

1 **Maturation of SARS-CoV-2 Spike-specific memory B cells drives resilience to viral**  
2 **escape**

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42 **SUMMARY**

43 Memory B cells (MBCs) generate rapid antibody responses upon secondary encounter with a  
44 pathogen. Here, we investigated the kinetics, avidity and cross-reactivity of serum antibodies  
45 and MBCs in 155 SARS-CoV-2 infected and vaccinated individuals over a 16-month  
46 timeframe. SARS-CoV-2-specific MBCs and serum antibodies reached steady-state titers with  
47 comparable kinetics in infected and vaccinated individuals. Whereas MBCs of infected  
48 individuals targeted both pre- and postfusion Spike (S), most vaccine-elicited MBCs were  
49 specific for prefusion S, consistent with the use of prefusion-stabilized S in mRNA vaccines.  
50 Furthermore, a large fraction of MBCs recognizing postfusion S cross-reacted with human  
51 betacoronaviruses. The avidity of MBC-derived and serum antibodies increased over time  
52 resulting in enhanced resilience to viral escape by SARS-CoV-2 variants, including Omicron  
53 BA.1 and BA.2 sub-lineages, albeit only partially for BA.4 and BA.5 sublineages. Overall, the  
54 maturation of high-affinity and broadly-reactive MBCs provides the basis for effective recall  
55 responses to future SARS-CoV-2 variants.

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57

58 **KEYWORDS**

59 Coronavirus, COVID-19, SARS-CoV-2, Spike, variants of concern, memory B cells, affinity  
60 maturation, cross-reactivity, antibodies, mRNA-vaccine

61

## 62 INTRODUCTION

63 Since its appearance in 2019, severe acute respiratory syndrome coronavirus 2 (SARS-  
64 CoV-2) has rapidly spread worldwide resulting in more than 500 million infections and 6.2  
65 million deaths. The virus has evolved into variants of concern (VOC), including the currently  
66 circulating Omicron (B.1.1.529) sublineages, which have infected many convalescent and  
67 vaccinated individuals (Tegally et al., 2022; Viana et al., 2022). These VOC have accrued  
68 several mutations, in particular in the Spike (S), resulting in the reduction or complete loss of  
69 neutralizing activity of polyclonal serum and monoclonal antibodies of individuals who were  
70 infected or vaccinated with the prototypic SARS-CoV-2 S (Bowen et al., 2022a; Cameroni et  
71 al., 2022; Cele et al., 2021; Chen et al., 2021; Collier et al., 2021; Garcia-Beltran et al., 2021;  
72 Hoffmann et al., 2020; McCallum et al., 2022; McCallum et al., 2021; Meng et al., 2022; Rees-  
73 Spear et al., 2021; Shen et al., 2021; Supasa et al., 2021; Walls et al., 2022; Wang et al., 2021a;  
74 Wang et al., 2022; Zhou et al., 2021). Besides serum antibodies, memory B cells (MBCs)  
75 induced by infection or vaccination play a major role in humoral immunity through recall  
76 responses to a second encounter with the same or a related pathogen. Although several studies  
77 reported a progressive decline of serum antibody titers over time in convalescent and  
78 vaccinated individuals, SARS-CoV-2-specific MBCs have been shown to increase or remain  
79 stable in number and to produce neutralizing antibodies (Dan et al., 2021; Gaebler et al., 2021;  
80 Goel et al., 2021a; Goel et al., 2021b; Rodda et al., 2021; Roltgen et al., 2020; Sokal et al.,  
81 2021b; Walls *et al.*, 2022).

82 In this study, we performed single-MBC repertoire analysis from longitudinal samples  
83 of convalescent or healthy individuals receiving up to three doses of the Pfizer/BioNtech  
84 BNT162b2 mRNA vaccine. We found that, while SARS-CoV-2-specific serum antibodies  
85 waned, MBCs increased progressively in frequency and avidity, reaching steady-state levels  
86 that remained stable up to 16 months after infection or after the third vaccine dose. Vaccination-  
87 induced MBCs mainly targeted the prefusion SARS-CoV-2 S conformation, while infection-  
88 induced MBCs recognized also the postfusion conformation and cross-reacted with the S of  
89 human betacoronaviruses HCoV-HKU1 and HCoV-OC43. We show that the increased avidity  
90 of MBC-derived antibodies provides a mechanism of resilience against emerging variants,  
91 including Omicron BA.1 and BA.2 sublineages.

92

## 93 RESULTS

### 94 Progressive maturation of MBCs and serum antibodies following SARS-CoV-2 infection

95 Blood samples were collected from 64 individuals diagnosed with COVID-19 between  
96 March and November 2020 and from 13 individuals diagnosed between December 2020 and  
97 January 2021 after an outbreak of the SARS-CoV-2 Alpha variant (**Table S1**). Peripheral blood  
98 mononuclear cells (PBMCs) were isolated for antigen-specific memory B cell repertoire  
99 analysis (AMBRA) (Pinna et al., 2009) (**Figure 1A**). PBMCs were stimulated in multiple  
100 cultures with IL-2 and the Toll-like receptor 7/8-agonist R848 to promote the selective  
101 proliferation and differentiation of MBCs into antibody-secreting cells. The culture  
102 supernatants were collected on day 10 and screened in parallel using multiple ELISA assays to  
103 detect antibodies of different specificities and to determine the frequencies of antigen-specific  
104 MBCs expressed as a fraction of total IgG<sup>+</sup> MBCs (**Figures S1A-S1C**).

105 Repertoire analysis of MBCs collected at early time points after infection with SARS-  
106 CoV-2 Wuhan-Hu-1 (13-65 days after symptom onset) showed that 90% of the donors had  
107 detectable MBCs specific for the prefusion-stabilized SARS-CoV-2 S ectodomain trimer  
108 (Walls et al., 2020) (**Figures 1B** and **S1D**). The magnitudes of MBC responses were  
109 heterogenous with frequencies ranging between 0 and 6.6% for S-specific MBCs across  
110 individuals. In most cases RBD-specific MBCs dominated the response to S, whereas NTD-  
111 and S<sub>2</sub>-specific MBCs were present at lower frequencies, concurring with the fact that most  
112 mAbs cloned from the memory B cells of previously infected subjects target the RBD  
113 (McCallum *et al.*, 2021; Piccoli et al., 2020). Overall, S-specific MBCs were present at higher  
114 frequencies than N-specific MBCs (median: 0.59% vs 0.06%, respectively). A similar  
115 magnitude and reactivity of the MBC response was observed in the individuals infected with  
116 the SARS-CoV-2 Alpha variant, with a higher frequency of MBCs specific for RBD carrying  
117 the N501Y mutation as compared to the Wuhan-Hu-1 RBD (**Figure S1E**).

118 By analyzing longitudinal samples collected up to 16 months after infection, we found  
119 that S-, RBD-, NTD-, S<sub>2</sub>- and N-specific MBCs progressively rose in frequency in the first 6-  
120 8 months, reaching up to 20% of total IgG MBCs in some cases, followed by a plateau (Dan *et*  
121 *al.*, 2021). Conversely, the frequency of MBCs specific for the S and N proteins of common  
122 cold coronaviruses remained largely constant over time (**Figure 1C** and **S1F**). The expansion  
123 of RBD-specific MBCs was accompanied by an increase of MBCs producing antibodies that  
124 blocked RBD binding to human ACE2, a correlate of neutralization (Piccoli *et al.*, 2020)  
125 (**Figures 1D** and **S1G**).

126 Analysis of longitudinal serum samples showed that IgG antibodies to SARS-CoV-2 S,  
127 RBD and N progressively decreased and reached a plateau, which paralleled the rise of MBCs,  
128 consistent with previous reports (Achiron et al., 2021; Gaebler *et al.*, 2021; Khoury et al., 2021)  
129 (**Figure 1E**). We observed a modest increase of IgG antibodies recognizing HCoV-HKU1 and  
130 HCoV-OC43 S early after SARS-CoV-2 infection, which rapidly dropped to levels maintained  
131 until the end of the observation period (**Figure 1E**). No correlation was observed between  
132 serum IgG S antibody levels and MBC frequencies in the subjects tested with both assays over  
133 the 500-day period analyzed (**Figure 1F**).

134 The binding avidity of serum- and MBC-derived specific antibodies was expressed as  
135 an avidity index by measuring antibody binding in presence of a chaotropic agent (**Figure**  
136 **S1H**). The avidity of serum IgG antibodies to SARS-CoV-2 S, RBD and N increased over time  
137 reaching levels comparable to those observed for serum IgG antibodies to HKU1 and OC43  
138 antigens (**Figure 1G**). Similarly, the frequency of high-avidity MBC-derived RBD-specific  
139 antibodies increased over time reaching a plateau after approximately 3-to-4 months after  
140 infection (**Figure 1H**).

141 The rapid decline of serum antibodies is consistent with an initial generation of many  
142 short-lived plasma cells from either naïve B cells or cross-reactive MBCs. However, the clonal  
143 analysis of serial samples reveals a rapid expansion of S- and RBD-specific MBCs followed  
144 by a progressive maturation consistent with a germinal center reaction leading to the generation  
145 of plasma cells and MBCs with increased affinity.

