

1 **Randomised controlled trial reveals no benefit to a 3-month delay**
2 **in COVID-19 mRNA booster vaccine**

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4 Wen Shi Lee*¹, Jennifer Audsley*², Mai-Chi Trieu^{1,3}, Arnold Reynaldi⁴, L Carissa
5 Aurelia¹, Palak H Mehta¹, Joanne Patterson⁵, Helen E Kent¹, Julie Nguyen¹, Thakshila
6 Amarasena¹, Robyn Esterbauer¹, Ebene R Haycroft¹, Pradhipa Ramanathan¹, Miles
7 P Davenport⁴, Timothy E Schlub⁶, Joseph Sasadeusz^{2,5}, Adam K Wheatley¹, Amy W
8 Chung¹, Jennifer A Juno^{†1}, Kevin J Selva^{†1}, Stephen J Kent^{†1,7}.

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10 Authorship note: *WSL and *JA contributed equally to this work and are co-first authors.

11 †JAJ, †KJS and †SJK are co-senior authors.

12

13 Address correspondence to:

14 Stephen J Kent, Peter Doherty Institute for Infection and Immunity, University of
15 Melbourne, 792 Elizabeth Street, Melbourne, Victoria, 3000, Australia. Phone: +61 3
16 8344 9939. Email: skent@unimelb.edu.au

17 Kevin J Selva, Peter Doherty Institute for Infection and Immunity, University of
18 Melbourne, 792 Elizabeth Street, Melbourne, Victoria, 3000, Australia. Phone: +61 3
19 9035 8640. Email: kevin.selva@unimelb.edu.au

20 Jennifer A Juno, Peter Doherty Institute for Infection and Immunity, University of
21 Melbourne, 792 Elizabeth Street, Melbourne, Victoria, 3000, Australia. Phone: + 61 3
22 8344 3791. Email: jennifer.juno@unimelb.edu.au

23

24 Affiliations

25 1 Department of Microbiology and Immunology, Peter Doherty Institute for Infection
26 and Immunity, University of Melbourne, Melbourne, Victoria, Australia.

27

28 2 Department of Infectious Diseases, Peter Doherty Institute for Infection and Immunity,
29 University of Melbourne and Royal Melbourne Hospital, Melbourne, Victoria, Australia.

30

31 3 Influenza Centre, Department of Clinical Science, University of Bergen, Bergen,
32 Norway.

33

34 4 Kirby Institute, University of New South Wales, Kensington, New South Wales,
35 Australia.

36

37 5 Victorian Infectious Diseases Service, Royal Melbourne Hospital, Melbourne, VIC,
38 Australia.

39

40 6 Sydney School of Public Health, Faculty of Medicine and Health, University of
41 Sydney, Sydney, New South Wales, Australia.

42

43 7 Melbourne Sexual Health Centre and Department of Infectious Diseases, Alfred
44 Hospital and Central Clinical School, Monash University, Melbourne, Victoria, Australia.

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46 **Conflicts-of-interest statement**

47 The full conflict-of-interest statement is available in the Supplemental Materials.

48

49 **40-word summary**

50 Delaying uptake of the bivalent Spikevax mRNA booster by 3 months did not

51 significantly improve neutralisation and IgG antibody responses against both ancestral

52 and Omicron strains, suggesting no substantial benefit from delaying COVID-19

53 mRNA booster vaccination in pre-immune populations.

54 **Abstract**

55 **Background**

56 There is uncertainty around the timing of booster vaccination against COVID-19 in
57 highly vaccinated populations during the present endemic phase of COVID-19.
58 Studies focused on primary vaccination have previously suggested improved immunity
59 after delaying immunisation.

60 **Methods**

61 We conducted a randomised controlled trial (Nov 2022 – Aug 2023) and assigned 52
62 fully vaccinated adults to an immediate or a 3-month delayed bivalent Spikevax mRNA
63 booster vaccine. Follow-up visits were completed for 48 participants (n = 24 per arm),
64 with saliva and plasma samples collected following each visit.

65 **Results**

66 The rise in neutralising antibody responses to ancestral and Omicron strains were
67 almost identical between the immediate and delayed vaccination arms. Analyses of
68 plasma and salivary antibody responses (IgG, IgA), plasma antibody-dependent
69 phagocytic activity, and the decay kinetics of antibody responses were similar between
70 the 2 arms. Symptomatic and asymptomatic SARS-CoV-2 infection occurred in 49%
71 (21/49) participants over the median 11.5 months of follow up and were also similar
72 between the 2 arms.

73 **Conclusions**

74 Our data suggests no benefit from delaying COVID-19 mRNA booster vaccination in
75 pre-immune populations during the present endemic phase of COVID-19

76 **Trial registration**

77 Australian New Zealand Clinical Trials Registry number 12622000411741.

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81 **Introduction**

82 Ancestral spike-based COVID-19 vaccines have reduced effectiveness at preventing
83 symptomatic Omicron variant infections due to progressive neutralising antibody
84 escape (1). As such, COVID-19 vaccines have been serially updated to include
85 Omicron spike variants. Bivalent COVID-19 mRNA vaccines (BA.1 first approved in
86 Australia late 2022, followed by BA.5) are superior to ancestral monovalent vaccines
87 at boosting Omicron neutralising antibodies (2-4), and preventing hospitalisation and
88 severe disease (1, 5). Despite this, the bivalent mRNA boosters have shown only
89 modest efficacy against infection with more recent XBB subvariants (6).

90

91 The durability of immunity afforded by bivalent booster vaccines and optimal timing of
92 receiving boosters following last vaccination or infection remains unclear. Guidance
93 on the timing of repeated boosters varies widely. Early studies found a longer interval
94 between the first and second vaccine doses (8-16 weeks) elicited higher binding and
95 neutralising antibody titres compared to the standard 3-4 week interval (7-9), possibly
96 due to improved maturation of antibody and B cell responses (10, 11). An extended
97 interval between vaccination and infection also enhanced neutralising antibody titres
98 (12). However, a third mRNA vaccine dose equalised this response, resulting in similar
99 neutralising antibody titres in individuals who had short or long intervals between the
100 first two doses (13).

101

102 The potential benefit of longer delays between subsequent boosters in highly
103 vaccinated populations in the endemic phase of COVID-19 is currently unclear, with a
104 fine balance between the potential for improved immunity with a longer duration

105 between doses, the serial escape of Omicron strains leading to transient protective
106 immunity, and vaccine fatigue within the population.

107

108 To determine whether there is an immunological benefit with a longer interval between
109 last vaccination/infection and subsequent booster vaccination, we undertook an open-
110 label randomised controlled trial administering the Moderna BA.1 bivalent mRNA
111 booster (mRNA-1273.214) upon recruitment (immediate arm) or 3 months following
112 recruitment (delayed arm). We found that antibody-mediated immunity to circulating
113 variants was not improved by delaying the booster.

