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Journal Pre-proof



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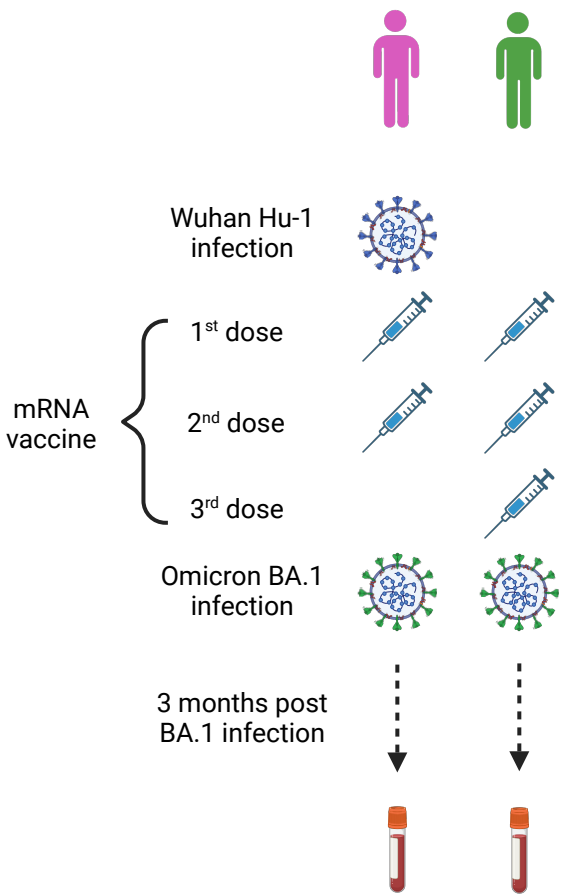
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Wuhan Hu-1 infection

mRNA vaccine

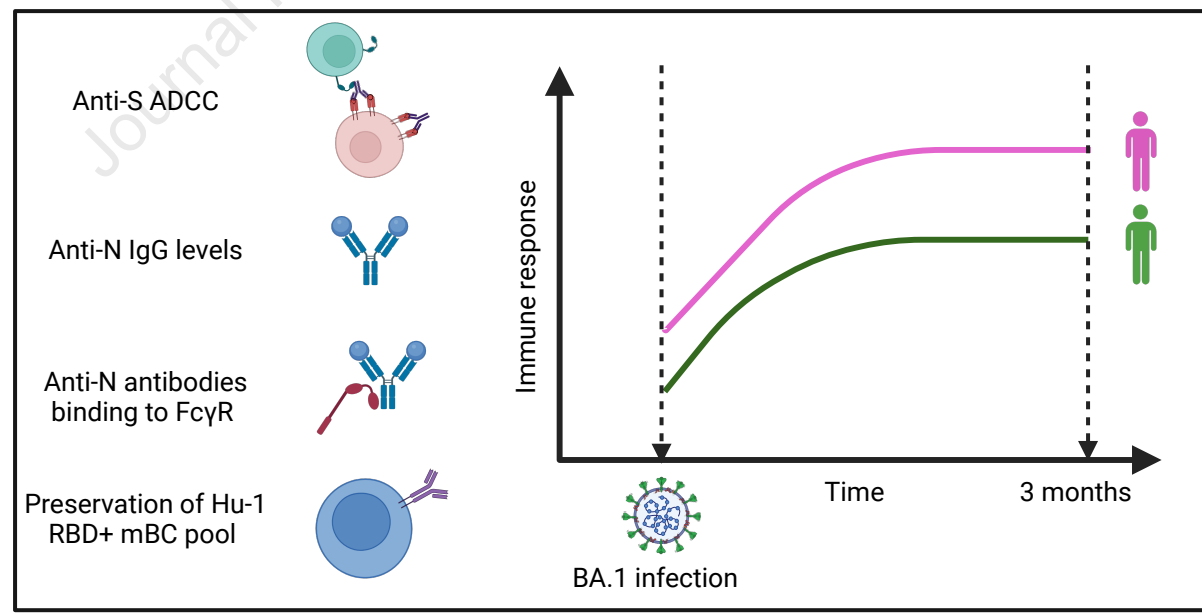
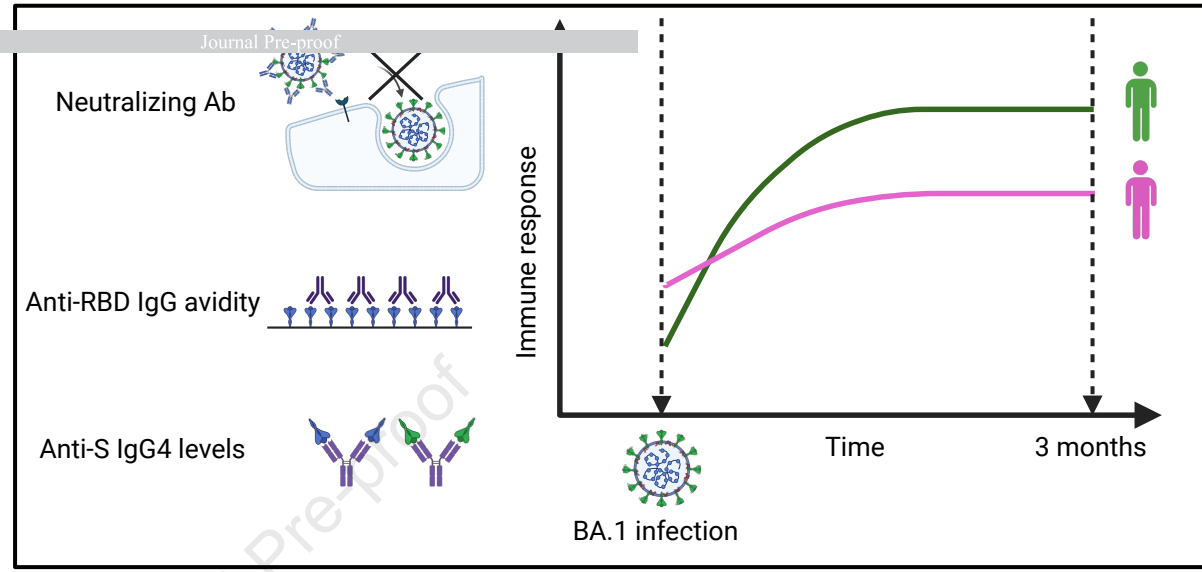
1st dose

2nd dose

3rd dose

Omicron BA.1 infection

3 months post BA.1 infection



1 **BA.1 breakthrough infection elicits distinct antibody and memory B cell responses in**
2 **vaccinated-only versus hybrid immunity individuals**

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42

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43 **Summary**

44 Immune memory is influenced by the frequency and type of antigenic challenges. Here, we
45 performed a cross-sectional comparison of immune parameters following a BA.1
46 breakthrough infection in individuals with prior hybrid immunity (conferred by infection and
47 vaccination) versus those solely vaccinated in a cohort of health care workers in Lyon, France.
48 The results showed higher levels of serum anti-RBD antibodies and neutralizing antibodies
49 against BA.1 post-infection in the vaccine-only group. Individuals in this group also showed a
50 decrease in memory B cells against the ancestral strain but an increase in those specific and
51 cross-reactive to BA.1, suggesting a more limited immune imprinting. Conversely, hybrid
52 immunity prevents the decrease in ADCC response, possibly by limiting IgG4 class-switching,
53 and enhanced anti-N responses post-infection. This highlights that BA.1 breakthrough
54 infection induces different immune responses depending on prior history of vaccination and
55 infection, which should be considered for further vaccination guidelines.

56 **Keywords:** SARS-CoV-2, breakthrough infection, hybrid immunity, immune response, IgG4
57 class-switching, ADCC function

58 Introduction

59 Immune memory against SARS-CoV-2 in the general population has been acquired through
60 cycles of infections and /or vaccinations, with great heterogeneity in this pattern across
61 individuals. Infection by SARS-CoV-2 results in broad cellular and humoral immunity against
62 various antigens, whereas most vaccines target only the viral Spike (S) protein. mRNA
63 vaccination induces strong levels of neutralizing antibodies, especially in previously infected
64 individuals, the latter situation defined as hybrid immunity.¹⁻³ Hybrid immunity is also
65 acquired if the infection occurs after vaccination, a situation that can be defined as
66 breakthrough immunity.⁴ Breakthrough infections have started to arise when variants of
67 concern (VOCs) have emerged, leading up to the current Omicron lineage.^{5,6} These VOCs differ
68 from the initial strain mainly at the level of the S protein, which accumulated immune evasive
69 mutations, making it much less sensitive to neutralizing antibodies directed against the so-
70 called Wild Type or Wuhan Hu-1 (Hu-1) strain. The emergence of these VOCs led to new
71 epidemic waves responsible for high infection rates, even in vaccinated individuals, prompting
72 health authorities to recommend vaccine boosters regardless of infection history.⁷⁻⁹ For
73 example, the WHO's Strategic Advisory Group on immunization (SAGE) recommends a
74 revaccination for healthcare workers (HCWs) 12 months after the most recent dose regardless
75 of their previous infection history.⁹

76 However, repeated vaccination may lead to the emergence of S-specific IgG4 that have less
77 efficient Fc-dependent immune properties than IgG1. This is especially true in individuals not
78 previously infected by SARS-CoV-2 before their vaccination scheme.¹⁰⁻¹² Reciprocally, a
79 dampening of the immune response post Omicron BA.1 breakthrough infection has been
80 observed among individuals with hybrid immunity in comparison to those with vaccination-
81 induced immunity.¹³ In particular, most individuals increase BA.1/BA.2-specific neutralizing
82 antibodies following infection, but the magnitude of increase is lower in individuals with
83 established hybrid immunity compared to infection-naïve vaccinated subjects.¹² This
84 phenomenon has already been described in influenza under the term of "original antigenic
85 sin", also called "immune imprinting", and can be explained by the strong response of memory
86 B cells (mBCs) induced by infection or vaccination competing with naïve B cells directed
87 against antigenic variants.¹⁴⁻¹⁸ Thus, SARS-CoV-2 infection occurring either before or after
88 vaccination can influence antiviral immunity in distinct ways, particularly regarding antibody
89 subclasses, antibody functions, and antigen specificity. To explore this further, we investigated
90 a cohort of HCWs from Lyon Hospital. We conducted a comprehensive analysis of humoral
91 and cellular immunity parameters targeting S and N antigens across five groups of individuals
92 with diverse vaccination and infection histories. Our goal was to identify the key factors
93 shaping immunity after a BA.1 breakthrough infection, with a particular focus on prior SARS-
94 CoV-2 infection before vaccination and the cumulative number of antigenic exposures.

95

96 Results

97 Clinical characteristics of study population

98 To understand how antiviral immunity evolves after a BA.1 breakthrough infection and how
99 prior infection and vaccination history influence this evolution, we leveraged a cohort of 714
100 HCWs that we previously described¹⁹, and identified five groups of individuals.

101 First, we selected two groups of individuals who had a breakthrough infection during the
102 Omicron BA.1 wave:

- 103 i) the first group consisted of individuals who were previously infected during the Hu-
104 1 wave of infection and then received two doses of a monovalent mRNA vaccine
105 before the BA.1 breakthrough infection (Hu-1/2RNA/BA.1),
- 106 ii) the second group consisted of individuals with vaccination-induced immunity who
107 were previously COVID-19-naïve and received three doses of a monovalent mRNA
108 vaccine before the BA.1 breakthrough infection (3RNA/BA.1).

109 Then, we selected three other groups did not undergo breakthrough infection:

- 110 iii) the third and fourth groups consisted of individuals infected during the Hu-1 wave
111 of the pandemic then received 2 or 3 doses of a monovalent mRNA vaccine (Hu-
112 1/2RNA and Hu-1/3RNA respectively),
- 113 iv) the fifth group consisted of individuals who were COVID-19-naïve and received
114 three doses of a monovalent mRNA vaccine (3RNA).

115 Clinical and demographic characteristics of selected individuals are detailed in Table 1. Each
116 group is composed of 15 individuals.

117 All infections, whether during the Hu-1 wave (established hybrid immunity) or the BA.1 wave
118 (breakthrough infection), were mildly symptomatic. Importantly, blood sampling was
119 performed 3 months (median [Interquartile range, IQR] of 91 [71-104] days) post last exposure
120 to viral antigens, i.e. 3 months post last infection (groups Hu-1/2RNA/BA.1 and 3RNA/BA.1) or
121 vaccination (groups Hu-1/2RNA, Hu-1/3RNA and 3RNA). Figure S1 summarizes the different
122 groups, highlighting the timing of infections/vaccination and sampling events.

