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Neutralizing monoclonal antibodies

elicited by mosaic RBD nanoparticles

bind conserved sarbecovirus epitopes

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Summary

Protection from SARS-related coronaviruses with spillover potential and SARS-CoV-2 variants could prevent and/or end pandemics. We show that mice immunized with nanoparticles co-displaying spike receptor-binding domains (RBDs) from eight sarbecoviruses (mosaic-8 RBD-nanoparticles) efficiently elicit cross-reactive anti-sarbecovirus antibodies against conserved class 1/4 and class 3 RBD epitopes. Monoclonal antibodies (mAbs) identified from initial screening of <10,000 single B-cells secreting IgGs binding two or more sarbecovirus RBDs showed cross-reactive binding and neutralization of SARS-CoV-2 variants and animal sarbecoviruses. Single-particle cryo-EM structures of antibody–spike complexes, including a Fab-Omicron complex, mapped neutralizing mAbs to conserved class 1/4 RBD epitopes and revealed neutralization mechanisms, potentials for intra-spike trimer crosslinking by single IgGs, and induced changes in trimer upon Fab binding. In addition, we identified a mAb resembling Bebtelovimab, an EUA-approved human class 3 anti-RBD mAb. These results support using mosaic RBD-nanoparticles to identify therapeutic pan-sarbecovirus and pan-variant mAbs and to elicit them by vaccination.

Keywords

Coronavirus, SARS-CoV-2, COVID-19, mosaic nanoparticle, neutralizing antibodies, vaccine design, cryo-electron microscopy

Introduction

Spillover of animal SARS-like betacoronaviruses (sarbecoviruses) resulted in two human health emergencies in the past 20 years: the SARS-CoV epidemic in the early 2000s and the current COVID-19 pandemic caused by SARS-CoV-2. Large coronavirus reservoirs in bats are predictive of future cross-species transmission (Menachery et al., 2015; Menachery et al., 2016; Zhou et al., 2021), necessitating a vaccine that could protect against emerging coronaviruses. In addition, SARS-CoV-2 variants have been discovered throughout the current pandemic, with the Alpha, Beta, Delta, Gamma, and Omicron lineages designated as variants of concern (VOCs) due to apparent increased transmissibility and/or resistance to neutralizing antibodies elicited by infection or vaccination (Burki, 2021; Liu et al., 2021; Planas et al., 2021; Washington et al., 2021). In the case of Omicron VOCs, a large number of substitutions in the SARS-CoV-2 spike protein receptor-binding domain (RBD), the major target of neutralizing antibodies (Barnes et al., 2020a; Brouwer et al., 2020; Cao et al., 2020; Kreer et al., 2020; Liu et al., 2020b; Piccoli et al., 2020; Pinto et al., 2020; Robbiani et al., 2020; Rogers et al., 2020; Seydoux et al., 2020; Zost et al., 2020) and detectable cross-variant neutralization (Bowen et al., 2021), results in reduced efficacies of vaccines and therapeutic monoclonal antibodies (mAbs) (Liu et al., 2021; Starr et al., 2021).

Comparison of the variability of RBDs across sarbecoviruses (Figure S1) and within SARS-CoV-2 VOCs and variants of interest (VOIs) suggest that vaccines and mAbs targeting the more conserved neutralizing antibody epitopes (class 4 and class 1/4; nomenclature from (Barnes et al., 2020a; Jette et al., 2021)) (Figure 1A,B) could protect against present and future SARS-CoV-2 VOCs and prevent future sarbecovirus spillover events from causing another epidemic or pandemic. By contrast, antibodies targeting the less conserved class 1 and class 2 RBD epitopes that directly overlap with the binding footprint for human ACE2, the SARS-CoV-2 host receptor, recognize a portion of the RBD that exhibits sequence variability between sarbecoviruses (Barnes et al., 2020a) (Figure 1A), which is also where VOC and VOI substitutions accumulate (Figure 1B; Figure S2). Class 3 RBD epitopes are more conserved than class 1 and class 2 epitopes but exhibit some variation across sarbecoviruses (Figure 1B; Figure S2), suggesting the potential for continued variability amongst SARS-CoV-2 VOCs.

We previously described a vaccine approach involving simultaneous display of sarbecovirus RBDs on protein-based nanoparticles (Figure 1C) that showed enhanced heterologous binding, neutralization, and protection from sarbecovirus challenges compared with homotypic (SARS-CoV-2 RBD–only) nanoparticles in animal models (Cohen et al., 2021; Cohen

71   et al., 2022). The hypothesis behind enhanced elicitation of cross-reactive antibodies by mosaic

72   RBD-nanoparticles is that B cell receptors (BCRs) that recognize conserved, but not readily

73   accessible, RBD epitopes would be stimulated to proliferate to produce cross-reactive Abs through

74   bivalent binding of BCRs to adjacent RBDs, whereas BCRs that bind to strain-specific epitopes

75   could not bind bivalently to adjacent RBDs since these epitopes differ when RBDs are arranged

76   randomly on a nanoparticle (Figure 1D) (Cohen et al., 2022). By contrast, homotypic RBD-

77   nanoparticles are predicted to stimulate BCRs against readily accessible, immunodominant strain-

78   specific epitopes presented on all RBDs. Of relevance to the mAb potentials for sarbecovirus cross-

79   reactivity, the more conserved class 4 and class 1/4 epitopes targeted by polyclonal antibodies in

80   mosaic-8 RBD-nanoparticle antisera are unlikely to vary in SARS-CoV-2 VOCs because they

81   contact other portions of the spike trimer, by contrast to the class 1 and 2 RBD epitope regions

82   targeted by homotypic SARS-CoV-2 RBD-nanoparticle antisera, which (in common with the class

83   3 RBD region) are not involved in contacts with non-RBD portions of spike (Cohen et al., 2022).

84   Although the epitopes of both class 4 and class 1/4 anti-RBD mAbs fall outside of the ACE2-

85   binding footprint within class 1 and class 2 RBD epitopes, these mAbs can directly compete with

86   ACE2 for binding through steric effects, thereby rationalizing their increased neutralization

87   potencies (Jette et al., 2021; Liu et al., 2020a; Tortorici et al., 2021).

88   Here we investigated the RBD epitopes of mAbs isolated from mosaic RBD- and

89   homotypic RBD-immunized mice to characterize the antibody response to RBD nanoparticles.

90   Binding and neutralization results, together with cryo-EM structures of antibody Fab-spike trimer

91   complexes, suggested that the mosaic RBD-nanoparticle vaccine approach works as designed to

92   target conserved epitopes, and could be used both for more broadly protective vaccines and as a

93   method to produce therapeutic neutralizing mAbs that would not be affected by Omicron or future

94   SARS-CoV-2 VOC substitutions.

95

96   **Results**

97   **The majority of mosaic-8 elicited mouse mAbs identified as binding two or more RBDs are**

98   **cross-neutralizing**

99   We made multimerized RBD-nanoparticles using the SpyCatcher-SpyTag system (Brune

100   et al., 2016; Zakeri et al., 2012) to covalently attach RBDs with C-terminal SpyTag003 sequences

101   to a 60-mer nanoparticle (SpyCatcher003-mi3) (Keeble et al., 2019). As previously described, we

102   produced and characterized nanoparticles presenting randomly arranged RBDs from SARS-CoV-

103   2 and seven animal sarbecovirus spikes (mosaic-8 RBD-mi3) as well as nanoparticles presenting

104   only SARS-CoV-2 RBDs (homotypic SARS-CoV-2 RBD-mi3) (Cohen et al., 2021) (Figure S1).

105   Mice were primed and boosted with either mosaic-8 or homotypic SARS-CoV-2 RBD-

106   nanoparticles in AddaVax adjuvant. We used a Berkeley Lights Beacon Optofluidic system to

107   screen a subset of B cells for binding to one or more labeled RBDs (Figure S3). B cells secreting

108   IgGs that bound to at least one RBD were exported from the instrument, and the variable domains

109   of heavy and light chain genes were sequenced and subcloned into expression vectors containing

110   genes encoding human IgG C_H1-C_H2-C_H3 domains, human C_H1, or human C_L domains. From 39

111   exported cells, we isolated genes for 15 RBD-binding mAbs (Table S1) that were expressed as

112   IgGs and corresponding Fabs. The 15 unique IgG sequences included 13 that were derived from

113   mosaic-8 immunized mice and identified during the screen as binding to two or more labeled RBDs

114   (six mAbs) or to one labeled RBD (seven mAbs), and two were derived from homotypic RBD-

115   nanoparticle immunized mice and identified as binding to two or more labeled RBD (Figure 2A)

116   (Table S1). Two mAbs from mosaic-8 immunized mice were excluded from analyses after

117   showing no detectable binding to purified RBDs (Table S1).

118   We first evaluated binding of the 13 purified mAb IgGs to RBDs from SARS-CoV-2

119   variants and other sarbecoviruses using enzyme-linked immunosorbent assays (ELISAs). RBDs

120   were included from sarbecoviruses clades 1a, 1b, 2, and 3 clades (as defined in (Starr et al., 2022))

121   (Figure 2A). We compared the mAb binding profiles to four human anti-RBD IgGs with known

122   epitopes: C118, a broadly cross-reactive class 1/4 mAb isolated from a convalescent COVID-19

123   donor (Jette et al., 2021; Robbiani et al., 2020), S309 (Sotrovimab), a cross-reactive class 3 mAb

124   isolated from a SARS-CoV–infected donor (Pinto et al., 2020), and mAbs isolated from COVID-

125   19 donors that bind to more variable RBD epitopes overlapping with the ACE2-binding footprint

126   (Robbiani et al., 2020): C102 (class 1) and C144 (class 2) (Figure 2A). Of the seven mAbs isolated

127   from B cells from vaccinated mice that were identified during screening as secreting IgGs that

128   bound to more than one RBD (Figure 2A), five were isolated from mosaic-8 RBD-nanoparticle-

129   immunized mice (names with M8a prefixes), and two were isolated from homotypic RBD-

130   nanoparticle-immunized mice (names with HSW prefixes). These seven mAbs showed binding to

131   SARS-CoV-2 spike trimer and SARS-CoV-2 RBDs including Beta, Delta, and Omicrons BA.1

132   and BA.2 in addition to the SARS-CoV-2 WA1 variant included in the mosaic-8 RBD

133   nanoparticles, as well as cross-reactive binding to animal sarbecovirus RBDs (Figure 2A). The

134   half maximal effective concentrations (EC_{50} values) for binding of these mAbs to most of the

135   RBDs ranged from 1 to 10,000 ng/mL (Figure 2A). By comparison, six mAbs isolated from B

136   cells that secreted IgGs that bound only one RBD during screening recognized a smaller subset of

137   RBDs evaluated in our ELISA panel, and none bound to SARS-CoV-2 spike (Figure 2A). Some

138   of these mAbs might prove useful for detecting whether particular RBDs are present on a mosaic

139   RBD-nanoparticle: for example, M8a-7 and M8a-29 could be used to detect WIV1 or Rs4081,

140   respectively, on mosaic-8 RBD-nanoparticles. We proceeded with further analysis of the seven

141   mAbs that were isolated from B cells secreting IgGs that bound to two or more RBDs during

142   screening (Figure 2A).

143   The five M8a IgGs and two HSW IgGs that showed cross-reactive RBD binding during

144   screening and by ELISA shared amino acid sequence identities of ~50% to 90% in their V_H and

145   V_L domains (Figure S4A,B) and had varied lengths for their complementarity-determining regions

146   3 (CDR3s), which are often critical in antigen recognition (Davies and Metzger, 1983): the mAb

147   CDR3s ranged from 9-16 residues for the heavy chain CDR3 (CDRH3) and all were 9 residues for

148   the light chain CDR3 (CDRL3) (Figure S4C), compared with 11 (IgH) and 9 (Igκ) for average

149   C57Bl/6 mouse antibody CDR3s (Rettig et al., 2018). The CDRH1, CDRH2, and CDRL2 regions

150   were the same lengths across the seven mAbs, whereas the CDRL1 ranged from 6-12 residues

151   (Figure S4). M8a-34 and HSW-1 both had long (16 residues) CDRH3s, and M8a-31 had the

152   shortest (9 residues) CDRH3. By contrast to its shorter than average CDRH3, M8a-31 had the

153   longest CDRL1 (12 residues) compared with M8a-3, M8a-6, M8a-28, and HSW-2, which all

154   included six-residue CDRL1s (Figure S4C). M8a-3 and M8a-6, related by high sequence identities

155   (87.6% for V_H and 89.7% for V_L) (Figure S4B) and the shared V gene segments (IgH V1-69 and

156   Igκ V6-25) (Figure S4A, Table S1), both contained 14-residue CDRH3s and six-residue CDRL1s

157   (Figure S4C), yet M8a-3 showed a broader RBD binding profile by ELISA, such that it bound all

158   RBDs evaluated except for the clade 2 Rf1 and clade 3 BM4831 RBDs, whereas M8a-6 did not

159   bind detectably to any of the three clade 3 RBDs or to three of the clade 1a and clade 2 RBDs

160   (Figure 2A). M8a-28 showed weak binding to some non-SARS-2 RBDs of clade 1b (RsSTT200

161   and Pang17), clade 1a (SHC014 and LyRa3) and clade 2 (Rs4081, RmYN02 and Yun11), and

162   weak or no binding to RBDs of clade 3 (weak for BtKY72 and Khosta-2, and no binding to

163   BM4831 RBD of clade 3 (Figure 2A). In contrast, HSW-2 showed binding to RBDs from all clades

164   except SARS-CoV from clade 1a (Figure 2A). M8a-31 and M8a-34 recognized all RBDs in the

165   ELISA panel (Figure 2A). Although M8a-34 and HSW-1 shared a sequence identity of 75.3% for

166   V_H and 88.3% for V_L with the same light chain IgκV3-5 V gene segment (Figure S4A, Table S1),

167   and both had 16-residue CDRH3s and 10-residue CDRL1s (Figure S4B,C), HSW-1 was not as

168   broadly cross-reactive by ELISA, showing no detectable binding to RBDs of SARS-CoV (clade

169   1a), Yun 11 (clade 2), or BM4831 and Khosta2 (clade 3) (Figure 2A).

170   We next measured neutralization potencies using a pseudovirus neutralization assay

171   (Crawford et al., 2020) against sarbecoviruses known to use human ACE2 for target cell entry,

172   including SARS-CoV-2 WA1 D614G, SARS-CoV-2 Beta, SARS-CoV-2 Delta, SARS-CoV-2

173   Omicron BA.1 and BA.2, SARS-CoV, WIV1, SHC014, a modified BtKY72 (Starr et al., 2022)

174   Khosta2/SARS-CoV Chimera, and LyRa3/SARS-CoV Chimera (Figure 2B). Among all, M8a-3

175   was the most consistently cross-reactively potent, with low half-maximal inhibitory concentrations

176   (IC_{50} values) against all pseudoviruses evaluated (Figure 2B). Despite sharing high sequence

177   identity, the same V gene segments, and similar CDR characteristics with M8a-3 (Figure S4),

178   M8a-6 showed no neutralizing activity except for weak activity against SHC014 and BtKY72. A

179   less related mAb, M8a-28, was a potent neutralizer, but only against SARS-CoV-2 variants. The

180   M8a-31 and M8a-34 mAbs were less potent against SARS-CoV-2 variants, but were more broadly

181   cross-reactive, correlating with their ELISA profiles (Figure 2B). By contrast to the five M8a

182   mAbs, the HSW-1 and HSW-2 mAbs isolated from homotypic SARS-CoV-2 RBD nanoparticle-

183   immunized mice identified as binding two or more RBDs during screening showed overall weaker

184   neutralizing potencies, with eight of 18 assays showing no neutralizing activity and most of the

185   remaining assays showing IC_{50} values above 10 µg/mL (Figure 2B).

186   To identify RBD epitopes for the mAbs, we used a binding assay to assess potential

187   competition with proteins that bind to known RBD epitopes. For this competition assay, we used

188   the four human anti-RBD mAbs with known epitopes that were used as controls for ELISAs

189   (Figure 2A): C118 (class 1/4), S309 (class 3), C102 (class 1), and C144 (class 2), along with other

190   potential competitor mAbs or control mAbs: C022 (class 1/4) (Jette et al., 2021; Robbiani et al.,

191   2020), CR3022 (class 4) (Huo et al., 2020), COVA1-16 (Liu et al., 2020a), C135 (class 3), C110

192   (class 3), C105 (class 1) (Robbiani et al., 2020), and a soluble human ACE2-Fc construct (Jette et

193   al., 2021). The ELISA revealed the expected competition for the characterized human mAbs

194   (Figure S5), validating its use for mapping RBD epitopes for the seven mouse mAbs elicited by

195   RBD-nanoparticle immunization. Three of the five m8a mAbs (M8a-3, M8a-31, and M8a-34)

196   mapped to class 1/4 and/or class 4 epitopes, M8a-28 mapped to the class 3 RBD region, and Ma-

197   6 did not compete with any of the labeled anti-RBD IgGs. The identification of a class 3 RBD

198   epitope for M8a-28 rationalized its potent neutralization of SARS-CoV-2 WA1 and VOCs and

199   limited cross-reactive neutralization of animal sarbecoviruses (Figure 2B), by contrast with the

200   class 1/4 RBD epitope identification for the remaining less potently neutralizing M8a mAbs (M8a-

201   3, M8a-31, and M8a-34), since this class of anti-RBD mAb tends to show less potent

202   neutralization, but broader sarbecovirus cross-reactivity, than other classes due to the more

203   occluded nature of the class 1/4 epitope (Cohen et al., 2022; Jette et al., 2021; Tortorici et al.,

204   2021). Of the two HSW mAbs, HSW-1 did not exhibit any detectable competition, and HSW-2

205   showed competition with CR3022, a class 4 anti-RBD mAb. Overall, these results demonstrated

206   that the majority of the mAbs identified during Beacon screening mapped to the more conserved

207   class 1/4, 4, and 3 RBD epitopes.

208

209   **Cryo-EM structures of Fab-spike trimer complexes reveal cross-reactive recognition and**

210   **rationalize neutralization results**

211   To deduce recognition and neutralization mechanisms, we performed structural analyses

212   of the seven mAbs identified as binding two or more RBDs during the Beacon isolation (Figure

213   2A). Single-particle cryo-EM was used to solve structures of complexes of a SARS-CoV-2 6P

214   spike trimer (Hsieh et al., 2020) and a mAb Fab, five of which were derived from M8a IgGs

215   isolated in mosaic-8 nanoparticle–immunized mice (Figure 3, Figure 4A) and two from HSW IgGs

216   elicited in homotypic SARS-CoV-2 RBD nanoparticle–immunized mice (Figure 5) (Figure S6-13,

217   Table S2).

218   Each of the five M8a mAb Fab structures were solved as complexes with the SARS-CoV-

219   2 WA1 spike trimer, and the M8a-31 Fab was also solved complexed with the Omicron BA.1 spike

220   (Figure 3A-F; Figure S6-S11; Table S2). For five of the six M8a-spike structures (WA1 spike plus

221   Fabs from M8a-3, M8a-31, M8a-34, M8a-28 and Omicron BA.1 spike plus M8a-31 Fab), we

222   observed one Fab bound to each of the three RBDs, which were in 'up' positions in all cases except

223   for the M8a-28–spike structure in which all three RBDs were in the 'down' conformation (Figure

224   3C). In the remaining structure (M8a-6–spike), we found only one well-resolved Fab per trimer.

225   A 3.1 Å resolution M8a-3 Fab–spike complex structure revealed Fab V_H-V_L interactions

226   with 'up' RBDs using all six CDRs along with residues within the light chain framework region 2

227   and 3 (FWRL2 and FWRL3) (Figure 3A, 4B, Figure S6, S14A). Consistent with the competition

228   ELISA results (Figure S5), comparison of the M8a-3 Fab-RBD interaction with previously-

229   characterized representative anti-RBD antibodies in different structural classes (Barnes et al.,

230   2020a; Jette et al., 2021) showed overlap with the class 1 and class 4 RBD epitopes (Figure S14A)

231   and a binding footprint adjacent to that of ACE2 (Figure 3A, 4A), similar to the human mAb C118,

232   a class 1/4 anti-RBD antibody that blocks ACE2 binding without substantially overlapping with

233   the ACE2 binding footprint (Jette et al., 2021) and that competes for binding to RBD with M8a-3

234   (Figure S5). The M8a-3–spike structure showing recognition of a largely conserved region of the

235   RBD (Figure 4B) was consistent with ELISA and neutralization results demonstrating that M8a-3

236   neutralized and/or bound to most of the sarbecoviruses and the SARS-CoV-2 variants tested

237   (Figure 2).

238   A 3.2 Å spike trimer structure complexes with the related, but mostly non-neutralizing

239   M8a-6 mAb, showed three 'up" RBDs, but only one well-resolved Fab (Figure 3B; Figure S7,

240   S14B), which we modeled using information from a partially-refined 3.0 Å M8a-6 Fab–RBD

241   crystal structure. The M8a-6 Fab shared a similar RBD binding epitope and approach angle as

242   M8a-3 (Figure 3A, 4A, Figure S14B), interacting with the RBD using all six CDRs plus framework

243   regions FWRH2, FWRL2, and FWRL3 (Figure 4C). Furthermore, M8a-6 also recognized a similar

244   epitope as the C118 (Jette et al., 2021) and M8a-3 mAbs, involving mostly conserved RBD

245   residues (Figure 4C, Figure S14B). Despite sharing high sequence identity and similar binding

246   epitopes on SARS-CoV-2 RBD with M8a-3, M8a-6 was non-neutralizing against SARS-CoV-2

247   and only weakly neutralizing against SHC014, whereas M8a-3 neutralized SARS-CoV-2 D614G

248   with a 0.18 µg/mL IC_{50} (Figure 2B). These different neutralization profiles likely result from a

249   weaker interaction of M8a-6 as compared with M8a-3 with CoV spikes, as demonstrated by

250   incomplete binding of Fabs in the M8a-6–spike complex cryo-EM structure and the lack of

251   competition of M8a-6 IgG with any of the biotinylated IgGs, including C118, with known epitopes

252   (Figure S5).

253   In common with M8a-3, M8a-31 exhibited broadly cross-reactive binding and

254   neutralization across SARS-CoV-2 variants and other sarbecoviruses (Figure 2) and competed

255   with class 1/4 and class 4 anti-RBD antibodies (Jette et al., 2021) (Figure S5). Single-particle cryo-

256   EM structures were determined for M8a-31 Fab bound to SARS-CoV-2 WA1 (Figure 3C, Figure

257   S8) and to Omicron BA.1 (Figure 3D, Figure S9) spike trimers at resolutions of 2.9 Å and 3.5 Å,

258   respectively. In both structures, three M8a-31 Fabs interacted with 'up' RBDs (Figure 3C,D,

259   Figure S14C,D). Despite the 15 substitutions in the Omicron BA.1 RBD compared with the WA1

260   RBD, the epitope and binding pose of the M8a-31 Fab in both structures were similar (Figure

261   S14C,D) with a root mean square deviation (RMSD) of 1.0 Å (calculated using all 1,267 resolved

262   Cα atoms in each Fab-spike protomer structure). The binding of M8a-31 Fab to both the SARS-

263 CoV-2 WA1 and Omicron BA.1 RBDs was mainly stabilized through interactions with all CDRs

264 except for CDRL3 along with FWRH1, FWRH2, FWRL2, and FWRL3 (Figure 4D). The M8a-31

265 epitope overlapped with class 4 anti-RBD antibodies, but was shifted towards the ACE2 binding

266 site compared with the CR3022 class 4 mAb (Figure S14C,D), consistent with its competition with

267 the C118 class 1/4 mAb (Figure S5). The mainly conserved nature of the M8a-31 epitope (Figure

268 4D) is consistent with its broadly cross-reactive binding and neutralization properties (Figure 2).

269 In common with M8a-3 and M8a-31, M8a-34 bound and neutralized most sarbecoviruses

270 across different clades and SARS-CoV-2 variants (Figure 2) and exhibited a similar competition

271 profile (Figure S5). To map its epitope, we determined a single-particle cryo-EM structure of M8a-

272 34 Fab bound to the SARS-CoV-2 WA1 spike trimer at 3.5 Å resolution (Figure 3E, Figure S10),

273 revealing interactions of three Fabs with three 'up' RBDs (Figure 3E, Figure S14E) that were

274 modeled using an M8a-34 Fab–RBD crystal structure (Table S3). M8a-34 Fab interacted with the

275 RBD through all three CDRHs as well as CDRL1 and CDRL3 (Figure 4E). The M8a-34 Fab

276 epitope was similar to epitopes of other class 1/4 mAbs including M8a-3, M8a-6 and M8a-31,

277 which overlapped with the binding epitopes of CR3022 (class 4) and C118 (class 1/4) (Figure 4A,

278 Figure S14E), again consistent with its broad binding and neutralizing properties (Figure 2) and

279 competition ELISA results (Figure S5).