114 **Results**

115 **Study design**

116 We recruited 52 adults over the period 11/09/2022-30/01/2023, of whom 49 completed
117 follow up (Figure 1) in this open-label RCT. The follow-up period was 11/09/2022-
118 02/12/2023. Recruitment was stopped prior to reaching the pre-defined sample size in
119 the immediate arm (n=29, compared with n=25 recruited), due to the withdrawal and
120 replacement of the BA.1 bivalent vaccine with the BA.4/5 bivalent vaccine. Forty-eight
121 subjects (24 randomised to the immediate vaccine arm and 24 to the 3-month delay
122 arm) were analysed for immunologic outcomes since one subject tested positive for
123 SARS-CoV-2 1 day after receiving the vaccine (Supplemental Table 1). The relative
124 immunogenicity of the Moderna Spikevax BA.1 bivalent vaccine booster in Australia
125 was unknown at study initiation and the primary outcome was achieving a plasma
126 neutralising antibody titre of >1:100 to Omicron BA.1 two weeks after vaccination in
127 the immediate arm. Key additional endpoints included comparisons of antibody
128 responses in plasma and saliva (mean titre of SARS-CoV-2 antibodies) between the
129 two arms (Supplemental Figure 1 and 2), safety analyses (number of self-reported
130 adverse events collected at day 3 and day 7 post-vaccination) and breakthrough
131 COVID-19 infections during the study. Subjects were evenly matched for age, gender,
132 number of prior vaccinations and number of COVID-19 infections (Supplemental Table
133 1). Median time since last vaccination or COVID-19 infection at enrolment was similar
134 between the groups at 8.0 and 10.5 months for the immediate and delayed arms
135 respectively. The delayed arm was boosted a median of 3.1 months later than the
136 immediate arm. Three subjects randomised to the delayed arm acquired COVID-19
137 while waiting for vaccination and as per protocol waited 4 months after infection for
138 their booster vaccine – two of these subjects received the BA.4/5 bivalent Spikevax

139 vaccine since the BA.1 bivalent vaccine had been withdrawn in the interim. One
140 additional subject in the delayed arm also received the BA.4/5 bivalent Spikevax
141 vaccine. There were 102 vaccine adverse events reported (Supplemental Table 2),
142 with no statistically significant difference in reporting between the 2 arms. None of the
143 adverse events were serious, and all were consistent with reactions reported
144 previously (2).

145

146 **Bivalent vaccine boosts immune responses similarly in immediate and delayed** 147 **arms**

148 Neutralising antibodies are a key correlate of protective immunity against COVID-19
149 (14). Plasma neutralising titres to BA.1 (in the booster) and XBB.1.5 (a dominant
150 circulating Omicron strain during the study) were relatively low prior to vaccination in
151 both groups (median IC₅₀ 219 and 269 for delayed and immediate arms for BA.1; 24
152 and 29 respectively for XBB.1.5 in a live virus neutralisation test, Figures 2, A-C, F-H)
153 despite a median of 3 prior COVID-19 vaccinations and 69.4% having at least 1 prior
154 self-reported COVID-19 infection previously. For the delayed arm, BA.1 and XBB.1.5
155 neutralising titres were similar from study recruitment (3 months pre-booster) to the
156 day of vaccination (Day 0) (Figure 2, B and G). After receiving the booster, all
157 immediate arm subjects achieved an Omicron BA.1 neutralisation titre of >1:100 by 2
158 weeks ($P \leq 0.0001$), meeting the study primary endpoint (Figure 2C). Neutralising
159 titres at day 14 post-booster were almost identical between the immediate and delayed
160 arms to both Omicron BA.1 and XBB.1.5 (Figure 2, D and I), reaching median IC₅₀
161 titres of 1548 and 1583 for BA.1, and 313 and 356 for XBB.1.5 in the delayed and
162 immediate arms respectively. At day 84 post-vaccination, neutralising titres decayed
163 ~1.4-2.1 fold from day 14 but remained similar between both arms (Figure 2, E and J).

164

165 Neutralising activity against ancestral and XBB.1.5 strains across all sampled
166 timepoints from both arms was also analysed using a surrogate bead-based Spike-
167 ACE2 inhibition assay (Supplemental Figure 3, A-G). Percent inhibition against both
168 ancestral and XBB.1.5 spikes peaked at Day 14 for both arms at similar levels and
169 gradually decayed over time.

170

171 Since antibodies in the upper airways may be important in preventing SARS-CoV-2
172 infection (15), we measured neutralising antibody responses in saliva using an ELISA-
173 based surrogate virus neutralisation test (16) (Supplemental Figures 3, H-J). Salivary
174 neutralising antibodies to the ancestral strain were boosted in most subjects at Day 14
175 ($P \leq 0.001$) and were similar in both immediate and delayed arms (Figure 2, K-N).

176

177 Spike-specific T cells were recently implicated to be a predictor of protection against
178 symptomatic infection in vaccinated children (17). In addition to quantifying the
179 serological response to booster vaccination, we assessed the frequency of spike-
180 specific CD4 and CD8 T cells at day 0 and day 7 post-vaccination in a subset of the
181 cohort (Supplemental Figure 4A). Immunisation drove a significant expansion of spike-
182 specific memory for both CD4 and CD8 T cells, as measured by production of IFN γ ,
183 IL-2 and/or TNF (Supplemental Figure 4, B and C; $p=0.007$ for CD4 Tmem, $p=0.016$
184 for CD8 Tmem). We did not detect any substantial spike-specific cTFH responses,
185 likely due to poor cytokine production by cTFH relative to other T cell subsets (18)
186 (Supplemental Figure 4B). Spike-specific T cell frequencies at day 7 were comparable
187 between the immediate and delayed vaccination arms for both CD4 and CD8

188 populations (Supplemental Figure 4, D and E; Supplemental Table 3), consistent with
189 the serological data.

190

191 **Decay kinetics of vaccine-induced antibodies**

192 Beyond peak antibody titres following vaccination, an important parameter of vaccine-
193 induced antibodies is how fast they decay, leaving subjects vulnerable to breakthrough
194 infection (19). Differences in decay kinetics of various antibody parameters were
195 analysed across the immediate and delayed vaccination arms. Here, we studied not
196 only plasma neutralising antibody responses (Figure 3, A-C), but also total IgG and
197 IgA in plasma (Figure 3, D-F, G-I) and saliva respectively (Figure 3, P-R, S-U).
198 Furthermore, as Fc-effector functions have been implicated in assisting antibody-
199 mediated immunity to SARS-CoV-2 (20, 21), we also examined Fc-gamma receptor
200 2a (FcγR2a) engagement and antibody-dependent cellular phagocytosis (ADCP) in
201 plasma (Figure 3, J-O; Supplemental Figure 5, A-E). While the decay analyses
202 focused on antibody responses to Omicron XBB.1.5 (Figure 3, A-U) as this was a
203 major circulating strain during our study, we also examined total IgG and IgA, and
204 FcγR2a binding responses in plasma (Supplemental Figure 6, 8, 9, 11, 13) and saliva
205 (Supplemental Figure 7, 8, 10, 12, 13) respectively against ancestral, Omicron BA.1
206 and Omicron BA.5 strains.