123 Hybrid immunity preserves Fc receptor-dependent anti-S IgG function post breakthrough 124 infection

125 We first compared serological immunity against S or RBD antigens post BA.1 breakthrough
126 infection, in groups Hu-1/2RNA/BA.1 and 3RNA/BA.1 (Figure 1A). These two groups had an
127 equivalent cumulative number of exposures to the S antigen through infection or vaccination.
128 Higher anti-RBD IgG levels were found in the 3RNA/BA.1 group (median [IQR] of 5013 [2783-
129 7030] binding antibody unit (BAU)/mL) in comparison to the Hu-1/2RNA/BA.1 group (2740
130 [1118-4379] BAU/mL, $p=0.1150$, Figure 1B). Anti-RBD IgG from individuals in the 3RNA/BA.1
131 group also had a significantly increased avidity compared to those from the Hu-1/2RNA/BA.1

132 group, ($p=0.0238$, Figure 1C). We then analyzed serum IgG subtypes against Hu-1 and BA.1 S
133 proteins in both groups. The results were similar for both proteins, with individuals in the
134 3RNA/BA.1 group having a higher level of anti-S IgG4 ($p<0.0001$ for both proteins, Figure 1D).
135 In particular, there was a 1.63-fold and a 1.48-fold increase in IgG4 levels, in these individuals
136 compared to those from the Hu-1/2RNA/BA.1 group for the Hu-1 S and BA.1 S protein,
137 respectively (Figure 1D).

138 Serum neutralizing antibody titers against SARS-CoV-2 isolates 19A (B38 lineage), BA.1, BA.4,
139 BA.5, and XBB.1.5 were then assessed. For all tested isolates, there was no significant
140 difference in neutralizing antibody titers between serum samples from the Hu-1/2RNA/BA.1
141 group and those from the 3RNA/BA.1 group ($p>0.05$, Figure 1E). Yet, for the BA.1 isolate, the
142 results showed a tendency towards higher neutralizing titers among the 3RNA/BA.1 subjects
143 with a median [IQR] titer of 480 [120-960] in comparison to 240 [80-480] for the Hu-
144 1/2RNA/BA.1 ones (Figure 1E).

145 ADCC was then assessed against the D614G, BA.1, and BA.4/5 S proteins. ADCC was 5.22-,
146 4.43- and 4.25-fold higher among individuals belonging to the Hu-1/2RNA/BA.1 group in
147 comparison to those belonging to the 3RNA/BA.1 group for the D614G, BA.1 and BA.4/5 S
148 proteins respectively ($p=0.0039$, $p=0.0855$ and $p=0.0038$, respectively, Figure 1F).

149 Together, these results demonstrate that a prior infection before the mRNA vaccine regimen
150 significantly increases the ADCC activity of antibodies against S proteins carried by the tested
151 VOCs post BA.1 reinfection, but slightly limits the emergence of neutralizing antibodies against
152 all tested Omicron subvariants.

153 **BA.1 breakthrough infection in individuals with prior hybrid immunity recalls anti-N** 154 **serological immunity**

155 Next, we monitored serological humoral immunity against the N protein, an antigen not
156 present in COVID-19 vaccines available in France. Anti-N IgG levels were higher among
157 individuals from the Hu-1/2RNA/BA.1 group in comparison to the 3RNA/BA.1 group (median
158 [IQR] index of 7.09 [5.94-8.58] and 1.33 [0.58-2.23]; $p<0.0001$) (Figure 2A).

159 Anti-N IgG subtyping was performed and higher IgG1, IgG2, and IgG4 levels were found among
160 individuals in the Hu-1/2RNA/BA.1 group in comparison to those in the 3RNA/BA.1 group
161 ($p=0.0046$, $p<0.0001$, $p<0.0001$, respectively, Figure 2B). No significant difference was
162 observed regarding anti-N IgG3 levels between the two groups ($p=0.5490$, Figure 2B). Serum
163 anti-N IgG binding to Fc γ RIIa and Fc γ RIIIa was then assessed using a Luminex assay. Sera
164 collected from individuals in the Hu-1/2RNA/BA.1 group had antibodies with a higher capacity
165 to bind both receptors in comparison to the 3RNA/BA.1 group ($p<0.0001$ for both receptors,
166 Figure 2C). The difference in the magnitude of the serological response against the N protein
167 confirms that subjects in the Hu-1/2RNA/BA.1 group who were infected twice with the virus,
168 exhibit a recall response against this protein.

169 **BA.1 breakthrough infection induces different immune responses based on prior history of**
170 **vaccination and infection**

171 The preserved ADCC function, along with the robust anti-N response observed after a BA.1
172 breakthrough infection in individuals with prior hybrid immunity, led us to investigate whether
173 these serological responses were driven by the BA.1 breakthrough infection itself or were a
174 consequence of the pre-existing hybrid immunity. To address this question, we leveraged the
175 groups without breakthrough infection that consisted of subjects infected during the Hu-1
176 wave of the pandemic and that received 2 (Hu-1/2RNA) or 3 (Hu-1/3RNA) doses of a
177 monovalent mRNA vaccine (Figure 3A, Figures S2 and S3). Serum IgG were analyzed in
178 individuals from these groups 3 months post vaccination, in comparison to those from the Hu-
179 1/2RNA/BA.1 group. No significant difference was observed between the 3 groups in terms of
180 ADCC, as well as other anti-S serological parameters ($p>0.05$, Figure 3B). Nevertheless, a
181 tendency towards higher neutralizing antibody titers against BA.1 was observed among
182 individuals from the Hu-1/2RNA/BA.1 group (median [IQR] of 240 [80-480]) in comparison to
183 those from the Hu-1/2RNA (160 [30-320]) and Hu-1/3RNA (60 [30-120]) groups (Figure 3B,
184 Figure S2F). As for the anti-N immune response, anti-N IgG levels, subtypes, and Fc γ R binding
185 were significantly higher in subjects from the Hu-1/2RNA/BA.1 group compared to the other
186 2 groups ($p<0.001$, Figure 3B, Figure S3). Thus, although a BA.1 breakthrough infection does
187 not significantly impact the anti-S serological response among individuals with established
188 hybrid immunity, it leads to an increase in neutralization capacity against BA.1. Additionally, a
189 breakthrough infection triggers a recall of pre-existing immunity targeting the N protein
190 among individuals with established hybrid immunity.

191 In parallel, we examined whether the dampening of ADCC associated with IgG4 class-switching
192 in individuals with a BA.1 breakthrough infection after vaccination was a result of the
193 breakthrough infection itself or a consequence of repeated mRNA vaccinations. For this, we
194 compared the serological immune response induced by 3 mRNA vaccine doses between
195 subjects with (3RNA/BA.1) or without (3RNA) BA.1 breakthrough infection (Figure 3A, Figures
196 S2 and S3). No significant difference was observed between the groups in terms of anti-RBD
197 IgG levels, anti-S ADCC and anti-N IgG subtypes ($p>0.05$, Figure 3C, Figures S2 and S3).
198 Interestingly, significantly higher anti-S IgG4 levels were observed among individuals in the
199 3RNA/BA.1 group, for both the Hu-1 and BA.1 S proteins ($p=0.0097$ and $p=0.0052$ respectively,
200 Figure 3C, Figure S2D-E). In addition, there was a tendency towards higher BA.1 and XBB.1.5
201 neutralizing antibody titers in the 3RNA/BA.1 group ($p=0.0542$ and $p=0.0457$ respectively,
202 Figure 3C, Figure S2F). Thus, among individuals with vaccination-induced immunity, a BA.1
203 breakthrough infection resulted in an increase in neutralization capacity against BA.1 despite
204 an increase in anti-S IgG4 levels.

205 Furthermore, we observed an inverse trend between anti-S IgG4 levels and anti-S ADCC
206 activity and sought to further investigate this relationship. Correlation analyses were
207 performed between anti-Hu-1 S IgG4 levels and D614G S ADCC activity as well as anti-BA.1 S
208 IgG4 levels and BA.1 S ADCC activity across all individuals in the cohort's five groups. As

209 illustrated in Figure S2J, both IgG4 levels showed a significant negative association with ADCC
210 activity, supporting a potential causal link between these parameters. It has been reported
211 that IgG4 levels tend to increase following a third dose of vaccination in COVID-19-naïve
212 patients.^{10,11} In our study, we aimed to compare anti-S IgG4 levels between triple vaccinated
213 individuals infected before or after the vaccination scheme; i.e. Hu-1/3RNA and 3RNA/BA.1
214 groups. Our results showed that despite both groups having the same number of vaccinations
215 and infections, individuals in the 3RNA/BA.1 group had significantly higher anti-S IgG4 levels
216 ($p=0.003$ for Hu-1 S, Figure S2D, and $p=0.0380$ for BA.1 S Figure S2E). This would suggest that
217 hybrid immunity limits the increase in IgG4 levels following repeated exposure to viral
218 antigens in comparison to vaccination alone.

219 In summary, a BA.1 breakthrough infection enhanced neutralization capacity against BA.1
220 regardless of prior history of vaccination and infection. For individuals with established hybrid
221 immunity, a BA.1 breakthrough infection recalled the anti-N serological response. As for
222 individuals with vaccination-induced immunity, a BA.1 breakthrough infection led to an
223 increase in anti-S IgG4 levels. Hence, a BA.1 breakthrough infection does not induce the same
224 immune response depending on prior history of vaccination and infection.

225 **Prior Hybrid immunity limits the emergence of mBCs specific to BA.1 RBD upon** 226 **breakthrough infection**

227 Next, we examined the pool of mBCs specific to the Hu-1 and BA.1 RBD for subjects belonging
228 to the Hu-1/2RNA/BA.1, Hu-1/2RNA, Hu-1/3RNA and 3RNA/BA.1 groups (Figure 4A). RBD-
229 specific mBCs were analyzed by multiparameter flow cytometry approach using fluorescently
230 labelled tetrameric Hu-1 and BA.1 RBDs as previously described.² The gating strategy to
231 identify mBCs is depicted in Figure S4A. Two distinct couples of fluorophores were used to
232 detect Hu-1 and BA.1 RBD specific mBCs allowing the identification of mBCs only specific to
233 either the Hu-1 or BA.1 RBD and those cross-reactive to both RBDs (Figure S4B-C).

234 Figure 4B and 4C illustrate the percentage of mBCs specific to the Hu-1 RBD and mBCs specific
235 and cross-reactive to the BA.1 RBD (BA.1 RBD+), respectively, as a proportion of the total mBC
236 population. The results showed a significantly lower percentage of Hu-1 RBD-specific mBCs
237 and a significantly higher percentage of BA.1 RBD+ mBCs following a breakthrough infection
238 in individuals previously only vaccinated, compared to those with prior hybrid immunity
239 ($p=0.095$, Figure 4B-C). This would suggest that established hybrid immunity restricts the
240 diversification of the RBD+ mBCs pool by preserving the Hu-1 RBD-specific mBCs and limiting
241 the expansion of BA.1 RBD+ mBCs.

242 To investigate this hypothesis further, we compared the pool of RBD+ mBCs between the
243 three groups with established hybrid immunity, with or without a breakthrough infection. The
244 results indicate no significant differences among the three groups for either Hu-1 RBD-specific
245 mBCs or BA.1 RBD+ mBCs ($p>0.05$, Figure 4D). This suggests a similar prevalence of Hu-1 RBD
246 specific and BA.1 RBD+ mBCs across the three groups with established hybrid immunity.

247 Taken together, these results suggest that a BA.1 breakthrough infection increases the
248 frequency of BA.1 RBD+ mBCs among individuals previously only vaccinated in comparison to
249 those with established hybrid immunity. These results suggest that hybrid immunity results in
250 stronger immune imprinting than sole vaccination, limiting the emergence of mBCs specific or
251 cross-reactive to the BA.1 RBD upon breakthrough infection.

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252 **Discussion**

253 Previous studies have reported contradictory results regarding the impact of prior hybrid
254 immunity on the immune response post breakthrough infection.^{10,13,20–22} We show here that
255 prior hybrid immunity is generally not a situation that prevents the development of the
256 response against Omicron post breakthrough infection. It is even rather beneficial as it
257 maintains a strong ADCC capacity of anti-S IgG possibly by preventing the class-switch to IgG4,
258 and it significantly increases the humoral immunity against N, without altering the level of
259 neutralizing antibodies against different variants contrary to findings reported in earlier
260 studies.¹³ Conversely, we report that prior hybrid immunity slightly limits the emergence of
261 BA.1 RBD-specific mBCs linked to the preservation of Hu-1 RBD-specific mBCs compartment.