280 The M8a-28 mAb showed the lowest degree of cross-reactive RBD binding (Figure 2A).

281 M8a-28 mapped to the class 3 epitope instead of the more conserved class 1/4 and class 4 epitopes

282 (Figure S5), and except for M8a-6 (a weakly/non-neutralizing mAb), it showed the lowest levels

283 of cross-reactive sarbecovirus neutralization of the five mAbs isolated from mosaic-8 immunized

284 mice (Figure 2B). Single-particle cryo-EM structures of the M8a-28 Fab in complex with the

285 SARS-CoV-2 spike were determined in two conformational states: a 2.8 Å structure with each of

286 three Fabs binding to a 'down' RBD (Figure 3F) and a 3.1 Å structure with two Fabs bound to

287 adjacent 'down' RBDs and a third Fab at lower occupancy bound to a flexible 'up' RBD (Figure

288 S11). The Fab-RBD interaction was mediated by all six CDRs, plus FWRH3 and FWRL1 (Figure

289 4F). Notably, the M8a-28 Fab approached the RBD from the opposite direction compared with

290 Fabs from the other M8a mAbs (Figure 4A, Figure S14F), interacting with more variable RBD

291 regions (Figure 4F) that overlapped with the epitope of the class 3 anti-RBD mAb, S309 (Pinto et

292 al., 2020) (Figure S14F). Although M8a-28 potently neutralized SARS-CoV-2 WA1 D614G,

293 Beta , Delta, Omicron BA.1 and BA.2, it was only weakly neutralizing or non-neutralizing against

294   other sarbecoviruses (Figure 2B), consistent with its epitope spanning more variable RBD residues

295   than epitopes of class 4 and class 1/4 anti-RBD mAbs (Jette et al., 2021) .

296   The HSW-1 and HSW-2 mAbs, isolated from homotypic SARS-CoV-2 RBD nanoparticle-

297   immunized mice, were each isolated from B cells secreting IgGs that bound to all four RBDs used

298   during screening. Accordingly, they exhibited broad cross-reactive binding to sarbecovirus RBDs

299   by ELISA, with HSW-2 binding to all tested RBDs except for SARS-CoV RBD and HSW-1 also

300   not interacting detectably with SARS-CoV-1 RBD and three other RBDs from clade 2 and 3

301   sarbecoviruses (Figure 2A). Despite broad recognition of sarbecovirus RBDs, the HSW mAbs

302   especially HSW-2, exhibited overall weaker neutralization potencies across the sarbecoviruses

303   tested, with all IC_{50} values being above $10\,\mu g/mL$ (Figure 2B). To compare recognition properties

304   with the M8a Fabs, we determined a single-particle cryo-EM structure of HSW-1 bound to SARS-

305   CoV-2 WA1 spike trimer at 3.1 Å resolution, revealing a single well-ordered Fab bound to a trimer

306   with two 'up' RBDs and one 'down' RBD (Figure 5A, Figure S12, Figure S15A). In common with

307   the M8a-6 mAb, for which the Fab-spike structure also revealed only a single bound Fab per trimer

308   (Figure 3B), HSW-1 showed no detectable competition with anti-RBD mAbs with known epitopes

309   (Figure S5). The bound HSW-1 Fab interacted with two RBDs: one 'up' RBD (1° RBD) and the

310   adjacent 'down' RBD (2° RBD) (Figure 5A, Figure S15A). Interactions between the HSW-1 Fab

311   and 1° RBD were mediated by both the heavy chain through FWRH1, CDRH1 and CDRH3, and

312   the light chain through CDRL1, CDRL2, CDRL3 and FWRL2 (Figure 5A,B). The interactions

313   between HSW-1 and the 2° RBD were mediated by the HSW-1 light chain (Figure 5A). Structural

314   comparisons showed the epitope of HSW-1 overlapped somewhat with the binding epitopes of

315   C118 (class 1/4) and CR3022 (class 4) and included mostly conserved residues (Figure S15A).

316   We next used single-particle cryo-EM to investigate the interactions of HSW-2 with spike

317   trimer. We observed two main populations of particles, one population of unliganded intact spike

318   trimer and a second HSW-2 Fab complexed with spike S1 domain protomers (Figure S13). From

319   the second population, we obtained an EM reconstruction at 4.1 Å resolution of the HSW-2 Fab

320   bound to the S1 domain of the SARS-CoV-2 WA1 spike (Figure 5C, Figure S15B) using a crystal

321   structure of an HSW-2 Fab–RBD complex (Table S3) to derive detailed interactions. HSW-2 used

322   its six CDRs plus FWRH2, FWRL1, FWRL2 and FWRL3 to recognize the bottom of the RBD

323   (Figure 5D,E), consistent with its competition with the class 4 anti-RBD antibody CR3022 (Figure

324   S5). Although their binding poses differed, the HSW-2 epitope overlapped with the largely-

325   conserved epitope of the class 4 mAb, CR3022 (Figure S15B), a mAb isolated from a SARS-CoV

326   patient that also induces dissociation of the SARS-CoV-2 spike trimer (Huo et al., 2020). Although

327   S1 shedding resulting from mAb binding has been suggested as a possible neutralization

328   mechanism for CR3022 and other class 4 anti-RBD mAbs (Huo et al., 2020; Piccoli et al., 2020;

329   Wec et al., 2020), HSW-2 was largely non-neutralizing (Figure 2B). To determine whether HSW-

330   2 was raised against an RBD epitope that is inaccessible in an intact spike trimer, we aligned the

331   RBD portion of the HSW-2 Fab-RBD structure to RBDs from a spike trimer structure with all 'up'

332   RBDs and to a spike structure with all 'down' RBDs, finding steric clashes in both cases (Figure

333   5F,G). The inability of the HSW-2 Fab to access either 'up' or 'down' RBDs in the context of an

334   intact spike trimer is consistent with the observation that although HSW-2 bound to almost all

335   RBDs evaluated by ELISA (Figure 2A), it showed weak or no neutralization activity against the

336   tested strains (Figure 2B).

337

338   **Class 1/4 anti-RBD mAbs induce spike trimer opening and exhibit different potentials for**

339   **intra-spike crosslinking and susceptibility to mutations**

340   To address potential effects of mAb binding on spike trimer conformation, we compared

341   the Fab-bound spike trimer structures reported here to other trimer structures. We previously used

342   the distances between the Cα atoms of residue 428 in each of three 'up' RBDs within an intact

343   trimer to assess spike openness, with inter-protomer distances of 39 Å or less indicating a typical

344   prefusion spike trimer conformation (Figure 6A) (either unliganded, bound to ACE2, or bound to

345   a class 1, 2, or 3 anti-RBD mAb), and binding of class 4 and class 1/4 anti-RBD mAbs resulting

346   in increases in the inter-RBD distances (Jette et al., 2021). For example, inter-RBD distances for

347   spike trimers bound to C118 or to S2X259, human donor-derived mAbs recognizing class 1/4

348   epitopes (Jette et al., 2021; Robbiani et al., 2020; Tortorici et al., 2021), were 53 Å (Jette et al.,

349   2021) (C118) or 43 Å (S2X259), demonstrating opening of the trimer to accommodate a Fab

350   interacting with the occluded class 1/4 region of the RBD (Figure 6B). In the present study, we

351   found inter-protomer distances ranging from 48-69 Å for trimers bound to Fabs from M8a-3

352   (Figure 6C), M8a-6 (Figure 6D), M8a-31 (Figure 6E,F), M8a-34 (Figure 6G) and HSW-1 (Figure

353   f16H), consistent with increased openness of trimers bound to class 1/4 and class 4 anti-RBD

354   antibodies. By contrast, the comparable inter-protomer distance was 31 Å in the M8a-28–spike

355   structure with all RBDs in the 'down' conformation (Figure 6I), consistent with M8a-28

356   recognition of the non-occluded class 3 RBD epitope.

357   To understand how substitutions in recent VOCs might affect binding of the mAbs for

358 which we had Fab-spike structures, we mapped their binding epitopes compared to the locations

359 of Omicron BA.1 and BA.2 substitutions on the RBD (Figure 4B-F, 5B,D). Most of the Omicron

360 BA.1 an BA.2 substitutions were in the ACE2 binding region (Figure 4A, Figure S2B), which

361 includes mainly variable residues (Figure 1A), with fewer substitutions in conserved regions

362 (Figure 1A, 4B-F, 5B,D, Figure S2B). The Omicron substitutions were mainly at the peripheries

363 of the RBD epitopes of the m8a mAbs isolated from mosaic-8 nanoparticle-immunized mice

364 (Figure 4B-F), and there were no Omicron BA.1 or BA.2 substitutions within the binding epitopes

365 of the two HSW mAbs isolated from homotypic SARS-CoV-2 nanoparticle-immunized mice

366 (Figure 5B,D). Despite the Omicron BA.1 or BA.2 substitutions not greatly affecting RBD binding

367 by the seven mAbs (Figure 2A), some of the class 1/4 M8a mAbs showed somewhat reduced

368 neutralization potencies (Figure 2B). Evaluation of potent class 1, 2 and 3 anti-RBD human mAbs

369 also revealed that, except for LY-CoV1404 (Bebtelovimab), most showed diminished binding and

370 neutralizing activities against Omicron BA.1 (Zhou et al., 2022). Epitope analysis showed that

371 LY-CoV1404 targets a class 3 RBD epitope adjacent to Omicron BA.1 and BA.2 mutations that

372 is only somewhat conserved with respect to variability within sarbecoviruses, but unlike other

373 EUA-approved class 3 anti-RBD therapeutic mAbs, it was minimally impacted by those

374 substitutions (Figure 7).

375   Although we found that RBD binding correlated with neutralization potencies for

376 polyclonal antisera from RBD-nanoparticle immunized animals (Cohen et al., 2021), this is not

377 always true for mAbs; e.g., mAbs such as CR3022 bind to SARS-CoV-2 RBD, but neutralize only

378 weakly or not at all (Niu et al., 2021). One mechanism by which Omicron or other RBD

379 substitutions could indirectly affect neutralization potencies of mAbs without affecting binding to

380 isolated RBDs is by changing the dynamics of the conversion between 'up' and 'down'

381 conformations of RBDs on spike trimers. Some classes of anti-RBD mAbs have a strong or

382 absolute preference for binding an 'up' versus a 'down' RBD; for example, most class 1 and class

383 4 anti-RBD mAbs only recognize 'up' RBDs (Barnes et al., 2020a). To address the ability of the

384 mAbs investigated here to potentially recognize both 'up' and 'down' RBDs, we evaluated the

385 accessibility of their epitopes on a spike trimer by mapping each binding epitope onto an

386 unliganded trimer structure with one 'up' and two 'down' RBDs (PDB 6VYB) (Figure S16) and

387 a trimer with all 'up' RBDs (PDB 7RKV) (Figure S17). The class 4 and 1/4 epitopes of M8a-3,

388 M8a-6, M8a-31, M8a-34, and HSW-1 were buried when RBDs adopted the 'down' conformation

389 (Figure S16A-D, F), but fully exposed in the 'up' RBDs (Figure S17A-D, F). Although the class

390 4 epitope of HSW-2 was buried in 'down' RBD conformation (Figure S16G) and could be partially

391 exposed in an 'up' RBD conformation (Figure S17G), structural alignments showed that HSW-2

392 cannot bind to 'up' or 'down' RBDs in the context of a spike trimer (Figure 5F,G). By contrast,

393 the class 3 epitope of M8a-28 was exposed in both RBD conformations (Figure S16E, S17E).

394 Likely related to these observations, only the M8a-28–bound trimer structure showed an inter-

395 protomer RBD distance of 31 Å (Figure 6I) that is equivalent to that of an unliganded trimer (28-

396 40 Å) (Figure 6A). The other class 4 and 1/4 mAb Fab-bound trimer structures showed larger inter-

397 protomer RBD distances (up to ~70 Å), corresponding to ~ 11 to 34 Å more outward displacement

398 of RBDs in comparison to unliganded or class 1- or ACE2-liganded spike trimer structures (Figure

399 6B-H) (Barnes et al., 2020a). This outward displacement of RBDs could result in spike trimer

400 destabilization that could lead to S1 shedding (Huo et al., 2020; Jette et al., 2021; Piccoli et al.,

401 2020; Pinto et al., 2020).

402   Another property of IgG antibodies that could affect their neutralization potencies relates

403 to their ability to utilize bivalency. Since IgG antibodies have two identical Fab arms, they can

404 increase their apparent affinities for binding to tethered viral antigens through avidity effects,

405 which can occur through either inter-spike crosslinking (simultaneous binding of two neighboring

406 spike trimers) or intra-spike crosslinking (simultaneous binding of two neighboring RBDs within

407 the same spike trimer). Inter-spike crosslinking is likely possible for most anti-RBD antibodies

408 based on the numbers and densities of SARS-CoV-2 spikes (Barnes et al., 2020b), and intra-spike

409 crosslinking could also occur for some anti-RBD antibodies, depending on their epitopes and

410 binding poses (Barnes et al., 2020a). To evaluate whether the M8a or HSW mAbs could enhance

411 their binding through intra-spike crosslinking, we measured distances between neighboring Fabs

412 in the Fab-spike structures to predict if simultaneous binding of two Fabs within an intact IgG to

413 adjacent RBDs on a trimer would be possible. We previously defined a distance of ≤65 Å between

414 the C-termini of the C_H1 domains of adjacent bound RBD-bound Fabs as being required to allow

415 the N-termini of the two chains of an IgG hinge to each of the C-termini of two bound Fabs (Barnes

416 et al., 2020a) and compared that to the distances measured in a previously characterized class 1/4

417 mAb, C118, in complex with the spike trimer (Figure 6B) (Jette et al., 2021). The measured

418 distances in spike trimers complexed with the M8a-3 (126 Å, 130 Å, and 159 Å) (Figure 6C), M8a-

419 34 (107 Å, 110 Å, and 150 Å) (Figure 6G), or M8a-28 (144 Å) (Figure 6I) Fabs were too large to

420 permit intra-spike crosslinking. Although we could not measure analogous distances in the M8a-

421   6–spike structure because only one Fab was bound (Figure 6D), the similar epitope and pose for

422   M8a-3 and M8a-6 (Figure 3A,B; 4B,C) suggest that an IgG version of M8a-6 would also be unable

423   to crosslink adjacent RBDs. Thus, the weak binding of M8a-6 to a spike trimer could not be

424   improved by intra-spike crosslinking avidity effects, again rationalizing its lack of neutralizing

425   activity (Figure 2B). For spike trimers in complex with M8a-31 Fab (Figure 6E,F), the distances

426   between the C-termini of adjacent C_H1 domains were measured as 52 Å and 49 Å for M8a-31 Fab

427   bound to the WA1 and Omicron BA.1 spikes, respectively, suggestive of potential intra-spike

428   crosslinking. We could not evaluate the potential for intra-spike crosslinking for the HSW-1 or

429   HSW-2 mAbs because either only one Fab was bound per spike (HSW-1) (Figure 5A) or the

430   reconstructions showed Fab binding to dissociated S1 monomer only (HSW-2) (Figure 5C).

431   We also used modeling to assess how the RBD-nanoparticles used to elicit the mAbs

432   investigated here might engage with bivalent B cell receptors. To address this issue, we asked

433   whether the geometric arrangement of RBDs on mosaic-8 RBD-mi3 nanoparticles would permit

434   bivalent engagement of neighboring RBDs by IgGs, here representing membrane-bound B cell

435   receptors hypothesized to engage adjacent RBDs (Figure 1D). We first constructed IgG models of

436   each of the Fabs in the M8a and HSW Fab-spike structures (Figure 3,5). Next, we asked if it was

437   sterically possible for both Fabs of an IgG to interact with the epitope identified from its cryo-EM

438   structure on adjacent RBDs on a modeled RBD-mi3 nanoparticle. For each of the seven mAb

439   epitopes, we found that the RBD-mi3 nanoparticle geometry was predicted to allow simultaneous

440   recognition of adjacent RBDs by both Fabs of an IgG (Figure S18), thus confirming that the

441   geometric arrangement of RBD attachment sites on SpyCatcher-mi3 would allow B cell receptor

442   engagement through avidity effects (Figure 1D).

443

444   **mAbs elicited by mosaic-8 RBD-nanoparticles resemble EUA-approved therapeutics or a**

445   **potent cross-reactive human class 1/4 anti-RBD antibody**

446   Although our B cell cloning study from mosaic-8 RBD-nanoparticle immunized mice

447   started with <10,000 cells, the five M8a mAbs identified as binding two or more RBDs during

448   screening all target the desired more conserved epitopes (class 3 and class 1/4) rather than the class

449   1 and class 2 RBD epitopes more commonly elicited by vaccination or infection (Greaney et al.,

450   2021a; Greaney et al., 2021b; Piccoli et al., 2020) (Figure 3,4), and three of the five mAbs were

451   potently and/or broadly neutralizing (Figure 2B). By contrast, the only two mAbs isolated from

452   homotypic SARS-CoV-2 RBD-nanoparticle immunized mice that were identified as binding two

453   or more RBDs during screening targeted different epitopes (Figure 5) and were only weakly- or

454   non-neutralizing (Figure 2B). This is consistent with our previous results showing that mosaic-8

455   RBD-nanoparticles are more likely to elicit cross-reactive and cross-neutralizing anti-sarbecovirus

456   antibodies against conserved epitopes than homotypic RBD-nanoparticles (Cohen et al., 2022) and

457   argues against use of homotypic RBD-nanoparticles as immunogens to elicit cross-reactive

458   neutralizing antibodies.

459   Human mAbs that received Emergency Use Authorization for COVID-19 treatment

460   include class 1 and class 2 anti-RBD mAbs that are no longer effective against SARS-CoV-2

461   variants, and class 3 anti-RBD mAbs, two of which, Bebtelovimab and Cilgavimab, retain at least

462   partial efficacy against Omicron variants (Figure 7A,B). The class 3 RBD epitope, although less

463   affected by SARS-CoV-2 VOC substitutions than the immunodominant class 1 and class 2 RBD

464   epitopes, nevertheless encompasses an RBD region that includes variability across sarbecovirus

465   sequences (Figure 1A,B, 7C-G). The ability of the class 3 epitope to accumulate substitutions is

466   likely because the class 3 RBD region is accessible on both 'up' and 'down' RBD conformations,

467   thus not being constrained to avoid substitutions because of contacts with other regions of a

468   sarbecovirus spike trimer (Figure 7A) (Cohen et al., 2022). The epitope identified for M8a-28

469   (Figure 7C) resembles epitopes of the class 3 anti-RBD therapeutic mAbs (Figure 7D-G). Some

470   of these mAbs, including M8a-28 (Figure 2B), neutralize Omicron VOCs, but their epitope

471   locations within a region that varies among sarbecoviruses suggests that future SARS-CoV-2

472   variants are likely to include substitutions that reduce or completely abrogate their efficacies

473   (Figure 7C-G). By contrast, the more occluded class 1/4 RBD epitope (Figure 7A), to which bound

474   mAbs can inhibit ACE2 binding (Jette et al., 2021; Liu et al., 2020a; Tortorici et al., 2021), exhibits

475   less variability across sarbecoviruses, likely because substitutions that affect its contacts as a

476   'down' RBD with other spike trimer regions limit its variability between SARS-CoV-2 VOCs and

477   other sarbecoviruses (Cohen et al., 2022). The fact that four of five mouse mAbs identified as

478   binding to two or more different RBDs during B cell screening after mosaic-8 immunization target

479   the class 1/4 epitope, in common with the potent, cross-reactive, and protective S2X259 human

480   mAb (Tortorici et al., 2021) (Figure 7H), supports the potential for using mosaic RBD-

481   nanoparticles as immunogens to efficiently elicit cross-reactive and potent neutralizing mAbs

482   against SARS-CoV-2 variants and animal sarbecoviruses that could spill over to infect humans. In

483   addition, together with previous challenge and serum epitope mapping (Cohen et al., 2022), these

484   results further validate mosaic-8 RBD-nanoparticles as a broadly protective vaccine candidate.

16

485

486 **Discussion**

487   Here, we characterized mouse mAbs elicited using mosaic RBD-nanoparticles (M8a

488 mAbs) or homotypic RBD-nanoparticles (HSW mAbs) using both structural and functional

489 analyses, showing that mosaic RBD-nanoparticles induce potently neutralizing antibodies that

490 cross-react between animal sarbecoviruses and SARS-CoV-2 VOCs (Figure 2). Although we

491 identified only five mAbs that bound to two or more RBDs from mosaic-8 nanoparticle immunized

492 mice in these first experiments, one mAb (M8a-3) was both cross-reactive and strongly

493 neutralizing, and two others (M8a-31 and M8a-34) were less potently neutralizing but were cross-

494 reactive (Figure 2), demonstrating the potential for the mosaic-8 immunogen to efficiently elicit

495 potentially useful therapeutic antibodies that could be identified in larger-scale experiments and

496 developed as future therapeutic mAbs. Another mAb (M8a-28) potently neutralized SARS-CoV-

497 2 variants and resembled therapeutic antibodies in current use (Figure 7C-G).

498   Structural studies of Fab complexes with SARS-CoV-2 spike trimers, including one with

499 Omicron BA.1, demonstrated that four of the five mAbs isolated from mosaic-8 immunized mice

500 recognized conserved epitopes (Figure 4), as designed in the immunization approach (Figure 1D)

501 and as shown for polyclonal antisera raised in mice by mosaic-8 RBD-nanoparticle immunization

502 (Cohen et al., 2022). By contrast, antibodies raised in homotypic SARS-CoV-2 RBD-nanoparticle

503 immunized mice more commonly recognize variable class 1 and class 2 RBD epitopes (Cohen et

504 al., 2022), likely explaining why it was more difficult in the current study to isolate single B cells

505 from homotypic RBD-nanoparticle immunized mice secreting IgGs that bound two or more

506 labeled RBDs (Figure 2A, Figure S3). The two cross-RBD binding mAbs we were able to isolate

507 from homotypic RBD-nanoparticle immunized mice showed binding to multiple sarbecovirus

508 RBDs (Figure 2A) but were only weakly- or non-neutralizing (Figure 2B). Structural studies

509 rationalized the weak neutralization profiles of the HSW mAbs: the HSW-1–spike structure

510 showed only one bound Fab per trimer (Figure 5A) as compared with three bound Fabs per trimer

511 in the structures of more potently neutralizing mAbs (Figure 3), and the HSW-2 Fab epitope

512 (Figure 5D) was incompatible with binding to its RBD epitope on intact spike trimer (Figure 5F,G),

513 resulting in a trimer dissociation and a Fab–monomeric S1 subunit structure (Figure 5C).

514   Together with previous studies (Cohen et al., 2021; Cohen et al., 2022), the current results

515 support the mosaic-8 RBD-nanoparticles as a promising vaccine candidate to protect against

516 SARS-CoV-2, including future variants, and against potential spillover sarbecoviruses from

517   animal reservoirs. In addition, the fact that potent cross-reactive mAbs were identified from

518   relatively few B cells suggest that high-throughput screening of larger samples from animals

519   immunized with mosaic-8 RBD-mi3 could be used to identify many new therapeutic mAbs, which

520   could then be used to prevent or treat infections of Omicron and future SARS-CoV-2 variants.

521

522

Materials and methods

Preparation of homotypic and mosaic-8 RBD-mi3 nanoparticles

Mammalian expression vectors encoding RBDs of SARS-CoV-2 and other sarbecoviruses were constructed as described (Cohen et al., 2021) in two versions: one with a C-terminal 6x-His tag and a SpyTag003 (RGVPHIVMVDAYKRYK) (Keeble et al., 2019) for the 8 RBDs that were coupled to SpyCatcher003-mi3 nanoparticles (Keeble et al., 2019) and other versions with only a 6x-His tag or with a His tag plus an Avi tag for ELISAs. Expression vectors encoding RBDs were constructed similarly for the following sarbecoviruses: BM4831-CoV (GenBank NC014470; spike residues 310-530), BtKY72-CoV (GenBank KY352407; spike residues 309-530), C028 (GenBank AAV98001.1; spike residues 306-523), Khosta2 (GenBank QVN46569.1; spike residues 307-526), LYRa3 (GenBank AHX37569.1; spike residues 310-527), Pangolin17-CoV (GenBank QIA48632; spike residues 317-539), RaTG13-CoV (GenBank QHR63300; spike residues 319-541), Rf1-CoV (GenBank DQ412042; spike residues 310-515), RmYN02-CoV (GISAID EPI_ISL_412977; spike residues 298-503), Rs4081-CoV (GenBank KY417143; spike residues 310-515), RshSTT200 (GISAID EPI_ISL_852605; spike residues 306-519), SARS-CoV (GenBank AAP13441.1; spike residues 318-510), SARS-CoV-2 WA1 (GenBank MT246667.1; spike residues 319-539), SHC014-CoV (GenBank KC881005; spike residues 307-524), W1V1-CoV (GenBank KF367457; spike residues 307-528), and Yun11-CoV (GenBank JX993988; spike residues 310-515). SARS-CoV-2 variants, including Beta, Delta, Omicron BA.1, and Omicron BA.2 with C-terminal 6x-His tag were also constructed similarly as the SARS-CoV-2 WA1 RBD construct for ELISA. All RBD proteins were expressed by transient transfection of Expi293F cells and purified by Ni-NTA and size exclusion chromatography (SEC) using a HiLoad 16/600 Superdex 200 column (Cytiva, Marlborough, MA) (Barnes et al., 2020b). Peak fractions were pooled, concentrated, and stored at 4˚C until use.