207

208 Decay kinetics of plasma neutralising antibodies (Figure 3, A-C), as well as total IgG
209 and IgA against XBB.1.5 spike in plasma (Figure 3, D-I) and saliva (Figure 3, P-U)
210 respectively were very similar between the immediate and delayed arms out to 84
211 days post-booster. Of note, spike-specific salivary IgA responses were not induced by
212 the vaccine, consistent with the known poor mucosal immunity induced by

213 intramuscular vaccines (Figure 3, S and T; Supplemental Figure 10) (22, 23). FcγR2a-
214 binding antibodies to spike in plasma were elicited by the vaccine and had a modestly
215 faster decay rate in the delayed arm ($t_{1/2}$ of 45 vs 88 days, $P \leq 0.05$; Figure 3, J-L;
216 Supplemental Figure 10). However, this difference diminished when we compared Fc-
217 effector responses of plasma antibodies using a cell-based phagocytosis assay
218 (ADCP) (Figure 3, M-O). Overall, our results suggest delaying vaccination in the
219 context of our study has no substantial benefit in terms of preserving long-term
220 antibody immunity.

221

222 We also modelled the time required for the various XBB.1.5 antibody responses to
223 decrease to pre-booster levels (Figure 3, C, F, I, L, O, R, U). Plasma neutralising titres
224 against XBB.1.5 took an average of 240 days to decay to baseline levels. Saliva IgG
225 took the longest time to decay (1225 days) while plasma IgA took the shortest time
226 (162 days).

227

228 **COVID-19 infections during the study**

229 Australia has experienced multiple waves of COVID-19, including during the current
230 study. Although not powered for efficacy, we documented symptomatic COVID-19
231 infections over the course of follow-up. We identified 14 symptomatic infections out to
232 a maximum follow up of 12.4 months (Supplemental Table 4). This included 2 subjects
233 who reported two symptomatic infections (one subject in each arm). The symptomatic
234 infections were evenly distributed between the immediate and delayed arms with
235 similar Kaplan-Meier lines (Figure 4A, Log-rank Mantel-Cox test; $P = 0.109$). The
236 apparent reduction in COVID-free survival in the delayed arm was due last subject in

237 follow-up acquiring COVID-19. All documented infections were mild in severity
238 consistent with multiple prior vaccinations.

239

240 Analyses of serial immune responses following breakthrough COVID-19 has been
241 informative regarding the recall of immunity that helps control infection (24-26). Little
242 is known about serial salivary antibody responses following breakthrough COVID-19
243 with recent Omicron strains. We were able to obtain nasal swab samples for four
244 subjects with breakthrough COVID-19 during the trial and found that three of four
245 subjects acquired the XBF strain (viral sequencing was unsuccessful in the last nasal
246 swab). We also obtained additional serial saliva and blood samples and analysed
247 antibody responses (Figure 4, B-G; Supplemental Figure 14). We detected transient
248 rises in XBB.1.5-specific total IgG and IgA, and FcγR2a binding responses in both
249 plasma and saliva in 3 of the 4 subjects (Figure 4, B-G), confirming that breakthrough
250 COVID-19 can boost mucosal immunity.

251

252 As asymptomatic SARS-CoV-2 infections are also common, we analysed non-vaccine
253 elicited antibodies to the N protein. We identified 10 subjects without symptomatic
254 COVID-19 during our study with a clear and sustained rise in N antibodies (>4-fold
255 increase over previous sampling timepoint; Figure 4, H and I) and a rise in XBB.1.5
256 neutralisation titres. Combined cases of symptomatic and asymptomatic infection
257 were evenly divided between the arms and similar over time (Figure 4J, Log-rank
258 Mantel-Cox test; $P = 0.838$).

259 **Discussion**

260 Timing of SARS-CoV-2 booster vaccination is contentious in high vaccinated
261 populations in the present endemic phase of COVID-19, with (i) waning immunity, (ii)
262 changing escape profiles of new variants, and (iii) booster fatigue all factors to consider.
263 We randomised healthy adults to receive an immediate or 3 month delayed COVID-
264 19 booster. The booster improved antibody and T cell immunity in all subjects. We
265 found no difference in booster-induced antibody-based immunity to either ancestral,
266 vaccine (BA.1) or circulating strains of SARS-CoV-2 (XBB.1.5) between the immediate
267 and delayed arms. Further, the decay kinetics of spike-specific antibodies over the
268 subsequent 12 weeks were not improved in the delayed arm, suggesting no longer
269 term benefit from delaying vaccination. Remarkably, over 40% of participants (21 of
270 49) completing the study had symptomatic or asymptomatic COVID-19 during the
271 mean 11.5-month study follow-up but the rates of infection were similar in both arms.
272 Taken together, our results suggest no substantial benefit in delaying booster
273 vaccination to improve antibody-based immunity to SARS-CoV-2.

274

275 The changing landscape of SARS-CoV-2 Omicron variants is a major factor driving
276 poor immunity and breakthrough COVID-19 infections. Levels of neutralising
277 antibodies against Omicron XBB.1.5 (which was a common circulating strain during
278 our study) were low pre-booster (median IC_{50} of 24 or 29 respectively, with 75% being
279 $<1:100$). XBB.1.5 titres reached a median of 346 across the whole cohort 2 weeks
280 after vaccination, consistent with a previous study showing BA.1 bivalent vaccines
281 boosted neutralising titres against XBB.1.5 (27), despite the poor effectiveness
282 afforded against symptomatic XBB.1.5 infection (6). XBB.1.5 titres waned to a median
283 of 186 by 84 days and were estimated to return to the low pre-booster baseline levels

284 by an average of 240 days after receiving the booster. This illustrates the relatively
285 short-lived effect current mRNA booster vaccines.

286

287 Although the BA.1 bivalent vaccine we studied has been superseded with a XBB.1.5
288 monovalent vaccine (28), recent dominant Omicron strains such as JN.1 have
289 continued to escape neutralising antibody responses (29). Maintaining high levels of
290 neutralising antibodies to circulating and emerging variants with the current process
291 of updating vaccines is inefficient, resulting in increasing cases of COVID-19
292 breakthrough infections, as we observed. Nonetheless, delaying booster vaccination
293 with the hope of improving the peak or durability of antibody immunity during the
294 present endemic phase of COVID-19 does not work nor prevent COVID-19. There is
295 a need for vaccines that elicit broader and more durable protective immunity against
296 SARS-CoV-2.

