

1 Therapeutic and vaccine-induced cross-reactive antibodies with 2 effector function against emerging Omicron variants

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43 **Currently circulating SARS-CoV-2 variants acquired convergent mutations at receptor-**
44 **binding domain (RBD) hot spots¹. Their impact on viral infection, transmission, and**
45 **efficacy of vaccines and therapeutics remains poorly understood. Here, we demonstrate**
46 **that recently emerged BQ.1.1. and XBB.1 variants bind ACE2 with high affinity and promote**
47 **membrane fusion more efficiently than earlier Omicron variants. Structures of the BQ.1.1**
48 **and XBB.1 RBDs bound to human ACE2 and S309 Fab (sotrovimab parent) explain the**
49 **altered ACE2 recognition and preserved antibody binding through conformational**
50 **selection. We show that sotrovimab binds avidly to all Omicron variants, promotes Fc-**
51 **dependent effector functions and protects mice challenged with BQ.1.1, the variant**
52 **displaying the greatest loss of neutralization. Moreover, in several donors vaccine-elicited**
53 **plasma antibodies cross-react with and trigger effector functions against Omicron variants**
54 **despite reduced neutralizing activity. Cross-reactive RBD-directed human memory B cells**
55 **remained dominant even after two exposures to Omicron spikes, underscoring persistent**
56 **immune imprinting. Our findings suggest that this previously overlooked class of cross-**
57 **reactive antibodies, exemplified by S309, may contribute to protection against disease**
58 **caused by emerging variants through elicitation of effector functions.**

59

60 The emergence of the Omicron variant of concern at the end of 2021 marked a new phase
61 of the COVID-19 pandemic². Omicron lineages accumulated tens of amino acid mutations in their
62 spike (S) glycoprotein that enhanced receptor engagement, altered the preferred internalization
63 route in cells and promoted immune evasion from neutralizing antibodies of unprecedented
64 magnitude³⁻¹⁵. As a result, most countries experienced repeated waves of infections in 2021 and
65 2022, driven by successive Omicron lineages (e.g., BA.1/BA.1.1, BA.2, BA.5), including in
66 individuals who received multiple COVID-19 vaccine doses.

67 Currently, many different Omicron variant lineages are co-circulating worldwide. Due to
68 convergent evolution, many of these lineages independently acquired identical or similar amino
69 acid mutations at key antigenic sites in the RBD and in the NTD, relative to their presumed BA.2
70 and BA.5 ancestors¹. The BA.2.75.2 lineage rose in frequency in multiple countries (e.g., India)
71 and has the following RBD residue mutations relative to BA.2: D339H, R346T, G446S, N460K,
72 F486S and R493Q. CH.1.1 emerged in Asia in November of 2022 and currently accounts for
73 ~12% of infections in Europe and carries the K444T and L452R RBD residue mutations relative
74 to BA.2.75.2. XBB.1 is an inter-Omicron recombinant lineage, derived from BJ.1 and BA.2.75,
75 and harbors the following RBD residue mutations relative to BA.2: D339H, R346T, L368I, V445P,
76 G446S, N460K, F486S, F490S, R493Q (**Fig. 1a**). Furthermore, the XBB.1.5 lineage, which
77 contains a proline at position 486 instead of a serine (F486 in Wuhan), is currently rising in the
78 US with ~85% of cases attributed to the variant as of 25 February 2023. BQ.1 and BQ.1.1 are
79 dominant in several Western countries and account for 56% of all sequenced SARS-CoV-2
80 genomes in the US (<https://covid.cdc.gov/covid-data-tracker/#variant-proportions>). BQ.1.1 has the
81 following residue mutations relative to BA.5: R346T, K444T and N460K (**Fig. 1a**). Several of the
82 mutated amino acid positions present in the XBB.1(.5) and BQ.1.1 variant S glycoproteins were
83 previously detected in cryptic lineages through wastewater sequencing¹⁶ and virtually all of them
84 map to key NTD and RBD antigenic sites.

85 SARS-CoV-2 S recognizes angiotensin-converting enzyme 2 (ACE2) as its main entry
86 receptor, leading to membrane fusion and viral entry^{17–20}. RBD-directed antibodies account for
87 most neutralizing activity against vaccine-matched and vaccine-mismatched viruses, whereas the
88 N-terminal domain is mostly targeted by variant-specific neutralizing antibodies^{21–34}. Here, we set
89 out to understand how the constellation of mutations in the BQ.1.1, XBB.1(.5) and BA.2.75.2 S
90 variants affect the functional properties of SARS-CoV-2, humoral and memory immunity in
91 humans, and recognition by therapeutic antibodies.

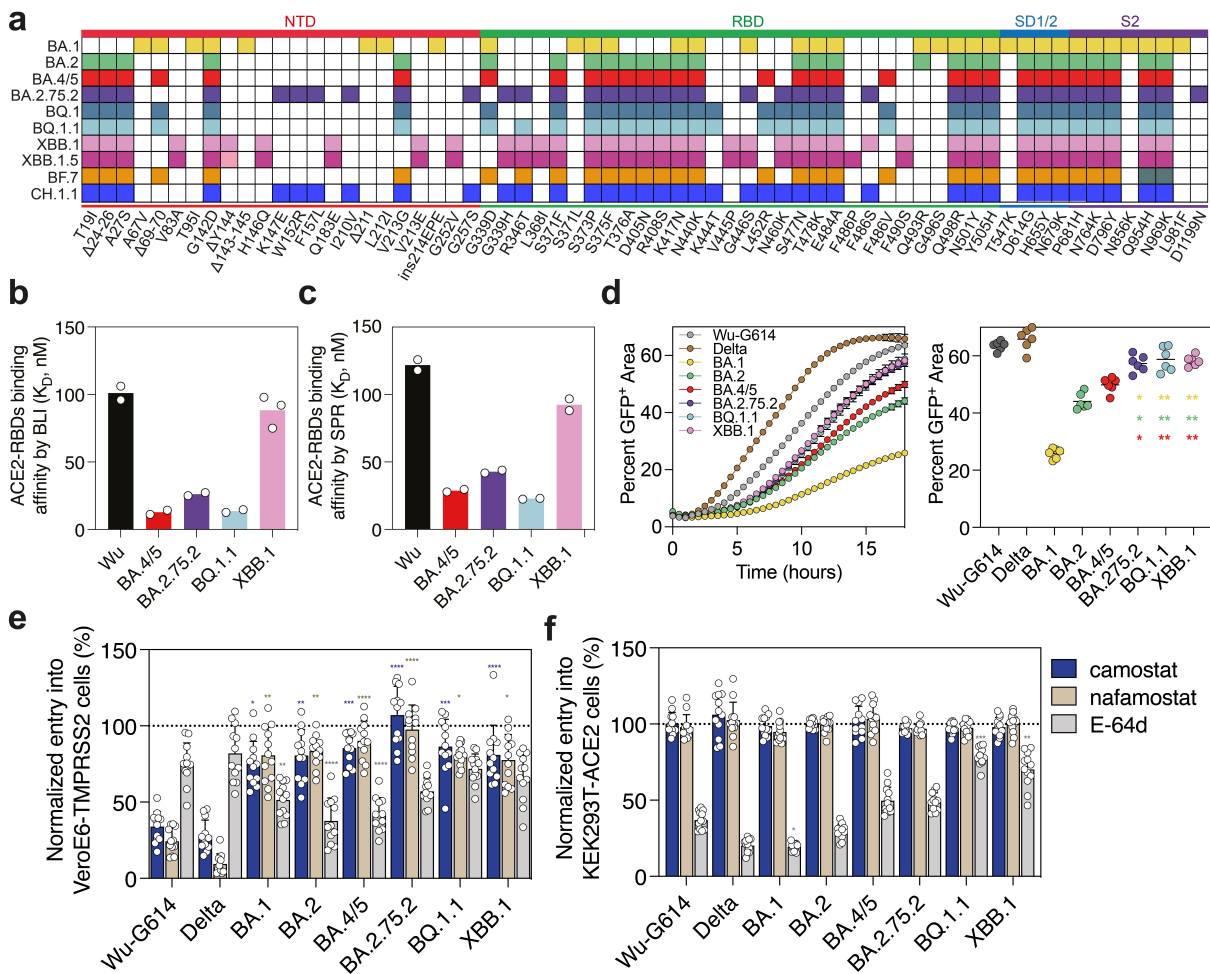
92 **Functional properties of BQ.1.1, XBB.1 and BA.2.75.2 S**

93 We first determined the binding kinetics and affinity of the monomeric human ACE2
94 ectodomain to immobilized variant RBDs using biolayer interferometry (**Fig. 1b, Extended Data**
95 **Fig. 1** and **Supplementary Table 1**). We measured identical equilibrium dissociation constants
96 for the BQ.1.1 and BA.5 RBDs ($K_D= 12.8$ nM and 13.7 nM, respectively), indicating that the
97 additional BQ.1.1 mutations, which map outside the ACE2-binding interface, do not influence
98 receptor engagement (**Fig. 1b, Extended Data Fig. 1** and **Supplementary Table 1**). The
99 enhanced ACE2 binding affinity of the BA.2.75.2 RBD ($K_D= 26.2$ nM), relative to BA.2, results
100 from the R493Q reversion, as G446S has a negligible effect and F486S has a deleterious effect
101 on ACE2 engagement based on mutagenesis and deep-mutational scanning data³⁵. We also
102 determined that ACE2 bound to the XBB.1 RBD with an affinity comparable to the Wuhan-Hu-1
103 (Wu) RBD ($K_D= 88.4$ nM and $K_D= 101.1$ nM, respectively). As V445P does not change the
104 conformation of the ACE2-bound XBB.1 RBD, relative to BQ.1.1, and none of the three residue
105 substitutions compared to BA.2.75.2 involve side chain-mediated contacts with the host receptor,
106 it is possible that the V445P mutation alters the backbone conformational dynamic of the free
107 XBB.1 RBD and dampens ACE2 binding. We observed a similar ranking of these variant RBDs
108 using surface plasmon resonance (SPR) to determine ACE2 binding affinities (**Fig. 1c, Extended**
109 **Data Fig. 1** and **Supplementary Table 2**). Modulation of ACE2 binding resulted from off-rate
110 differences, whereas on-rates remained comparable across all variants tested, in agreement with
111 observations made with previous variants^{3,5,35–37}. Collectively these data indicate that BQ.1.1,
112 BA.2.75.2 and BA.5 have comparably high ACE2 binding affinity, suggesting that the BQ.1.1 and
113 BA.2.75.2 viral fitness is not limited by this step of host cell invasion, whereas the lower ACE2-
114 binding affinity of XBB.1 might have limited its spread.

115 We next compared the kinetics and magnitude of cell-cell fusion promoted by the Wu-
116 G614, Delta, BA.1, BA.2, BA.5, BQ.1.1, BA.2.75.2 and XBB.1 S glycoproteins. This live cell
117 imaging assay uses a split green fluorescent protein (GFP) system with VeroE6 target cells stably
118 expressing TMPRSS2 and GFP β_{1-10} strands and BHK-21 effector cells stably expressing GFP
119 β_{11} strand and transiently transfected with S^{3,38}. We observed slower and reduced fusogenicity for
120 the BA.5, BA.2 and BA.1 S glycoproteins compared with Wu-G614 and even more so relative to
121 Delta S¹² (**Fig. 1d** and **Extended Data Fig. 2**), in line with previous findings and the lack of
122 syncytia formation observed with authentic viruses^{3,7}. BQ.1.1, BA.2.75.2 and XBB.1 S, however,
123 promoted membrane fusion more efficiently than the earlier Omicron variants (**Fig. 1d** and
124 **Extended Data Fig. 2**), suggesting enhanced fusogenic potential of these recently emerged
125 variants.

126 We and others previously showed that BA.1, BA.2 and BA.5 had an altered cell entry
127 pathway relative to previous SARS-CoV-2 strains, with Omicron variants entering preferentially

128 through the endosomal entry route (cathepsin-mediated) as opposed to plasma membrane fusion
129 (TMPRSS2-mediated)^{6-8,19,39}. To assess the preferred cell entry route of the emerging Omicron
130 variants, we investigated the impact of protease inhibitors on entry of non-replicative vesicular
131 stomatitis virus (VSV) pseudotyped with S glycoproteins into VeroE6-TMPRSS2 cells (enabling
132 both plasma membrane and endosomal entry routes) and HEK293T-ACE2 cells (enabling
133 endosomal entry only). The serine protease (TMPRSS2) inhibitors camostat and nafamostat
134 potently blocked entry of Wu-G614 and Delta S VSV pseudoviruses in VeroE6-TMPRSS2 cells,
135 but had limited effect on any of the Omicron variants, including BQ.1.1, BA.2.75.2 and XBB.1
136 (**Fig. 1e**). Reciprocally, the cathepsin B and L inhibitor E64d more significantly inhibited the entry
137 of BA.1, BA.2 and BA.5 S VSV pseudoviruses relative to Wu-G614, whereas no significant
138 difference in entry was measured for Delta, BA.2.75.2, BQ.1.1 or XBB.1 S VSV compared to Wu-
139 G614 in VeroE6-TMPRSS2 cells (**Fig. 1e**). Furthermore, BQ.1.1 and XBB.1 S-mediated entry
140 was also less affected by E-64d than all other variant S evaluated in HEK293T-ACE2 cells (**Fig.**
141 **1f** and **Extended Data Fig. 2**). These findings indicate a possible alteration of protease
142 requirements for BQ.1.1 and XBB.1 S-mediated entry, relative to other Omicron lineages
143 assessed. The inefficient use of TMPRSS2 observed, however, concurs with the identical BQ.1.1,
144 BA.2.75.2 and XBB.1 S₂ subunit sequences, particularly the presence of the N969K substitution,
145 which was previously identified as the key change accounting for the switch from plasma
146 membrane to endosomal cell entry routes of Omicron variants⁶⁻⁸.
147



148
149 **Fig. 1. Functional properties of the newly emerged BQ.1.1, XBB.1 and BA.2.75.2 variant S**
150 **glycoproteins.** **a**, Schematic view of S mutations carried by SARS-CoV-2 variants used in this
151 study. **b**, **c**, Equilibrium dissociation constants (K_D) measured by biolayer interferometry (**b**) and
152 surface plasmon resonance (**c**) for binding of the monomeric human ACE2 ectodomain to the
153 immobilized Wu, BA.4/5, BA.2.75.2, BQ.1.1, or XBB.1 RBDs. **d**, Cell-cell fusion (expressed as the
154 percentage of GFP⁺ area) between cells expressing the Wu-G614, Delta, BA.1, BA.2, BA.4/5,
155 BA.2.75.2, BQ.1.1, or XBB.1 S glycoproteins and VeroE6-TMPRSS2 cells measured over an 18-
156 hour time course experiment using a split GFP system (left panel). The mean magnitude of cell-
157 cell fusion at 18 hours is shown on the right panel. Data are from 6 fields of view from a single
158 experiment and representative of results from two independent biological replicates. Comparisons
159 of fusogenicity mediated by BA.1, BA.2, or BA.4/5 S to BA.2.75.2, BQ.1.1, or and XBB.1 S were
160 completed using the Dunnett's test with the colors of the asterisks indicating which group the
161 comparison is done with (BA.1: gold; BA.2: green; BA.4/5: red). ** $P < 0.01$. **e**, **f**, Relative entry of
162 VSV pseudotyped with the Wu-G614, Delta, BA.1, BA.2, BA.4/5, BA.2.75.2, BQ.1.1, or XBB.1 S
163 in VeroE6-TMPRSS2 (**e**) or HEK293T-ACE2 (**f**) cells treated with 50 μ M of camostat, nafamostat,
164 or E-64d. Normalized entry was calculated based on entry values obtained for VeroE6-TMPRSS2
165 or HEK293T-ACE2 cells treated with DMSO only for each pseudovirus. Mean normalized entry
166 and standard deviation are presented for each pseudovirus. Twelve technical replicates were

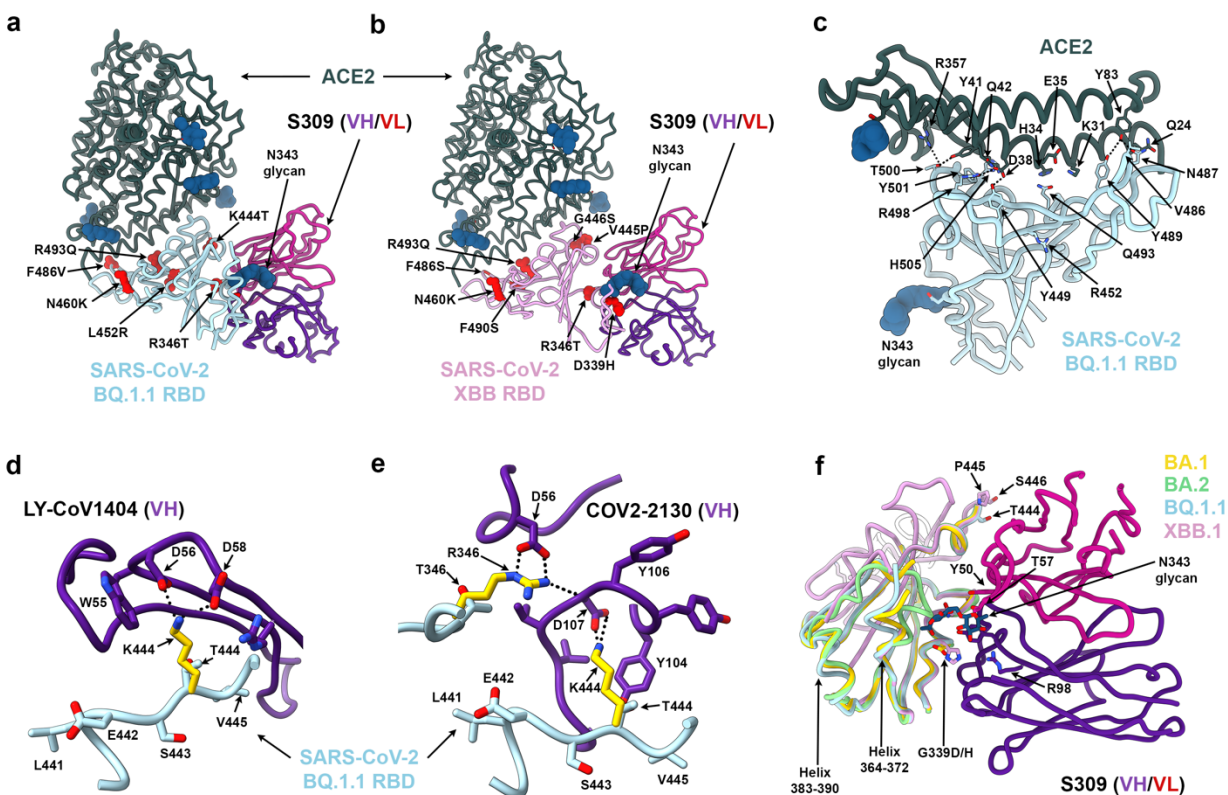
167 performed for each pseudovirus and inhibitor and one experiment representative of two
168 independent biological replicates is shown. Comparison of relative entry values were made
169 between Wu-G614 S VSV pseudovirus and each of the examined SARS-CoV-2 variant S VSV
170 pseudoviruses using the Dunnett's test. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

171
172 **Structural analysis of the impact of BQ.1.1, XBB.1 and BA.2.75.2 RBD mutations on**
173 **recognition by ACE2 and therapeutic antibodies**

174 To reveal how amino acid substitutions in the BQ.1.1 and XBB.1 RBDs alter receptor
175 recognition and key antigenic sites, we determined cryo-electron microscopy (cryoEM) structures
176 of each RBD bound to the human ACE2 ectodomain and to the Fab fragment of the S309
177 monoclonal antibody (sotrovimab parent) (**Fig. 2a-b, Extended Data Fig. 3a-e** and
178 **Supplementary Table 3**). In both structures, the R493Q reversion likely relieves repulsion with
179 ACE2 residue K31 and restores a network of local interactions similar to that made with the Wu
180 RBD⁴⁰ (**Fig. 2c**). Regarding interactions with therapeutic antibodies, the BQ.1.1 RBD structure
181 shows that the K444T substitution would abrogate salt bridges with the carboxyl side chains of
182 the LY-CoV1404 (bebtelovimab parent) and heavy chain residues D56 and D58 or of the COV2-
183 2130 (cilgavimab parent) heavy chain residue D107 (**Fig 2d, e**). Moreover, R346T (BQ.1.1/XBB.1)
184 would abrogate a salt bridge with the COV2-2130 heavy chain residue D56; G446S (XBB.1) is
185 expected to reduce COV2-2130 binding sterically and V445P (XBB.1) is expected to reduce
186 binding due to a loss of van der Waals interactions with LY-CoV1404 (**Extended Data Fig. 3f**).
187 These data explain the decreased LY-CoV1404 binding observed by deep-mutational scanning
188 of yeast-displayed mutant RBDs and the markedly reduced neutralization of LY-CoV1404, COV2-
189 2130 or the COV2-2130/COV2-2196 (Evusheld parent) cocktail against the BQ.1.1 and XBB.1
190 variants^{1,35,41}.

191 The structures demonstrate that S309 binds to both the BQ.1.1 and XBB.1 RBDs and
192 reveal the molecular basis for accommodation of the H339 residue in the XBB.1 epitope (**Fig. 2f**).
193 The S309 binding pose is indistinguishable from that observed when bound to the Wu RBD⁴² or
194 to the BA.1 RBD (**Fig. 2f**). We recently described that the S371F mutation, which is present in
195 BA.2, BA.5, BQ.1.1, XBB.1 and BA.2.75.2, leads to conformational changes of the RBD helix
196 comprising residues 364-372 that are sterically incompatible with the glycan N343 conformation
197 observed in S309-bound spike structures⁴³. In the BQ.1.1 structure, helix 364-372 is weakly
198 resolved in the cryoEM map and adopts a conformation similar to that observed in the S309-
199 bound BA.1 structure but distinct from apo BA.2⁴⁴ or apo BA.5 S⁴⁵ structures (**Fig. 2f**). Residues
200 368-373 are disordered in the XBB.1 RBD cryoEM map, as is the case for the adjacent residues
201 380-392 (**Fig. 2f**). These findings are strongly indicative of conformational frustration of helix 364-
202 372 which is constrained to adopt an energetically disfavored conformation upon S309 binding
203 and could explain the reduced neutralizing activity of this antibody against these variants^{1,14,41,46,47}
204 (**Fig. 3a**).