146

### 147 **Three mRNA-vaccine doses induce high-avidity MBCs and serum antibodies.**

148 The frequency and fine specificity of MBCs were analyzed after the first, second and  
149 third administration of the Pfizer/BioNtech BNT162b2 mRNA vaccine in two cohorts of  
150 healthy individuals. Vaccine recipients were either naïve (n=46) or immune (n=32) to SARS-  
151 CoV-2 due to infection occurring 53-389 days before the first dose. In most naïve individuals,  
152 the first vaccine dose induced SARS-CoV-2 S-specific MBCs at frequencies comparable to  
153 those found in samples collected from convalescent individuals at similar timepoints post  
154 antigen exposure (**Figure 2A**). The second and third doses resulted in a further 5-fold and 10-  
155 fold increase of median MBC frequency, respectively (**Figure 2A**). As expected, vaccination  
156 of naïve donors did not elicit N-specific MBCs, the few exceptions likely reflecting cross-  
157 reactivity with other betacoronaviruses (**Figure S2A**). Remarkably, the first vaccine dose  
158 induced very high MBC S-specific frequencies in previously infected donors, exceeding by  
159 ~40-fold those found in naïve vaccinated or convalescent individuals. Additional vaccine doses

160 did not result in further MBC increase and did not alter the frequency of MBCs specific for the  
161 S of the common cold coronaviruses HCoV-HKU1 and HCoV-OC43 (**Figures 2B** and **S2B**).

162 After administration of two vaccine doses, the response in both naïve and infected  
163 donors was dominated by RBD-specific MBCs, while MBCs specific for the NTD or the S<sub>2</sub>  
164 subunit were present at low to undetectable levels (**Figures 2C**, **2D** and **S2C**). Interestingly,  
165 NTD-specific, but not S<sub>2</sub>-, MBCs increased over time in naïve donors (**Figure 2C** and **S2C**).  
166 High-avidity RBD-specific and ACE2-blocking antibodies were detected only after the second  
167 dose in naïve individuals, whereas these antibodies were detected after one dose in all the  
168 previously infected donors and were not further boosted upon subsequent immunizations  
169 (**Figure 2E** and **2F**).

170 When we analyzed longitudinal samples of serum antibodies against SARS-CoV-2 S  
171 and RBD, we observed that a single immunization induced highly heterogenous antibody levels  
172 in naïve donors that were further increased in all samples after the second and the third  
173 immunization (**Figures 2G** and **S2D**). In infected donors, the titers of serum antibodies had  
174 reached the maximal level after the first immunization, with no further increase after the second  
175 or the third immunization. The serum titers of S-specific antibodies declined similarly over  
176 time in naïve and infected donors with a half-life of 4 months. As expected, no overall variation  
177 in N-specific antibody titers was observed (**Figure S2E**). While the avidity of SARS-CoV-2  
178 S- and RBD-specific serum antibodies in naïve donors rapidly increased after vaccination, the  
179 avidity in infected donors was found to be high before vaccination with no further increase  
180 over time (**Figures 2I**, **S2F** and **S2G**). In both naïve and infected donors, vaccination did not  
181 increase the presence or avidity of antibodies specific for the S of the human betacoronaviruses  
182 HCoV-HKU1 and HCoV-OC43, concurring with recent data (Walls *et al.*, 2022) (**Figures 2H**,  
183 **2J**, **S2H** and **S2I**).

184 Collectively, these findings indicate that, while in infected donors a single dose of an  
185 mRNA vaccine is sufficient to boost a high-avidity antibody response to SARS-CoV-2 S, in  
186 naïve donors such response is elicited only upon three rounds of immunization.

187

188 **The antibody response in naïve vaccinated individuals is skewed towards prefusion**  
189 **SARS-CoV-2 S.**

190 The Pfizer/BioNtech BNT162b2 mRNA vaccine was designed to express the full-  
191 length SARS-CoV-2 S stabilized in its prefusion conformation through the 2P mutations  
192 (Vogel *et al.*, 2021; Wrapp *et al.*, 2020) and recent data suggest that vaccination induce high  
193 titers of prefusion S-specific plasma antibodies (Bowen *et al.*, 2021). To assess whether

194 vaccination also induced a MBC response preferentially targeting the prefusion conformation  
195 of S as compared to that elicited by natural infection, we analyzed the MBC-derived antibodies  
196 for their binding to either the prefusion-stabilized SARS-CoV-2 S, postfusion S<sub>2</sub>, or both in  
197 cohorts of convalescent donors and in naïve or infected vaccinated individuals (**Figure 3A**).  
198 We found a strong correlation between antibodies binding to postfusion S<sub>2</sub> and a structurally-  
199 validated postfusion SARS-CoV-2 S<sub>2</sub> (Bowen *et al.*, 2021), thus supporting the use of S<sub>2</sub> as a  
200 proxy for the postfusion conformation of S (**Figure S3**). Across all individuals, most MBCs  
201 induced by natural infection and/or vaccination were specific for the prefusion conformation  
202 (**Figure 3B-D**). However, while convalescent and infected vaccinated individuals had 10% and  
203 5% of their MBCs specific for S<sub>2</sub>, respectively, only 1% of MBCs from naïve vaccinated donors  
204 were postfusion S<sub>2</sub>-specific. A fraction of MBCs recognized S epitopes shared between  
205 prefusion and postfusion conformations. Collectively, these data show that mRNA vaccines  
206 primarily induce MBC responses skewed to the prefusion conformation of SARS-CoV-2 S.

207

### 208 **Infection- and vaccine-induced MBCs show variable degrees of cross-reactivity against** 209 **other betacoronaviruses.**

210 Unlike serological analyses, the AMBRA method is suitable to dissect the cross-  
211 reactivity of individual MBC-derived antibodies, at the single clone level, against a panel of  
212 human and animal betacoronaviruses (Forster *et al.*, 2020). Analysis of MBCs from SARS-  
213 CoV-2-convalescent and from naïve or infected donors receiving two vaccine doses revealed  
214 a high frequency (35-52%) of SARS-CoV-2 S-specific MBCs that cross-reacted with SARS-  
215 CoV S, consistent with the high level of S sequence similarity with SARS-CoV-2 (**Figures 4A**  
216 **and 4B**). Conversely, we observed a low frequency (2-19%) of SARS-CoV-2 S-specific MBCs  
217 that cross-reacted with the more divergent S of MERS-CoV, HCoV-HKU1 and HCoV-OC43  
218 betacoronaviruses. A similar trend was observed at late time points in convalescent donors as  
219 well as after the third vaccine dose in vaccinated individuals (**Figure S4**). Deconvolution of  
220 SARS-CoV-2 S-reactivity revealed a higher frequency of cross-reactive antibodies among the  
221 subset of S<sub>2</sub>-specific MBCs (**Figures 4A and 4B**).

222 The same analysis was performed on RBDs of sarbecoviruses representative of clades  
223 1a (SARS-CoV), 1b (Pangolin Guangxi), 2 (bat ZC45) and 3 (bat BM48-31/BGR/2008)  
224 comparing the reactivity of MBCs from SARS-CoV-2-convalescent and from naïve or infected  
225 donors receiving two vaccine doses. We found that the frequencies of antibodies cross-reactive  
226 to heterologous sarbecovirus RBDs, including those inhibiting binding of RBD to human  
227 ACE2, were progressively lower as a function of the decreasing sequence identity with SARS-

228 CoV-2 (**Figure 5A, 5B, S5A and S5B**). Infected vaccinees had the highest frequency of cross-  
229 reactive MBCs against diverse RBDs, suggesting that increased avidity resulting from multiple  
230 and diverse antigenic stimulations may have also contributed to broadening the reactivity  
231 towards heterologous sarbecoviruses.

232

### 233 **Affinity maturation of RBD-specific MBCs leads to resilience to viral escape by SARS-** 234 **CoV-2 variants of concern.**

235 In view of the continuing emergence of SARS-CoV-2 VOC, we analyzed at a clonal  
236 level the extent to which MBCs elicited by infection with Wuhan-Hu-1-related viruses in early  
237 2020 or by mRNA vaccines could cross-react with RBDs of progressively diverging VOC. We  
238 therefore tested MBC-derived antibodies for their ability to retain binding (here measured as  
239 less than 2-fold loss compared to Wuhan-Hu-1) to RBD of different VOC, including Beta  
240 (B.1.351), Delta (B.1.617.2) and Omicron (B.1.1.529) BA.1, BA.2, BA.3 and BA.4/BA.5 sub-  
241 lineages (**Figures 6A, 6B and S6A**). In naïve or infected individuals receiving two doses of the  
242 Pfizer/BioNtech BNT162b2 mRNA vaccine, mutations in the VOC RBDs were poorly  
243 tolerated with the greatest loss of binding observed against Omicron sublineages. Importantly,  
244 the third vaccine dose substantially increased the resilience to VOC escape from binding  
245 antibodies in both naïve and infected individuals to a degree similar to that observed in  
246 convalescent individuals more than one year after infection, in line with analysis of neutralizing  
247 antibody responses (Bowen et al., 2022b; Cameroni *et al.*, 2022; Park et al., 2022; Walls *et al.*,  
248 2022). Interestingly, in individuals given three vaccine doses, the higher fraction of MBCs  
249 cross-reactive with VOC RBDs was characterized by high-avidity and ACE2-blocking activity  
250 (**Figures 6A, 6B, S6A and S6B**). However, in all cohorts analyzed, the binding of MBC-  
251 derived antibodies was substantially reduced when tested against BA.4/BA.5 RBDs (**Figures**  
252 **6A**).

