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## **Broadly potent anti-SARS-CoV-2 antibody shares 93% of epitope with ACE2 and provides full protection in monkeys**

Craig Fenwick, Priscilla Turelli, Yoan Duhoo, Kelvin Lau, Cécile Herate, Romain Marlin, Myriam Lamrayah, Jérémy Campos, Line Esteves-Leuenberger, Alex Farina, et al.

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1 *Title:* Broadly potent anti-SARS-CoV-2 antibody shares 93% of epitope with ACE2 and  
2 provides full protection in monkeys

3 *Running title:* Potent antibody protects from XBB.1.5 infection in NHPs

4 Craig Fenwick,<sup>a,†</sup> Priscilla Turelli,<sup>b,†</sup> Yoan Duhoo,<sup>c,‡</sup> Kelvin Lau,<sup>b,‡</sup> Cécile Herate,<sup>d,‡</sup> Romain Marlin,<sup>d,‡</sup>  
5 Myriam Lamrayah,<sup>b,‡</sup> Jérémy Campos,<sup>a</sup> Line Esteves-Leuenberger,<sup>a</sup> Alex Farina,<sup>a</sup> Charlène Raclot,<sup>b</sup>  
6 Vanessa Genet,<sup>b</sup> Flurin Fiscalini,<sup>a</sup> Julien Cesborn,<sup>a</sup> Laurent Perez,<sup>a</sup> Nathalie Dereuddre-Bosquet,<sup>d</sup>  
7 Vanessa Contreras,<sup>d</sup> Kyllian Lheureux,<sup>d</sup> Francis Relouzat,<sup>d</sup> Rana Abdelnabi,<sup>e</sup> Pieter Leyssen,<sup>e</sup> Yves  
8 Lévy,<sup>f,g,h</sup> Florence Pojer,<sup>b</sup> Roger Le Grand,<sup>d</sup> Didier Trono,<sup>b,\*</sup> Giuseppe Pantaleo,<sup>a,i,\*</sup>

9

- 10 a- Service of Immunology and Allergy, Department of Medicine, Lausanne University Hospital  
11 and University of Lausanne, Lausanne, Switzerland  
12 b- School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.  
13 c- School of Basic Sciences, Ecole Polytechnique Fédérale de Lausanne and Faculty of Biology  
14 and Medicine, UNIL, Lausanne, Switzerland.  
15 d- CEA, Université Paris Sud 11, INSERM U1184, Center for Immunology of Viral Infections and  
16 Autoimmune Diseases, IDMIT Department, IBFJ, Fontenay-aux-Roses, France.  
17 e- KU Leuven Department of Microbiology, Immunology and Transplantation, Rega Institute for  
18 Medical Research, Laboratory of Virology and Chemotherapy, B-3000 Leuven, Belgium.  
19 f- VRI, Université Paris-Est Créteil, Faculté de Médecine, INSERM U955, 94010 Créteil, France.  
20 g- Inserm U955, Equipe 16, Créteil, France.  
21 h- AP-HP, Hôpital Henri-Mondor Albert-Chenevier, Service d'Immunologie Clinique et Maladies  
22 Infectieuses, Créteil, France.  
23 i- Swiss Vaccine Research Institute, Lausanne University Hospital and University of Lausanne,  
24 Switzerland.  
25

26 *Key words:* SARS-CoV-2, neutralizing antibodies, Variants of concern, Omicron, ACE2 mimetic

27 *Highlights:*

- 28 • Anti-SARS-CoV-2 neutralizing antibody with ACE2 mimetic binding properties  
29 • Broad-spectrum activity against all past and current SARS-CoV-2 variants of concern  
30 • Mutations escaping mAb activity bind ACE2 poorly and are rare in circulating virus  
31 • Sterilizing protection from XBB.1.5 infection in an NHP challenge model  
32

33 †These authors contributed equally and ‡ authors contributed equally.

34 \*Lead and co-corresponding authors: Prof. Giuseppe Pantaleo  
35 Service of Immunology and Allergy  
36 Lausanne University Hospital  
37 1011 Lausanne, Switzerland  
38 giuseppe.pantaleo@chuv.ch

39 Prof. Didier Trono  
40 School of Life Sciences  
41 Ecole Polytechnique Fédérale de Lausanne  
42 Lausanne, Switzerland  
43 didier.trono@epfl.ch  
44

45

46 **Abstract**

47 *Objectives:* Due to the rapid evolution of SARS-CoV-2 to variants with reduced sensitivity to  
48 vaccine-induced humoral immunity and the near complete loss of protective efficacy of licensed  
49 therapeutic monoclonal antibodies, we isolated a potent, broad-spectrum neutralizing antibody  
50 that could potentially provide prophylactic protection to immunocompromised patient  
51 populations.

52 *Methods:* Spike-specific B-cell clones isolated from a vaccinated post-infected donor were  
53 profiled for those producing potent neutralizing antibodies against a panel of SARS-CoV-2  
54 variants. The P4J15 antibody was further characterized to define the structural binding epitope,  
55 viral resistance and *in vivo* efficacy.

56 *Results:* The P4J15 mAb shows <20 ng/ml neutralizing activity against all variants including  
57 the latest XBB.2.3 and EG.1 sub-lineages. Structural studies of P4J15 in complex with Omicron  
58 XBB.1 Spike show that the P4J15 epitope shares ~93% of its buried surface area with the ACE2  
59 contact region, consistent with an ACE2 mimetic antibody. *In vitro* selection of SARS-CoV-2  
60 mutants escaping P4J15 neutralization showed reduced infectivity, poor ACE2 binding, and  
61 mutations are rare in public sequence databases. Using a SARS-CoV-2 XBB.1.5 monkey  
62 challenge model, P4J15-LS confers complete prophylactic protection with an exceptionally  
63 long *in vivo* half-life of 43 days.

64 *Conclusions:* The P4J15 mAb has potential as a broad-spectrum anti-SARS-CoV-2 drug for  
65 prophylactic protection of at-risk patient populations.

66

67 **MAIN**

68 The SARS-CoV-2 virus which lead to the global COVID-19 pandemic is responsible for >767  
69 million confirmed infections and almost 7 million fatalities worldwide (WHO Coronavirus  
70 (COVID-19) Dashboard <https://covid19.who.int/>) (1). Enormous efforts by the scientific and  
71 medical communities in vaccine, antiviral drugs and monoclonal antibody development have  
72 allowed most people to return to normal lives after the peak of the pandemic, typified in most  
73 parts of the world by lockdowns, isolation, and overwhelmed health care networks. However,  
74 these hard-fought victories are being eroded by the continued regional and international spread  
75 of variants of concern (VOC), which are both more transmissible and more resistant to immune  
76 responses (2-4). SARS-CoV-2 infection leading to COVID-19 disease is of increased concern

77 due to the rapidly waning immunity in the general population, the apathy that has developed  
78 for receiving vaccine boosts and the reduced levels of neutralizing antibodies generated by even  
79 the most recent bivalent vaccines (which include the ancestral SAR-CoV-2 and Omicron BA.1  
80 or BA.4/5 Spike) against current VOCs (5). Alarming, these factors contribute to SARS-CoV-  
81 2 infection being a leading cause of death in children and adolescents up to 19 years of age,  
82 accounting for 2% of all deaths in this age group in the year prior to August 2022 (6). The  
83 greatest unmet medical need is the >30 million immunocompromised individual in the US and  
84 Europe alone that are at high risk of infection due to their inability to mount a protective  
85 humoral immune response following vaccination (7, 8). These at-risk individuals include  
86 people with blood and immune cell cancers, transplantation patients and recipients of  
87 immunosuppressive drugs, all of which account for >40% of hospitalizations with breakthrough  
88 SARS-CoV-2 infections (9, 10). Since the emergence of the BQ.1, BQ.1.1 and XBB.1 lineages  
89 in the fall of 2022, all authorized therapeutic mAbs have become almost completely ineffective,  
90 including Evusheld, the combination of tixagevimab (AZD8895) and cilgavimab (AZD1061)  
91 mAbs that was designed for therapeutic and prophylactic purposes (11). Although there are  
92 recent reports in the literature identifying mAbs with some breadth of neutralizing activity, most  
93 are significantly less potent against the circulating variants than antibodies that previously  
94 demonstrated protection in the clinic (12-15). Furthermore, the SARS-CoV-2 virus will  
95 continue to evolve due to both the tremendous pool of circulating viruses and selective pressures  
96 exerted by immune responses present in the general population, necessitating that new mAbs  
97 be developed to counter currently circulating variants and anticipate future adaptations. Here  
98 we report the isolation of the human mAb, P4J15, that binds the Spike receptor binding domain  
99 (RBD) and through blocking ACE2 receptor binding, exerts a potent neutralizing activity  
100 against all current SARS-CoV-2 variants. Structural studies reveal that this broad activity can  
101 be attributed to the high binding surface area of the P4J15 epitope, which includes residues  
102 essential for efficient ACE2 binding and infection. Live virus resistance studies confirmed that  
103 P4J15 escape mutants selected in cell culture were poorly infectious, owing to RBD mutations  
104 strongly reducing affinity for the ACE2 receptor. Accordingly, the corresponding escape  
105 mutations were found only at low frequency in the GISAID sequence database, confirming their  
106 detrimental effect on virus fitness. Finally, P4J15 conferred near complete protection from  
107 infection in hamster and monkey live virus challenge studies performed with Omicron BA.5  
108 and XBB.1.5 variants, respectively. With this demonstrated *in vivo* efficacy against the most  
109 recent variants, neutralizing potency and breadth of protection, we propose that P4J15 could be  
110 a strong candidate for clinical development.

111 **RESULTS**

112 **P4J15 is a potent and broadly neutralizing anti-SARS-CoV-2 monoclonal antibody**

113 As part of a longitudinal study to monitor the waning humoral immune response in a cohort of  
114 20 donors, we performed routine serum testing of anti-Spike and anti-Nucleocapsid antibodies.  
115 One donor with weak neutralizing antibody levels consistent with prior SARS-CoV-2 infection,  
116 received two doses of the mRNA-1273 vaccine in late 2021 and became SARS-CoV-2 positive  
117 four weeks later during the Omicron BA.1 wave. Two months later, this donor had among the  
118 highest serum antibody levels in all donors tested, with excellent breadth of neutralization  
119 against Omicron BA.1 and a panel of other pre- and post- Omicron SARS-CoV-2 variants in a  
120 trimeric Spike-ACE2 surrogate neutralization assay (16). To focus our mAb screening efforts,  
121 we sorted Omicron BA.1 Spike-binding memory B cells and identified a panel of 16 Spike  
122 binding antibodies by profiling antibody supernatants from B cell clones. The P4J15 mAb,  
123 produced by expression of paired heavy and light chains in ExpiCHO cells, showed the highest  
124 affinity for the SARS-CoV-2 ancestral, Alpha, Beta, Gamma, Delta, Omicron BA.1, BA.1.1,  
125 BA.2, BA.2.75.2, BQ.1, BQ.1.1, XBB.1 and the RaTG13 sarbecovirus Spike trimer proteins,  
126 while showing only low levels of binding to SARS-CoV-1 Spike (**Supplementary Data Fig.**  
127 **1a-b**). Profiling studies in a Luminex Spike binding assay showed that the purified P4J15 mAbs  
128 bound SARS-CoV-2 Spike proteins in our panel with IC<sub>50</sub> values ranging from 0.6 to 1.5 ng/ml  
129 (**Supplementary Data Fig. 1b**) and RaTG13 Spike with an IC<sub>50</sub> of 11 ng/ml. Using our Spike-  
130 ACE2 surrogate neutralization assay, we also found that P4J15 had the most potent and broadest  
131 activity in blocking ACE2-binding to Spike trimers from our extensive panel of SARS-CoV-2  
132 variants, with IC<sub>50</sub> values below 5 ng/ml (**Supplementary Data Fig. 2a-b**). These studies  
133 indicate that P4J15 has a superior affinity in binding and Spike-ACE2 blocking profile  
134 compared to a panel of approved or clinically advanced anti-SARS-CoV-2 mAbs including  
135 AZD8895 and AZD1061 from AstraZeneca (17), ADG-2 from Adagio (18), bebtelovimab from  
136 Eli Lilly (19) and S309/sotrovimab from Vir/GSK(20).

137 Cross-competitive Spike trimer binding studies performed with our panel of comparator anti-  
138 SARS-CoV-2 mAbs and P2G3/P5C3 mAbs previously described by our group (21, 22)  
139 revealed that P4J15 binds an overlapping epitope with AZD8895 and P5C3 class 1 mAbs,  
140 although neither of these mAbs identified early in the pandemic significantly binds to post-  
141 BA.4/5 Omicron variants. No cross-competitive binding was observed between P4J15 and the  
142 class 3 mAbs sotrovimab, bebtelovimab and P2G3, whereas the class 4 mAb ADG-2 showed

143 mixed competition results with P4J15, depending on which antibody was bound to Spike first.  
144 **(Supplementary Data Fig. 3).**

### 145 **P4J15 outperforms clinically relevant antibodies in pseudovirus-based neutralization** 146 **assays**

147 We next evaluated the activity of P4J15 compared with a panel of clinically approved mAbs in  
148 pseudotyped lentiviral and SARS-CoV-2 virus-like particle (VLP) cell-based neutralization  
149 assays. P4J15 demonstrated potent neutralizing activity against lentiviruses pseudotyped with  
150 Spike from the ancestral 2019-nCoV (D614G), Alpha, Beta, Delta, Omicron BA.1, BA.4/5 and  
151 BA.2.75.2 VOCs with EC<sub>50</sub> values of 41, 14, 19, 16, 9, 5 and 9 ng/ml, respectively (**Fig. 1a-b**).  
152 In contrast to all other reference antibodies, P4J15 strongly neutralized the Omicron BQ.1,  
153 BQ.1.1, XBB.1 Spike pseudoviruses with an EC<sub>50</sub> values of 6, 9 and 14 ng/ml, respectively  
154 (**Fig. 1b-c**) and showed no significant loss of activity as compared to the other VOCs. In parallel  
155 testing, P4J15 was 13- to 300-fold more potent than sotrovimab, while the Evusheld dual  
156 combination of neutralizing mAbs, and bebtelovimab were almost inactive against BQ.1.1 and  
157 XBB.1 VOCs with EC<sub>50s</sub> >8700 ng/ml. As Spike is incorporated at the plasma membrane in  
158 pseudotyped lentivectors and in the ER-Golgi intermediate compartment (ERGIC) in SARS-  
159 CoV-2 viruses, we decided to use a SARS-CoV-2-based particle (VLPs) as a second system to  
160 profile P4J15 efficacy. These VLP-based assays confirmed results obtained in the lentiviral  
161 assay pseudotyped with Spike from Delta BA.1, BA.4/5, BA.2.75.2, BQ.1, BQ.1.1 and XBB.1  
162 (EC<sub>50</sub> values of 15, 2, 4, 10, 10, 13 and 13 ng/ml, respectively) (**Fig. 1b** and **1d**). VLP  
163 neutralizing assays also revealed that P4J15 retained full neutralizing activity against the latest  
164 circulating Omicron sub-lineages XBB.1.5, CH.1.1, XBB.1.16, XBB.1.16.1, XBB.2.3 and  
165 EG.1 with EC<sub>50</sub> values of 6, 10, 9, 13 18, and 16 ng/ml (**Fig. 1e** with results summarized in **Fig.**  
166 **1b**).