205



206
 207 **Fig. 2. Structural analysis of the BQ.1.1 and XBB.1 RBDs.** **a, b,** CryoEM structures of the
 208 BQ.1.1 RBD (**a**, cyan) or the XBB.1 RBD (**b**, pink) bound to the human ACE2 ectodomain (green)
 209 and the S309 Fab fragment (purple/magenta for the heavy/light chains). Amino acid residues
 210 mutated relative to Omicron BA.2 are shown as red spheres. **c,** Zoomed-in view of the BQ.1.1
 211 RBD interactions formed with human ACE2 with select amino acid residue side chains shown as
 212 sticks. N-linked glycans are shown as dark blue spheres in (**a-c**). **d, e,** Superimposition of the LY-
 213 CoV1404-bound Wu RBD structure (**d**, purple, PDB 7MMO) or of the COV2-2130-bound Wu RBD
 214 structure (**e**, purple, PDB 7L7E) onto the BQ.1.1 RBD cryoEM structure presented here
 215 highlighting the expected disruptions of electrostatic interactions with the monoclonal antibodies
 216 resulting from the K444T and the R346T RBD mutations. **f,** RBD-based superimpositions of the
 217 S309-bound BA.1 S (gold, PDB 7TLY), apo BA.2 S (green, PDB 7UB0), S309- and ACE2-bound
 218 BQ.1.1 (cyan) and XBB.1 (pink) RBD cryoEM structures (this study). S309 is rendered purple and
 219 magenta for heavy and light chains, respectively, and the N343 glycan along with select
 220 side chains are rendered as sticks. The expected N343 glycan clashes with BA.2 residues N370 and
 221 F371 (sticks) are indicated with a red/orange star.

222

223 S309 activates effector functions and protects mice against BQ.1.1 challenge

224 Based on the cryoEM visualization of S309 binding to the BQ.1.1 and XBB.1 RBDs, we
 225 investigated the binding kinetics and affinity of the S309 Fab to the immobilized Wu, BA.1, BA.2
 226 BA.2.75.2, BQ.1, BQ.1.1 and XBB.1 RBDs using SPR (**Fig. 3b**). The binding affinity (K_D) of S309
 227 against these variants decreased up to ~ 100 fold, primarily as a result of a slower association rate
 228 as compared to that against Wu RBD. Little to no dissociation could be observed within the
 229 timeframe of our experiment with any RBD evaluated (**Extended Data Fig. 4**). Structural
 230 frustration of the RBD helix 364-372, in F371-harboring SARS-CoV-2 Omicron variants, combined

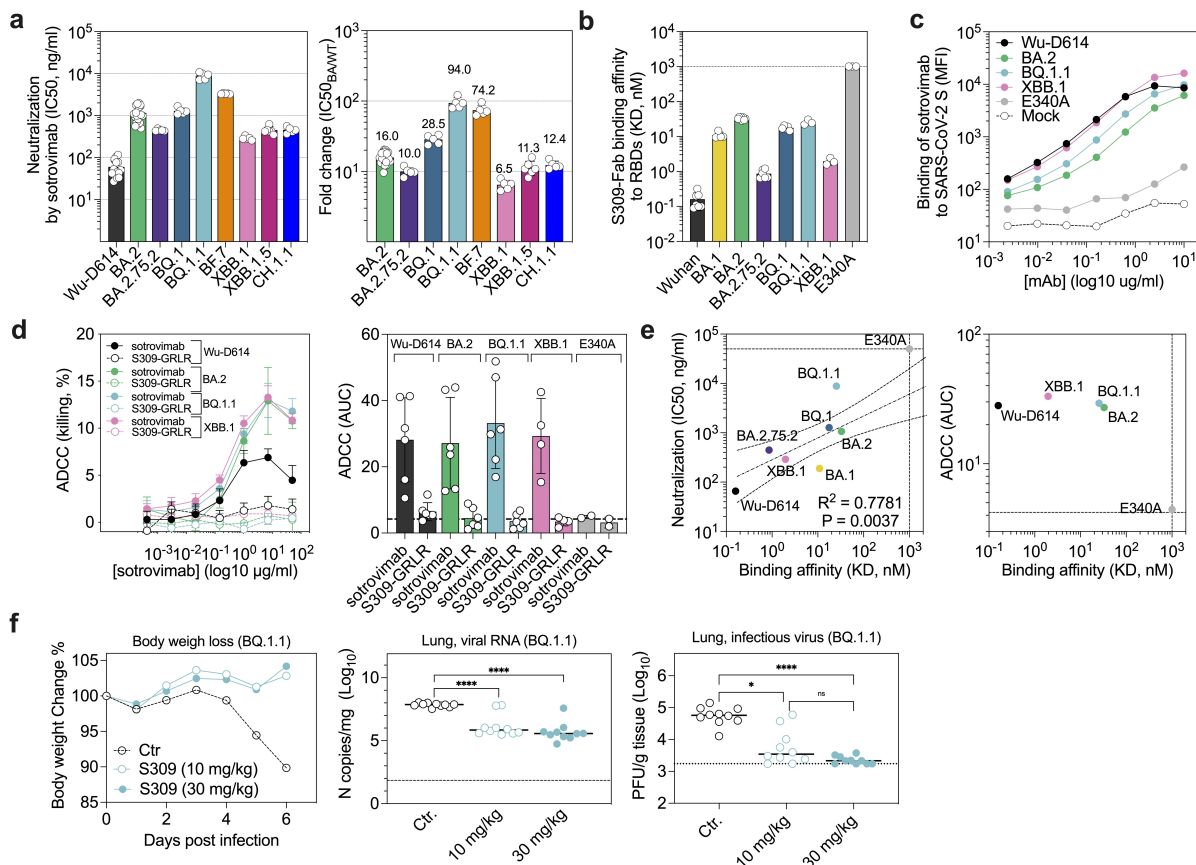
231 with substitutions at position 339 (G339D/H) likely modulate the observed on-rates through S309-
232 mediated backbone conformational and amino acid side chain rotameric selection. We also
233 observed that the sotrovimab IgG (derivative of S309) efficiently cross-reacted with full-length,
234 cell-surface expressed BQ.1.1 and XBB.1 S trimers, to levels comparable or greater than those
235 observed for BA.2 S, but not with the E340A S (negative control⁴⁸) escape mutant (**Fig. 3c** and
236 **Extended Data Fig. 5a**). These data show that sotrovimab IgG binds avidly to all currently
237 dominant Omicron SARS-CoV-2 variants. However, the neutralizing potency of sotrovimab varied
238 widely against these variants, ranging from a 6.5-fold loss against XBB.1 up to a 94-fold loss
239 against BQ.1.1 relative to Wu (**Fig. 3a** and **Extended Data Fig. 6a-b**), in line with recent
240 reports^{1,14,41,46,47}.

241 Given the reduced neutralization potency of sotrovimab against these variants despite
242 retaining binding avidity, we evaluated the ability of this therapeutic antibody to activate antibody-
243 dependent cell cytotoxicity (ADCC) using primary natural killer (NK) effector cells and CHO target
244 cells expressing SARS-CoV-2 S of the different Omicron variants at their surface. Sotrovimab
245 efficiently promoted ADCC of cells expressing Wu-D614, BA.2, BQ.1.1 or XBB.1 S in a
246 concentration- and Fc-dependent manner (**Fig. 3d** and **Extended Data Fig. 5b**). Although we
247 observed a linear relationship between the S309 Fab binding affinity and in vitro neutralization
248 potency, this correlation was not apparent with respect to the magnitude of ADCC, which was
249 equivalent against all variants in spite of up to 100-fold differences in binding affinity (**Fig. 3e**).

250 To better understand the physiological relevance of these findings, we administered S309
251 to mice which were subsequently challenged with BQ.1.1, the variant associated with the greatest
252 loss of in vitro neutralizing activity (i.e., 94-fold loss relative to Wu-D614 S pseudovirus). S309
253 was given as prophylaxis at 10 or 30 mg/kg doses to K18/hACE2 mice one day prior to challenge
254 with SARS-CoV-2 BQ.1.1. Notably, S309 completely protected K18-hACE2 transgenic mice from
255 weight loss at both doses, whereas animals receiving a control antibody lost greater than 10% of
256 body weight by day 6 (**Fig. 3f**). Furthermore, S309 administration reduced both viral RNA and
257 infectious virus titers in the lung in a statistically significant manner at both 10 and 30 mg/kg doses
258 relative to control mice (**Fig. 3f**). These data indicate that, despite its marked reduction of in vitro
259 neutralizing activity, S309 can protect mice from BQ.1.1 challenge (consistent with a recent pre-
260 print on BQ.1.1-challenged hamsters⁴⁹).

261

262



263
 264 **Fig 3. S309 triggers antibody-dependent cell cytotoxicity (ADCC) in vitro and protects mice**
 265 **against BQ.1.1 challenge in vivo.** **a**, Sotrovimab-mediated neutralization of Wu-D614, BA.2,
 266 BA.2.75.2, BQ.1, BQ.1.1, BF.7, XBB.1, XBB.1.5 and CH.1.1 S VSV pseudoviruses. The
 267 sotrovimab neutralizing potency is represented by its IC₅₀ (left panel) or fold change relative to
 268 neutralization of the Wu-D614 VSV pseudovirus (right panel). Each symbol represents IC₅₀ or
 269 fold-change values from biological replicates. **b**, Single-cycle kinetics surface plasmon resonance
 270 (SPR) analysis of S309 Fab binding to the indicated SARS-CoV-2 RBD variants. Each symbol
 271 represents K_D values from independent experiments. **c**, Binding of sotrovimab to SARS-CoV-2 S
 272 variants transiently expressed at the surface of Expi-CHO cells as determined by flow-cytometry.
 273 Similar levels of variant S expression were confirmed by staining with a monoclonal antibody
 274 (S2V29) that retains equal and potent neutralizing activity against Wu-D614, BA.2, BQ.1.1, XBB.1
 275 and E340A (**Extended Data Fig. 5a**). **d**, ExpiCHO-S cells transiently transfected with Wu-D614,
 276 BA.2, BQ.1.1 or XBB.1 S were incubated with the indicated concentrations of sotrovimab
 277 (derivative of S309) or S309-GRLR mAb and mixed with purified NK cells isolated from healthy
 278 donors. Cell lysis was determined by a lactate dehydrogenase release assay. Data are presented
 279 as mean values ± standard deviations (SD) from one representative donor (left panel). Area under
 280 the curve (AUC) analyses from two, four or six NK donors (right panel). **e**, Correlation of
 281 sotrovimab Fab binding affinity with neutralizing activity or ADCC. The neutralization IC₅₀ values
 282 from (a) or the ADCC AUC values from (d) are plotted on the y-axis and the binding affinity to
 283 each RBD variant obtained in (b) is plotted on the x-axis. The neutralization data of S309 against

284 BA.1 were adapted from Cameroni et al. Dotted lines indicate the limit of detection. Best-fit lines
285 were calculated using a simple linear regression. Two-tailed Pearson correlation was used to
286 calculate the R^2 and P values. **f**, Eight-week-old female K18-hACE2 mice received 30 mg/kg of a
287 control isotype-matched monoclonal antibody (anti-West Nile virus hE16), or 10 or 30 mg/kg of
288 S309 (parent of sotrovimab) by intraperitoneal injection one day before intranasal inoculation with
289 10^4 FFU of SARS-CoV-2 BQ.1.1. Body weight loss was monitored daily, and tissues were
290 collected at six days after inoculation. Body weight loss (left panel), lung viral RNA determined by
291 RT-qPCR (middle panel), and infectious lung virus titers was measured by plaque assay (right
292 panel) (lines indicate the median; $n = 10$ mice per group; Kruskal-Wallis ANOVA with Dunn's post-
293 test between isotype and S309 treatment; ns, not significant; * $P < 0.05$, ****, $P < 0.0001$).

294

295 **Bivalent mRNA vaccines enhance neutralizing antibody responses against emerging** 296 **SARS-CoV-2 variants**

297 To assess the impact of the BQ1.1, XBB.1 and BA.2.75.2 S mutations on vaccine-elicited
298 antibody responses, we quantified plasma neutralizing activity using VSV pseudotyped with Wu-
299 G614, BA.1, BA.5, BF.7, BQ.1.1, XBB.1 or BA.2.75.2 S. We compared plasma from eight cohorts
300 of individuals obtained 15-30 days post vaccination or PCR-confirmed breakthrough (BT) infection
301 corresponding to subjects: (i) mRNA vaccinated four times with the prototype vaccine encoding
302 Wuhan-1 spike and without known infection ("Wu₄ vaccinated"); (ii) mRNA vaccinated four times
303 without known infection, the last dose being the Wu/BA.5 bivalent booster ("Wu/BA.5 bivalent
304 vaccinated"); (iii) previously infected in 2020 (with a WA1/2020-like SARS-CoV-2 strain) and then
305 mRNA vaccinated four to five times, the last dose being Wu/BA.5 mRNA bivalent booster ("pre-
306 Omicron infected-Wu/BA.5 bivalent vaccinated"); (iv) mRNA vaccinated before experiencing an
307 Omicron BA.1, BA.2, BA.2.12.1 or BA.5 BT infection and vaccinated again with the Wu/BA.5
308 mRNA bivalent booster ("Omicron BT-Wu/BA.5 bivalent vaccinated"). As an alternative to the
309 Wu/BA.5 bivalent mRNA booster, Switzerland and a few other countries, offer a Wu/BA.1 bivalent
310 mRNA booster. We therefore analyzed neutralization in additional cohorts: (v) mRNA vaccinated
311 three times with the Wu monovalent vaccine without known infection ("Wu₃ vaccinated"); (vi)
312 mRNA vaccinated three times with the Wu monovalent vaccine after pre-Omicron BT infection
313 ("pre-Omicron infected-Wu vaccinated"); (vii) mRNA vaccinated four times without known
314 infection, the last dose being the Wu/BA.1 bivalent booster ("Wu/BA.1 bivalent vaccinated"); (viii)
315 mRNA vaccinated four times with a BA.1 or a BA.2 BT infection, the last dose being the Wu/BA.1
316 bivalent booster ("Omicron BT-Wu/BA.1 bivalent vaccinated"). In addition, we analyzed plasma
317 samples obtained from kidney transplant recipients (KTR) vaccinated with four Wu monovalent
318 doses with or without pre-Omicron infection.

319 Wu/BA.5 or Wu/BA.1 bivalent mRNA vaccination elicited comparable neutralizing
320 antibody titers against Wu-G614 S VSV with that observed in matched Wu vaccinated cohorts
321 but higher neutralization of BA.1 S and BA.5 S VSV pseudoviruses (**Fig. 4 a, b**). Cohorts that
322 received the Wu/BA.5 or the Wu/BA.1 bivalent mRNA vaccines had detectable neutralizing
323 activity against the vaccine-mismatched XBB.1, BA.2.75.2 and BQ.1.1 S VSV pseudoviruses,
324 irrespective of prior infection status, whereas little to no neutralization of these variants was
325 detected for the Wu-only vaccinated subjects (**Fig. 4 a, b**). We did not detect plasma neutralizing
326 activity against circulating Omicron variants in subjects that were vaccinated four times with the
327 Wu monovalent booster but were immunosuppressed following kidney transplantation,

328 underscoring the difficulties associated with protecting these at-risk populations (**Extended Data**
329 **Fig. 7**). Overall, these data suggest that bivalent (Wu/BA.1 or Wu/BA.5) mRNA vaccination elicits
330 more potent and broader antibody responses against vaccine-matched and mismatched Omicron
331 variants than Wu mRNA vaccination in healthy subjects.

332

333 **Plasma antibodies maintain binding and Fc-mediated effector function against emerging** 334 **Omicron variants**

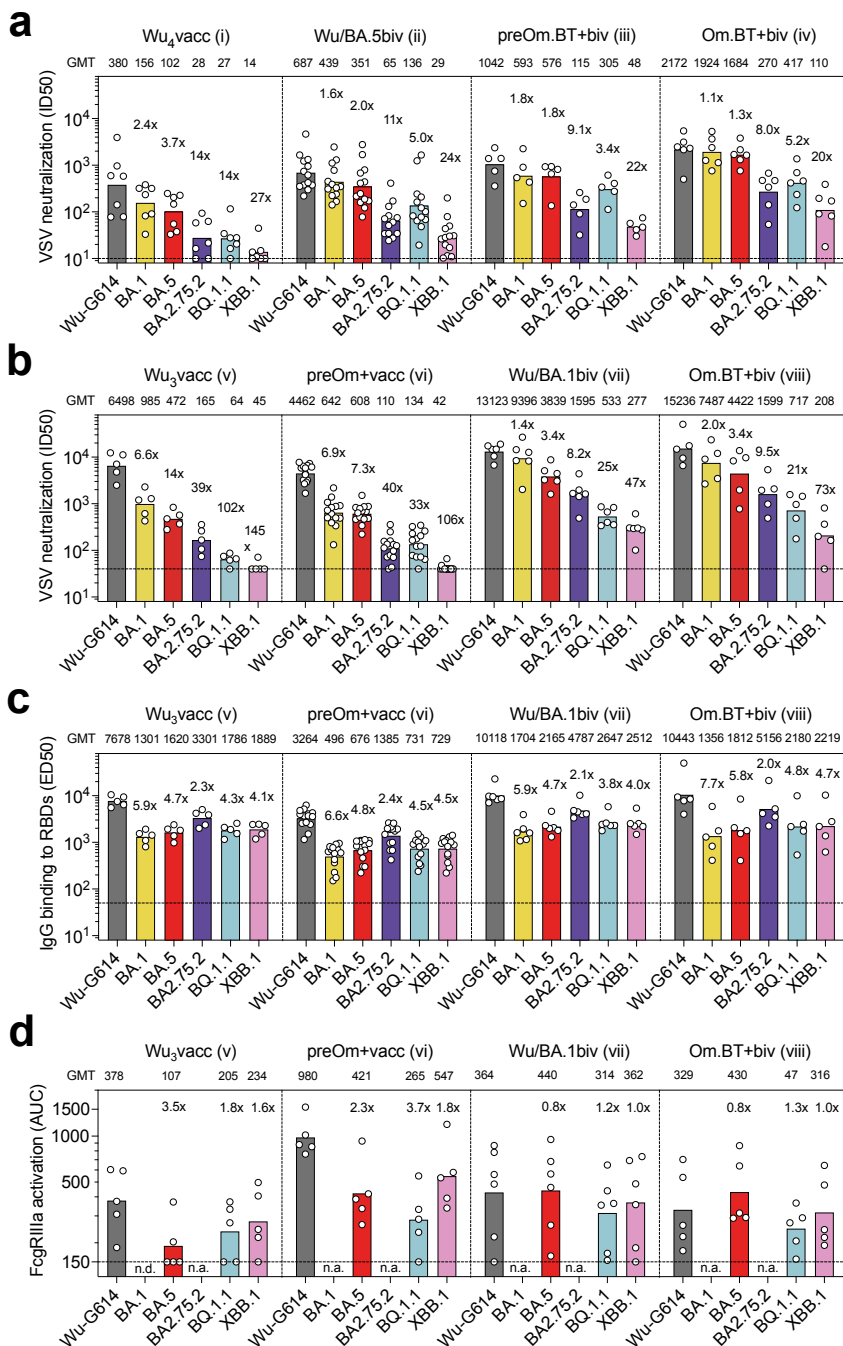
335 Next, we investigated binding and Fc-mediated effector function of plasma antibodies from
336 cohorts v to viii. The progressive reduction of neutralizing antibody titers against currently
337 dominant Omicron variants overtime was not paralleled by a similar decrease in IgG binding titers
338 to matched RBDs (**Fig. 4c**), as binding titers remained comparable across all Omicron variants.
339 This finding is consistent with data on pre-Omicron variants⁵⁰ and with the notion that SARS-CoV-
340 2 is evolving primarily to escape from neutralizing antibodies that exert the greatest selective
341 pressure.

342 Fc-mediated effector function of plasma antibodies was evaluated by measuring activation
343 of the Fc γ R11a (V158 allele) as a surrogate of ADCC using Jurkat reporter cells and Wu-G614,
344 BA.5, BQ.1.1 and XBB.1 SARS-CoV-2 S-expressing ExpiCHO as target cells (**Fig. 4d** and
345 **Extended Data Fig. 8**). Each cohort exhibited high individual variability in the ability of plasma to
346 trigger activation of Fc γ R11a, with some donors lacking measurable activity despite the presence
347 of high titers of RBD-binding antibodies (**Fig. 4c**). Samples that scored positive for Fc γ R11a
348 triggering against Wu retained activity against BA.5, BQ.1.1 and XBB.1 S variants. These results
349 suggest that antibodies capable of triggering Fc effector functions vary widely amongst
350 individuals, but, when present, these antibodies are broadly reactive against Omicron variants.