253 Taken together, these findings indicate that long-lasting affinity maturation upon  
254 infection by Wuhan-Hu-1 SARS-CoV-2 and multiple vaccinations can drive the development  
255 of MBCs with greater resilience to viral escape.

256



## 257 **DISCUSSION**

258 Kinetics of serum- and MBC-derived antibodies to SARS-CoV-2 after infection and  
259 vaccination have been extensively described (Dan *et al.*, 2021; Gaebler *et al.*, 2021; Goel *et*  
260 *al.*, 2021a; Goel *et al.*, 2021b; Rodda *et al.*, 2021; Roltgen *et al.*, 2020; Sokal *et al.*, 2021b;  
261 Walls *et al.*, 2022). In this study, we provide an in depth characterization of the maturation of  
262 the memory B cell response to SARS-CoV-2, supporting evidence of how the MBC repertoire  
263 is shaped to broaden recall responses to other betacoronaviruses and future variants of concern.  
264 Compared to flow-cytometry-based methods (Dan *et al.*, 2021; Goel *et al.*, 2021a; Rodda *et*  
265 *al.*, 2021; Sokal *et al.*, 2021b; Wang *et al.*, 2021b), the antigen-specific memory B cell  
266 repertoire analysis (AMBRA) has the advantage of analyzing very large numbers of MBCs at  
267 the single-cell level, thus allowing unbiased direct comparisons of multiple specificities and  
268 functional properties of MBC-derived antibodies.

269 Documenting the reciprocal kinetics of serum antibodies and MBCs to SARS-CoV-2  
270 antigens illustrates a fundamental aspect of the antibody response. While serum antibodies  
271 produced by the first wave of short-lived plasma cells decline over time, MBCs increase in  
272 numbers reaching up to 20% of total IgG MBCs in a few months after SARS-CoV-2 infection  
273 before their frequencies stabilize. Importantly, this time-dependent increase of MBCs is  
274 accompanied by affinity maturation and breadth expansion. As a consequence, while serum  
275 antibodies decline, the immune system builds up the capacity to mount a very potent secondary  
276 memory response. Accordingly, high numbers of MBCs and breadth against VOC are  
277 characteristic of donors who had hybrid immunity due to infection followed by vaccination  
278 (Crotty, 2021; Rodda *et al.*, 2022). Conversely, naïve donors require a longer time and multiple  
279 immunizations to develop an MBC response of magnitude and breadth, which are comparable  
280 to those of infected individuals (Goel *et al.*, 2022; Muecksch *et al.*, 2022). The increased avidity  
281 resulting from multiple antigenic stimulations may therefore contribute to broaden the  
282 reactivity towards heterologous sarbecoviruses as well as to generate resilience to new VOC  
283 (Goel *et al.*, 2022; Sokal *et al.*, 2021a; Stamatatos *et al.*, 2021), including Omicron sublineages.  
284 This is consistent with the notion that cross-reactive MBCs are primarily induced by repeated  
285 antigenic stimulations leading to epitope spread and affinity maturation, which are fundamental  
286 for an effective and long-lasting recall response to future SARS-CoV-2 variants. However, the  
287 less pronounced resilience observed against the recently emerging BA.4 and BA.5 variants  
288 suggests that immune escape, even from high-avidity antibodies, may be a major driver for  
289 the evolution of Omicron sublineages.

290

291 **SUPPLEMENTAL INFORMATION**

292

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302

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304 Experiment design: R.M., J.B., C.S.-F., D.C. and L.Pi.

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314

315 **DECLARATION OF INTERESTS**

316 R.M., J.B., C.S.-F., I.B., F.M., K.C., N.S., G.L., C.S., E.C., E.A.D.J., J.R.D., N.C., A.T.,  
317 H.W.V., A.L., D.C. and L.Pi. are employees of Vir Biotechnology Inc. and may hold shares in  
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321

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500

501 **STAR METHODS**

502 **KEY RESOURCES TABLE**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
CD3	BioLegend	OKT3; Cat#317328
CD4	BioLegend	RM4-5; Cat#100526
CD19	BioLegend	SJ25C1; Cat#363024
CD16	BioLegend	3G8; Cat#302044
CD14	BioLegend	M5E2; Cat#301838
IgG	BD Biosciences	G18-145; Cat#555786
Goat F(ab') <sub>2</sub> Anti-Mouse IgG(H+L), Human ads-AP	Bioconcept	1032-04
<b>Biological samples</b>		
Donors' PBMCs	This study	
Donors' sera	This study	
<b>Chemicals, Peptides, and Recombinant Proteins</b>		
R848 (Resiquimod)	InvivoGen	Cat#tlrl-r848-5
Recombinant human IL-2	ImmunoTools	Cat#11340027
RBD, mouse Fc Tag	Sino Biological	Cat#40592-V05H
pNPP	Sigma-Aldrich	Cat#71768-25G
SARS-CoV-2 S2	The Native Antigen Company	Cat#REC31807-500
SARS-CoV-2 S1	The Native Antigen Company	Cat#40150-V08B1
SARS-CoV-2 N	The Native Antigen Company	Cat#REC31812
HCoV-OC43 N	The Native Antigen Company	Cat#REC31857
SARS-CoV-2 NTD	(McCallum <i>et al.</i> , 2021)	
SARS-CoV-2 S	(Walls <i>et al.</i> , 2020)	
SARS-CoV S	(Walls <i>et al.</i> , 2019)	
HCoV-OC43 S	(Pinto <i>et al.</i> , 2021)	
MERS-CoV S	(Walls <i>et al.</i> , 2019)	
HCoV-HKU1 S	(Pinto <i>et al.</i> , 2021)	
SARS-CoV-2 RBD	(Piccoli <i>et al.</i> , 2020)	
BM48-31/BGR/2008 RBD	This study	
Pangolin GX RBD	This study	
ZC45 RBD	This study	
SARS-CoV RBD	This study	
Beta RBD	This study	

Delta RBD	This study	
Omicron BA.1 RBD	This study	
Omicron BA.2 RBD	This study	
Omicron BA.3 RBD	This study	
Omicron BA.4/5 RBD	This study	
Human ACE2	(Piccoli <i>et al.</i> , 2020)	
Blocker™ Casein in PBS	Thermo Fisher Scientific	Cat#37528
Tween 20	Sigma Aldrich	Cat#93773-1KG
Goat Anti-Human IgG-AP	Bioconcept	Cat#2040-04
Sodium thiocyanate	Sigma-Aldrich	Cat#251410-2.5KG
Zombie Aqua Fixable Viability Kit	BioLegend	Cat#423101
Ficoll-Paque PLUS (6x500ml)	VWR International	Cat#17-1440-03
RPMI-1640 W/O L-Glutamine (10x500ml)	Life Technologies Europe BV	Cat#31870074
HyClone Fetal Bovine Serum, US Origin 500ml,	VWR International	Cat#SH30070.03
DPBS w/o Ca and Mg (500ml),	Chemie Brunschwig	Cat#P04-36500 Pan Biotech
MEM NEAA Solution 100x, 100ml,	Bioconcept	Cat#5-13K00-H
Stable Glutamine solution (L-Ala/L-Gln)100x, 100ml	Bioconcept	Cat#5-10K50-H
Penicillin-Streptomycin	Bioconcept	Cat#4-01F00-H
Kanamycin (5,000ug/ml), 100ml	Bioconcept	Cat#4-08F00-H
Transferrin (Holo) from human serum	LuBioscience	Cat#0905-100
2-Mercaptoethanol 50MM	Bioconcept	Cat#5-69F00-E
Sodium Pyruvate (100mM, 100 ml)	Bioconcept	Cat#5-60F00-H
<b>Cell lines</b>		
FreeStyle™ 293-F Cells	ThermoFisher Scientific	Cat# R79007
Expi293F™ Cells	ThermoFisher Scientific	Cat# A14527
ExpiCHO-S™	ThermoFisher Scientific	Cat# A29127
<b>Recombinant DNA</b>		
SARS-CoV-2 NTD pCMV plasmid	(McCallum <i>et al.</i> , 2021)	
SARS-CoV-2 S phCMV1 plasmid	(Starr <i>et al.</i> , 2021; Walls <i>et al.</i> , 2020)	
SARS-CoV S phCMV1 plasmid	(Walls <i>et al.</i> , 2019)	
HCoV-OC43 S phCMV1 plasmid	(Pinto <i>et al.</i> , 2021)	
MERS-CoV S phCMV1 plasmid	(Walls <i>et al.</i> , 2019)	
HCoV-HKU1 S phCMV1 plasmid	(Pinto <i>et al.</i> , 2021)	
SARS-CoV-2 RBD phCMV1 plasmid	(Piccoli <i>et al.</i> , 2020)	
BM48-31/BGR/2008 RBD phCMV1 plasmid	This study	
Pangolin GX RBD phCMV1 plasmid	This study	
ZC45 RBD phCMV1 plasmid	This study	