167

### 168 **Cryo-electron microscopy structure of P4J15 Fab in complex with the Spike trimer**

169 To understand the structural basis of P4J15 potent neutralization of SARS-CoV-2 variants of  
170 concern, the complex formed by Omicron XBB.1 SARS-CoV-2 Spike trimer and P4J15 Fab  
171 fragments was characterized using single particle cryo-electron microscopy (cryo-EM). The  
172 single particle cryo-EM reconstruction of the Omicron XBB.1 Spike trimeric ectodomain  
173 bound to the Fab at a 3.01 Å resolution and the P4J15 Fab binding RBD in the up- or open-  
174 conformation (**Fig. 2a** and **Supplementary data Fig. 4a-e** and **6**). P4J15 binds RBD with a

175 buried surface area of approximately 828 Å<sup>2</sup> as a Class 1 neutralizing mAb, recognizing an  
176 epitope on the SARS-CoV-2 RBD that largely overlaps with the ACE2 receptor binding motif.  
177 To characterize the P4J15 paratope and epitope interface in detail, we performed local  
178 refinement of the P4J15 Fab-RBD interacting region and reached a resolution of 3.85 Å with  
179 well-defined density, allowing clear interpretation of sidechain positions at the interface. We  
180 also used EMReady (23), a deep learning tool, to enhance the quality even further (**Fig. 2b-c**  
181 **and Supplementary data Fig. 4-5**). The P4J15 paratope interactions are mediated mainly  
182 through electrostatic and hydrophobic contacts and involve 26 residues of the RBD, bound by  
183 the three heavy chain CDRs, two light chain CDRs and residues of the heavy chain Frame  
184 region 3 (FRH3) of the P4J15 mAb. The CDRH1, CDRH2 and FRH3 loops of P4J15 (**Fig. 2d-**  
185 **f and Supplementary data Fig. 7-8**) form contacts with the Gly447-Phe456 and Gly485-  
186 His505 saddle-like region of the RBD that encompasses the β5 and β6 antiparallel beta sheet of  
187 the ACE2 binding region (**Fig. 2e**). CDRH3 sits upon the RBD Arg454-Lys458 loop and  
188 together with the CDRL1 and CDRL3 forms additional contacts with the Tyr473-Lys478 and  
189 Ser486-Tyr489 of the ACE2 interface region (**Fig. 2f**). These contact residues are further  
190 illustrated in **Figure 2g** with a structural model of the RBD viewed from above and the P4J15  
191 contact buried surface on the RBD shaded in dark grey and compared to the ACE2 contact  
192 residues. It is important to underscore that 22 of these P4J15 contact residues on the RBD are  
193 shared with key contacts formed between the RBD (**Fig. 2g**) and the ACE2 receptor, which is  
194 essential for virus binding and infection of target cells. The common area on the RBD for these  
195 22 residues that contact P4J15 and ACE2 is on average 774 Å<sup>2</sup>, which is almost 93% of the  
196 P4J15 epitope and 87% of the 887 Å<sup>2</sup> contact area with ACE2. Based on these observations,  
197 we propose that P4J15 may act as an ACE2 mimetic antibody and that it will be difficult for  
198 the virus to develop resistance mutations that completely disrupt the P4J15-RBD interaction  
199 without adversely affecting the ACE2-RBD interaction.

200

### 201 **P4J15 viral escape mutations have reduced infectivity and ACE2 binding**

202 To gain insight into the predicted clinical value of P4J15 and variant residues in the SARS-  
203 CoV-2 Spike that may affect the mAbs neutralizing activity, we characterized the emergence  
204 of escape mutants in live virus tissue culture studies. For this, we grew SARS-CoV-2 Omicron  
205 BA.2.75.2 and Omicron BQ.1 variants in the presence of sub-optimal neutralizing doses of  
206 P4J15 for three passages to generate a heterogeneous viral population, before switching to  
207 stringent mAb concentrations in order to select authentic escapees (**Fig. 3a**). Viral genome

208 sequencing of these mAb-resistant mutants pointed to the importance of Spike substitutions  
209 F456S, A475D, G476D, N477D/K, N487S/D/K escaping P4J15 in the BA.2.75.2 selection  
210 studies and G476D, N487S/T/D/K, Y489H substitutions identified with BQ.1 virus studies  
211 (**Fig. 3b**). The identified mutations were then generated by site-directed mutagenesis in a Spike  
212 BA.2.75.2 and Spike BQ.1 expression vectors and used to generate mutant Spike virus-like  
213 particles (VLPs), allowing us to measure the impact of these mutations on the neutralizing  
214 capacity of P4J15 and on viral infectivity. Spike mutations that conferred a near complete loss  
215 of neutralizing activity in the BA.2.75.2 VLP assay were F456S, A475D, G476D, N487D,  
216 N487K, and N487T while N477D, N477K and N487S conferred a 14- to 29-fold loss of activity  
217 (**Fig. 3c**). Similarly, in the BQ.1 Spike VLP assay, G476D, N487D, N487K, and N487T  
218 induced resistance to neutralization by P4J15 along with the Y489H mutation, while the N487S  
219 mutation conferred only partial resistance (**Fig. 3c**). However, the infectivity of select VLPs  
220 was reduced with Spike proteins harbouring many of the escapee mutations (**Fig. 3d**) including  
221 A475D and N487D in the BA.2.75.2 Spike and N487D, N487K and N487T in BQ.1 Spike.  
222 Furthermore, using the ACE2-RBD interactive tool developed by Jesse Bloom's lab(24), it was  
223 found that all the Spike escape mutations in RBD induced a strong, 1- to 3-log reduction in  
224 binding affinity for ACE2 relative to the Omicron BA.2 Spike reference strain (**Fig. 3e**). The  
225 reduced binding affinity of ACE2 for the Spike RBD escape mutations was expected based on  
226 our structural data in **Figure 2e-g**, as these residues are important for both P4J15 and ACE2  
227 binding to the RBD. Furthermore, we examined the GISAID EpiCoV database to determine the  
228 frequency of the Spike mutations mediating escape to P4J15 neutralization. They were all found  
229 to be extremely rare and present in less than 0.0051% of the >15'6 million sequences deposited  
230 as of June 2023 (**Fig 3c**). Therefore, escape mutations to P4J15 are only present at very low  
231 frequency in viruses isolated from infected individuals, consistent with the marked reduction in  
232 infectivity and/or ACE2 binding measured *in vitro* for the corresponding viruses and Spike  
233 proteins (**Fig. 3d-e**). Finally, to confirm the barrier to P4J15 viral resistance imposed by the  
234 obligatory Spike-ACE2 interaction, we bioinformatically identified the rare but most common  
235 amino acid substitutions at positions identified at the P4J15-RBD contact site and in our  
236 resistance studies. As shown in **Figure 3f**, with substitutions made to the Omicron BA.5 or  
237 BQ.1 Spike, none of the mutations tested, including N417D, V445D, G446D, N450D, L455F,  
238 F456L, K458H, S459P, A475V, G476S, N477G, T478R, G485D, P491S, S494P and G504D  
239 that are within or adjacent to the P4J15-RBD contact site, significantly reduced the neutralizing  
240 potency of P4J15. These studies support the hypothesis that the large, buried surface area bound



241 by P4J15 translates into the antibody's ability to lose some of these contacts without affecting  
242 the overall binding and neutralizing properties.

243

#### 244 **Prophylactic use of P4J15 in the hamster Omicron BA.5 infection model**

245 To further validate the potency of P4J15, *in vivo* live virus challenge experiments were  
246 performed in a prophylactic hamster challenge model of SARS-CoV-2 infection. Animals were  
247 dosed with 5, 1 or 0.5 mg/kg of P4J15, 5 mg/kg of bebtelovimab or a human IgG1 control,  
248 challenged two days later with an intranasal inoculation of the Omicron BA.5 SARS-CoV-2  
249 virus (**Fig. 4a**) and lung tissue from the hamsters were examined four days later for infectious  
250 virus and viral RNA. Infectious virus was undetectable in the lungs of all but one P4J15 treated  
251 hamsters in the lowest dose 0.5 mg/kg group, which still had a >2-log reduction in levels of  
252 infectious virus compared to the isotype mAb-treated control animals (**Fig. 4b**). In comparison,  
253 1 out of 5 hamsters in the 5 mg/kg bebtelovimab group had detectable levels of infectious virus.  
254 Importantly, protective plasma levels of P4J15 in Omicron BA.5 challenged hamsters were  
255 shown to be ~7 µg/ml, whereas in the bebtelovimab arm of the study, the one treated animal  
256 with detectable infection virus in the lungs had mAb plasma levels of 83 µg/ml. Interestingly,  
257 although all P4J15 treatment groups showed a significant reduction in genomic RNA levels,  
258 relatively high levels were detected in two and three hamsters for the 1 and 0.5 mg/kg dose  
259 arms (**Fig. 4c**). This indicates that although P4J15 treatments virtually eliminated infectious  
260 virus, RNA, presumably from inactivated virus, was still detectable in select animals four days  
261 after challenge.

262

#### 263 **P4J15 shows full prophylactic therapeutic efficacy in cynomolgus macaques**

264 We next evaluated P4J15 LS with M428L/N434S half-life extension mutations in the Fc  
265 domain in mediating protection from live SARS-CoV-2 Omicron XBB.1.5 virus infection in a  
266 pre-exposure challenge study in cynomolgus macaques. Non-human primates (NHP, n=6) were  
267 administered 5 mg/kg of P4J15 intravenously and challenged 72 hours later with  $1 \times 10^5$  TCID<sub>50</sub>  
268 of SARS-CoV-2 Omicron XBB.1.5 virus via a combined intranasal and intratracheal route (**Fig.**  
269 **5a**). Following viral challenge, control animals (n=4, tested in parallel and n=2 historical  
270 controls) showed similar genomic (g)RNA levels and kinetics with median peak viral loads  
271 (VL) of 7.3, 7.1 and 6.4-log<sub>10</sub> copies/ml gRNA in nasopharyngeal swabs, tracheal swabs and  
272 bronchoalveolar lavage (BAL) samples, respectively, at 2-3 days post challenge (**Fig. 5b-d**).

273 In comparison, the six P4J15 LS treated NHPs had essentially undetectable levels of gRNA at  
274 all sample and time points tested. This complete protection provided by P4J15 LS was further  
275 confirmed by evaluating signs of active viral replication, as assessed by subgenomic (sg)RNA  
276 levels, which peaked in control animals at 3-4 days post-challenge with nasopharyngeal swabs,  
277 tracheal swabs and BAL showing median values of 5.2- 5.2 and 4.2-log<sub>10</sub> copies per ml,  
278 respectively (**Fig. 5e-g**). As expected with the almost complete viral suppression, area under  
279 the curve analysis (AUC) for P4J15 LS treated NHPs showed a significant reduction in gRNA  
280 compared to controls in nasopharyngeal samples collected from left and right nostrils  
281 throughout the study and tracheal samples ( $P < 0.0001$  and  $0.0022$ , respectively) (**Fig. 5h**).  
282 Similar significantly reduced levels of sgRNA in the AUC analysis was observed in P4J15 LS  
283 treated NHPs where sgRNA was undetected in all samples analysed throughout the study period  
284 (**Fig. 5i**). This indicates the absence of any replicating virus and the complete prophylactic  
285 protection provided by P4J15 LS in a non-human primate model. Finally, pharmacokinetic  
286 analysis of the NHPs dosed with P4J15 LS showed a long *in vivo* half-life of 43 days and a  
287 mean serum concentration of 75 µg/ml in the six animals 46 days after dosing (**Fig. 5j**). In  
288 comparison, the Evusheld antibodies AZD1061 and AZD8895 with the YTE extended half-life  
289 mutations showed an NHP *in vivo* half-life of 19 days (25).

## 290 **DISCUSSION**

291 As the WHO declares that the emergency phase of the SARS-CoV-2 pandemic has ended, the  
292 strain on the health care system continues with hospitalization rates from new infections still  
293 reaching >15'000 patients per week across North America and Europe (Supplementary Table  
294 1). Furthermore, it was recently reported that 10% of individuals suffer from long COVID after  
295 Omicron infection, with clinical symptoms that can include fatigue, brain fog, and dizziness  
296 lasting for upwards of six months (26). Newly emerged SARS-CoV-2 variants, including  
297 BQ.1.1 and XBB variants, now up to XBB.2.3, are increasingly infectious and immune evasive,  
298 significantly eroding the protection conferred by vaccines and previous infections. In addition,  
299 almost all licensed monoclonal antibodies for SARS-CoV-2 are inactive against currently  
300 circulating VOCs.

301 Here we report the isolation of the fully human P4J15 antibody from a vaccinated, convalescent  
302 donor with superior breadth and neutralizing potency to all other reported antibodies against  
303 SARS-CoV-2 VOCs up to the most recent XBB.2.3 (12-15). This unique antibody has been  
304 extensively optimized *in vivo* by somatic hypermutation, as evidenced by the low 82.5% and  
305 90.5% identity of the P4J15 heavy and light chain sequences, respectively, relative to the

306 IGHV4-34\*01 and IGKV1-33\*01 germline genes. The uniqueness of P4J15 is also illustrated  
307 by the low identity of 53.3% with the closest match of the 12016 anti-spike HCDR3 sequences  
308 reported to date.

309 Cryo-EM performed with the antibody Fab bound to Omicron XBB.1 Spike revealed that P4J15  
310 binds as a Class 1 mAb to the up-RBD conformation of the Spike trimer with a large, buried  
311 surface area of 828 Å<sup>2</sup>. Importantly, of the 26 RBD residues that make up the P4J15 binding  
312 epitope, 22 are shared with those used for ACE2 binding, representing 93% of the P4J15 contact  
313 site. Conversely, ~87% of the 887 Å<sup>2</sup> ACE2 binding epitope on RBD is shared by P4J15, which  
314 accounts for its potent neutralizing mechanism of action through blockade of ACE2 binding to  
315 all SARS-CoV-2 Spike variants tested to date. Interestingly, there are 10 RBD residues shared  
316 between P4J15 and ACE2 that have undergone evolutionary fine-tuning to evade neutralizing  
317 antibody responses while optimizing ACE2 binding and/or viral infectivity. Through the  
318 pandemic, these substitutions relative to the ancestral Spike include S477N, T478K/R,  
319 E484K/A, F486V/S/P, F490S, Q493R/Q, G496S, Q498R, N501Y and Y505H. Selection of  
320 these mutations over the last 42 months has contributed to the XBB.1 and BQ.1.1 variants  
321 exhibiting a 7.6- and 17-fold increase in infectivity relative to Omicron BA.2, respectively, as  
322 monitored in pseudovirus and cell fusion assays (27, 28). Indeed, similar to ACE2 and  
323 consistent with having ACE2 mimetic properties, we see that P4J15 has improved neutralizing  
324 activities against post-Omicron variants (EC<sub>50</sub> values of 5 to 14 ng/ml) compared to the  
325 ancestral 2019-nCoV (EC<sub>50</sub> of 41 ng/ml) in the Spike pseudotyped lentiviral neutralization  
326 assays.

327 Even with the highly overlapping binding epitope of P4J15 and ACE2 on the Spike RBD, we  
328 confirmed that the development of resistance is inevitable when a virus is under selective  
329 pressure, at least *in vitro*. Mutations centred at F456, A475, G476, N477, N487 and Y489 did  
330 confer escape to neutralization by P4J15 but also reduced binding to ACE2 by 1- to 3-logs,  
331 indicating that the virus incurs a significant fitness penalty in developing resistance. Although  
332 epistasis is always possible to complement for deleterious mutations (29), the almost complete  
333 absence of these specific resistance mutations within the GISAID database confirms that the  
334 virus cannot easily escape the type of inhibition imposed by P4J15 without compromising its  
335 ability to spread in the population. Of note, substitutions at some of the incriminated positions  
336 are detectable in public sequence databases, albeit at very low frequency, but these mutations  
337 do not confer resistance to neutralization by P4J15, which requires very specific amino acid  
338 substitutions. We note that in select Spike mutations, there are discrepancies between the

339 infectivity data obtained in the VLP-based assay and the predicted binding affinity of the  
340 resistant Spike for the ACE2 receptor, illustrating the limitations of any *in vitro* assay. The  
341 relative ACE2 binding data were obtained from Jesse D. Bloom's online interactive tool. While  
342 this deep mutational scanning approach is a highly valuable and well controlled approach for  
343 predicting the potential effects of mutations in the RBD, it has shortcomings, as acknowledged  
344 by its inventors themselves. These include i) differences in glycan structures on the Spike  
345 surface in yeast vs. human cells, ii) use of only the RBD portion of the Spike protein to assess  
346 the impact of mutations, iii) assessment of the effects of mutations in the BA.2 background, and  
347 iv) information limited to the affinity of the tested Spike protein for its ACE2 receptor, without  
348 consideration of any other step important for viral entry, let alone genome  
349 replication/expression. The infectivity measurements were performed with of HEK 293T cell  
350 produced VLPs as tested in HEK 293T ACE2/TMPRSS2 target cells. The results indeed  
351 indicate that some of the escape mutants, in the VLP assay, are slightly more infectious than  
352 the wild-type control whereas the said mutations alter ACE2 recognition when tested in yeast  
353 with an RBD-only construct. While on the one hand, the VLP assay is predicted to reflect more  
354 fully the multiple steps leading to the delivery of the viral genetic content into its target cells,  
355 on the other hand, the rarity of these mutations in nature tells us that this system still misses  
356 features important for viral fitness. Part of this difference may be virological, including the  
357 concentration of receptor and entry accessory mediators on HEK 293T cells overexpressing  
358 ACE2 and TMPRSS2, which cannot be expected to mimic that found on the respiratory  
359 epithelium, and the stoichiometry of Spike on VLPs and SARS-CoV-2 virions, which is not  
360 identical, and other differences are immunological, including the sensitivity to the prevailing  
361 immunity in the population.