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354
 355 **Fig. 4. Neutralization, binding and Fc-dependent effector functions of vaccine-elicited**
 356 **plasma antibodies against the BA.2.75.2, BQ.1.1 and XBB.1 variants.** a, b, Neutralization of
 357 SARS-CoV-2 pseudotyped VSV carrying Wu-G614, Omicron BA.1, BA.5, BA.2.75.2, BQ.1.1 and
 358 XBB.1 S by plasma samples of cohorts i-iv (a) and v-viii (b). Plasma neutralizing titers expressed
 359 as ID₅₀s from n = 2 biological (a) and technical (b) replicates are shown. Bars and values on top
 360 represent geometric mean ID₅₀ titers (GMT). Fold-loss of neutralization against Omicron variants
 361 as compared to Wu-G614 is shown above each corresponding bar. Horizontal dashed lines
 362 indicate the limit of detection in these assays (ID₅₀ = 10 in panel a and 40 in panel b). c, Binding
 363 of plasma IgG antibodies to SARS-CoV-2 RBDs from Wu, Omicron BA.1, BA.5, BA.2.75.2, BQ.1.1

364 and XBB.1 variants as measured by ELISA. Shown are ED₅₀ values from n = 2 technical replicates
365 of samples from cohorts v-viii. Bars represent geometric mean ED₅₀ binding titers (GMT). Fold-
366 loss of binding titers to Omicron variants as compared to Wu is shown above each corresponding
367 bar. Horizontal dashed line indicates the limit of detection (ED₅₀ = 50). **d**, Activation of FcγRIIIa
368 (V158 allele) measured using Jurkat reporter cells and Wu-G614, BA.5, BQ.1.1 and XBB.1 SARS-
369 CoV-2 S glycoprotein-expressing ExpiCHO as target cells. Shown are AUC values from one
370 experiment with plasma samples from cohorts v-viii (n=5 donors for cohort v, n=5 for cohort vi,
371 n=6 for cohort vii and n=5 for cohort viii). Bars and values on top represent geometric mean AUC
372 titers (GMT). Fold-change of activation with Omicron variants as compared to Wu-G614 is shown
373 above each corresponding bar. The horizontal dashed line indicates the limit of detection (AUC =
374 150). n.a., not assayed. Demographics of cohorts are summarized in **Supplementary Table 5**.

375

376 **Cross-reactive RBD-specific memory B cells dominate bivalent vaccine-elicited responses**

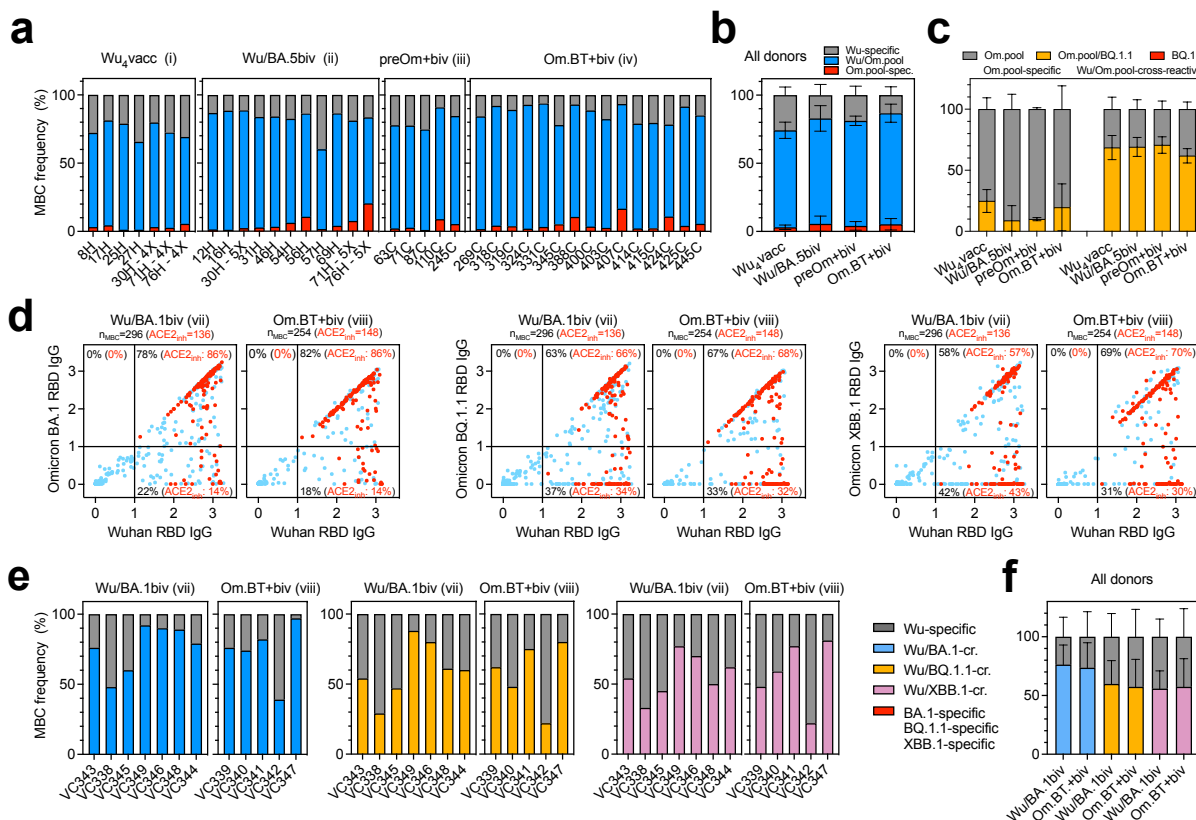
377 We next compared memory B cell (MBC) populations in the Wu₄ vaccinated, Wu/BA.5
378 bivalent vaccinated (including one individual [31H] who received the Janssen COVID-19 vaccine
379 as their first vaccine series), pre-Omicron infected-Wu/BA.5 bivalent vaccinated, and Omicron
380 BT-Wu/BA.5 bivalent vaccinated subjects (cohorts i-iv) by enumerating the frequency of Wu RBD-
381 specific, Omicron RBD-specific (pool of BA.1/BA.2/BA.5), and cross-reactive MBCs by flow
382 cytometry. Individuals who were exposed only to Wu S through vaccination (Wu₄ vaccinated) had
383 the highest frequency of Wu RBD-specific MBCs (mean frequency: 25.8%) and the lowest
384 frequency of cross-reactive MBCs (mean frequency: 71.1%) of the four cohorts examined (**Fig.**
385 **5a, b** and **Extended Data Fig. 9**). Individuals who were exposed only once to Omicron S through
386 vaccination had few Omicron RBD-specific MBCs (mean frequency: 5.1%), regardless of whether
387 they had experienced a pre-Omicron SARS-CoV-2 infection (Wu/BA.5 bivalent and pre-Omicron
388 bivalent). Most RBD-specific MBCs in these cohorts cross-reacted with the Wu RBD and the
389 Omicron RBD pool (BA.1/BA.2/BA.5), with uninfected individuals having similar frequencies of
390 cross-reactive MBCs (mean: 77.4%) when compared to individuals who had experienced a pre-
391 Omicron infection (mean frequency: 77.1%) (**Fig. 5a, b** and **Extended Data Fig. 9**). These data
392 are consistent with previous analyses of MBC populations in Wu vaccinated individuals who had
393 experienced an Omicron BT infection and suggest that immunological imprinting limits the
394 development of new Omicron-specific MBCs, although there is efficient recall of cross-reactive
395 MBCs after a single exposure to Omicron S^{1,43,51}. Although Omicron BT-Wu/BA.5 bivalent
396 vaccinated subjects had two exposures to an Omicron S (one through infection and one through
397 vaccination), they still had relatively few Omicron-specific MBCs (mean: 5.3%) similar to those
398 individuals who received only the bivalent booster. The Wu/Omicron (BA.1/BA.2/BA.5) RBD pool
399 cross-reactive MBCs were further enriched (mean frequency: 81.5%) for this cohort compared to
400 the Wu/BA.5 bivalent vaccinated cohort (**Fig. 5a, b** and **Extended Data Fig. 9**).

401 To assess the ability of MBCs to recognize the currently circulating Omicron variants, we
402 examined whether MBCs recognizing the Omicron RBD pool (including those that are cross-
403 reactive with the Wu RBD and those that are not) could bind the BQ.1.1 RBD (**Fig. 5c** and
404 **Extended Data Fig. 10a, b**). Most Wu/Omicron RBD pool cross-reactive memory B cells
405 recognized the BQ.1.1 RBD (mean frequency: 66.3%), whereas only a small fraction of MBCs
406 binding to the Omicron RBD pool and not the Wu RBD also recognized the BQ.1.1 RBD (mean
407 frequency: 16.9%), regardless of infection and vaccination status. These Omicron-specific MBCs

408 were likely elicited de novo upon Omicron S exposure (through infection and/or vaccination) and
 409 their breadth towards currently dominant Omicron variants may improve over time through affinity
 410 maturation, similar to the maturation of the antibody response observed after infection with the
 411 SARS-CoV-2 Wu or Washington-1 strain or vaccination^{30,52–59}.

412 We next determined if the BA.5 versus BA.1 formulations of bivalent boosters differentially
 413 affected the composition of the RBD-specific MBC population (**Fig. 5d-f** and **Extended Data Fig.**
 414 **10 c,d**). The cross-reactivity of IgGs secreted by in vitro stimulated memory B cells to the Wu,
 415 BA.1, BQ.1.1 and XBB.1 RBDs was assessed following Wu/BA.1 bivalent vaccination for
 416 uninfected individuals and individuals who had experienced an Omicron BT infection (cohorts vii
 417 and viii). Vaccination with the Wu/BA.1 bivalent booster did not elicit Omicron RBD-specific MBCs
 418 in either the uninfected or Omicron-BT cohorts. Most RBD-specific antibodies were found to be
 419 cross-reactive with BA.1 regardless of infection status (mean frequencies: 76.3% in uninfected
 420 and 73.6% in Omicron BT infected subjects), whereas a lower fraction cross-reacted with BQ.1.1
 421 (59.9% and 57.4%) and XBB.1 RBDs (55.9% and 57.4%).

422 Collectively, these data suggest that two Omicron S exposures are not sufficient to
 423 overcome the immunological imprinting induced by repeated Wu S exposures but does continue
 424 to enrich for MBCs cross-reacting with multiple RBD variants.
 425



426
 427 **Figure 5. Cross-reactivity of vaccine-elicited SARS-CoV-2 RBD-specific MBCs. a, b,**
 428 **Frequency of Wu RBD-specific (grey), Omicron (BA.1/BA.2/BA.5) RBD pool-specific (red) and**
 429 **Wu/Omicron RBD pool-cross-reactive (blue) MBCs from donors of cohorts i-iv, as measured by**
 430 **flow cytometry. Individual frequencies and mean ± SD are shown in panels a and b, respectively.**

431 **c**, Analysis of cross-reactivity with the BQ.1.1 RBD of Omicron RBD pool-specific (red bars of
432 panel b) and Wu/Omicron RBD-cross-reactive (blue bars of panel b) MBCs. Om.pool, MBCs
433 recognizing the Omicron (BA.1/BA.2/BA.5) RBD pool. **d**, Cumulative cross-reactivity of IgGs
434 secreted from in vitro stimulated MBCs between the Wu RBD and the Omicron BA.1, BQ.1.1 or
435 XBB.1 RBDs as measured by ELISA. Data represent average OD values with blank subtracted
436 from n = 2 replicates of MBC cultures analyzed from donors of cohorts vii and viii. RBD-specific
437 IgGs showing inhibition of binding to ACE2 are depicted in red. Number of total and ACE2-
438 inhibiting (ACE2_{inh}) RBD-directed IgG positive cultures are indicated on top of each graph.
439 Percentages of Wu-specific, Omicron-specific and Wu/Omicron-cross-reactive IgG positive
440 cultures are indicated within each quadrant. **e**, **f**, Individual frequencies and mean frequencies ±
441 SD of Wu RBD-specific (grey), Omicron-specific (red) and Wu/Omicron RBD cross-reactive (blue
442 for BA.1, yellow for BQ.1.1 and purple for XBB.1) IgG positive cultures from donors of cohorts vii
443 and viii are shown in panels **e** and **f**, respectively.

444

445 Discussion

446 SARS-CoV-2 evolution is driven by viral immune escape and transmissibility^{60,61}. The
447 Omicron BA.1, BA.2 and BA.5 lineages, which led to successive waves of infection over the past
448 year, were characterized by immune evasion from Wu S-elicited neutralizing antibodies, higher
449 ACE2-binding affinity, impaired S-mediated fusogenicity and a change in entry pathway due to
450 reduced TMPRSS2-dependence^{3-15,39}. Here, we report that the recently emerged SARS-CoV-2
451 Omicron variants have an unprecedented level of immune evasion with reduction of neutralizing
452 antibody titers reaching up to 100-fold for XBB.1. Whereas BQ.1.1 and BA.2.75.2 retained the
453 high ACE2 binding affinity, similar to earlier Omicron variants, XBB.1 has a lower affinity,
454 comparable to that of the Wu RBD. Although XBB.1 is the most immune evasive of these three
455 Omicron variants, its reduced affinity for ACE2 relative to other co-circulating strains may have
456 hindered its spread. The enhanced ACE2 binding affinity of the newly emerging XBB.1.5, which
457 harbors the S486P RBD mutation (relative to BA.2), may explain the current spread of this rapidly
458 rising variant⁴⁷. We also demonstrate that BQ.1.1 and XBB.1 S display an increased resistance
459 to protease inhibitors blocking membrane or endosomal viral entry relative to other Omicron
460 lineages, suggesting peculiar alterations in the viral entry process of these variants. Collectively,
461 these findings illustrate the complex interplay of immune evasion, fusogenicity and ACE2 affinity
462 in driving SARS-CoV-2 evolution.

463 There is growing evidence that multiple mechanisms can contribute to protection from
464 infection and severe disease, including humoral and cellular responses^{26,62-65}. In particular,
465 antibody-dependent cell cytotoxicity (ADCC) and antibody-dependent opsonophagocytosis are
466 Fc-mediated effector functions that can promote virus clearance and enhance adaptive immune
467 responses in vivo, independently of direct viral neutralization. In this context, it is intriguing that
468 both neutralizing and binding antibody titers were reported as correlates of protection in a phase
469 3 study⁶⁶. We recently showed that sotrovimab (S309) retained in vitro effector functions against
470 BA.2 and conferred Fc-dependent protection in the lungs of mice infected with BA.2⁶⁷. This
471 preclinical finding is supported by the accumulation of real-world evidence suggesting that
472 adverse clinical outcomes, such as hospitalization and mortality, remained consistently low during
473 the BA.2 wave in patients treated with sotrovimab⁶⁸⁻⁷². Here, we show that sotrovimab retained in
474 vitro effector functions against all Omicron variants at levels comparable to those observed with

475 Wu. Importantly, despite 94-fold reduced in vitro neutralizing activity, prophylactic administration
476 of S309 (sotrovimab parent) protected mice against BQ.1.1 challenge, suggesting that Fc-
477 dependent effector functions can compensate for substantial losses of neutralizing activity. Our
478 observation that vaccine-elicited polyclonal plasma antibodies cross-react and trigger Fc γ R11a
479 signaling, as a surrogate of ADCC, upon recognition of the BQ.1.1 and XBB.1 S glycoproteins,
480 concur with observations made with prior Omicron variants⁷³⁻⁷⁵ and further hint at a protective
481 role for broadly reactive antibodies with effector functions. Our findings are consistent with a
482 model in which the erosion of neutralizing antibody titers due to viral immune evasion is
483 associated with increased frequency of breakthrough infections, while the persistence of
484 functional cross-reactive antibodies contributes to protection against severe COVID-19⁷⁶.

485 Immune imprinting, also termed original antigenic sin, was first described in 1960 based
486 on the observation that influenza virus infection with a strain distinct from prior exposures
487 preferentially boosted antibody responses against epitopes shared with the original strain instead
488 of inducing responses against the new strain⁷⁷. Analysis of the specificity of antibodies secreted
489 by plasmablasts obtained approximately 1-2 weeks following infection with the antigenically-
490 shifted 2009 H1N1 swine origin pandemic influenza virus revealed a recall of pre-existing, cross-
491 reactive memory B cells^{78,79}. Conversely, antibodies secreted by plasma cells and memory B
492 cells⁸⁰ obtained from the same individuals after seasonal influenza vaccination, corresponding to
493 a second antigenic exposure, were subtype-specific (i.e., targeting non-conserved epitopes). We
494 and others previously showed that Omicron breakthrough infections of Wu-vaccinated subjects
495 primarily recall cross-reactive memory B cells specific for epitopes shared by multiple SARS-CoV-
496 2 variants rather than priming naïve B cells recognizing Omicron RBD-specific epitopes^{1,43,51}.
497 Unexpectedly, we observed a low to undetectable number of MBCs specific for Omicron RBDs,
498 and not cross-reacting with the Wu RBD, even after two exposures to Omicron S antigens,
499 including with Wu/BA.5 or Wu/BA.1 bivalent mRNA vaccination. This observation may be
500 explained by a combination of strong immune imprinting and by the antigenic dominance of Wu
501 antigens in bivalent vaccines. This mechanism is reminiscent of current efforts to develop
502 universal influenza vaccines to elicit cross-reactive antibodies by serial stimulation with
503 heterologous chimeric hemagglutinins⁸¹. As expected, however, we found that bivalent Wu/BA.5
504 mRNA vaccination enriches for MBCs that are cross-reactive with the vaccine-matched and
505 mismatched RBD variants, relative to monovalent Wu mRNA vaccination. The identification of
506 suitable strategies to design broadly protective vaccines against sarbecoviruses^{21,22,82-88} and the
507 development of mucosal vaccines⁸⁹⁻⁹¹ may result in the development of variant-proof SARS-CoV-
508 2 vaccines through the elicitation of functional cross-reactive antibodies.

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517 **Methods**

518 **Cells and Viruses**

519 Cell lines used in this study were obtained from ATCC (HEK293T and VeroE6), Thermo Fisher
520 Scientific (Expi-CHO-S cells, FreeStyle 293-F cells and Expi293F cells), Takara (Lenti-X 293T
521 cells), kindly gifted from Jesse Bloom (HEK293T-ACE2), or generated in lab (BHK-21-GFP₁₋₁₀,
522 VeroE6-TMPRSS2 or VeroE6-TMPRSS2-GFP₁₁). None of the cell lines used were authenticated
523 nor tested for mycoplasma contamination. SARS-CoV-2 isolates used in this study were obtained
524 through BEI Resources, NIAID, NIH: (Wild type isolate hCoV-19/USA-WA1/2020, NR-52281
525 deposited by the Centers for Disease Control and Prevention ; Lineage B.1.1.529, BA.2; Omicron
526 Variant Isolate hCoV-19/USA/CO-CDPHE-2102544747/2021, NR-56520; Lineage XBB.1.5;
527 Omicron Variant Isolate hCoV-19/USA/MD-HP40900/2022, NR-59104, contributed by Dr. Andrew
528 S. Pekosz). Viruses were propagated and titered on Vero-TMPRSS2 cells in house. The genomic
529 sequences of all strains were confirmed by Sanger and/or NGS sequencing.
530

531 **Sample donors**

532 Samples were obtained from SARS-CoV-2 convalescent and vaccinated individuals under study
533 protocols approved by the local institutional review boards (Canton Ticino and Canton Aargau
534 Ethics Committees, Switzerland). All donors provided written informed consent for the use of
535 blood and blood derivatives (such as peripheral blood mononuclear cells, sera or plasma) for
536 research. Sera and peripheral blood mononuclear cells (PBMCs) from individuals who received
537 the Wuhan-Hu-1/BA.5 bivalent mRNA vaccines were obtained from the HAARVI study approved
538 by the University of Washington Human Subjects Division Institutional Review Board
539 (STUDY00000959). Demographic data for these individuals is presented in **Supplementary**
540 **Tables 5 and 6.**

541 **Constructs**

542
543 The full-length Wuhan-Hu-1/G614, Delta, BA.1, BA.2, and BA.4/5 S constructs with a 21 amino
544 acid C-terminal deletion used for pseudovirus were previously described elsewhere (). The full-
545 length BA.2.75.2 and XBB.1 S constructs containing a 21 amino acid C-terminal deletion were
546 codon-optimized, synthesized, and inserted the HDM vector by Genscript. The full-length BQ.1.1
547 S construct containing a 21 amino acid C-terminal deletion was generated by mutagenesis of the
548 BA.4/5 S construct by Genscript.

549 The SARS-CoV-2 Wuhan-Hu-1 RBD construct containing an N-terminal mu-phosphatase
550 secretion signal and a C-terminal octa-histidine tag followed by flexible linker and Avi tag was
551 previously described elsewhere. The BA.4/5 RBD construct containing an N-terminal BM40
552 secretion tag and a C-terminal octa-histidine tag followed by flexible linker and Avi tag was
553 previously described elsewhere. The BA.2.75.2, BQ.1.1, and BA.4/5 RBD constructs containing
554 an N-terminal BM40 secretion tag and a C-terminal octa-histidine tag followed by flexible linker
555 and Avi tag were codon optimized, synthesized, and inserted into the pcDNA3.1(+) vector by
556 Genscript. The boundaries of the construct are N-328RFPN331 and 528KKST531-C.