262 Our results reveal significantly higher anti-S IgG4 levels following a BA.1 breakthrough
263 infection in individuals with vaccination-only immunity compared to those with prior hybrid
264 immunity, whereas IgG1 levels were similar between the two groups. These findings align with
265 previous findings indicating high anti-S IgG4 levels among COVID-19-naïve individuals upon
266 repeated vaccination or breakthrough infection^{10,23} and low anti-S IgG4 levels in individuals
267 with hybrid immunity.^{23,24} Interestingly, we also show that low levels of anti-S IgG4 are
268 maintained post BA.1 breakthrough infection among those individuals, suggesting a sustained
269 protective effect of the initial infection. IgG4 have been described as anti-inflammatory
270 antibodies secreted in response to chronic antigen exposure with a reduced capacity to induce
271 Fc-mediated antibody effector functions such as ADCC.^{25,26} Here, we demonstrate that
272 alongside a significant increase in anti-S IgG4 levels among individuals experiencing a
273 breakthrough infection post-vaccination alone, there is a significantly reduced anti-S ADCC
274 function, despite a slight improvement in BA.1 neutralization capacity. This indicates that
275 hybrid immunity maintains ADCC function, possibly by preventing the IgG4 class-switch post
276 breakthrough infection and maintaining IgG1, that have the highest ADCC potential.²⁷

277 In the context of COVID-19, most studies have focused on neutralizing antibodies, with limited
278 attention given to ADCC.^{28–32} Nevertheless, a study demonstrated that in an animal model,
279 the ADCC function of antibodies was essential for their protective effect when used as a
280 treatment for SARS-CoV-2 infection.³³ Moreover, in the context of Respiratory Syncytial Virus
281 (RSV) infections, ADCC function has been described as essential for the control of respiratory
282 infections.^{34,35} Bartsch *et al.* showed that individuals with higher IgG4 levels and lower Fc-
283 mediated antibody effector functions were more susceptible to infection after RSV
284 challenge.³⁴ Therefore, the influence of additional vaccinations on ADCC function should also
285 be considered when establishing recommendations for COVID-19 vaccination.

286 In the present study, we also show that vaccination-induced immunity leads to a decrease in
287 the proportion of Hu-1 RBD specific mBCs paralleled by an increase in the proportion of BA.1
288 RBD+ mBCs, indicating a diversification of the RBD+ mBCs pool post BA.1 breakthrough
289 infection. Conversely, hybrid immunity preserves the pool of Hu-1 RBD specific mBCs. These
290 results are in line with previous studies reporting the limited *de novo* B cell response in
291 individuals with prior hybrid immunity post Omicron breakthrough infection, described as

292 “hybrid immune damping”.¹³ This would indicate that prior hybrid immunity limits the
293 diversification of the RBD+ mBCs pool due to immune imprinting.

294 Regarding the humoral response against the N protein, an antigen not present in most
295 vaccines, the effect of a breakthrough infection was significant in individuals with established
296 hybrid immunity, compared to those with vaccination-induced immunity. It has been
297 previously reported that a breakthrough infection in previously infection-naïve vaccinated
298 individuals does not lead to a robust anti-N humoral response with only 40% of individuals
299 with a PCR-confirmed SARS-CoV-2 infection seroconverting to anti-N antibodies.³⁶ In addition,
300 Allen *et al.* compared the anti-N humoral response between individuals who were first
301 infected and then vaccinated, and those who were infected after vaccination. Their findings
302 revealed that 26% of previously vaccinated individuals seroconverted to anti-N antibodies,
303 compared to 82% of individuals who were first infected.³⁷ These results would suggest that a
304 single exposure to antigens other than the S protein, such as N, might not be sufficient to
305 induce a robust immune response in previously vaccinated individuals. Nevertheless, the
306 possibility of vaccinating with antigens other than the S to avoid immune fatigue against the
307 S protein should still be considered. Indeed, previous studies have described that a two-dose
308 vaccination regimen targeting the N protein in animal models as well as clinical trials results
309 in long-lasting N specific humoral and T cell immune responses in humans and decreased
310 infection severity in animals.^{38–41} The N protein exhibits high conservation across SARS-CoV-2
311 variants. The XBB.1.5 variant, for example, carries 43 mutations in the S protein and only 7
312 mutations in the N protein in comparison to Hu-1 strain.⁴² Together these data would suggest
313 that combining S antigens with more conserved antigens such as N in vaccines may enhance
314 and diversify the immune response against SARS-CoV-2 in the face of a plateau in the anti-S
315 immune response. Such recommendation would particularly apply to individuals with high
316 SARS-CoV-2 exposure levels, such as HCWs.

317 In conclusion, our results highlight that a breakthrough infection induces different immune
318 responses depending on prior history of vaccination and infection, which should be considered
319 for further vaccination guidelines.

320 **Limitation of the study**

321 One of the limitations of this study is the low number of individuals included in each group,
322 and the inclusion of individuals with co-morbidities. The latter point could not be avoided due
323 to the constraints imposed by the selection criteria for the five groups. Moreover, it remains
324 to be assessed whether the limitation of IgG4 class-switch is beneficial to immune protection
325 against SARS-CoV-2 and its viral escape mutants. Our study did not include COVID-19-naïve
326 individuals vaccinated with only two doses in order to evaluate the impact of IgG4 levels with
327 repeated vaccination. Due to the unavailability of PBMC samples from the 3RNA group, we
328 were unable to perform a comparison with the 3RNA/BA.1 group to assess the impact of a
329 breakthrough infection on the RBD+ mBC pool. Another limitation of our study is the absence
330 of an analysis of the T cell response, particularly against the N antigen, as well as the mucosal

331 response, both of which play crucial roles in protection.^{43,44} These parameters were addressed
332 in a recent report, which specifically demonstrated that T cell responses to non-S antigens
333 increase significantly in vaccinated individuals following a BA.1 breakthrough infection,
334 regardless of their prior infection status.¹² Studying the T cell response could also have allowed
335 us to confirm that all individuals classified as naïve for COVID-19 were truly unexposed prior
336 to vaccination. Several studies have shown that in some convalescent individuals, serology
337 may be negative after infection, whereas the T cell response is detectable^{45,46} and does not
338 wane⁴⁷ over time.

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361 Author contributions

362 All authors read and approved the final version of the manuscript. Data collection and data
363 analysis: C Saade, T Bruel, M Killian, B Pozzetto, A Drouillard, L Khoryati, N Mariano, C Mignon,
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366 the samples and performed experiments. Supervision: V Barrateau, M Espi, O Thauinat, O
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369 design and writing of original draft: C Saade, S Paul, T Bruel, T Walzer and S Trouillet-Assant.

370 Declaration of interests

371 The authors declare no competing interests.

372 Ethics statement

373 For the Covid-Ser cohort, ethics approval was obtained from the national review board for
374 biomedical research in April 2020 (Comité de Protection des Personnes Sud Méditerranée I,
375 Marseille, France; ID RCB 2020-A00932-37), and the study was registered on ClinicalTrials.gov
376 (NCT04341142). For the COVIMMUNITY cohort, ethics approval was obtained from the
377 national review board (ClinicalTrials.gov: NCT04648709). For the ABCOVID cohort, ethics

378 approval was obtained from ILE DE FRANCE IV ethical committee (ClinicalTrials.gov:
379 NCT04750720). Written informed consent was obtained from all participants.

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382 **Table 1. Clinical characteristics of individuals included in this study.**

	Hu-1/2RNA/BA.1	Hu-1/2RNA	Hu-1/3RNA	3RNA/BA.1	3RNA	Adjusted p-value
n	15	15	15	15	15	-
Age at blood sampling (years), median [IQR]	34 [31-42]	55 [38-58]	55 [28-61]	44 [40-53]	51 [33-60]	0.303
Female, n (%)	12 (80)	12 (80)	12 (80)	12 (80)	10 (66.66)	1
Time elapsed between the last vaccination or infection event and blood sampling (days)	73 [65-86]	92 [86-97]	110 [94-112]	107 [65-120]	84 [70-98]	0.035
Body mass index ^a , n	15/15	14/15	15/15	15/15	13/15	-
Body mass index, median [IQR]	23.81 [21.60-25.47]	25.01 [20.72-27.76]	23.88 [20.20-27.72]	25.10 [21.94-32.89]	22.00 [19.50-25.00]	0.868
Currently smoker, n (%)	1/15 (6.66)	2/15 (13.33)	1/15 (6.66)	4/15 (26.66)	2/15 (13.33)	1
Alcohol consumption ^a (daily), n (%)	0/15 (0)	0/15 (0)	0/15 (0)	0/15 (0)	0/13 (0)	-
Comorbidities						-
Presence of comorbidities, n (%)	2/15 (13.33)	9/15 (60)	3/15 (20)	3/15 (20)	1/15 (6.66)	0.208
Neurological disorders, n (%)	1 (6.66)	1 (6.66)	1 (6.66)	1 (6.66)	0 (0)	1

Cardiovascular disorders, n (%)	1 (6.66)	0 (0)	1 (6.66)	0 (0)	0 (0)	1
Hypertension, n (%)	0 (0)	1 (6.66)	0 (0)	0 (0)	0 (0)	1
Heart failure, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1
Diabetes, n (%)	0 (0)	1 (6.66)	0 (0)	0 (0)	1 (6.66)	1
Immune deficiency, n (%)	0 (0)	1 (6.66)	0 (0)	0 (0)	0 (0)	1
Liver disease, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1
Kidney disease, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1
History of cancer, n (%)	0 (0)	1 (6.66)	0 (0)	1 (6.66)	0 (0)	1
Hypothyroidy, n (%)	0 (0)	1 (6.66)	0 (0)	1 (6.66)	0 (0)	1
Rheumatic disease, n (%)	0 (0)	2 (13.33)	1 (6.66)	0 (0)	0 (0)	1
Chronic lung disease, n (%)	0 (0)	3 (20)	1 (6.66)	0 (0)	0 (0)	0.472
Autoimmune and inflammatory disease, n (%) ^b	1 (6.66)	2 (13.33)	0 (0)	0 (0)	0 (0)	1

383 IQR, interquartile range; ^amissing data; ^bautoimmune and inflammatory disease include: Adisson's disease, Celiac disease, and Crohn's disease.

384 Comparison of clinical parameters between all groups were carried out using a Bonferroni's test for multiple comparisons.

385 **Figure 1. Prior hybrid immunity status prevents the decline of Fc receptor-dependent anti-**
386 **Spike IgG functions post breakthrough infection.** (A) Serum samples were collected from
387 individuals with established hybrid (Hu-1/2RNA/BA.1, purple, n=15) or vaccination-induced
388 (3RNA/BA.1, green, n=15) immunity 3 months post BA.1 breakthrough infection. (B) IgG levels
389 specific to the Wuhan Hu-1 (Hu-1) RBD were assessed and concentrations were expressed in
390 binding antibody unit (BAU)/mL. Each serum sample was evaluated as a single measurement.
391 The data are represented using a \log_2 scale. The dotted line represents the positivity threshold
392 (≥ 20.33 BAU/mL). (C) Purified total IgGs from each individual were assayed for Hu-1 RBD-
393 specific IgG off-rate measurement using bilayer interferometry. K_{off} values are expressed as
394 s^{-1} and each sample was assayed as a single measurement. (D) Subtyping of Hu-1 and BA.1 S-
395 specific IgG was carried out on serum samples. The data are expressed using a \log_2 scale. Each
396 sample was assayed as a single measurement. (E) Antibody neutralization capacity was
397 evaluated against the 19A, BA.1, BA.4, BA.5 and XBB.1.5 live isolates. The data are represented
398 using a \log_2 scale. Serum samples were tested as technical duplicates. The dotted line
399 represents the positivity threshold (PRNT₅₀ ≥ 20). (F) The induction of the CD16 pathway was
400 used as a surrogate of ADCC using the ADCC reporter assay. 293T cells stably expressing
401 D614G, BA.1 and BA.4/5 S proteins, or a control plasmid, were used as target cells. The fold
402 change of CD16 activation was calculated in each condition, and the control condition (cells
403 transduced with an empty vector) was subtracted to account for interindividual variations of
404 the background. The y-axis indicates the calculated ADCC induction, with a value of zero
405 indicating no induction. Each sample was assayed as a single measurement. Data for all
406 parameters are expressed as box-and-whiskers plots according to the Tukey method with the
407 line inside the box indicating the median, the upper and lower line of the box indicating the
408 interquartile range [IQR]. Multiple regressions (ANCOVA) were realized for each parameter,
409 adjusted on covariate "age", to compare the results obtained between the two groups. Exact
410 p-values are indicated for each parameter. MFI: mean fluorescence intensity.

411 **Figure 2. BA.1 breakthrough infection in individuals with prior hybrid immunity recalls anti-**
412 **nucleocapsid serological immunity.** Serum samples were collected from individuals with
413 established hybrid- (Hu-1/2RNA/BA.1, purple, n=15) or vaccination-induced (3RNA/BA.1,
414 green, n=15) immunity 3 months post BA.1 breakthrough infection. (A) anti-N total IgG levels
415 were quantified and expressed as an index. Each sample was assayed as a single
416 measurement. The dotted line represents the positivity threshold (index \geq 1.4) according to
417 manufacturer's instruction. (B) Subtyping of N-specific IgG was carried out on serum samples.
418 The data are expressed using a \log_2 scale. Each sample was assayed as a single measurement.
419 (C) Antibodies specific to the N protein were assessed for their ability to bind the Fc γ RIIIa and
420 Fc γ RIIIa receptors. Each sample was assayed as a single measurement. Data for all parameters
421 are expressed as box-and-whiskers plots according to the Tukey method with the line inside
422 the box indicating the median, the upper and lower line of the box indicating the interquartile
423 range [IQR]. Multiple regressions (ANCOVA) were realized for each parameter, adjusted on
424 covariate "age", to compare the results obtained between the two groups. Exact p-values are
425 indicated for each parameter. MFI: mean fluorescence intensity.