SpyCatcher003-mi3 (Keeble et al., 2019) were expressed in *E. coli* BL21 (DE3)-RIPL (Agilent Technology) and purified as described previously (Cohen et al., 2021). Briefly, *E. coli* transduced with a SpyCatcher003-mi3 expression plasmid (Addgene, Watertown, MA) were lysed with a cell disrupter in the presence of 2 mM PMSF. After spinning at 21,000 x *g* for 30 minutes, supernatant containing SpyCatcher003-mi3 particles was passed over a pre-packed Ni-NTA column. The eluent was concentrated and further purified by SEC using a HiLoad 16/600 Superdex 200 column (Cytiva, Marlborough, MA). Peak fractions were pooled and stored at 4˚C until use.

554   SpyCatcher003-mi3 particles were used for SpyTagged RBD conjugation for up to a month after

555   clarification by filtering using a 0.2 µm filter or spinning at 21,000 x g for 10 min.

556   For conjugation, purified SpyCatcher003-mi3 was incubated with purified SpyTagged

557   RBDs (either 8 different RBDs to make mosaic-8 RBD-mi3 or SARS-CoV-2 RBD only to make

558   homotypic RBD-mi3) at a molar ratio of 1:1.2 overnight at room temperature. Conjugation

559   efficiencies of individual RBDs to SpyCatcher003-mi3 were verified as shown in Figure S2 of

560   (Cohen et al., 2021). Conjugated mi3-RBD particles were purified by SEC using a Superose 6

561   10/300 column (Cytiva, Marlborough, MA). Peak fractions pooled and the concentrations of

562   conjugated mi3 particles were determined using a Bio-Rad Protein Assay (Bio-Rad, Hercules,

563   CA). Conjugated nanoparticles were characterized by electron microscopy imaging and SEC as

564   shown in Figure S1C-E, and by electron microscopy, SEC and dynamic light scattering as shown

565   in Figure S1 of (Cohen et al., 2022).

566   For negative-stain electron microscopy imaging of mosaic-8 and homotypic SARS-CoV-

567   2 RBD-nanoparticles: ultrathin, holey carbon-coated, 400 mesh Cu grids (Ted Pella, Inc.) were

568   glow discharged (60 s at 15 mA), and a 3 µL aliquot of SEC-purified RBD-nanoparticles was

569   diluted to ~40-100 µg/mL and applied to grids for 60 s. Grids were negatively stained with 2%

570   (w/v) uranyl acetate for 30 s, and images were collected with a 120 keV FEI Tecnai T12

571   transmission electron microscope at 42,000x magnification.

572

573   **Immunizations**

574   Immunizations were done using protocols, #19023, approved by the City of Hope IACUC

575   committee. Experiments were conducted using 4–6-week-old female C57BL/6 mice (Charles

576   River Laboratories, Wilmington, MA). Immunizations were carried out as previously described

577   (Cohen et al., 2021) using intraperitoneal injections of 5 µg of conjugated RBD-mi3 nanoparticle

578   (calculated as the mass of the RBD, assuming 100% efficiency of conjugation to SpyCatcher003-

579   mi3) in 100 µL of 50% v/v AddaVax^{TM} adjuvant (Invivogen, San Diego, CA). Animals were

580   boosted 4 weeks after the prime with the same quantity of antigen in adjuvant. A final booster was

581   administered intraperitoneally 3 days before mouse spleen harvest.

582

583   **Beacon**

584   Plasma B cells were isolated from immunized animals for characterization on a Berkeley Lights

585   Beacon instrument. Spleens were isolated from two immunized mice per condition and prepared

586   into single cell suspensions as described (Cohen et al., 2021). Plasma B cells were isolated by

587   CD138$^+$ cell enrichment (Miltenyi Biotec CD138$^+$ plasma cell isolation kit, catalog no. 130-092-

588   530). Enriched plasma B cell samples were loaded onto an OptoSelect 11k chip (Berkeley Lights,

589   Inc., Emeryville, CA) in BLI Mouse Plasma Cell Media (Berkeley Lights, Inc., catalog no. 750-

590   70004). Single cells were then isolated in individual nanoliter-volume compartments (Nanopens

591   using light-based OptoElectro Positioning (OEP) manipulation with settings optimized for plasma

592   B cells. From Mosaic-8 RBD-nanoparticle immunized animals, 9,695 cells were penned in one

593   chip, of which 7,747 were single cell pens. For homotypic SARS-CoV-2 RBD-nanoparticle

594   immunized animals, 9,130 cells were penned in a second chip, of which 7699 were single cell pens

595   (Figure S3A). On chip fluorescence assays were used to identify cells secreting antibodies specific

596   to RBD antigens. Briefly, C-terminally Avi-tagged RBDs were modified with site-specific

597   biotinylation (Avidity, LLC, Aurora, CO) according to the manufacturer's protocol and

598   immobilized on streptavidin-coated beads (Berkeley Lights, Inc. catalog no. 520-00053). Assays

599   were conducted by mixing beads coupled with one of four RBDs used for screening with a

600   fluorescently labeled goat anti-mouse secondary antibody Alexa568 at 1:2500 dilution and

601   importing this assay mixture into the OptoSelect 11k chip. Assays were conducted post 30 minutes

602   incubation after cell penning at 36 ˚C. Images were acquired every 5 minutes for 9 cycles while

603   the beads remained stationary in the main channel above the Nanopens of the OptoSelect chip.

604   Antibodies specific for the immobilized RBD bound the antigen-coupled beads, which sequestered

605   the fluorescent secondary antibody, creating a "bloom" of fluorescent signal immediately above

606   Nanopens containing plasma B cells. Beads were washed out of the chip, and this assay was

607   conducted for each of the four RBDs. After completion of all assays, RBD-specific cells of interest

608   were exported using OEP from individual nanopen chambers to individual wells of a 96-well PCR

609   plate containing lysis buffer.

610   After running assays and selecting positive blooms with single cells, we ran the OptoSeq

611   BCR Export workflow, which performs reverse transcription overnight on the chip and exports

612   cell lysates containing cDNA on capture beads onto a 96 well plate. cDNA amplification and

613   chain-specific PCR were performed the following day and run on an agarose gel to confirm that

614   bands of the correct size were present. PCR products were then purified using AMPure XP

615   magnetic beads and submitted for Sanger sequencing at the City of Hope Sequencing Core.

616

617   **Cloning**

618   Sequences for V_H and V_L domains were codon optimized using GeneArt (Thermo Fisher

619   Scientific, Waltham, MA) and gene blocks for each domain were purchased from Integrated DNA

620   Technologies (IDT, Coralville, IA). Expression constructs were assembled using Gibson reactions

621   (Gibson et al., 2010; Gibson et al., 2009). The heavy chain for IgG expression was constructed by

622   subcloning the V_H gene into a p3BNC expression vector encoding the human IgG C_H1, C_H2, and

623   C_H3 domains, and the heavy chain for Fab expression was constructed by assembling the V_H gene

624   into a p3BNC expression vector encoding a human C_H1 and a C-terminal 6x-His tag. The

625   expression plasmid for the light chain was constructed by subcloning the V_L gene into a p3BNC

626   vector that also encoded kappa human C_L. The numbering of V_H and V_L protein sequences and the

627   identification of the V gene segments were determined using the ANARCI server (Dunbar et al.,

628   2016).

629

630   **IgG and spike trimer production and purification**

631   Proteins were expressed in Expi293 cells by transient transfection. IgGs and a previously-

632   described human ACE2-Fc construct (Jette et al., 2021) were purified from cell supernatants using

633   MabSelect SURE columns (Cytiva, Marlborough, MA), and His-tagged Fabs were isolated from

634   cell supernatants using Ni-NTA columns (Qiagen, Hilden, Germany). IgGs, ACE2-Fc, and Fabs

635   were further purified by SEC using a Superdex 200 16/600 column (Cytiva, Marlborough, MA).

636   Purified proteins were concentrated using a 100 kDa and 30 kDa cutoff concentrator (EMD

637   Millipore, Burlington, MA), respectively, to 10 to 15 mg/mL, and final concentrated proteins were

638   stored at 4 ˚C until use. 6P versions (Hsieh et al., 2020) of soluble SARS-CoV-2 WA1 and SARS-

639   CoV-2 Omicron BA.1 spike trimers were isolated from cell supernatants using a Ni-NTA column

640   (Qiagen, Hilden, Germany). Eluents from Ni-NTA purifications were subjected to SEC using a

641   HiLoad Superdex 200 16/600 column followed by a Superose 6 10/300 (Cytiva, Marlborough,

642   MA) column. Peak fractions were pooled and concentrated to ~6 mg/ml, flash frozen in 50 µL

643   aliquots, and stored at -80 ˚C until use.

644

645   **ELISAs**

646   Nunc® MaxiSorp™ 384-well plates (Millipore Sigma, St. Louis, MO) were coated with 10 µg/mL

647   of purified RBD in 0.1 M $NaHCO_3$ pH 9.8 and stored overnight at 4 ˚C. After blocking with 3%

648   bovine serum albumin (BSA) for an hour at room temperature, plates were washed with Tris-

649   buffered saline including 0.1% Tween 20 (TBST). After removing blocking solution from the

650   plates, 100 μg/mL of purified IgGs were serially diluted by 4-fold using TBST with 3% BSA and

651   incubated with plates at room temperature for 3 hours. Plates were then washed with TBST and

652   incubated with secondary HRP-conjugated goat anti-human IgG (Southern Biotech, Birmingham,

653   AL) at a 1:15,000 dilution for 45 minutes at room temperature. Plates were washed with TBST,

654   developed using SuperSignal ELISA Femto Maximum Sensitivity Substrate (Thermo Fisher

655   Scientific, Waltham, MA), and read at 425 nm. ELISA data were collected in duplicate, and each

656   assay was conducted at least twice for the seven mAbs that were structurally characterized. Curves

657   were plotted and integrated to obtain half-maximal effective concentrations (EC_{50}) using Graphpad

658   Prism v9.3.1.

659   Competition ELISAs were performed using a Tecan Evo liquid handling robot using

660   modifications of a previously described protocol (Escolano et al., 2021). IgGs were randomly

661   biotinylated at primary amines using EZ-link NHS-PEG4 Biotinylation Kit according to the

662   manufacturer's protocol (Thermo Fisher Scientific, Waltham, MA). SARS-CoV-2 RBD (2.5

663   μg/mL) was adsorbed overnight at 4ºC to a 384-well Nunc MaxiSorp ELISA plate (Millipore

664   Sigma, St. Louis, MO). The RBD was removed via aspiration and the plate blocked with 3% BSA

665   in TBST for 1 hour at room temperature. The blocking was removed via aspiration and 10 μg/mL

666   unlabeled IgG was added and incubated for 2 hours, followed by addition of 0.25 μg/mL

667   biotinylated IgG. The plate was incubated for 2 hours at room temperature, and bound biotinylated

668   IgG was detected using horseradish peroxidase-conjugated streptavidin (Southern Biotech,

669   Birmingham, AL) (1 hour, room temperature) and developed with SuperSignal ELISA Femto

670   Substrate (Thermo Fisher Scientific, Waltham, MA). Relative light units (RLU) were measured

671   and the signal for each competition pair was normalized to the signal for the biotinylated IgG when

672   unlabeled IgG was not present. Measurements were performed in technical quadruplicates. Data

673   presented are representative of two independent experiments.

674

675   **Neutralization assays**

676   SARS-CoV-2, SARS-CoV-2 VOCs, SARS-CoV, WIV1, SHC014, BtKY72 (including mutations

677   allowing human ACE2 binding (Starr et al., 2022)), Khosta2/SARS-CoV Chimera, and

678   LyRa3/SARS-CoV Chimera pseudoviruses based on HIV lentiviral particles were prepared as

679   described (Crawford et al., 2020; Robbiani et al., 2020). Khosta2/SARS-CoV and LyRa3/SARS-

680   CoV Chimeras were constructed by replacing the RBD of SARS-CoV Spike with the RBDs of

681   Khosta2 and LyRa3 Spikes separately. Assays were done using 4-fold dilutions of purified IgGs

682   at a starting concentration of 100 μg/mL by incubating with a pseudovirus at 37 ˚C for an hour.

683   After incubating with 293TACE2 target cells for 48 hours at 37 ˚C, cells were washed 2 times with

684   phosphate-buffered saline (PBS) and lysed with Luciferase Cell Culture Lysis 5x reagent

685   (Promega, Madison, WI). Using the Nano-Glo Luciferase Assay System (Promega, Madison, WI),

686   the NanoLuc Luciferase activity in lysates was measured. Relative luminescence units (RLUs)

687   were normalized to values derived from cells infected with pseudovirus in the absence of IgG.

688   Data were collected at each IgG concentration in duplicate and reported data come from assays

689   performed at least twice (except for the M8a-28 against Omicron BA.1 assay, which was

690   performed once). Half-maximal inhibitory concentrations (IC$_{50}$ values) were determined using

691   nonlinear regression in AntibodyDatabase (West et al., 2013).

692

693   X-ray crystallography

694   RBD-Fab complexes were formed by incubating SARS-CoV-2 RBD with a 1.1x molar excess of

695   Fab for an hour at room temperature. Complexes were purified by SEC using a Superdex 200

696   10/300 Increase column (Cytiva, Marlborough, MA). Peak fractions containing RBD-Fab

697   complexes were pooled and concentrated to ~15 mg/ml. Crystallization trials were set up using

698   commercially available screens by mixing 0.2 μL of RBD-Fab complex and 0.2 μL well solution

699   using a TTP LabTech Mosquito instrument via the sitting drop vapor diffusion method at room

700   temperature. Crystals of M8a-6 Fab–RBD complex were obtained from Proplex screen (Molecular

701   Dimensions, Holland, OH), containing 0.1 M sodium citrate pH 5.5 and 15 % PEG 6,000. Crystals

702   of M8a-34 Fab–RBD complex were obtained from a PEGion screen (Hampton Research, Aliso

703   Viejo, CA), containing 2% v/v tacsimate pH 4.0, 0.1 M sodium acetate trihydrate pH 4.6, 16 %

704   PEG 3,350. Crystals of RBD–HSW-2 complexes were obtained from a Proplex screen (Molecular

705   Dimensions, Holland, OH), containing 0.2 M sodium chloride, 0.1 M sodium/potassium phosphate

706   pH 6.5, 25 % PEG 1,000. All crystals were cryoprotected in well solution mixed with 20% glycerol

707   or PEG 400 before freezing in liquid nitrogen.

708   X-ray diffraction data were collected at the Stanford Synchrotron Radiation Lightsource

709   (SSRL) beamline 12-2 with a Pilatus 6M pixel detector (Dectris, Philadelphia, PA) using the Blu-

710   ice interface (McPhillips et al., 2002) (Table S3). All X-ray datasets were indexed and integrated

711   with XDS (Kabsch, 2010) and scaled with Aimless (Winn et al., 2011). The M8a-6 Fab–RBD

712   structure was solved by molecular replacement using a structure of a Fab-RBD complex from a

713   single-particle cryo-EM structure (PDB 7SC1) as the input model for *Phaser* in Phenix

714   (Liebschner et al., 2019). During the refinement of the M8a-6 Fab–RBD structure, we observed

715   electron density for a second RBD and the variable domains of M8a-6 Fab, but no Fab constant

716   domains were found. Refinement of a model containing the original M8a-6 Fab–RBD complex, a

717   second copy of RBD and the variable domains resulted in no improvements in the refinement

718   statistics. We thus only partially refined the coordinates for the M8a-6 Fab–RBD crystal structure,

719   which were then docked and refined in the cryo-EM M8a-6–spike reconstruction. The M8a-34

720   Fab–RBD structure was solved by molecular replacement using the partially refined model of

721   M8a-6–RBD complex structure as the input model for *Phaser* in Phenix (Liebschner et al., 2019).

722   The HSW-2 Fab–RBD structure was solved by molecular replacement using the partially refined

723   model of M8a-34–RBD complex structure as the input model for *Phaser* in Phenix (Liebschner et

724   al., 2019). Iterative refinement and model-building cycles were carried out with phenix.refine in

725   Phenix (Liebschner et al., 2019) and *Coot* (Emsley et al., 2010), respectively. The refined models

726   were subsequently used as input models for docking into cryo-EM maps of Fab-spike complexes.

727

728   **Cryo-EM Sample Preparation**

729   SARS-CoV-2 S–Fab complexes were formed by incubating purified spike trimer and Fabs at a

730   1.1x molar excess of Fab per spike protomer at room temperature for 30 minutes to a final

731   concentration of ~2 mg/mL. Fluorinated octylmaltoside solution (Anatrace, Maumee, OH) was

732   added to the spike-Fab complex to a final concentration of 0.02% (w/v) prior to freezing, and 3 µL

733   of the complex/detergent mixture was immediately applied to QuantiFoil 300 mesh 1.2/1.3 grids

734   (Electron Microscopy Sciences, Hatfield, PA) that had been freshly glow discharged with PELCO

735   easiGLOW (Ted Pella, Redding, CA) for 1 min at 20 mA. Grids were blotted for 3 to 4 seconds

736   with 0 blot force using Whatman No.1 filter paper and 100% humidity at room temperature and

737   vitrified in 100% liquid ethane using a Mark IV Vitrobot (Thermo Fisher Scientific, Waltham,

738   MA).

739

740   **Cryo-EM data collection and processing**

741   Single-particle cryo-EM datasets for complexes of SARS-CoV-2 WA1 spike 6P with M8a-3 Fab,

742   M8a-6 Fab, M8a-28 Fab, M8a-31 Fab, M8a-34 Fab or HSW-1 Fab and SARS-CoV-2 Omicron

743   BA.1 spike 6P with M8a-31 Fab were collected using SerialEM automated data collection software

744   (Mastronarde, 2005) on a 300 keV Titan Krios (Thermo Fisher Scientific, Waltham, MA) cryo-

745   electron microscope equipped with a K3 direct electron detector camera (Gatan, Pleasanton, CA).

746   For SARS-CoV-2 WA1 spike 6P complexed with HSW-2, a dataset was collected with SerialEM

747   (Mastronarde, 2005) on a 200 keV Talos Arctica cryo-electron microscope (Thermo Fisher

748   Scientific, Waltham, MA) equipped with a K3 camera (Gatan, Pleasanton, CA). Movies were

749   recorded with 40 frames, a defocus range of -1 to -3 µm, and a total dosage of 60 e$^-$/Å^2 using a 3x3

750   beam image shift pattern with 3 exposures per hole in the superresolution mode with a pixel size

751   of 0.416 Å for the collections on the Krios and a single exposure per hole in the superresolution

752   mode with a pixel size of 0.4345 Å for the collection on the Talos Arctica. Detailed data processing

753   workflows for each complex structure are outlined in figs. S6-13. All datasets were motion

754   corrected with patch motion correction using a bining factor of 2, and CTF parameters were

755   estimated using Patch CTF in cryoSPARC v3.2 (Punjani et al., 2017). Particle picking was done

756   with blob picker in cryoSPARC using a particle diameter of 100 to 200 Å, and movies and picked

757   particles were inspected before extraction. Particles were extracted and classified using 2D

758   classification in cryoSPARC (Punjani et al., 2017). After discarding ice and junk particles, the

759   remaining particles were used for *ab initio* modeling with 4 volumes, which were futher refined

760   with heterogenerous refinement in cryoSPARC (Punjani et al., 2017). Subsequent homogeneous

761   and non-uniform refinements were carried out for final reconstructions in cryoSPARC (Punjani et

762   al., 2017). Because Fab interactions with 'up' RBDs are generally not well resolved in Fab-spike

763   complex structures (Pinto et al., 2020), we used masks to locally refine and improve the interfaces

764   of Fabs bound to 'up' RBDs when necessary. For local refinements, masks were generated using

765   UCSF Chimera (Pettersen et al., 2004) and refinements were carried out in cryoSPARC (Punjani

766   et al., 2017).

767

768   **Cryo-EM Structure Modeling and Refinement**

769   An initial model of the M8a-3 Fab–spike trimer complex was generated by docking a single-

770   particle cryo-EM Fab-SARS-CoV-2 spike 6P complex structure (PDB 7SC1) into the cryo-EM

771   density using UCSF Chimera (Pettersen et al., 2004). The model was refined using real space

772   refinement in Phenix (Liebschner et al., 2019). The Fab amino acid seqence was manually

773   corrected in *Coot* (Emsley et al., 2010). The model of the M8a-3 Fab–spike complex was

774   subsequently used for docking and model generation for remaining Fab-spike trimer complexes.

775   For the Fab-spike complexes that we have RBD-Fab crystal structures for (M8a-6 Fab-RBD, M8a-

776   34 Fab–RBD and HSW-2 Fab–RBD structures), we first docked the spike trimer (PDB 7SC1) in

777   the EM density map, manually fitted the RBDs in *Coot* (Emsley et al., 2010) and refined the spike

778   trimer using phenix.real_space_refine (Liebschner et al., 2019). The RBD-Fab structures were then

779   aligned to each of the RBDs in the corresponding Fab–spike complexes, and the RBD regions in

780   the EM model were replaced by the RBDs from crystal structures upon structural alignments in

781   *Coot* (Emsley et al., 2010). The final model containing the spike trimer and the Fabs were

782   subsequently refined with phenix.real_space_refine (Liebschner et al., 2019). Iterative real space

783   refinement and model building were separately carried out in Phenix (Liebschner et al., 2019) and

784   *Coot* (Emsley et al., 2010). Single-particle cryo-EM refinement statistics are reported in Table S2.

785

786   **Structure Analyses**

787   Structure figures were made using UCSF ChimeraX (Goddard et al., 2018; Pettersen et al., 2021).

788   Distances were measured using PyMol (Schrödinger and DeLano, 2020). Interacting residues

789   between a Fab and RBD were analyzed by PDBePISA (Krissinel and Henrick, 2007) using the

790   following interaction definitions: potential H bonds were defined as a distance less than 3.9 Å

791   between the donor and acceptor residues when H was present at the acceptor and there was an A-

792   D-H angle between 90˚ and 270˚; potential salt bridges and van der Waals interactions were

793   defined between residues that were less than 4 Å (Krissinel and Henrick, 2007). Sequence

794   alignments were done using Geneious (https://www.geneious.com/).

795   To evaluate the potential for intra-spike crosslinking by the two Fabs of a single IgG

796   binding to adjacent RBDs within a single spike trimer, we measured the distances between the $C\alpha$

797   atoms of the C-terminal residues of the C_H1 domains of adjacent RBD-binding Fabs in the

798   structures of mAb-spike complexes as described previously (Barnes et al., 2020a). A cut-off of no

799   more than 65 Å was used to identify IgGs whose binding orientation could allow for both Fabs to

800   bind simultaneously to adjacent RBDs in a single spike trimer. This cut-off was larger than the

801   distance measured between comparable residues of C_H1 domains in intact IgG crystal structures

802   (42Å, PDB 1HZH; 48Å, PDB 1IGY; 52Å, PDB 1IGT) to account for potential influences of crystal

803   packing, flexibilities in the elbow bend angle relating the V_H-V_L and C_H1-C_L, and uncertainties in

804   the placements of C_H1-C_L domains in cryo-EM structures of the Fab-spike complexes (Barnes et

805   al., 2020a).

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Acknowledgments

Author contributions

Competing interests

Data and materials availability: Atomic models and cryo-EM maps generated from cryo-EM studies of the M8a-3–WA1 spike 6P, M8a-6–WA1 spike 6P, M8a-28–WA1 spike 6P, M8a-31–WA1 spike 6P, M8a-31–Omicron BA.1 spike 6P, M8a-34–WA1 spike 6P, HSW-1–WA1 spike 6P, and HSW-2–WA1 spike S1 domain complexes have been deposited at the Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB) under the following accession codes: PDB

1027   7UZ4, 7UZ5, 7UZ6, 7UZ7, 7UZ8, 7UZ9, 7UZA, and 7UZB; EMDB EMD-26878, EMD-26879,
1028   EMD-26880, EMD-26881, EMD-26882, EMD-26883, EMD-26884, and EMD-26885. Atomic
1029   models generated from crystal structures of M8a-34–RBD and HSW-2–RBD complexes have been
1030   deposited at the PDB under accession codes 7UZC and 7UZD, respectively. Materials are available
1031   upon request to the corresponding author with a signed material transfer agreement. This work is
1032   licensed under a Creative Commons Attribution-NonCommercial 2.0 Generic (CC BY-NC 2.0)
1033   license, which permits unrestricted use, distribution, and reproduction in any medium, provided
1034   the original work is properly cited and not for commercial purposes. To view a copy of this license,
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1036   figures/photos/artwork or other content included in the article that is credited to a third party;
1037   obtain authorization from the rights holder before using such material.