297

298 Our study had limitations. First, our study had 24 subjects per arm analysed for
299 antibody immunity owing to intercurrent COVID-19 infections and the updating of the
300 bivalent vaccine. Although subject numbers were adequate for most analyses, our
301 ability to detect small differences in peak or waning of antibodies between the 2 arms
302 was less robust. However, the virtually identical levels of neutralising antibody
303 responses, confirmed with multiple other analyses of antibody responses, suggests
304 any real difference between immediate or delayed vaccination would be very small
305 and of doubtful clinical significance. Second, there were many intercurrent
306 asymptomatic and symptomatic SARS-CoV-2 infections, and presumably many more
307 exposures to SARS-CoV-2 that did not lead to overt infections during our study. These
308 COVID-19 breakthrough infections also modulate antibody responses (24-26), as

309 documented here in several cases (Figure 4, B-G; Supplemental Figure 14). While
310 these infections and exposures could confound some of our antibody analyses, the
311 infections were evenly distributed between the 2 arms and unavoidable given
312 circulating SARS-CoV-2 levels during our study. Third, our subjects had an average of
313 3 prior vaccinations and an average time from prior vaccination or COVID-19 infection
314 of 9.4 months. There might be scenarios with less prior vaccinations and/or COVID-
315 19 infections, or different timing of booster vaccination that could reveal differences in
316 immediate or delayed vaccination. Too short a time between a COVID-19 infection and
317 a booster vaccine has been shown to be suboptimal (30). However, pre-booster
318 neutralising antibodies to the circulating XBB.1.5 variant were low in our study and a
319 significant proportion of our study population acquired SARS-CoV-2 infection during
320 our trial. This suggests we studied a relevant population in efforts to improve immunity
321 and protection from infection. Fourth, we studied a group of healthy adults who were
322 less than 65 years old, while immunocompromised or elderly groups – key target
323 groups for vaccination – may respond differently and have a larger benefit from more
324 frequent booster vaccination (31). Lastly, our assays to date are largely focused on
325 antibody immunity, while cellular immunity could theoretically be modulated to a
326 greater degree by vaccination timing and potentially play an important role in long term
327 immunity (32). Nevertheless, neutralising antibodies have emerged as a robust
328 correlate of immunity to SARS-CoV-2 and guide most vaccine recommendations (14,
329 19).

330

331 In summary, this randomised controlled trial of highly vaccinated healthy adults during
332 the present endemic phase of COVID-19 showed no benefit in the induction of

- 333 protective antibodies against SARS-CoV-2 by delaying booster vaccination 3 months.
- 334 Regular booster SARS-CoV-2 vaccinations are supported by this study.

335 **Methods**

336 **Sex as a biological variable**

337 This study was open to all sexes, and male and female participants were recruited.

338 Randomisation included matching for sex.

339

340 **Study participants**

341 Adults (18-65 years) who had received 2-3 doses of COVID-19 vaccines at least 4

342 months prior were eligible. Exclusion criteria included prior COVID-19 infection within

343 4 months, immunosuppression and previous significant adverse events to COVID-19

344 vaccines. A SARS-CoV-2 Omicron blood neutralising titre of >1:100 in >90% of

345 participants was considered a successful outcome since this level is predicted to be

346 reliably protective against the Omicron strain. Based on this, power calculations were

347 carried out using G*Power version 3.1.9.7 using a one-tailed the Exact Generic

348 Binomial Test. 29 participants in the immediate vaccine group was estimated to be

349 required for a proportion of participants with a neutralisation titre of >1:100 greater

350 than 90%. Dynamic (adaptive) randomisation with minimisation to promote balance

351 in age, sex and timing of initial vaccines was used to allocate participants to either

352 interventional group. Age was stratified by 10-year intervals and time since 2nd

353 vaccine by monthly intervals, using equal weighting of covariate factors. This achieved

354 using R: A language and environment for statistical computing, library Minirand,

355 function Minirand using equal weighting of covariate factors and high probability of

356 assignment = 0.90. Participants were recruited in Melbourne, Australia and were

357 randomised to receive a Moderna BA.1 bivalent mRNA vaccine booster dose (0.5ml)

358 administered intramuscularly upon enrolment (immediate arm) or three months later

359 (delayed arm). Most participants received the Moderna BA.1 bivalent vaccine,

360 however during the study the Moderna BA.4/5 bivalent vaccine replaced the BA.1
361 formulation and three participants received the BA.4/5 vaccine. Participants were
362 randomised into the two arms and matched for age (10-year intervals), sex (male,
363 female, other) and timing of last COVID-19 vaccine dose (2 month intervals, from a
364 minimum of 4 months). The study was open-labelled.

365

366 Serial blood plasma samples and saliva samples (SalivaBio, Salimetrics) were
367 collected and stored at -80°C. Salivary samples from both delayed and immediate
368 arms had comparable levels of total secretory IgA between respective timepoints
369 (Supplemental Figure 1). Peripheral blood mononuclear cells (PBMC) were isolated
370 from whole blood by Ficoll-Paque separation and cryopreserved in 10% DMSO/90%
371 fetal calf serum (FCS).

372

373 **Variant Spike multiplex bead assay**

374 SARS-CoV-2 specific total IgG, IgA, and FcγR2a dimer (Bruce Wines, Burnet Institute)
375 engagement in plasma (1:25600, 1:6400, 1:6400) and saliva (1:50, 1:50, 1:12.5) from
376 the booster cohort were assessed using a customised multiplex bead-based array
377 consisting of ancestral and Omicron spike trimers (BA.1, BA.5, XBB.1.5, Sino
378 Biological) as previously described (26) (Supplemental Figure 2). SARS-CoV-2
379 nucleocapsid (N) protein was included to screen for asymptomatic infections.
380 SIVgp120, H1Cal2009 (Sino Biological) and tetanus toxoid (MilliporeSigma) were
381 included as controls. Briefly, spike-coupled beads were first incubated with samples
382 overnight at 4°C, then washed and incubated with biotinylated detectors (isotype
383 detection antibodies, MabTech; FcγR2a dimers) for 2 hours at room temperature (RT).
384 After washing, beads were incubated with Streptavidin-R-Phycoerythrin

385 (ThermoFisher Scientific) for 2 hours at RT. Beads were washed again and read on
386 the Intelliflex (Luminex). Assays were repeated in duplicates.

387

388 **Virus neutralisation assay**

389 Plasma live virus neutralisation assay with viability dye readout was performed against
390 Omicron BA.1 and XBB.1.5 viruses as previously described (33). Infectivity of virus
391 stocks was determined by titration on HAT-24 cells (a clone of transduced HEK293T
392 cells stably expressing human ACE2 and TMPRSS2) (34). Virus stocks were titrated
393 in quintuplicate in three independent experiments to obtain mean 50% infectious dose
394 (ID_{50}) values.

395

396 To determine serum neutralization activity, heat-inactivated plasma samples were
397 diluted 3-fold (1:20–1:43,740) in duplicate and incubated with SARS-CoV-2 virus at a
398 final concentration of $2 \times ID_{50}$ at 37°C for 1 h. Next, 40,000 freshly trypsinized HAT-24
399 cells in DMEM with 5% FCS were added and incubated at 37°C. “Cells only” and
400 “Virus+Cells” controls were included to represent 0% and 100% infectivity respectively.
401 After 48 h, 10 μ L of alamarBlue Cell Viability Reagent (ThermoFisher) was added into
402 each well and incubated at 37°C for 1 h. The reaction was then stopped with 1% SDS
403 and read on a FLUOstar Omega plate reader. The relative fluorescent units (RFU)
404 measured were used to calculate %neutralization with the following formula: (“Sample”
405 - “Virus+Cells”) \div (“Cells only” - “Virus+Cells”) \times 100. IC_{50} values were determined
406 using four-parameter non-linear regression in GraphPad Prism with curve fit
407 constrained to have a minimum of 0% and a maximum of 100% neutralization.