557

558 **Generation of VSV pseudovirus**

559 Replication defective VSV pseudovirus expressing SARS-CoV-2 spike proteins corresponding to
560 the ancestral Wuhan-Hu-1 virus and the VOCs were generated as previously described (3), with
561 some modifications. Lenti-X 293T cells (Takara) were seeded in 15-cm² dishes at a density of
562 10 × 10⁶ cells per dish and the following day were transfected with 25 µg of spike expression
563 plasmid with TransIT-Lenti (Mirus, 6600) according to the manufacturer's instructions. One day
564 after transfection, cells were infected with VSV-luc (VSV-G) with a multiplicity of infection (MOI)
565 of 3 for 1 h, rinsed three times with PBS containing Ca²⁺ and Mg²⁺, then incubated for an
566 additional 24 h in complete medium at 37 °C. The cell supernatant was clarified by centrifugation,
567 aliquoted, and frozen at -80 °C. Spike expression plasmids used for the generation of VSV
568 pseudoviruses carry the following mutations: BA.1: A67V, Δ69-70, T95I, G142D, Δ143-145,
569 Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N,
570 T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H,
571 N764K, D796Y, N856K, Q954H, N969K, L981F; BA.2: T19I, L24-, P25-, P26-, A27S, G142D,
572 V213G, G339D, S371L, S373P, S375F, D405N, R408S, K417N, N440K, S477N, T478K, E484A,
573 Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K,
574 Q954H, N969K; K417N, N440K, G446S, N460K, S477N, T478K, E484A, Q498R, N501Y, Y505H,
575 D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K; BA.2.75.2: T19I, L24-, P25-,
576 P26-, A27S, G142D, K147E, W152R, F157L, I210V, V213G, G257S, G339H, R346T, S371F,
577 S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A,
578 F486S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K,
579 D1199N; BQ.1: T19I, L24-, P25-, P26-, A27S, Δ69-70, G142D, V213G, G339D, S371F, S373P,
580 S375F, T376A, D405N, R408S, K417N, N440K, K444T, L452R, N460K, S477N, T478K, E484A,
581 F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K;
582 BQ.1.1: T19I, L24-, P25-, P26-, A27S, Δ69-70, G142D, V213G, G339D, R436T, S371F, S373P,
583 S375F, T376A, D405N, R408S, K417N, N440K, K444T, L452R, N460K, S477N, T478K, E484A,
584 F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K;
585 BF.7: T19I, L24-, P25-, P26-, A27S, Δ69-70, G142D, V213G, G339D, R436T, S371F, S373P,
586 S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R,
587 N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K; XBB.1: T19I,
588 L24-, P25-, P26-, A27S, V83A, G142D, Y144-, H146Q, Q183E, V213E, G252V, G339H, R346T,
589 L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K,
590 S477N, T478K, E484A, F486S, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H,
591 N764K, D796Y, Q954H, N969K; XBB.1.5: T19I, L24-, P25-, P26-, A27S, V83A, G142D, Y144-,
592 H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N,
593 R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486P, F490S, Q498R,
594 N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K; CH.1.1: T19I,
595 del24-26, A27S, G142D, K147E, W152R, F157L, I210V, V213G, G257S, G339H, R346T, S371F,
596 S373P, S375F, T376A, D405N, R408S, K417N, N440K, K444T, G446S, L452R, N460K, S477N,
597 T478K, E484A, F486S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y,
598 Q954H, N969K

599 Pseudotyped VSV was produced as previously described. Briefly, HEK293T were split into poly-
600 D-lysine coated 15 cm plates and grown overnight until they reached approximately 70-80%
601 confluency. The cells were washed 3 times with Opti-MEM (Gibco) and transfected with either the
602 Wuhan-Hu-1(D614), Wu-G614, Delta, BA.1, BA.2, BA.4/5, BA.2.75.2, BQ.1.1, or XBB.1 S

603 constructs using Lipofectamine 2000 (Life Technologies). After 4-6 hours, the media was
604 supplemented with an equal volume of DMEM supplemented with 20% FBS and 2% PS. The
605 cells were incubated for 20-24 hours, washed 3 times with DMEM, and infected with VSVΔG-luc.
606 Two hours after VSVΔG-luc infection, the cells were then washed an additional five times with
607 DMEM. The cells were grown in DMEM (for virus stocks used in sotrovimab neutralization assays)
608 or in DMEM supplemented with anti-VSV-G antibody (11-mouse hybridoma supernatant diluted
609 1:25, from CRL-2700, ATCC) for 18-24 hours, after which the supernatant was harvested and
610 clarified by low-speed centrifugation at 2,500 g for 10 min. The supernatant was then filtered (0.45
611 μm) and some virus stocks were concentrated 10 times using a 30 kDa centrifugal concentrator
612 (Amicon Ultra). The pseudotyped viruses were then aliquoted and frozen at -80°C.

613

614 **VSV pseudovirus neutralization**

615 Vero E6 cells were grown in DMEM supplemented with 10% FBS and seeded into white-walled
616 96 well plates (PerkinElmer, 6005688) at a density of 20,000 cells per well. The next day,
617 monoclonal antibodies were serially diluted in pre-warmed complete medium, mixed with
618 pseudoviruses and incubated for 1 h at 37 °C in round bottom polypropylene plates. Medium from
619 cells was aspirated and 50 μl of virus–monoclonal antibody complexes were added to cells, which
620 were then incubated for 1 h at 37 °C. An additional 100 μl of pre-warmed complete medium was
621 then added on top of complexes and cells were incubated for an additional 16–24 h. Conditions
622 were tested in duplicate or triplicate wells on each plate and 6-8 wells per plate contained
623 untreated infected cells (defining the 0% of neutralization, 'MAX RLU' value) and uninfected cells
624 (defining the 100% of neutralization, 'MIN RLU' value). Virus–monoclonal antibody-containing
625 medium was then aspirated from cells and 50 or 100 μl of a 1:2 dilution of SteadyLite Plus
626 (PerkinElmer) or BioGlo (Promega) in PBS with Ca²⁺ and Mg²⁺ was added to cells. Plates were
627 incubated for 15 min at room temperature and then analyzed on the Synergy-H1 (Biotek). The
628 average relative light units (RLUs) of untreated infected wells (MAX RLU_{ave}) were subtracted by
629 the average of MIN RLU (MIN RLU_{ave}) and used to normalize percentage of neutralization of
630 individual RLU values of experimental data according to the following formula: $(1 - (\text{RLU}_x - \text{MIN RLU}_{\text{ave}}) / (\text{MAX RLU}_{\text{ave}} - \text{MIN RLU}_{\text{ave}})) \times 100$. Data were analyzed with Prism (v.9.1.0).
631 IC₅₀ values were calculated from the interpolated value from the log(inhibitor) versus response,
632 using variable slope (four parameters) nonlinear regression with an upper constraint of <100.
633 Each neutralization experiment was conducted on at least two independent experiments – that is,
634 biological replicates – in which each biological replicate contains a technical duplicate or triplicate.
635
636 VeroE6-TMPRSS2 were split into white-walled, clear bottom 96 well plates (Corning) and grown
637 overnight until they reached approximately 70% confluency. Sera (or plasma) were diluted in
638 DMEM starting at a 1:33 or 1:66 dilution and serially diluted in DMEM at a 1:3 dilution thereafter.
639 Pseudotyped VSV was diluted at a 1:25 to 1:100 ratio in DMEM and an equal volume was added
640 to the diluted sera. The virus-sera mixture was incubated for 30 minutes at room temperature and
641 added to the VeroE6-TMPRSS2 cells. After two hours, an equal volume of DMEM supplemented
642 with 20% FBS and 2% PS was added to the cells. After 20-24 hours, ONE-Glo EX (Promega)
643 was added to each well and the cells were incubated for 5 minutes at 37°C. Luminescence values
644 were measured using a BioTek plate reader. Luminescence readings from the neutralization
645 assays were normalized and analyzed using GraphPad Prism 9. The relative light unit (RLU)
646 values recorded from uninfected cells were used to define 100% neutralization and RLU values

647 recorded from cells infected with pseudovirus without sera or antibodies were used to define 0%
648 neutralization. ID50 were determined from the normalized data points using a [inhibitor] vs.
649 normalized response – variable slope model using at least two technical repeats to generate the
650 curve fits. At least two biological replicates with two distinct batches of pseudovirus were
651 conducted for each sample.

652

653 **Neutralization of authentic SARS-CoV-2 viruses**

654 Vero-TMPRSS2 cells were seeded into black-walled, clear-bottom 96-well plates at 20,000
655 cells/well and cultured overnight at 37°C. The next day, 9-point 4-fold serial dilutions of mAbs
656 were prepared in growth media (DMEM + 10% FBS). The different SARS-CoV-2 strains were
657 diluted in infection media (DMEM + 2% BSA) at a final MOI of 0.01 PFU/cell, added to the mAb
658 dilutions and incubated for 30 min at 37°C. Media was removed from the cells, mAb-virus
659 complexes were added and incubated at 37°C for 18h (wild-type, XBB.1.5) or 24h (BA.2). Cells
660 were fixed with 4% PFA (Electron Microscopy Sciences, #15714S), permeabilized with Triton X-
661 100 (SIGMA, #X100-500ML) and stained with an antibody against the viral nucleocapsid protein
662 (Sino Biologicals, #40143-R001) followed by a staining with the nuclear dye Hoechst 33342
663 (Fisher Scientific, # H1399) and a goat anti-rabbit Alexa Fluor 647 antibody (Invitrogen, #A-
664 21245). Plates were imaged on a Cytation5 plate reader. Whole well images were acquired (12
665 images at 4X magnification per well) and nucleocapsid-positive cells were counted using the
666 manufacturer's software.

667

668 **Pseudotyped VSV entry assays with protease inhibitors**

669 VeroE6-TMPRSS2 or HEK293T-ACE2 were split into white-walled, clear bottom 96 well plates
670 (Corning) at a density of 18,000 or 36,000 cells, respectively, and grown overnight. The following
671 day, the growth media was removed and, for assays conducted with VeroE6-TMPRSS2, the cells
672 washed once with DMEM. The cells were incubated for 2 hours with DMEM containing 50 µM of
673 Camostat (Sigma), Nafamostat (Sigma), E-64d (Sigma), or 0.5% DMSO. All three protease
674 inhibitors were dissolved in DMSO to a concentration of 10 mM and diluted in DMEM. The
675 protease inhibitors were removed and pseudovirus diluted 1:50 or 1:200 in DMEM was added to
676 the cells. After two hours, an equal volume of DMEM supplemented with 20% FBS and 2% PS
677 was added to the cells. After 20-24 hours, ONE-Glo EX (Promega) was added to each well and
678 the cells were incubated for 5 minutes at 37°C. Luminescence values were measured using a
679 BioTek plate reader. Luminescence readings from the neutralization assays were normalized and
680 analyzed using GraphPad Prism 9. The RLU values recorded from uninfected cells were used to
681 define 0% infectivity and RLU values recorded from cells incubated with 0.5% DMSO only and
682 infected with pseudovirus were used to define 100% infectivity. Twelve technical replicates were
683 performed for each inhibitor and pseudovirus and at least two biological replicates with two distinct
684 batches of pseudovirus were conducted.

685

686 **Recombinant protein production for BLI, FACS, and cryoEM**

687 SARS-CoV-2 RBD proteins were produced and purified from Expi293F cells as previously
688 described. In brief, cells were grown to a density of 3×10^6 cells/mL and transfected using the
689 ExpiFectamine 293 Transfection Kit (ThermoFisher Scientific). Three to 5 days post-transfection,

690 proteins were purified from clarified supernatants using HisTrap HP affinity columns (Cytiva) and
691 washed with ten column volumes of 20 mM imidazole, 25 mM sodium phosphate pH 8.0, and 300
692 mM NaCl before elution on a gradient to 500 mM imidazole, 25 mM sodium phosphate pH 8.0,
693 and 300 mM NaCl. Proteins were buffer exchanged into 20 mM sodium phosphate pH 8 and 100
694 mM NaCl and concentrated using centrifugal filters (Amicon Ultra) before being flash frozen. For
695 cryo-EM, recombinant ACE2 (residues 19-615 from Uniprot Q9BYF1 with a C-terminal thrombin
696 cleavage site-TwinStrep-10xHis-GGG-tag, and N-terminal signal peptide) was expressed in
697 ExpiCHO-S cells at 37°C and 8% CO₂ with kifunensine added to 10 μM. Cell culture supernatant
698 was collected eight days post transfection, supplemented with buffer to a final concentration of 80
699 mM Tris-HCl pH 8.0, 100 mM NaCl, and then incubated with BioLock (IBA GmbH) solution. ACE2
700 was purified using a 5 mL StrepTrap HP column (Cytiva) followed by isolation of the monomeric
701 ACE2 by size exclusion chromatography using a Superdex 200 Increase 10/300 GL column
702 (Cytiva) pre-equilibrated in PBS. Recombinant S309 Fab used for cryo-EM was expressed in
703 HEK293 suspension cells, purified using CaptureSelect IgG-CH1 resin and buffer exchanged into
704 PBS (ATUM Bio).

705

706 **Biolayer interferometry**

707 Biotinylated Wu, BA.4/5, BA.2.75.2, BQ.1.1, and XBB.1 RBDs were diluted to a concentration of
708 5 ng/μL in 10X kinetics buffer and loaded onto pre-hydrated streptavidin biosensors to a 1 nm
709 total shift. The loaded tips were dipped into a 1:3 dilution series of monomeric human ACE2
710 starting at 900 nM or 300 nM for 300 seconds followed by dissociation in 10X kinetics buffer for
711 300 seconds. All steps of the affinity measurements using biolayer interferometry were carried
712 out at 30°C with a shaking speed of 1,000 rpm.

713

714 **Surface plasmon resonance to measure binding of RBDs with ACE2**

715 Measurements were performed using a Biacore T200 instrument. Experiments were performed
716 at 25°C, with the samples held at 15°C in the instrument prior to injection. A CM5 chip with
717 covalently immobilized anti-Avi polyclonal antibody (GenScript, Cat #: A00674-40) was used for
718 surface capture of His-Avi tag containing RBDs. Running buffer was 1x HBS-EP+ pH 7.4 (10 mM
719 HEPES, 150 mM NaCl, 3 mM EDTA and 0.05% v/v Surfactant P20) (Cytiva, Cat #: BR100669).
720 Experiments were performed with a 4-point dilution series of monomeric S309 Fab or monomeric
721 Strep-tagged ACE2 starting at 300nM. The dilution factors are 3-fold (300, 100, 33.3, 11,1nM),
722 3.25-fold (300, 92.3, 28.4, 8.7nM) or 4-fold (300, 75, 18.8, 4.7nM). Experiments were run as
723 single-cycle kinetics, n=2-14 for each RBD ligand. Data were double reference-subtracted and fit
724 to a binding model using Biacore Evaluation software. The 1:1 binding model was used to
725 determine the kinetic parameters. Kinetic values out of the instrument's limit were omitted. K_D
726 values are reported as the average of all replicates with the corresponding standard deviation
727 (**Supplementary Table 2**).

728

729 **In vivo studies**

730 Animal studies were carried out in accordance with the recommendations in the Guide for the
731 Care and Use of Laboratory Animals of the National Institutes of Health. The protocols were
732 approved by the Institutional Animal Care and Use Committee at the Washington University

733 School of Medicine (assurance number A3381–01). Virus inoculations were performed under
734 anesthesia that was induced and maintained with ketamine hydrochloride and xylazine, and all
735 efforts were made to minimize animal suffering. Heterozygous K18-hACE2 C57BL/6 J mice
736 (strain: 2B6.Cg-Tg(K18-ACE2)2PrImn/J) were obtained from The Jackson Laboratory. All animals
737 were housed in groups of 3 to 5 and fed standard chow diets. The photoperiod was 12 h on:12 h
738 off dark/light cycle. The ambient animal room temperature was 70° F, controlled within $\pm 2^\circ$ and
739 the room humidity was 50%, controlled within $\pm 5\%$. Eight- to ten-week-old female K18-hACE2
740 mice were administered indicated doses of S309 or control (anti-WNV hE16) mAb dose by
741 intraperitoneal injection one day before or after intranasal inoculation with 10^4 FFU of BQ.1.1.
742 Weight was recorded daily, and animals were euthanized on day +6 after virus inoculation.

743

744 **Measurement of viral RNA levels**

745 Tissues were weighed and homogenized with zirconia beads in a MagNA Lyser instrument
746 (Roche Life Science) in 1 mL of DMEM medium supplemented with 2% heat-inactivated FBS.
747 Tissue homogenates were clarified by centrifugation at approximately $10,000 \times g$ for 5 min and
748 stored at -80°C . RNA was extracted using the MagMax mirVana Total RNA isolation kit (Thermo
749 Fisher Scientific) on the Kingfisher Flex extraction robot (Thermo Fisher Scientific). RNA was
750 reverse transcribed and amplified using the TaqMan RNA-to-CT 1-Step Kit (Thermo Fisher
751 Scientific). Reverse transcription was carried out at 48°C for 15 min followed by 2 min at 95°C .
752 Amplification was accomplished over 50 cycles as follows: 95°C for 15 s and 60°C for 1 min.
753 Copies of SARS-CoV-2 *N* gene RNA in samples were determined using a previously published
754 assay⁴². Briefly, a TaqMan assay was designed to target a highly conserved region of the *N* gene
755 (Forward primer: ATGCTGCAATCGTGCTACAA; Reverse primer: GACTGCCGCCTCTGCTC;
756 Probe: /56-FAM/TCAAGGAAC/ZEN/AACATTGCCAA/3IABkFQ/). This region was included in an
757 RNA standard to allow for copy number determination down to 10 copies per reaction. The
758 reaction mixture contained final concentrations of primers and probe of 500 and 100 nM,
759 respectively.

760

761 **Viral plaque assay**

762 VeroE6-TMPRSS2-ACE2 cells were seeded at a density of 1×10^5 cells per well in 24-well tissue
763 culture plates. The following day, medium was removed and replaced with 200 μL of material to
764 be titrated diluted serially in DMEM supplemented with 2% FBS. One hour later, 1 mL of
765 methylcellulose overlay was added. Plates were incubated for 72 h, and then fixed with 4%
766 paraformaldehyde (final concentration) in PBS for 20 min. Plates were stained with 0.05% (w/v)
767 crystal violet in 20% methanol and washed twice with distilled, deionized water.

768

769 **Transient expression of recombinant SARS-CoV-2 protein and flow cytometry.**

770 ExpiCHO-S cells were seeded at 6×10^6 cells/mL in a volume of 5 mL in a 50 mL bioreactor. The
771 following day, cells were transfected with SARS-CoV-2 spike glycoprotein-encoding pcDNA3.1(+)
772 plasmids (BetaCoV/Wuhan-Hu-1/2019, accession number MN908947, Wu-D614; Omicron BA.2,
773 BQ.1.1, XBB.1 and BA.2-E340A generated by overlap PCR mutagenesis of the Wuhan D614
774 plasmid) harboring the $\Delta 19$ C-terminal truncation. Spike encoding plasmids were diluted in cold

775 OptiPRO SFM (Life Technologies, 12309-050), mixed with ExpiFectamine CHO Reagent (Life
776 Technologies, A29130) and added to cells. Transfected cells were then incubated at 37°C with
777 8% CO₂ with an orbital shaking speed of 250 RPM (orbital diameter of 25 mm) for 24 to 48 h.
778 Transiently transfected ExpiCHO-S cells were harvested and washed twice in wash buffer (PBS
779 2% FBS, 2mM EDTA). Cells were counted and distributed into round bottom 96-well plates
780 (Corning, 3799) and incubated with serial dilutions of mAb starting at 10 µg/mL. Alexa Fluor647-
781 labelled Goat anti-human IgG secondary Ab (Jackson ImmunoResearch, 109–606–098) was
782 prepared at 2 µg/mL and added onto cells after two washing steps. Cells were then washed twice
783 and resuspended in wash buffer for data acquisition at Ze5 cytometer (BioRad).

784

785 **Fc-mediated effector functions (ADCC).**

786 Primary cells were collected from healthy human donors with informed consent and authorization
787 via the Comitato Etico Canton Ticino (Switzerland). ADCC assays were performed using
788 ExpiCHO-S cells transiently transfected with SARS-CoV-2 spike glycoproteins (Wuhan D614,
789 BA.2, BQ.1.1 or XBB.1) as targets. NK cells were isolated from fresh blood of healthy donors
790 using the MACSxpress NK Isolation Kit (Miltenyi Biotec, cat. no. 130-098-185). Target cells were
791 incubated with titrated concentrations of mAbs for 10 min and then with primary human NK cells
792 at an effector to target ratio ranging from 7.75:1 to 9:1. ADCC was measured using the LDH
793 release assay (Cytotoxicity Detection Kit (LDH) (Roche; cat. no. 11644793001) after 4 h
794 incubation at 37°C.

795

796 **Measurement of plasma effector functions.**

797 Antibody-dependent activation of human FcγRIIIa by plasma antibodies was performed with a
798 bioluminescent reporter assay. ExpiCHO-S cells transiently expressing full-length SARS-CoV-2
799 S from Wuhan-D614, BA.5, BQ.1.1 and XBB.1 (target cells) were incubated with serial dilutions
800 of plasma from immune donors. After a 20-minute incubation, Jurkat reporter cells stably
801 expressing FcγRIIIa V158 and NFAT-driven luciferase gene (effector cells) were added at an
802 effector to target ratio of 6:1 for FcγRIIIa. Signaling was quantified by the luciferase signal
803 produced via activation of the NFAT pathway. Luminescence was measured after 22 hours of
804 incubation at 37°C with 5% CO₂ with a luminometer using the Bio-Glo-TM Luciferase Assay
805 Reagent according to the manufacturer's instructions (Promega).

806

807 **Antigen-specific memory B cell repertoire analysis (AMBRA) of secreted IgGs**

808 Replicate cultures of total unfractionated PBMCs obtained from SARS-CoV-2 infected and/or
809 vaccinated individuals were seeded in 96 U-bottom plates (Corning) in RPMI1640 supplemented
810 with 10% fetal calf serum (Hyclone), sodium pyruvate, MEM non-essential amino acids, stable
811 glutamine, 2-mercaptoethanol, Penicillin-Streptomycin, Kanamycin and Transferrin. Memory B
812 cell stimulation and differentiation was induced by adding 2.5 µg/ml R848 (3 M) and 1000 U/ml
813 human recombinant IL-2 at 37 °C and 5% CO₂. After 10 days, the cell culture supernatants were
814 collected for ELISA analysis.

