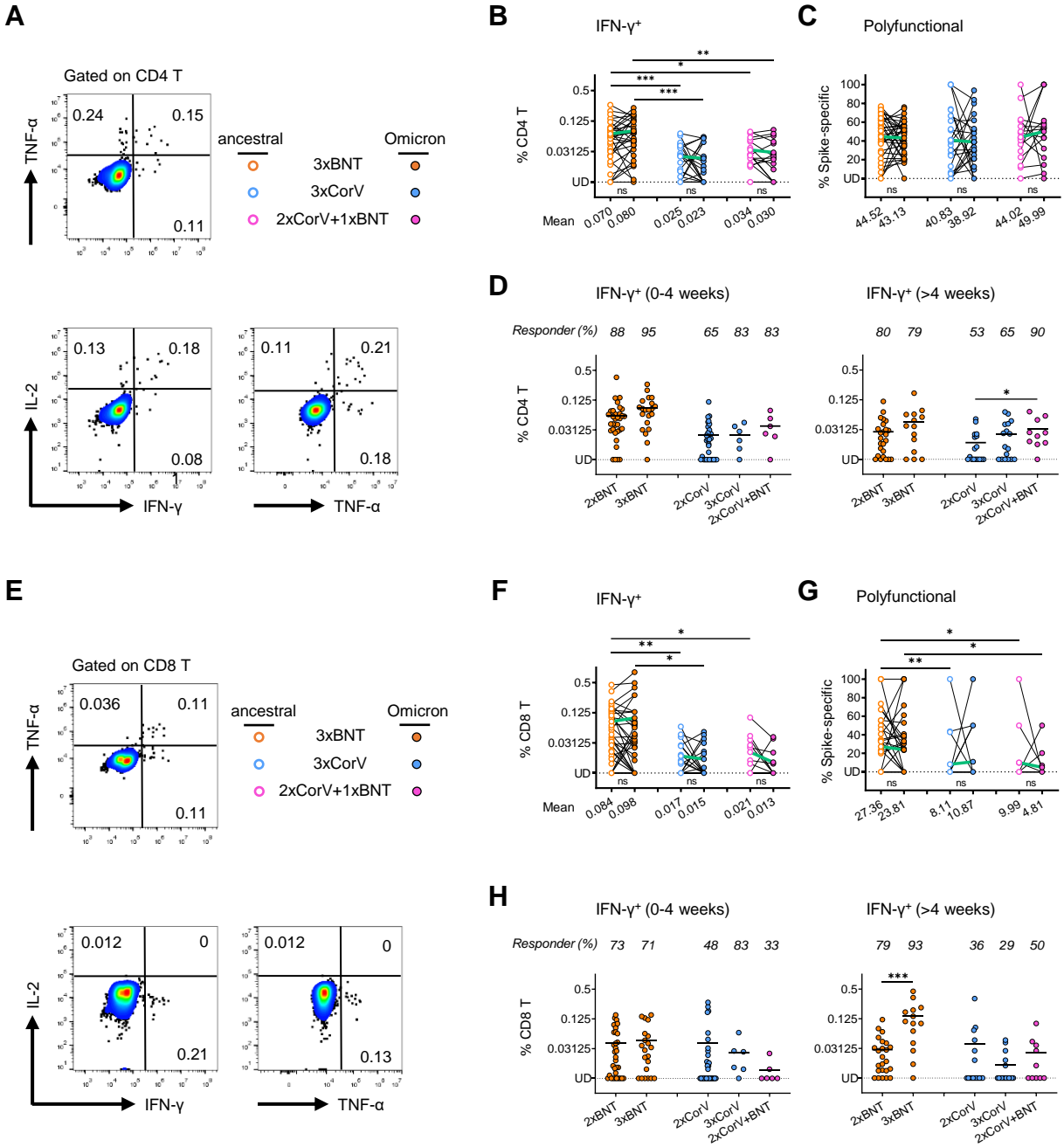


Figure 4