408

409 **Surrogate virus neutralisation test (sVNT)**

410 Neutralising activity of plasma (final dilutions 1:6400) were also assessed using an
411 adapted surrogate spike-ACE2 inhibition assay (35) (Supplemental Figure 3, A-G).
412 Briefly, ancestral or Omicron XBB.1.5 variant S1-coupled beads were incubated with
413 diluted plasma overnight at 4°C. Avi-tagged biotinylated ACE2 (Nicholas Gherardin,
414 University of Melbourne) was added and beads incubated for 1 hour at RT. After
415 washing, beads were incubated with streptavidin-PE for 1 hour at RT, then R-
416 Phycoerythrin Biotin-XX conjugate (ThermoFisher Scientific) was added incubated for
417 another hour at RT. Beads were washed and read on the Intelliflex. Assays were
418 repeated in duplicates. Saliva neutralising activity against the ancestral virus in saliva
419 (final dilutions 1:2) and plasma (final dilutions 1:200) samples were measured using
420 the sVNT kit (GenScript cPass) as per manufacturer's directions. Readings above the
421 recommended 30% cut-off are positive for neutralising activity (Figure 3, H-J).

422

423 **Bead-based THP-1 ADCP assay**

424 A bead-based ADCP assay was performed as previously described (Supplemental
425 Figure 5A) (36). Briefly, SARS-CoV-2 XBB.1.5 Spike trimer (Sino Biological) was
426 biotinylated and coupled to 1 µM fluorescent NeutrAvidin Fluospheres (beads;
427 Invitrogen) overnight at 4°C. Antigen-coated beads were washed and diluted in 1%
428 BSA/PBS and incubated with plasma (final dilutions 1:1600; Supplemental Figure 5B)
429 for 2 hours at 37°C in a 96-well U-bottom plate. THP-1 monocytes (100,000/well) were
430 added to opsonised beads and incubated for 16 h under cell culture conditions. THP-
431 1 monocytes were then fixed and acquired by flow cytometry on a BD LSR Fortessa
432 with a high-throughput sampler. The data was analysed using FlowJo 10.9.0 (see
433 Supplemental Figure 5A for gating strategy) and a phagocytosis score was calculated

434 as previously described using the formula: (% bead-positive cells x mean fluorescent
435 intensity).

436

437 **Spike-specific T cell Assays**

438 Cryopreserved PBMC were thawed and rested for 4 hours in RPMI-1640
439 supplemented with 10% FCS and penicillin-streptomycin (RF10). 2×10^6 PBMC were
440 seeded per well in a 96-well U bottom plate and stimulated with 1ug/mL of a peptide
441 pool covering the spike protein (PepTivator SARS-CoV-2 Prot_S Complete) or an
442 equivalent volume of vehicle control (sterile H₂O). After 1 hour, Brefeldin A (Golgi Plug,
443 BD Biosciences) was added to the cell culture. PBMC were cultured for a total of 16
444 hours before being washed with PBS. Cells were stained with live/dead (Invitrogen)
445 for 3 minutes at room temperature and then incubated with the surface antibody
446 cocktail for 30min at 4C. The surface antibody cocktail included: CD20 BV510, 2H7;
447 CD3 BUV395, SK7; CD27 BUV737, L128; CXCR5 BB515, RF8B2 (all from BD
448 Biosciences); CD4 BV605, RPA-T4; CD8 BV650, RPA-T8; and CD45RA PerCP-Cy5.5,
449 HI100 (all from BioLegend). After fixation and permeabilization (BD CytoFix/CytoPerm)
450 for 20 minutes at 4C, cells were incubated with the intracellular antibody cocktail (IFN γ
451 APC, B27; TNF BV421, Mab11; IL-2 PE, MQ1-17H12; all from BioLegend). Cells were
452 washed in Perm/Wash buffer, resuspended in PBS+1%FCS, and acquired on a BD
453 LSR Fortessa.

454

455 **Modelling**

456 A piecewise model was used to estimate the growth and decay rate of various immune
457 responses following vaccination. The model of the immune response y for subject i at
458 time y_i can be written as:

459
$$y_i(t) = \begin{cases} Be^{gt}; & t < T_{peak} \\ Be^{gT_{peak}} \times e^{-d(t-T_{peak})}; & t \geq T_{peak}. \end{cases}$$

460 The model has 4 parameters; B, g, T_{peak} , and d . We assumed a constant baseline
461 value B for the immune response pre vaccination. The immune response will grow at
462 a rate of g until T_{peak} . From T_{peak} , the immune response will decay at a rate of d . For
463 each subject, the parameters were taken from a normal distribution, with each
464 parameter having its own mean (fixed effect). A diagonal random effect structure was
465 used, where we assumed there was no correlation within the random effects. The
466 model was fitted to the log-transformed data values, with a constant error model
467 distributed around zero with a standard deviation σ . We also censored the data from
468 below (left-censoring) if it was less than the threshold for detection. Model fitting was
469 performed using Monolix2023R1.

470

471 **Statistics**

472 Statistical analysis was performed with GraphPad Prism 10.2.0 (GraphPad Software).
473 Antibody responses between cohorts/timepoints/variants were presented as medians
474 and compared using 2-tailed Mann-Whitney U tests, Kruskal-Wallis test followed by
475 Dunn's test for multiple comparisons, Friedman test followed by Dunn's test for
476 multiple comparisons or Wilcoxon matched-paired signed rank test where appropriate.
477 P values ≤ 0.05 were considered significant.

478

479 **Study Approval**

480 The study was approved by Ethics Committees at the Royal Melbourne Hospital
481 (Study number 2021/272) and University of Melbourne (Approvals 13793 and 23497).
482 Written informed consent was obtained from all participants prior to enrolment. This

483 study was registered with the Australian New Zealand Clinical Trials Registry
484 (anzctr.org.au, #12622000411741).

485

486 **Data Availability**

487 All the data and methods are presented in the manuscript or in the Supplemental
488 Materials. All individual values for figures are available in the Supplemental Supporting
489 Data Values file.

490

491 **Author contributions**

492 SJK conceived and designed the study. JS, JP and HEK recruited subjects. TES
493 generated the random allocation sequence and assigned participants to the
494 interventions. KJS, WSL, LCA, PHM, JA, MCT, JP, HEK, JN, TA, RE, ERH, PR, TES,
495 JS, AWC, AKW, SJK and JAJ were responsible for the acquisition of data. KJS, WSL,
496 JA, AR, MPD, JAJ and SJK performed the analyses and interpreted the results. SJK,
497 KJS, WSL and JA wrote the first draft. All authors critically revised the report and
498 approved the final version. The order of co-first authors was assigned based on their
499 experimental and editorial contributions to this study.

500

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506 Neither the Australian Government nor Moderna played any role in the study.

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510 Godfrey, Nicholas Gherardin and Samuel Redmond (University of Melbourne) for
511 sharing their recombinant biotinylated-ACE-2 protein. We thank Hayley Page and
512 Tseng Lau (Royal Melbourne Hospital) for additional nursing and logistical support.
513 We thank Julian Druce, Leon Caly and Thomas Tran at the Victorian Infectious
514 Diseases Reference Laboratory for isolating and distributing SARS-CoV-2 virus
515 isolates.