362 Finally, *in vivo* efficacy studies performed with two animal models demonstrate that P4J15  
363 provides exceptional levels of protection against infection. In hamsters challenged with the  
364 Omicron BA.5 virus, animals pre-dosed with 0.5 mg/kg of mAb resulting in serum  
365 concentrations as low as 7 µg/ml were strongly protected from infection with almost complete  
366 suppression of infectious virus in the lungs. A cynomolgus monkey challenge performed with  
367 the latest available XBB.1.5 SARS-CoV-2 variant further revealed that P4J15 at 5 mg/kg  
368 produced near sterilizing protection in all six treated animals, with only two positive samples  
369 with viral genomic RNA near the detection limit of the RT-PCR assay out of a total of 192  
370 nasal, tracheal and BAL samples tested. These results are among the most definitive protection  
371 results for SARS-CoV-2 non-human primate challenge studies (25, 30, 31). In addition to

372 complete *in vivo* efficacy, the P4J15 antibody produced with the LS extended half-life  
373 mutations in the Fc domain showed an extremely long terminal *in vivo* half-life of 43 days,  
374 more than twice that observed for Evusheld in a similar NHP study. Given that Evusheld  
375 antibodies achieved an *in vivo* half-life of 90 days in human clinical trials (25), it is expected  
376 that P4J15 LS could provide a similar duration of prophylactic protection of more than six  
377 months following a single dose of antibody administered by intravenous, intramuscular or  
378 intraperitoneal injection (32, 33). With its impressive *in vivo* protection, P4J15 LS could form  
379 a strong basis for prophylactic therapy in immunocompromised patients. To supplement this  
380 activity and protect against the development of resistance (34), P4J15 could potentially be  
381 combined as a cocktail with a second neutralizing antibody that binds a distinct epitope on  
382 Spike. For example, although sotrovimab is only weakly to moderately active against some of  
383 the newer SARS-CoV-2 variants, this antibody would help to suppress any low-level, poorly  
384 fit viral quasi-species that are resistant to P4J15.

385         The world is at a critical juncture in the SARS-CoV-2 pandemic where humoral  
386 protection afforded by vaccines is waning, the public at large has become complacent and new  
387 variants with ever-increasing infectivity and immune resistance are emerging regularly. As a  
388 result of these factors, the most vulnerable in our population, the immunocompromised, those  
389 with comorbidities such as cancer and the elderly, who are unable to mount a strong protective  
390 humoral immune response after vaccination (7, 35), are at significantly increased risk of  
391 hospitalization and death. Given the potent neutralizing activity of P4J15, its ACE2 mimetic  
392 properties that may help to limit the development of resistant virus and the impressive complete  
393 *in vivo* protection in the non-human primate model, this monoclonal antibody has the potential  
394 to be a superior anti-SARS-CoV-2 mAb for prophylactic and/or potentially therapeutic  
395 interventions. Furthermore, the breadth of potent neutralizing activity against all current VOCs  
396 and variant quasi-species within public SARS-CoV-2 sequence databases suggests that it may  
397 be able to neutralize many future VOCs that could emerge in the months and years ahead,  
398 providing a sustainable and long-lasting solution to protect the most vulnerable in our  
399 population.

400

## 401 **Materials and methods**

### 402 **Study COVID-19 donors**

403 Serum and blood mononuclear cell samples were from donors participating in the ImmunoCov  
404 and ImmunoVax studies performed by the Immunology and Allergy Service, Lausanne  
405 University Hospital with all participants being adults of varying ages and having signed  
406 informed consent forms for the use of biological samples. Study design and use of subject  
407 samples were approved by the Institutional Review Board of the Lausanne University  
408 Hospital and the ‘Commission d’éthique du Canton de Vaud’ (CER-VD with trial reference  
409 numbers 2020-00620 and 2021-00041, respectively).

#### 410 **Production of SARS-CoV-2 Spike proteins**

411 SARS-CoV-2 Spike mutations are listed in Supplementary Table 1. Production of 2019-nCoV  
412 (D614G), Alpha, Beta, Delta and Omicron BA.1 variants has already been described (21). BA.2  
413 and further Omicron sub-lineages ORFs were cloned by 1kb gBlocks assembly (IDT DNA) and  
414 In-Fusion cloning into the nCoV-BA.1 plasmid described earlier (36). Single mutations were  
415 further introduced by PCR-mediated mutagenesis in each sub-lineage. The full Omicron ORFs  
416 were sequence verified for all the clones. The final constructs encode the Spike ectodomains,  
417 containing a native signal peptide, the 2P and furin cleavage site mutations, a C-terminal T4  
418 foldon fusion domain to stabilize the trimer complex followed by C-terminal 8x His and 2x  
419 Strep tags for affinity purification. The trimeric Spike variants were produced and purified as  
420 previously described (16). The purity of Omicron Spike trimers used for cryo-EM was  
421 determined to be >98% pure by SDS-PAGE analysis. Biotinylation of Spike or RBD proteins  
422 was performed using the EZ-Link™ NHS-PEG4-Biotin (Life Technologies, USA) using a 3-  
423 fold molar excess of reagent and using the manufacturer’s protocol. Biotinylated proteins were  
424 buffer exchanged with PBS using an Amicon Ultra-0.5 with a 3 kDa molecular weight cut-off.  
425 Spike and RBD tetramers were prepared fresh before use and formed by combining  
426 biotinylated proteins with PE-conjugated Streptavidin (BD Biosciences, USA) at a molar ratio  
427 of 4:1.

#### 428 **Binding and ACE2 blocking studies with SARS-CoV-2 Spike**

429 Luminex beads used for the serological and purified antibody binding assays were prepared by  
430 covalent coupling of SARS-CoV-2 proteins with MagPlex beads using the manufacturer’s  
431 protocol with a Bio-Plex Amine Coupling Kit (Bio-Rad, France). Each of the SARS-CoV-2  
432 Spike proteins expressed with different mutations were coupled with different colored MagPlex  
433 beads so that tests could be performed with a single protein bead per well or in a multiplexed  
434 Luminex binding assay. Binding curves for antibody affinity measurements and the Spike-

435 ACE2 interaction assay were performed as previously described (16, 37) using anti-human IgG-  
436 PE secondary antibody (OneLambda ThermoFisher; Cat # H10104; 1:100 dilution) for antibody  
437 detection in Spike Luminex binding assay and anti-mouse IgG-PE secondary antibody  
438 (OneLambda ThermoFisher; Cat# P-21129; 1:100 dilution) in the Spike-ACE2 surrogate  
439 neutralization assay. Competitive binding studies were performed by pre-incubating 25 µg/ml  
440 of the indicated competitor antibody with the original 2019-nCoV Spike trimer protein coupled  
441 Luminex beads for 30 minutes. Biotinylated P4J15, P2G3, AZD8895, AZD1061,  
442 bebtelovimab, ADG-2 or sotrovimab antibodies (prepared as described above) were added to  
443 each well at 1 µg/ml followed by a further 20-minute incubation. Biotinylated antibody bound  
444 to RBD in the presence of competitor was stained with Streptavidin-PE at a 1:1000 dilution  
445 (BD Biosciences) and analysed on a 200 Bioplex instruments. COVID-19 serum samples from  
446 20 donors were monitored for levels of IgG antibody binding to the SARS-CoV-2 Spike trimer  
447 proteins from 2019-nCoV, D614G, Alpha, Beta, Delta, Omicron BA.1 and BA.4 in the  
448 Luminex bead-based assay.

#### 449 **Anti-Spike B cell sorting, immortalization and cloning**

450 The bloods from ImmunoVax study donors were collected in EDTA tubes and the isolation of  
451 blood mononuclear cells was performed using Leucosep centrifuge tubes (Greiner Bio-one)  
452 prefilled with density gradient medium (Ficoll-Paque™ PLUS, GE Healthcare) according to  
453 the manufacturer's instructions. Freshly isolated cells were stained with the cocktail of  
454 fluorescent conjugated antibodies containing mouse anti-human CD19 APC-Cy7 (BD  
455 Biosciences; Cat#557791; Clone SJ25C1; 5 µl titration in 100 µl), mouse anti-human CD3-  
456 BV510 (BD Biosciences; Cat#563109; Clone UCHT1; 1 µl titration), mouse anti-human IgM-  
457 FITC (Biolegend; Cat#314506; clone MHM-88; 2 µl titration), mouse anti-human IgD PE-  
458 CF594 (BD Biosciences; Cat#562540; Clone IA6-2; 3 µl titration), mouse anti-human CD27-  
459 APC (BD Biosciences; Cat#558664; Clone: M-T271; 5 µl titration), mouse anti-human CD38-  
460 V450 (BD Biosciences; Cat#646851; Clone HB7; 5 µl titration) mAbs were used for antigen  
461 specific B cell sorting along with the pre-complexed Omicron BA.1 variant Spike tetramer (2  
462 µg in 100µl) coupled to PE-streptavidin (BD Biosciences; Cat#SA10044; 4:1 molar ratio). All  
463 other aspects with cell sorting, immortalization protocol using EBV positive supernatants from  
464 B95-8 cells and cloning were as described in Fenwick et al (21). Sequences for mAbs P4J15  
465 are provided in PDB submissions PDB-8PQ2 and EMD-17819.

#### 466 **SARS-CoV-2 live virus stocks**

467 All biosafety level 3 procedures were approved by the Swiss Federal Office of Public Health.  
468 The SARS-CoV-2 BA.2.75.2 (EPI\_ISL\_14795784) and BQ.1 (EPI\_ISL\_15369810) isolates  
469 were a kind gift from I. Eckerle, Geneva University Hospitals. Viral stocks were prepared in  
470 EpiSerf (ThermoFisher Scientific, USA) with a single passage on VeroE6 cells, aliquoted,  
471 frozen, titrated on VeroE6 cells by conventional plaque forming units and sequence verified.  
472 BA.2.75.2 isolate differed from our cloned BA.2.75.2 ORF by 1 supplemental mutation  
473 (G181V) already found in the original virus isolated from the patient.

#### 474 **Selection of resistant virus in presence of mAbs**

475 The day before infection, HEK293T ACE2/TMPRSS2 cells previously described (22) were  
476 seeded in 6-well plates coated with poly-L-lysine at a density of  $1 \times 10^6$  cells per well. To  
477 generate a viral population under mAb pressure, early passage virus was diluted in 1.5 ml  
478 EpiSerf 2% FCS and incubated with 0.5 ng/ml mAb for 1 hr at 37°C in duplicates. Each mixture  
479 was added to the cells and P1 (passage 1) supernatants were harvested 3 days later, clarified on  
480 0.45  $\mu$ m SPIN-X centrifuge tube filters at 4000 $\times$ g for 4 minutes. Aliquots of cleared P1  
481 supernatants were diluted 1:40 in DMEM 2%, incubated with mAbs as described above and  
482 used to infect fresh cells for 4 days. P2 supernatants were treated as P1 and P3 supernatants  
483 were collected for RNA extraction and subsequent selection step. To select for mAb resistant  
484 viruses, 200  $\mu$ l of the cleared undiluted P3 heterogeneous viral population was incubated with  
485 200  $\mu$ l mAbs at 2.5 or 10  $\mu$ g/ml final concentration for 1 hr at 37°C. Mixture was then applied  
486 on cells in 800  $\mu$ l DMEM 2% (1:2 volume) for 3 to 4 days. Viruses were selected one more  
487 time and aliquots of passage 5 were used for RNA extraction and sequencing. Virus produced  
488 in absence of mAb was collected and treated the same way in parallel to control for appearance  
489 of mutations due to cell culture adaptation.

#### 490 **Spike-pseudotyped vectors production and neutralization assays**

491 HDM-IDT Spike-fixK plasmid (BEI catalogue number NR-52514, obtained from J.D. Bloom,  
492 Fred Hutchinson Cancer Research Centre) was used as backbone for all the cloning. The  
493 cloning of D614G, Alpha, Beta and Delta clones have previously been described (22).  
494 Pseudoviruses were alternatively produced with the original 2019-nCoV (Cat #100976), Alpha  
495 / B.1.1.7 (Cat #101023) and Beta/B.1.351 (Cat #101024) pCAGGS-SARS2-Spike vectors  
496 obtained from NIBSC. Omicron ORFs have been cloned with 1kb gBlocks assembly (IDT  
497 DNA) followed by In-Fusion cloning in the same plasmid or were generated by gene synthesis  
498 with a codon-optimized Spike ORF (Twist Biosciences). Escape mutations have been further



499 introduced by PCR-mediated mutagenesis. Pseudoviruses were produced by co-transfection  
500 with pMDL p.RRE, pRSV.Rev and pUltra-Chili-Luc vectors (Addgene) into HEK 293T cells  
501 as previously described (22).

502 SARS-CoV-2 pseudotyped VLPs have been produced by co-transfection of HDM-IDT Spike-  
503 fixK, CoV-2 N, CoV-2-M-IRES-E and Luc-PS9 plasmids as described in Syed et al (38).  
504 Briefly, for a 10-cm plate, plasmids CoV-2-N (0.67), CoV-2-M-IRES-E (0.33), HDM-  
505 IDTSpike-fixK (0.03) and Luc-PS9 (1.0) at indicated mass ratios for a total of 20 µg of DNA  
506 were diluted in 1 ml Opti-MEM (Gibco, ThermoFisher Scientific, USA). Then, 60 µg TransIT-  
507 LT1 transfection reagent (Mirus Bio, USA) was added to plasmid dilution to complex the DNA,  
508 according to the provider's instructions. Transfection mixture was incubated for 15 minutes at  
509 room temperature and then added dropwise on HEK 293T cells in 10 ml of DMEM containing  
510 10% foetal bovine serum. Media was changed after 18 hours of transfection. VLPs containing  
511 media was collected 36 and 48 hours post transfection, pooled, centrifuged 5 minutes at 500 ×  
512 g and the supernatants filtered using a 0.45 µm syringe filter. Samples were aliquoted and stored  
513 at 4°C if used immediately or at -80°C for further analyses.

#### 514 **RNA genome quantification**

515 Viral RNA was extracted from the supernatants with EZNA viral RNA extraction kit, DNase-  
516 treated when particles were produced by transfection, reverse transcribed with Maxima H  
517 Minus cDNA Synthesis Master Mix (ThermoFisher Scientific, USA) as recommended by the  
518 manufacturer and the genome quantified by RT-qPCR performed in triplicates using the  
519 following primers to detect either the Luciferase gene for VLPs or the RdP gene for viruses:  
520 Luc(s): 5- GTG GTG TGC AGC GAG AAT AG -3' ; Luc(as): 5- CTG TTC AGC AGC TCG CGC  
521 TC -3'; RdP(s): 5-AGC TTG TCA CAC CGT TTC-3', RdP(as): 5'-AAG CAG TTG TGG CAT  
522 CTC-3' .

523 Absence of DNA contamination was always verified with a control amplification performed in  
524 parallel in absence of reverse transcription step.