SARS-CoV RBD phCMV1 plasmid	This study	
Beta RBD phCMV1 plasmid	This study	
Delta RBD phCMV1 plasmid	This study	
Omicron BA.1 RBD phCMV1 plasmid	This study	
Omicron BA.2 RBD phCMV1 plasmid	This study	
Omicron BA.3 RBD phCMV1 plasmid	This study	
Omicron BA.4/5 RBD phCMV1 plasmid	This study	
Human ACE2 phCMV1 plasmid	(Piccoli <i>et al.</i> , 2020)	
<b>Software</b>		
Flowjo (v10.7.1)	FlowJo	<a href="https://www.flowjo.com">https://www.flowjo.com</a>
GraphPad Prism (v9.3.1)	GraphPad	<a href="https://www.graphpad.com">https://www.graphpad.com</a>
Everest (v3.0)	Bio-Rad	<a href="https://www.bio-rad.com">https://www.bio-rad.com</a>
Microsoft Excel for Microsoft 365 MSO (Version 2204 Build 16.0.15128.20240)	Microsoft	<a href="https://www.microsoft.com">https://www.microsoft.com</a>

503

## 504 **RESOURCE AVAILABILITY**

### 505 **Lead contact**

506 Further information and requests for resources and reagents should be directed to and will be  
507 fulfilled by the Lead Contact, Luca Piccoli ([lpiccoli@vir.bio](mailto:lpiccoli@vir.bio)).

### 508 **Materials availability**

509 Materials generated in this study will be made available on request and may require a material  
510 transfer agreement.

### 511 **Data and code availability**

512 Data and code generated in this study will be made available on request and may require a  
513 material transfer agreement.

514

## 515 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

### 516 **Cell lines**

517 Cell lines used in this study were obtained from ThermoFisher Scientific (FreeStyle<sup>TM</sup> 293-F  
518 Cells, Expi293F<sup>TM</sup> Cells and ExpiCHO-S<sup>TM</sup>).

519

### 520 **Study participants and ethics statement**

521 Samples were obtained from 3 cohorts of Wuhan SARS-CoV-2-infected individuals, 1 cohort  
522 of Alpha SARS-CoV-2-infected individuals and 2 cohorts of individuals vaccinated with  
523 Pfizer/BioNtech BNT162b2 mRNA COVID-19 vaccine under study protocols approved by the

524 local Institutional Review Boards (Canton Ticino Ethics Committee, Switzerland and the  
525 Ethical committee of Luigi Sacco Hospital, Milan, Italy). COVID-19 was diagnosed by PCR  
526 with primers specific for the detection of Wuhan or Alpha SARS-CoV-2 in nasal swabs. All  
527 donors provided written informed consent for the use of blood and blood components (such as  
528 PBMCs, sera or plasma) and were recruited at hospitals or as outpatients. Based on their  
529 availability, participants were enrolled and allocated to either single blood draws or  
530 longitudinal follow-up.

531

## 532 **METHOD DETAILS**

### 533 **Isolation of peripheral blood mononuclear cells (PBMCs), plasma and sera**

534 PBMCs and plasma were isolated from blood draw performed using tubes or syringes pre-filled  
535 with heparin or sodium EDTA, followed by Ficoll-Paque PLUS (6x500ml) (VWR  
536 International, 17-1440-03) density gradient centrifugation. Sera were obtained from blood  
537 collected using tubes containing clot activator, followed by centrifugation. PBMCs, plasma  
538 and sera were stored in liquid nitrogen and -80°C freezers until use, respectively.

539

### 540 **Immunophenotyping**

541 PBMCs were thawed and washed twice with RPMI-1640 W/O L-Glutamine (10x500ml) (Life  
542 Technologies Europe BV, 31870074) 10% HyClone Fetal Bovine Serum, US Origin 500ml  
543 (VWR International, SH30070.03), and incubated in the same medium for 2 h at 37°C. Live  
544 PBMCs were counted post thawing and seeded at 1 million into round-bottom 96-well plates  
545 (Corning, 3799). PBMCs were stained with LIFE/DEAD marker (Zombie Aqua Fixable  
546 Viability Kit, BioLegend 423101) in Dulbecco's phosphate-buffered saline (DPBS) w/o Ca and  
547 Mg (500ml), (Chemie Brunschwig, P04-36500 Pan Biotech) for 30' at RT, washed in MACS  
548 buffer (PBS 2% HyClone, 2 mM EDTA), and stained with antibodies to CD3, CD4, CD19,  
549 CD16, CD14, (BioLegend), IgG (BD Bioscience) (Key Resources Table) for 30' at 4°C. Cells  
550 were then washed and resuspended in MACS buffer for data acquisition at ZE5 cytometer (Bio-  
551 Rad). Data were analysed with FlowJo software.

552

### 553 **Memory B cell culture**

554 Replicate cultures of total PBMCs were set at different cell densities (10,000-30,000  
555 cells/culture) in 96 U-bottom plates (Corning, 3799). Cells were cultured at 37°C in RPMI 10%  
556 Hyclone, 1% Stable Glutamine, 1% Sodium Pyruvate, 1% MEM NEAA, 1% Pen-Strep, 1%  
557 Kanamycin, 30 µg/ml Transferrin Holo, 50 µM 2-Mercaptoethanol (50 mM), and stimulated

558 with 2.5 µg/mL R848 (Invivogen, tlr1-r848-5) and 1,000 U/mL human recombinant IL-2  
559 (ImmunoTools, 11340027). Supernatants were harvested after 10 days.

560

### 561 **Plasmid design**

562 The SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-HKU1 and HCoV-OC43 prefusion S and  
563 the SARS-CoV-2 postfusion S ectodomains were synthesized by Genscript or GeneArt and  
564 cloned in the pHCMV1 vector, as previously described (Bowen *et al.*, 2021; Lempp *et al.*, 2021;  
565 Pallesen *et al.*, 2017; Pinto *et al.*, 2021; Starr *et al.*, 2021; Walls *et al.*, 2020; Walls *et al.*, 2019).  
566 The Wuhan-Hu-1 SARS-CoV-2 RBD plasmid, which encodes for the S residues 328-531, was  
567 synthesized by Genscript and cloned in the pHCMV1 vector, as previously described (Piccoli  
568 *et al.*, 2020). Plasmids encoding for RBDs of different sarbecoviruses were synthesized by  
569 Genscript and cloned in the pHCMV1 vector. Plasmids encoding for the RBD of SARS-CoV-  
570 2 Beta, Delta, Omicron BA.1, BA.2, BA3 and BA.4/5 variants were generated by overlap PCR  
571 (Collier *et al.*, 2021). The SARS-CoV-2 NTD plasmid, which encodes for the S residues 14-  
572 307, was synthesized by GeneArt and cloned in the pCMV vector, as previously described  
573 (McCallum *et al.*, 2021).

574

### 575 **Recombinant glycoprotein production**

576 All SARS-CoV-2 S ectodomains were produced in 500-mL cultures of FreeStyle™ 293-F cells  
577 (ThermoFisher Scientific) grown in suspension using FreeStyle 293 expression medium  
578 (ThermoFisher Scientific) at 37°C in a humidified 8% CO<sub>2</sub> incubator rotating at 130 r.p.m.  
579 Cells grown to a density of 2.5 million cells per mL were transfected using PEI (9 µg/mL) or  
580 293fectin and respective plasmids, and cultivated for 3-4 days. The supernatant was harvested  
581 and, for some productions, cells were resuspended for another three days, yielding two  
582 harvests. SARS-CoV-2 S ectodomain was purified from clarified supernatants using a 5-mL  
583 C-tag affinity matrix column (Thermo-Fischer). SARS-CoV, MERS-CoV and HCoV-HKU1  
584 S ectodomains were purified using a cobalt affinity column (Cytiva, HiTrap TALON crude).  
585 HCoV-OC43 S ectodomain was purified using a 1-ml StrepTrap column (GE Healthcare). All  
586 purified proteins were then concentrated using a 100 kDa centrifugal filter (Amicon Ultra 0.5  
587 mL centrifugal filters, MilliporeSigma). Concentrated SARS-CoV-2 S was further purified by  
588 a sizing step, using a Superose 6 Increase 10/300 GL column (Cytiva) with 50 mM Tris pH 8,  
589 200 mM NaCl as a running buffer. Peak fractions corresponding to homogeneous spike trimer  
590 were pooled. All the proteins were flash frozen in liquid nitrogen and stored for further usage  
591 at -80°C.