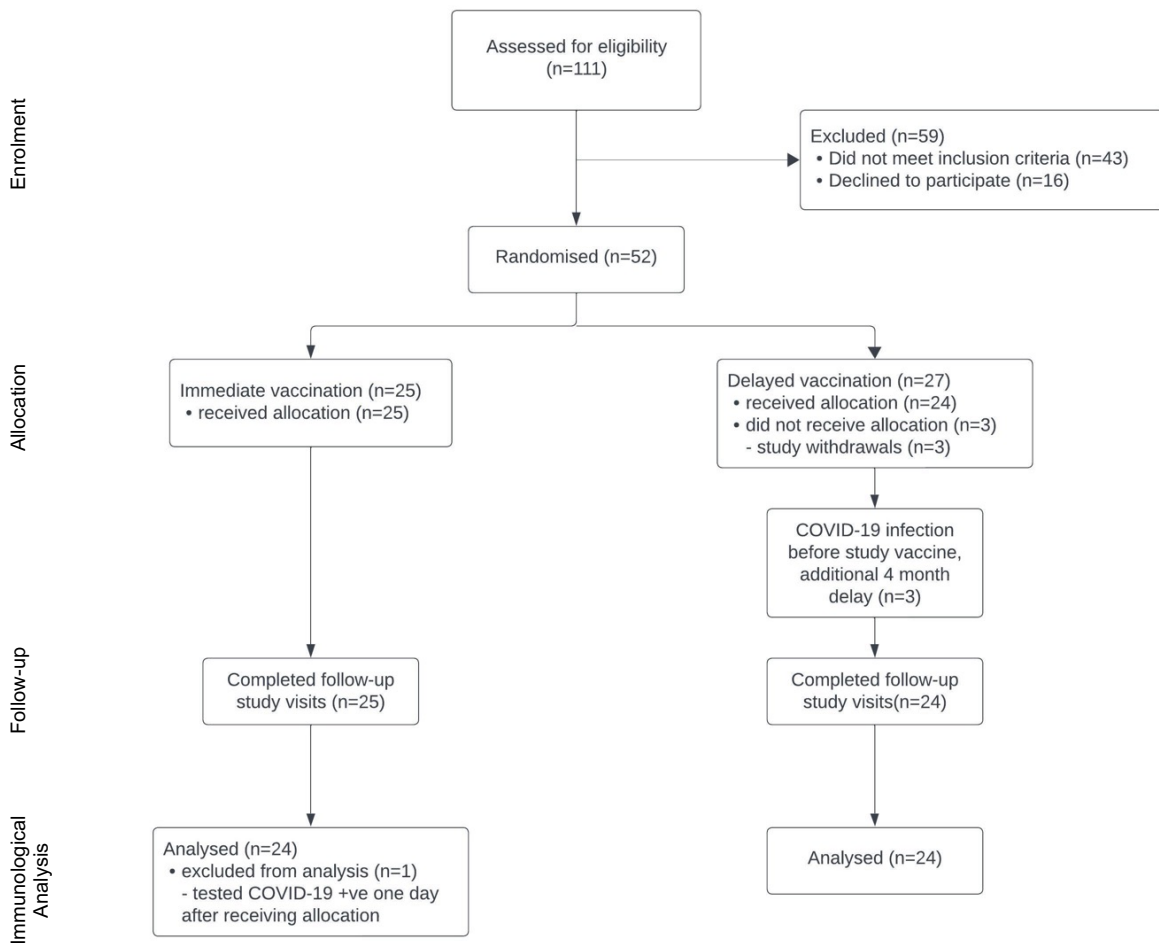
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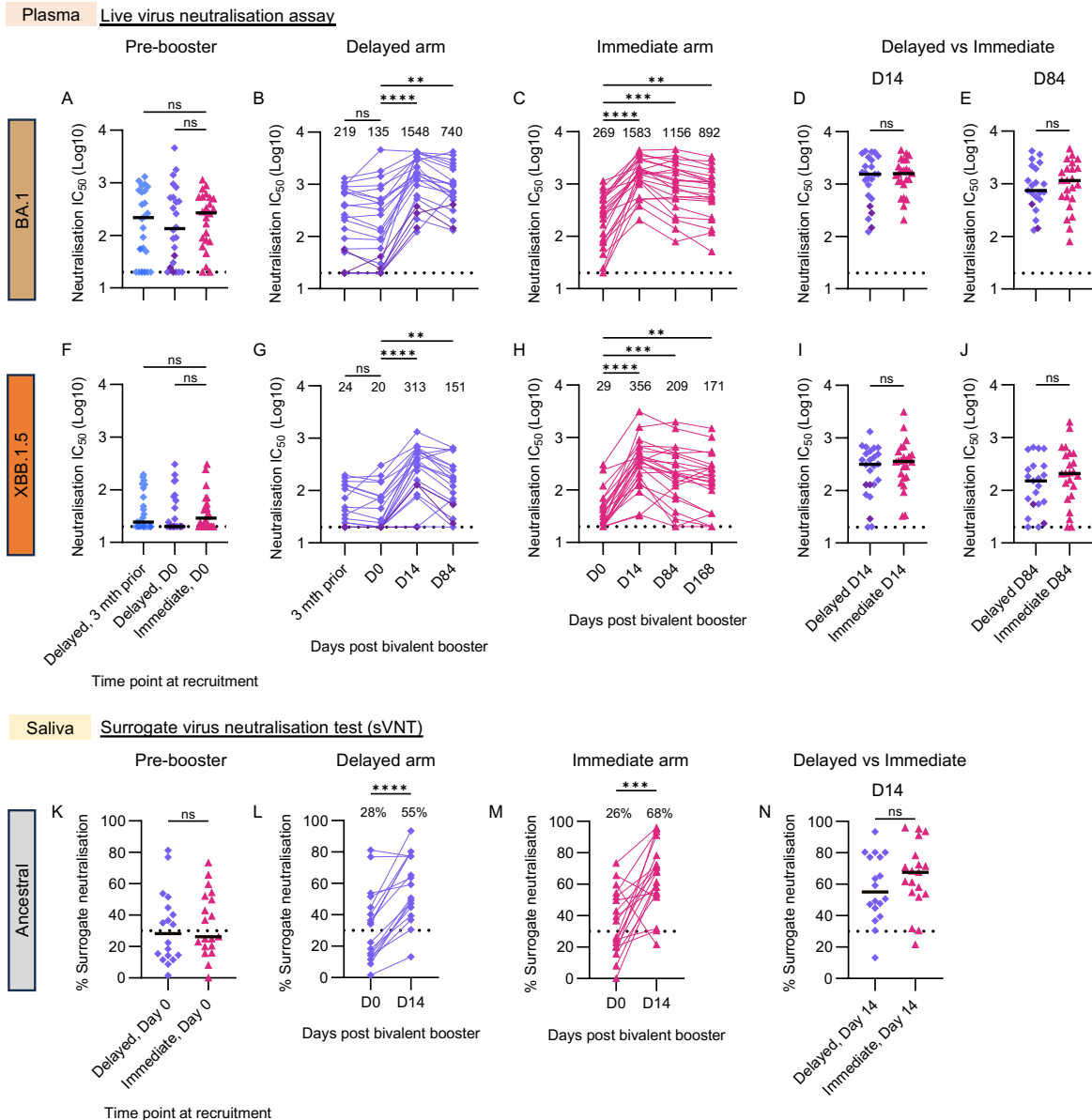
640 **Figures**