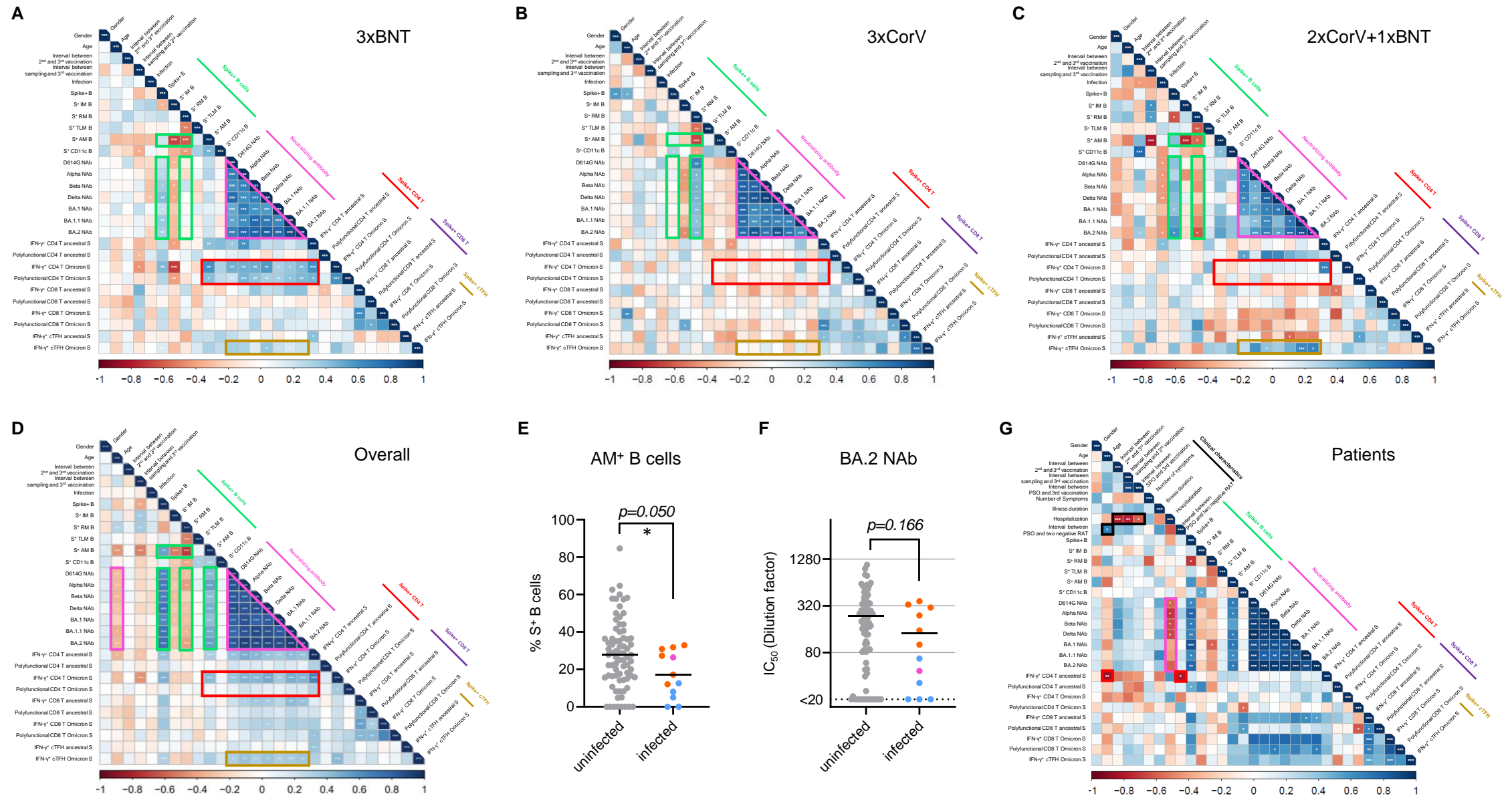


Figure 5

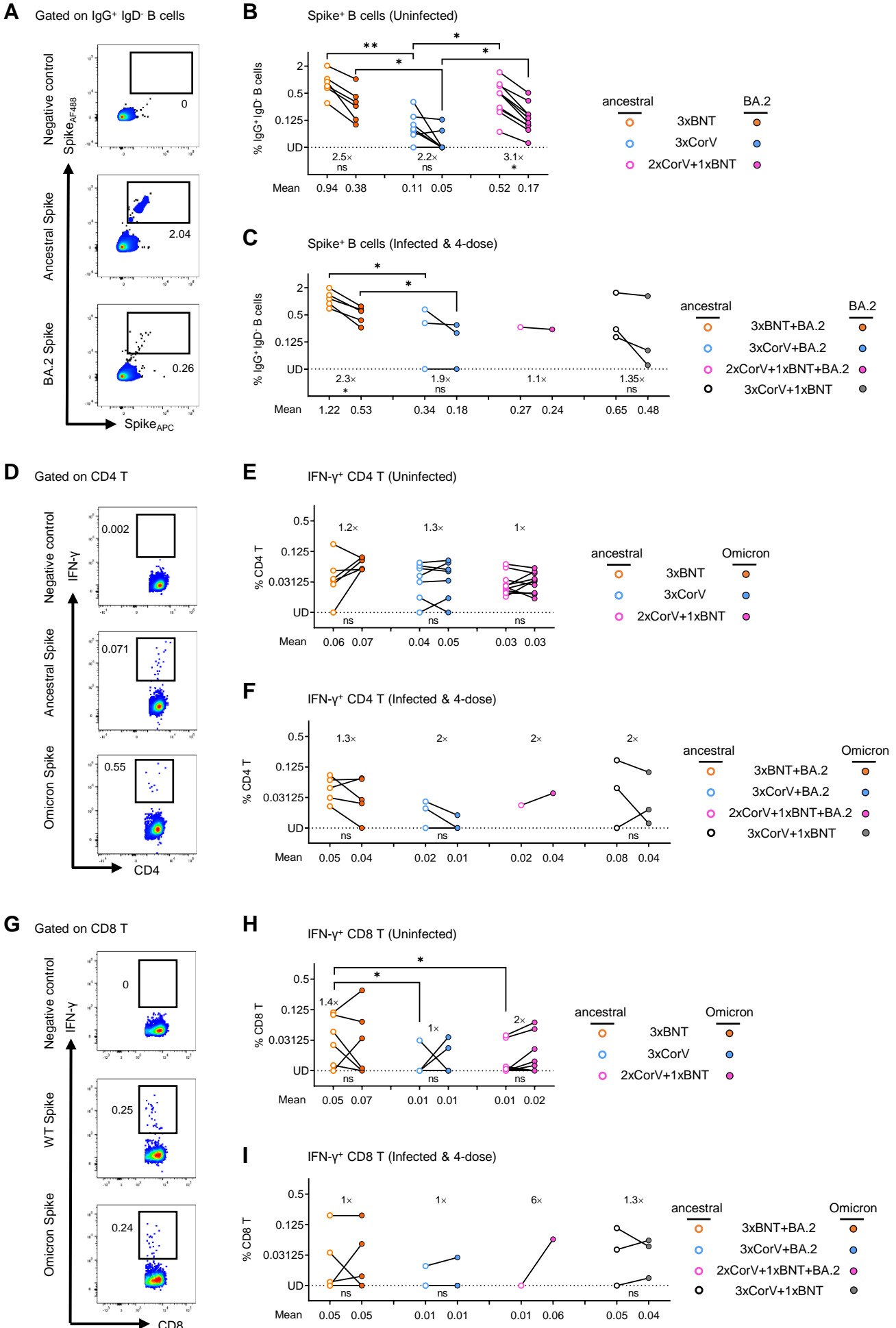