426 **Figure 3. BA.1 breakthrough infection changes immune responses based on previous history**
427 **of vaccination/infection.** (A) Serum samples were collected from individuals with established
428 hybrid immunity with (Hu-1/2RNA/BA.1, purple, n=15) or without (Hu-1/2RNA, red, n=15; Hu-
429 1/3RNA, orange; n=15) BA.1 breakthrough infection. Additionally, serum samples were
430 collected from individuals with vaccination-induced immunity with (3RNA/BA.1, green, n=15)
431 or without (3RNA, black, n=15) BA.1 breakthrough infection. All blood sampling was
432 performed 3 months post last infection or vaccination. (B) Bubble plot representing a
433 comparison of the measured humoral parameters between individuals with established
434 hybrid immunity. The bubble plot was generated by scaling and centering the values. (C)
435 Bubble plot representing a comparison of the measured humoral parameters between
436 individuals with vaccination-induced immunity. The bubble plot was generated by scaling and
437 centering the values. The intensity of the color as well as the size of the circles illustrated at
438 the right of the bubble plots is proportional to the level of different immune parameters
439 measured. To compare immune parameters between the groups, a statistical analysis was
440 carried out using a two-way ANOVA mixed model followed by a Bonferroni's test to correct
441 for multiple comparisons.

442 **Figure 4. Hybrid immunity preserves anti-Hu-1 RBD memory B cell pool post BA.1**
443 **breakthrough infection and limits the emergence of BA.1-specific memory.** (A) PBMC
444 samples were collected from individuals with established hybrid immunity with (Hu-
445 1/2RNA/BA.1, purple, n=10) or without (Hu-1/2RNA, red, n=9; Hu-1/3RNA, orange; n=10) a
446 BA.1 breakthrough infection. Additionally, PBMC samples were collected from individuals with
447 vaccination-induced immunity with a BA.1 breakthrough infection (3RNA/BA.1, green, n=10).
448 All blood sampling was performed 3 months post last infection or vaccination. We then used
449 flow cytometry to analyze the percentage of mBCs specific to the Hu-1 RBD (B) and mBCs
450 specific and cross-reactive to the BA.1 RBD+ (C) relative to total memory B cells recognizing
451 the RBD in individuals with vaccination-induced immunity and those with prior hybrid
452 immunity, post breakthrough infection. (D) Stacked bar plot representing the mean
453 proportion of Hu-1 RBD-specific, Hu-1 and BA.1 RBD cross-reactive and BA.1 RBD-specific
454 mBCs relative to all RBD+ mBCs among individuals with established hybrid immunity. Data are
455 expressed as box-and-whiskers plots according to the Tukey method with the line inside the
456 box indicating the median, the upper and lower line of the box indicating the interquartile
457 range. Multiple regressions (ANCOVA) were realized for each parameter, adjusted on
458 covariate "age", to compare the results obtained between the Hu-1/2RNA/BA.1 and
459 3RNA/BA.1 groups. A Kruskal-Wallis test followed by Dunn's multiple comparison test were
460 carried out to compare the percentages of RBD+ mBCs between the three groups with
461 established hybrid immunity. Exact p-values are indicated for each parameter.

462

463 STAR methods**464 RESOURCE AVAILABILITY****465 Lead contact**

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470 Materials availability

471 This study did not generate new unique reagents.

472 Data and code availability

473 All data associated with this study are a result of quantification and functional tests carried
474 out as described below. All data described herein are present in the paper or the supplemental
475 materials, in addition to the key resources table. Raw data have been deposited on Mendeley
476 Data.

477 This paper does not report original code.

478 Any additional information required to reanalyze the data reported in this paper is available
479 from the lead contact upon request

480 EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

481 For the Covid-Ser cohort, clinical data were recorded by a trained clinical research associate
482 using the Clinsight software (version _ Csonline 7.5.720.1). Seven hundred and fourteen with
483 different histories of vaccination and infection were included in a prospective longitudinal
484 cohort study conducted in Hospices Civils de Lyon (HCL; Lyon, France). Blood sampling was
485 performed 3 months after the end of the vaccination schedule or the last infection event, and
486 PBMC and serum samples were collected and stored. Written informed consent was obtained
487 from all participants; ethics approval was obtained from the national review board for
488 biomedical research in April 2020 (Comité de Protection des Personnes Sud Méditerranée I,
489 Marseille, France; ID RCB 2020-A00932-37), and the study was registered on ClinicalTrials.gov
490 (NCT04341142).¹⁹ The inclusion criteria for this study were as follows: (i) seropositivity for
491 anti-SARS-CoV-2 Abs or record of COVID-19 vaccination; (ii) age > 18; (iii) written consent ; and
492 (iv) affiliation to social security. The exclusion criteria of this study were pregnancy or lactation
493 for women.

494 COVID-19-naive vaccinated individuals were from two different cohorts. The first cohort,
495 COVIMMUNITY, aimed to characterize the immune response in HCWs. Ethics approval was
496 obtained from the national review board (ClinicalTrials.gov: NCT04648709).⁴⁸ The second
497 cohort, ABCOVID, aimed to study the kinetics of COVID-19 antibodies in patients with

498 confirmed SARS-CoV-2 infection as well as the kinetics of neutralizing antibodies post
499 vaccination.⁴⁹ This study was approved by the ILE DE FRANCE IV ethical committee
500 (ClinicalTrials.gov: NCT04750720). Written informed consent was collected at enrolment for
501 all subjects.

502 The occurrence of an infection, since the start of the COVID-19 pandemic and up until the
503 Omicron BA.1 wave, was monitored in our cohorts by assessing anti-RBD IgG titers through
504 longitudinal blood sampling. Additionally, HCWs underwent frequent COVID-19 testing due to
505 hospital regulations. A breakthrough infection was identified by a rebound of anti-RBD Ab
506 titers thus excluding the subject from the present study.

507 All individuals in the three cohorts received either the BNT162b2 or the mRNA-1273 vaccine.
508 SARS-CoV-2 infection before vaccination occurred during the Wuhan Hu-1 wave of the
509 pandemic, before the emergence of variants of concern (VOCs). SARS-CoV-2 infection after
510 vaccination occurred during the Omicron BA.1 wave.⁸ All selected individuals were divided
511 into 5 groups, according to their infection and vaccination history. Each group is composed of
512 15 individuals.

513

514 **METHOD DETAILS**

515 **Quantification of IgG titers**

516 Serum samples were immediately stored at -80°C after blood sampling. Anti-RBD IgG were
517 measured using the bioMérieux Vidas SARS-CoV-2 IgG II (9COG) diagnosis kit (BioMérieux,
518 #424114), according to the manufacturer's recommendation. For standardization of these
519 assays to the first WHO international standard, the concentrations were transformed in
520 Binding Antibody Unit (BAU)/mL using the conversion factors provided by the manufacturer.
521 Anti-N IgG were quantified using the Abbott Architect instrument using the Abbott SARS-CoV-
522 2 IgG assay (ref. 6R86-20) following the manufacturer's instructions. The results are expressed
523 as an index.

524 **Anti-RBG IgG avidity**

525 Avidity of RBD-specific IgG from patient sera is based on off-rate constant (K_{off} , s^{-1})
526 measurement because of its inverse relationship with the stability of the Ag/Ab complex,
527 independent of antibody concentration. Total IgG were purified from 100 μ L of each individual
528 serum with Magne Protein G Beads (Promega, #G7471) according to manufacturer's
529 instructions. BLI studies were performed on purified IgG with an Octet R8 instrument
530 (Sartorius) using Octet[®] amine-reactive biosensors (AR2G; Sartorius, #18-5092). Kinetics
531 assays were carried out at 30°C using Standard Kinetics Acquisition rate settings (5.0 Hz,
532 averaging by 20, 1000 rpm). Briefly, after hydration in water, AR2G sensors were activated 10
533 min in 20 mM EDC and 10 mM s-NHS before antigen loading. RBD-His pre-fusion S (Sino
534 Biological, cat 40592-V08B), 10 μ g/ml in 10 mM sodium acetate pH6 was amine coupled to
535 AR2G sensors during 10 min using Octet[®] amine-coupling reagents (Sartorius, #18-5095).

536 Then, the sensor surface was inactivated with a solution of 1 M ethanolamine pH 8.5. RBD
537 loaded AR2G sensors were dipped in 1X PBS (pH7.4) for 2 min to establish a baseline time
538 course before 10 min association in purified serum IgG. Purified IgG were assayed from
539 undiluted to diluted 1:50 in PBS depending on RBD-specific IgG titers of each sample. The
540 dissociation step was monitored for 10 min by dipping sensors in 1X PBS into the wells used
541 to collect the baseline. Dissociation rates were determined using Octet Analysis studio 12.2
542 software by Local full fitting of association and dissociation steps according to a 1:1 binding
543 model.

544 **Anti-S and anti-N IgG subtyping and anti-N FcγR binding**

545 A custom SARS-CoV-2 multiplex assay was designed with the Wuhan Hu-1 S (Sino Biological,
546 #40591-V08H) and N (Sino Biological, #40588-V08B) and the BA.1 S (Sino Biological, #40589-
547 V08H30) antigens. Tetanus toxoid (Sigma Aldrich, #582231) and influenza hemagglutinin
548 H1Cal2009 (Sino Biological, #11085-V08H) were also added to the assay as positive controls,
549 while BSA-blocked beads were included as negative controls. The antigens were covalently
550 coupled to Bio-Plex Pro Magnetic COOH beads (Bio-Rad) using a two-step carbodiimide
551 reaction as per manufacturer's instructions, in a ratio of 10 million beads-to-100 μg of antigen.
552 The antigen-coupled beads were resuspended in a storage buffer (PBS, 0.05% sodium azide)
553 as one million beads per 100 μL, and stored in the dark at 4°C before use.

554 The IgG1-4 subclasses of antigen-specific antibodies present in the collected serum were
555 assessed using a multiplex assay as previously described.⁵⁰ Briefly, the antigen-coupled beads
556 were mixed, resuspended in 0.1% PBS-BSA and added to wells of a 96-well Bio-Plex Pro flat
557 bottom plate (Bio-Rad, #171025001) containing serum in a 1:100 final dilution in 0.1% PBS-
558 BSA. The sealed plates were incubated on a plate shaker overnight at 4°C. After incubation,
559 the plates were centrifuged and washed with PBS-0.05% Tween 20 using a magnetic Bio-Plex
560 Pro wash station (Bio-Rad, #30034376).

561 For IgG subtyping, mouse anti-human IgG1, IgG2, IgG3, IgG4 antibodies conjugated with
562 phycoerythrin (PE, Southern Biotech) were added at a final concentration of 1.3 μg/mL and
563 the mixture was incubated for 2h at room temperature on a plate shaker. For the detection
564 of antigen-specific FcγR binding, soluble recombinant FcγRIIa (higher affinity polymorphisms
565 FcγRIIa-H131) and FcγRIIIa (higher affinity polymorphisms FcγRIIIa-V158) dimers were
566 biotinylated and added at a final concentration of 1.3 μg/mL.⁵¹ After incubation at room
567 temperature for 2h on a plate shaker, the plate was washed with PBS-0.05% Tween 20, and
568 streptavidin-PE conjugate (SAPE, Invitrogen) was added at 1 μg/ml for another 2h incubation
569 time on a plate shaker.

570 After the last washing with PBS-0.05% Tween 20, the beads were resuspended in MAGPIX®
571 Drive Fluid PLUS (Luminex, #40-50030), the plates were read on a Bio-Plex MAGPIX® multiplex
572 reader (Bio-Rad), and analyzed using Bio-Plex Manager software (Bio-Rad). The binding of the
573 PE-detectors was measured to calculate the median fluorescence intensity (MFI). Double

574 background subtraction was conducted, first removing background of blank (buffer only) wells
575 followed by removal of BSA-blocked control bead background signal for each well.

576 IgG subtype quantifications are relative and thus the method does not allow for comparisons
577 between IgG subtype levels.