641

642 **Figure 1: CONSORT flow diagram.**

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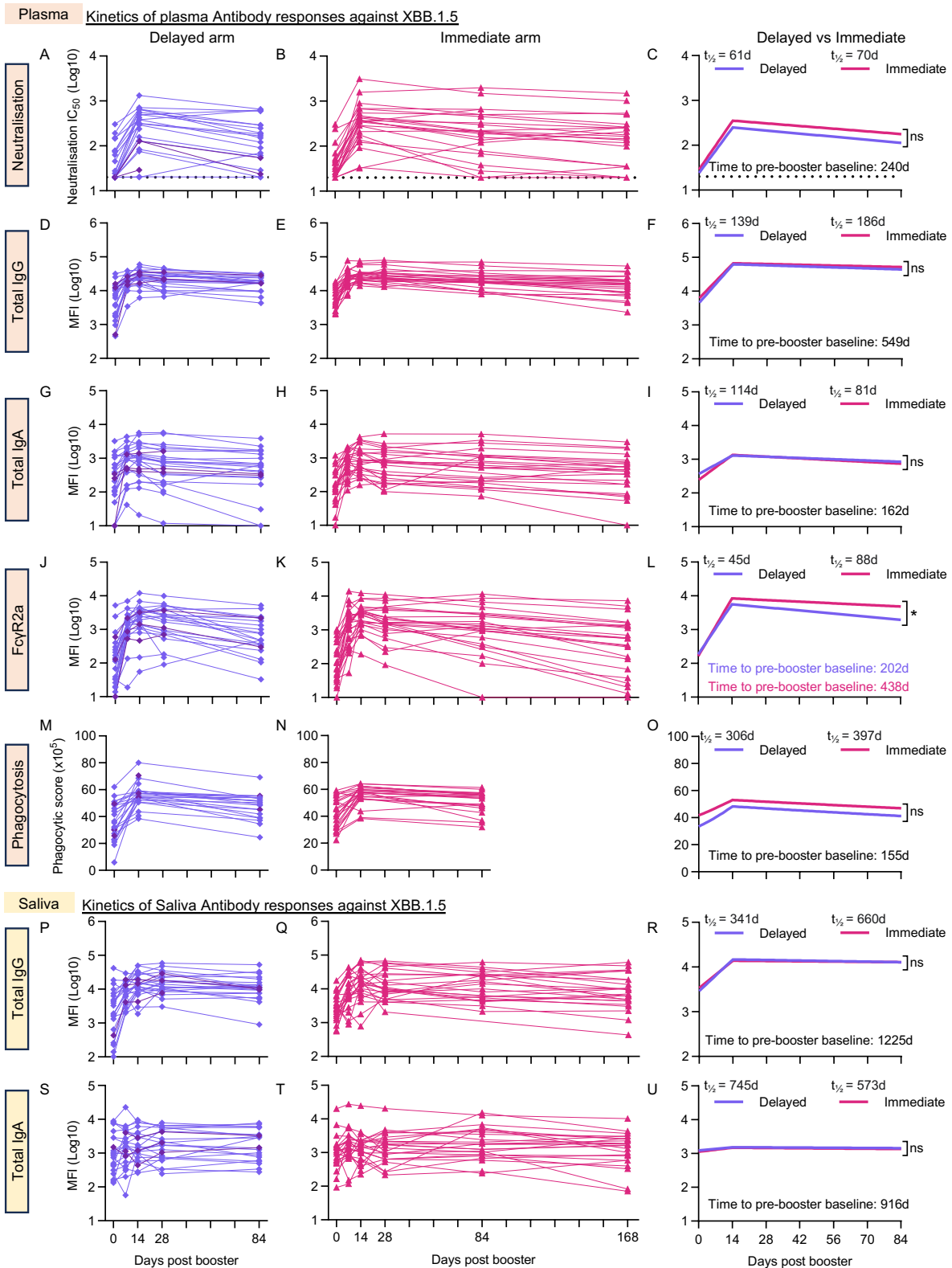
644

645 **Figure 2: Neutralising antibodies following bivalent mRNA booster vaccination.**

646 Plasma neutralising activity was measured using a live virus neutralisation assay
 647 against SARS-CoV-2 Omicron BA.1 (**A-E**) and XBB.1.5 (**F-J**) variants. Pre-booster (**A**
 648 **and F**) and post-booster (Day 14; **D and I**) (Day 84; **E and J**) neutralising antibody
 649 responses were compared between the delayed (blue/purple diamond, n=24) and
 650 immediate arms (pink triangle, n=24) at the respective sampling timepoints. Line
 651 graphs describe the kinetics of plasma neutralisation activity of the delayed (**B and G**)
 652 and immediate (**C and H**) arms after receiving the bivalent booster. Numbers above

653 each timepoint describe the respective median neutralisation IC_{50} against each viral
654 variant. Dotted lines depict the detection threshold for the assay (neutralisation IC_{50}
655 =20). Dark purple diamonds and lines show the antibody responses of the 3 individuals
656 who received the BA.5 bivalent booster in the delayed arm. Saliva neutralising activity
657 against ancestral SARS-CoV-2 was measured using the surrogate virus neutralisation
658 test (sVNT; Genscript). Pre-booster (**K**) and post-booster (Day 14; **N**) neutralising
659 antibody responses are compared between the delayed (purple diamond, n=18) and
660 immediate arms (pink triangle, n=19) respectively. Line graphs describe the change in
661 saliva neutralisation activity following the bivalent booster (**L and M**). Numbers
662 describe the % surrogate neutralisation observed at each timepoint. Dotted lines
663 depict the sVNT cutoff for neutralising activity (30%). Statistical significance was
664 calculated between cohorts and timepoints using the 2-tailed Mann-Whitney *U* test, or
665 Kruskal-Wallis test followed by Dunn's test for multiple comparisons. Paired saliva
666 analysis (D0 vs D14) was performed using Wilcoxon matched-paired signed rank test.
667 Experiments were performed in duplicates. Graphs are displayed as median, and
668 where significant, *P* values were reported ($*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$; $****P$
669 ≤ 0.0001).