592 Postfusion SARS-CoV-2 S, all RBDs and the NTD were produced in Expi293F™ Cells  
593 (ThermoFisher Scientific) grown in suspension using Expi293™ Expression Medium  
594 (ThermoFisher Scientific) at 37°C in a humidified 8% CO<sub>2</sub> incubator rotating at 130 r.p.m.  
595 Cells grown to a density of 3 million per mL were transfected using the respective plasmids  
596 with the ExpiFectamine™ 293 Transfection Kit (ThermoFisher Scientific) and cultivated for 5  
597 days. SARS-CoV-2 S (used to prepare postfusion S) was purified using a nickel HisTrap HP  
598 affinity column (Cytiva) and then incubated with 1:1 w/w S2X58-Fab (Starr *et al.*, 2021) and  
599 10 µg/mL trypsin for one hour at 37°C before size exclusion on a Superose 6 Increase column  
600 (Cytiva). Supernatants containing RBDs were harvested five days after transfection,  
601 equilibrated with 0.1 M Tris-HCl, 0.15 M NaCl, 10 mM EDTA, pH 8.0 and supplemented with  
602 a biotin blocking solution (IBA Lifesciences). RBDs were purified by affinity chromatography  
603 on a Strep-Trap HP 5 ml column followed by elution with 50 mM biotin and buffer exchange  
604 into PBS. The NTD domain was purified from clarified supernatants using 2 ml of cobalt resin  
605 (Takara Bio TALON), washing with 50 column volumes of 20 mM HEPES-HCl pH 8.0 and  
606 150 mM NaCl and eluted with 600 mM imidazole. Purified protein was concentrated using a  
607 30 kDa centrifugal filter (Amicon Ultra 0.5 mL centrifugal filters, MilliporeSigma), the  
608 imidazole was washed away by consecutive dilutions in the centrifugal filter unit with 20 mM  
609 HEPES-HCl pH 8.0 and 150 mM NaCl, and finally concentrated to 20 mg/ml and flash frozen.  
610 Recombinant human ACE2 was expressed in Expi293F™ or ExpiCHO-S™ cells transiently  
611 transfected with a plasmid encoding for ACE2 residues 19-615, as previously described  
612 (Piccoli *et al.*, 2020). ). Supernatant was collected 6-8 days after transfection, supplemented  
613 with buffer to a final concentration of 80 mM Tris-HCl pH 8.0, 100 mM NaCl, and then  
614 incubated with BioLock (IBA GmbH) solution. ACE2 was purified using StrepTrap High  
615 Performance columns (Cytiva) followed by isolation of the monomeric ACE2 by size exclusion  
616 chromatography using a Superdex 200 Increase 10/300 GL column (Cytiva) pre-equilibrated  
617 in PBS or 20 mM Tris-HCl pH 7.5, 150 mM NaCl.

618

### 619 **Enzyme-linked immunosorbent assay (ELISA)**

620 Spectraplate-384 with high protein binding treatment (custom made from Perkin Elmer) were  
621 coated overnight at 4°C with 1 µg/ml of SARS-CoV-2 S (produced in house), SARS-CoV-2  
622 S2 (The Native Antigen Company, REC31807-500), S1 (The Native Antigen Company,  
623 40150-V08B1), NTD (produced in house), N (The Native Antigen Company, REC31812),  
624 SARS-CoV S (produced in house), MERS-CoV S (produced in house), HCoV-HKU1 S  
625 (produced in house), HCoV-OC43 S (produced in house), HCoV-OC43 N (The Native Antigen

626 Company, REC31857), RBD SARS-CoV-2 (produced in house), RBD SARS-CoV (produced  
627 in house), RBD PangolinGX (produced in house), RBD ZC45 (produced in house), RBD  
628 BM48-31/BGR/2008 (produced in house), Beta RBD (produced in house), Delta RBD  
629 (produced in house), Omicron BA.1, BA.2 and BA.3 RBD (produced in house) in PBS pH 7.2  
630 or PBS alone as control. Plates were subsequently blocked with Blocker Casein (1%) in PBS  
631 (Thermo Fisher Scientific, 37528) supplemented with 0.05% Tween 20 (Sigma Aldrich,  
632 93773-1KG). The coated plates were incubated with diluted B cell supernatant for 1 h at RT.  
633 Plates were washed with PBS containing 0.05 % Tween20 (PBS-T), and binding was revealed  
634 using secondary goat anti-human IgG-AP (Southern Biotech, 2040-04). After washing, pNPP  
635 substrate (Sigma-Aldrich, 71768-25G) was added and plates were read at 405 nm after 1 h or  
636 30'. For chaotropic ELISA, after incubation with B-cell supernatants, plates were washed and  
637 incubated with 1 M solution of sodium thiocyanate (NaSCN) (Sigma-Aldrich, 251410-2.5KG)  
638 for 1 h. Avidity Index was calculated as the ratio (%) between the ED50 in presence and the  
639 ED50 in absence of NaSCN.

640

#### 641 **Blockade of RBD binding to human ACE2**

642 Plasma or memory B cell culture supernatants were diluted in PBS and mixed with SARS-  
643 CoV-2 RBD mouse Fc-tagged antigen (Sino Biological, 40592-V05H, final concentration 20  
644 ng/ml) and incubated for 30 min at 37°C. The mix was added for 30 min to ELISA 384-well  
645 plates (NUNC, P6366-1CS) pre-coated overnight at 4°C with 4 µg/ml human ACE2 (produced  
646 in house) in PBS. Plates were washed with PBS containing 0.05 % Tween20 (PBS-T), and  
647 RBD binding was revealed using secondary goat anti-mouse IgG-AP (Southern Biotech, 1032-  
648 04). After washing, pNPP substrate (Sigma-Aldrich, 71768-25G) was added and plates were  
649 read at 405 nm after 1h. The percentage of inhibition was calculated as follow:  $(1 - (\text{OD sample} - \text{OD neg ctr}) / (\text{OD pos ctr} - \text{OD neg ctr})) \times 100$ .

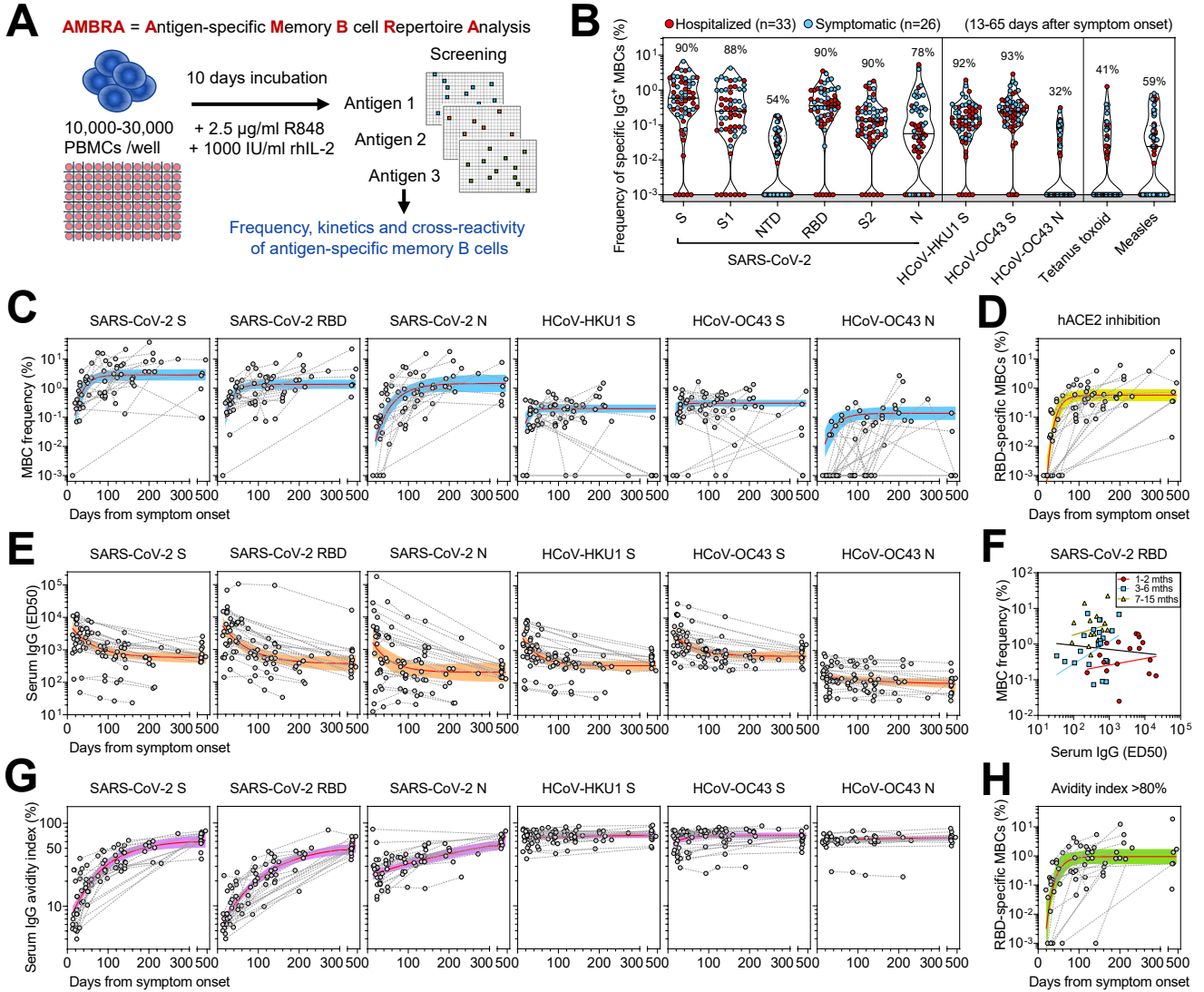
650

#### 651 **QUANTIFICATION AND STATISTICAL ANALYSIS**

652 Data management and statistical analysis were carried out by in-house software based on  
653 PostgreSQL and Scala (Odersky et al., 2004). Positive cultures of antigen-specific MBCs were  
654 identified from those showing OD values >0.5 by ELISA. This cut-off was determined from 3  
655 times the average OD of pre-pandemic controls. The frequency of B cells precursors specific  
656 for a given antigen was calculated assuming a Poisson distribution with the following equation:  
657 % of antigen-specific MBCs =  $-100 * (\text{LN}(\text{number of negative wells} / \text{number of total seeded}$   
658  $\text{wells})) / \text{number of IgG}^+ \text{ MBCs per well}$ . Other statistical and data analyses were performed  
659

660 using GraphPad Prism (v9.3.1) and Microsoft Excel for Microsoft 365 MSO (Version 2204  
661 Build 16.0.15128.20240). Nonparametric Kruskal-Wallis test was used to analyze statistical  
662 differences between groups analyzed. Correction for multiple comparison was performed with  
663 Dunn's test. Statistical significance was defined as  $p < 0.05$ . ED50 values were determined by  
664 non-linear regression analysis (log(agonist) versus response - Variable slope (four  
665 parameters)). Variation of frequencies and serum titers or avidity over time was determined by  
666 one-phase association or decay kinetics models from all the non-null values of each sample.