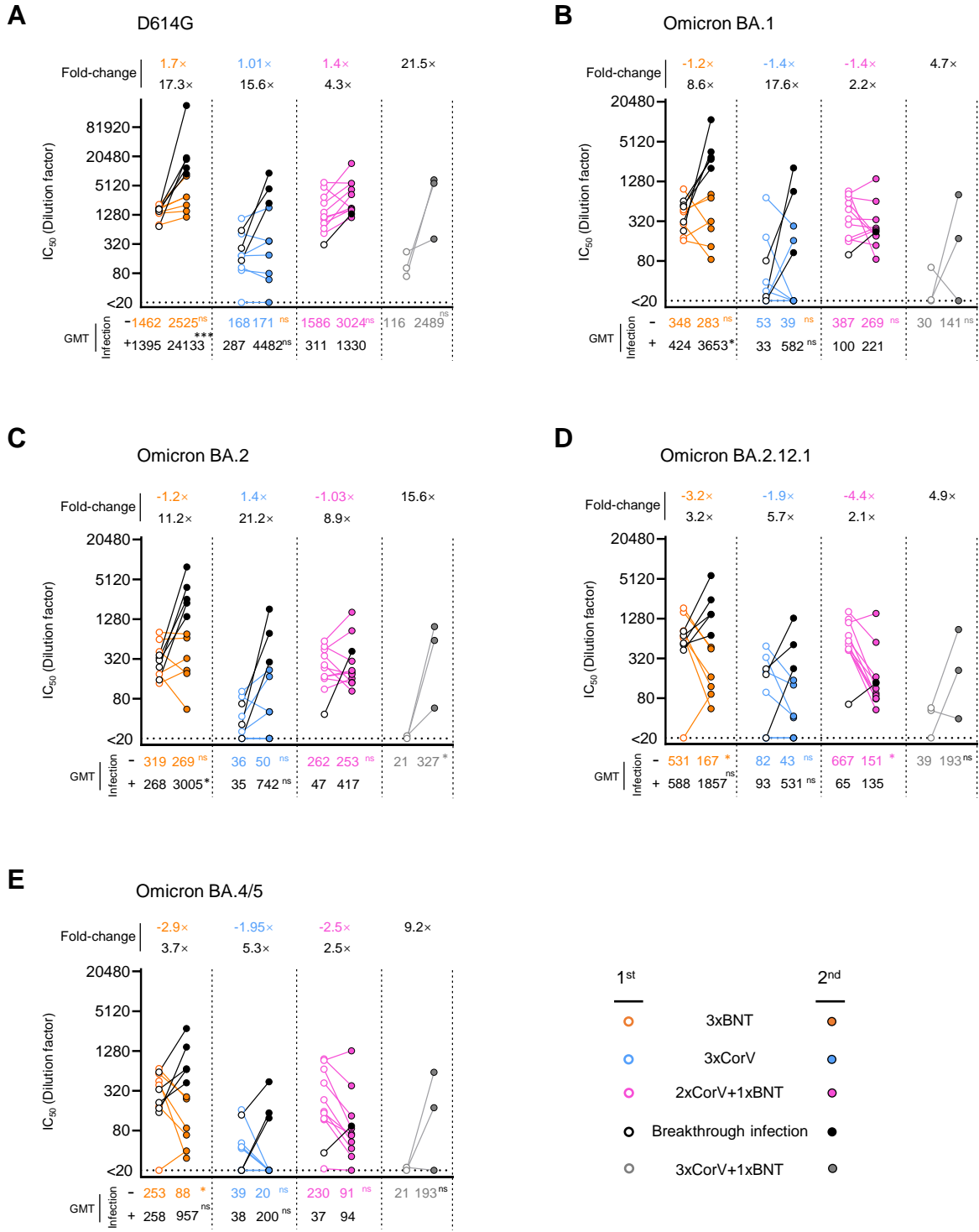