670



671

672 **Figure 3: Antibody kinetics following bivalent mRNA booster vaccination.**

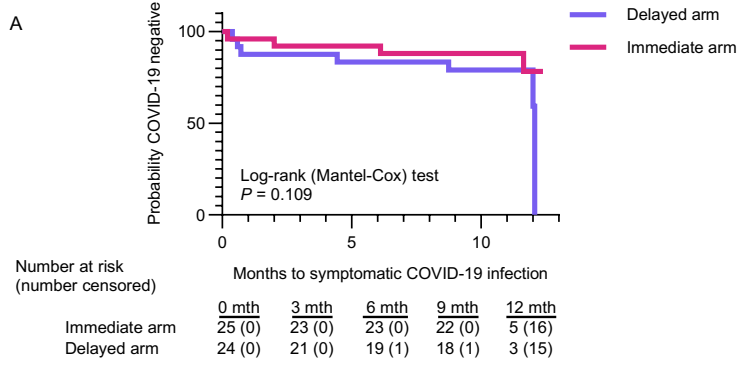
673 Kinetics of plasma (A-O) and saliva (P-U) antibody responses against SARS-CoV-2

674 variant Omicron XBB.1.5. Line graphs depict the plasma neutralisation responses in

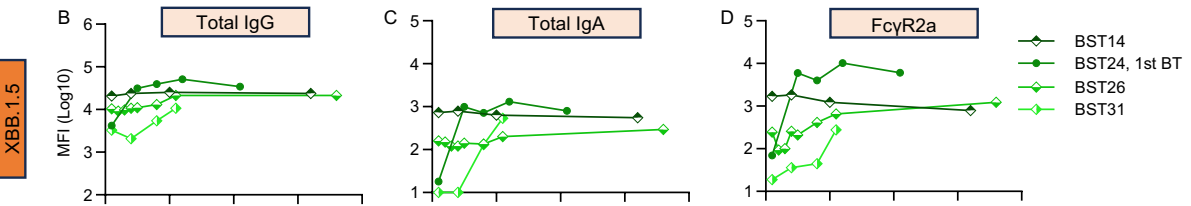
675 the delayed (purple diamond, n=24; **A**) and immediate (pink triangle, n=24; **B**) arms
676 as previously described in Figure 2G-H. Line graphs also illustrate the rise and decay
677 of plasma total IgG (**D and E**), total IgA responses (**G and H**), Fc-gamma receptor 2a
678 binding (**J and K**) and antibody-dependent phagocytic activity (**M and N**), as well as
679 salivary total IgG (**P and Q**) and total IgA (**S and T**) responses in the delayed (purple
680 diamond, n=24; **D, G, J, M, P, S**) and immediate (pink triangle, n=24; **E, H, K, N, Q, T**)
681 arms respectively. Dark purple diamonds and lines show the antibody responses of
682 the 3 individuals who received the BA.5 bivalent booster in the delayed arm. Modelled
683 decay slopes (**C, F, I, L, O, R, U**) describe the half-life and time taken for the respective
684 antibody responses to return to pre-booster baseline levels. Statistical significance
685 was calculated between cohorts using the likelihood ratio test and where significant,
686 *P* values were reported ($*P \leq 0.05$). Experiments were performed in duplicates.

687

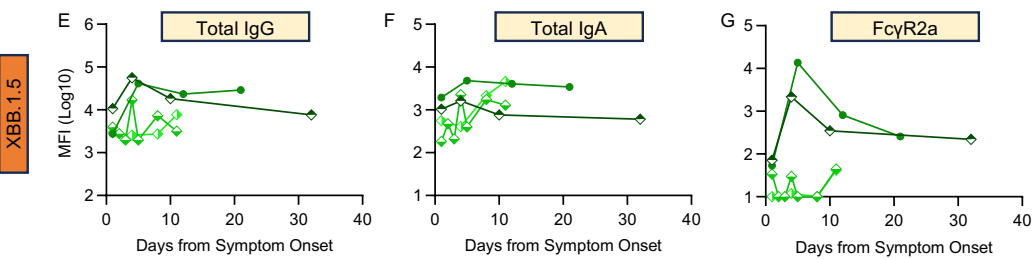
Kaplan-Meier plot for symptomatic breakthrough COVID-19



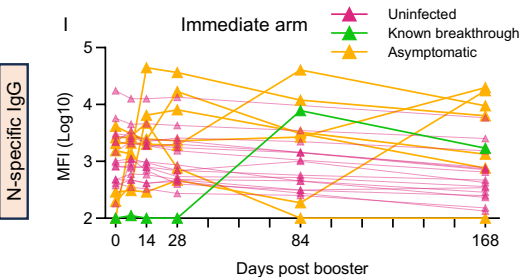
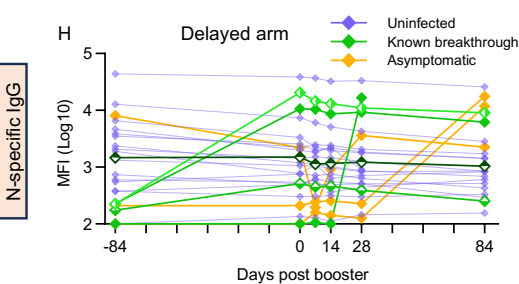
Plasma Antibody responses following breakthrough infections



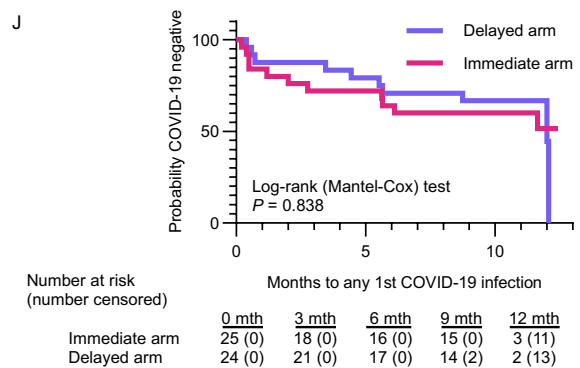
Saliva Antibody responses following breakthrough infections



Plasma N-specific IgG responses across timepoints



Kaplan-Meier plot for entire cohort (known breakthroughs and asymptomatics)



688

689 **Figure 4: Breakthrough COVID-19.** Kaplan-Meier probability of remaining
 690 symptomatic COVID-19 negative during the study in the delayed (purple) and
 691 immediate (pink) arms (A). Includes all first on-study COVID-19 symptomatic

692 infections (pre and post study vaccination, self-reported). Probability for the delayed
693 arm reaches zero because the final three delayed arm subjects are positive/censored
694 just after 12 months, while there are 5 final immediate arm participants remaining at
695 risk. The numbers below the graph show the remaining number at risk (number
696 censored) during the study at baseline (0 mth), month 3 (3 mth), month 6 (6 mth),
697 month 9 (9 mth) and month 12 (12 mth). Statistical significance between survival
698 curves were calculated via Log-rank Mantel-Cox test. Line graphs show the plasma
699 (**B-D**) and salivary (**E-G**) antibody responses against Omicron XBB.1.5 from 4
700 representative individuals (green) with COVID-19 breakthrough infections (RATs
701 positive). Total IgG (**B and E**), Fc-gamma receptor 2a binding (**C and F**), and total IgA
702 responses (**D and G**) against Omicron XBB.1.5 are shown following their symptom
703 onset. Line graphs also depict the kinetics of N-specific IgG for both the delayed
704 (purple diamonds; **H**) and immediate arms (pink triangles; **I**) across sampling
705 timepoints, highlighting Individuals with known symptomatic (RATs positive; green)
706 and asymptomatic breakthrough infections (>4-fold rise in N-specific IgG from
707 previous timepoint; yellow). Experiments were performed in duplicates. Kaplan-Meier
708 probability of remaining COVID-19 negative during the study in the delayed (purple)
709 and immediate (pink) arms (**J**). Includes all first on-study COVID-19 infections (pre and
710 post study vaccination, self-reported and asymptomatic laboratory diagnosed).
711 Probability for the delayed arm reaches zero because the final two delayed arm
712 subjects are positive/censored just after 12 months, while there are 3 final immediate
713 arm participants remaining at risk.

714

715