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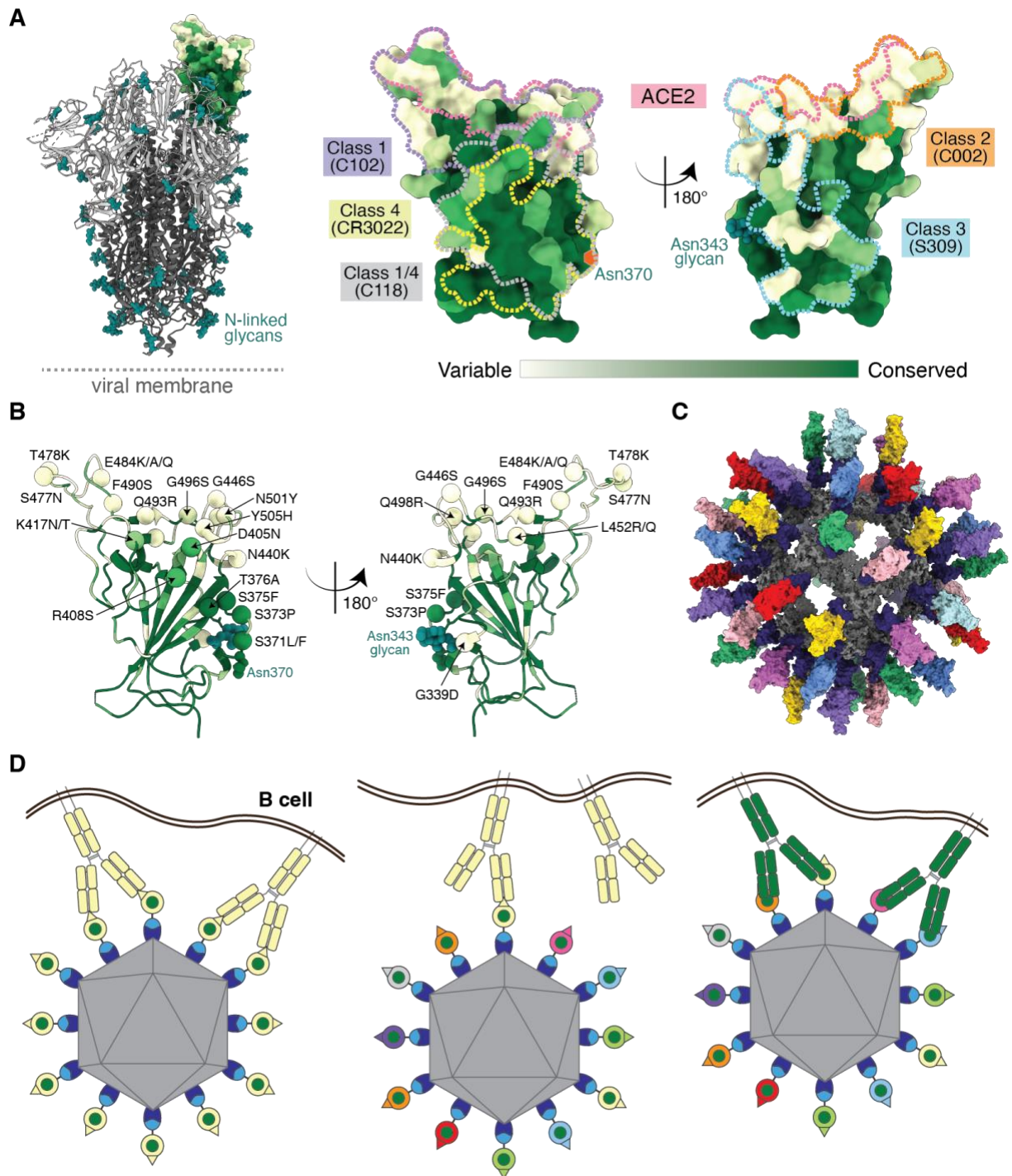
Figure 1. Utilizing antibody avidity effects suggests a strategy to target antibodies to conserved regions of sarbecovirus RBDs. (**A**) Left: Structure of SARS-CoV-2 spike trimer (PDB 6VYB) with one RBD in an "up" position. Right: Sequence conservation of 16 sarbecovirus RBDs (Figure S1) calculated by the ConSurf Database (Landau et al., 2005) plotted on a surface representation of the RBD structure (PDB 7BZ5). Class 1, 2, 3, 4, and 1/4 epitopes are outlined in different colored dots using information from structures of the representative monoclonal antibodies bound to RBD or spike trimer (C102: PDB 7K8M; C002: PDB 7K8T, S309: PDB 7JX3; CR3022: PDB 7LOP; C118: PDB 7RKV). (**B**) RBD mutations of 13 SARS-CoV-2 VOCs and

1049   VOIs (https://viralzone.expasy.org/9556) plotted onto the RBD structure (PDB 7BZ5) as spheres
1050   that are colored according to the variability gradient in panel A. The N-linked glycan at position
1051   343 of SARS-CoV-2 RBD is shown as teal spheres, and a potential N-linked glycosylation site at
1052   position 370 (SARS-CoV-2 numbering) found in some sarbecovirus RBDs but not in the SARS-
1053   CoV-2 RBD is indicated by an orange hexagon. (**C**) Structural model of mosaic-8 nanoparticle
1054   formed by SpyCatcher-mi3 and eight SpyTagged RBDs made using coordinates of an RBD (PDB
1055   7SC1), mi3 (PDB 7B3Y), and SpyCatcher (PDB 4MLI). (**D**) Hypothesis for preferential
1056   stimulation of B cells with that encode cross-reactive antibodies by mosaic (right) versus
1057   homotypic (left) RBD nanoparticles. Left: Yellow B cell receptors recognizing an accessible
1058   strain-specific epitope (yellow triangle) can crosslink between adjacent RBDs on a homotypic
1059   nanoparticle to enhance binding through avidity effects. Middle: Yellow B cell receptors against
1060   a strain-specific orange epitope cannot crosslink between adjacent RBDs on a mosaic RBD
1061   nanoparticle that presents different versions of the epitope (colored triangles). Right: Green cross-
1062   reactive B cell receptors can crosslink between a conserved epitope (green circles) on adjacent
1063   RBDs in a mosaic RBD nanoparticle to enhance binding to a more occluded, but conserved,
1064   epitope through avidity effects.

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Figure 2. A subset of mAbs elicited in mosaic-8 and homotypic SARS-CoV-2 RBD nanoparticle-immunized mice show cross-reactive binding and neutralization properties. (A) Top four rows: RBDs used for screening of single B cells. Red indicates binding; dark gray indicates no binding. Remaining rows: ELISA EC$_{50}$ values for mouse mAb binding to sarbecovirus RBDs from different clades. RBDs that were included on the mosaic-8 RBD-nanoparticles are

1074   shaded in green. EC_{50} values were derived from ELISAs conducted with duplicate samples at least

1075   twice (for first seven mAbs) or once (for remaining mAbs). M8a-11 and M8a-26 shared the same

1076   protein sequences, the same EC_{50} values were presented twice. (**B**) Neutralization potencies (IC_{50}

1077   values) of mAbs against SARS-CoV-2 variants and indicated sarbecoviruses. IC_{50} values are

1078   reported from neutralization assays that were conducted using duplicate samples at least twice

1079   except for a single assay for M8a-28 against Omicron BA.1.

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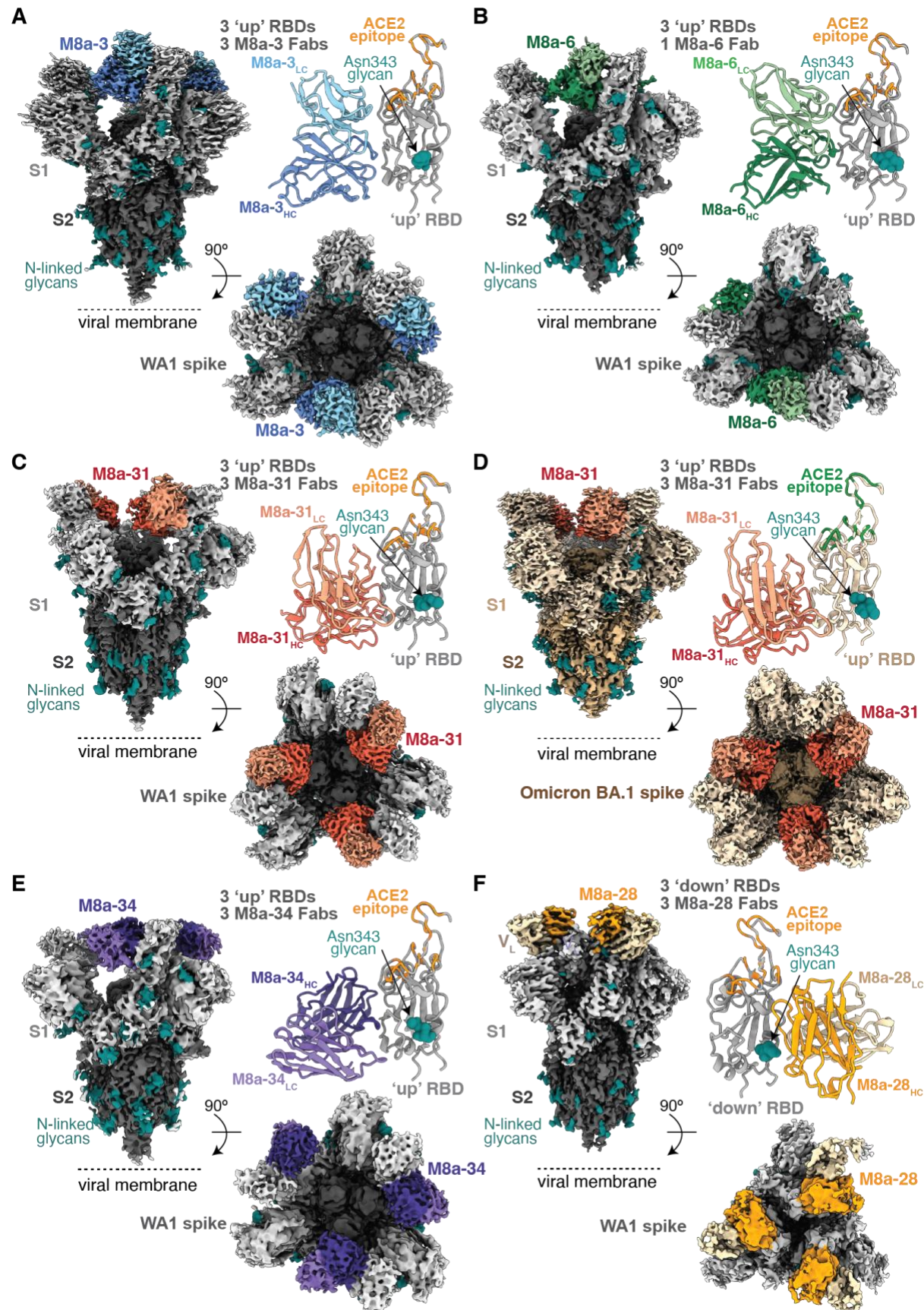
Figure 3. mAbs isolated from mice immunized with mosaic-8 nanoparticles target epitopes outside of the ACE2 binding footprint. EM densities of single-particle cryo-EM structures of Fab-spike trimer complexes are shown from the side (upper left), top (lower right), and as cartoon diagrams of the Fab V_H-V_L interaction with the RBD (upper right; RBD residues involved in ACE2 binding are in colored in orange for complexes with WA1 spike and in green for complex with Omicron BA.1 spike). Only V_H-V_L domains are shown for each Fab. (**A**) WA1 spike complexed with M8a-3. (**B**) WA1 spike complexed with M8a-6. (**C**) WA1 spike complexed with M8a-31. (**D**) Omicron BA.1 spike complexed with M8a-31. (**E**) WA1 spike complexed with M8a-34. (**F**) WA1 spike complexed with M8a-28.
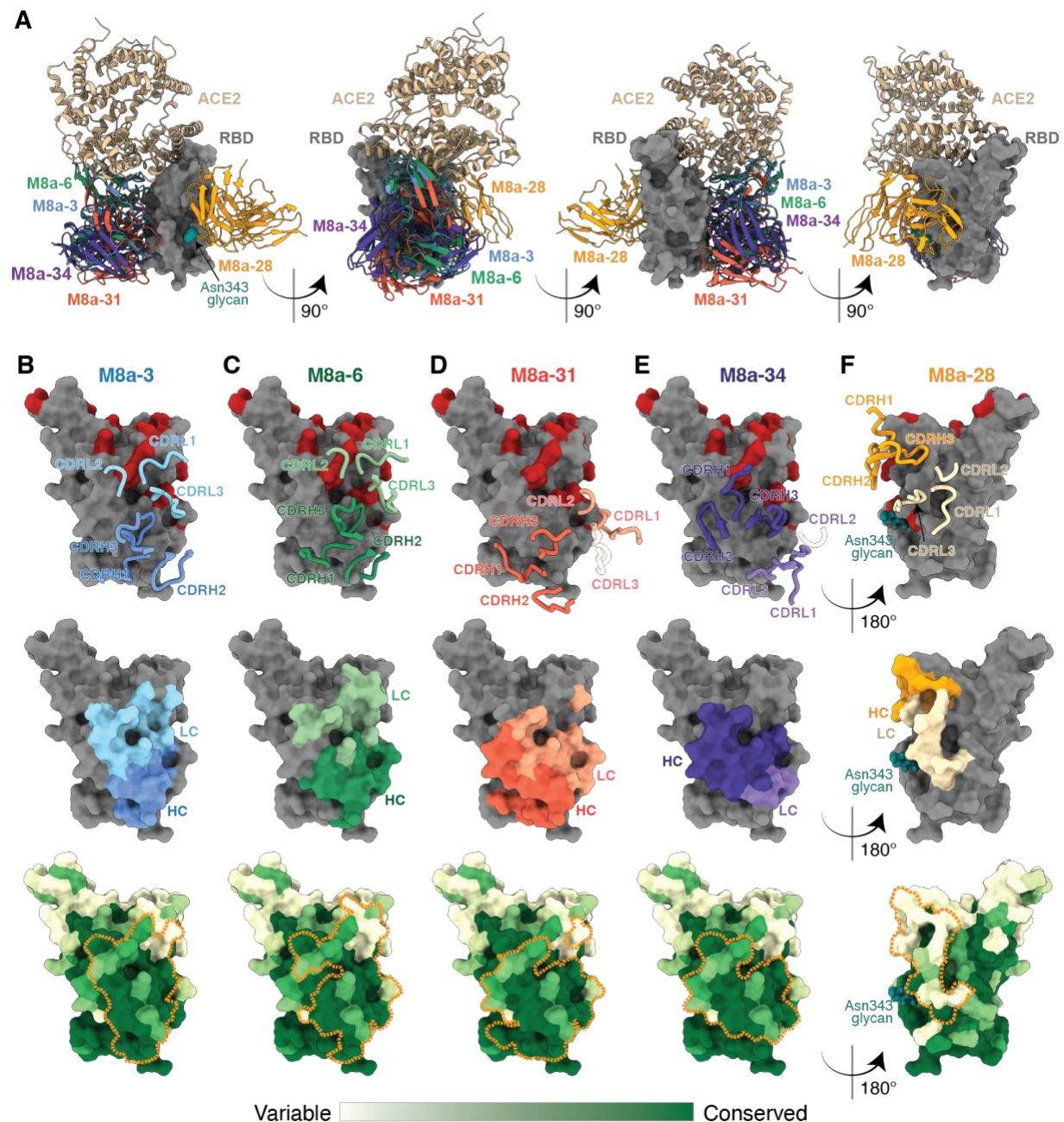
Figure 4. Epitopes of mAbs elicited by mosaic-8 immunization demonstrate targeting of non-class 1/class 2 RBD epitopes. (**A**) Four views of RBD surface (dark gray) with overlay of mAb V_H-V_L domains (different colored cartoon representations) from Fab-spike structures. ACE2 (tan cartoon representation) complexed with RBD (PDB 6M0J) is shown for comparison. (**B-F**) mAb epitopes on RBD surface shown with overlaid heavy and light chain CDRs (top, CDRs that do not interact with the RBD are shown in transparent cartoons; all CDRs were defined based on the IMGT definition), as colored areas for heavy and light chains (middle) and outlined with orange dotted lines on a sequence conservation surface plot (bottom; calculated using the 16 sarbecovirus RBD sequences shown in Figure S1). The N-glycan at RBD position Asn343 was shown as

1101   spheres. (**B**) M8a-3. (**C**) M8a-6. (**D**) M8a-31 from complex with WA1 spike trimer. (**E**) M8a-34.

1102   (**F**) M8a-28. Omicron BA.1 and BA.2 substitutions were colored red in the top panels of (**B**-**F**).

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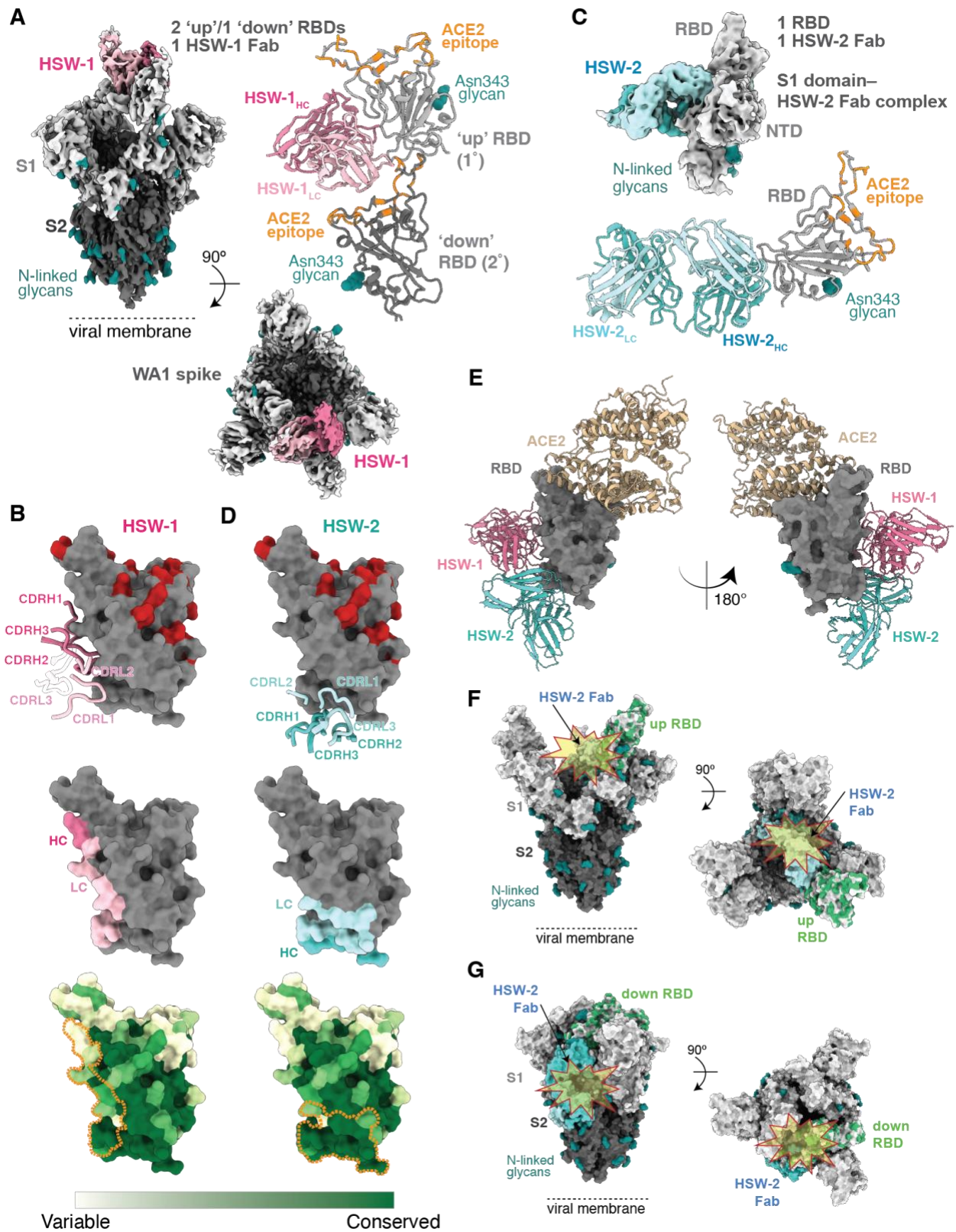
Figure 5. Epitopes of mAbs isolated from mice immunized with homotypic SARS-CoV-2 nanoparticles that target conserved RBD epitopes. (**A**) EM density of single-particle cryo-EM structure of HSW-1 Fab–spike trimer complex shown from the side (upper left), the top (lower right), and as a cartoon diagram of the HSW-1 V_H-V_L interaction with two adjacent RBDs (1˚ and 2˚) (upper right). HSW-1 interacts mainly with an 'up' RBD (1˚ RBD, light grey) but also includes V_L interactions with a 'down' RBD (2˚ RBD, dark grey). (**B**) HSW-1 epitope on RBD surface shown with overlaid heavy and light chain CDRs (top, CDRs that do not interact with the RBD are shown in transparent cartoons; all CDRs were defined based on the IMGT definition), as colored areas for heavy and light chains (middle) and outlined with orange dotted lines on a sequence conservation surface plot (bottom; calculated using the 16 sarbecovirus RBD sequences in Figure S1). Omicron BA.1 and BA.2 substitutions were colored red in the top panel. (**C**) EM density of single-particle cryo-EM structure of HSW-2–Fab S1 domain complex (top) and cartoon diagram of the HSW-2 V_H-V_L interaction with the RBD (bottom). (**D**) HSW-1 epitope on RBD surface shown with overlaid heavy and light chain CDRs (top, all CDRs were defined based on the IMGT definition), as colored areas for heavy and light chains (middle) and outlined with orange dotted lines on a sequence conservation surface plot (bottom; calculated using the 16 sarbecovirus RBD sequences shown in Figure S1). Omicron BA.1 and BA.2 substitutions were colored red in the top panel. (**E**) Two views of RBD surface (dark gray) with overlays of mAb V_H-V_L domains (different colored cartoon representations) from HSW Fab-spike structures and ACE2 (tan cartoon representation from PDB 6M0J). (**F,G**) Superpositions of HSW-2–RBD structure onto the RBD from a spike trimer structure showing that HSW-2 Fab is sterically hindered from binding to either an 'up' or 'down' RBD on an intact spike trimer due to clashes (indicated as starbursts) with the spike S2 domain. (**F**) HSW-2 Fab-RBD interaction modeled onto an 'up' RBD from the M8a-31–spike complex structure. (**G**) HSW-2 Fab-RBD interaction modeled onto a 'down' RBD from the M8a-28–spike complex structure.
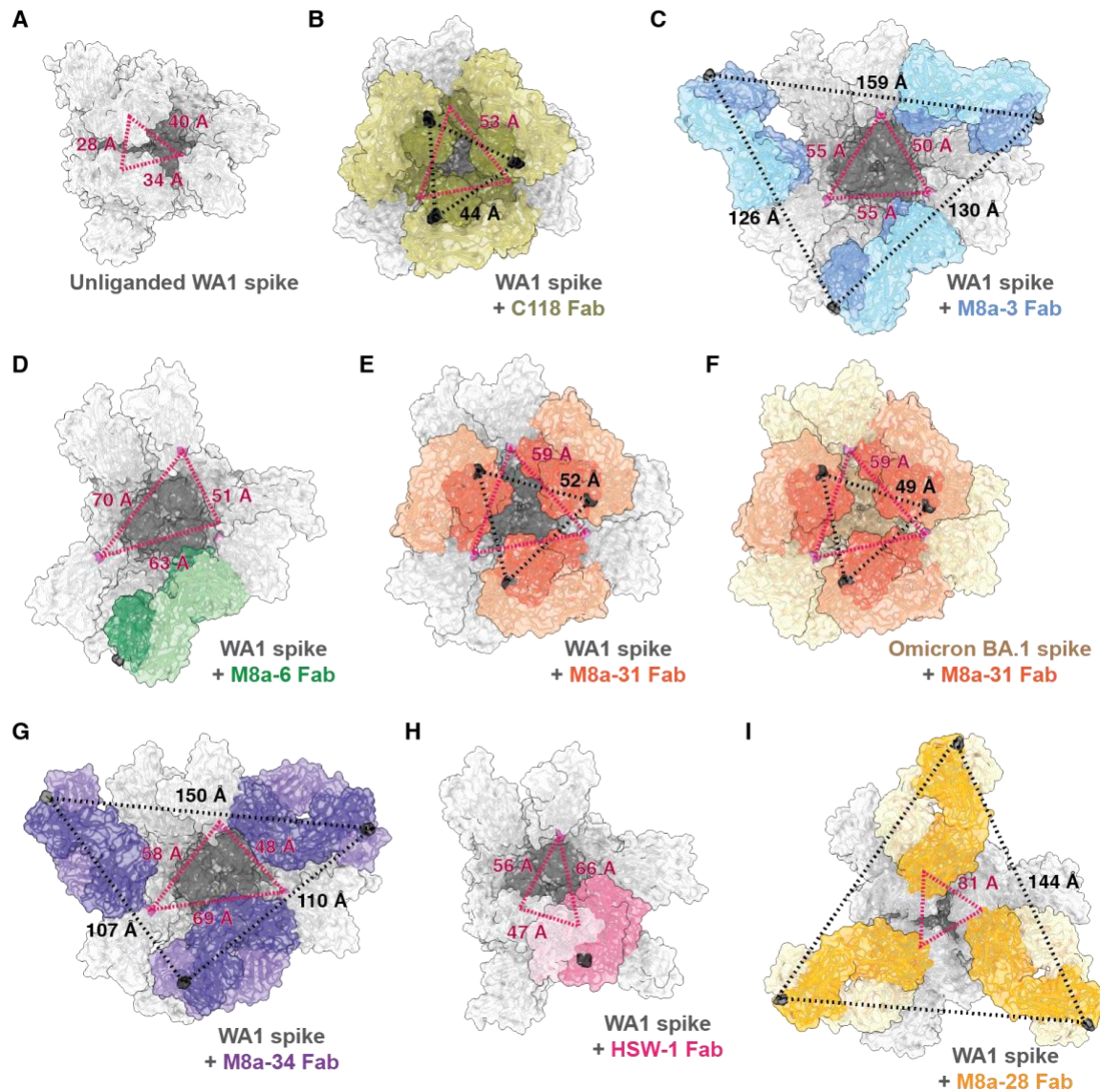
Figure 6. Spike-mAb complex structures show increased trimer openness and the potential for intra-spike IgG crosslinking. Red dotted lines: Trimer openness was assessed by measuring distances between the Cα atoms of RBD residue 428 (pink) in each RBD of a spike trimer from the indicated Fab-spike complex structures (top-down views with mAb Fabs shown in colors on a gray spike trimer (WA1) or an orange spike trimer (Omicron BA.1). Distances of 39 Å or less indicate a typical closed, prefusion spike trimer conformation (Barnes et al., 2020a) (panel A). Binding of class 1/4 anti-RBD antibodies such as C118 and S2X259 result in larger inter-RBD distances: 53 Å for C118 (panel B) and 43 Å for S2X259 (PDB 7RA8), indicating a more open trimer conformation. Black dotted lines: The potential for intra-spike crosslinking by the two Fabs of a single bound IgG was assessed by measuring distances between the Cα atoms of C-terminal C$_H$1 residues (black) on adjacent bound Fabs on the RBDs of a spike trimer. Distances less than 65 Å are considered compatible with the potential for intra-spike crosslinking (Barnes et al., 2020a). (**A**) Unliganded spike (PDB 6VYB): closed prefusion conformation. (**B**) C118 Fab–WA1 spike (PDB 7RKV): open trimer conformation with potential for intra-spike crosslinking by C118 IgG. (**C**) M8a-3 Fab–WA1 spike: open trimer confirmation with no potential for intra-spike