578 **Live virus neutralization experiments**

579 A 50% Plaque Reduction Neutralization Test (PRNT₅₀) was used for the detection and titration
580 of neutralizing Abs as previously described.^{2,52} A 10-fold dilution of each serum specimen in
581 Dulbecco's Modified Eagle's (DMEM) high glucose (Sigma-Aldrich, #D6429) culture medium
582 was first heated for 30 min at 56°C to avoid complement-linked reduction of the viral activity.
583 Two percent of heat inactivated Fetal Bovine Serum (FBS, Eurobio scientific, #CVFSF06-01) and
584 1% of penicillin (10,000 UI/mL) and streptomycin (10,000 UI/mL) (Eurobio scientific,
585 #CABPES01-0U) were added to the culture medium. Serial 2-fold dilutions (tested in duplicate)
586 of the serum specimens in culture medium were mixed in equal volume with the live SARS-
587 CoV-2 virus. After gentle shaking and a contact of 30 min at room temperature in plastic
588 microplates, 150 µL of the mix was transferred into 96-well microplates covered with Vero E6
589 cells (ATCC CRL-1586). The plates were incubated at 37°C in a 5% CO₂ atmosphere. Infection
590 efficiency was evaluated by microscopy 5 days later when the cytopathic effect of the virus
591 control reached 100 to 500 Tissue Culture Infectious Dose (TCID₅₀/150 µL). Neutralization was
592 recorded if more than 50% of the cells present in the well were preserved. The neutralizing
593 titer was expressed as the inverse of the higher serum dilution that exhibited neutralizing
594 activity; a threshold of 20 was used (PRNT₅₀ titer ≥20). All experiments were performed in a
595 biosafety level 3 laboratory. The different viral strains that were used were sequenced and
596 deposited on GISAID [GISAID accession numbers: EPI_ISL_1707038, 19A (B.38);
597 EPI_ISL_7608613, Omicron BA.1 (B.1.1.529), EPI_ISL_12396843, BA.4; EPI_ISL_12852091,
598 BA.5 and EPI_ISL_16335276, XBB.1.5.

599 **Antibody dependent cellular cytotoxicity (ADCC)**

600 ADCC was quantified using the ADCC Reporter Bioassay (Promega) as previously described.⁵³
601 Briefly, 293T cells stably expressing the indicated S proteins (3x10⁴ per well) were co-cultured
602 with Jurkat-CD16-NFAT-rLuc cells (3x10⁴ per well) in presence or absence of mAbs at the
603 indicated concentration. Luciferase was measured after 18 h of incubation using an EnSpire
604 plate reader (PerkinElmer). ADCC was measured as the fold induction of Luciferase activity
605 compared to the "no serum" condition. Sera were tested at a 1:30 dilution. For each serum,
606 the control condition (cells transduced with an empty vector) was subtracted to account for
607 interindividual variations of the background. We previously reported correlations between
608 the ADCC Reporter Bioassay titers and an ADCC assay based on primary NK cells and cells
609 infected with an authentic virus.⁵⁴

610 **Flow cytometry analysis of SARS-CoV-2 RBD-specific B cells**

611 Flow cytometry was carried out as previously described.² Cryopreserved PBMCs were thawed,
612 then centrifuged and suspended in PBS + 0.5% FBS and 2 mM EDTA (PEB buffer) and incubated
613 with Fc receptor block (Miltenyi Biotec 130-059-901, 1:10 dilution) for 15 minutes at 4°C. Cells
614 were then washed in PEB and stained for 30 minutes in brilliant stain buffer at 4°C in the dark
615 using the following Abs: anti-CD45-PerCP (BD Biosciences, ref. 368506, 1:10 dilution), anti-
616 CD3-APC Fire 810 (BioLegend, ref. 344858, 1:10 dilution), anti-CD19-PE Vio770 (Miltenyi
617 Biotec, ref. 130-113-170, 1:25 dilution), anti-CD20-BV421 (BD Biosciences, ref. 562873, 1:10
618 dilution), anti-CD27-PerCPVio700 (Miltenyi Biotec, ref. 130-113-632, 1:25 dilution), anti-IgM-
619 PE- CF594 (BD Biosciences, ref. 562539, 1:20 dilution), anti-IgD-BV605 (BioLegend 348232,
620 1:25 dilution) together with both the PE- and APC-conjugated recombinant Wuhan Hu-1 RBD
621 tetramers as well as BUV737- and BB515-conjugated recombinant BA.1 RBD tetramers.
622 Fluorescent SARS-CoV-2 RBD tetramers were prepared as follows. Biotinylated recombinant
623 RBD domains of SARS-CoV-2 (Wuhan Hu-1 and BA.1 strains) were purchased from Miltenyi
624 Biotec (Wuhan Hu-1 strain ref. 130-127-457, BA.1 strain ref. 130-130-419) and tetramerized
625 either with streptavidin-PE (BD Biosciences ref. 554061), streptavidin-allophycocyanin (APC)
626 (BioLegend, ref. 405243), streptavidin-BUV737 (BD Biosciences ref. 612775) or with
627 streptavidin-BB515 (BD Biosciences ref. 564453). PE and APC were used to label Hu-1 RBD
628 while BUV737 and BB515 were used to label BA.1 RBD. The identification of mBCs was based
629 on the exclusion of IgD⁺ CD27⁻ naive B cells (Figure S3A). Gating of PE and APC double positive
630 mBCs enabled the identification of Hu-1 RBD-specific mBCs while gating of BUV737 and BB515
631 double positive mBCs enabled the identification of BA.1 RBD + mBCs (Figure S3B and S3C
632 respectively).

633

634 **QUANTIFICATION AND STATISTICAL ANALYSIS**

635 Comparison of clinical parameters between all groups was carried out using a Kruskal-Wallis
636 or a Fisher's exact test, both followed by Bonferroni's test for multiple comparisons. For the
637 comparisons of immune parameters between the Hu-1/2RNA/BA.1 and the 3RNA/BA.1
638 groups, multiple regressions (ANCOVA) were realized for each variable, incorporating age as
639 a covariate, given the age difference between the two groups, using R software. When
640 comparing immune parameters between the 3RNA/BA.1 and 3RNA groups and between the
641 Hu-1/2RNA/BA.1, Hu-1/2RNA and Hu-1/3RNA groups, a two-way ANOVA mixed model was
642 conducted followed by a Bonferroni's test for multiple comparisons. Comparisons of the
643 percentage of RBD⁺ mBCs between the 3 groups with established hybrid immunity were
644 conducted using the Kruskal-Wallis test followed by Dunn's multiple comparisons test.
645 Comparisons for immune parameters between all 5 groups were carried out using a Kruskal-
646 Wallis test followed by a Dunn's multiple comparisons test. Analyses were conducted using
647 GraphPad Prism[®] software (version 8; GraphPad software) and R software ([https://cran.r-](https://cran.r-project.org/)
648 [project.org/](https://cran.r-project.org/)). Exact p-values are represented on all graphs. All statistical details can be found
649 in the figure legends.

650 KEY RESOURCES TABLE

651

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse anti-Human IgG1 Fc-PE	Southern Biotech	Cat#9054-09; RRID: AB_2796628
Mouse anti-Human IgG2 Fc-PE	Southern Biotech	Cat#9070-09; RRID: AB_2796639
Mouse anti-Human IgG3 Hinge-PE	Southern Biotech	Cat#9210-09; RRID: AB_2796701
Mouse anti-Human IgG4 Fc-PE	Southern Biotech	Cat#9200-09; RRID: AB_2796693
anti-IgG AlexaFluor647	Jackson ImmunoResearch	Cat#A-21445
Anti-CD3 APC Fire 810	Biolegend	Ref# 344858
Anti-CD19 PE-Vio770	Miltenyi	Ref# 130-113-170
Anti-CD20 BV421	BD	Ref# 562873
Anti-CD27 PerCP-Vio® 700	Miltenyi	Ref# 130-113-632
Anti-CD45 PerCP	Biolegend	Ref# 368506
Anti-IgM PE-CF594	BD	Ref# 562539
Anti-IgD BV605	Biolegend	Ref# 348232
Fixable Viability Dye eFluor™ 780	ebiosciences	65-0865-18
Bacterial and virus strains		
Biological samples		
Chemicals, peptides, and recombinant proteins		
SARS-CoV-2 (2019-nCoV) Spike Protein (RBD, His Tag)	Sino Biological	Cat #40592-V08B
Tetanus toxoid	Sigma Aldrich	Cat#582231
Influenza hemagglutinin H1Cal2009	Sino Biologicals	Cat#11085-V08H
Wuhan Hu-1 Spike	Sino Biologicals	Cat#40591-V08H
Wuhan Hu-1 Nucleocapsid	Sino Biologicals	Cat#40588-V08B
BA.1 Spike	Sino Biologicals	Cat#40589-V08H30
FcγRIIa-H131 dimer	Wines et al. ⁴⁵	N/A
FcγRIIIa-V158 dimer	Wines et al. ⁴⁵	N/A
Hoechst 33342	Invitrogen	Cat#H3570
Paraformaldehyde 4%	Alfa Aesar	Cat#J19943.K2

Recombinant SARS-CoV-2 RBD (HEK)-Biotin 50ug	Miltenyi	130-127-457
Recombinant SARS-CoV-2 RBD B.1.1.529/BA.1 omicron (HEK)-Biotin 50ug	Miltenyi	130-130-419
Critical commercial assays		
Magne Protein G Beads	Promega	Cat #G7471
Octet® amine-reactive biosensors, AR2G	Sartorius	Cat #18-5092
Octet® amine-coupling reagents	Sartorius	Cat #18-5095
ADCC Reporter Bioassay	Promega	Cat#G7010
Bright-Glo Luciferase Assay System	Promega	Cat#E2620
Deposited data		
Raw and analyzed data	Mendeley data	https://data.mendeley.com/datasets/8tpxx5cx dg/1
Experimental models: Cell lines		
Vero E6	ATCC	Cat#CRL-1586
293T	ATCC	Cat#CRL-3216
Experimental models: Organisms/strains		
Oligonucleotides		
Recombinant DNA		
pLV-Empty-PuroR	This paper	N/A
pLV- Spike(D614G)-PuroR	This paper	N/A
pLV- Spike(BA.1)-PuroR	This paper	N/A
pLV- Spike(BA.4/5)-PuroR	This paper	N/A
Software and algorithms		
Octet Analysis studio 12.2 software	Sartorius	Octet® Software Download Sartorius
Harmony High-Content Imaging and Analysis Software	PerkinElmer	Cat#HH17000012
Excel 365	Microsoft	https://www.microsoft.com/en-ca/microsoft-365/excel

Prism 8	Graphpad	https://www.graphpad.com/
FlowJo v10	Tree Star	https://www.flowjo.com/
R software v4.4.1	R Foundation for Statistical Computing	https://www.r-project.org/
Other		
Octet R8 instrument	Sarorius	OCTET-R8
Bio-Plex Pro Magnetic COOH beads	Bio-Rad	Cat#MC100XX-01
Streptavidin-PE conjugate	Invitrogen	Cat#S866
PE Streptavidin	BD	554061
APC Streptavidin	BD	554067
BUV737 Streptavidin 100ug	BD	612775
BB515 Streptavidin 0,1mg	BD	564453
Brilliant Stain Buffer	BD	566349
FcR Blocking Reagent human	Miltenyi	130-059-901