815

816 **Enzyme-linked immunosorbent assay (ELISA)**

817 Ninety-six half area well-plates (Corning, 3690) were coated overnight at 4 °C with 25 µl of
818 sarbecovirus RBD proteins prepared at 5 µg/ml in PBS pH 7.2. Plates were then blocked with
819 PBS 1% BSA (Sigma-Aldrich, A3059) and subsequently incubated with mAb serial dilutions for 1
820 h at room temperature. After 4 washing steps with PBS 0.05% Tween 20 (PBS-T) (Sigma-Aldrich,
821 93773), goat anti-human IgG secondary antibody (Southern Biotech, 2040-04) was added and
822 incubated for 1 h at room temperature. Plates were then washed four times with PBS-T and 4-
823 nitrophenyl phosphate (pNPP, Sigma-Aldrich, 71768) substrate was added. After 30 min
824 incubation, absorbance at 405 nm was measured by a plate reader (Biotek) and data were plotted
825 using Prism GraphPad 9.1.0. To test MBC-derived antibodies, Spectraplate-384 with high protein
826 binding treatment (custom made from Perkin Elmer) were coated overnight at 4 °C with 3 µg/ml of
827 RBD (produced in house), SARS-CoV RBD (produced in house), Omicron BA.1, BQ.1.1 and
828 XBB:1 RBDs (produced in house) in PBS pH 7.2 or PBS alone as control. Plates were
829 subsequently blocked with Blocker Casein (1%) in PBS (Thermo Fisher Scientific, 37528)
830 supplemented with 0.05% Tween 20 (Sigma Aldrich, 93773-1KG). The coated plates were
831 incubated with diluted B cell supernatant for 1h at RT. Plates were washed with PBS containing
832 0.05 % Tween20 (PBS-T), and binding was revealed using secondary goat anti-human IgG-AP
833 (Southern Biotech, 2040-04). After washing, pNPP substrate (Sigma-Aldrich, 71768-25G) was
834 added and plates were read at 405 nm after 1 h or 30 minutes.
835

836 **Blockade of RBD binding to human ACE2**

837 Memory B cell culture supernatants were diluted in PBS and mixed with SARS-CoV-2 RBD mouse
838 Fc-tagged antigen (Sino Biological, 40592-V05H, final concentration 20 ng/ml) and incubated for
839 30 min at 37 °C. The mix was added for 30 min to ELISA 384-well plates (NUNC, P6366-1CS)
840 pre-coated overnight at 4 °C with 4 µg/ml human ACE2 (produced in house) in PBS. Plates were
841 washed with PBS containing 0.05 % Tween20 (PBS-T), and RBD binding was revealed using
842 secondary goat anti-mouse IgG-AP (Southern Biotech, 1032-04). After washing, pNPP substrate
843 (Sigma-Aldrich, 71768-25G) was added and plates were read at 405 nm after 1h.
844

845 **Recombinant protein production for SPR binding assays and AMBRA ELISA**

846 SARS-CoV-2 RBD plasmids encode for residues 328-531 of the spike protein from GenBank
847 NC_045512.2 with an N-terminal signal peptide and a C-terminal 8xHis-AviTag or thrombin
848 cleavage site-8xHis-AviTag. Proteins were expressed in Expi293F cells (Thermo Fisher Scientific)
849 at 37 °C and 8% CO₂. Transfections were performed using the ExpiFectamine 293 Transfection
850 Kit (Thermo Fisher Scientific). Cell culture supernatants were collected four to five days after
851 transfection and supplemented with 10x PBS to a final concentration of 2.5x PBS (342.5 mM
852 NaCl, 6.75 mM KCl and 29.75 mM phosphates). SARS-CoV-2 RBDs were purified by IMAC using
853 Cobalt or Nickel resin followed by buffer exchange into PBS using Amicon centrifugal filters
854 (Milipore Sigma) or by size exclusion chromatography using a Superdex 200 Increase 10/300 GL
855 column (Cytiva). For SPR binding measurements, recombinant ACE2 (residues 19-615 from
856 Uniprot Q9BYF1 with a C-terminal thrombin cleavage site-TwinStrep-10xHis-GGG-tag, and N-
857 terminal signal peptide) was expressed in Expi293F cells at 37 °C and 8% CO₂. Transfection was
858 performed using the ExpiFectamine 293 Transfection Kit (Thermo Fisher Scientific). Cell culture
859 supernatant was collected seven days after transfection, supplemented to a final concentration of
860 80 mM Tris-HCl pH 8.0, 100 mM NaCl, and then incubated with BioLock solution (IBA GmbH).

861 ACE2 was purified using a 1 mL StrepTrap HP column (Cytiva) followed by isolation of the
862 monomeric ACE2 by size exclusion chromatography using a Superdex 200 Increase 10/300 GL
863 column (Cytiva) pre-equilibrated in PBS. Recombinant S309 Fab used for SPR binding studies
864 was produced in ExpiCHO-S cells and purified using a Capture Select CH1-XL MiniChrom
865 Column (ThermoFisher), followed by buffer exchange into PBS using a HiPrep 26/10 Desalting
866 Column (Cytiva).

867

868 **Surface plasmon resonance (SPR) assays to measure binding of the S309 Fab to RBDs**

869 Measurements were performed using a Biacore T200 instrument. A CM5 chip with covalently
870 immobilized StrepTactin XT was used for surface capture of Twin-Strep Tag-containing RBDs.
871 Running buffer was HBS-EP+ pH 7.4 (Cytiva) and measurements were performed at 25°C.
872 Experiments were performed with a 3-fold dilution series of monomeric S309 Fab at 300, 100, 33
873 and 11 nM and were run as single-cycle kinetics. Data were double reference-subtracted and fit
874 to a binding model using Biacore Evaluation software. The 1:1 binding model was used to
875 estimate the kinetics parameters. The experiment was performed twice with two biological
876 replicates for each ligand (RBDs). K_D values are reported as the average of two replicates with
877 the corresponding standard deviation.

878

879 **Cell-cell fusion assay**

880 Cell-cell fusion assays using a split GFP system was conducted as previously described. In brief,
881 VeroE6-TMPRSS2-GFP₁₁ cells were split into 96-well, glass bottom, black walled plates (CellVis)
882 at a density of 36,000 cells per well. BHK-21-GFP₁₋₁₀ cells were split into 6-well plates at a density
883 of 1×10^6 cells per well. The following day, the growth media was removed and replaced with
884 DMEM containing 10% FBS and 1% PS and the cells were transfected with 4 µg of S protein
885 using Lipofectamine 2000. Twenty-four hours after transfection, BHK-21-GFP₁₋₁₀ expressing the
886 S protein were washed three times using FluoroBrite DMEM (Thermo Fisher) and detached using
887 an enzyme-free cell dissociation buffer (Gibco). The VeroE6-TMPRSS2-GFP₁₁ were washed
888 three times with FluoroBrite DMEM and 9,000 BHK-21-GFP₁₋₁₀ cells were plated on top of the
889 VeroE6-TMPRSS2-GFP₁₁ cells. The cells were incubated at 37°C and 5% CO₂ in a Cytation 7
890 plate Imager (Biotek) and both brightfield and GFP images were collected every 30 minutes for
891 18 hours. Fusogenicity was assessed by measuring the area showing GFP fluorescence for each
892 image using Gen5 Image Prime v3.11 software.

893 To measure surface expression of the variant SARS-CoV-2 S protein, 1×10^6 transiently
894 transfected BHK-21-GFP₁₋₁₀ cells were collected by centrifugation at 1,000 x g for 5 min. The cells
895 were washed once with PBS and fixed with 2% paraformaldehyde. The cells were washed twice
896 with flow staining buffer (1% BSA, 1 mM EDTA, 0.1% NaN₃ in PBS) and labeled with 250 µg/mL
897 of S2L20, an NTD-directed antibody that recognizes all currently and previously circulating SARS-
898 CoV-2 variants, for 45 minutes. The cells were washed three times with flow staining buffer and
899 labeled with a PE-conjugated anti-Human IgG Fc antibody (Thermo Fisher) for 30 mins. The cells
900 were washed an additional three times and resuspended in flow staining buffer. The labeled cells
901 were analyzed using a BD FACSymphony A3. Cells were gated on singleton events and a total of
902 10,000 singleton events were collected for each sample. The fraction of S-positive cells was
903 determined in FlowJo 10.8.1 by gating singleton events for the mock transfected cells on PE
904 intensity.

905

906 **Flow cytometry analysis of SARS-CoV-2 RBD-reactive MBCs**

907 RBD-streptavidin tetramers conjugated to fluorophores were generated by incubating biotinylated
908 Wu, BA.1, BA.2, BA.4/5, or BQ.1.1 with streptavidin at a 4:1 molar ratio for 30 mins at 4°C. Excess
909 free biotin was then added to the reaction to bind any unconjugated sites in the streptavidin
910 tetramers. The RBD-streptavidin tetramers were washed once with PBS and concentrated with a
911 30 kDa centrifugal concentrator (Amicon). An additional streptavidin tetramer conjugated to biotin
912 only was generated and included in the staining.

913 Approximately 5 to 15 million PMBCs were collected 5-72 days post-vaccination for individuals
914 who received either the Wu monovalent mRNA booster or Wu/BA.5 bivalent mRNA booster. The
915 cells were collected by centrifugation at 1,000 x g for 5 mins at 4°C and washed twice with PBS.
916 The cells were then stained with Zombie Aqua dye (Biolegend; diluted 1:100 in PBS) for 30 mins
917 at room temperature after which the cells were washed twice with FACS staining buffer (0.1%
918 BSA, 0.1% NaN₃ in PBS). The cells were then stained with antibodies for CD20-PECy7 (BD),
919 CD3-Alexa eFluor780 (ThermoFisher), CD8-Alexa eFluor780 (ThermoFisher), CD14-Alexa
920 eFluor780 (ThermoFisher), CD16-Alexa eFluor780 (ThermoFisher), IgM-Alexa Fluor647
921 (BioLegend), IgD-Alexa Fluor647 (BioLegend), and CD38-Brilliant Violet 785 (BioLegend), all
922 diluted 1:200 in Brilliant Stain Buffer (BD), along with the RBD-streptavidin tetramers for 30 mins
923 at 4°C. The cells were washed three times, resuspended in FACS staining buffer, and passed
924 through a 35 µm filter. The cells were examined using a BD FACSAria III and FACSDiva for
925 acquisition and FlowJo 10.8.1 for analysis. Single live CD20⁺/CD3⁻/CD8⁻/CD14⁻/CD16⁻
926 /IgM^{lo}/IgD^{lo}/CD38^{lo}/RBD⁺ cells were sorted based on reactivity to the Omicron and Wu RBDs into
927 RNAlater and stored at -80°C.

928

929 **CryoEM sample preparation, data collection and data processing**

930 CryoEM grids of BQ.1.1 RBD-ACE2-S309 or XBB.1 RBD-ACE2-S309 complex were prepared
931 fresh after SEC purification. For BQ.1.1 RBD-ACE2-S309 complex, three microliters of 0.25
932 mg/ml BQ.1.1 RBD-ACE2-S309 were loaded onto freshly glow discharged R 2/2 UltrAuFoil grids,
933 prior to plunge freezing using a vitrobot MarkIV (ThermoFisher Scientific) with a blot force of 0
934 and 6 sec blot time at 100 % humidity and 22°C. Data were acquired using an FEI Titan Krios
935 transmission electron microscope operated at 300 kV and equipped with a Gatan K3 direct
936 detector and Gatan Quantum GIF energy filter, operated in zero-loss mode with a slit width of 20
937 eV. For BQ.1.1 RBD-ACE2-S309 data set, automated data collection was carried out using
938 Legion at a nominal magnification of 105,000x with a pixel size of 0.843 Å and stage tilt angle of
939 30°. 6,487 micrographs were collected with a defocus range comprised between -0.5 and -2.5
940 µm. For XBB.1 RBD-ACE2-S309 complex, samples were prepared using the Vitrobot Mark IV
941 (Thermo Fisher Scientific) with R 2/2 UltrAuFoil grids and with a Chameleon (SPT Labtech) with
942 self-wicking nanowire Cu R1.2/0.8 holey carbon grids. For XBB.1 RBD-ACE2-S309 data set,
943 6,355 micrographs from UltrAuFoil grids and 2,889 micrographs from chameleon grids were
944 collected with a defocus range comprised between -0.2 and -3 µm. The dose rate was adjusted
945 to 15 counts/pixel/s, and each movie was acquired in super-resolution mode fractionated in 75
946 frames of 40 ms. Movie frame alignment, estimation of the microscope contrast-transfer function
947 parameters, particle picking, and extraction were carried out using Warp.

948 Two rounds of reference-free 2D classification were performed using cryoSPARC to select well-
949 defined particle images. These selected particles were subjected to two rounds of 3D
950 classification with 50 iterations each (angular sampling 7.5° for 25 iterations and 1.8° with local
951 search for 25 iterations) using Relion with an initial model generated with ab-initio reconstruction
952 in cryoSPARC. 3D refinements were carried out using non-uniform refinement along with per-
953 particle defocus refinement in CryoSPARC. Selected particle images were subjected to the
954 Bayesian polishing procedure implemented in Relion3.0 before performing another round of non-
955 uniform refinement in cryoSPARC followed by per-particle defocus refinement and again non-
956 uniform refinement. To further improve the density of the BQ.1.1 RBD and XBB.1 RBD, the
957 particles were subjected to focus 3D classification without refining angles and shifts using a soft
958 mask encompassing the ACE2, RBD and S309 variable domains using a tau value of 60 in Relion.
959 Particles belonging to classes with the best resolved local density were selected and subjected
960 to non-uniform refinement using cryoSPARC. Local resolution estimation, filtering, and
961 sharpening were carried out using CryoSPARC. Reported resolutions are based on the gold-
962 standard Fourier shell correlation (FSC) of 0.143 criterion and Fourier shell correlation curves
963 were corrected for the effects of soft masking by high-resolution noise substitution.

964

965 **Model building and refinement**

966 UCSF Chimera and Coot were used to fit atomic models into the cryoEM maps. Spike RBD
967 domain, ACE2, S309 Fab models were refined and relaxed using Rosetta using sharpened and
968 unsharpened maps.

969

970 **Statistical Analysis**

971 All statistical tests were performed as described in the indicated figure legends using Prism v9.0.
972 The number of independent experiments performed are indicated in the relevant figure legends.

973

974 **Data Availability**

975 All datasets generated and information presented in the study are available from the
976 corresponding authors on reasonable request. Materials generated in this study can be available
977 on request and may require a material transfer agreement.

978

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990

991 **Author Contributions**

992 A.A, L.P., D.C. and D.V. designed the experiments; A.A, K.S., M.B., H.D., J.B., C.S.F., F.M., D.P.,
993 C.Sa., M.G., R.A., D.J., C.M., E.D., C.S, C.Y., A.R., S.Su., J.Z., N.F., D.B. and J.N. performed
994 binding, neutralization assays, biolayer interferometry and surface plasmon resonance binding
995 measurements. L.A.P, G.Sc., F.A.L. and D.V. supervised in vitro neutralization assays. E.C. and
996 K.C. designed and performed mutagenesis for the spike mutant expression plasmids. G.L., G.Le.,
997 B.G., L.V. and M.A.S. performed experiments to measure effector functions; A.A., C.S.F., M.B.,
998 F.M., J.B. and L.P performed memory B cell repertoire analysis. A.A. carried out membrane fusion
999 and protease inhibition experiments. O.G., A.C., P.F., N.F., M.D., O.G., A.C., P.F., A.F.P., M.B.,
1000 C.G., S.Z., L.B., M.J.K., J.K.L., N.F., and H.C. contributed to the recruitment of donors and
1001 collection of plasma samples. J.B.C., S.S. and B.W. performed mouse experiments and viral
1002 burden analyses. Y.J.P. carried out cryoEM specimen preparation, data collection and
1003 processing. Y.J.P. and D.V. built and refined the atomic models. C.S. purified recombinant
1004 glycoproteins. A.A, L.P., G.S., A.L., M.S.D., D.C. and D.V. analyzed the data and wrote the
1005 manuscript with input from all authors; M.S.D, D.C., and D.V. supervised the project.

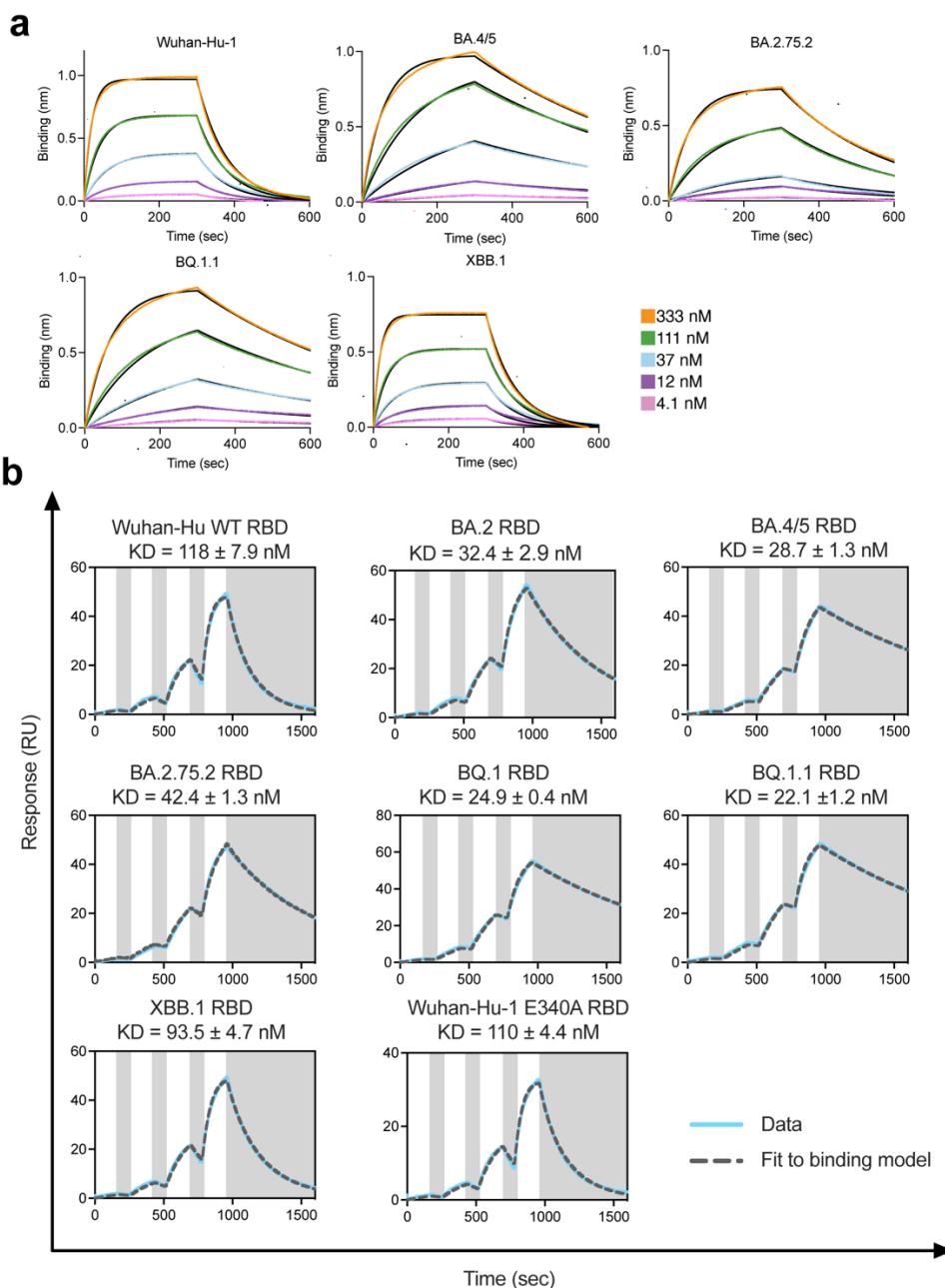
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1007 **Competing Interests**

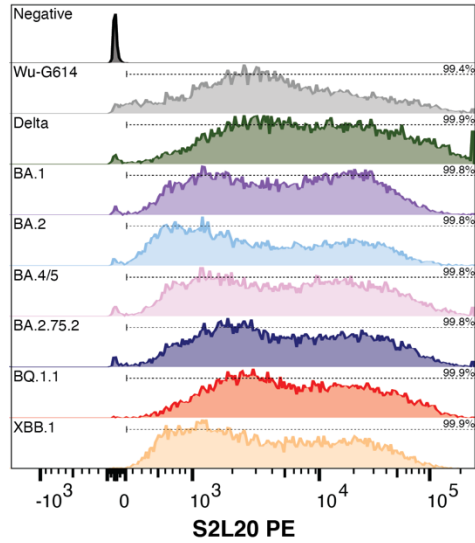
1008 L.P., M.B., B.G., H.D., J.B., C.S.F., F.M., M.D., D.P., L.V., C.Sa., M.G., G.L., G.Le., C.M., E.D.,
1009 A.R., R.A., D.J., S.S., K.C., E.C., G.Sc., J.Z., N.F., D.B. and J.N., F.A.L., N.C., M.A.S., L.A.P.,
1010 G.S., A.L. and D.C. are employees of and may hold shares in Vir Biotechnology Inc. L.A.P. is a
1011 former employee and shareholder of Regeneron Pharmaceuticals and is member of the Scientific
1012 Advisory Board AI-driven Structure-enabled Antiviral Platform (ASAP). Regeneron provided no
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1014 Generate Biomedicines, Moderna, and Immunome. The Diamond laboratory has received
1015 unrelated funding support in sponsored research agreements from Moderna and Emergent
1016 BioSolutions. The remaining authors declare that the research was conducted in the absence of
1017 any commercial or financial relationships that could be construed as a potential conflict of interest.