Figure 6



Supplementary Table 1. Significance in demographic characteristics among each vaccine cohort.

	P value						
	2×BNT vs 3×BNT	2×CorV vs 3×CorV	2×CorV vs 2×BNT	3×CorV vs 3×BNT	2×CorV +1×BNT vs 2×CorV	2×CorV +1×BNT vs 3×CorV	2×CorV +1×BNT vs 3×BNT
Vaccinations							
Infection rate %	<0.0001	0.009	0.828	0.22	<0.0001	0.159	0.426
Age	<0.0001	0.2654	0.0006	0.1024	0.6595	0.9274	0.4714
Gender	0.21	1.0	0.294	1.0	0.515	0.524	1.0
Median interval days between latest vaccination and symptom onset	<0.0001	<0.0001	0.4355	0.3926	0.0027	0.1598	0.2162
Asymptomatic rate %	0.821	ns	1.0	ns	ns	ns	ns
Number of symptoms	0.3009	0.2634	0.7435	0.6130	0.8218	0.3983	0.5026
Presentation to hospital %	0.183	1.0	1.0	0.541	0.426	0.371	0.163
Duration of illness, days	0.8024	0.4392	0.1780	0.8306	0.9264	0.6639	0.8108
The interval days between symptom onset and two negative RAT	0.9501	0.474	0.3277	0.8324	0.9645	0.7541	0.5315