#### 525 **Viral escapees sequencing and mapping**

526 Viral RNA was extracted from passage 5 supernatants and deep-sequenced. Sequencing reads  
527 were mapped to the SARS-CoV-2 WuhCor1 strain downloaded from the UCSC database, using  
528 botwie2 in sensitive mode with read gap penalties 5,1.9. The perbase package

529 (<https://github.com/sstadick/perbase>) was then used to obtain the nucleotide depth for each base  
530 in the genome. Only mutations found in more than 20% of the reads were taken into accounts.

531

### 532 **Infectivity and neutralization assays of pseudotyped particles**

533 In each well of a black 96-well previously coated with poly-L-lysine (0.01% w/v solution,  
534 Sigma-Aldrich USA), 50 µl of VLP-containing supernatants were added to 50 µl of cell  
535 suspension containing 100 000 receiver cells (HEK293T ACE2/TMPRSS2 cells), in n=8  
536 replicates. 24 hours later, supernatant was removed, then 30 µl of DMEM medium was added  
537 with 30 µl of reconstituted luciferase assay buffer (Bright-Glo luciferase assay, Promega, USA)  
538 and mixed. Luminescence was measured 5 minutes after using a Hidex Sense microplate reader  
539 (Hidex Oy, Finland).

540 For lentiviral containing supernatants, the protocol is identical except the incubation time is 72  
541 hrs instead of 24 hrs with assays performed as previously described (21).

542

### 543 **NHP challenge model for SARS-COV-2 Omicron BA.1 infection**

544 Cynomolgus macaques (*Macaca fascicularis*) originating from Mauritian AAALAC certified  
545 breeding centres were used in this study. All animals were housed within IDMIT animal  
546 facilities at CEA, Fontenay-aux-Roses under BSL-3 containment when necessary (Animal  
547 facility authorization #D92-032-02, Préfecture des Hauts de Seine, France) and in compliance  
548 with European Directive 2010/63/EU, the French regulations and the Standards for Human Care  
549 and Use of Laboratory Animals, of the Office for Laboratory Animal Welfare (OLAW,  
550 assurance number #A5826-01, US). Animals tested negative for *Campylobacter*, *Yersinia*,  
551 *Shigella* and *Salmonella* before being use in the study.

552 The protocols were approved by the institutional ethical committee “Comité d’Ethique en  
553 Expérimentation Animale du Commissariat à l’Energie Atomique et aux Energies Alternatives”  
554 (CEtEA #44) under statement number A20-011. The study was authorized by the “Research,  
555 Innovation and Education Ministry” under registration number APAFIS#29191-  
556 2021011811505374 v1. All information on the ethics committee is available at  
557 [https://cache.media.enseignementsup-recherche.gouv.fr/file/utilisation\\_des\\_animaux\\_fins\\_](https://cache.media.enseignementsup-recherche.gouv.fr/file/utilisation_des_animaux_fins_scientifiques/22/1/comiteethiqueea17_juin2013_257221.pdf)  
558 [scientific/22/1/comiteethiqueea17\\_juin2013\\_257221.pdf](https://cache.media.enseignementsup-recherche.gouv.fr/file/utilisation_des_animaux_fins_scientifiques/22/1/comiteethiqueea17_juin2013_257221.pdf).

559 In the prophylactic protection study, ten female cynomolgus macaques aged 26-27 months at  
560 the beginning of the study were randomly assigned between the control and treated groups to  
561 evaluate the efficacy of P4J15 LS in protecting from challenge with the SARS-CoV-2 XBB.1.5  
562 virus (NIH/BEI reference: NR-59105; hCoV-19/USA/MD-HP40900/2022). The treated group  
563 (n = 6) received one dose at 5 mg/kg of P4J15 LS human IgG1 monoclonal antibody delivered  
564 by intravenous slow bolus injection over 3-8 minutes three day prior to challenge, while control  
565 animals (n = 4 in parallel and n=2 historical) received no treatment. The two historical control  
566 animals were male and infected three weeks before the study with P4J15 LS. All animals were  
567 then exposed to a total dose of  $10^5$  TCID<sub>50</sub> of Omicron XBB.1.5 SARS-CoV-2 virus produced  
568 in Vero-ACE2-TMPRSS2 (NIH/BEI reference: NR-59105) via the combination of intranasal  
569 and intratracheal routes with sample collection and testing performed as previously described  
570 (39). Tracheal swabs, nasopharyngeal swabs and bronchoalveolar lavages were performed on  
571 all NHPs collected throughout the study to monitor levels of both genomic and subgenomic  
572 RNA for the SARS-COV-2 virus as previously described (40). All animals and data points were  
573 included in the analysis. The NHP sample size was selected based on the large, 1- to 2-log  
574 reduction in viral RNA anticipated in the trachea, nasopharyngeal and/or BAL with an effective  
575 therapy that can provide statistically significant differences between treated and untreated  
576 NHPs. These sample size assumptions were confirmed with the statistical differences observed  
577 in viral RNA in viral RNA levels was evaluated using the Mann-Whitney two-sided tests to  
578 compare control and treatment groups.

### 579 **Hamster challenge model SARS-CoV-2 infection**

580 KU LEUVEN R&D has developed and validated a SARS-CoV-2 Syrian Golden hamster  
581 infection model that is suitable for the evaluation of potential antiviral activity of novel  
582 antibodies (41-43). The SARS-CoV-2 strain used in this study was the Omicron BA.5 variant  
583 (BA.5.2.1) [EPI\_ISL\_14782497] was isolated from nasopharyngeal swabs taken from patient  
584 in Belgium. The variant was isolated in Vero E6 then passage 2 virus on Calu-3 cells was used  
585 for the study described here and the genetic sequence of the stock was confirmed by deep  
586 sequencing analysis. The titer of the virus stock was determined by end-point dilution on Vero  
587 E6 cells by the Reed and Muench method. Live virus-related work was conducted in the high-  
588 containment A3 and BSL3+ facilities of the KU Leuven Rega Institute (3CAPS) under licenses  
589 AMV 30112018 SBB 219 2018 0892 and AMV 23102017 SBB 219 20170589 according to  
590 institutional guidelines. Vero E6 cells (African green monkey kidney, ATCC CRL-1586) were  
591 cultured in minimal essential medium (Gibco) supplemented with 10% fetal bovine serum

592 (Integro), 1% L- glutamine (Gibco) and 1% bicarbonate (Gibco). End-point titrations were  
593 performed with medium containing 2% fetal bovine serum instead of 10%.

594 The hamster infection model of SARS-CoV-2 has been described before (41, 43). In brief,  
595 wild-type Syrian Golden hamsters (*Mesocricetus auratus*) were purchased from Janvier  
596 Laboratories and were housed per two in ventilated isolator cages (IsoCage N  
597 Biocontainment System, Tecniplast) with ad libitum access to food and water and cage  
598 enrichment (wood block). The animals were acclimated for 4 days prior to study start.  
599 Housing conditions and experimental procedures were approved by the ethics committee  
600 of animal experimentation of KU Leuven (license P065-2020). Female hamsters of 6-8 weeks  
601 old were administered IgG1 isotype control (5 mg/kg), P4J15 LS (5 mg/kg, 1 mg/kg or 0.5  
602 mg/kg) or bebtelovimab (5 mg/kg) by intraperitoneal injection. Two days later, hamsters were  
603 anesthetized with ketamine/xylazine/atropine, blood samples were collected, and animals were  
604 inoculated intranasally with 100  $\mu$ L containing  $1 \times 10^4$  n tissue culture infectious dose (TCID<sub>50</sub>)  
605 of SARS-CoV-2 Omicron BA.5 (day 0). Hamsters were monitored for appearance, behaviour  
606 and weight. Antibody concentrations present in the hamster plasma on day 0 of the study were  
607 performed using the Luminex assay described above with Spike trimer coupled beads and using  
608 purified P4J15 LS antibody to generate a standard curve. In these studies, no control animals  
609 were excluded. In treated groups, animals with undetectable levels of serum antibodies (one  
610 hamsters in the 5 mg/kg P4J15 LS group and 2 hamsters in the 1 mg/kg P4J15 LS group) were  
611 excluded from the analysis as this indicated a technical failure in the drug administration. At  
612 day 4 post infection, hamsters were sacrificed, and lung tissues were homogenized using bead  
613 disruption (Precellys) in 350  $\mu$ l TRK lysis buffer (E.Z.N.A. Total RNA Kit, Omega Bio-tek)  
614 and centrifuged (10,000 rpm, 5 min) to pellet the cell debris. RNA was extracted according to  
615 the manufacturer's instructions. Of 50  $\mu$ l eluate, 4  $\mu$ l was used as a template in RT-qPCR  
616 reactions. RT-qPCR was performed on a LightCycler96 platform (Roche) using the iTaq  
617 Universal Probes One-Step RT-qPCR kit (BioRad) with N2 primers and probes targeting the  
618 nucleocapsid (41). Standards of SARS-CoV-2 cDNA (IDT) were used to express viral genome  
619 copies per mg tissue. For end-point virus titrations, lung tissues were homogenized using bead  
620 disruption (Precellys) in 350  $\mu$ l minimal essential medium and centrifuged (10,000 rpm, 5min,  
621 4°C) to pellet the cell debris. To quantify infectious SARS- CoV-2 particles, endpoint titrations  
622 were performed on confluent Vero E6 cells in 96- well plates. Viral titers were calculated by  
623 the Reed and Muench method using the Lindenbach calculator and were expressed as 50%  
624 tissue culture infectious dose (TCID<sub>50</sub>) per mg tissue. The hamster sample size was selected

625 based on the large, >1-log reduction in viral RNA and infectious virus anticipated in the lung  
626 tissue with an effective therapy that can provide statistically significant differences between  
627 treated and untreated animals. These sample size assumptions were confirmed in our statistical  
628 analysis. Statistical differences in viral RNA levels and infectivity were evaluated using the  
629 Mann-Whitney two-sided tests to compare control and treatment groups.

### 630 **Cryo-electron microscopy**

631 Cryo-EM grids were prepared with a Vitrobot Mark IV (ThermoFisher Scientific). Quantifoil  
632 R1.2/1.3 Au 400 holey carbon grids were glow-discharged for 90s at 15mA using a GloQube  
633 Plus Glow-Discharge System (Quorum, Inc.). 2.0  $\mu$ l of a 2.1 mg/ml XBB.1 Spike was mixed  
634 with 2.0  $\mu$ l of a 0.28 mg/ml P4J15 Fab fragments (Final 11.1  $\mu$ M XBB.1 Spike:5.6  $\mu$ M P4J15  
635 Fab) and 3.0  $\mu$ l of the fresh complex was applied to the glow-discharged grids, blotted for 4s  
636 under blot force 10 at 95% humidity, wait time 10s and 10 °C in the sample chamber, and then  
637 the blotted grid was plunge-frozen in liquid nitrogen-cooled liquid ethane.

638 Grids were transferred in a ThermoFisher Scientific Titan Krios G4 transmission electron  
639 microscope, equipped with a Cold-FEG on a Falcon IV detector (Dubochet Center for Imaging,  
640 Lausanne) in electron counting mode. Falcon IV gain references were collected just before data  
641 collection. Data was collected using TFS EPU v2.12.1 using aberration-free image shift  
642 protocol (AFIS), recording 4 micrographs per ice hole. A total of 24 814 micrographs in EER  
643 format were recorded at magnification of 165kx, corresponding to the 0.83Å pixel size at the  
644 specimen level, with defocus values ranging from -0.6 to -2.0  $\mu$ m. Exposures were adjusted  
645 automatically to 50  $e^-/\text{Å}^2$  total dose.

646

### 647 **Cryo-EM image processing**

648 During the data acquisition phase, on-the-fly processing was employed to assess the data quality  
649 for screening purposes, utilizing cryoSPARC live v3.3.1 (44). Raw stacks were subjected to  
650 motion correction without binning, utilizing cryoSPARC's implementation of motion correction  
651 and contrast transfer function estimation (45). A total of 6,769,807 particles were automatically  
652 template-picked. Following several rounds of 2D classification, 362,329 particles were selected  
653 and utilized for ab-initio reconstruction and 3D classifications. Within this dataset, multiple  
654 conformers were identified; however, after thorough validation, a subset of 95,910 particles  
655 corresponding to a P4J15 fragment bound to an XBB1 trimer was deemed reliable.

656 Homogeneous refinement using the selected particles resulted in a 3D reconstruction at a  
657 resolution of 3.01 Å (FSC 0.143) with C1 symmetry. To further enhance the map quality,  
658 focused refinement was performed using a soft mask volume encompassing an RBD-up region  
659 and its bound Fab. This refinement process yielded a final Coulomb map at 3.85 Å resolution  
660 (FSC 0.143) with C1 symmetry (**Supplementary Fig. 4**). The soft mask volumes were  
661 manually generated in UCSF ChimeraX (46) and the Cryosparc Volume tool. Post-processing  
662 polishing was conducted with EMReady (23) to improve the map quality and aid in resolving  
663 any atom position ambiguities. Finally, the building and refinement steps were exclusively  
664 carried out using CryoSPARC maps.

### 665 **Cryo-electron microscopy model building**

666 To generate initial models of the P4J15 Fab and XBB1 spike, various approaches were  
667 employed. These included utilizing models from the Spike trimer (PDB ID 7QO7), AlphaFold2  
668 (implemented through ColabFold), and ModelAngelo 0.3 (47) for sequence-based generation.  
669 The cryo-EM maps were fitted with the Spike trimer using UCSF ChimeraX, serving as the  
670 starting point for further manual refinement. Manual extension and building of the docked  
671 models were carried out using Coot 0.9.8 (48). To refine the models, Phenix 1.20 (49) was  
672 employed. The generated figures depicting the models were prepared using UCSF ChimeraX.  
673 The numbering scheme for the full-length Spike models within the global map is based on  
674 Omicron numbering. For models containing only the RBD within the local maps, wild-type  
675 numbering is utilized. In the case of the P4J15 Fab, the numbering of both the heavy and light  
676 chains start from one, beginning with the CH1 and CL domains, respectively. For additional  
677 analysis, buried surface area measurements were calculated using ChimeraX. Predictions  
678 regarding hydrogen bonds and salt bridges were performed using PDBePISA.

### 679 **Statistical analysis**

680 Statistical parameters including the exact value of n, the definition of centre, dispersion, and  
681 precision measures (Mean or Median  $\pm$  SEM) and statistical significance are reported in the  
682 Figures and Figure Legends. Data were judged to be statistically significant when  $p < 0.05$ . In  
683 Figures, asterisks denote statistical significance as calculated using the two-tailed non-  
684 parametric Mann-Whitney U test for two groups' comparison or with Kruskal-Wallis tests with  
685 Dunn's multiple-comparison correction. Analyses were performed in GraphPad Prism  
686 (GraphPad Software, Inc.) and Microsoft Excel.

687

## 688 **Data availability**

689 All data supporting the findings of this study are available within the paper and in the Source  
690 Data. The reconstructed maps of the global Omicron Spike with Fabs bound are available from  
691 the EMDB database, C1 symmetry, EMD-17819. The atomic model for the RBD-up with one  
692 Fabs bound in the locally refined map is available from the PDB database, PDB-8PQ2. All  
693 plasmids made in this study are available upon request to the corresponding authors.

694

## 695 **Declaration of Competing Interest**

696 C.F., G.P., P.T. and D.T. are co-inventors on a patent application that encompasses the  
697 antibodies and data described in this manuscript (EP 22199188.8). DT and GP are amongst the  
698 founders of and own equity in Aerium Therapeutics, which has rights to and is pursuing the  
699 development of the antibodies described in the publication and has a Sponsored Research  
700 Agreements with the Lausanne University Hospital (CHUV) and the Ecole Polytechnique  
701 Fédérale de Lausanne (EPFL). The remaining authors declare no competing interests.