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 1021 **Extended Data Fig. 1. Evaluation of human ACE2 binding to SARS-CoV-2 variant RBDs. a,**
 1022 Biolayer interferometry binding curves obtained for monomeric human ACE2 binding to
 1023 biotinylated Wu, BA.4/5, BA.2.75.2, BQ.1.1, or XBB.1 RBDs immobilized on the surface of
 1024 streptavidin biosensors. Kinetic rate constants and affinities are shown in **Supplementary Table**
 1025 **1**. Fits are shown as solid black lines. **b**, SPR sensorgrams of monomeric human ACE2 binding
 1026 to the Wu (Wild-type, WT), BA.2, BA.2.75.2, BA.5, BQ.1, BQ.1.1, XBB.1 and Wu E340A RBDs
 1027 immobilized at the surface of a SPR chip coated with anti-Avi polyclonal antibody. Experiments
 1028 were performed with serial dilutions of Fabs and run as single-cycle kinetics. Gray blocks denote
 1029 the dissociation phase. Fits are shown as dashed grey lines. Kinetic rate constants and affinities
 1030 are shown in **Supplementary Table 2**.
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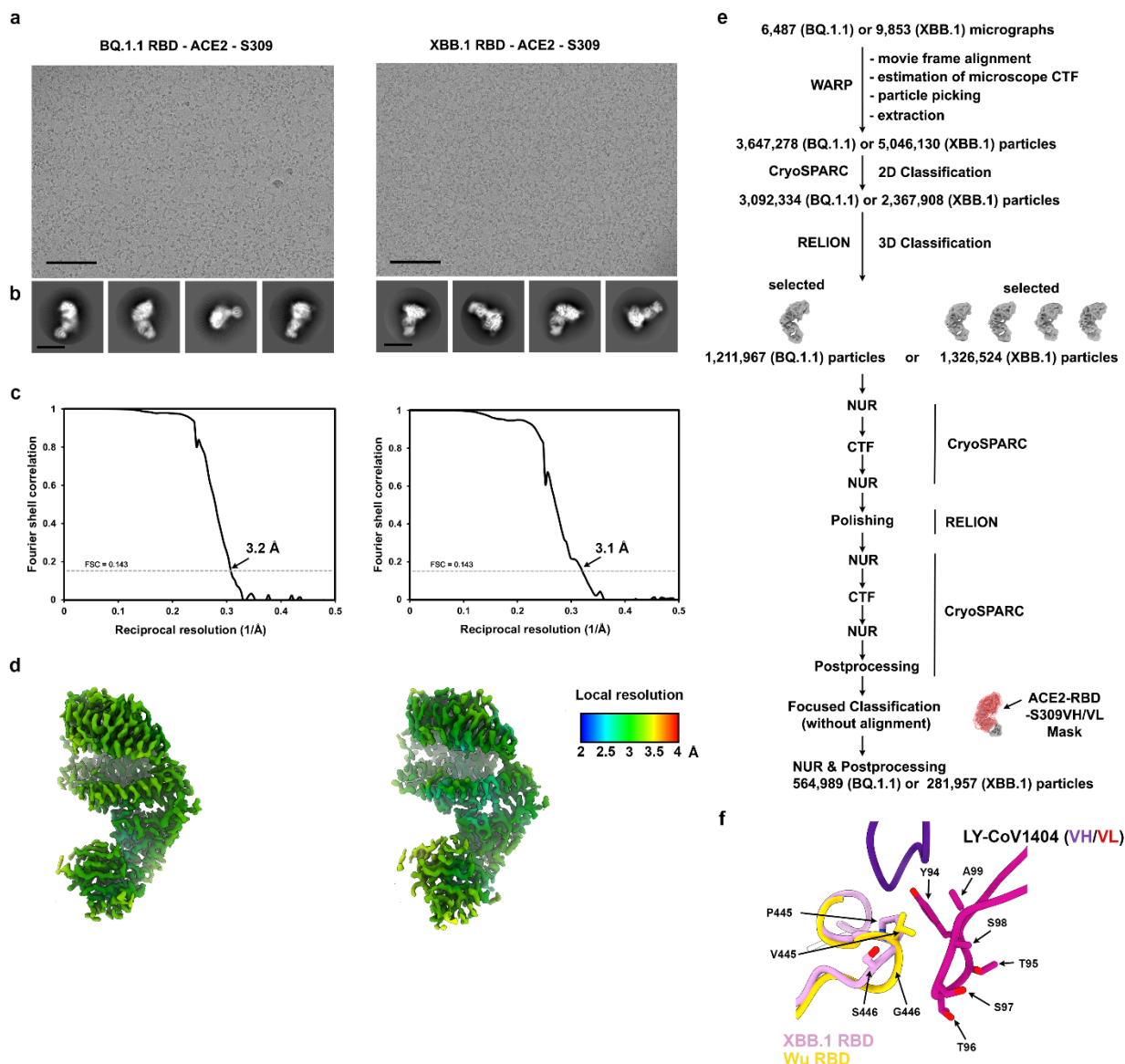
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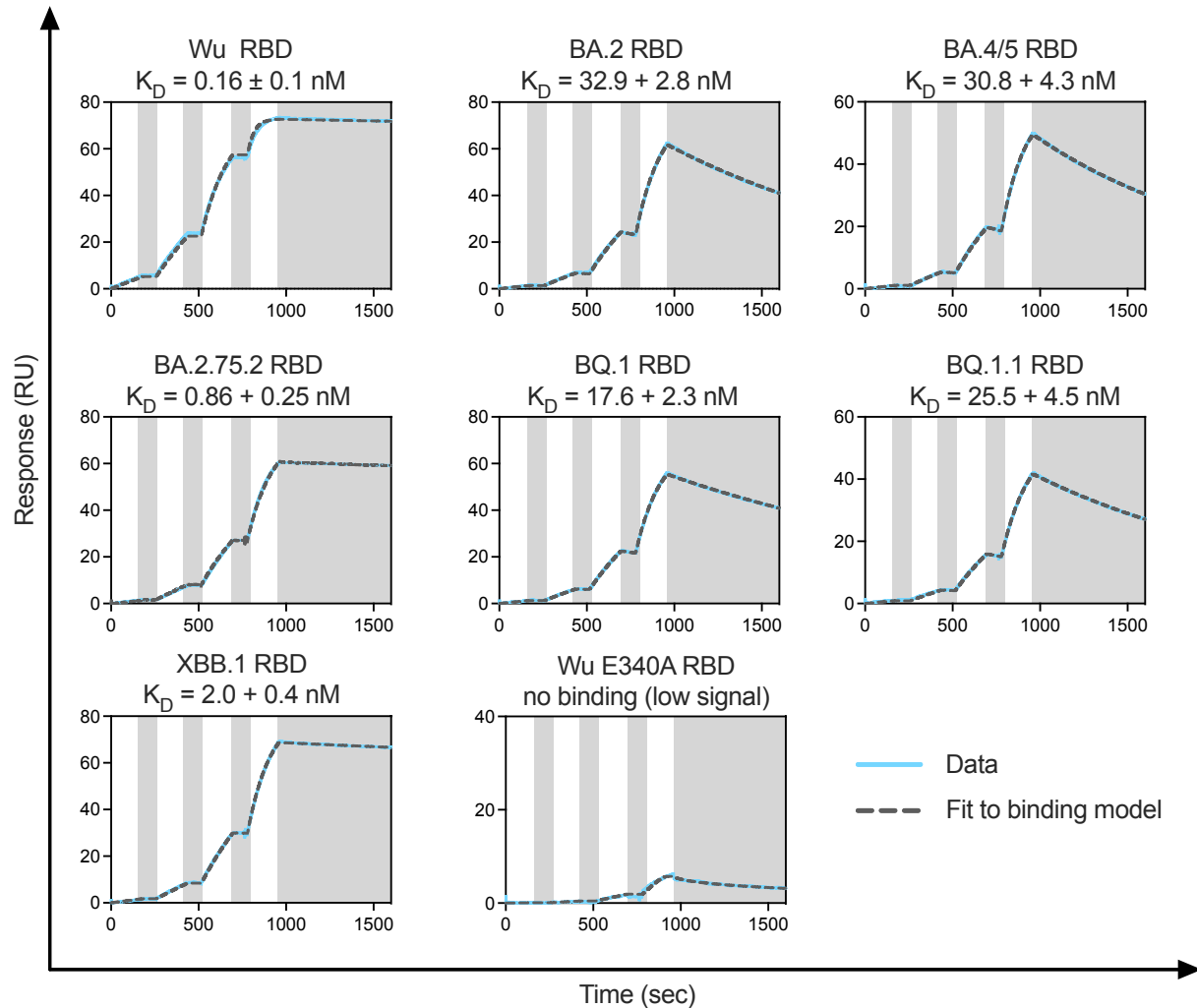
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Extended Data Fig. 2. Membrane fusion assay. Quantification of SARS-CoV-2 S surface expression by flow cytometry for BHK-21 GFP₁₋₁₀ cells transfected with Wu-G614, Delta, BA.1, BA.2, BA.4/5, BA.2.75.2, BQ.1.1, or XBB.1 S proteins using the NTD-directed antibody S2L20^{24,37}. The y-axis is present as a modal scale scaled to maximum singleton events for that plot. The percentage of S-positive cells is based on the PE intensity for mock transfected (negative) cells and represented by the dashed line above each plot.



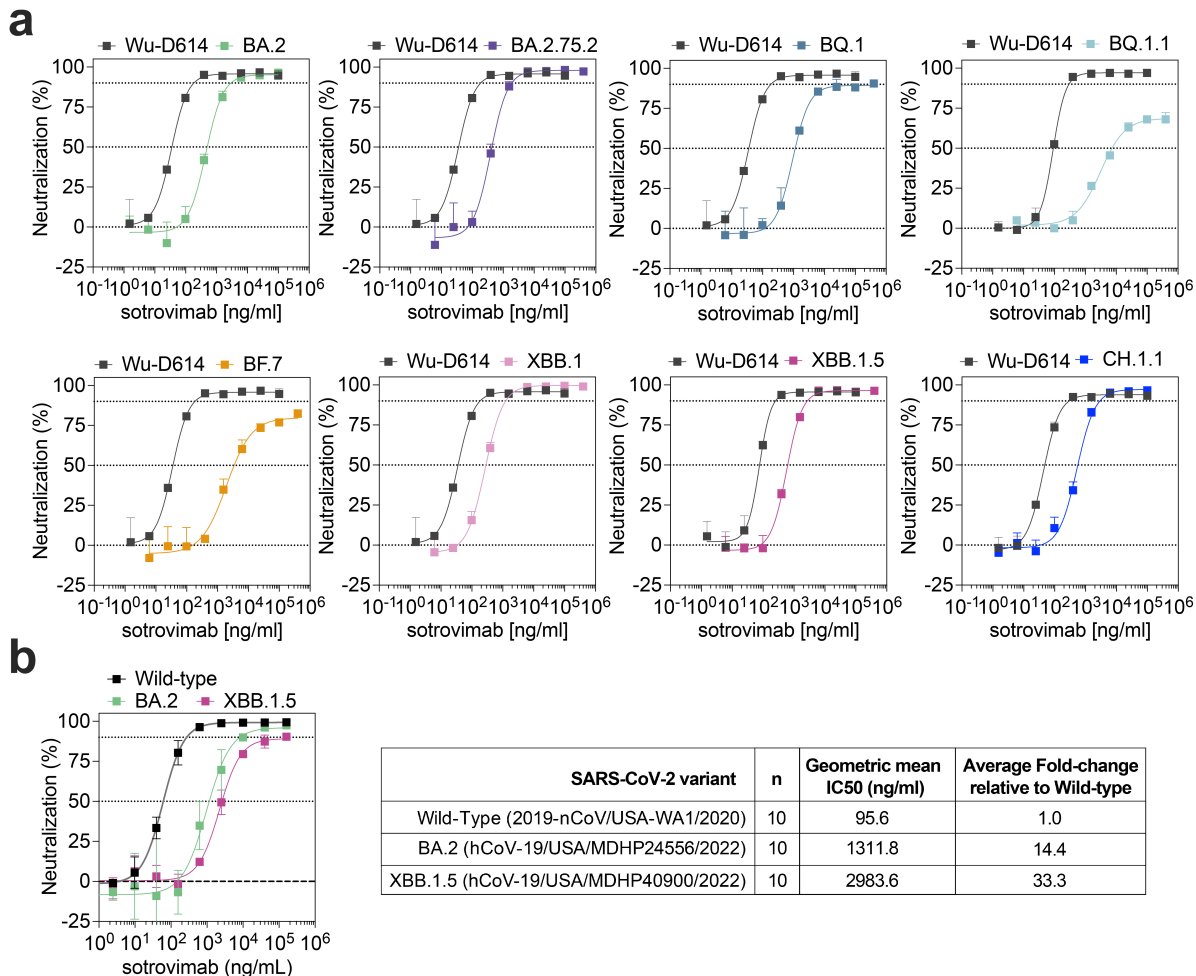
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 1042 **Extended Data Fig. 3. CryoEM data processing of the BQ.1.1 or the XBB.1 RBDs bound to**
 1043 **ACE2 and S309. a-b,** Representative electron micrograph (a) and 2D class averages (b) of the
 1044 BQ.1.1 RBD (left) or the XBB.1 RBD (right) bound to the human ACE2 ectodomain and the S309
 1045 Fab fragment embedded in vitreous ice. The scale bar represents 100nm (a) or 100 Å (b). **c-d,**
 1046 Gold-standard Fourier shell correlation curves (c) and local resolution maps calculated using
 1047 cryoSPARC (d) for the 3D reconstructions of the BQ.1.1 RBD (left) or the XBB.1 RBD (right)
 1048 bound to the human ACE2 ectodomain and the S309 Fab fragment. The 0.143 cutoff is indicated
 1049 by a horizontal dashed line. **e,** Data processing flowchart. CTF: contrast transfer function; NUR:
 1050 non-uniform refinement. **f,** Superimposition of the LYCoV1404-bound Wu RBD (gold, PDB
 1051 7MMO) crystal structure to the ACE2- and S309-bound XBB.1 RBD (pink) cryoEM structure (S309
 1052 and ACE2 are not shown for clarity).

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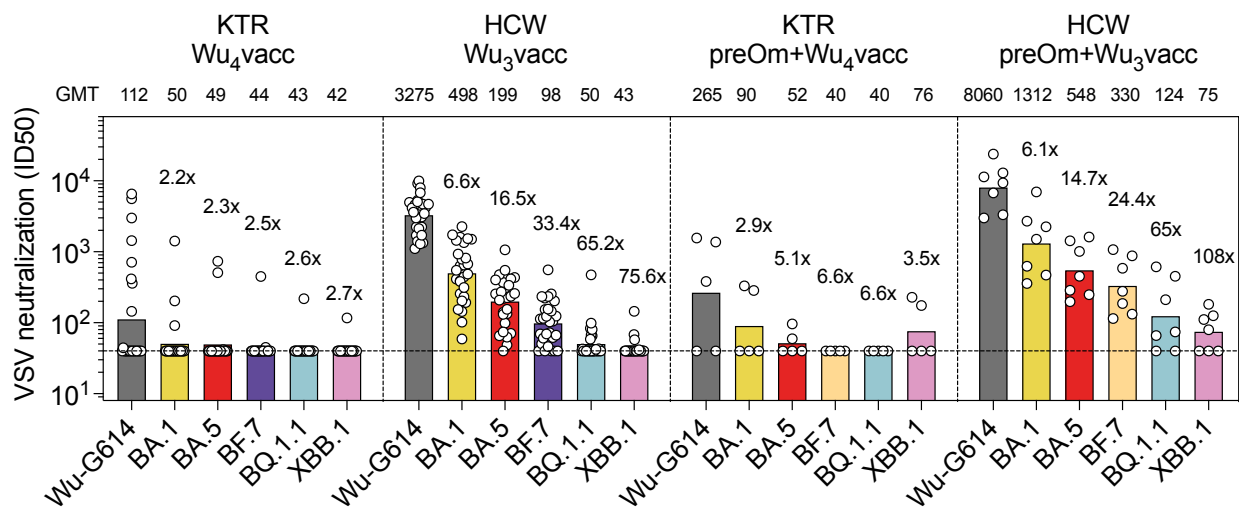
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Extended Data Fig. 4. Cross-reactivity of S309 with SARS-CoV-2 variant RBDs. Representative sensograms of S309 Fab binding to the SARS-CoV-2 Wu, BA.2, BA.2.75.2, BA.5, BQ.1, BQ.1.1, XBB.1 and Wu E340A RBDs immobilized at the surface of a SPR chip coated with anti-Avi polyclonal antibody. Experiments were performed with serial dilutions of Fabs and were run as single-cycle kinetics. Gray blocks denote the dissociation phase. Fits are shown as dashed grey lines. Kinetic rate constants and affinities are shown in **Supplementary Table 4**.



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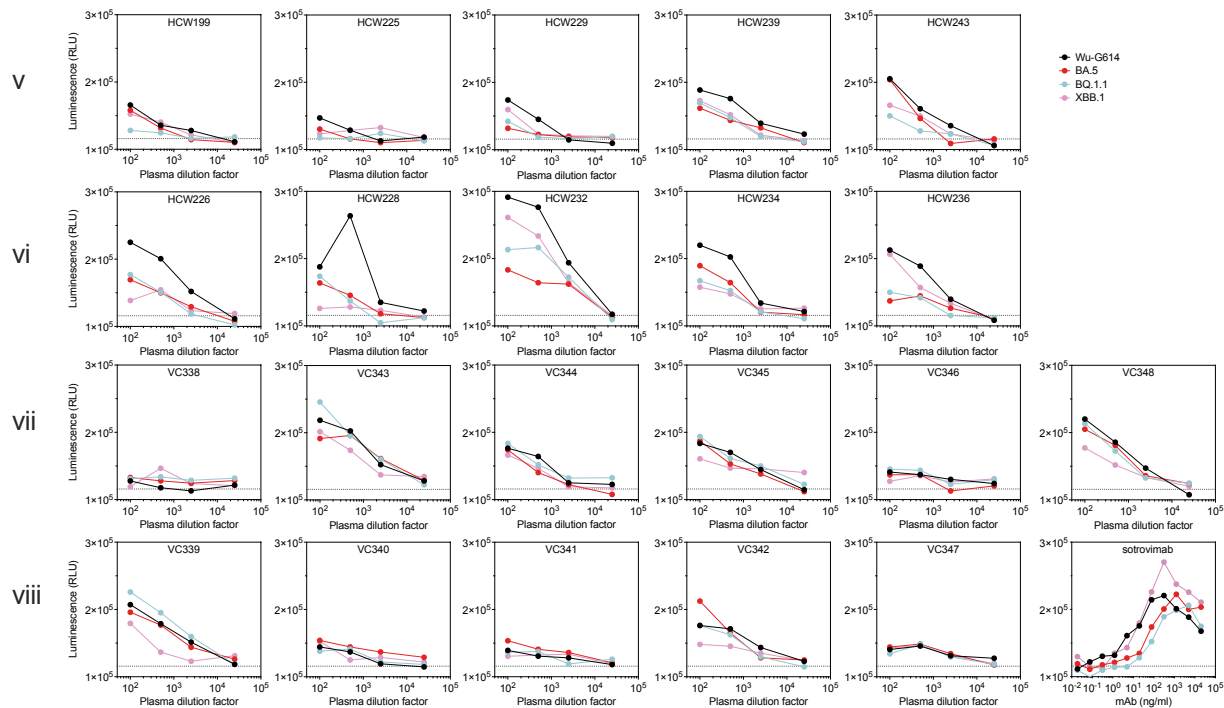
Extended Data Fig. 6. Sotrovimab neutralization of SARS-CoV-2 Omicron variants. a, Sotrovimab-mediated neutralization of Wu-D614, BA.2, BA.2.75.2, BQ.1, BQ.1.1, BF.7, XBB.1, XBB.1.5, and CH.1.1 S VSV pseudoviruses using VeroE6 as target cells. Dose-response curves of one representative experiment out of at least 5 experiments are shown. **b,** Sotrovimab-mediated neutralization of Wild-Type (2019-nCoV/USA-WA1/2020), Omicron BA.2 (hCoV-19/USA/MDHP24556/2022) and Omicron XBB.1.5 (hCoV-19/USA/MDHP40900/2022) authentic viruses using VeroE6-TMPRSS2 as target cells. Neutralization data (left panel) represent the means of triplicates \pm standard deviation from one representative of $n = 10$ biologically independent experiments. Shown is also the geometric mean IC₅₀ and average fold-change relative to wild-type of the 10 performed experiments (right panel).