# Figure 1



**Figure 1. Early response, RBD immunodominance, kinetics and affinity maturation of memory B cells primed by Wuhan SARS-CoV-2**

(A) Scheme of the AMBRA method used in this study. PBMC, peripheral blood mononuclear cells. R848, agonist of Toll-like receptors 7 and 8. rhIL2, recombinant human interleukin 2.

(B) Frequency of SARS-CoV-2-specific MBCs isolated between 13 and 65 days after symptom onset from  $n = 59$  donors (33 hospitalized, red, and 26 symptomatic, blue) after the analysis of 5,664 MBC cultures. Shown is the reactivity to antigens of SARS-CoV-2 and other betacoronaviruses (HCoV-HKU1 and HCoV-OC43): Spike (S), S1 domain, N-terminal domain (NTD), receptor-binding domain (RBD), S2 domain, Nucleoprotein (N). Reactivities to Tetanus toxoid and to Measles virus (lysate) are included as controls. Median and quartiles are shown as plain and dotted lines, respectively. Percentages of donors with detectable specific MBCs are indicated above each set of data.

(C) Frequency of MBCs specific for SARS-CoV-2 S, RBD and N, HCoV-HKU1 S, HCoV-OC43 S and N from  $n = 23$  donors followed-up up to 469 days after symptom onset. Frequencies were obtained from the analysis of 6,336 MBC cultures (66 samples, minimum 2 samples per donor). Black dotted lines connect samples from the same donor. A one-phase association kinetics model (red line) was calculated from all the non-null values of each sample. The area within 95% confidence bands is shown in blue.

(D) Frequency of SARS-CoV-2 RBD-specific MBC producing antibodies showing inhibition of RBD binding to ACE2 from  $n = 23$  donors. A one-phase association kinetics model (red line) was calculated from all the non-null values of each sample and the area within 95% confidence bands is shown in yellow.

(E) Serum IgG ED50 titers to SARS-CoV-2 S, RBD and N, HCoV-HKU1 S, HCoV-OC43 S and N of samples collected from 29 donors analyzed up to 469 days after symptom onset. A one-phase decay kinetics model (red line) was calculated from all the non-null values of each sample and the area within 95% confidence bands is shown in orange.

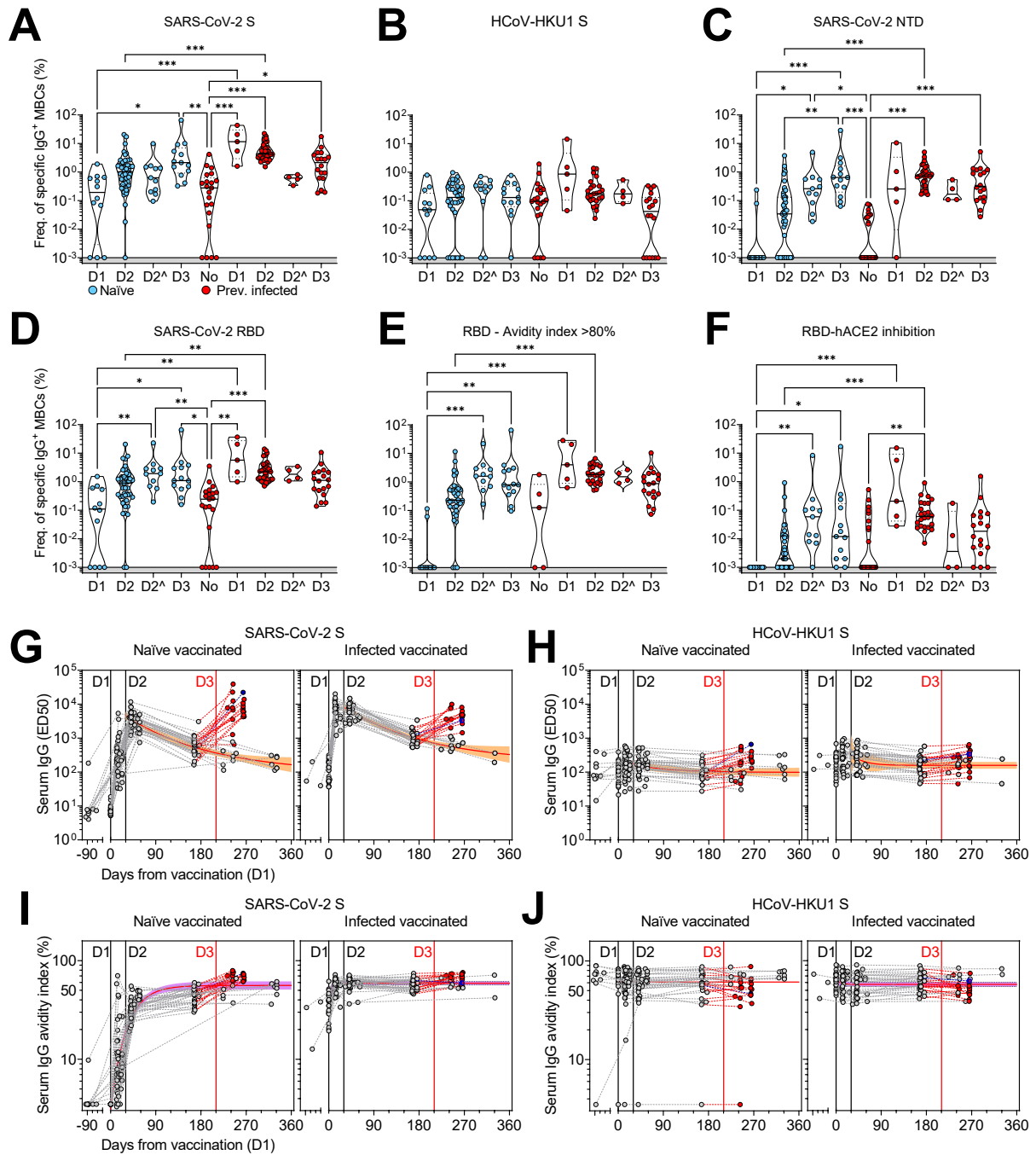
(F) Correlation analysis between frequency of SARS-CoV-2 RBD-specific MBCs and serum RBD-specific IgG titers of  $n = 56$  samples from  $n = 18$  donors collected at different time points. All samples (black line): Spearman  $r = -0.102$  (95% confidence interval  $-0.363$  to  $0.173$ ; non-significant  $P = 0.45$ ). Samples at 1-2 months ( $n = 18$ , red line): Spearman  $r = 0.112$  (95% confidence interval  $-0.387$  to  $0.561$ ; non-significant  $P = 0.66$ ). Samples at 3-6 months ( $n = 23$ , blue line): Spearman  $r = 0.214$  (95% confidence interval  $-0.229$  to  $0.584$ ; non-significant  $P = 0.33$ ). Samples at 7-15 months ( $n = 15$ , yellow line): Spearman  $r = 0.221$  (95% confidence interval  $-0.343$  to  $0.668$ ; non-significant  $P = 0.43$ ).

(G) Serum IgG avidity indexes to SARS-CoV-2 S, RBD and N, HCoV-HKU1 S, HCoV-OC43 S and N of samples collected from 29 donors analyzed up to 469 days after symptom onset. A one-phase association kinetics model (red line) was calculated from all the non-null values of each sample and the area within 95% confidence bands is shown in violet.

(H) Frequency of SARS-CoV-2 RBD-specific B cells with an avidity index greater than 80%. A one-phase association kinetics model (red line) was calculated from all the non-null values of each sample and the area within 95% confidence bands is shown in green.



# Figure 2



**Figure 2. Characterization of vaccine-induced MBC- and serum-derived antibody response in naïve and SARS-CoV-2 immune donors**

(A-D) Frequency of MBCs specific for SARS-CoV-2 S (A), HCoV-HKU1 S (B), SARS-CoV-2 NTD (C) and RBD (D) of n = 12, 45 and 13 naïve donors and 5, 31, and 18 previously infected donors 10-35 days after the first (D1), the second (D2) and the third dose (D3) of Pfizer/BioNtech BNT162b2 mRNA vaccine, respectively. Shown are also 11 naïve and 4 immune donors whose MBCs were isolated 125-293 days after the second dose (D2<sup>^</sup>). Median frequencies are compared within donor groups and between respective vaccine doses as well as to a group of n = 21 convalescent donors at 18-30 days after symptom onset. Significant differences are indicated as \*\*\* (p-value < 0.001); \*\* (p < 0.002), \* (p < 0.033), ns (non-significant, p > 0.12).