Supplementary Table 2. Characteristics of the two and three doses of SARS-CoV-2 vaccinee cohorts who included for comparison of immune responses

Characteristics	2xBNT (n=27)	2xCorV (n=16)	3xBNT (n=41)	3xCorV (n=28)	2xCorV+1xBNT (n=21)
Age	30 (22-66)	27 (22-33)	46 (27-55)	51 (40-58)	47 (32-53)
Gender					
Male (n)	11	10	29	15	19
Female (n)	16	6	12	13	2
Days between the 1st and 2nd dose	21 (20-31)	28 (22-35)	23 (21-36)	28 (28-71)	29 (28-97)
Days between the 2nd and 3rd dose	-	-	236 (180-283)	236 (191-287)	240 (189-284)
Days between last vaccination and blood collection	31 (7-47)	27 (10-105)	23 (7-75)	56 (13-77)	47 (7-77)
Number of Infection after last vaccination	0	0	6	5	1

Values displayed are medians, with ranges in parentheses

Supplementary Table 3. Comparison in neutralizing antibody titers between 2-dose and 3-dose vaccinations.

Vaccinations	Homologous BNT162b2			Homologous CoronaVac			Heterologous BNT162b2	
	2xBNT	3xBNT		2xCorV	3xCorV		2xCorV+1xBNT	
0-4 weeks after vaccination	n=9	n=24		n=9	n=8		n=6	
Median time (days) post-vaccination	14 (7-26)	16 (7-28)	ns	25 (10-28)	20 (13-28)	ns	18.5 (7-24)	ns
	†NAb IC ₅₀ GMT (95% CI)		‡Fold	†NAb IC ₅₀ GMT (95% CI)		€Fold	†NAb IC ₅₀ GMT (95% CI)	δFold
D614G	736 (334-1621)	1393 (1061-1830)	1.9 ns	80 (39-165)	181 (106-308)	2.3 ns	1242 (481-3204)	15.6 **
Alpha	589 (242-1434)	1545 (1099-2171)	2.6 ns	80 (30-215)	90 (43-185)	1.1 ns	1000 (387-2582)	12.5 **
Beta	143 (41-491)	923 (608-1400)	6.5 **	24 (15-38)	109 (49-244)	4.5 *	788 (253-2459)	32.8 **
Delta	241 (86-677)	586 (425-807)	2.4 ns	28 (20-38)	46 (19-116)	1.6 ns	324 (123-852)	11.6 ***
BA.1	66 (32-140)	295 (215-404)	4.5 **	22 (18-28)	36 (20-66)	1.6 ns	238 (105-535)	10.8 *
BA.1.1	30 (15-58)	411 (287-589)	12.1 **	21 (19-22)	58 (27-124)	2.8 **	257 (106-626)	12.2 **
BA.2	43 (17-109)	273 (202-370)	13.7 **	20 (20-20)	28 (17-46)	1.4 ns	202 (87-465)	10.1 ***
BA.2.12.1	50 (20-126)	707 (514-971)	14.1 **	20 (20-20)	65 (26-164)	3.3 *	521 (155-1744)	26.1 **
BA.4/5	67 (24-187)	339 (232-496)	5.1 *	20 (20-20)	37 (17-81)	1.9 ns	298 (37-1062)	14.9 **
>4 weeks after vaccination	n=18	n=17		n=7	n=20		n=15	
Median time (days) post-vaccination	31 (30-47)	45 (30-75)	ns	40 (32-105)	66 (30-77)	ns	59 (35-77)	ns
	†NAb IC ₅₀ GMT (95% CI)		‡Fold	†NAb IC ₅₀ GMT (95% CI)		€Fold	†NAb IC ₅₀ GMT (95% CI)	δFold
D614G	399 (297-536)	1106 (832-1471)	2.8 ***	21 (25-105)	122 (77-194)	5.8 ns	1443 (1018-2044)	68.7 **
Alpha	687 (44-1051)	1140 (760-1709)	1.7 ns	44 (20-96)	121 (82-179)	2.8 ns	1044 (726-1501)	23.7 **
Beta	132 (72-243)	762 (448-1296)	5.8 ***	20 (20-20)	64 (38-107)	3.2 ns	718 (503-1025)	35.9 ***
Delta	110 (79-153)	584 (407-839)	5.3 ***	23 (16.8-32)	41 (26-63)	1.8 ns	387 (293-511)	16.8 ***
BA.1	37 (24-58)	326 (219-484)	8.8 ***	20 (20-20)	31 (21-46)	1.6 ns	310 (203-473)	15.5 ***
BA.1.1	31 (22-41)	284 (485-435)	9.2 ***	20 (20-20)	29 (21-40)	1.5 ns	274 (186-402)	13.7 **
BA.2	28 (21-38)	284 (199-406)	10.1***	20 (20-20)	27 (21-34)	1.4 ns	218 (152-311)	10.9 **
BA.2.12.1	36 (25-53)	488 (285-836)	13.6***	20 (20-20)	47 (28-78)	2.4 ns	550 (361-838)	27.5 ***
BA.4/5	36 (23-56)	214 (135-340)	5.9 ***	20 (20-20)	25 (20-32)	1.3 ns	193 (104-357)	9.7 *