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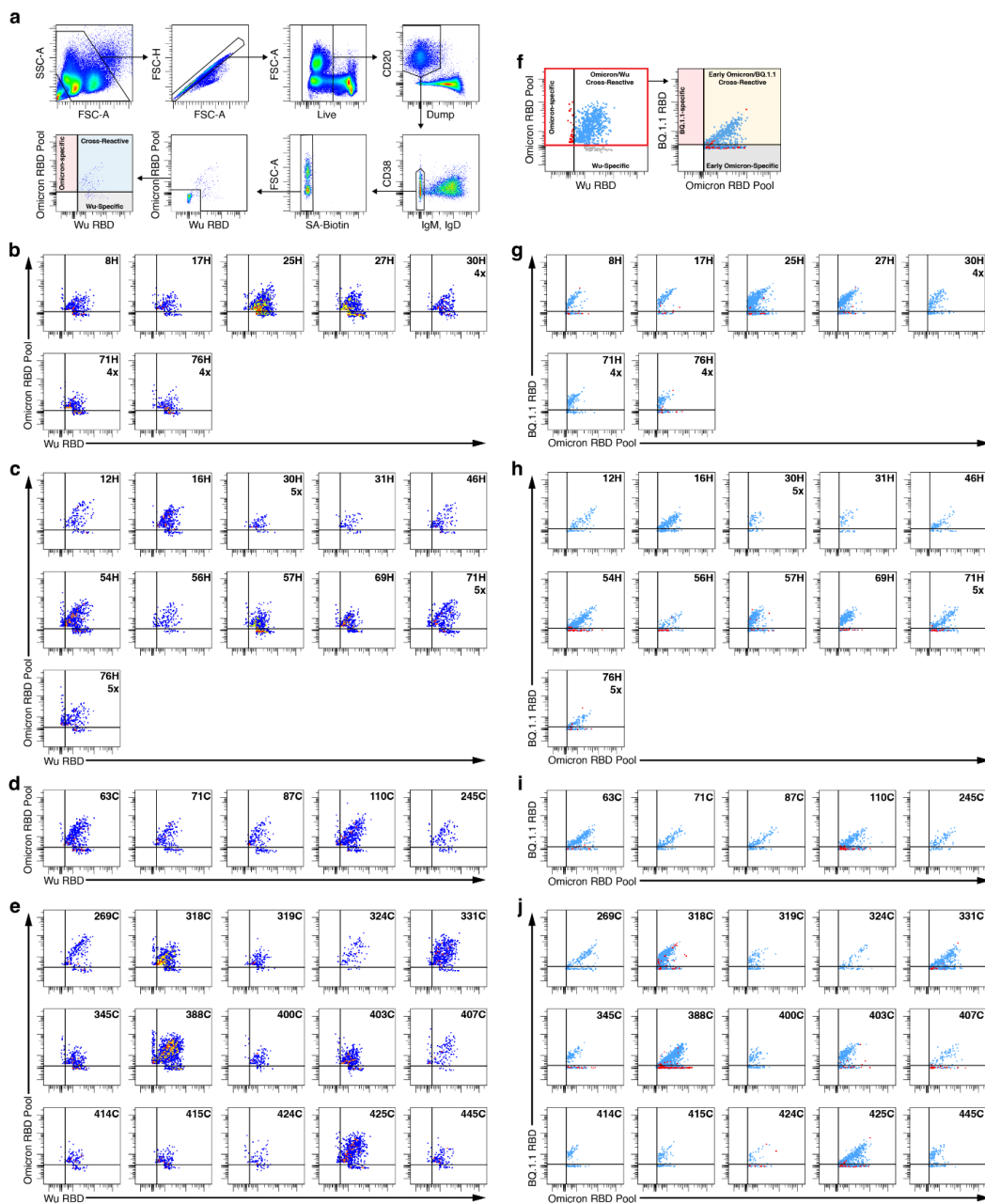
1093 **Extended Data Fig. 7 Vaccine-elicited plasma neutralizing antibodies against emerging**
 1094 **Omicron variants in kidney transplant recipients.** Neutralization of SARS-CoV-2 pseudotyped
 1095 VSV carrying Wu-G614, Omicron BA.1, BA.5, BF.7, BQ.1.1 and XBB.1 by serum samples from
 1096 kidney transplant recipients (KTR) or healthcare workers (HCW) collected 2-4 months after
 1097 receiving 4 (Wu₄vacc) or 3 doses (Wu₃vacc) of monovalent Wu vaccine, respectively. Shown are
 1098 ID50 values from n = 2 technical replicates. Bars and values on top represent geometric mean
 1099 ID50 titers (GMT). Fold-loss of neutralization against Omicron variants as compared to Wu-G614
 1100 is shown above each corresponding bar. Horizontal dashed lines indicate the lowest limit of
 1101 detectable neutralization in these assays (ID50 = 40). Cohort demographics are summarized in
 1102 **Supplementary Table 6.**

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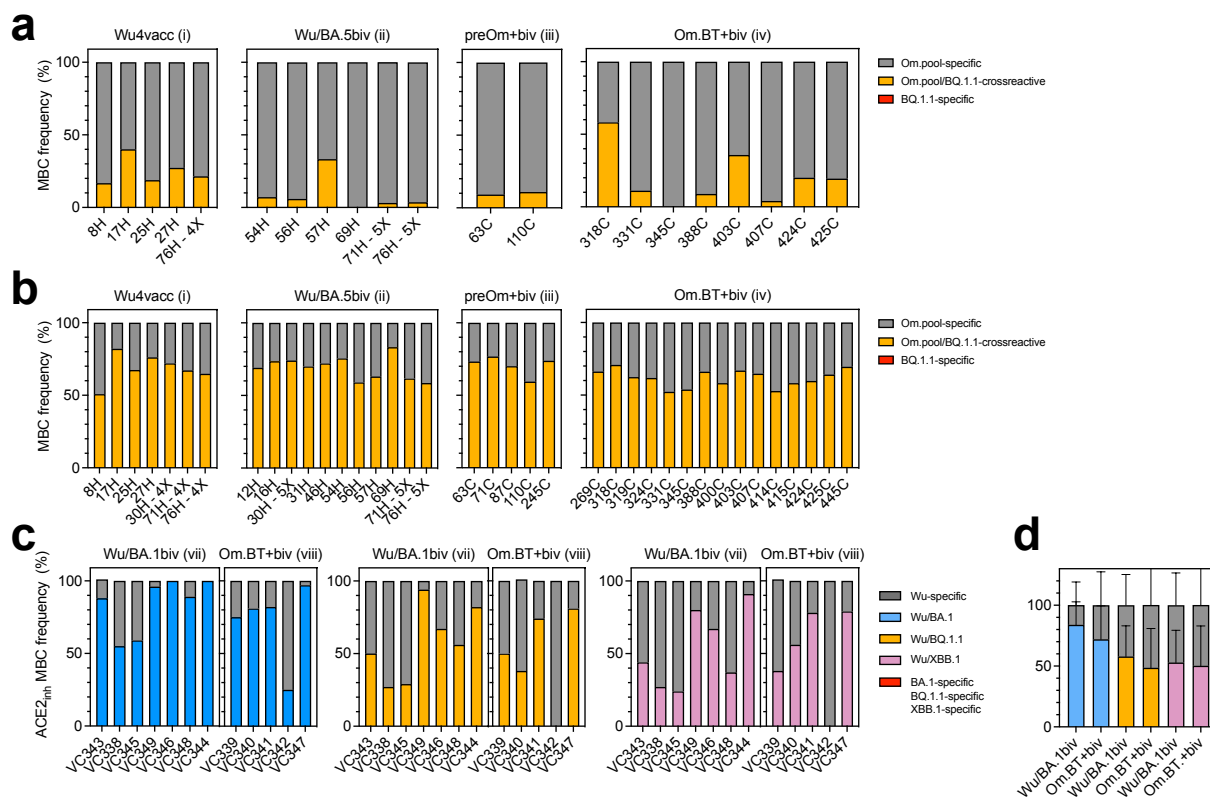
1107 **Extended Data Fig 8. Analysis of individual plasma samples activating Fc γ RIIIa.** Activation
1108 of high-affinity (V158) Fc γ RIIIa measured using Jurkat reporter cells and Wu-G614, BA.5, BQ.1.1
1109 and XBB.1 SARS-CoV-2 S glycoprotein-expressing ExpiCHO as target cells. Luminescence
1110 (RLU) values from one experiment are shown with plasma samples from cohorts v-viii (n=5 donors
1111 for cohort v, n=5 for cohort vi, n=6 for cohort vii and n=5 for cohort viii) and compared to
1112 sotrovimab. Horizontal dotted line indicates the lowest limit of detectable activation (RLU =
1113 115,737).
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Extended Data Fig. 9 MBC analysis by flow cytometry. **a**, Gating strategy to identify Omicron (BA.1/BA.2/BA.5) pool RBD- and Wu RBD-recognizing MBCs. Dump includes markers for CD3, CD8, CD14, and CD16. Gating for RBD-positive memory B cells was based on staining of PBMCs from healthy donors collected in 2019 prior to the SARS-CoV-2 pandemic. Individual plots showing Omicron (BA.1/BA.2/BA.5) pool RBD- and Wu RBD-positive MBCs for Wu₄ vaccinated (b), Wu/BA.5 bivalent vaccinated (c), pre-Omicron infected-Wu/BA.5 bivalent vaccinated (d), and Omicron BT-Wu/BA.5 bivalent vaccinated individuals (e). **f**, Gating strategy to determine whether (BA.1, BA.2, and BA.4/5) pool RBD-positive MBCs recognize the BQ.1.1 RBD. Individual plots showing Omicron (BA.1, BA.2, and BA.4/5) RBD pool and BQ.1.1 RBD-recognizing memory B cells for Wu₄ vaccinated (g), Wu/BA.5 bivalent vaccinated (h), pre-Omicron infected-Wu/BA.5 bivalent vaccinated (i), and Omicron BT-Wu/BA.5 bivalent vaccinated individuals (j).

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 1129 **Extended Data Figure 10. Subanalysis of cross-reactivity of vaccine-elicited MBCs. a-c,**
 1130 Analysis of cross-reactivity with BQ.1.1 of Omicron-specific (a) and Wu/Omicron-cross-reactive
 1131 (b). Om.pool, MBCs reactive to Omicron BA.1, BA.2 or BA.5 RBDs in cohorts i-iv. **c, d**, Individual
 1132 (c) and mean±sd (d) frequencies of Wu-G614-specific (grey), Omicron-specific (red) and
 1133 Wu/Omicron-cross-reactive (blue for BA.1, yellow for BQ.1.1 and purple for XBB.1) MBCs
 1134 showing inhibition of binding of ACE2 to RBD from donors of cohorts vii and viii.

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1138 **Supplementary Table 1. Kinetics of monomeric human ACE2 binding to immobilized**
1139 **SARS-CoV-2 variant RBDs as measured by biolayer interferometry.**

	K_D (nM)	k_{on} (M⁻¹s⁻¹)	k_{off}(s⁻¹)
Wuhan-Hu-1	101.1 ± 7.3	1.30 x 10 ⁵	1.31 x 10 ⁻²
BA.4/5	12.8 ± 2.2	1.45 x 10 ⁵	1.82 x 10 ⁻³
BA.2.75.2	26.2 ± 1.7	1.35 x 10 ⁵	3.52 x 10 ⁻³
BQ.1.1	13.7 ± 1.4	1.39 x 10 ⁵	1.89 x 10 ⁻³
XBB.1	88.4 ± 11.9	1.47 x 10 ⁵	1.29 x 10 ⁻²

1140 Values are presented as mean ± standard deviation

1141 **Supplementary Table 2. Kinetics of monomeric human ACE2 binding to immobilized**
1142 **SARS-CoV-2 variant RBDs as measured by Surface Plasmon Resonance.**

RBD	average KD (nM)	stdev (KD) (nM)	averaged ka (1/Ms)	stdev(ka) (1/Ms)	averaged kd (1/s)	stdev(kd) (1/s)	Number of replicates
Wu-Hu- WT	117.93	7.86	6.09E+04	6.65E+03	6.09E+04	6.65E+03	3
Wu-Hu E340A	110.25	4.35	5.42E+04	1.36E+04	5.95E-03	1.26E-03	3
BA.2	32.40	2.90	2.27E+04	1.92E+03	7.34E-04	8.47E-05	3
BA.4/5	28.72	1.28	2.69E+04	4.17E+02	7.73E-04	2.26E-05	2

1143 Averaged binding affinity (KD in nM unit), corresponding standard deviation, and number of replicates for
1144 each RBD-ACE2 pair. N/A: standard deviation calculation not applicable because only one replicate
1145 produces data within the instrument's limit. NB: no binding; N/A: not applicable.

1146 **Supplementary Table 3. CryoEM data collection and refinement statistics.**

	SARS-CoV-2 BQ.1.1 RBD - ACE2-S309 PDB EMD	SARS-CoV-2 XBB.1 RBD - ACE2-S309 PDB EMD
Data collection and processing		
Magnification	105,000	105,000
Voltage (kV)	300	300
Electron exposure (e ⁻ /Å ²)	60	60
Defocus range (µm)	-0.5 - -2.5	-0.2 - -3.0
Pixel size (Å)	0.843	0.843
Symmetry imposed	C1	C1
Final particle images (no.)	564,989	281,957
Map resolution (Å)	3.2	3.1
FSC threshold	0.143	0.143
Map sharpening <i>B</i> factor (Å ²)	-150	-120
Validation		
MolProbity score	0.95	1.51
Clashscore	1.07	5.94
Poor rotamers (%)	0.46	0.48
Ramachandran plot		
Favored (%)	97.26	96.87
Allowed (%)	2.64	2.71
Disallowed (%)	0.1	0.42

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1148 **Supplementary Table 4. Kinetics of S309 Fab binding to immobilized SARS-CoV-2 variant**
1149 **RBDs as measured by Surface Plasmon Resonance.**

RBD	average K_D (nM)	stdev(K_D) (nM)	averaged k_{on} (1/Ms)	stdev(k_{on}) (1/Ms)	averaged k_{off} (1/s)	stdev(k_{off}) (1/s)	Number of replicates
Wuhan-Hu-WT	0.16	0.09	9.12E+04	9.86E+03	1.41E-05	7.06E-06	7
Wuhan-Hu E340A	NB	N/A	NB	N/A	NB	N/A	3
BA.1	10.88	2.51	2.29E+04	2.49E+03	2.45E-04	3.23E-05	4
BA.2	32.88	2.79	2.16E+04	2.01E+03	7.05E-04	5.25E-05	8
BA.4/5	30.82	4.33	2.49E+04	1.97E+03	7.60E-04	7.08E-05	14
BA.2.75.2	0.86	0.25	2.87E+04	3.14E+03	2.43E-05	5.52E-06	6
BQ.1	17.63	2.31	2.51E+04	1.39E+03	4.40E-04	3.60E-05	5
BQ.1.1	25.49	4.46	2.34E+04	1.79E+03	5.92E-04	6.60E-05	3
XBB.1	1.96	0.44	2.73E+04	1.60E+03	5.32E-05	1.06E-05	3

1150 Averaged binding affinity (K_D in nM unit), corresponding standard deviation, and number of replicates for each RBD-
1151 S309 Fab pair. N/A: standard deviation calculation not applicable because only one replicate produces data within the
1152 instrument's limit. NB: no binding; N/A: not applicable.
1153

1154 **Supplementary Table 5. Donors' demographics.**

Donor ID	Gender	Vaccine doses	COVID diagnosis	SARS-CoV-2 variant	D sample-vacc (days)	Cohort
15H	M	4	no	/	5	Wu ₄ vacc
17H	M	4	no	/	21	Wu ₄ vacc
25H	M	4	no	/	16	Wu ₄ vacc
27H	M	4	no	/	42	Wu ₄ vacc
30H - 4X	F	4	no	/	17	Wu ₄ vacc
46H	M	4	no	/	15	Wu ₄ vacc
71H - 4x	F	4	no	/	48	Wu ₄ vacc
76H - 4x	F	4	no	/	13	Wu ₄ vacc
8H	M	4	no	/	72	Wu ₄ vacc
12H	F	4	no	/	34	Wu/BA.5biv
16H	F	4	no	/	33	Wu/BA.5biv
17H	M	5	no	/	18	Wu/BA.5biv
29H	M	5	no	/	37	Wu/BA.5biv
30H - 5x	F	5	no	/	30	Wu/BA.5biv
31H*	F	3	no	/	54	Wu/BA.5biv
46H	M	5	no	/	16	Wu/BA.5biv
51H	F	4	no	/	33	Wu/BA.5biv
54H	F	4	no	/	27	Wu/BA.5biv
56H	M	4	no	/	28	Wu/BA.5biv
57H	M	5	no	/	46	Wu/BA.5biv
69H	F	5	no	/	42	Wu/BA.5biv
71H - 5x	F	5	no	/	20	Wu/BA.5biv
76H - 5x	F	5	no	/	60	Wu/BA.5biv
110C	M	5	yes	WA-1	38	preOm+biv
245C	F	4	yes	WA-1	25	preOm+biv
63C	F	4	yes	WA-1	33	preOm+biv
71C	F	5	yes	WA-1	28	preOm+biv
87C	M	5	yes	WA-1	30	preOm+biv
269C	M	4	yes	Gamma/P.1 & Om. BA.1	30	Om.BT+biv
318C	F	4	yes	Omicron	42	Om.BT+biv
319C	M	4	yes	Omicron	28	Om.BT+biv
324C	F	4	yes	Omicron	32	Om.BT+biv
331C	M	5	yes	Omicron	33	Om.BT+biv
345C	M	4	yes	Omicron	28	Om.BT+biv
388C	M	4	yes	Omicron BA.5	45	Om.BT+biv
400C	F	4	yes	Omicron BA.2	22	Om.BT+biv
403C	F	4	yes	Omicron BA.2.12.1	33	Om.BT+biv
407C	F	4	yes	Omicron BA.2	34	Om.BT+biv
414C	M	4	yes	Omicron BA.4	51	Om.BT+biv
415C	F	4	yes	Omicron BA.2	30	Om.BT+biv
424C	F	4	yes	Omicron BA.2.12.1	29	Om.BT+biv
425C	M	4	yes	Omicron BA.2.12.1	31	Om.BT+biv
428C	F	4	yes	Omicron BA.5	13	Om.BT+biv
445C	F	4	yes	Omicron BA.5	36	Om.BT+biv
HCW199	M	3	no	/	18	Wu ₃ vacc
HCW225	F	3	no	/	25	Wu ₃ vacc
HCW229	F	3	no	/	20	Wu ₃ vacc
HCW239	F	3	no	/	26	Wu ₃ vacc
HCW243	F	3	no	/	27	Wu ₃ vacc
HCW198	M	3	yes	pre Omicron	18	preOm+vacc
HCW200	F	3	yes	pre Omicron	13	preOm+vacc
HCW202	M	3	yes	pre Omicron	13	preOm+vacc
HCW221	F	3	yes	pre Omicron	20	preOm+vacc
HCW224	F	3	yes	pre Omicron	18	preOm+vacc
HCW226	F	3	yes	pre Omicron	25	preOm+vacc
HCW228	M	3	yes	pre Omicron	20	preOm+vacc

HCW232	F	3	yes	pre Omicron	25	preOm+vacc
HCW234	M	3	yes	pre Omicron	19	preOm+vacc
HCW236	F	3	yes	pre Omicron	27	preOm+vacc
HCW237	F	3	yes	pre Omicron	20	preOm+vacc
HCW238	M	3	yes	pre Omicron	26	preOm+vacc
HCW240	F	3	yes	pre Omicron	26	preOm+vacc
HCW242	F	3	yes	pre Omicron	25	preOm+vacc
VC338	F	4	no	/	13	Wu/BA.1biv
VC343	F	4	no	/	13	Wu/BA.1biv
VC344	M	4	no	/	12	Wu/BA.1biv
VC345	M	4	no	/	13	Wu/BA.1biv
VC346	M	4	no	/	13	Wu/BA.1biv
VC348	M	4	no	/	13	Wu/BA.1biv
VC349	F	4	no	/	19	Wu/BA.1biv
VC339	F	4	yes	Omicron BA.1/BA.2	13	Om.BT+biv
VC340	F	4	yes	Omicron BA.1/BA.2	13	Om.BT+biv
VC341	F	4	yes	Omicron BA.1/BA.2	13	Om.BT+biv
VC342	F	4	yes	Omicron BA.1/BA.2	12	Om.BT+biv
VC347	M	4	yes	Omicron BA.1/BA.2	13	Om.BT+biv

*Donor 31H received the Janssen COVID-19 vaccination as primary vaccine series.

1156 **Supplementary Table 6. Kidney transplant recipients' and healthcare workers'**
 1157 **demographics.**

Donor ID	Gender	No. of immunosuppressive drugs*	Vaccine doses	COVID diagnosis	SARS-CoV-2 variant	D sample-vaccine (days)	Cohort
KTR-004	M	2	4	no	/	83	KTR Wu ₄ vacc
KTR-007	M	3	4	no	/	140	KTR Wu ₄ vacc
KTR-009	M	2	4	no	/	46	KTR Wu ₄ vacc
KTR-010	F	3	4	no	/	51	KTR Wu ₄ vacc
KTR-011	M	2	4	no	/	80	KTR Wu ₄ vacc
KTR-013	F	1	4	no	/	92	KTR Wu ₄ vacc
KTR-026	M	2	4	no	/	63	KTR Wu ₄ vacc
KTR-027	F	3	4	no	/	35	KTR Wu ₄ vacc
KTR-030	M	2	4	no	/	71	KTR Wu ₄ vacc
KTR-039	M	2	4	no	/	77	KTR Wu ₄ vacc
KTR-042	M	3	4	no	/	40	KTR Wu ₄ vacc
KTR-047	M	2	4	no	/	76	KTR Wu ₄ vacc
KTR-050	M	2	4	no	/	84	KTR Wu ₄ vacc
KTR-054	M	2	4	no	/	18	KTR Wu ₄ vacc
KTR-056	M	2	4	no	/	70	KTR Wu ₄ vacc
KTR-059	M	2	4	no	/	78	KTR Wu ₄ vacc
KTR-060	M	2	4	no	/	71	KTR Wu ₄ vacc
KTR-061	M	2	4	no	/	54	KTR Wu ₄ vacc
KTR-071	F	3	4	no	/	35	KTR Wu ₄ vacc
KTR-083	M	3	4	no	/	82	KTR Wu ₄ vacc
KTR-085	F	2	4	no	/	71	KTR Wu ₄ vacc
KTR-094	M	2	4	no	/	41	KTR Wu ₄ vacc
KTR-095	M	3	4	no	/	74	KTR Wu ₄ vacc
KTR-096	M	2	4	no	/	43	KTR Wu ₄ vacc
KTR-101	M	2	4	no	/	72	KTR Wu ₄ vacc
KTR-102	M	3	4	no	/	133	KTR Wu ₄ vacc
KTR-017	F	2	4	yes	pre Omicron	58	KTR preOm+Wu ₄ vacc
KTR-021	M	2	4	yes	pre Omicron	84	KTR preOm+Wu ₄ vacc
KTR-031	F	2	4	yes	pre Omicron	59	KTR preOm+Wu ₄ vacc
KTR-084	F	3	4	yes	pre Omicron	65	KTR preOm+Wu ₄ vacc
KTR-099	M	2	4	yes	pre Omicron	93	KTR preOm+Wu ₄ vacc
HCW-001	M	/	3	no	/	90	HCW Wu ₃ vacc
HCW-002	F	/	3	no	/	78	HCW Wu ₃ vacc
HCW-003	F	/	3	no	/	76	HCW Wu ₃ vacc
HCW-004	M	/	3	no	/	90	HCW Wu ₃ vacc
HCW-005	F	/	3	no	/	83	HCW Wu ₃ vacc
HCW-008	F	/	3	no	/	86	HCW Wu ₃ vacc
HCW-009	F	/	3	no	/	81	HCW Wu ₃ vacc
HCW-011	M	/	3	no	/	93	HCW Wu ₃ vacc
HCW-012	F	/	3	no	/	94	HCW Wu ₃ vacc
HCW-013	F	/	3	no	/	55	HCW Wu ₃ vacc
HCW-016	F	/	3	no	/	6	HCW Wu ₃ vacc
HCW-017	F	/	3	no	/	50	HCW Wu ₃ vacc
HCW-018	M	/	3	no	/	110	HCW Wu ₃ vacc
HCW-019	F	/	3	no	/	29	HCW Wu ₃ vacc
HCW-020	F	/	3	no	/	90	HCW Wu ₃ vacc
HCW-021	M	/	3	no	/	28	HCW Wu ₃ vacc
HCW-022	F	/	3	no	/	28	HCW Wu ₃ vacc
HCW-023	F	/	3	no	/	22	HCW Wu ₃ vacc
HCW-024	M	/	3	no	/	35	HCW Wu ₃ vacc
HCW-025	F	/	3	no	/	94	HCW Wu ₃ vacc
HCW-026	F	/	3	no	/	28	HCW Wu ₃ vacc
HCW-027	F	/	3	no	/	38	HCW Wu ₃ vacc
HCW-028	F	/	3	no	/	29	HCW Wu ₃ vacc