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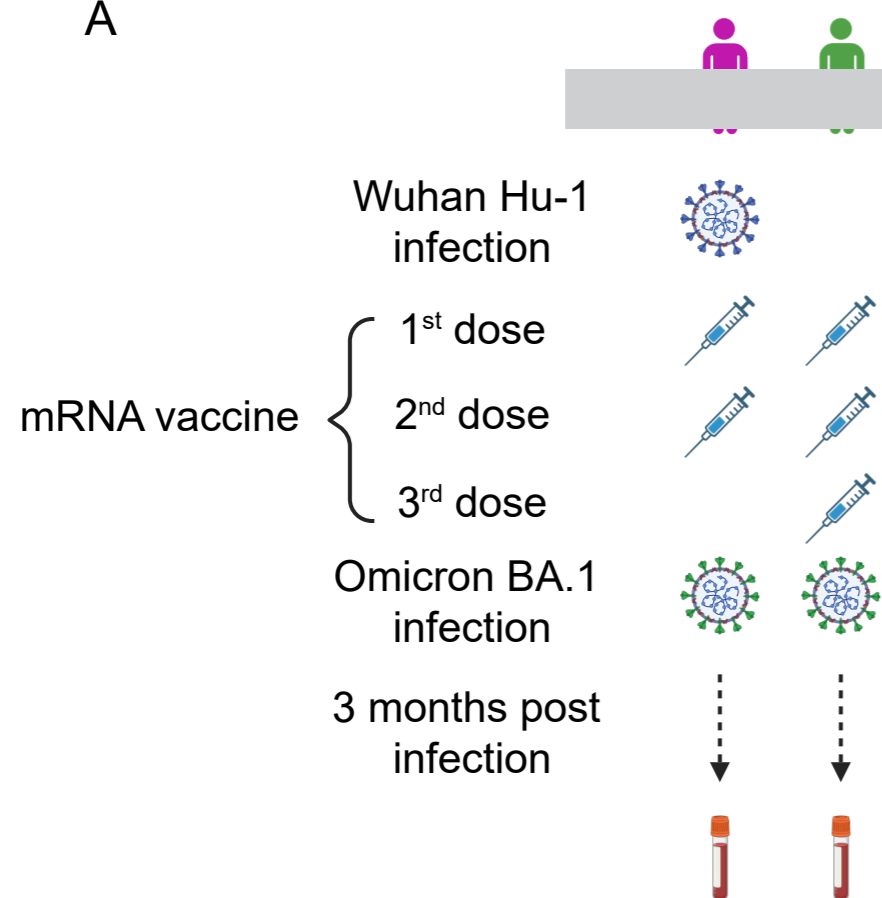
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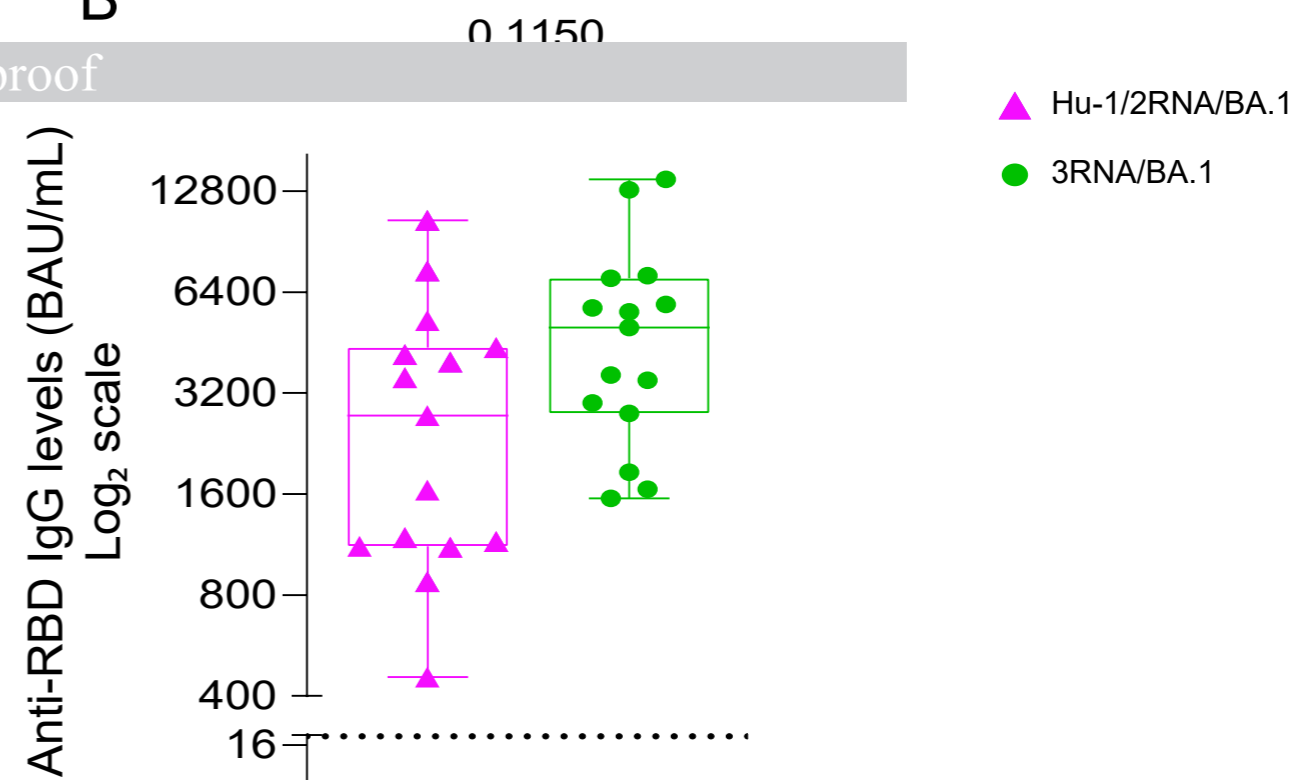
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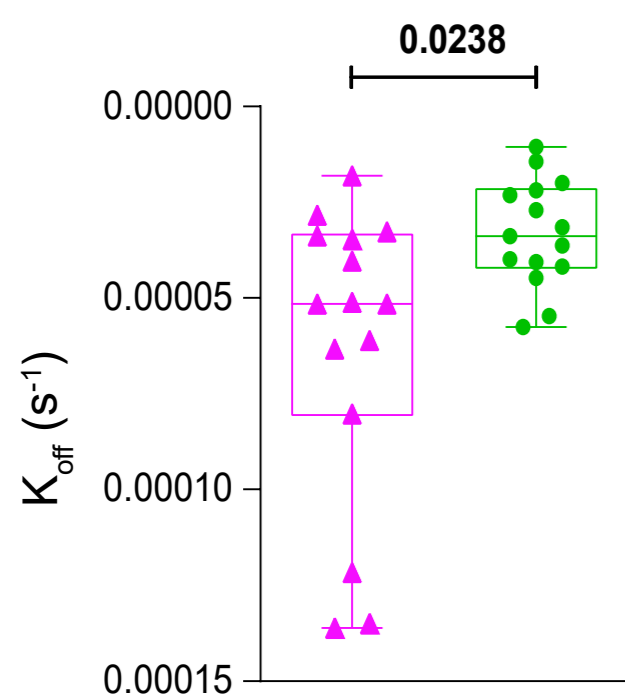
A