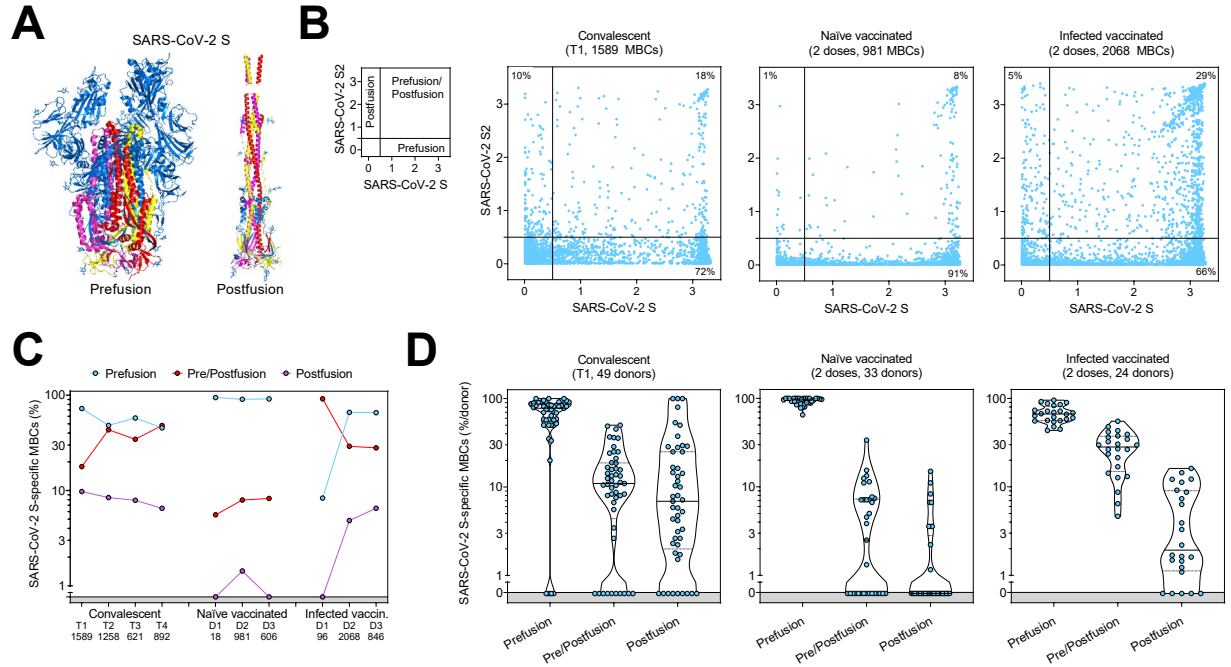
(E) Frequency of SARS-CoV-2 RBD-specific MBCs with an avidity index greater than 80%.

(F) Frequency of SARS-CoV-2 RBD-specific MBCs inhibiting binding of RBD to ACE2.

(G-H) Serum IgG ED50 titers to SARS-CoV-2 S (G) and HCoV-HKU1 S (H) of samples collected from n = 47 naïve (left) and 32 immune donors (right) 10-35 days after the first (D1), the second (D2) and the third dose (D3) of Pfizer/BioNtech BNT162b2 mRNA vaccine, respectively. A one-phase decay kinetics model (red line) was calculated from all the non-null values of each sample and the area within 95% confidence bands is shown in orange. 37 samples collected from individuals who received a third dose (red) or had a second SARS-CoV-2 infection (blue) were excluded from the decay analysis.

(I-J) Serum IgG avidity indexes to SARS-CoV-2 S (I) and HCoV-HKU1 S (J) of the same samples shown in panels G-H. A one-phase association kinetics model (red line) was calculated from all the non-null values of each sample and the area within 95% confidence bands is shown in violet.

# Figure 3



**Figure 3. Comparison of the prefusion and postfusion S-specific MBC responses after vaccination or natural infection**

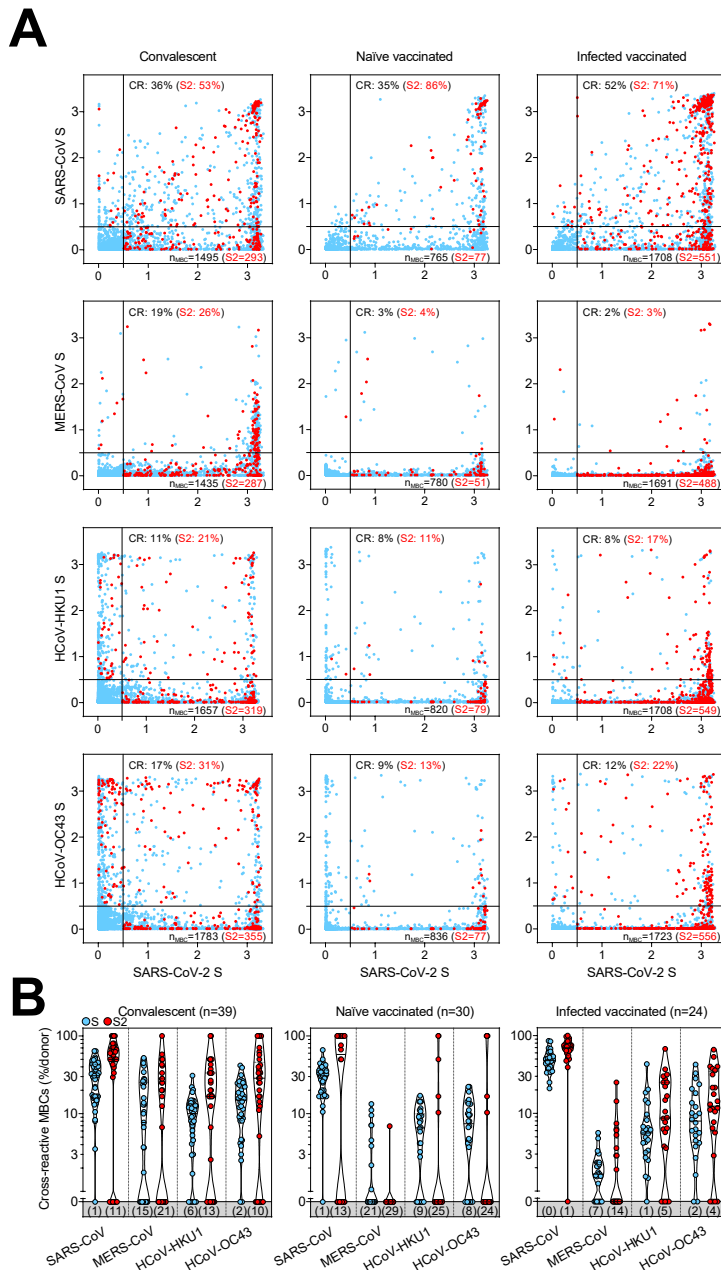
(A) Structural representation of SARS-CoV-2 S in its prefusion and postfusion conformation (adapted from PBD 7tat and 7e9t). The three S2 domains that are maintained in both conformations are colored in red, yellow and pink.

(B) MBC cross-reactivity between SARS-CoV-2 S (prefusion S) and S2 (postfusion S). Shown are average OD values as measured by ELISA with blank subtracted from  $n = 2$  replicates of 1589, 981 and 2068 MBC cultures analyzed from 49 convalescent, 33 naïve and 24 infected vaccinated donors. Cumulative fraction of MBCs reactive to either prefusion or postfusion S or both is indicated as percentage in the respective quadrant. The small panel on the left describes the distribution of prefusion and/or postfusion S-specific MBCs in the different quadrants.

(C) Cumulative fraction of S-specific MBCs reactive to prefusion and/or postfusion S at different timepoints after natural infection (T1, T2, T3 and T4) or vaccine doses (D1, D2, D3).

(D) Individual fractions of S-specific MBCs reactive to prefusion and/or postfusion S in 49 convalescent, 33 naïve and 24 infected vaccinated donors.