HCW-031	F	/	3	no	/	35	HCW Wu ₃ vacc
HCW-033	F	/	3	no	/	31	HCW Wu ₃ vacc
HCW-037	F	/	3	no	/	45	HCW Wu ₃ vacc
HCW-038	F	/	3	no	/	61	HCW Wu ₃ vacc
HCW-039	F	/	3	no	/	23	HCW Wu ₃ vacc
HCW-010	F	/	3	yes	pre Omicron	69	HCW preOm+Wu ₃ vacc
HCW-014	F	/	3	yes	pre Omicron	89	HCW preOm+Wu ₃ vacc
HCW-015	F	/	3	yes	pre Omicron	86	HCW preOm+Wu ₃ vacc
HCW-029	F	/	3	yes	pre Omicron	77	HCW preOm+Wu ₃ vacc
HCW-030	F	/	3	yes	pre Omicron	35	HCW preOm+Wu ₃ vacc
HCW-034	F	/	3	yes	pre Omicron	28	HCW preOm+Wu ₃ vacc
HCW-036	F	/	3	yes	pre Omicron	34	HCW preOm+Wu ₃ vacc

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*any of the following: cyclosporin, tacrolimus, MMF/MPA, azathioprine, everolimus/sirolimus, belatacept or glucocorticoids

1162 **References**

1163

- 1164 1. Cao, Y. *et al.* Imprinted SARS-CoV-2 humoral immunity induces convergent Omicron RBD
1165 evolution. *Nature* (2022) doi:10.1038/s41586-022-05644-7.
- 1166 2. Viana, R. *et al.* Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern
1167 Africa. *Nature* (2022) doi:10.1038/d41586-021-03832-5.
- 1168 3. Bowen, J. E. *et al.* Omicron spike function and neutralizing activity elicited by a
1169 comprehensive panel of vaccines. *Science* eabq0203 (2022).
- 1170 4. Cameroni, E. *et al.* Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron
1171 antigenic shift. *Nature* **602**, 664–670 (2022).
- 1172 5. McCallum, M. *et al.* Structural basis of SARS-CoV-2 Omicron immune evasion and receptor
1173 engagement. *Science* eabn8652 (2022).
- 1174 6. Meng, B. *et al.* Altered TMPRSS2 usage by SARS-CoV-2 Omicron impacts infectivity and
1175 fusogenicity. *Nature* **603**, 706–714 (2022).
- 1176 7. Peacock, T. P. *et al.* The altered entry pathway and antigenic distance of the SARS-CoV-2
1177 Omicron variant map to separate domains of spike protein. *bioRxiv* 2021.12.31.474653
1178 (2022) doi:10.1101/2021.12.31.474653.
- 1179 8. Willett, B. J. *et al.* SARS-CoV-2 Omicron is an immune escape variant with an altered cell
1180 entry pathway. *Nat Microbiol* **7**, 1161–1179 (2022).
- 1181 9. Planas, D. *et al.* Considerable escape of SARS-CoV-2 Omicron to antibody neutralization.
1182 *Nature* (2021) doi:10.1038/d41586-021-03827-2.
- 1183 10. Liu, L. *et al.* Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2.
1184 *Nature* (2021) doi:10.1038/d41586-021-03826-3.
- 1185 11. Cao, Y. *et al.* Omicron escapes the majority of existing SARS-CoV-2 neutralizing
1186 antibodies. *Nature* (2021) doi:10.1038/d41586-021-03796-6.
- 1187 12. Mlcochova, P. *et al.* SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion.
1188 *Nature* (2021) doi:10.1038/s41586-021-03944-y.
- 1189 13. Carreño, J. M. *et al.* Activity of convalescent and vaccine serum against SARS-CoV-2
1190 Omicron. *Nature* **602**, 682–688 (2022).
- 1191 14. Akerman, A. *et al.* Emergence and antibody evasion of BQ and BA.2.75 SARS-CoV-2
1192 sublineages in the face of maturing antibody breadth at the population level. *bioRxiv* (2022)
1193 doi:10.1101/2022.12.06.22283000.
- 1194 15. Aggarwal, A. *et al.* SARS-CoV-2 Omicron BA.5: Evolving tropism and evasion of potent
1195 humoral responses and resistance to clinical immunotherapeutics relative to viral variants
1196 of concern. *EBioMedicine* **84**, 104270 (2022).
- 1197 16. Gregory, D. A. *et al.* Genetic diversity and evolutionary convergence of cryptic SARS- CoV-
1198 2 lineages detected via wastewater sequencing. *PLOS Pathogens* vol. 18 e1010636 at
1199 <https://doi.org/10.1371/journal.ppat.1010636> (2022).
- 1200 17. Walls, A. C. *et al.* Structure, Function, and Antigenicity of the SARS-CoV-2 Spike
1201 Glycoprotein. *Cell* **181**, 281-292.e6 (2020).
- 1202 18. Wrapp, D. *et al.* Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.
1203 *Science* **367**, 1260–1263 (2020).
- 1204 19. Hoffmann, M. *et al.* SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is
1205 Blocked by a Clinically Proven Protease Inhibitor. *Cell* **181**, 271-280.e8 (2020).

- 1206 20. Letko, M., Marzi, A. & Munster, V. Functional assessment of cell entry and receptor usage
1207 for SARS-CoV-2 and other lineage B betacoronaviruses. *Nature Microbiology* (2020)
1208 doi:10.1038/s41564-020-0688-y.
- 1209 21. Arunachalam, P. S. *et al.* Durable protection against the SARS-CoV-2 Omicron variant is
1210 induced by an adjuvanted subunit vaccine. *Sci. Transl. Med.* **14**, eabq4130 (2022).
- 1211 22. Arunachalam, P. S. *et al.* Adjuvanting a subunit COVID-19 vaccine to induce protective
1212 immunity. *Nature* (2021) doi:10.1038/s41586-021-03530-2.
- 1213 23. Piccoli, L. *et al.* Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2
1214 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. *Cell* **183**,
1215 1024-1042.e21 (2020).
- 1216 24. McCallum, M. *et al.* N-terminal domain antigenic mapping reveals a site of vulnerability for
1217 SARS-CoV-2. *Cell* (2021) doi:10.1016/j.cell.2021.03.028.
- 1218 25. Cromer, D. *et al.* Neutralising antibody titres as predictors of protection against SARS-CoV-
1219 2 variants and the impact of boosting: a meta-analysis. *Lancet Microbe* **3**, e52–e61 (2022).
- 1220 26. Goldblatt, D., Alter, G., Crotty, S. & Plotkin, S. A. Correlates of protection against SARS-
1221 CoV-2 infection and COVID-19 disease. *Immunol. Rev.* **310**, 6–26 (2022).
- 1222 27. Khoury, D. S. *et al.* Neutralizing antibody levels are highly predictive of immune protection
1223 from symptomatic SARS-CoV-2 infection. *Nat. Med.* **27**, 1205–1211 (2021).
- 1224 28. McMahan, K. *et al.* Correlates of protection against SARS-CoV-2 in rhesus macaques.
1225 *Nature* **590**, 630–634 (2021).
- 1226 29. Corbett, K. S. *et al.* Immune correlates of protection by mRNA-1273 vaccine against SARS-
1227 CoV-2 in nonhuman primates. *Science* (2021) doi:10.1126/science.abj0299.
- 1228 30. Cho, A. *et al.* Anti-SARS-CoV-2 receptor binding domain antibody evolution after mRNA
1229 vaccination. *Nature* (2021) doi:10.1038/s41586-021-04060-7.
- 1230 31. Robbiani, D. F. *et al.* Convergent antibody responses to SARS-CoV-2 in convalescent
1231 individuals. *Nature* (2020) doi:10.1038/s41586-020-2456-9.
- 1232 32. Barnes, C. O. *et al.* SARS-CoV-2 neutralizing antibody structures inform therapeutic
1233 strategies. *Nature* **588**, 682–687 (2020).
- 1234 33. Amanat, F. *et al.* Murine monoclonal antibodies against the receptor binding domain of
1235 SARS-CoV-2 neutralize authentic wild-type SARS-CoV-2 as well as B.1.1.7 and B.1.351
1236 viruses and protect in vivo in a mouse model in a neutralization-dependent manner. *MBio*
1237 **12**, e0100221 (2021).
- 1238 34. Hastie, K. M. *et al.* Defining variant-resistant epitopes targeted by SARS-CoV-2 antibodies:
1239 A global consortium study. *Science* **374**, 472–478 (2021).
- 1240 35. Starr, T. N. *et al.* Deep mutational scans for ACE2 binding, RBD expression, and antibody
1241 escape in the SARS-CoV-2 Omicron BA.1 and BA.2 receptor-binding domains. *PLoS*
1242 *Pathog.* **18**, e1010951 (2022).
- 1243 36. Starr, T. N. *et al.* Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain
1244 Reveals Constraints on Folding and ACE2 Binding. *Cell* **182**, 1295-1310.e20 (2020).
- 1245 37. McCallum, M. *et al.* Molecular basis of immune evasion by the Delta and Kappa SARS-
1246 CoV-2 variants. *Science* eab18506 (2021).
- 1247 38. Kodaka, M. *et al.* A new cell-based assay to evaluate myogenesis in mouse myoblast
1248 C2C12 cells. *Exp. Cell Res.* **336**, 171–181 (2015).

- 1249 39. Meng, B. *et al.* SARS-CoV-2 spike N-terminal domain modulates TMPRSS2-dependent
1250 viral entry and fusogenicity. *Cell Rep.* **40**, 111220 (2022).
- 1251 40. Lan, J. *et al.* Structure of the SARS-CoV-2 spike receptor-binding domain bound to the
1252 ACE2 receptor. *Nature* (2020) doi:10.1038/s41586-020-2180-5.
- 1253 41. Wang, Q. *et al.* Alarming antibody evasion properties of rising SARS-CoV-2 BQ and XBB
1254 subvariants. *Cell* (2022) doi:10.1016/j.cell.2022.12.018.
- 1255 42. Pinto, D. *et al.* Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV
1256 antibody. *Nature* **583**, 290–295 (2020).
- 1257 43. Park, Y.-J. *et al.* Imprinted antibody responses against SARS-CoV-2 Omicron sublineages.
1258 *Science* eadc9127 (2022).
- 1259 44. Stalls, V. *et al.* Cryo-EM structures of SARS-CoV-2 Omicron BA.2 spike. *Cell Rep.* **39**,
1260 111009 (2022).
- 1261 45. Cao, Y. *et al.* BA.2.12.1, BA.4 and BA.5 escape antibodies elicited by Omicron infection.
1262 *Nature* **608**, 593–602 (2022).
- 1263 46. Planas, D. *et al.* Resistance of Omicron subvariants BA.2.75.2, BA.4.6 and BQ.1.1 to
1264 neutralizing antibodies. *bioRxiv* (2022) doi:10.1101/2022.11.17.516888.
- 1265 47. Yue, C. *et al.* Enhanced transmissibility of XBB.1.5 is contributed by both strong ACE2
1266 binding and antibody evasion. *bioRxiv* (2023) doi:10.1101/2023.01.03.522427.
- 1267 48. Starr, T. N. *et al.* SARS-CoV-2 RBD antibodies that maximize breadth and resistance to
1268 escape. *Nature* (2021) doi:10.1038/s41586-021-03807-6.
- 1269 49. Driouich, J.-S., Bernardin, O., Touret, F., de Lamballerie, X. & Nougairède, A. In vivo
1270 activity of Sotrovimab against BQ.1.1 Omicron sublineage. *bioRxiv* (2023)
1271 doi:10.1101/2023.01.04.522629.
- 1272 50. Carreño, J. M. *et al.* Evidence for retained spike-binding and neutralizing activity against
1273 emerging SARS-CoV-2 variants in serum of COVID-19 mRNA vaccine recipients.
1274 *EBioMedicine* **73**, 103626 (2021).
- 1275 51. Quandt, J. *et al.* Omicron BA.1 breakthrough infection drives cross-variant neutralization
1276 and memory B cell formation against conserved epitopes. *Sci. Immunol.* **7**, eabq2427
1277 (2022).
- 1278 52. Muecksch, F. *et al.* Increased memory B cell potency and breadth after a SARS-CoV-2
1279 mRNA boost. *Nature* **607**, 128–134 (2022).
- 1280 53. Wang, Z. *et al.* Naturally enhanced neutralizing breadth against SARS-CoV-2 one year
1281 after infection. *Nature* **595**, 426–431 (2021).
- 1282 54. Muecksch, F. *et al.* Affinity maturation of SARS-CoV-2 neutralizing antibodies confers
1283 potency, breadth, and resilience to viral escape mutations. *Immunity* **54**, 1853-1868.e7
1284 (2021).
- 1285 55. Marzi, R. *et al.* Maturation of SARS-CoV-2 Spike-specific memory B cells drives resilience
1286 to viral escape. *iScience* **26**, 105726 (2023).
- 1287 56. Gaebler, C. *et al.* Evolution of antibody immunity to SARS-CoV-2. *Nature* **591**, 639–644
1288 (2021).
- 1289 57. Zhang, Z. *et al.* Humoral and cellular immune memory to four COVID-19 vaccines. *Cell*
1290 **185**, 2434-2451.e17 (2022).
- 1291 58. Turner, J. S. *et al.* SARS-CoV-2 mRNA vaccines induce persistent human germinal centre
1292 responses. *Nature* **596**, 109–113 (2021).

- 1293 59. Dan, J. M. *et al.* Immunological memory to SARS-CoV-2 assessed for up to 8 months after
1294 infection. *Science* **371**, eabf4063 (2021).
- 1295 60. Markov, P. V., Katzourakis, A. & Stilianakis, N. I. Antigenic evolution will lead to new SARS-
1296 CoV-2 variants with unpredictable severity. *Nat. Rev. Microbiol.* **20**, 251–252 (2022).
- 1297 61. Kemp, S. A. *et al.* SARS-CoV-2 evolution during treatment of chronic infection. *Nature* **592**,
1298 277–282 (2021).
- 1299 62. Sette, A. & Crotty, S. Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell* **184**, 861–880
1300 (2021).
- 1301 63. Zhang, A. *et al.* Beyond neutralization: Fc-dependent antibody effector functions in SARS-
1302 CoV-2 infection. *Nat. Rev. Immunol.* (2022) doi:10.1038/s41577-022-00813-1.
- 1303 64. Kaplonek, P. *et al.* mRNA-1273 and BNT162b2 COVID-19 vaccines elicit antibodies with
1304 differences in Fc-mediated effector functions. *Sci. Transl. Med.* **14**, eabm2311 (2022).
- 1305 65. Kaplonek, P. *et al.* mRNA-1273 vaccine-induced antibodies maintain Fc effector functions
1306 across SARS-CoV-2 variants of concern. *Immunity* **55**, 355–365.e4 (2022).
- 1307 66. Feng, S. *et al.* Correlates of protection against symptomatic and asymptomatic SARS-CoV-
1308 2 infection. *Nat. Med.* **27**, 2032–2040 (2021).
- 1309 67. Case, J. B. *et al.* Resilience of S309 and AZD7442 monoclonal antibody treatments against
1310 infection by SARS-CoV-2 Omicron lineage strains. *Nat. Commun.* **13**, 3824 (2022).
- 1311 68. Zheng, B. *et al.* Comparative effectiveness of sotrovimab and molnupiravir for prevention of
1312 severe covid-19 outcomes in patients in the community: observational cohort study with the
1313 OpenSAFELY platform. *BMJ* **379**, e071932 (2022).
- 1314 69. Cheng, M. M. *et al.* Real-world effectiveness of sotrovimab for the early treatment of
1315 COVID-19 during SARS-CoV-2 Delta and Omicron waves in the USA. *Infect. Dis. Ther.*
1316 (2023) doi:10.1007/s40121-022-00755-0.
- 1317 70. Martin-Blondel, G. *et al.* Sotrovimab to prevent severe COVID-19 in high-risk patients
1318 infected with Omicron BA.2. *J. Infect.* **85**, e104–e108 (2022).
- 1319 71. Harman, K. *et al.* Comparison of the risk of hospitalisation among BA.1 and BA.2 COVID-
1320 19 cases treated with Sotrovimab in the community in England. *bioRxiv* (2022)
1321 doi:10.1101/2022.10.21.22281171.
- 1322 72. Young-Xu, Y. *et al.* Effectiveness of sotrovimab in preventing COVID-19-related
1323 hospitalizations or deaths among U.s. veterans. *bioRxiv* (2022)
1324 doi:10.1101/2022.12.30.22284063.
- 1325 73. Bartsch, Y. C. *et al.* Omicron variant Spike-specific antibody binding and Fc activity are
1326 preserved in recipients of mRNA or inactivated COVID-19 vaccines. *Sci. Transl. Med.* **14**,
1327 eabn9243 (2022).
- 1328 74. Richardson, S. I. *et al.* Antibody-dependent cellular cytotoxicity against SARS-CoV-2
1329 Omicron sub-lineages is reduced in convalescent sera regardless of infecting variant. *Cell*
1330 *Rep. Med.* 100910 (2022).
- 1331 75. Mackin, S. R. *et al.* Fcy receptor-dependent antibody effector functions are required for
1332 vaccine protection against infection by antigenic variants of SARS-CoV-2. *bioRxivorg*
1333 (2022) doi:10.1101/2022.11.27.518117.
- 1334 76. Bergwerk, M. *et al.* Covid-19 breakthrough infections in vaccinated health care workers. *N.*
1335 *Engl. J. Med.* **385**, 1474–1484 (2021).

- 1336 77. Francis, T. On the doctrine of original antigenic sin. *Proc Am Philos Soc* **104**, 572–578
1337 (1960).
- 1338 78. Corti, D. *et al.* A neutralizing antibody selected from plasma cells that binds to group 1 and
1339 group 2 influenza A hemagglutinins. *Science* **333**, 850–856 (2011).
- 1340 79. Wrammert, J. *et al.* Broadly cross-reactive antibodies dominate the human B cell response
1341 against 2009 pandemic H1N1 influenza virus infection. *J. Exp. Med.* **208**, 181–193 (2011).
- 1342 80. Cheung, C. S.-F. *et al.* Identification and structure of a multidonor class of head-directed
1343 influenza-neutralizing antibodies reveal the mechanism for its recurrent elicitation. *Cell Rep.*
1344 **32**, 108088 (2020).
- 1345 81. Nachbagauer, R. *et al.* A chimeric hemagglutinin-based universal influenza virus vaccine
1346 approach induces broad and long-lasting immunity in a randomized, placebo-controlled
1347 phase I trial. *Nat. Med.* **27**, 106–114 (2021).
- 1348 82. Walls, A. C. *et al.* Elicitation of broadly protective sarbecovirus immunity by receptor-
1349 binding domain nanoparticle vaccines. *Cell* (2021) doi:10.1016/j.cell.2021.09.015.
- 1350 83. Walls, A. C. *et al.* Elicitation of Potent Neutralizing Antibody Responses by Designed
1351 Protein Nanoparticle Vaccines for SARS-CoV-2. *Cell* **183**, 1367-1382.e17 (2020).
- 1352 84. Martinez, D. R. *et al.* Chimeric spike mRNA vaccines protect against Sarbecovirus
1353 challenge in mice. *Science* (2021) doi:10.1126/science.abi4506.
- 1354 85. Cohen, A. A. *et al.* Mosaic RBD nanoparticles protect against challenge by diverse
1355 sarbecoviruses in animal models. *Science* **377**, eabq0839 (2022).
- 1356 86. Cohen, A. A. *et al.* Mosaic nanoparticles elicit cross-reactive immune responses to zoonotic
1357 coronaviruses in mice. *Science* **371**, 735–741 (2021).
- 1358 87. Li, D. *et al.* Breadth of SARS-CoV-2 neutralization and protection induced by a nanoparticle
1359 vaccine. *Nat. Commun.* **13**, 6309 (2022).
- 1360 88. González-Domínguez, I. *et al.* Trivalent NDV-HXP-S vaccine protects against
1361 phylogenetically distant SARS-CoV-2 variants of concern in mice. *Microbiol. Spectr.* **10**,
1362 e0153822 (2022).
- 1363 89. Sano, K. *et al.* SARS-CoV-2 vaccination induces mucosal antibody responses in previously
1364 infected individuals. *Nat. Commun.* **13**, 5135 (2022).
- 1365 90. Mao, T. *et al.* Unadjuvanted intranasal spike vaccine elicits protective mucosal immunity
1366 against sarbecoviruses. *Science* **378**, eabo2523 (2022).
- 1367 91. Hassan, A. O. *et al.* An intranasal vaccine durably protects against SARS-CoV-2 variants in
1368 mice. *Cell Rep.* **36**, 109452 (2021).
- 1369
1370