1148   crosslinking. (**D**) M8a-6–WA1 spike: open trimer conformation. Black dotted lines between the
1149   Cα atoms of C-terminal C_H1 residues are not shown because the reconstruction included only one
1150   Fab. (**E**) M8a-31–WA1 spike: open trimer conformation with potential for intra-spike crosslinking
1151   by M8a-31 IgG. (**F**) M8a-31–Omicron BA.1 spike: open trimer conformation with potential for
1152   intra-spike crosslinking by M8a-31 IgG. (**G**) M8a-34–WA1 spike: open trimer conformation with
1153   no potential for intra-spike crosslinking by M8a-34 IgG. (**H**) HSW-1–WA1 spike: open trimer
1154   conformation. Black dotted lines between the Cα atoms of C-terminal C_H1 residues are not shown
1155   because the reconstruction included only one Fab. (**I**) M8a-28–WA1 spike: closed trimer
1156   conformation with no potential for intra-spike crosslinking.
1157

B

Antibody	Generic name	PDB ID
LY-CoV1404	Bebtelovimab	7MMO
S309	Sotrovimab	7JX3
REGN10987	Imdevimab	6XDG
AZD1061	Cilgavimab/ Evusheld	7L7E
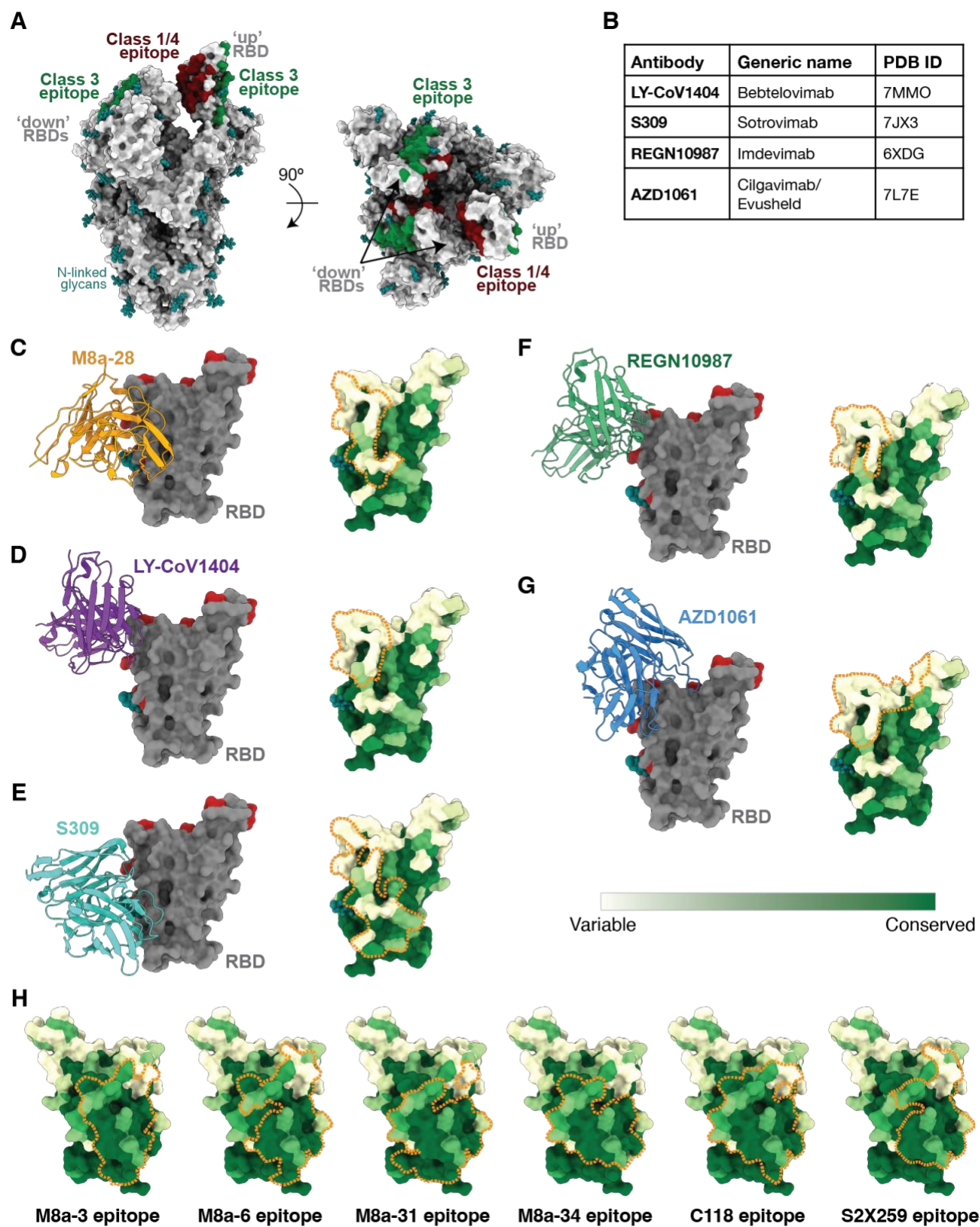
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Figure 7. Comparison of M8a epitopes with human mAbs targeting class 3 or class 1/4 RBD epitopes. (**A**) Locations of class 3 and class 1/4 RBD epitopes mapped on an unliganded spike trimer structure with two 'down' and one 'up' RBDs (PDB 6VYB) showing that the class 3 epitope is exposed, whereas the class 1/4 epitope is partially occluded in the context of the spike trimer. The binding epitopes of representative class 3 (S309/Sotrovimab, PDB 7JX3) and class 1/4 (C118, PDB 7RKV) anti-RBD antibodies were identified by PDBePISA (Krissinel and Henrick, 2007). (**B**) Class 3 anti-RBD mAbs that currently or previously received Emergency Use Authorization (EUA) approval for human administration by the US Food and Drug Administration (modified from (Zhou et al., 2022)). Of these mAbs, only LY-CoV1404/Bebtelovimab retains full neutralization potency against Omicron BA.1 (Zhou et al., 2022), and the NIH COVID-19 treatment guidelines recommend against use of Bamlanivimab plus Etesevimab, Casirivimab plus Imdevimab, or Sotrovimab for the treatment of COVID-19 (US Food and Drug Administration fact sheets listed below). (**C-G**) Left: V_H-V_L domains of M8a-28 and currently or previously EUA-approved class 3 anti-RBD mAbs (cartoon representations) shown interacting with an RBD (gray surface representation with Omicron BA.1 and BA.2 substitutions in red and the RBD Asn343 N-glycan shown as teal spheres). Right: mAb epitopes outlined with orange dotted lines on a sequence conservation surface plot (calculated using the 16 sarbecovirus RBD sequences shown in Figure S1). (**C**) M8a-28. (**D**) LY-CoV1404/Bebtelovimab (PDB 7MMO). (**E**) S309/Sotrovimab (PDB 7JX3). (**F**) REGN10987/Imdevimab (PDB 6XDG). (**G**) AZD1061/Cilgavimab (PDB 7L7E). (**H**) Comparison of the class 1/4 epitopes of M8a mouse mAbs isolated in these studies with the epitopes of C118 (PDB 7RKV), a broadly cross-reactive human mAb (Jette et al., 2021; Robbiani et al., 2020), and S2X259 (PDB 7RAL), a broadly reactive and potent human class 1/4 mAb (Tortorici et al., 2021) .

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Food and Drug Administration. Fact sheet for healthcare providers: emergency use authorization for Evusheld (tixagevimab co-packaged with cilgavimab). 2022. Available at: https://www.fda.gov/media/154701/download.

Food and Drug Administration. Fact sheet for health care providers: emergency use authorization (EUA) of bamlanivimab and etesevimab. 2022. Available at: https://www.fda.gov/media/145802/download.

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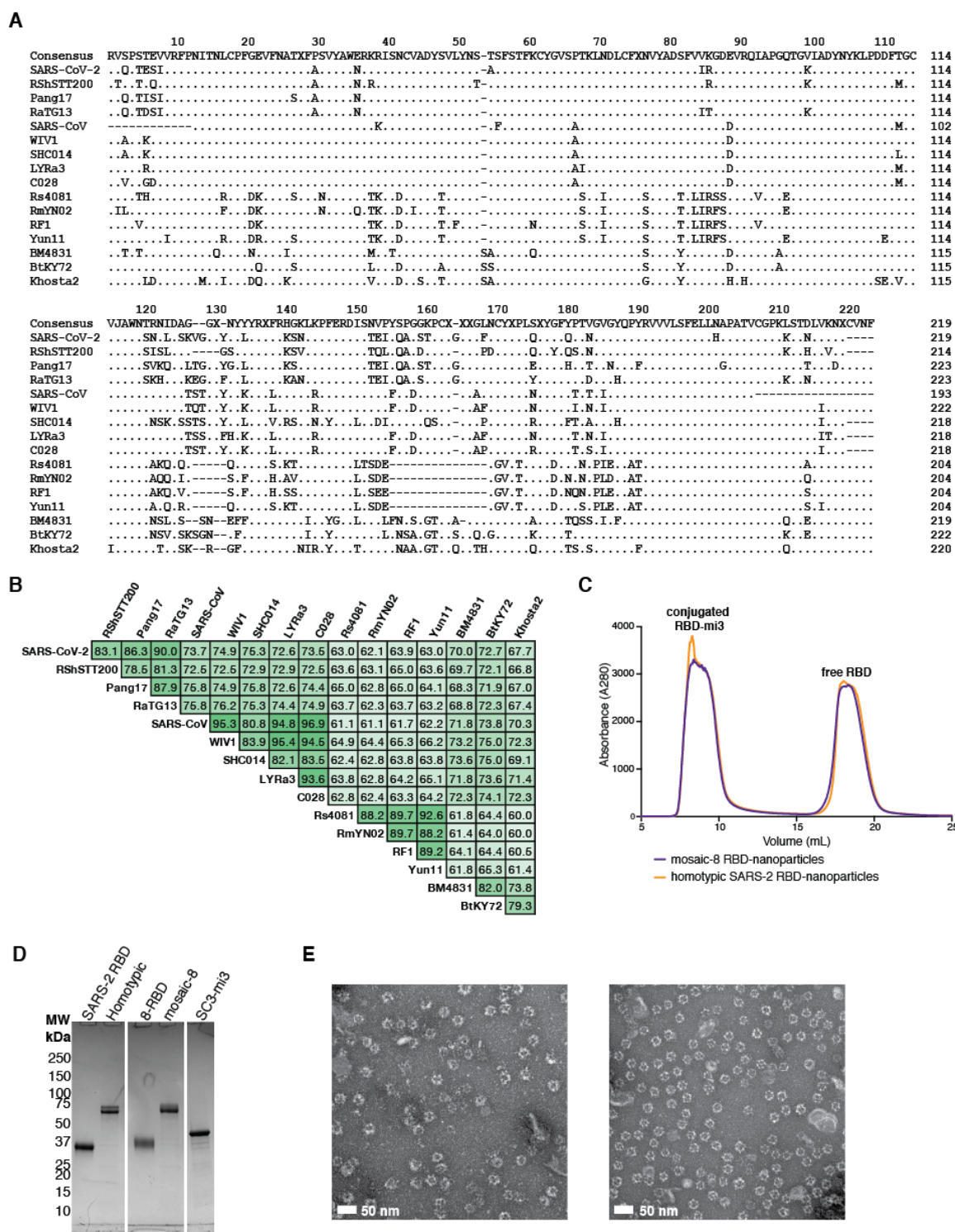
Figure S1. Sarbecovirus RBDs and construction of RBD-nanoparticles. (**A**) Sequence alignment of RBDs from 16 sarbecoviruses. (**B**) Pairwise sequence identities (%) relating the RBDs in panel A. (**C**) SEC profile showing separation of conjugated SpyCatcher003-mi3 nanoparticles from free RBDs. RBDs were added at a 2-fold molar excess over SpyCatcher003-mi3 subunits. (**D**) Reducing SDS-PAGE analysis of free RBD, purified conjugated RBD-

1205   nanoparticles, and unconjugated SpyCatcher-mi3 nanoparticles. (**E**) Negative stain EM of
1206   conjugated nanoparticles. Left: mosaic-8 RBD-nanoparticles. Right: homotypic SARS-CoV-2
1207   RBD-nanoparticles.
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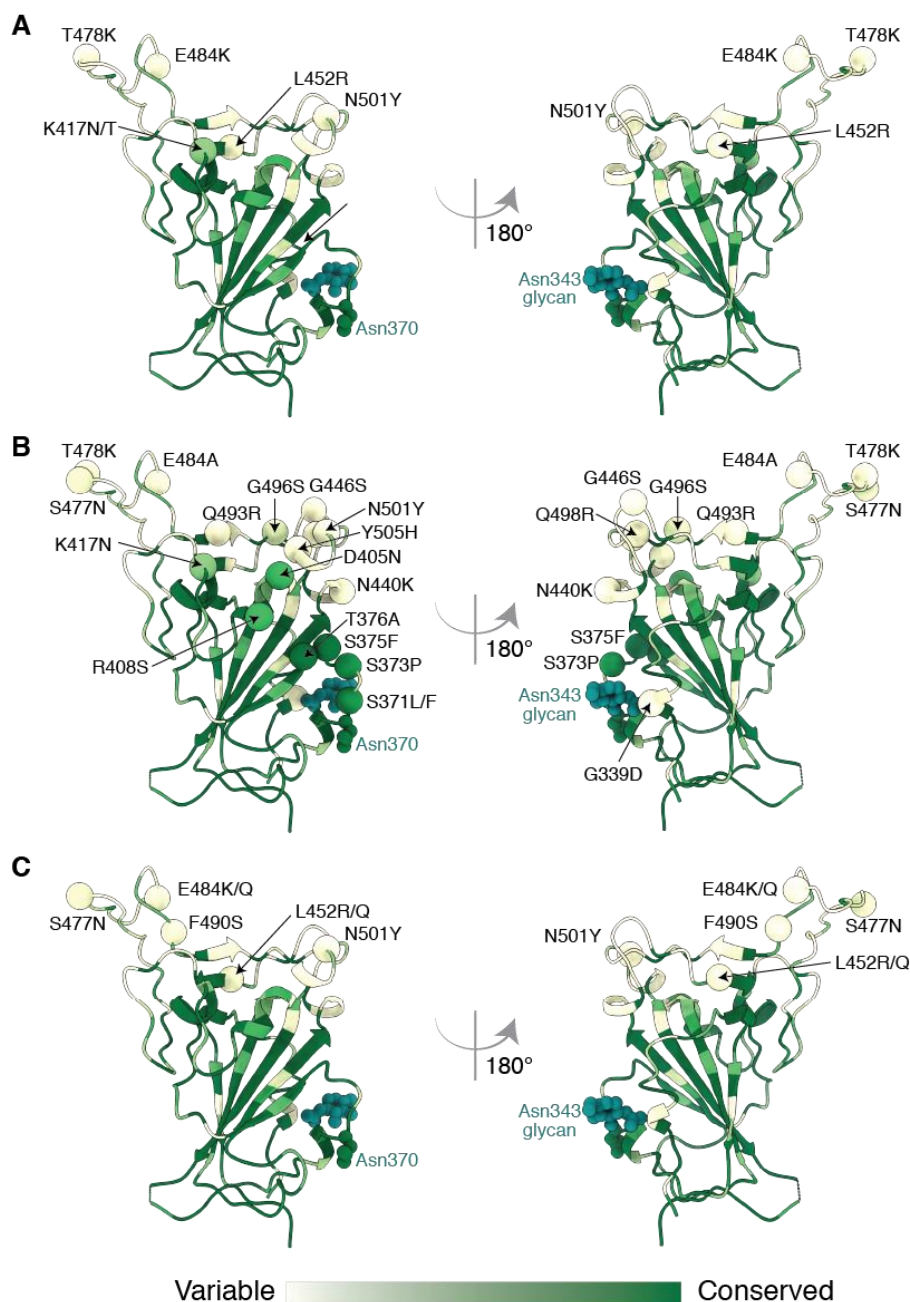
Figure S2. RBD VOC and VOI substitutions. Locations of RBD substitutions in VOCs and VOIs (https://viralzone.expasy.org/9556) are shown as spheres colored according to a variability gradient (bottom) on the WA1 RBD structure (PDB 7BZ5). The N-linked glycan at position 343 of SARS-CoV-2 RBD is shown as teal spheres, and a potential N-linked glycosylation site at position 370 (SARS-CoV-2 numbering) that is found in sarbecovirus RBDs but not in the SARS-CoV-2 RBD is also shown in spheres. (**A**) SARS-CoV-2 Alpha, Beta, Gamma and Delta variants. (**B**) SARS-CoV-2 Omicron BA.1 and BA.2 variants. (**C**) SARS-CoV-2 VOIs.

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A

Specificity assay for RBD binding

Loading outcome table

Loading outcome	Homotypic SARS-CoV-2 RBD-mi3 immunized mice	percent of chip	Mosaic-8 RBD-mi3 immunized mice	percent of chip
Pens with single cell	7699	70.0	7747	70.4
Pens with >1 cell	1431	13.0	1948	17.7
Total Pens with cells	9130	83.0	9695	88.1

B

Examples of assays for mosaic-8 RBD-mi3 immunized mice

| Clade 1b SARS-CoV-2 RBD | Clade 1a SARS-CoV RBD | Clade 2 Yun11 RBD | Clade 3 BtkY72 RBD |

Examples of assays for homotypic SARS-CoV-2 RBD-mi3 immunized mice

| Clade 1b SARS-CoV-2 RBD | Clade 1a SARS-CoV RBD | Clade 2 Yun11 RBD | Clade 3 BtkY72 RBD |

C

Mosaic-8 RBD-mi3 immunized mice assay results

Among positive pens with single cell	SARS-2 RBD	SARS-RBD	Yun11-RBD	BtKY72-RBD	Total
SARS-2-RBD		9	9	6	18
SARS-RBD	9		7	7	20
Yun11-RBD	9	7		5	24
BtKY72-RBD	6	7	5		9

Homotypic SARS-CoV-2 RBD-mi3 immunized mice assay results

Among positive pens with single cell	SARS-2 RBD	SARS-RBD	Yun11-RBD	BtKY72-RBD	Total
SARS-2-RBD		11	12	9	130
SARS-RBD	11		7	14	47
Yun11-RBD	12	7		17	39
BtKY72-RBD	9	14	17		36

Assay outcome	Immunization	
	Homotypic SARS-CoV-2 RBD-mi3 immunized mice	Mosaic-8 RBD-mi3 immunized mice
Positive for all 4 RBDs	3	5
Positive for 3 RBDs (negative for SARS-1-RBD)	3	0
Positive for 3 RBDs (negative for Yun11-RBD)	3	2
Positive for 3 RBDs (negative for BtKY72-RBD)	1	1
Positive for 3 RBDs (negative for SARS-2-RBD)	2	0

Figure S3. Beacon setup. Individual plasma cells were isolated, cultured, and assayed for RBD binding activities using the Beacon optofluidic system. (**A**) Antigen-specific binding activity was assayed using biotinylated RBDs immobilized on streptavidin-coated beads and loaded into the main channel of the Beacon microfluidic chip, which contained single plasma B cells in individual culture compartments (Nanopens). The specificity of secreted antibodies for the antigen presented in the channel was detected via the local concentration of a fluorescently-labeled secondary above a Nanopen secreting an antigen-specific antibody. (**B**) Examples of detection of secreted antibody binding to different RBDs. The Nanopen(s) from which the secreted antibody signal was emanating over the assay time course is indicated in yellow. (**C**) Summary of cross-reactive antigen-specificity assay results for four RBDs.