# Figure 4

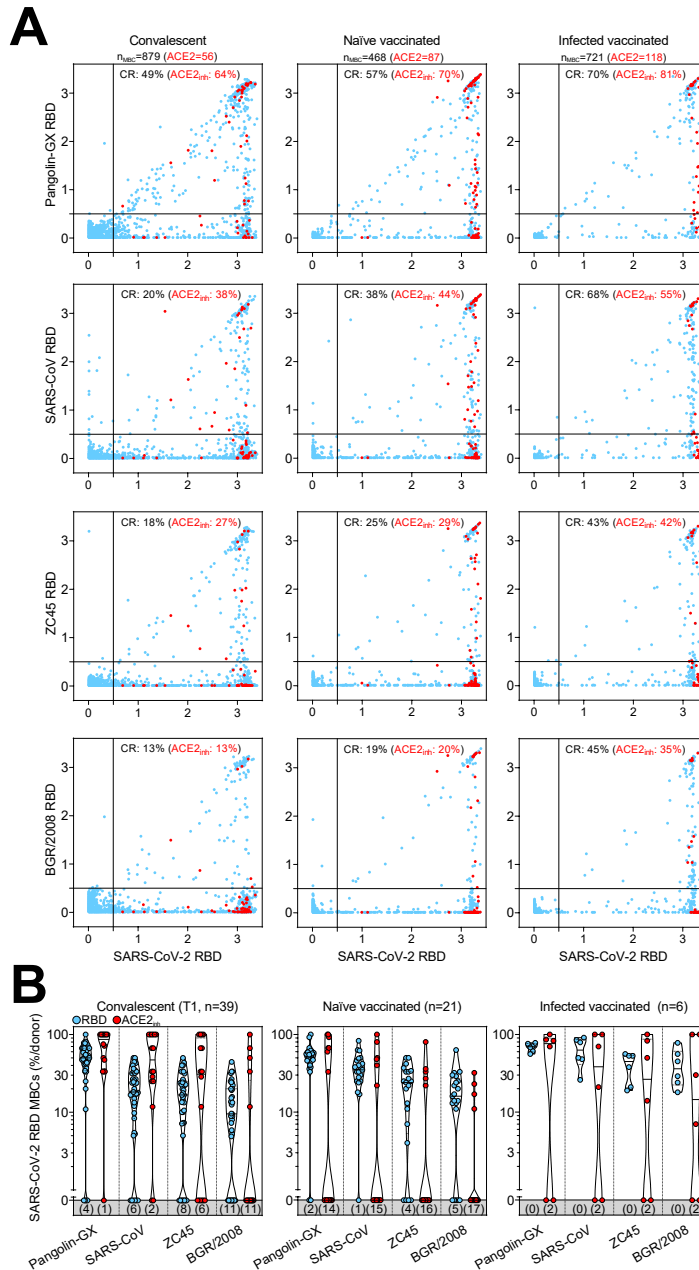


**Figure 4. Cross-reactivity to betacoronaviruses of MBCs primed by SARS-CoV-2 infection and/or vaccination**

(A) Cumulative MBC cross-reactivity between SARS-CoV-2 S and the four betacoronaviruses SARS-CoV, MERS-CoV, HCoV-HKU1 and HCoV-OC43. Shown are average OD values as measured by ELISA with blank subtracted from  $n = 2$  replicates of 3744, 2880 and 2304 MBC cultures analyzed from 39 convalescent, 30 naïve and 24 infected donors after two vaccine doses. S2-specific MBCs are shown in red. Numbers of S- and S2-specific MBCs are indicated in the bottom-right quadrants of each panel. Cumulative fractions of S- and S2-cross-reactive (CR) MBCs are indicated as percentage in the top-right quadrant.

(B) Individual fractions of SARS-CoV-2 S-specific MBCs that cross-react with the four betacoronaviruses in convalescent and vaccinated donors. Numbers in brackets indicate the donors with MBCs showing no cross-reactivity for the respective betacoronavirus S.

# Figure 5

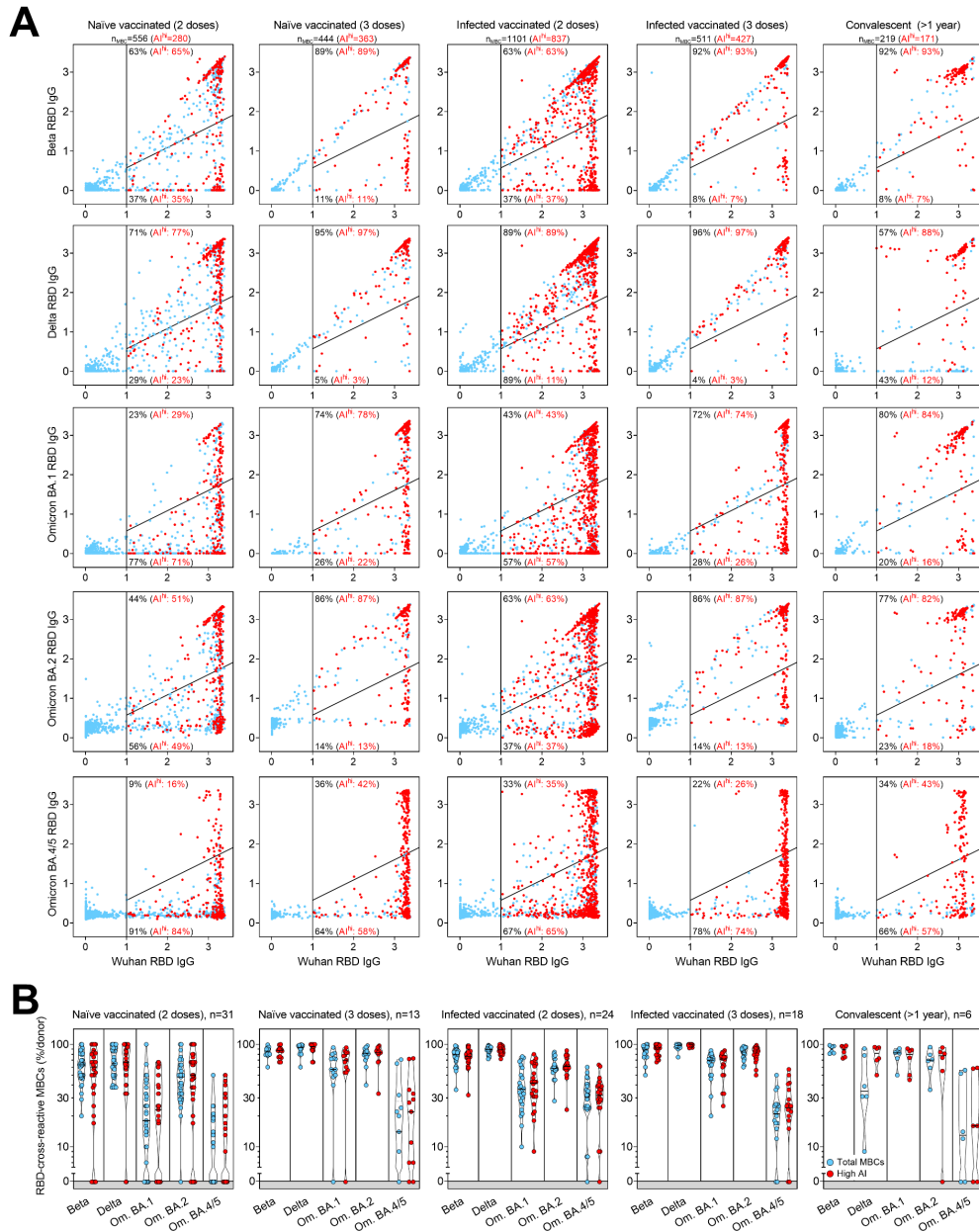


**Figure 5. Cross-reactivity to sarbecoviruses of MBCs primed by SARS-CoV-2 infection and/or vaccination**

(A) Cumulative MBC cross-reactivity between SARS-CoV-2 RBD and four sarbecoviruses representative of clades 1a (SARS-CoV), 1b (Pangolin Guangxi), 2 (ZC45) and 3 (BM48-31/BGR/2008). Shown are average OD values as measured by ELISA with blank subtracted from  $n = 2$  replicates of 3744, 2016 and 576 MBC cultures analyzed from 39 convalescent donors at two different timepoints and from 21 naïve and 6 infected donors after receiving two vaccine doses. RBD-specific MBCs showing inhibition of binding to ACE2 are shown in red. Cumulative fractions of total and ACE2-inhibiting RBD-cross-reactive (CR) MBCs are indicated as percentage in the top-right quadrant.

(B) Individual fractions of SARS-CoV-2 RBD-specific MBCs that cross-react with the four representative sarbecoviruses in convalescent and vaccinated donors. Numbers in brackets indicate the donors with MBCs showing no cross-reactivity for the respective sarbecovirus RBD.

# Figure 6



**Figure 6. Resilience to viral escape of VOC by high-avidity MBCs primed by SARS-CoV-2 infection and/or vaccination**

(A) Cumulative MBC cross-reactivity between RBD from Wuhan SARS-CoV-2 and Beta, Delta, Omicron BA.1, BA.2 and BA.4/5 VOC. Shown are average OD values as measured by ELISA with blank subtracted from  $n = 2$  replicates of 2976, 1248, 2304, 1728 and 576 MBC cultures analyzed from 31 and 13 naïve donors, 24 and 18 infected donors after receiving two and three vaccine doses and from 6 convalescent donors at 376-469 days from symptom onset. RBD-specific MBCs showing high avidity index ( $AI > 80\%$ ) are shown in red. Numbers of total and high-avidity RBD-specific MBCs are indicated in the top-left quadrants. Cumulative fractions of total and high-avidity RBD-specific MBCs maintaining or losing binding to the VOC RBD are indicated as percentage in the top-right and bottom-right quadrants.

(B) Individual fractions of total and high-avidity SARS-CoV-2 RBD-cross-reactive MBCs that maintain binding with the RBDs of different VOC in convalescent and vaccinated donors. Numbers on top indicate the donors analyzed for the different VOC.