†The neutralizing antibody titer was measured as the geometric mean titer (GMT) and 95% confidence interval (95% CI) of the 50% inhibitory concentrations (IC₅₀) against the series SARS-CoV-2 variants.

‡Fold indicates the change of neutralizing antibody titers in 3xBNT relative to 2xBNT.

€Fold indicates the change of neutralizing antibody titers in 3xCorV relative to 2xCorV.

δFold indicates the change of neutralizing antibody titers in 2xCorV+1xBNT relative to 2xCorV.

Significant differences in neutralizing antibody titers between 2-dose and 3-dose were performed using the 2-tailed Student's t test.

ns: no significance; *p < 0.05; **p < 0.01; ***p < 0.001.

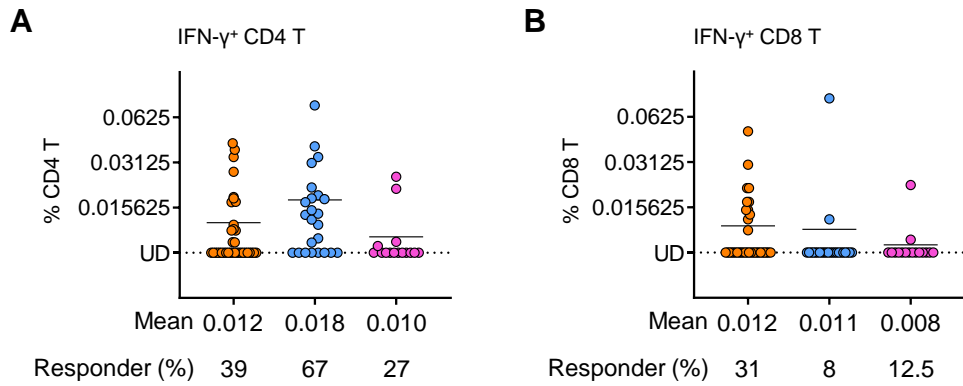
Supplementary Table 4. Comparison in antibody responder rates between 2-dose and 3-dose vaccination.