A

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                              CDRH1                    CDRH2
Consensus       QVQLQQPGAELVKPGASVKLSCKASGYTFTXYWMHWVKQRPGQGLEWIGXIYPXDGYTKYNE   62
M8a-3(IgHV1-69) ...........L.............N...........H......E.D.F.T.I.I.Q   62
M8a-6(IgHV1-69) ........T...M..........T.......H...........E......E.A.S.N.V...Q   62
M8a-28(IgHV1-55)...................M.....N.NH..IS..............D...LSHF.T...   62
M8a-31(IgHV14-3)E...K.SV....R......V..T...FNIKNIY........E...D...R.D.AN.NSR.AP   62
M8a-34(IgHV1-55)..................M......IT..IT..............D...GG.R.N...   62
HSW-1(IgHV1-64) ................T.M........S.................M.H.NS.S.....   62
HSW-2(IgHV1-82) ......S.P..........I.......V.STS..S........E.P....R...R..HSSSTG  62

                        CDRH3
Consensus       KFKXKATLTVDTSSSTAYMQLSSLTSEDSAVYYCARPDX---XXYXYFDYWGQGTTLTVSS   120
M8a-3(IgHV1-69) ...G.S.............................S--SGYPV....   121
M8a-6(IgHV1-69) ...G.S..S..R...................F.....N--SGYPV.........S.....   121
M8a-28(IgHV1-55)..TNR.........T.....N....D....F...W.YFD---SRT...   120
M8a-31(IgHV14-3)..QD...I.A....N...L.........T.I....DEGW-------G.AN.....LV...A  116
M8a-34(IgHV1-55)...S...............R...........Y.GNYVGY.YAM.......SV....   123
HSW-1(IgHV1-64) N..S........K........F.............V.SGSYYGTT.D...............   123
HSW-2(IgHV1-82) ...D.....A.K..N...IH............F...DYG-----.Y...............   118

                              CDRL1                    CDRL2
Consensus       DIVMTQSPASLSVSLGERVTISCRASQSV------GXFIAWYQQKPGQSPKLLIYXASTXXS   56
M8a-3(IgκV6-25) .......HKFM.T.V.D..S.T.K...D.-------.TY.................W...RHT   56
M8a-6(IgκV6-25) .......QKFM.T...D..S...K...D.-------.TTV................W...RHT  56
M8a-28(IgκV5-48)..LL..F..I....P....SF......TI-------.TN.H....RING..R...KY..ESI.  56
M8a-31(IgκV8-19).........S..T.TA..K..M..KS...LLNSGNQKNYLT.....V..P......W...RDP  62
M8a-34(IgκV3-5) ...L....V..A....Q.A........E..DFYG--NS..Y........A.....R..NLE.   60
HSW-1(IgκV3-5)  ...L.......A....Q.A........E..NIYG--NS.MH........P.....FR..NLE.  60
HSW-2(IgκV12-44)..Q.........A.V..A...T..L.EN.------YS.L......Q.K..Q..V.R.K.LAE  56

                        CDRL3
Consensus       GVPDRFSGSGSGTDFTLTIXSVZAEDLATYYCQQHYSTPXTFGGGTKLEIK   107
M8a-3(IgκV6-25) ......T......NY....S..Q.....L.H........Y.........   107
M8a-6(IgκV6-25) ......T.T.....Y....S..A.....L.....N..Y.........E   107
M8a-28(IgκV5-48).I.S...........S.S.NN.ES..I.D....IN.W.L...A....DL.   107
M8a-31(IgκV8-19)......T...F........S..Q.....V....ND..Y.L...A...V.L.   113
M8a-34(IgκV3-5) .I.A.......R......HP.E.D.V.....SIED.R.........   111
HSW-1(IgκV3-5)  .I.V.......R......NP.E.D.V.....H.SNED.F...S.......   111
HSW-2(IgκV12-44)...S.........Q.S.K.N.LQP..FG....H..G..P..........   107
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B

	M8a-6 V$_H$	M8a-28 V$_H$	M8a-31 V$_H$	M8a-34 V$_H$	HSW-1 V$_H$	HSW-2 V$_H$
M8a-3 V$_H$	87.6	70.7	58.7	74.8	76.4	69.4
M8a-6 V$_H$		67.5	53.7	72.4	72.4	68.6
M8a-28 V$_H$			55.8	76.4	70.7	66.7
M8a-31 V$_H$				56.9	55.3	60.2
M8a-34 V$_H$					75.6	69.1
HSW-1 V$_H$						67.5

	M8a-6 V$_L$	M8a-28 V$_L$	M8a-31 V$_L$	M8a-34 V$_L$	HSW-1 V$_L$	HSW-2 V$_L$
M8a-3 V$_L$	89.7	54.2	62.8	57.7	54.1	60.7
M8a-6 V$_L$		53.3	61.1	58.6	55.9	57.0
M8a-28 V$_L$			50.4	55.0	56.8	52.3
M8a-31 V$_L$				55.8	54.9	52.2
M8a-34 V$_L$					88.3	56.8
HSW-1 V$_L$						55.9

C

	CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3
M8a-3	8	8	14	6	3	9
M8a-6	8	8	14	6	3	9
M8a-28	8	8	13	6	3	9
M8a-31	8	8	9	12	3	9
M8a-34	8	8	16	10	3	9
HSW-1	8	8	16	10	3	9
HSW-2	8	8	11	6	3	9

Figure S4. Sequence alignment of seven mAbs isolated from RBD nanoparticle-immunized mice that bound two or more sarbecovirus RBDs. (**A**) Sequence alignment of V$_H$ and V$_L$ domains. CDRs were assigned using IMGT definitions (Lefranc et al., 2015). VH and VL gene segment assignments for each mAb were shown in parentheses. (**B**) Pairwise sequence identities (%) between V$_H$ and V$_L$ domains in (**A**). (**C**) Number of residues in CDRs.
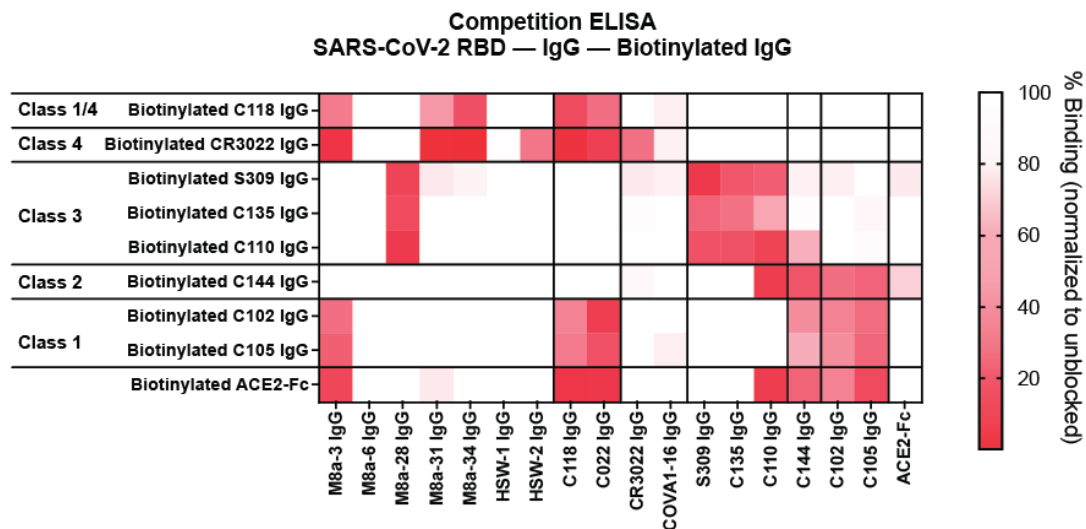
Figure S5. mAb epitope mapping. Competition ELISA experiment in which a test set of biotinylated IgGs of known epitopes or human ACE2-Fc (y-axis) were assayed for binding to SARS-CoV-2 RBD in the presence of unlabeled M8a, HSW, control IgGs, or ACE2-Fc (x-axis). The heat map shows shades of red indicating the percent binding of the tested IgGs or ACE2-Fc in the presence of competitor. M8a-3, M8a-31, M8a-34, and HSW-2 IgGs competed with biotinylated class 1/4 and/or class 4 anti-RBD, with HSW-2 competing with the biotinylated class 4 antibody only. M8a-3 IgG competed with biotinylated class 1 antibodies and ACE2-Fc in addition to the biotinylated class 1/4 and class 4 antibodies. M8a-28 IgG competed with biotinylated class 3 antibodies. M8a-6 and HSW-1 IgGs showed no competition in this assay.
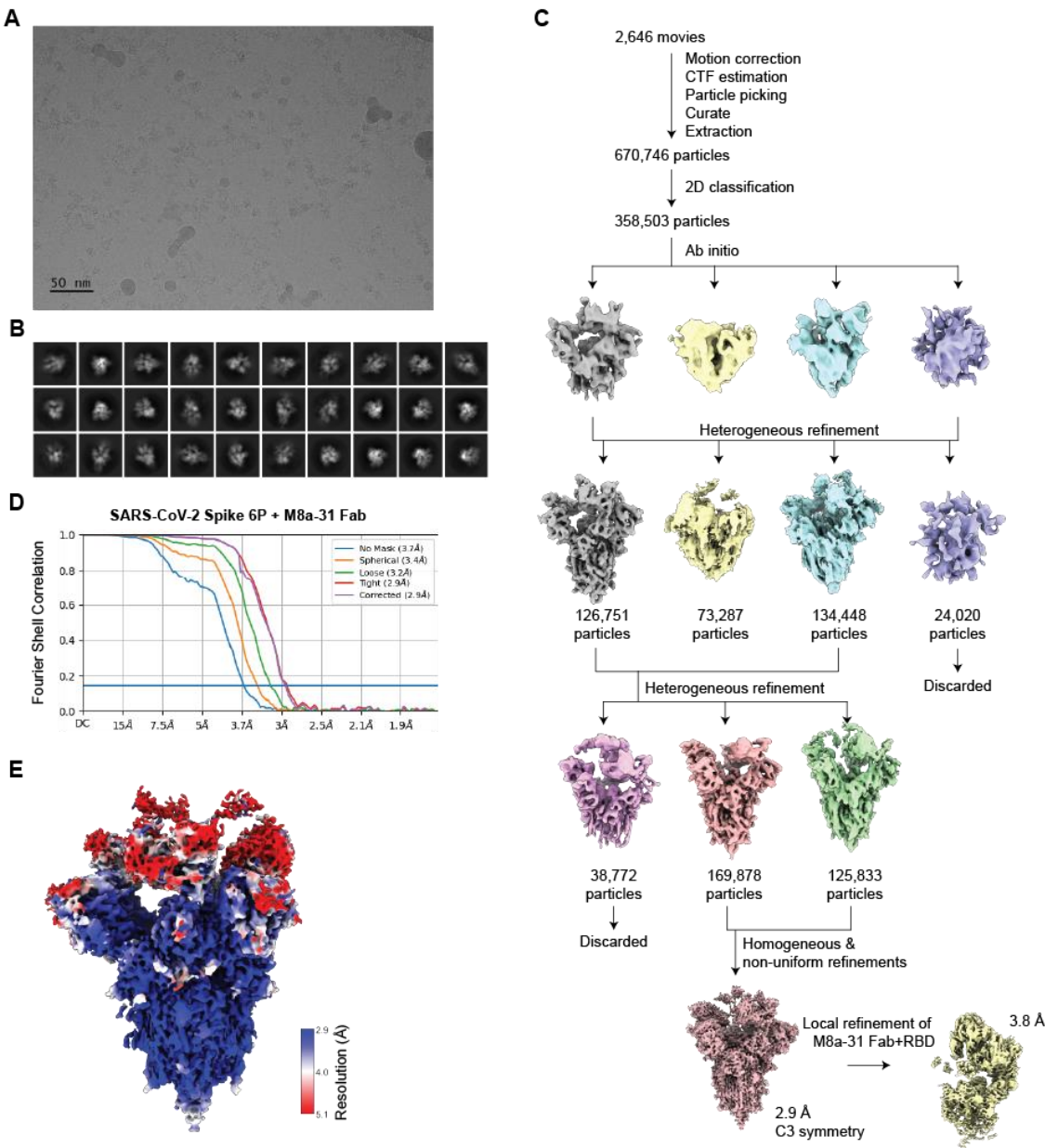
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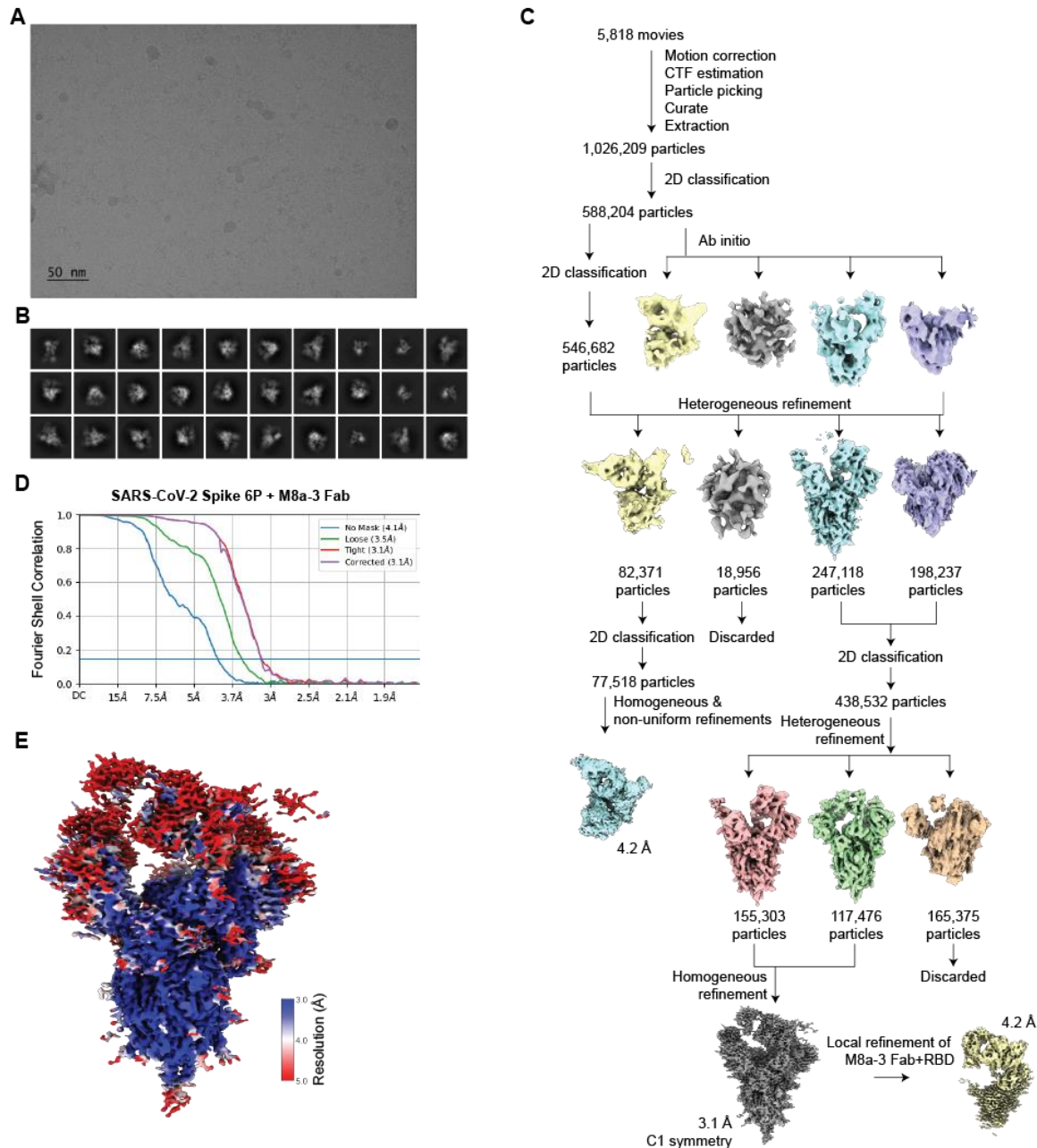
Figure S6. Cryo-EM data processing and validation for of M8a-3 Fab in complex with SARS-CoV-2 WA1 spike. (**A**) Representative micrograph. (**B**) Representative 2D classes. (**C**) Workflow of single-particle data processing. (**D**) Fourier shell correlation (FSC) plot of the final reconstruction. (**E**) Final reconstruction of M8a-3 Fab in complex with SARS-CoV-2 spike, colored by local resolution.
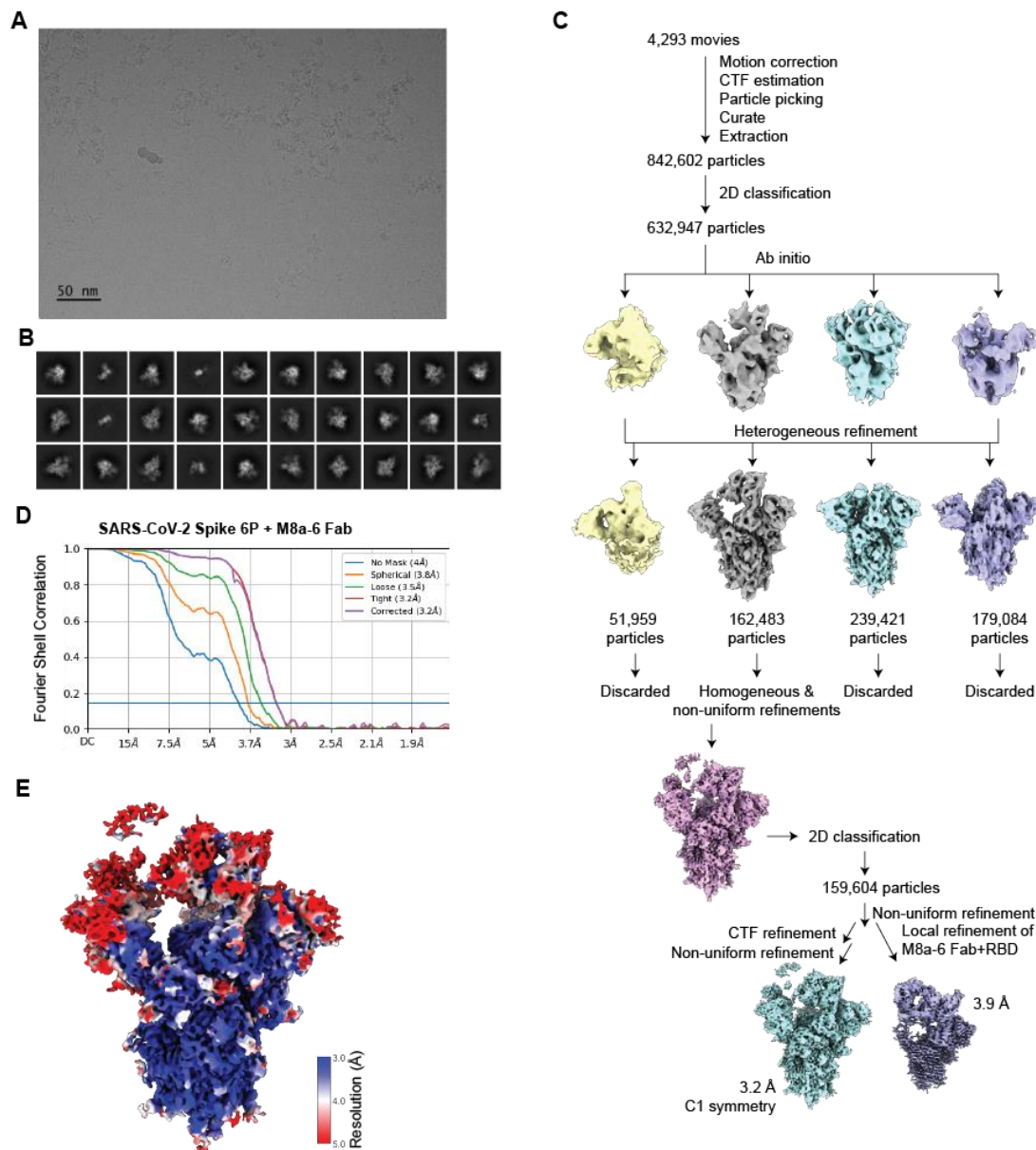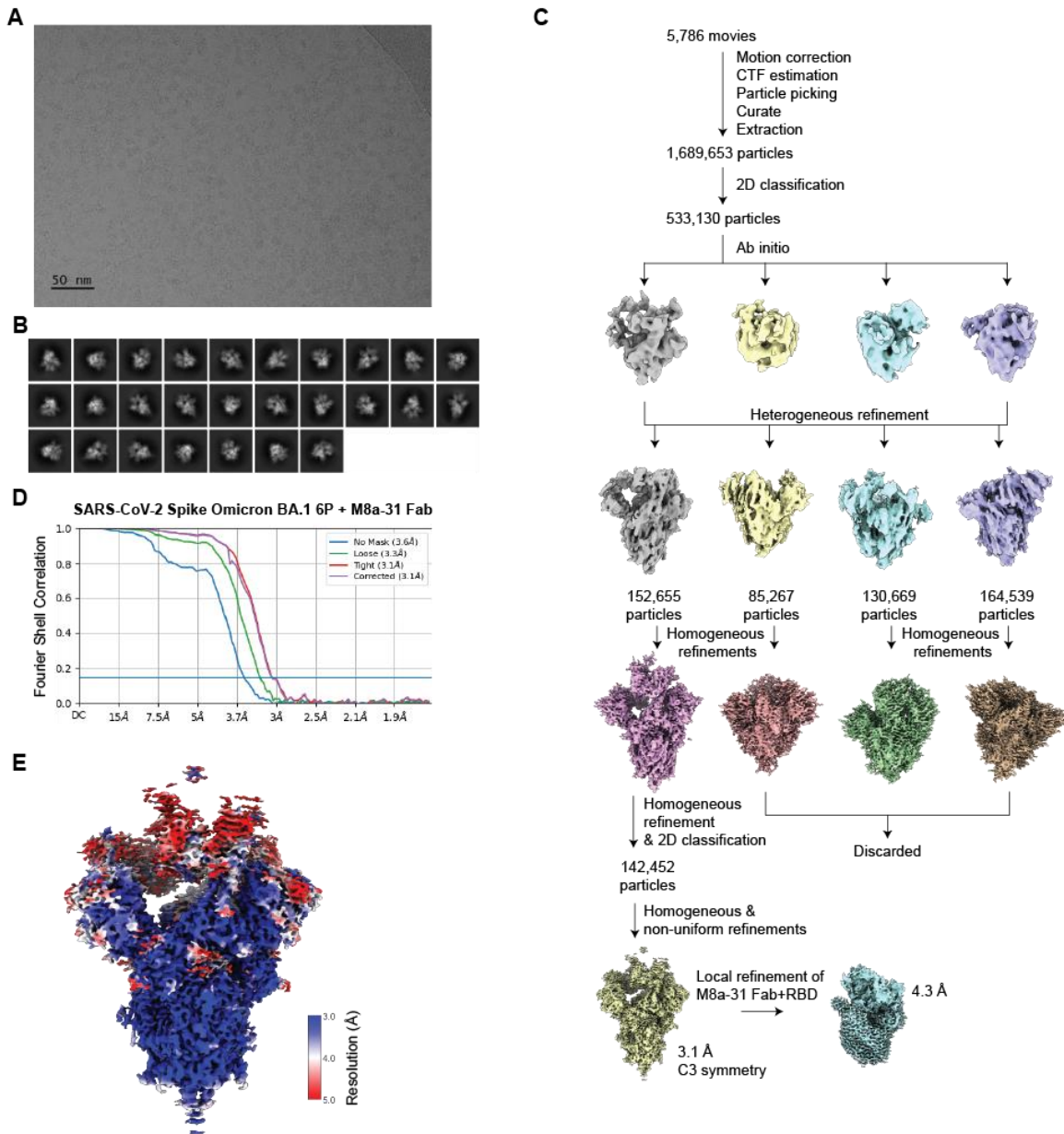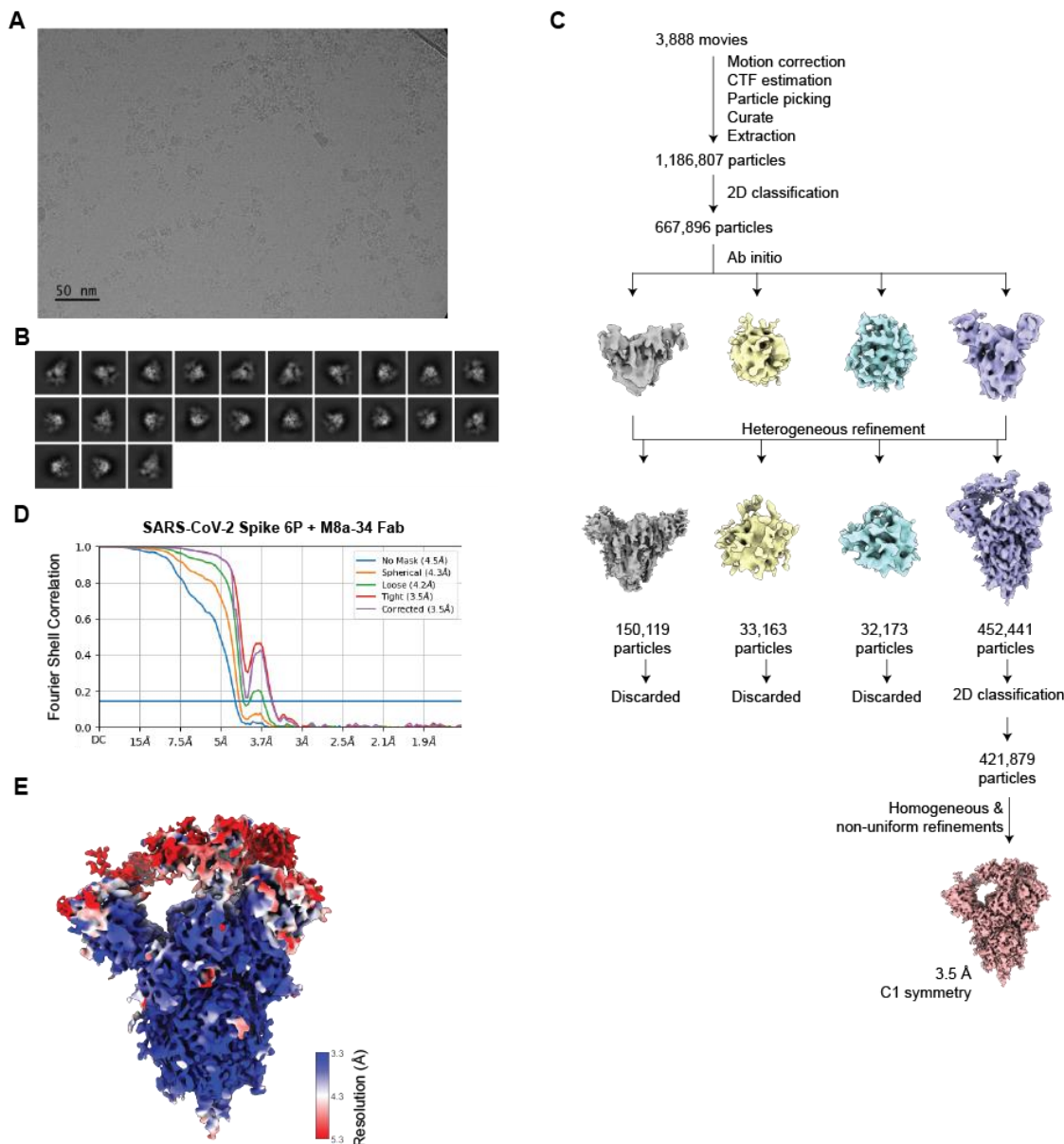
Figure S7. Cryo-EM data processing and validation for M8a-6 Fab in complex with SARS-CoV-2 WA1 spike. (**A**) Representative micrograph. (**B**) Representative 2D classes. (**C**) Workflow of single-particle data processing. (**D**) FSC plot of the final reconstruction. (**E**) Final reconstruction of M8a-6 Fab in complex with SARS-CoV-2 spike, colored by local resolution.
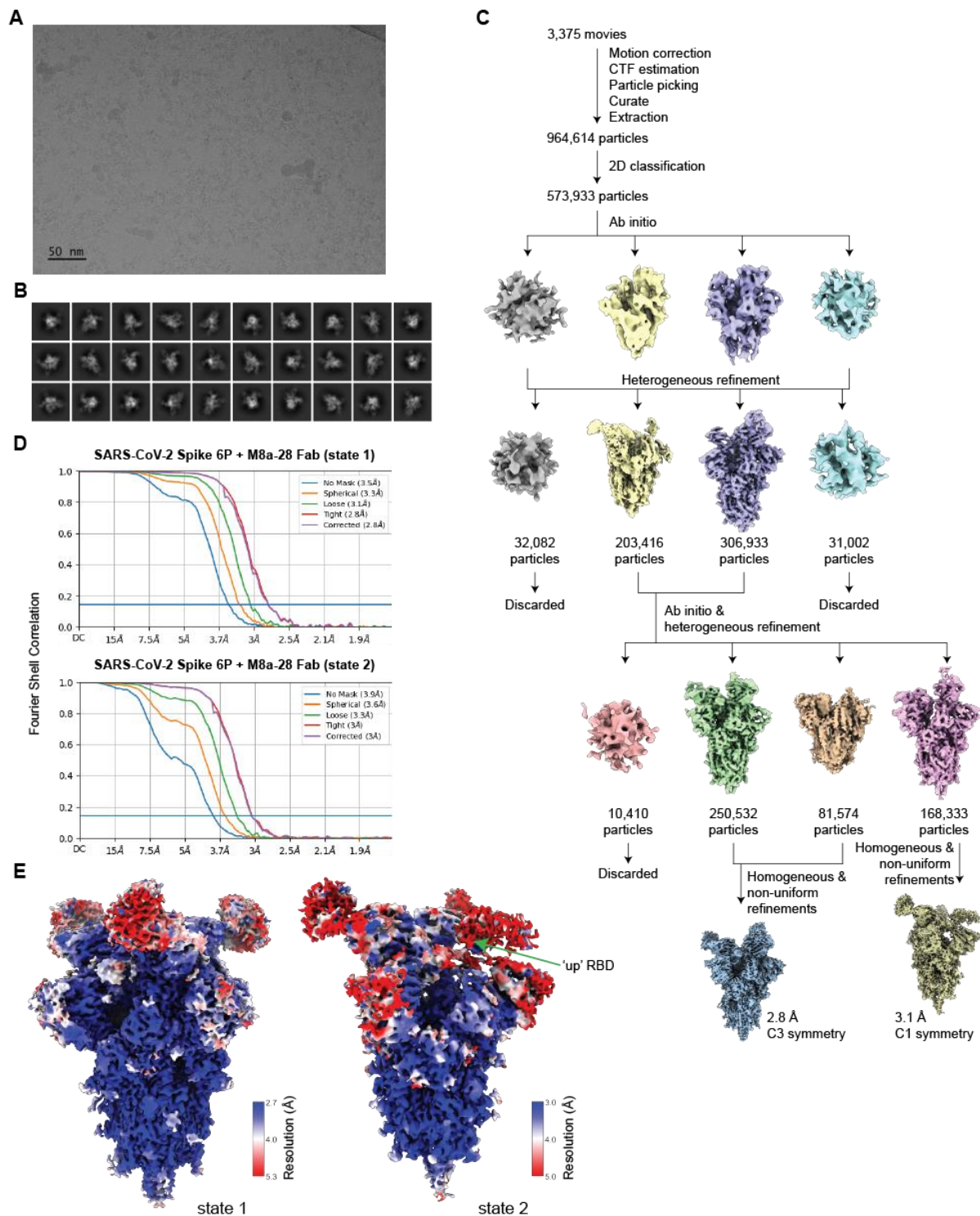
1261

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Figure S8. Cryo-EM data processing and validation for of M8a-31 Fab in complex with SARS-CoV-2 WA1 spike. (**A**) Representative micrograph. (**B**) Representative 2D classes. (**C**) Workflow of single-particle data processing. (**D**) FSC plot of the final reconstruction. (**E**) Final reconstruction of M8a-31 Fab in complex with SARS-CoV-2 spike, colored by local resolution.