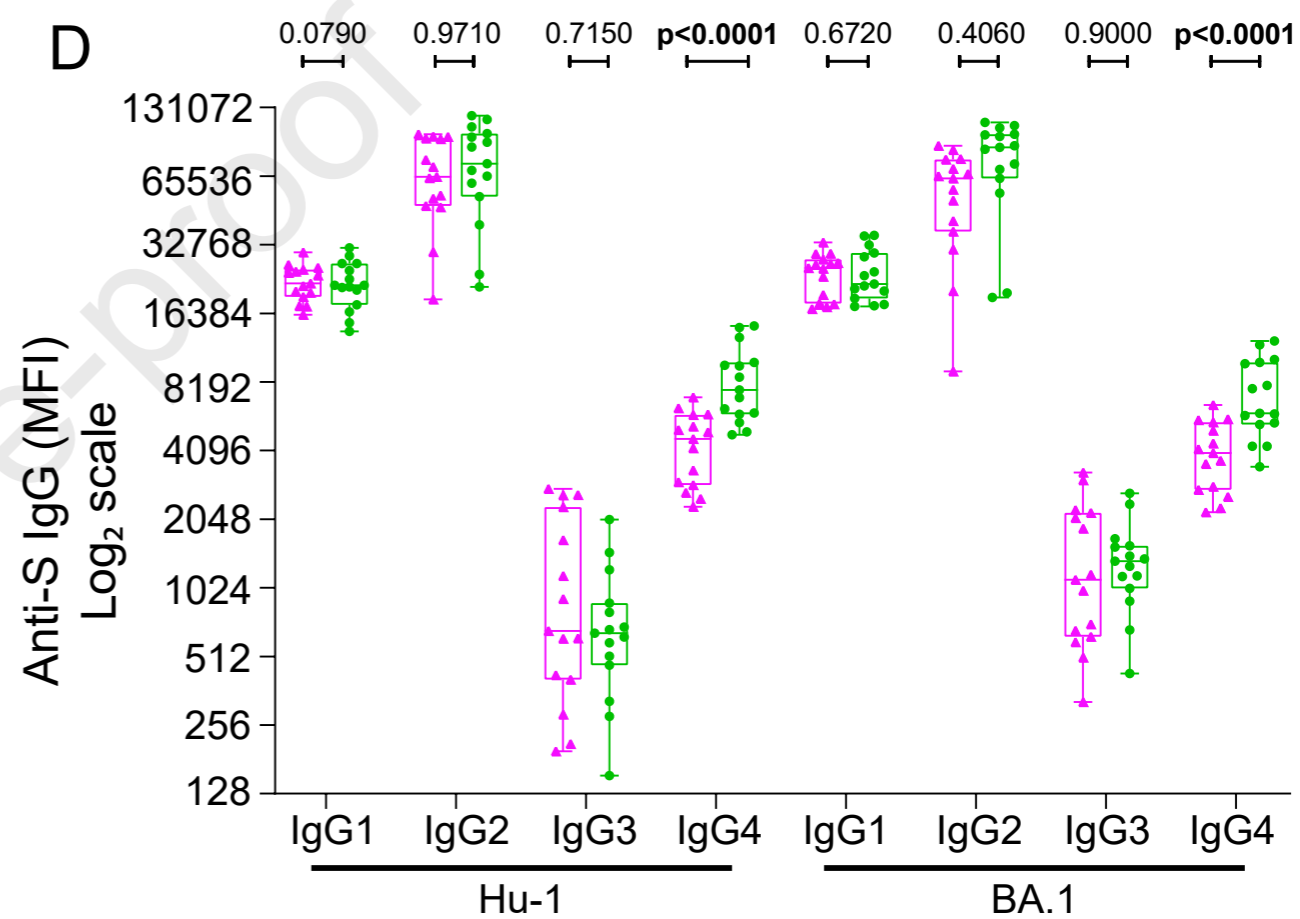
B



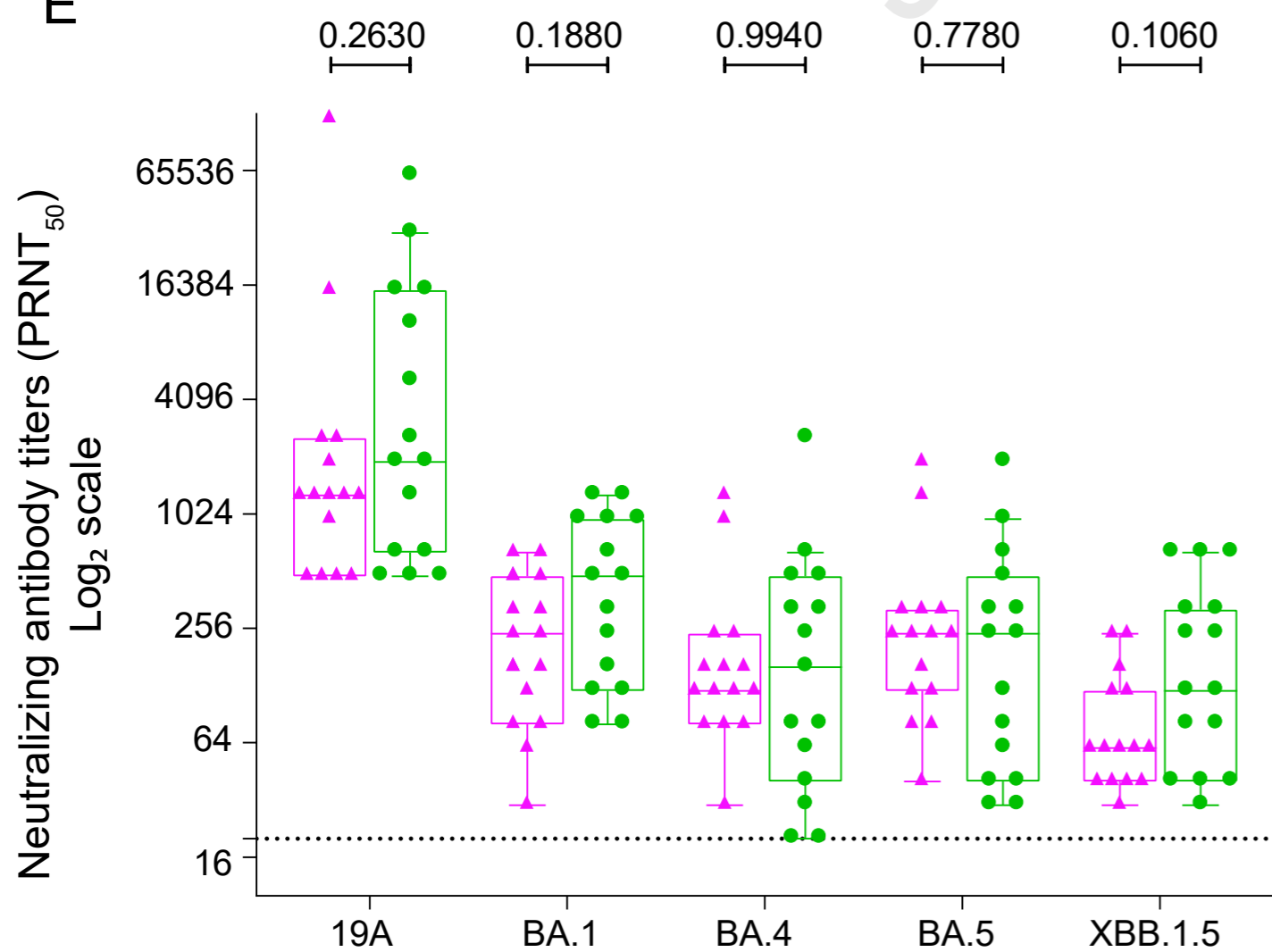
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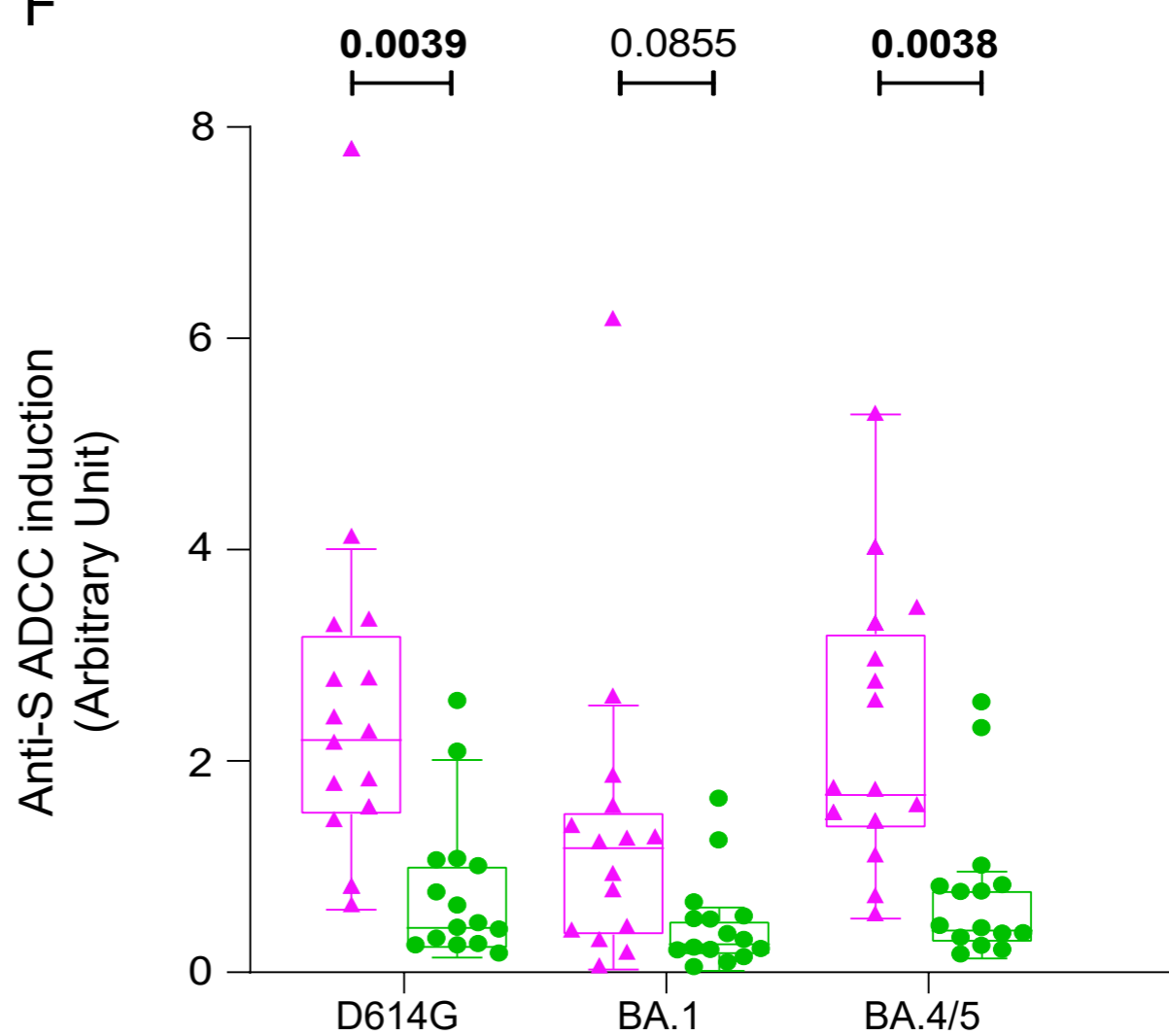
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E

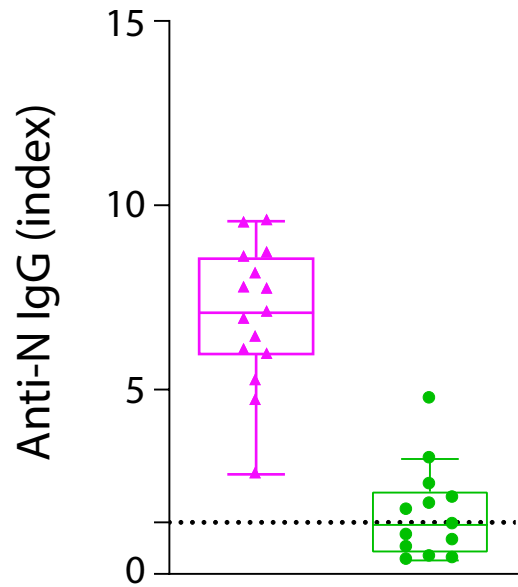


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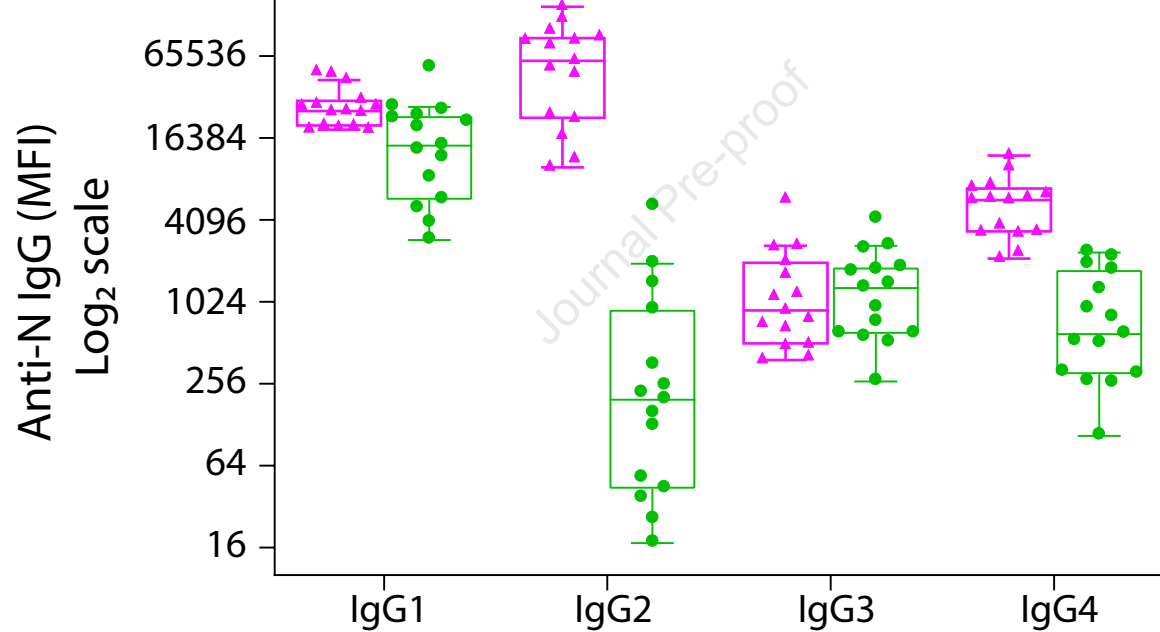


▲ Hu-1/2RNA/BA.1 ● 3RNA/BA.1

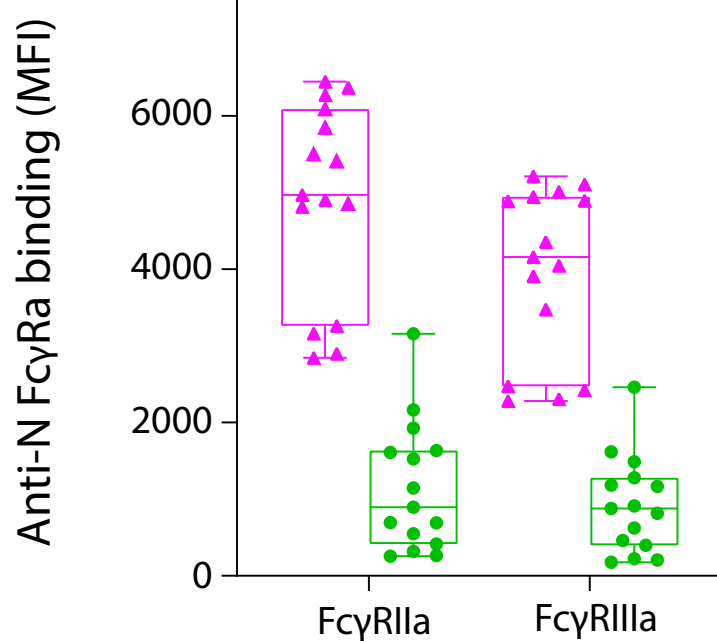
A



B



C



A



Wuhan Hu-1



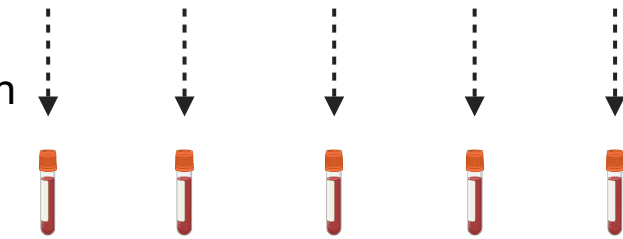
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1st dose2nd dose3rd dose

Omicron BA.1 infection



3 months post infection or vaccination



B

1 2 3



p. adjusted

1 vs 2 1 vs 3

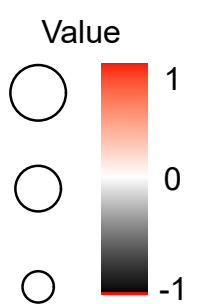
	1	2	3	1 vs 2	1 vs 3
Anti-RBD IgG				>0.9999	0.8066
K_{off} (s^{-1})				>0.9999	>0.9999
IgG1-Hu-1 S				>0.9999	>0.9999
IgG2-Hu-1 S				0.2452	>0.9999
IgG3-Hu-1 S				0.1612	0.2434
IgG4-Hu-1 S				0.0414	>0.9999
IgG1-BA.1 S				>0.9999	>0.9999
IgG2-BA.1 S				>0.9999	>0.9999
IgG3-BA.1 S				0.4032	0.7006
IgG4-BA.1 S				0.4673	>0.9999
PRNT ₅₀ -19A				>0.9999	>0.9999
PRNT ₅₀ -BA.1				>0.9999	0.0123
PRNT ₅₀ -BA.4				>0.9999	0.6354
PRNT ₅₀ -BA.5				>0.9999	0.3724
PRNT ₅₀ -XBB.1.5				0.9812	>0.9999
ADCC-D614G S				>0.9999	>0.9999
ADCC-BA.1 S				>0.9999	>0.9999
ADCC-BA.4/5 S				>0.9999	>0.9999
Anti-N IgG				<0.0001	<0.0001
IgG1-N				0.0003	<0.0001
IgG2-N				0.0003	0.0002
IgG3-N				0.1941	0.0382
IgG4-N				<0.0001	<0.0001
FcγRIIA-N				<0.0001	<0.0001
FcγRIIIA-N				<0.0001	<0.0001