Vaccinations	Homologous BNT162b2		Homologous CoronaVac		Heterologous BNT162b2
	2xBNT	3xBNT	2xCorV	3xCorV	2xCorV+1xBNT
0-4 weeks after vaccination					
Median time (days) post-vaccination	14 (7-26)	16 (7-28)	25 (10-28)	20 (13-28)	18.5 (7-24)
Responder rate % (No. participants with response/Total No.)					
D614G	100 (9/9)	100 (24/24)	89 (8/9)	100 (8/8)	100 (6/6)
Alpha	100 (9/9)	100 (24/24)	67 (6/9)	100 (8/8)	100 (6/6)
Beta	67 (6/9)	100 (24/24)	11 (1/9)	88 (7/8)	100 (6/6)
Delta	89 (8/9)	100 (24/24)	56 (5/9)	63 (5/8)	100 (6/6)
BA.1	67 (6/9)	100 (24/24)	11 (1/9)	88 (7/8)	100 (6/6)
BA.1.1	22 (2/9)	100 (24/24)	11 (1/9)	63 (5/8)	100 (6/6)
BA.2	33 (3/9)	100 (24/24)	0 (0/9)	38 (3/8)	100 (6/6)
BA.2.12.1	67 (6/9)	100 (24/24)	0 (0/9)	63 (5/8)	100 (6/6)
BA.4/5	56 (5/9)	100 (24/24)	0 (0/9)	38 (3/8)	100 (6/6)
>4 weeks after vaccination					
Median time (days) post-vaccination	31 (30-47)	45 (30-75)	40 (32-105)	66 (30-77)	59 (35-77)
Responder rate % (No. participants with response/Total No.)					
D614G	100 (18/18)	100 (17/17)	86 (6/7)	90 (18/20)	100 (15/15)
Alpha	100 (18/18)	100 (17/17)	71 (5/7)	100 (20/20)	100 (15/15)
Beta	89 (16/18)	100 (17/17)	0 (0/7)	70 (14/20)	100 (15/15)
Delta	94 (17/18)	100 (17/17)	71 (5/7)	50 (10/20)	100 (15/15)
BA.1	50 (9/18)	100 (17/17)	0 (0/7)	50 (10/20)	100 (15/15)
BA.1.1	39 (7/18)	100 (17/17)	0 (0/7)	30 (6/20)	100 (15/15)
BA.2	39 (7/18)	100 (17/17)	0 (0/7)	35 (7/20)	100 (15/15)
BA.2.12.1	56 (10/18)	94 (16/17)	0 (0/7)	50 (10/20)	100 (15/15)
BA.4/5	33 (6/18)	94 (16/17)	0 (0/7)	25 (5/20)	93 (14/15)

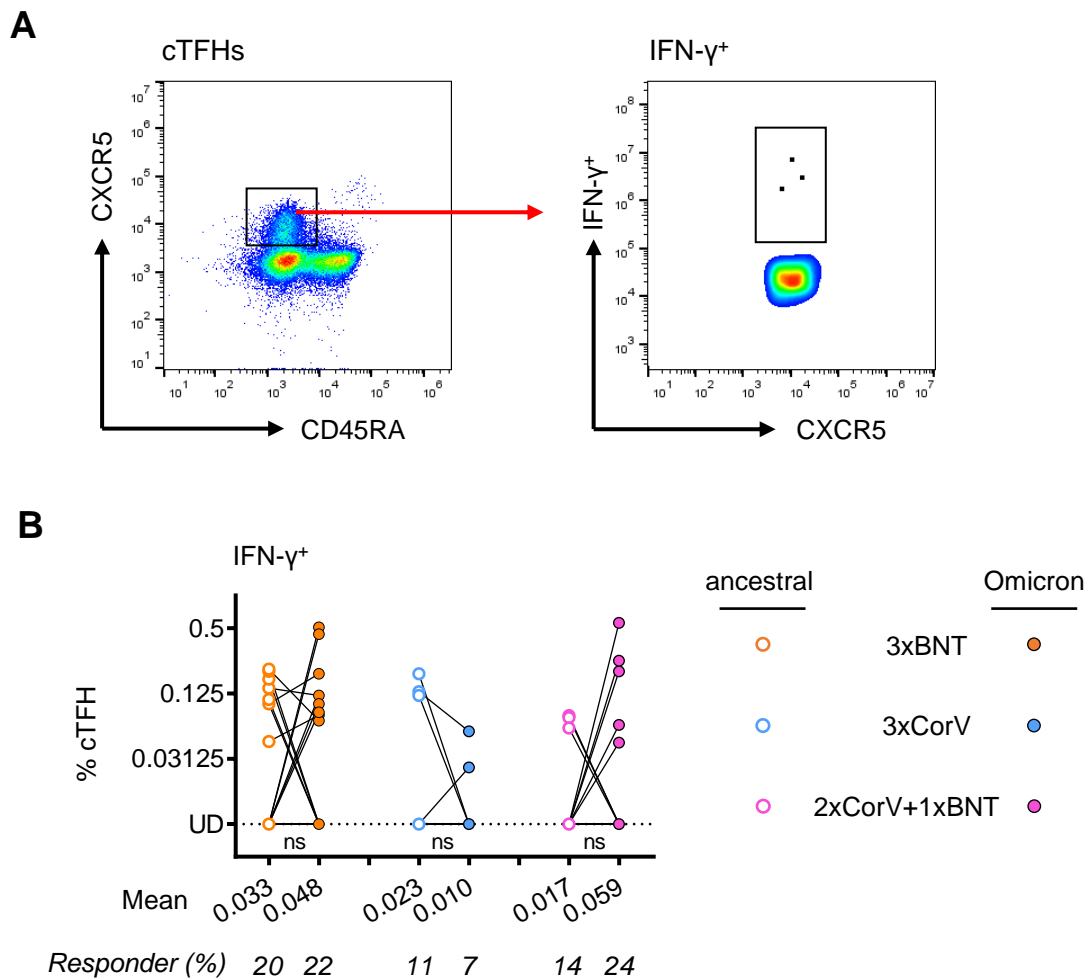
Supplementary Table 5. Characteristics of three doses and four doses of SARS-CoV-2 vaccinees with or without BA.2 infection