Figure S9. Cryo-EM data processing and validation for of M8a-31 Fab in complex with SARS-CoV-2 Omicron BA.1 spike. (**A**) Representative micrograph. (**B**) Representative 2D classes. (**C**) Workflow of single-particle data processing. (**D**) FSC plot of the final reconstruction. (**E**) Final reconstruction of M8a-3 Fab in complex with SARS-CoV-2 spike Omicron BA.1, colored by local resolution.
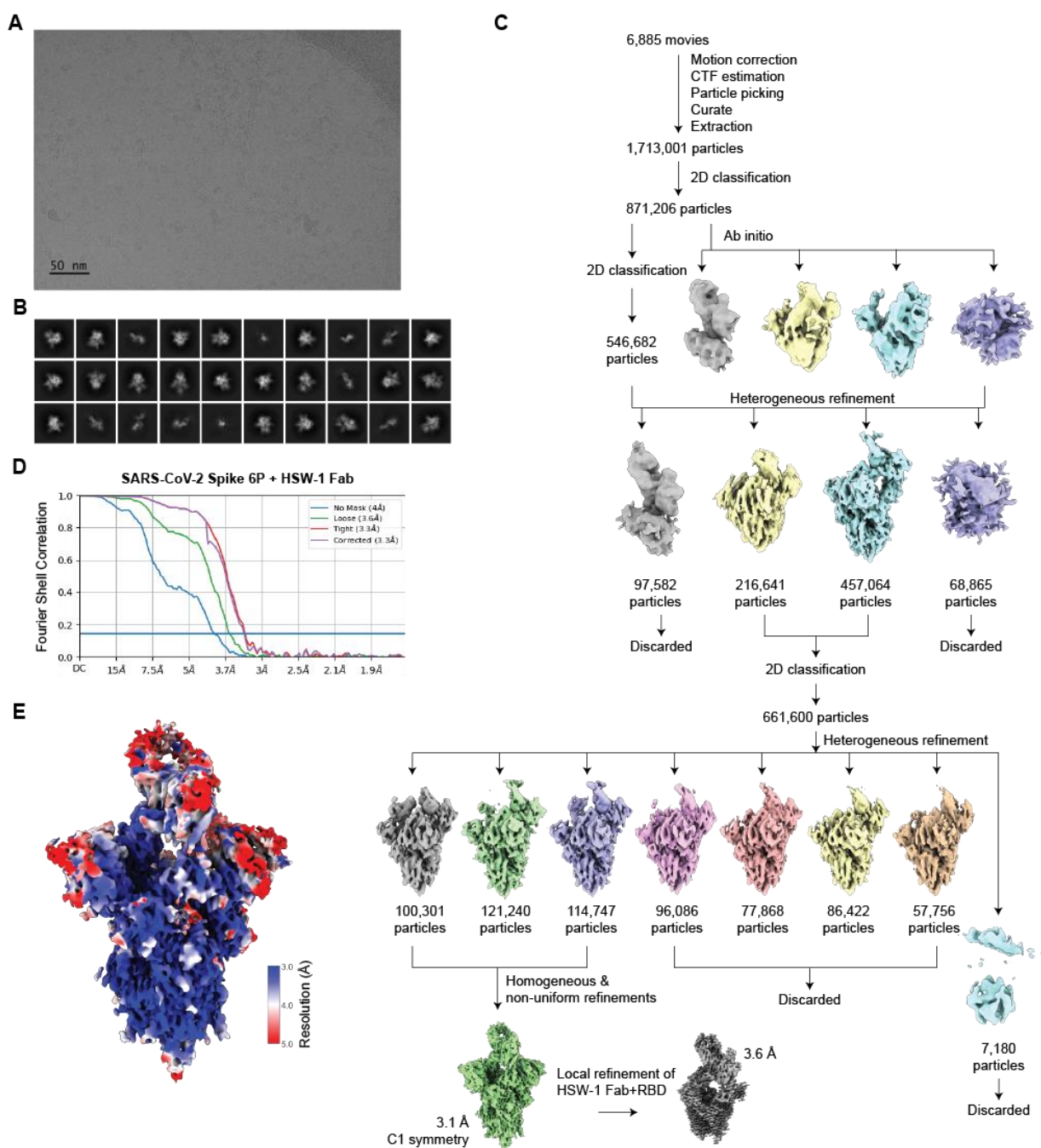
Figure S10. Cryo-EM data processing and validation for of M8a-34 Fab in complex with SARS-CoV-2 WA1 spike. (**A**) Representative micrograph. (**B**) Representative 2D classes. (**C**) Workflow of single-particle data processing. (**D**) FSC plot of the final reconstruction. (**E**) Final reconstruction of M8a-34 Fab in complex with SARS-CoV-2 spike, colored by local resolution.
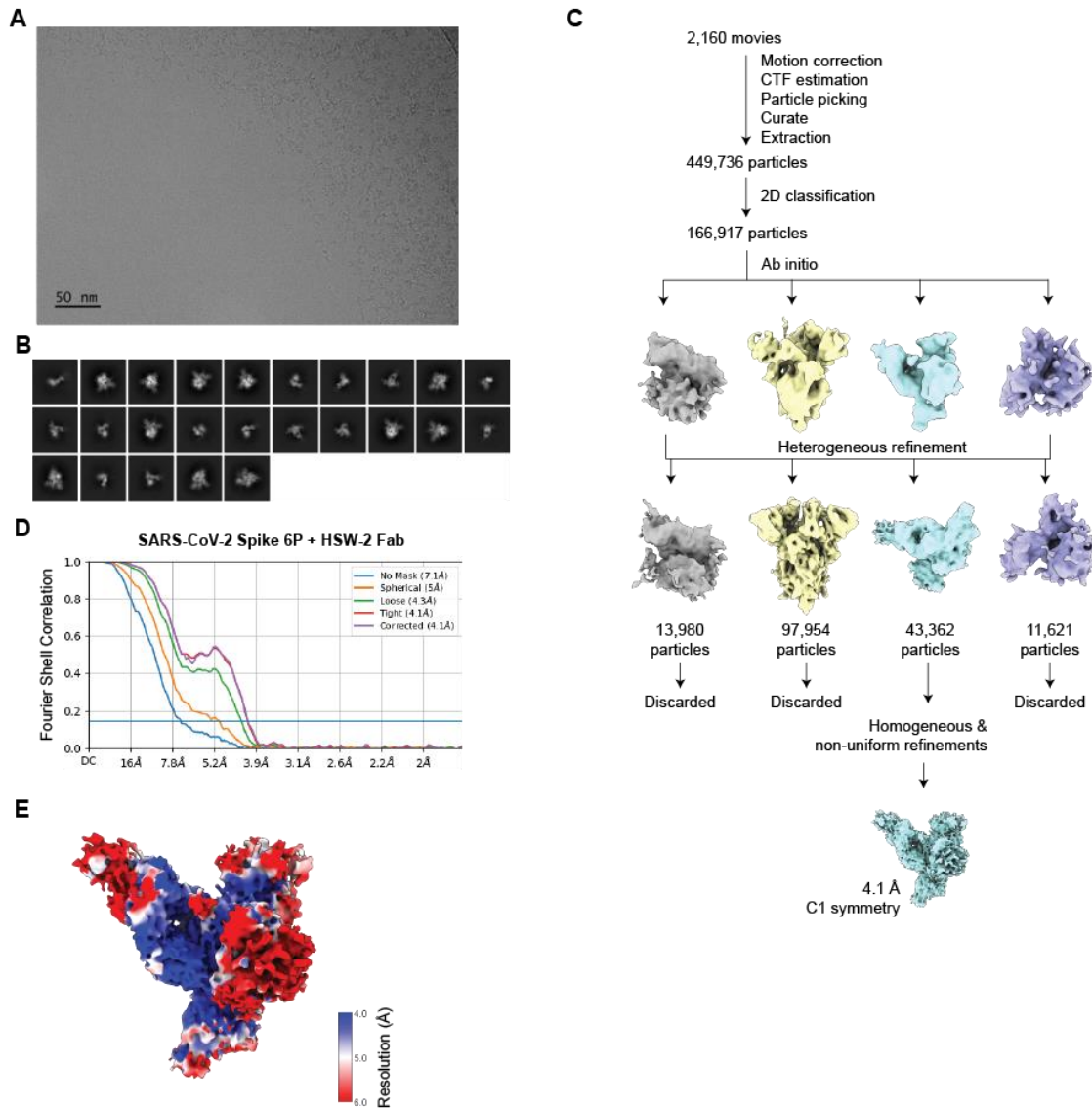
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Figure S11. Cryo-EM data processing and validation for M8a-28 Fab in complex with SARS-CoV-2 WA1 spike. (**A**) Representative micrograph. (**B**) Representative 2D classes. (**C**) Workflow of single-particle data processing. (**D**) FSC plot of the final reconstruction. (**E**) Final reconstructions of both states of M8a-28 Fab in complex with SARS-CoV-2 spike, colored by local resolution. An 'up' RBD in state 2 is labeled with an arrow.

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Figure S12. Cryo-EM data processing and validation for of HSW-1 Fab in complex with SARS-CoV-2 WA1 spike. (**A**) Representative micrograph. (**B**) Representative 2D classes. (**C**) Workflow of single-particle data processing. (**D**) FSC plot of the final reconstruction. (**E**) Final reconstruction of HSW-1 Fab in complex with SARS-CoV-2 spike, color by local resolution.

Figure S13. Cryo-EM data processing and validation for of HSW-2 Fab in complex with SARS-CoV-2 WA1 spike. (**A**) Representative micrograph. (**B**) Representative 2D classes. (**C**) Workflow of single-particle data processing. (**D**) FSC plot of the final reconstruction. (**E**) Final reconstruction of HSW-2 Fab in complex with SARS-CoV-2 spike S1 domain, color by local resolution.
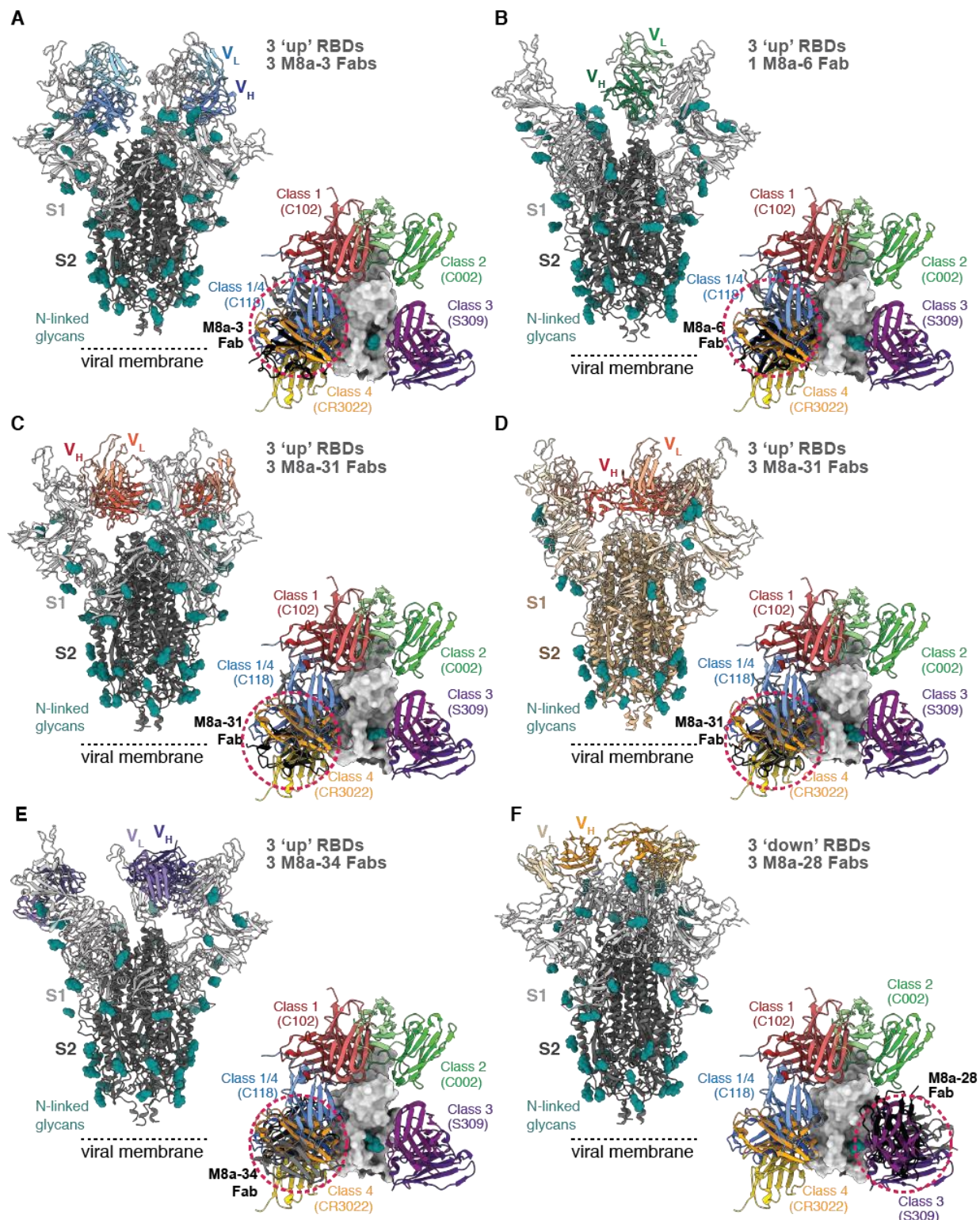
Figure S14. mAbs isolated from mice immunized with mosaic-8 nanoparticles. Cartoon representations of single particle cryo-EM structures of Fab-spike trimer complexes are shown from the side (left) with a comparison of binding epitopes of the Fab with representative anti-RBD antibodies: class 1 (C102, PDB 7K8M), class 2 (C002, PDB 7K8T), class 3 (S309, PDB 7JX3),

1307   class 4 (CR3022, PDB 7LOP), and class 1/4 (C118, PDB 7RKV)) aligned on an RBD in surface

1308   representation (right). Only V_H-V_L domains are shown for each Fab. Fabs of interests (colored in

1309   black and circled with a red dotted line) and the anti-RBD antibodies used for classification are

1310   aligned on a surface representation of the RBD. N-linked glycans are shown as teal spheres. (**A**)

1311   WA1 spike complexed with M8a-3. (**B**) SARS-CoV-2 WA1 spike complexed with M8a-6. (**C**)

1312   WA1 spike complexed with M8a-31. (**D**) Omicron BA.1 spike complexed with M8a-31. (**E**) WA1

1313   spike complexed with M8a-34. (**F**) WA1 spike complexed with M8a-28.