C

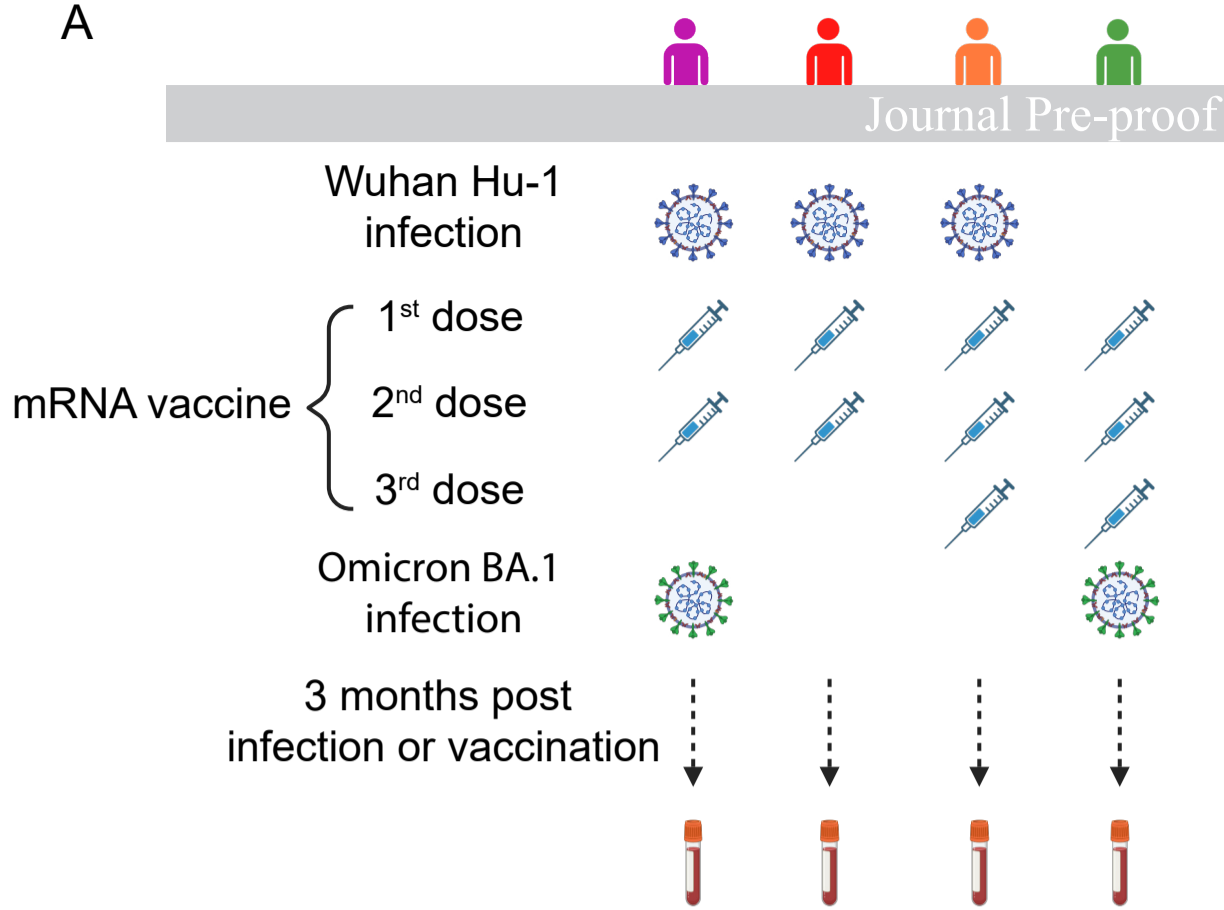


p. adjusted

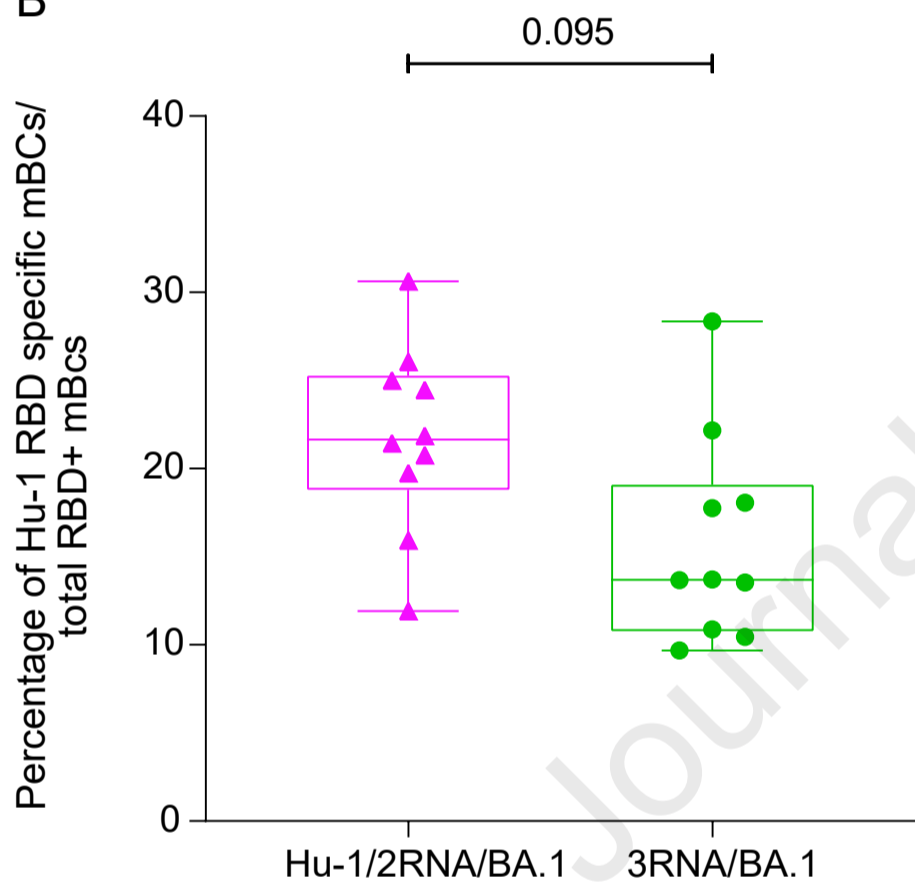
	4	5	p. adjusted
Anti-RBD IgG			0.0947
IgG1-Hu-1 S			>0.9999
IgG2-Hu-1 S			>0.9999
IgG3-Hu-1 S			>0.9999
IgG4-Hu-1 S			0.0097
IgG1-BA.1 S			>0.9999
IgG2-BA.1 S			0.0268
IgG3-BA.1 S			>0.9999
IgG4-BA.1 S			0.0052
PRNT ₅₀ -19A			>0.9999
PRNT ₅₀ -BA.1			0.0542
PRNT ₅₀ -BA.4			>0.9999
PRNT ₅₀ -BA.5			0.8820
PRNT ₅₀ -XBB.1.5			0.0457
ADCC-D614G S			>0.9999
ADCC-BA.1 S			>0.9999
ADCC-BA.4/5 S			>0.9999
IgG1-N			0.0990
IgG2-N			>0.9999
IgG3-N			0.2868
IgG4-N			>0.9999
FcγRIIA-N			>0.9999
FcγRIIIA-N			0.0999



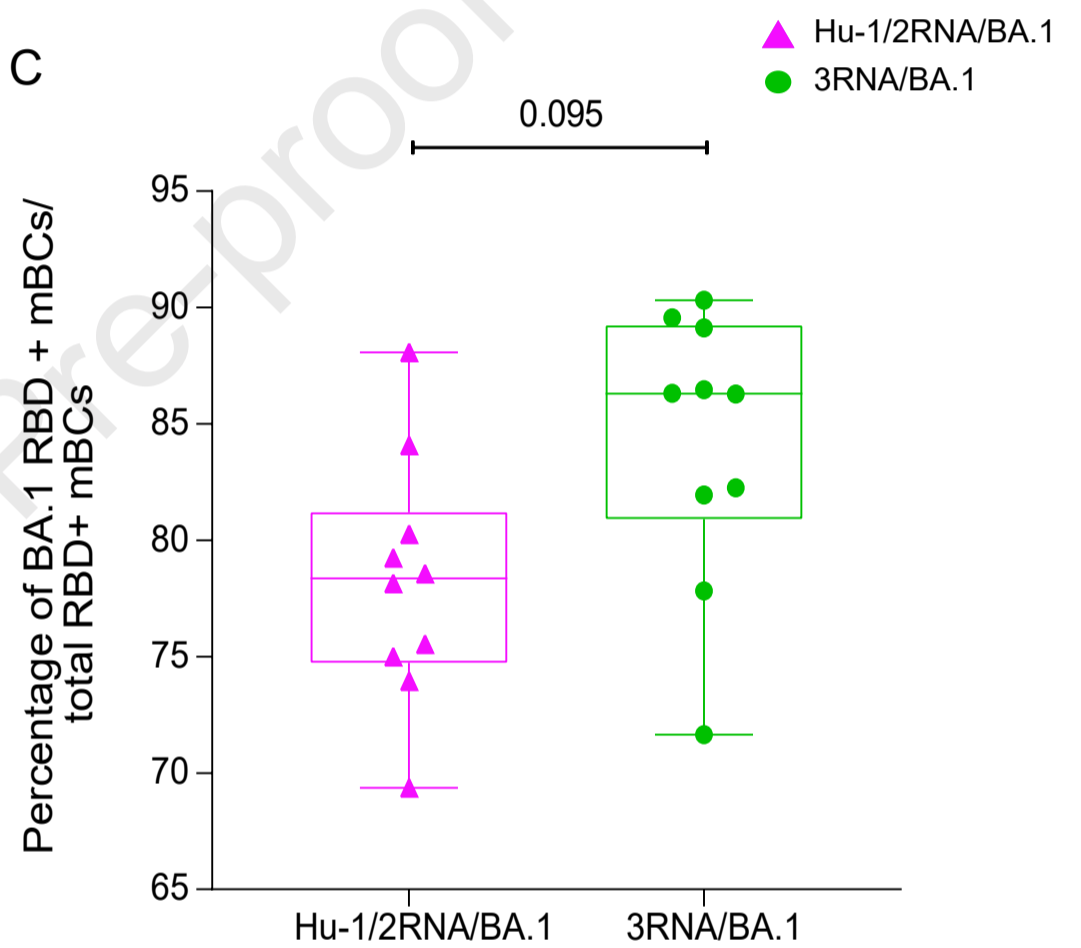
A



B



C

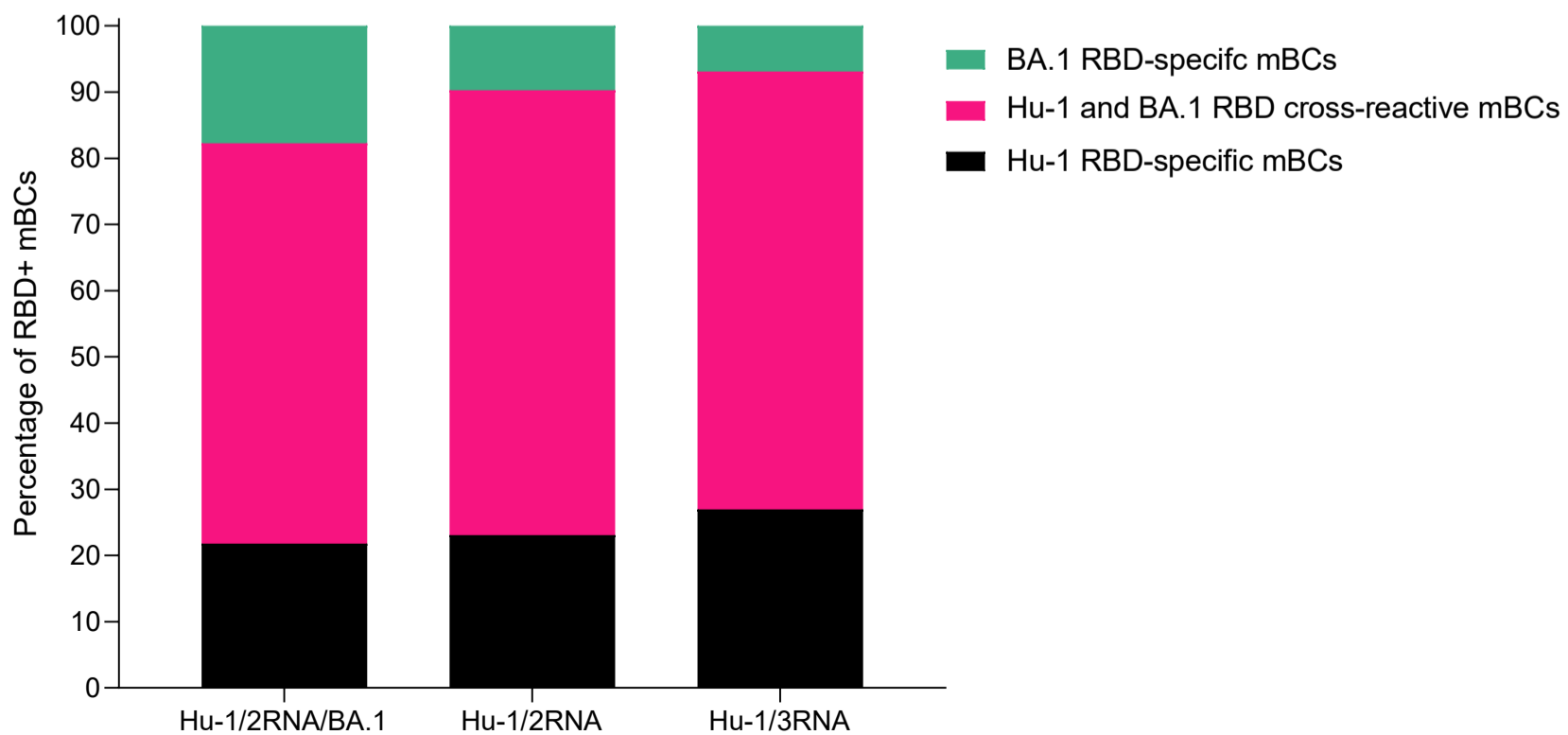


p>0.05

p>0.05

p>0.05

D



Highlights

- Hybrid immunity prevents decrease in the anti-S ADCC response post BA.1 infection
- Hybrid immunity limits increase in anti-S IgG4 levels post BA.1 infection
- Hybrid immunity boosts anti-N response despite anti-S imprinting post BA.1 infection

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