702

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713 UNIGE, UNIL initiative) for cryoEM grids preparation and data collection. We thank Julien  
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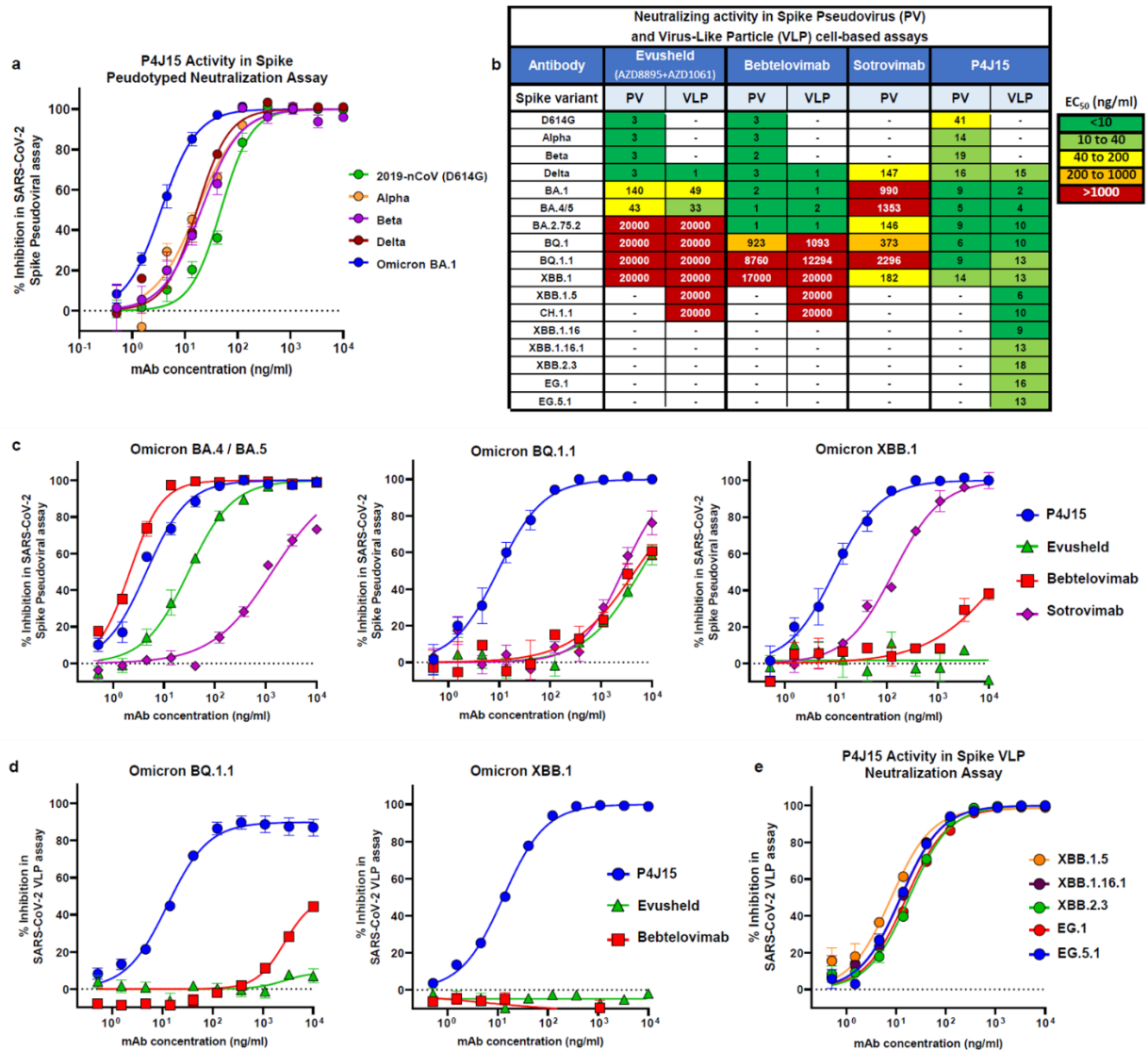
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#### 742 **Author contributions**

743 C.F. designed the strategy for isolating and profiling anti-Spike antibodies, coordinated the  
744 research activities, designed Spike variant constructs, analysed the data, wrote the initial draft  
745 and contributed to the editing of the manuscript. P.T. established, performed the experiments  
746 with live SARS-CoV-2 virus and designed the Spike protein mutations with the help of C.R.,  
747 analysed the results and contributed to the editing of the manuscript. C.R. and V.G. performed  
748 Spike cloning and VLP-based experiments. M.L. sets up the SARS-CoV-2 VLPs assay. Y.D.,  
749 K.L., F.P., L.P., and H.S. coordinated the cryo-EM analysis, analysed the structural data and  
750 contributed to the editing of the manuscript. Other contributed as follows: L.E.-L., performed  
751 the B cells sorting, immortalization, binding studies and mAb functional assays; A.F. and J.Ce.,  
752 cloning of mAb VH and HL, and generation of Omicron BA.5 mutations by site directed  
753 mutagenesis; J.Ca., binding studies, production of lentiviruses and pseudoviral assays; F.F.  
754 mAb purification, mAb characterization and molecular biology; F.P. coordinated production of  
755 recombinant Spike protein and mAb. P.L., Y.L. and R.L. designed the *in vivo* studies, which  
756 were executed by C.H, R.M., N. D.-B., F.R., R.A., C.S.F., G.V. and J.N. G.P. and D.T.  
757 conceived the study design, analysed the results and wrote the manuscript.

#### 758 **Figures**





759

760 **Figure 1: P4J15 exhibits potent and broad neutralizing activity against spike-coated**  
 761 **pseudoviruses**

762 **a)** Neutralization of lentiviral particles pseudotyped with SARS-CoV-2 Spike expressing the  
 763 ancestral 2019-nCoV (D614G), Alpha, Beta, Delta or Omicron BA.1 variants of concern in  
 764 HEK293T ACE2/TMPRSS2 cell infection assays. Replicates in concentration response curves  
 765 were n=6 for all Spike pseudoviruses. **b)** Heatmap table showing EC<sub>50</sub> neutralization potencies  
 766 for P4J15 and reference antibodies Evusheld (combination of AZD8895 and AZD1061),  
 767 bebtelovimab and sotrovimab evaluated in spike-coated pseudovirus and SARS-CoV-2 virus-  
 768 like particle cell-based assays. **c)** Concentration response inhibition curves for Omicron BA.4/  
 769 BA.5, BQ.1.1 and XBB.1 Spike pseudotyped lentivirus cell-based neutralization assays (n=6).  
 770 **d)** Concentration response inhibition curves for Omicron BQ.1.1 and XBB.1 Spike  
 771 pseudotyped SARS-CoV-2 VLP cell-based neutralization assays (n=8 for Evusheld and n=12

772 for remaining mAbs). e) Concentration response inhibition curves for P4J15 in XBB.1.5,  
773 XBB.1.16, XBB.1.16.1, XBB.2.3 and EG.1 Spike pseudotyped VLP cell-based neutralization  
774 assays (n=4). Mean values  $\pm$  SEM are shown.

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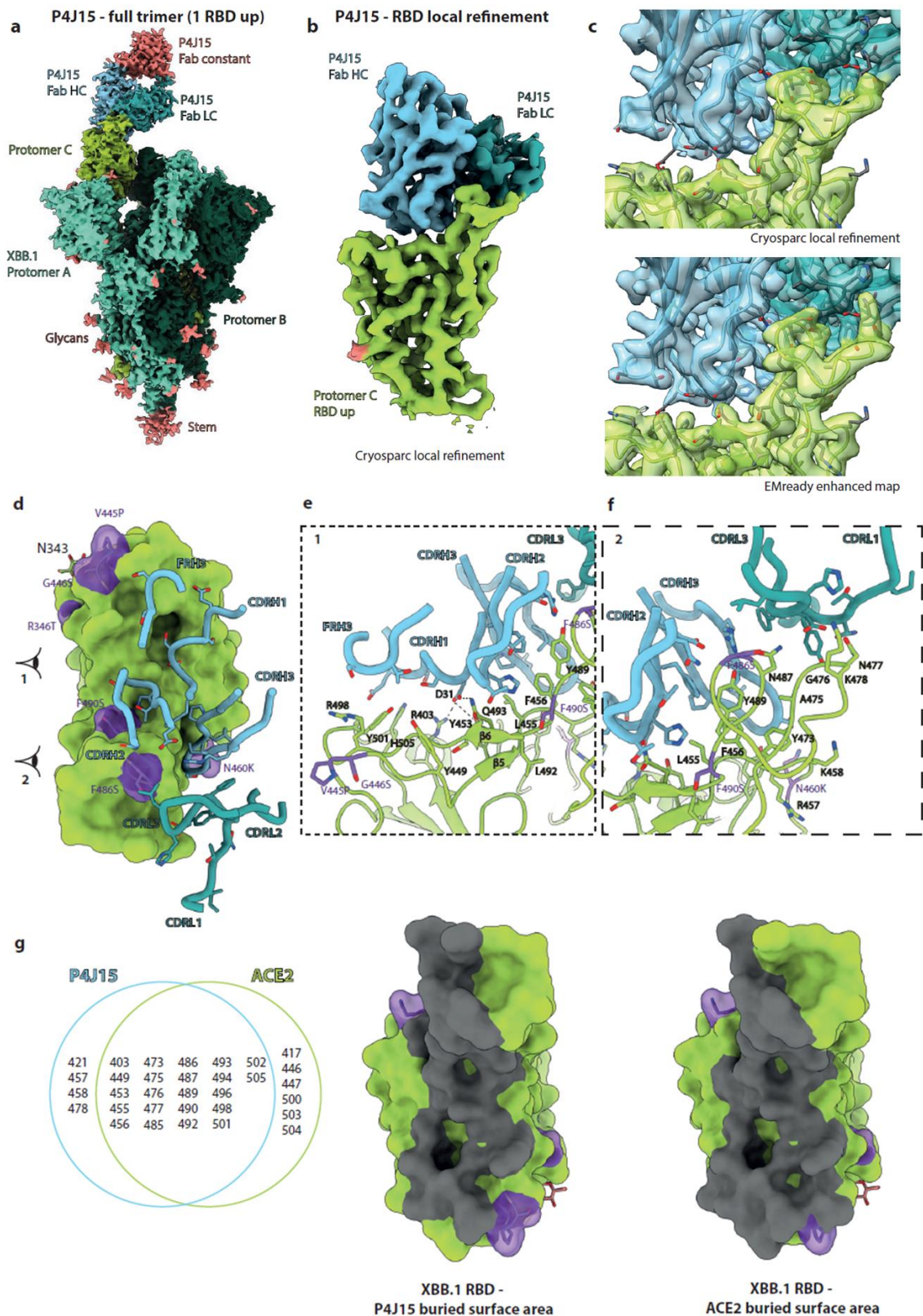
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791 **Figure 2. P4J15 binds the full-length Omicron Spike**

792 **a)** Cryo-EM composite density map of the full-length Omicron XBB.1 Spike bound to one  
 793 P4J15 Fab fragment. Spike protomers are colored in light green, green and dark green, while

794 P4J15 heavy chain variable region is cyan, the light chain variable region turquoise and Fab  
795 constant regions pink. **b)** Cryosparc local refinement map of RBD in the up configuration (light  
796 green) bound by P4J15 heavy and light chains. **c)** Zoomed-in view of RBD – P4J15 interaction  
797 with ribbon structure representation of both and semi-transparent surface representations of  
798 cryosparc local refinement and EMReady enhanced maps, shown in top and bottom panels,  
799 respectively. **d)** Top view representation of the RBD in green and P4J15 heavy and light chain  
800 contact loops shown in cyan and turquoise, respectively. **e)-f)** Front view of the RBD  
801 interaction region with P4J15 as indicated by the eye 1 and eye 2 as shown in panel d) for e)  
802 and f), respectively. RBD is represented as a ribbon structure in green, while P4J15 heavy chain  
803 CDRs and frame region 3 (FRH3) are shaded cyan and light chain CDRs are shaded turquoise.  
804 Key RBD and P4J15 contact residues are labelled and represented in stick format. **g)** Venn  
805 diagram showing common contact residues on RBD shared between P4J15 and ACE2. Buried  
806 surface area for P4J15 and ACE2 are shaded dark grey on the green space filled representation  
807 of the RBD. In panels d) and g), the XBB.1 mutations relative to Omicron BA.4 are shaded  
808 purple.

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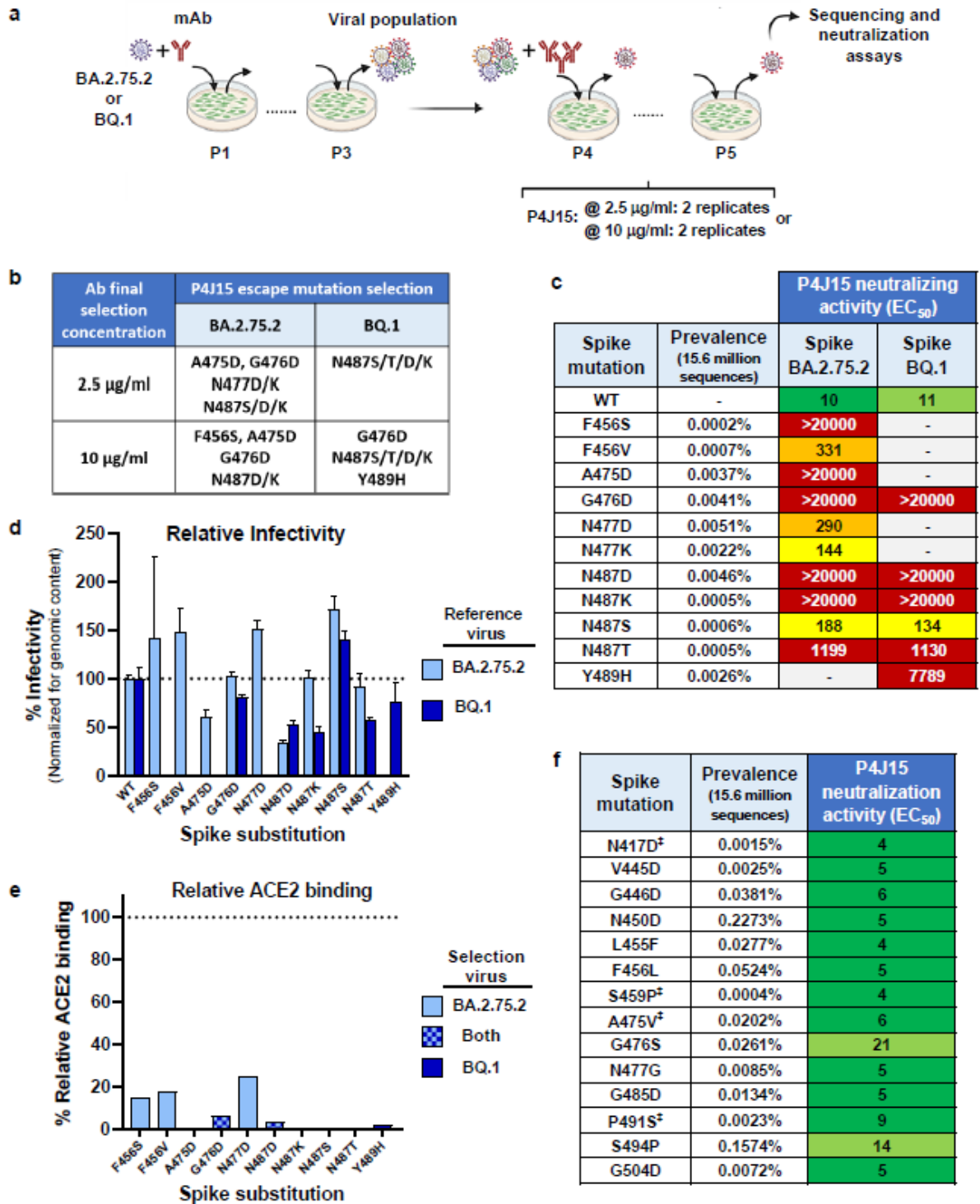
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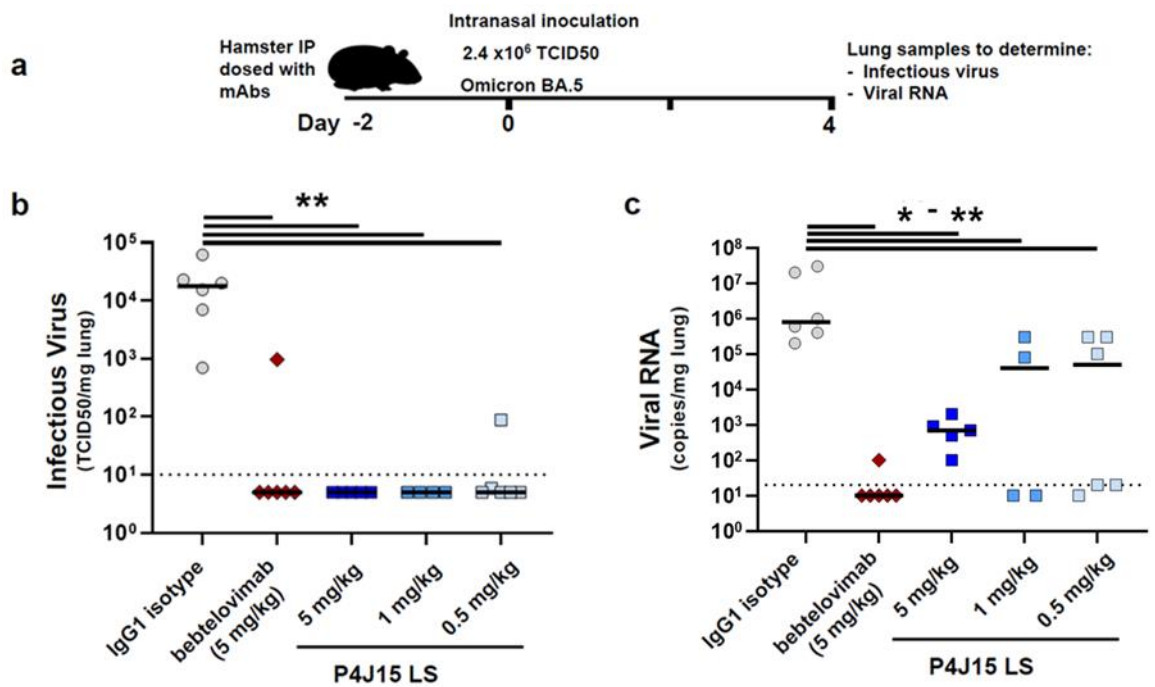
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821 **Figure 3: Identification and characterization of escape mutations to P4J15**