Characteristics	3xBNT (n=11)		3xCorV (n=10)		2xCorV+1xBNT (n=11)		3xCorV+1xBNT (n=3)
	Without (n=6)	With (n=5)	Without (n=7)	With (n=3)	Without (n=10)	With (n=1)	Without (n=3)
BA.2 infection							
Age	35 (30-42)	40 (40-49)	50 (42-57)	50 (48-58)	46 (32-52)	37	53 (50-56)
Gender							
Male (n)	4	4	3	1	9	1	2
Female (n)	2	1	4	2	1	0	1
Days between last vaccination and 1 st blood collection	31.5 (14-56)	31 (14-59)	56 (20-70)	47 (35-70)	38.5 (14-77)	7	63 (30-73)
Days between last vaccination and 2 nd blood collection	210.5 (193-235)	210 (193-238)	235 (199-249)	226 (214-249)	217.5 (193-256)	186	40 (14-47)
Days between symptom onset last vaccination and 2 nd blood collection	-	134 (133-148)	-	147 (123-165)	-	145	-

Values displayed are medians, with ranges in parentheses



Supplementary Figure 1. SARS-CoV-2 NP-specific T cell responses. PBMCs from vaccinees were subjected to the intracellular cytokine staining assay against NP peptide pool. IFN- γ ⁺ cells were gated on CD4 (**A**) and CD8 (**B**) T cells, respectively. Quantified results depict the percentage of IFN- γ ⁺ cells as background subtracted data from the same sample stimulated with negative control (anti-CD28/CD49d only). Each symbol represents an individual donor with a line indicating the mean of each group among the 3xBNT (orange), 3xCorV (blue) and 2xCorV+1xBNT (purple) vaccinees. The mean frequency of IFN- γ ⁺ cells and responder rates were depicted under x-axis (% of IFN- γ ⁺ cells > 0.00781% termed 'responder' after subtracted from percentage of unstimulated control). Undetected (UD): % of IFN- γ ⁺ cells < 0.00781%.



Supplementary Figure 2. SARS-CoV-2 spike-specific cTFH responses. PBMCs from vaccinees were subjected to the intracellular cytokine staining assay against Spike peptide pools from ancestral or Omicron SARS-CoV-2. (A) IFN- γ ⁺ cells were gated on cTFHs. (B) Quantified results depict the percentage of IFN- γ ⁺ cells as background subtracted data from the same sample stimulated with negative control (anti-CD28/CD49d only). Each symbol represents an individual donor with a line indicating the mean of each group to ancestral (open dots) or Omicron (solid dots) Spike among the 3xBNT (orange), 3xCorV (blue) and 2xCorV+1xBNT (purple) vaccinees. The mean frequency of IFN- γ ⁺ cells and responder rates were depicted under x-axis. Undetected (UD): % of IFN- γ ⁺ cells < 0.00781%. Statistics were generated by using 2-tailed Student's t test. Ns: no significance.