822 **a)** Schematic representation of escapees selection. Omicron BA.2.75.2 and BQ.1 replicative  
 823 isolates were used to infect VeroE6 cells (MOI of 0.2) each in duplicates in presence of  
 824 suboptimal concentrations of antibodies. Supernatants were collected, diluted 40-fold and used  
 825 to infect cells for two more passages in the same conditions (P1 to P3). Putative viral escapees  
 826 were further selected by serial passages of 2-fold diluted supernatants pre-incubated with high

827 concentrations of antibodies (two concentrations, each tested in duplicates). Viral RNA  
828 extracted from supernatants collected at passage 5 was deep-sequenced. **b)** Mutations identified  
829 across escape selection experiments are indicated in the table. **c)** Prevalence in GISAID  
830 sequence database is indicated for each identified mutation. BA.2.75.2 or BQ.1 Spikes were  
831 mutated accordingly with the identified residues and pseudotyped VLPs produced and tested in  
832 conventional neutralization assays. Heatmap table overviews the EC<sub>50</sub> value neutralizing  
833 potency of P4J15 against the different VLPs. **d)** Infectivity of the VLPs pseudotyped with the  
834 different Spike mutations is shown relative to the parent VLP produced with either BA.2.75.2  
835 or BA.1 Spike. Transduction efficiency was monitored by Luciferase activity in the VLPs  
836 transduced HEK293T ACE2/TMPRSS2 cells (n= 16 for all except for G476D and N487  
837 mutations where each have been tested in n=8 replicates). Infectivity is given for the same  
838 amount of each infectious VLP as determined by genome content of the stocks. Mean values ±  
839 SD are shown, and Kruskal-Wallis test shows significantly reduced infectivity of A475D,  
840 N487D (p=0.0065 and p<0.0001, respectively) for BA.2.75.2 VLPs and N487D, N487K,  
841 N487T (each p<0.0001) for BQ.1 VLPs. **e)** Relative binding of ACE2 to RBD with the indicated  
842 amino acid substitutions as determined using the ACE2-RBD interactive tool developed by  
843 Jesse Bloom's laboratory with Omicron BA.2 used as the reference variant. **f)** Pseudotyped  
844 lentiviruses or VLPs produced with rare but most common amino acid substitutions at positions  
845 identified at or near the P4J15-RBD contact site and in our resistance studies. Mutations were  
846 incorporated in the Omicron BA.4 /BA.5 Spike for lentiviruses and BQ.1 Spike for VLPs  
847 (indicated by ‡) with the prevalence in the GISAID sequence database indicated of each variant  
848 substitution. Heatmap tables (with same color ranges as in Figure 1b) overviews the neutralizing  
849 potency of P4J15 against the different pseudotyped lentiviruses (n=6) and VLPs (n=4).





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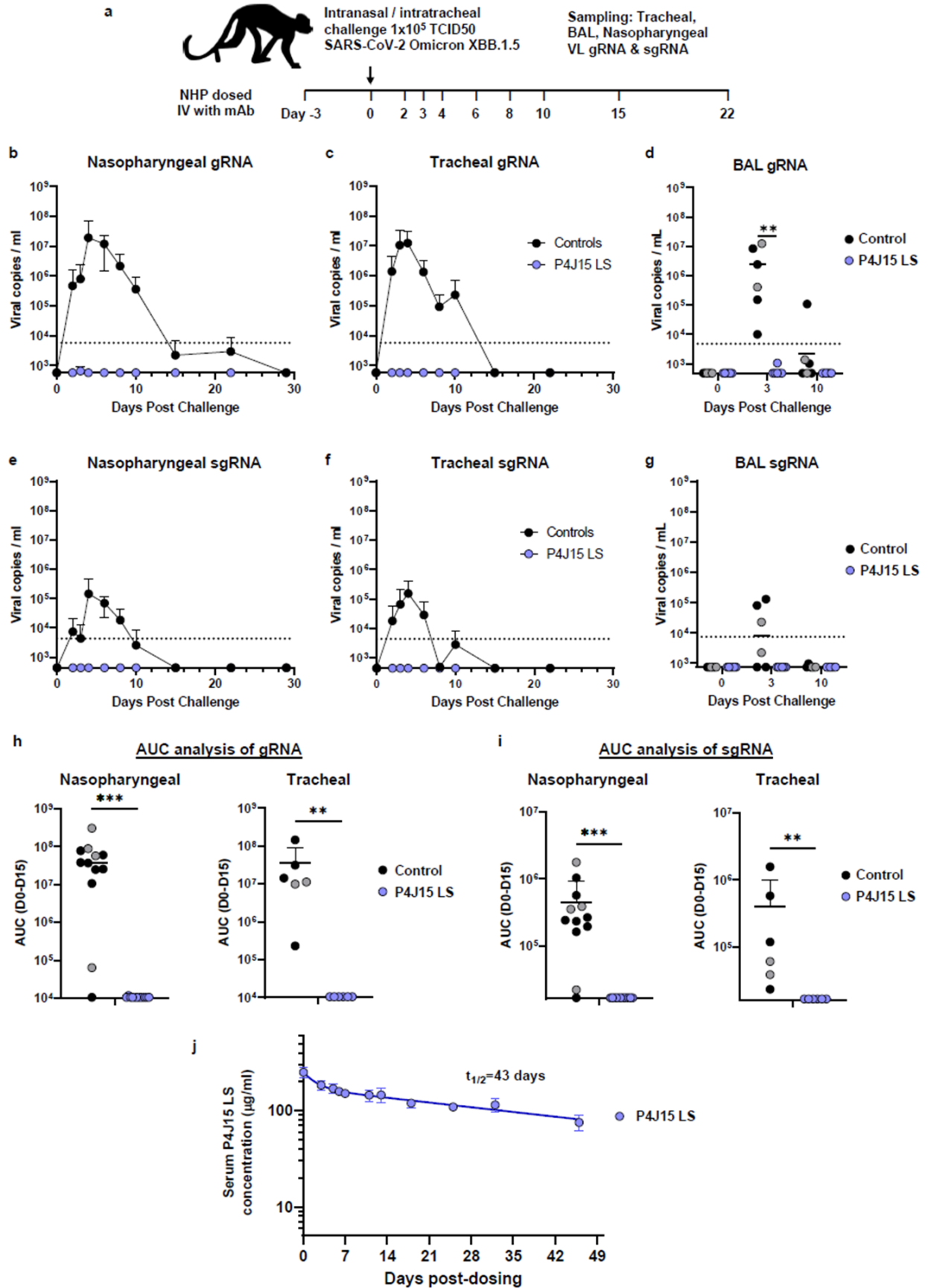
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852 **Figure 4: *In vivo* efficacy in the SARS-CoV-2 Omicron BA.5 hamster challenge model**

853 **a)** Overview of study design for the SARS-CoV-2 hamster challenge model. Animals were  
 854 administered intraperitoneally 5.0, 1.0 or 0.5 mg/kg of P4J15, 5 mg/kg of bebtelovimab positive  
 855 control or 5 mg/kg of an IgG1 isotype control and challenged two days later (Day 0) with an  
 856 intranasal inoculation of the Omicron BA.5 SARS-CoV-2 virus ( $2.4 \times 10^6$  TCID<sub>50</sub>). **b)** Median  
 857 levels of infectious virus and **c)** viral RNA copies/mg lung tissue in each of the study arms are  
 858 shown for day 4 post-inoculation with SARS-CoV-2 virus. A total of 4-6 hamsters were used  
 859 per P4J15 treatment arm. Non-parametric Mann-Whitney two-tailed tests were used to evaluate  
 860 the statistical difference between the treatment conditions with P= 0.0043, 0.0043, 0.0095 and  
 861 0.0022 (\*\*) in b (left to right) and P=0.0022, 0.0043, 0.0190 and 0.0087 (\* to \*\*) in c (left to  
 862 right).

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867 **Figure 5: *in vivo* efficacy of P4J15 against the SARS-COV-2 XBB.1.5 virus infection in**  
868 **the non-human primate (NHP) challenge model**

869 **a)** Overview of study design for the SARS-CoV-2 NHP challenge model. Six animals were  
870 administered intravenous 5 mg/kg of P4J15 LS and challenged three days later (Day 0) along  
871 with four control animals (in black) via intranasal and intratracheal inoculation of the Omicron  
872 XBB.1.5 SARS-CoV-2 virus ( $1 \times 10^5$  TCID<sub>50</sub>). Tracheal fluids, nasopharyngeal fluids and  
873 bronchoalveolar lavages (BAL) collected during the course of the study were evaluated for viral  
874 copies per ml of genomic (g)RNA **b)-d)** and subgenomic (sg)RNA **e)-g)** with data plotted to  
875 include two historical control animals (grey circles) infected with the same inoculum and batch  
876 of Omicron XBB.1.5 virus. **h)** Area under the curve (AUC) analysis of gRNA detected  
877 between days 0 and 15 of the study. Individual data for nasopharyngeal fluids collected from  
878 left and right nostril for each of the timepoints (n=12) and tracheal fluids (n=6) were plotted in  
879 left and right panels, respectively. **i)** AUC analysis of sgRNA detected between days 0 and 15  
880 of the study for samples as indicated in h. **j)** Pharmacokinetic evaluation of P4J15 LS serum  
881 concentrations with the terminal phase half-life determined using a two-phase exponential  
882 decay analysis. Mean values  $\pm$  SD are shown, and Mann-Whitney two-sided tests were  
883 performed to compare study groups in panels d, h, i with P values of 0.0022 (\*\*) for d; and  
884  $p < 0.0001$  (\*\*\*\*) and 0.0022 (\*\*) of h and i. Lower limit of detection were 2.76- and 2.63-log  
885 copies per ml for viral gRNA and sgRNA, respectively. Dotted line indicates lower limit of  
886 quantitation at 3.76- and 3.63-log copies per ml for viral gRNA and sgRNA, respectively.

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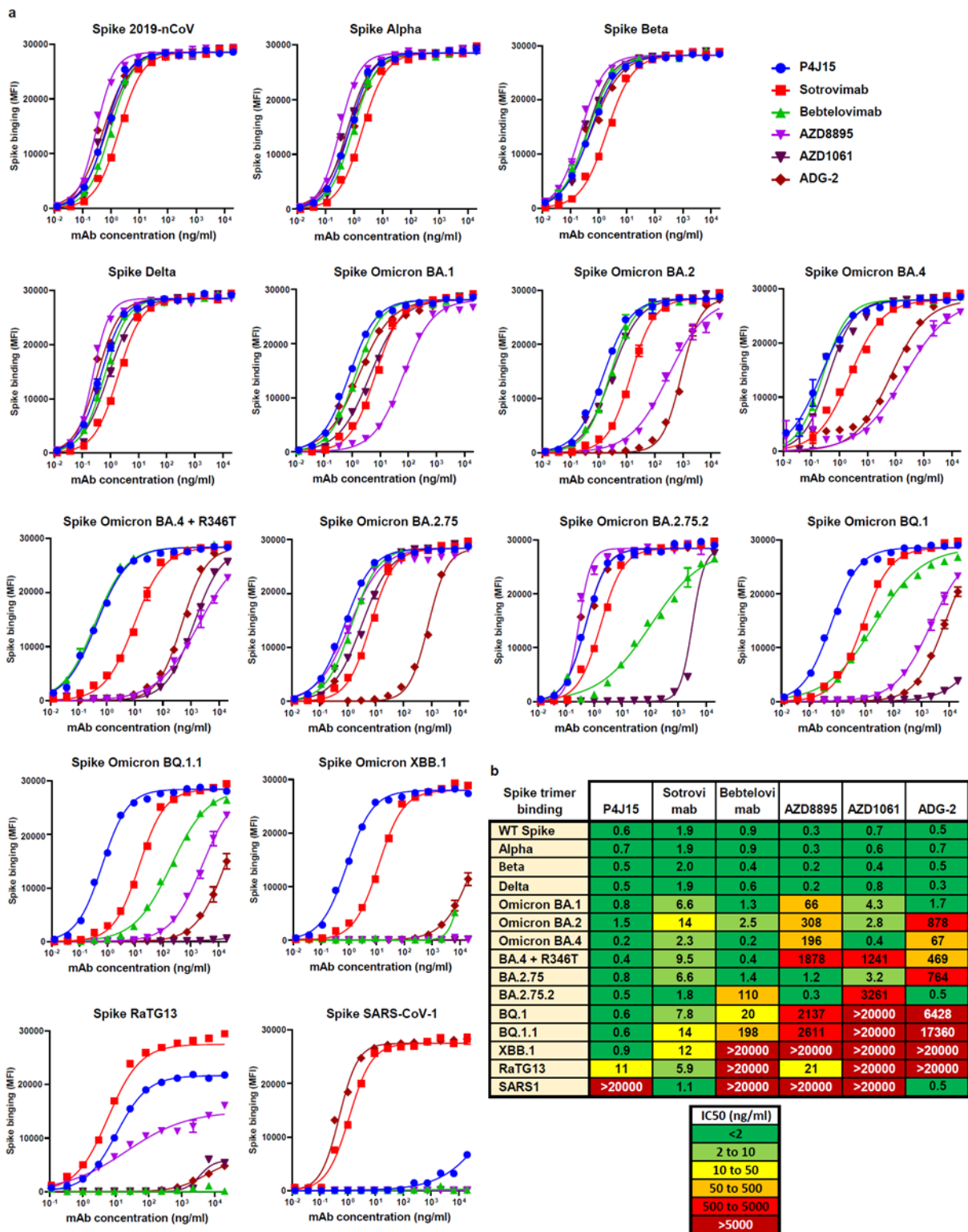
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897 **Supplementary Figures:**



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899 **Supplementary data Fig. 1 – Binding properties of P4J15 and other anti-SARS-CoV-2**  
 900 **antibodies for recombinant Spike trimer proteins from SARS-CoV-2 2019-nCoV to**  
 901 **Omicron XBB.1, and sarbecovirus RaTG13 and SARS-CoV-1 proteins.**

902 **a)** Spike binding curves performed in a Luminex bead-based assay. **b)** Heatmap table showing  
903 binding affinity IC50 values for our panel of mAbs to the indicated Spike trimer proteins. Data  
904 presented are representative of 2-4 independent experiments with each concentration response  
905 tested in duplicate. Mean values  $\pm$  SEM are shown.

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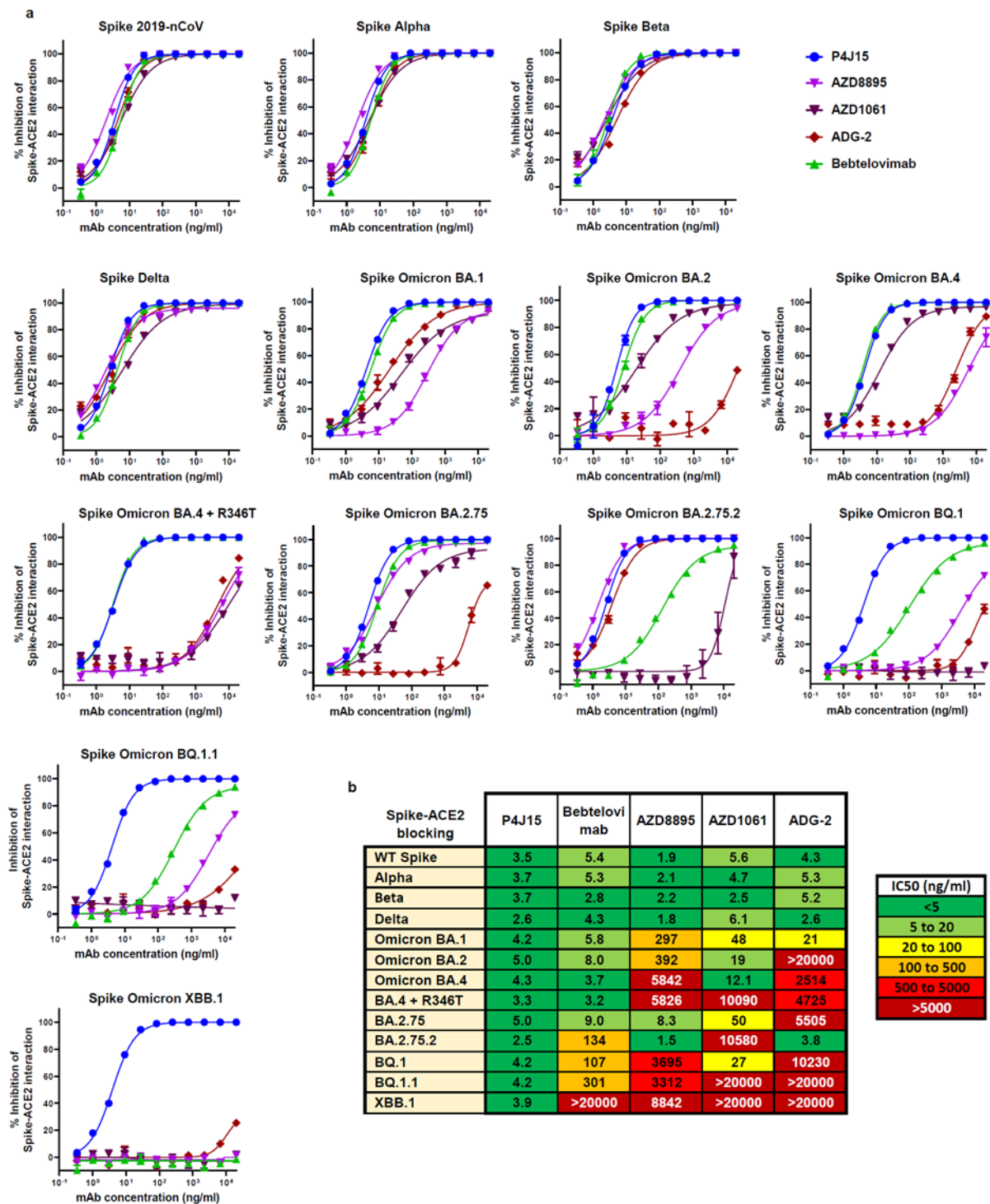
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924 **Supplementary data Fig. 2 – P4J15 is the most potent and broadly active antibody in a**  
 925 **Spike-ACE2 surrogate neutralization assay performed with trimeric Spike proteins from**  
 926 **a panel of SARS-CoV-2 variants of concern.**

927 **a) Spike-ACE2 blocking activity of P4J15 compared to a panel of authorized and clinically**  
 928 **advanced anti-Spike mAbs. b) Heatmap table showing IC<sub>50</sub> values for our panel of mAbs in the**  
 929 **Spike-ACE2 assay. Luminex based assays were performed with beads coupled with Spike**

930 trimer proteins from the original 2019-nCoV SARS-CoV-2, Alpha, Beta, Gamma, and the  
 931 different Omicron lineages listed. Sotrovimab was not included in this analysis as it binds the  
 932 RBD without blocking the Spike-ACE2 interaction. Data presented is representative of 2-4  
 933 independent experiments with each concentration response tested in duplicate. Mean values  $\pm$   
 934 SEM are shown.

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Detection of biotinylated mAbs binding to the Spike trimer								
Competition mAb pre-bound to Spike trimer	P5C3 - Biotin	AZD 8895	P4J15 - Biotin	ADG-2 - Biotin	bebtelovimab - Biotin	AZD1061 - Biotin	P2G3 - Biotin	Sotrovimab - Biotin
P4J15	Red	Red	Red	Red	White	White	White	White
P5C3	Red	Red	Red	Yellow	White	White	White	White
AZD8895	Red	Red	Red	White	White	White	White	White
ADG-2	Yellow	White	White	Red	Red	Red	Red	Yellow
Bebtelovimab	White	White	White	White	Red	Red	Red	Red
AZD1061	White	White	White	White	Red	Red	Red	Red
P2G3	White	White	White	White	Red	Red	Red	Red
Sotrovimab	White	White	White	White	Red	Red	Red	Red

Competitive <25%  
co-binding

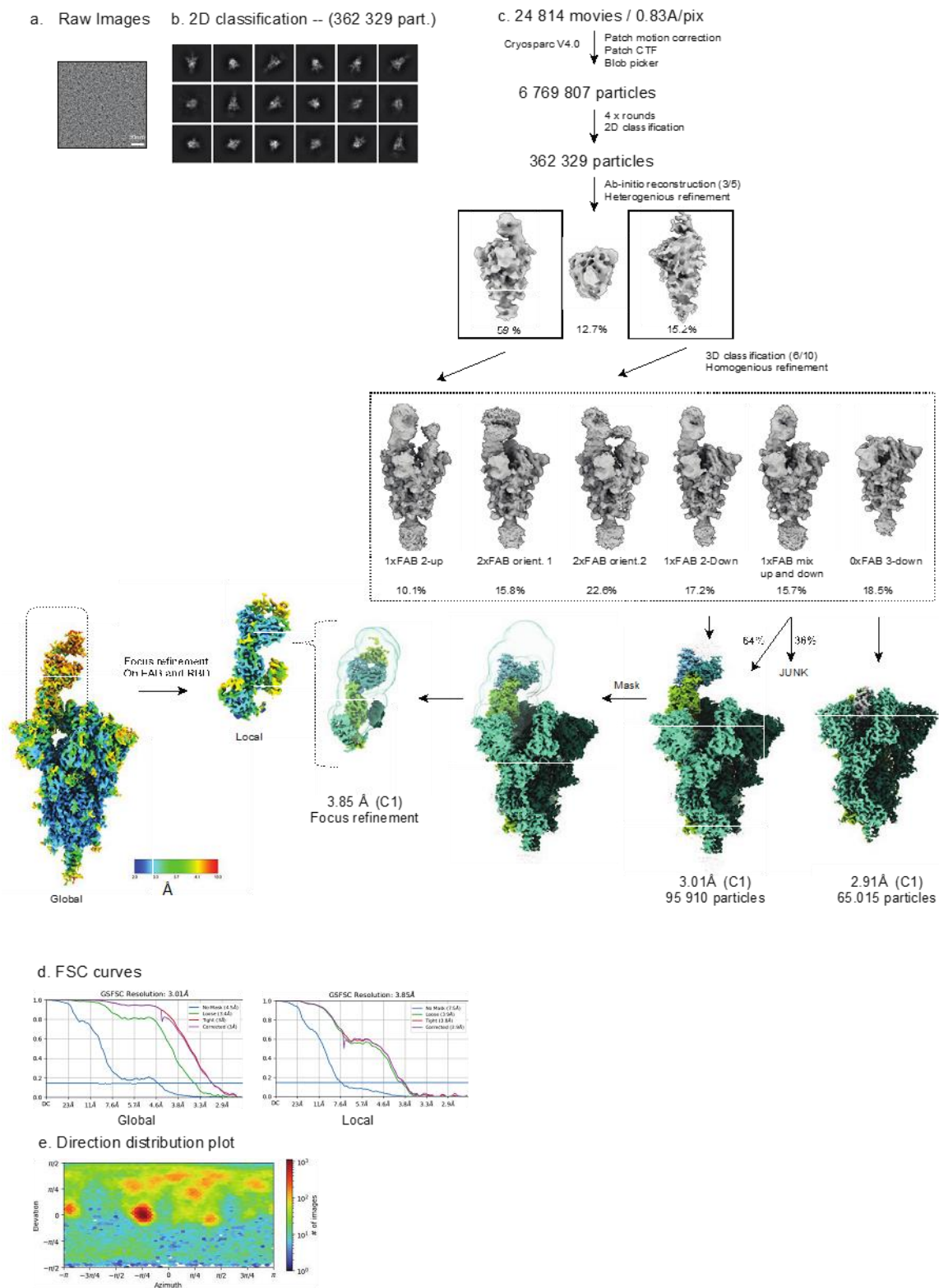
Partially competitive  
25-70% co-binding

Non-competitive >70%  
co-binding

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940 **Supplementary data Fig. 3 – P4J15 binds competitively to the Spike trimer with Class 1**  
 941 **neutralizing antibodies**

942 Competitive binding studies between antibodies binding to the 2019-nCoV Spike trimer  
 943 protein. Spike coupled beads pre-incubated with saturating concentrations of competitor  
 944 antibody were used for binding studies with the indicated biotinylated antibodies. Competitors  
 945 induced either strong blocking (Red boxes), partial competition (orange boxes) or non-  
 946 competitive (white boxes) binding with the corresponding antibody to Spike.

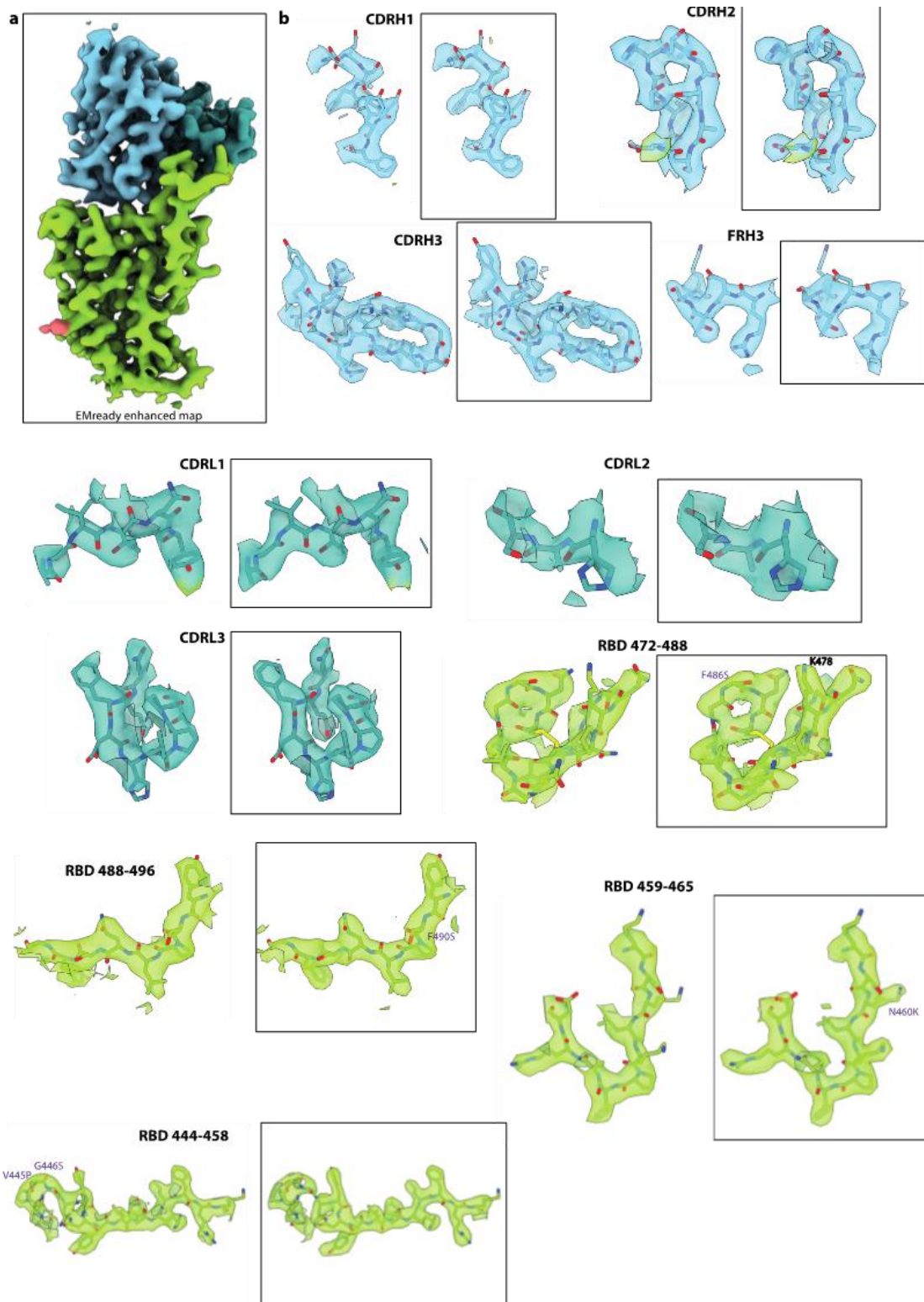


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948 **Supplementary Figure 4- Details of Cryo-EM processing and Resolution maps**

949 **a)** Raw representative micrograph. **b)** Representative 2D class averages. **c)** Cryo-EM  
 950 processing workflow performed in CryoSPARC **d)** FSC curves indicating a resolution of 3.01  
 951 Å of the full-length Omicron XBB.1 Spike bound to the P4J15 Fab and 3.85 Å for the focused  
 952 local refinement, **e)** Direction distribution plot

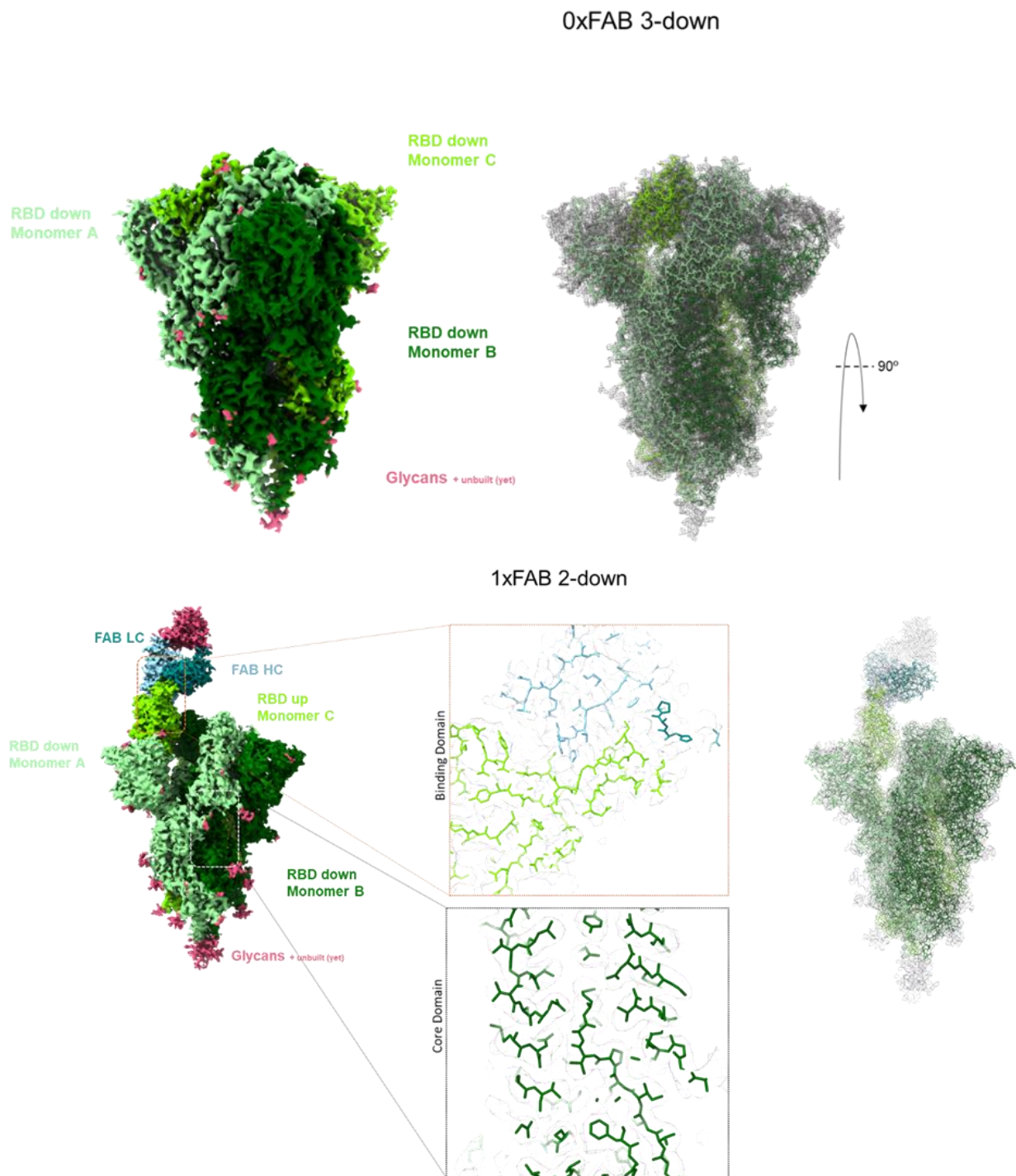




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954 **Supplementary Figure 5 - Highlights of regions of the XBB.1 RBD and P4J15 with**  
 955 **Cryo-EM density maps from Cryosparc and from EMReady.**

956 The Cryo-EM density is rendered as a mesh. The atomic model is shown as ribbon or stick  
 957 representation. Representations in a box are maps from EMReady.



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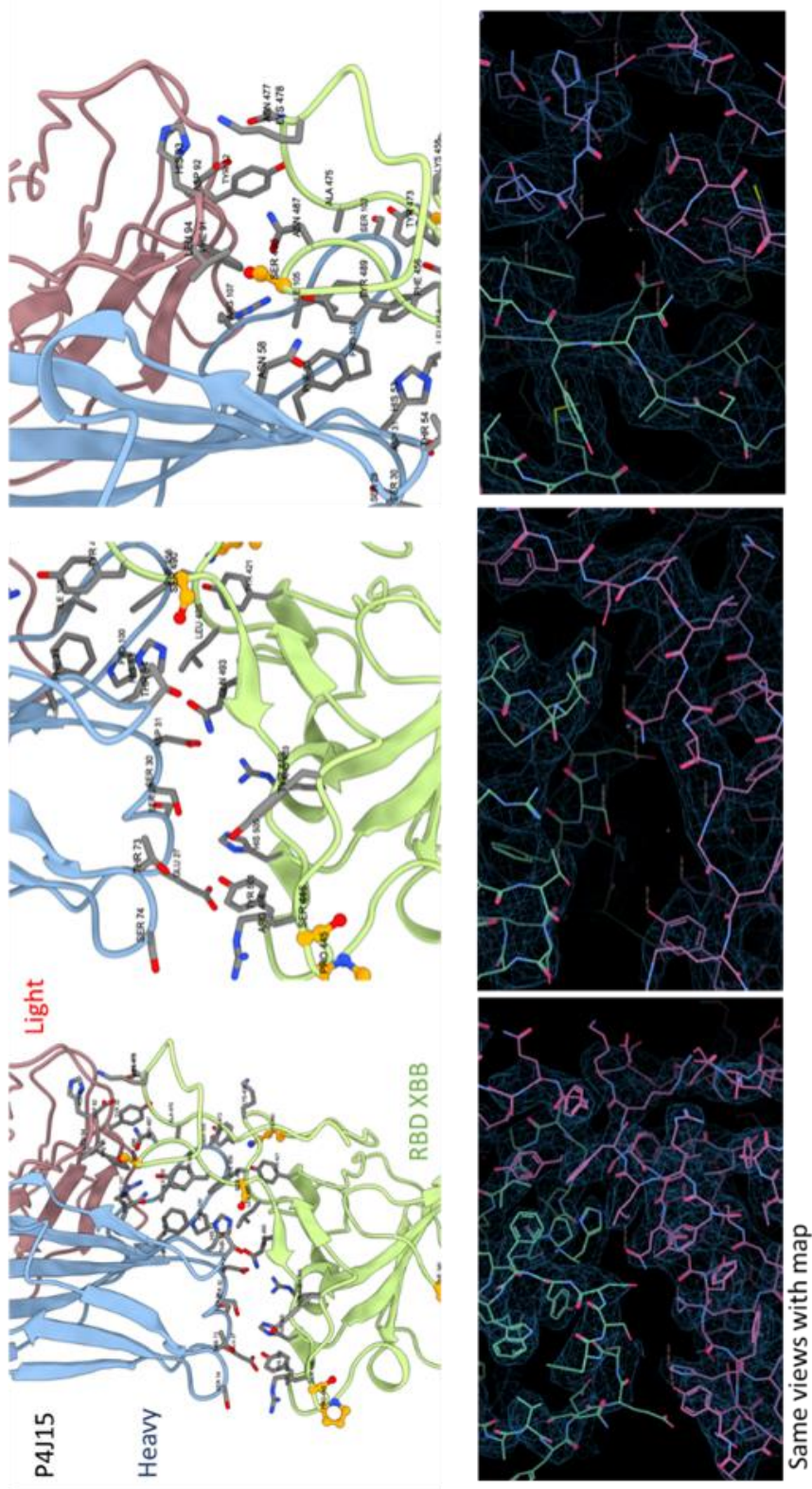
960 **Supplementary Figure 6- Details of Spike XBB.1 trimer 3D classifications.**

961 **a)** Spike trimer with all three RBDs in the down conformation at 2.91 Å resolution and **b)**  
 962 Spike trimer with P4J15 Fab bound in the RBD up conformation and the remaining two RBD  
 963 monomers in the down conformation.

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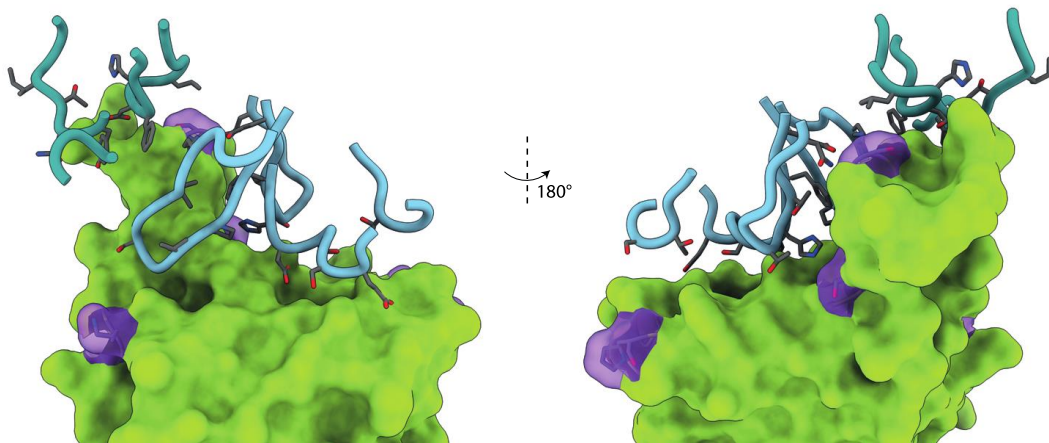


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967 **Supplementary Figure 7- Additional views of the P4J15 Fab-Omicron XBB.1 RBD**  
 968 **interactions.**

969 Ribbon structure with stick representation of contact residues for P4J15 and RBD from three  
 970 different views (top panels) with corresponding mesh representation of Cryo-EM density  
 971 (bottom panels).

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974 **Supplementary Figure 8- Back and front view representation of the RBD (green) and**  
975 **P4J15 heavy and light chain contact loops shown in cyan and turquoise**

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995 **Supplementary Table 1: Estimated new hospitalization rates in the United States and Europe**

Country / Region	Average weekly new hospitalizations: May 1 <sup>st</sup> to June 18 <sup>th</sup> , 2023, from Our World in Data	Population	New hospitalizations per week per 100000 individuals
Belgium	248	11'590'000	2,14
Czechia	81	10'709'000	0,76
Denmark	210	5'792'000	3,62
England	2133	55'977'000	3,81
Estonia	41	1'327'000	3,08
Germany	1330	83'784'000	1,59
Greece	500	10'423'000	4,80
Hungary	36	9'660'000	0,37
Ireland	207	4'938'000	4,20
Italy	777	60'462'000	1,29
Latvia	74	1'886'000	3,92
Northern Ireland	36	1'903'000	1,87
Slovakia	44	5'460'000	0,81
Spain	1867	46'755'000	3,99
Wales	4	3'210'000	0,13
Data available for Europe countries	7587	313'875'196	2,42
United States	8028	336'862'000	2,38
<b>Weekly new hospitalizations from Northern America and Europe</b>	<b>15615</b>		

996 <https://github.com/owid/covid-19-data/tree/master/public/data/hospitalizations>

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1011 **Supplementary Table 2: SARS-CoV-2 Spike variant substitutions**

<b>D614G</b>	D614G
<b>Alpha B.1.1.7</b>	Δ69-70, Δ144, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H
<b>Beta B.1.351</b>	L18F, D80A, D215G, Δ242-244, R246I, K417N, E484K, N501Y, D614G, A701V
<b>Gamma P.1</b>	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F
<b>delta B.1.617.2</b>	T19R, Δ156-157, R158G, L452R, T478K, D614G, P681R, D950N
<b>delta AY.4.2</b>	T19R, T95I, Y145H, Δ156-157, R158G, A222V, L452R, T478K, D614G, P681R, D950N
<b>Kappa B.1.617.1</b>	E154K, L452R, E484Q, D614G, P681R, Q1071H
<b>Lambda C.37</b>	G75V, T76I, R246N, del247-253, L452Q, F490S, D614G, T859N
<b>Iota B.1.526</b>	L5F, T95I, D253G, E484K, D614G, A701V
<b>Eta B.1.525</b>	Q52R, del69-70, E484K, Q677H, F888L
<b>Omicron BA.1</b>	A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F
<b>Omicron BA.1.1</b>	A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, G339D, R346K, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F
<b>Omicron BA.2</b>	T19I, Δ24-26, A27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
<b>Omicron BA.2.12</b>	T19I, Δ24-26, A27S, G142D, V213G G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, S704L, N764K, D796Y, Q954H, N969K
<b>Omicron BA.2.12.1</b>	T19I, Δ24-26, A27S, G142D, V213G G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452Q, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, S704L, N764K, D796Y, Q954H, N969K
<b>Omicron BA.2.75</b>	T19I, Δ24-26, A27S, G142D, K417E, W152R, F157L, I210V, V213G, G257S, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K

<b>Omicron BA.2.75.7</b>	T19I, Δ24-26, A27S, G142D, K417E, W152R, F157L, I210V, V213G, G257S, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A, F486S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
<b>Omicron BA.2.75.2</b>	T19I, Δ24-26, A27S, G142D, K417E, W152R, F157L, G181V, I210V, V213G, G257S, G339D, R346T, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A, F486S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, D1199N
<b>Omicron BA.4 and BA.5</b>	T19I, Δ24-26, A27S, Δ69-70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
<b>Omicron BF.7</b>	T19I, Δ24-26, A27S, Δ69-70, G142D, V213G, G339D, R346T, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
<b>Omicron BQ.1</b>	T19I, Δ24-26, A27S, Δ69-70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, K444T, L452R, N460K, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
<b>Omicron BQ.1.1</b>	T19I, Δ24-26, A27S, Δ69-70, G142D, V213G, G339D, R346T, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, K444T, L452R, N460K, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
<b>Omicron XBB.1/XBB.1.9</b>	T19I, Δ24-26, A27S, V83A, G142D, ΔY144, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486S, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
<b>Omicron XBB.1.5/ XBB.1.9.1</b>	T19I, Δ24-26, A27S, V83A, G142D, ΔY144, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486P, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
<b>Omicron XBB.1.16</b>	T19I, Δ24-26, A27S, V83A, G142D, ΔY144, H146Q, E180V, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478R, E484A, F486P, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
<b>Omicron XBB.1.16.1</b>	T19I, Δ24-26, A27S, V83A, G142D, ΔY144, H146Q, E180V, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478R, E484A, F486P, F490S, Q498R, N501Y, Y505H, T547I, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
<b>Omicron XBB.2.3</b>	T19I, Δ24-26, A27S, V83A, G142D, ΔY144, H146Q, E180V, Q183E, V213E, D253G, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478R, E484A, F486P, F490S, Q498R, N501Y, Y505H, P521S, T547I, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
<b>Omicron EG.1</b>	T19I, Δ24-26, A27S, V83A, G142D, ΔY144, H146Q, E180V, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478R, E484A, F486P, F490S, Q498R, N501Y, Y505H, T547I, Q613H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K

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1014 **Supplementary Table 3: Cryo-EM data collection and refinement statistics of SPIKE-FAB**  
 1015 **complex and SPIKE alone**

	<b>XBB - P4J15 (Closed)</b>	<b>XBB - P4J15 (Focus)</b>	<b>XBB - P4J15 FULL MAP</b>
<b>Data collection processing</b>	Awaiting AUTH	Awaiting AUTH	Awaiting AUTH
EMDB ID	17850	17819	Awaiting AUTH
PDB ID	8PSD	8PQ2	-
Detector	Falcon IV	Falcon IV	Falcon IV
Magnification	96 000	96 000	96 000
Voltage (kV)	300	300	300
Electron exposure (e-/A2)	50	50	50
Defocus range (um)	-0,25	-0,25	-0,25
Pixel size (A)	0,83	0,83	0,83
Symmetry imposed	C1	C1	C1
Initial particle images (no.)	6 769 807	6 769 807	6 769 807
Final particle images (no.)	65 015	95 910	95 910
Map resolution (A)	2,91	3,85	3,01
FSC threshold	0,143	0,143	0,143
Extraction box size (A)	457	457	457
Fourrier crop to box size (A)	299	299	299
Map pixel size (A)	1,268	1,268	1,268
<b>Refinement</b>			
Initial models used (PDB codes)	7q07	7q07	-
Model resolution (A)	2,9	3,7	-
FSC threshold	0,143	0,143	-
Map sharpening B factors (A2)	51,4	70,7	-
<b>Model composition</b>			
Non-hydrogen atoms	48535	5969	-
Protein residues	3076	390	-
Nucleotides	0	0	-
Ligands	36	1	-
<b>B factors (A2)</b>			
Protein *	74,67	69,96	-
DNA *	-	-	-
<b>R.m.s. deviation</b>			
Bond lenghts (A)	0,003	0,002	-
Bond angles (A)	0,47	0,501	-
Validation			
MolProbrity score	1,44	2,13	-
Clashscore	2,2	5,53	-
Poor rotamers (%)	1,6	2,73	-
<i>Ramachandran plot</i>			
Favored (%)	95,76	91,67	-
Allowed (%)	4,24	8,33	-
Disallowed (%)	0	0	-
<i>Model-to-data fit</i>			
Ccmask	0,81	0,79	-
Ccbox	0,64	0,6	-
Ccpeaks	0,6	0,44	-
CCvolume	0,79	0,66	-

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