1314
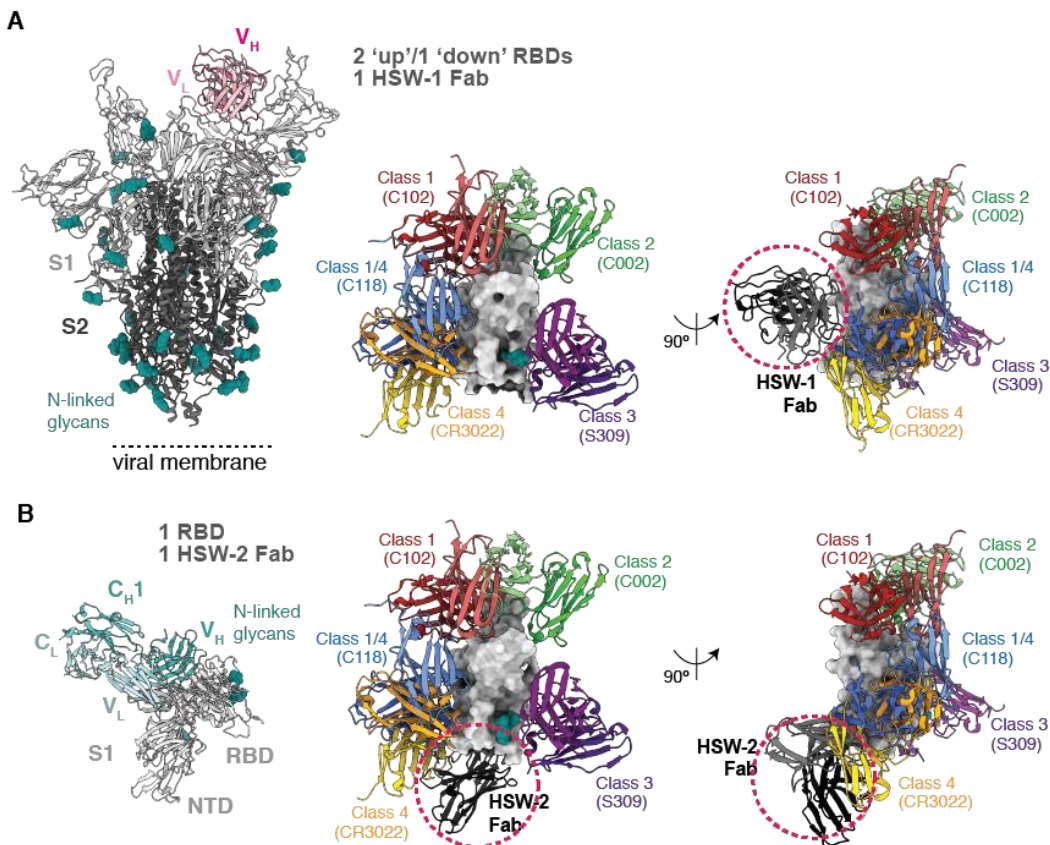
Figure S15. mAbs isolated from mice immunized with homotypic nanoparticles. Cartoon representations of single particle cryo-EM structures of Fab-spike trimer complexes are shown from the side (left) with a comparison of binding epitopes of the Fab with representative anti-RBD antibodies: class 1 (C102, PDB 7K8M), class 2 (C002, PDB 7K8T), class 3 (S309, PDB 7JX3), class 4 (CR3022, PDB 7LOP) and class 1/4 (C118, PDB 7RKV)) aligned on an RBD in surface representation (middle and right). Only V_H-V_L domains are shown for each Fab. Fabs of interests (colored in black and circled with a red dotted line) and the anti-RBD antibodies used for classification are aligned on a surface representation of the RBD. N-linked glycans are shown as teal spheres. (**A**) SARS-CoV-2 WA1 spike complexed with HSW-1. (**B**) WA1 spike S1 domain complexed with HSW-2.
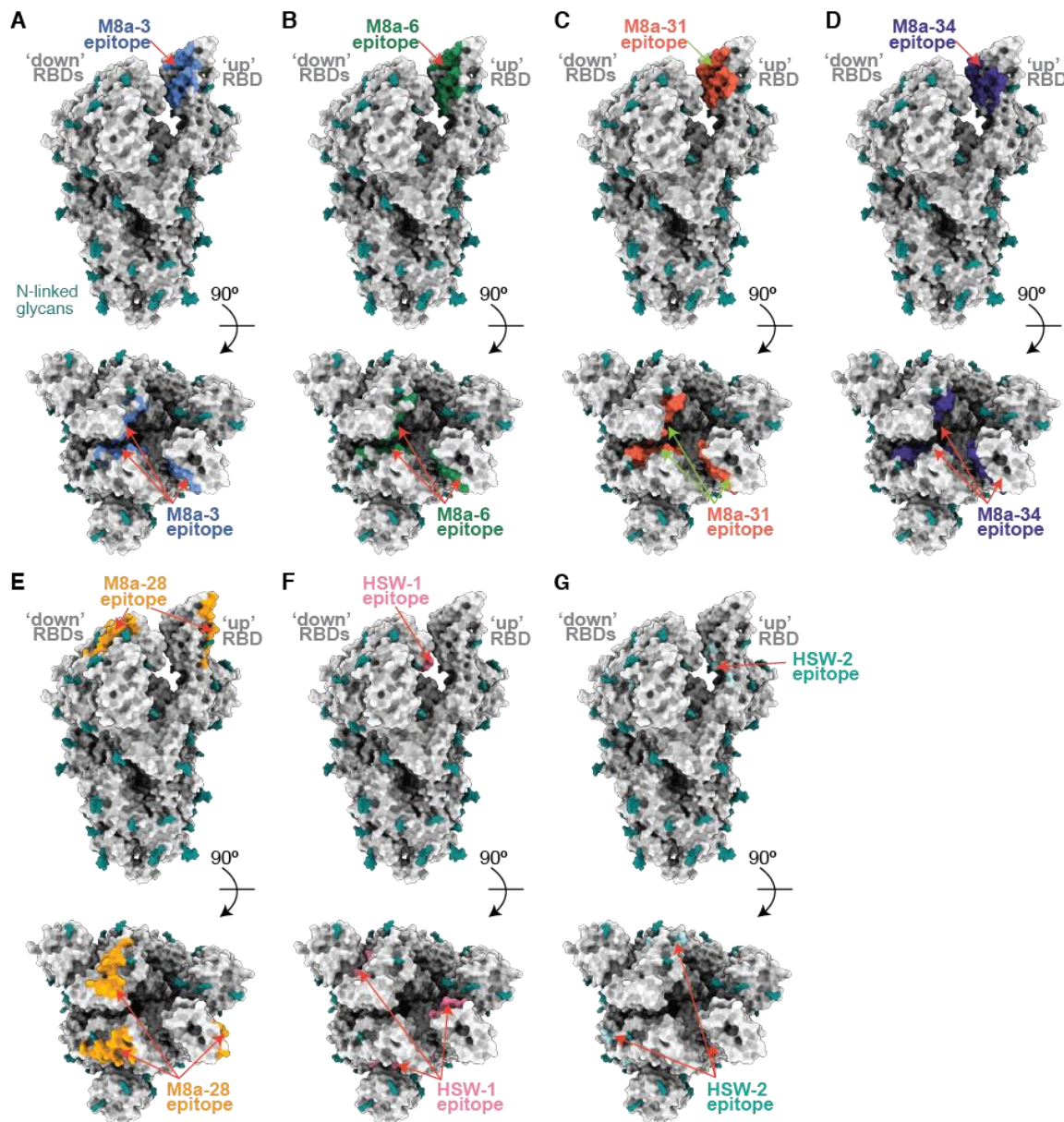
Figure S16. Epitopes of mAbs mapped on a unliganded SARS-CoV-2 spike trimer. Binding epitopes for all mAbs were identified by PDBePISA (Krissinel and Henrick, 2007) and then mapped on to a unliganded spike trimer with two 'down' and one 'up' RBDs (PDB 6VYB). The spike trimer is shown as a surface representation with N-linked glycans shown as teal spheres. The epitopes of (**A**) M8a-3, (**B**) M8a-6, (**C**) M8a-31 and (**D**) M8a-34 are blocked in the 'down' RBD conformation, but accessible in an 'up' RBD conformation. (**E**) The epitope of M8a-28 is accessible in both 'down' and 'up' RBD conformations. (**F**) The epitope of HSW-1 is blocked in the 'down' RBD conformation, but accessible in an 'up' RBD conformation. (**G**) The epitope of HSW-2 is blocked in both 'down' and 'up' RBD conformations.
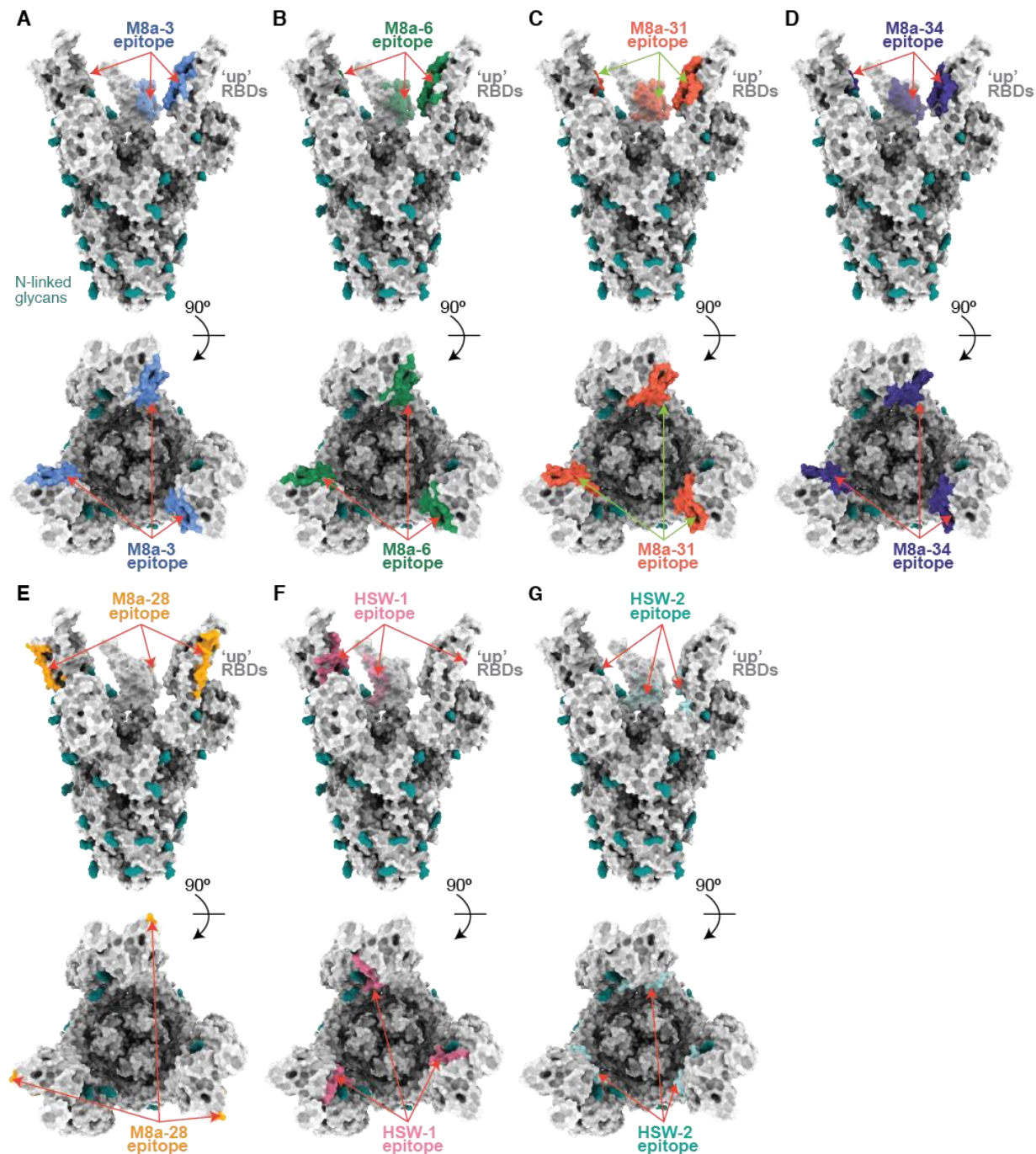
Figure S17. Epitopes of mAbs mapped on a SARS-CoV-2 spike trimer with all 'up' RBDs.
Binding epitopes for all mAbs were identified by PDBePISA (Krissinel and Henrick, 2007) and then mapped on to a spike trimer with three 'up' RBDs (PDB 7RKV; Fabs not shown). The spike trimer is shown as a surface representation with N-linked glycans shown as teal spheres. The epitopes of (**A**) M8a-3, (**B**) M8a-6, (**C**) M8a-31, and (**D**) M8a-34 are all accessible in an 'up' RBD conformation. (**E**) The epitope of M8a-28 is accessible in 'up' RBD conformation. (**F**) The epitope of HSW-1 is accessible in an 'up' RBD conformation. (**G**) The epitope of HSW-2 is sterically hindered in an 'up' RBD conformation.
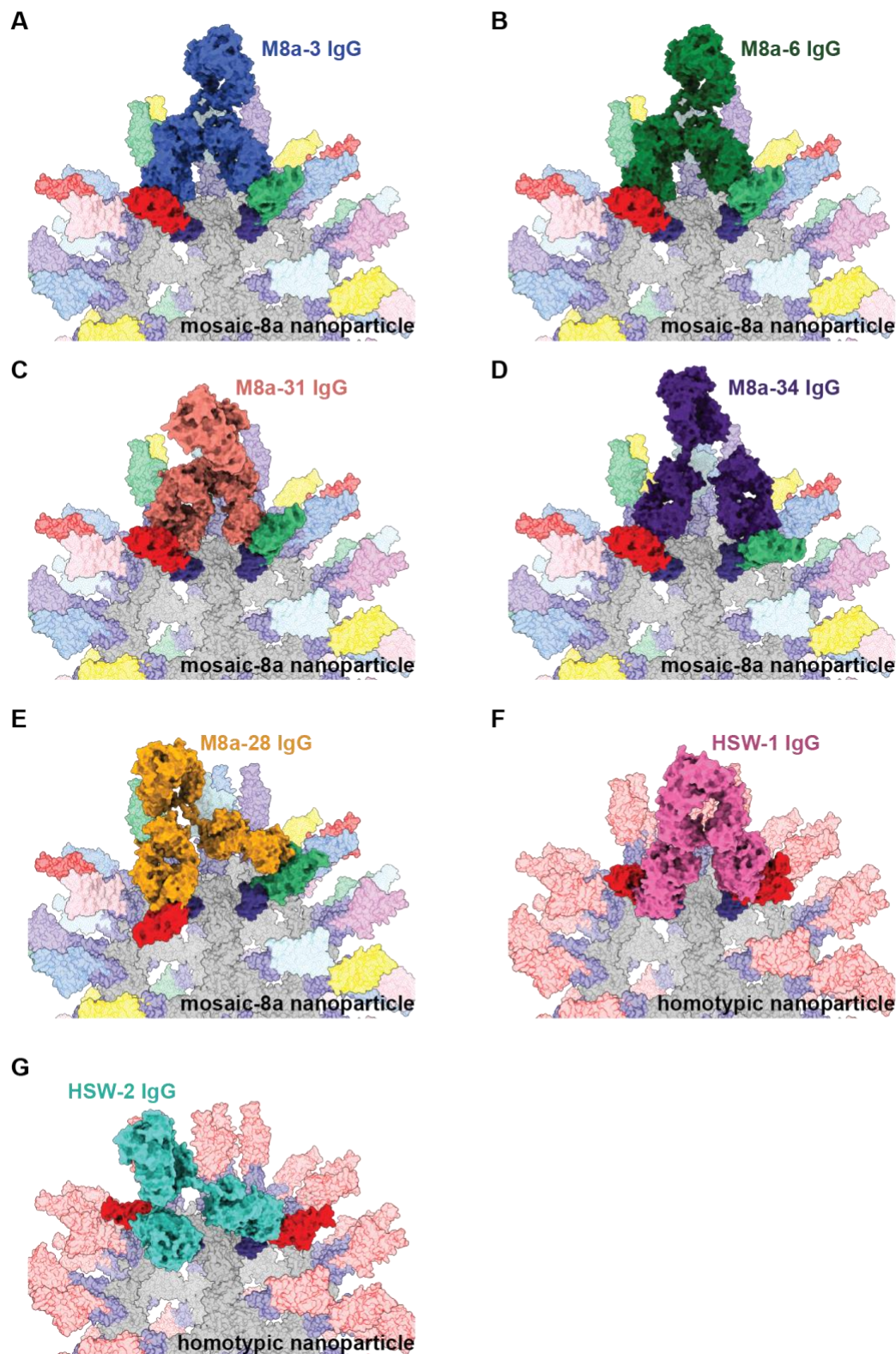
Figure S18. Models of M8a and HSW IgGs binding to adjacent RBDs on SpyCatcher-mi3 nanoparticles. Structural models of RBD-nanoparticles formed by SpyCatcher-mi3 and SpyTagged RBDs were made using coordinates of an RBD (PDB 7SC1) (represented in different

1350   colors for mosaic-8 nanoparticles, with two adjacent RBDs in red and blue, or in red for homotypic

1351   nanoparticles), mi3 (PDB 7B3Y) (gray), and SpyCatcher (PDB 4MLI) (dark blue). IgGs (various

1352   colors) were modeled using the coordinates of each mAb Fab based on an intact IgG crystal

1353   structure (PDB 1HZH), and the RBD binding epitope of each Fab in a modeled IgGs was

1354   determined based on the Fab-spike structures reported in this study. The two Fabs of each IgGs

1355   were positioned with distances between the C-termini of the Fab C_H1 domains to be less than 65

1356   Å, as described previously (Barnes et al., 2020a). Both the IgG Fc hinge and the linker region

1357   between a SpyTagged RBD and SpyCatcher were assumed to be flexible and adjusted accordingly.

1358   Models are shown for (**A**) M8a-3 IgG interacting with two adjacent RBDs on a mosaic-8 RBD-

1359   nanoparticle. (**B**) M8a-6 IgG interacting with two adjacent RBDs on a mosaic-8 RBD-

1360   nanoparticle. (**C**) M8a-31 IgG interacting with two adjacent RBDs on a mosaic-8 RBD-

1361   nanoparticle. (**D**) M8a-34 IgG interacting with two adjacent RBDs on a mosaic-8 RBD-

1362   nanoparticle. (**E**) M8a-28 IgG interacting with two adjacent RBDs on a mosaic-8 RBD-

1363   nanoparticle. (**F**) HSW-1 IgG interacting with two adjacent RBDs on a homotypic RBD-

1364   nanoparticle. (**G**) HSW-2 IgG interacting with two adjacent RBDs on a homotypic RBD-

1365   nanoparticle.

1366

1367

Tables S1 to S3

mAbs	V$_H$ (sequences)	V$_L$ (sequences)	Lineages
M8a-3	QVQLQQPGAELVLPGASVKLSCKASGYTFTNYWMHWVKQRP GHGLEWIGEIDPFDTYIKINQKFKGKSTLTVDTSSSTAYMQ LSSLTSEDSAVYYCARPDSSGYPVYFDYWGQGTTLTVSS	DIVMTQSHKFMSTSVGDRVSITCKASQDVGTYIAWYQQKPG QSPKLLIYWASTRHTGVPDRFTGSGSGTNYTLTISSVQAED LALYHCQQHYSTPYTFGGGTKLEIK	IgHV1-69 IgκV6-25
M8a-6	QVQLQQPGTELVMPGASVKLSCKTSGYTFTHYWMHWVKQRP GEGLEWIGEIAPSDNYVKYNQKFKGKSTLSVDRSSSTAYMQ LSSLTSEDSAVYFCARPDNSGYPVYFDYWGQGTSLTVSS	DIVMTQSQKFMSTSLGDRVSISCKASQDVGTTVAWYQQKPG QSPKLLIYWASTRHTGVPDRFTGTGSGTDYTLTISSVAAED LALYYCQQHYNTPYTFGGGTKLEIE	IgHV1-69 IgκV6-25
M8a-7	QAYLQQSGAEMVRPGASVKMSCKASGYTFNNYNMHWVKQTP SQGLEWIGGFYPGNDDTAYSQKFKGKATLTVDKSSSTAFMH LSSLTSEDSAVYFCARSLGRYYAMDYWGQGTSVTVSS	DIVLTQSPASLAVSLGQRATISCRASESVDDFGISYMNWFQ QKPGQTPKLLIYGASNQGSGVPARFSGSGSGTDFSLNIHPM EEDDPAMYFCQQSKEVPYTFGGGTKLEIK	IgHV1-12 IgκV3-2
M8a-9	QVQLQQPGAELVRPGSSVKLSCKASGYTFTSYWIHWVRQRP IEGPEWIGMIDPSDSGNHFNQNFKDKATWTVDKSSNTAYMQ LSSLTTEDSAVYYCARGSGSTYRGYFDYWGHGTTLTVSS	DIQMTQSSSSYLSVSLGGRVTITCKASDHINNWLAWYQQKPG NTPRLLISGATNLETGVPSRFSGSGSGKDYTLSITSLQTED VATYYCQQYWSSPLTFGAGTKLELK	IgHV1-52 IgκV13-85
M8a-11	EVQLQQSGPELVKPGASVKIPCKASGYTFTDYNMDWVKQSH EKSLEWIGEIDPNNGDTIYNQKFKGKASLTVDKSSSTAYME LRSLTSEDTAVYYCAKRGYYGSSLWWYFDVWGTGTTVTVSS	DIQMTQSSSSFSVSLGDRVTITCKASEDIYIRLAWYQQRPG NAPRLLISNAISLETGVPSRFSGSGSGKDYTLSITSLQTED VATYYCQQYWSTPWTFGGGTKLEIK	IgHV1-18 IgκV13-84
M8a-15	QVQLQQSGPELARPGASVKLSCRASGYTVTSFGLSWMKQRT GQGLEWIGEIYPTSKNTYYNDKFRTKATLTADKSSSTAYME LRSLTSEDSAVYFCVLYDYFDYWGQGTTLTVSS	QIVLTQSPAIMSASPGEKVTISCSASSSVSYMYWYQQKTGS SPKPWIYRTSNLASGVPVRFSGSGSGTSYSLTISSMEAEDA ATYYCQQYQSYPRTFGGGTKLEIK	IgHV1-81 IgκV4-61
M8a-25	EVQLQQSVAELVRPGASVKLSCTASGFNIKNTYMHWVKQRP EQGLEWIGRIDPSIDHTRYAPKFQGKAVITAFTSSNTAYLQ LSSLTSEDTAIYYCAREGGGNYPYYYAIDYWGQGTSVTVSS	DIQMTQSSSSFSVSLGDRVTITCKASEDIYIRLAWYQQRPG NAPRLLISNAISLETGVPSRFSGSGFGKDHTLSITSLQTED VATYYCQQYWSTPWTFGGGTKLEIK	IgHV14-3 IgκV13-84
M8a-28	QVQLQQPGAELVKPGASVKMSCKASGYNFNHYWISWVKQRP GQGLEWIGDIYPLSHFTTYNEKFTNRATLTVDTSSTTAYMQ LNSLTSDDSAVFYCARWDYFDSRTFDYWGQGTTLTVSS	DILLTQFPAILSVSPGERVSFSCRASQTIGTNIHWYQQRIN GSPRLLIKYASESISGIPSRFSGSGSGTDFSLSINNVESED IADYYCQQINSWPLTFGAGTKLDLK	IgHV1-55 IgκV5-48
M8a-29	EVQLQQSVAELVRPGASVKLSCTASGFNIKNTYMHWVKQRP EQGLEWIGRIDPSIDHTRYAPKFQGKAVITAFTSSNTAYLQ LSSLTSEDTAIYYCAREGGGNYPYYYAIDYWGQGTSVTVSS	DIVMTQAAFSNPVTLGTSASISCRSTKSLLHSNGITYLYWY LQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISR VEAEDVGVYYCAQNLELPYTFGGGTKLEIK	IgHV14-3 IgκV2-109
M8a-30	QVHLQQSGPELVKPGASVKISCKASGYGFSSSWMNWVKQRP GKGLEWIGRIYPGDGDTNYNDKFQGKATLTADRSSSTAYMH LTSLTSADSAVYFCARSLLYSFDYWGQGTTLTVSS	DVVVTQTPLSLPVSFGDQVSISCRSSQSLAGSYGHTYLSWY LHKSGQSPQLLIYGISNRFSGVPDRFSGSGSGTDFTLKIST IKPEDLGMYYCLQGTHQPLTFGAGTKLELK	IgHV1-82 IgκV1-88
M8a-31	EVQLKQSVAELVRPGASVKVSCTASGFNIKNIYMHWVKQRP EQGLDWIGRIDPANGNSRYAPKFQDKATITADTSSNTAYLQ LSSLTSEDTAIYYCADEGWGFANWGQGTLVTVSA	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTW YQQKVGQPPKLLIYWASTRDPGVPDRFTGSGFGTDFTLTIS SVQAEDLAVYYCQNDYSYPLTFGAGTKVELK	IgHV14-3 IgκV8-19
M8a-34	QVQLQQPGAELVKPGASVKMSCKASGYTFITYWITWVKQRP GQGLEWIGDIYPGGGRTNYNEKFKSKATLTVDTSSSTAYMQ LRSLTSEDSAVYYCARYDGNYVGYYYAMDYWGQGTSVTVSS	DIVLTQSPVSLAVSLGQRATISCRASESVDFYGNSFIYWYQ QKPGQAPKLLIYRASNLESGIPARFSGSGSRTDFTLTIHPV EADDVATYYCQQSIEDPRTFGGGTKLEIK	IgHV1-55 IgκV3-5
M8a-36	QVQLQQTGAELVKPGASVKMSCKASGYTFTSYWIIWVKKRP GQGLEWIGDIYPGSGSTNYNEKFKSKATLTVDTSSSTAYMQ LSSLTSEDSAVYYCTRGGSRFAMDYWGQGTSVTVSS	DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGNTYLYWF LQRPGQSPQLLIYRMSNLASGVPDRFSGSGSGTAFTLRISR VEAEDVGVYYCMQHLEYPYTFGGGTKLEIK	IgHV1-55 IgκV2-137
HSW-1	QVQLQQPGAELVKPGTSMKLSCKASGYTFTSYWMHWVKQRP GQGLEWIGMIHPNSGSTKYNENFKSKATLTVDKSSSTAYMQ FSSLTSEDSAVYYCVRSGSYYGTTYDYFDYWGQGTTLTVSS	DIVLTQSPASLAVSLGQRATISCRASESVNIYGNSFMHWYQ QKPGQPPKLLIFRASNLESGIPVRFSGSGSRTDFTLTINPV EADDVATYYCHQSNEDPFTFGSGTKLEIK	IgHV1-64 IgκV3-5
HSW-2	QVQLQQSGPELVKPGASVKISCKASGYVFSTSWMSWVKQRP GEGPEWIGRIYPRDGHSSSTGKFKDKATLTADKSSNTAYIH LSSLTSEDSAVYFCARDYGYYYFDYWGQGTTLTVSS	DIQMTQSPASLSASVGEAVTITCRLSENVYSFLAWYQQKQG KSPQLLVYRAKTLAEGVPSRFSGSGSGTQFSLKINSLQPED FGTYYCQHHYGTPPTFGGGTKLEIK	IgHV1-82 IgκV12-44

Table S1. Sequences and V gene segment lineages for 16 mAbs identified as binding at least one RBD during screening. M8a-9 and M8a-36 did not exhibit binding to purified RBDs by ELISA (data not shown). M8a-11 and M8a-26 are identical sequences.

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Structures	WA1 spike in complex with							Omicron BA.1 spike in complex with M8a-31 Fab
	M8a-3 Fab	M8a-6 Fab	M8a-28 Fab	M8a-31 Fab	M8a-34 Fab	HSW-1 Fab	HSW-2 Fab	
Data Collection and processing								
Microscope	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Talos Arctica	Titan Krios
Camera	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3	Gatan K3
Magnification	105,000	105,000	105,000	105,000	105,000	105,000	45,000	105,000
Voltage (keV)	300	300	300	300	300	300	200	300
Exposure (e/Å^2)	60	60	60	60	60	60	60	60
Pixel size (Å)	0.832	0.832	0.832	0.832	0.832	0.832	0.869	0.832
Defocus Range (μm)	-1 to -3	-1 to -3	-1 to -3	-1 to -3	-1 to -3	-1 to -3	-1 to -3	-1 to -3
Initial Particle Image (no.)	1,026,209	842,602	964,614	670,746	1,186,807	1,713,001	449,736	1,689,653
Final Particle Image (no.)	272,779	159,604	332,106	295,711	421,879	336,288	43,362	142,452
Symmetry Imposed	C1	C1	C3	C3	C1	C1	C1	C3
Map Resolution (Å)	3.1	3.2	2.8	2.9	3.5	3.1	4.1	3.1
FSC Threshold	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143
Map Resolution Range (Å)	3.0 - 3.5	3.1 - 3.5	2.7 to 3.1	2.9 - 3.2	3.5 - 4.3	3.0 to 3.4	3.9 - 4.3	2.9 - 3.3
Refinement								
Initial Model Used	PDB 7SC1	PDB 7SC1	PDB 7SC1	PDB 7SC1	PDB 7SC1	PDB 7SC1	PDB 7SC1	PDB 7SC1
Model Resolution (Å)	3.1	3.2	2.8	2.9	3.5	3.1	4.1	3.1
FSC Threshold	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143
Model composition								
non-hydrogen atoms	30,762	27,051	30,942	30,552	30,669	26,992	8,392	30,075
protein residues	3,867	3,397	3,906	3,861	3,873	3,409	1,077	3,801
ligands	51	48	39	45	42	38	4	33
Average B-factors (Å^2)								
protein	135.2	157.5	110.4	130.4	205.7	158.8	197.9	92.4
ligands	116.3	139.9	96.8	111.6	167.3	143.6	213.5	82.5
R.m.s. deviations								
Bond length (Å)	0.005	0.004	0.003	0.004	0.003	0.004	0.003	0.004
Bond angles (º)	0.562	0.582	0.536	0.572	0.586	0.593	0.589	0.594
Validation								
MolProbity score	1.85	1.63	1.74	1.72	1.75	1.83	1.9	1.77
Clashscore	10.3	9.4	9.5	9.1	11.1	12.3	14.6	10.8
Rotamer outliers	0.06	0.07	0.03	0	0.03	0	0	0.06
Ramachandran plot								
Ramachandran favored (%)	95.4	97.3	96.4	96.5	96.9	96.5	96.4	96.6
Ramachandran allowed (%)	4.6	2.7	3..6	3.5	3.1	3.5	3.6	3.4
Ramachandran outliers (%)	0	0	0	0	0	0	0	0
PDB ID	7UZ4	7UZ5	7UZ6	7UZ7	7UZ9	7UZA	7UZB	7UZ8

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1378 **Table S2. Single-particle cryo-EM data collection, processing, and refinement**

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Structures	SARS-CoV-2 RBD + M8a-34 Fab	SARS-CoV-2 RBD + HSW-2 Fab
Wavelength (Å)	0.97946	0.97946
Resolution range (Å)	39.23 - 2.2 (2.279 - 2.2)	39.08 - 3.0 (3.108 - 3.0)
Space group	$P2_1$	$P4_32_12$
Unit cell (Å, °)	54.5 165.2 93.7 90 106.6 90	123.6 123.6 145.6 90 90 90
Total reflections (no.)	553,434 (52,800)	592,837 (59,309)
Unique reflections (no.)	79,107 (7,859)	23,174 (2,178)
Multiplicity	7.0 (6.7)	25.6 (26.2)
Completeness (%)	98.5 (97.9)	99.5 (96.1)
Mean I/sigma(I)	12.2 (1.24)	19.6 (2.6)
Wilson B-factor (Å^2)	41.4	81.1
R-merge	0.105 (1.378)	0.147 (1.856)
R-meas	0.113 (1.494)	0.150 (1.893)
R-pim	0.0425 (0.568)	0.030 (0.366)
CC1/2	0.998 (0.642)	0.999 (0.842)
CC*	1 (0.884)	1 (0.956)
Reflections used in refinement (no.)	79,082 (7,845)	23,082 (2,178)
Reflections used for R-free (no.)	1,997 (195)	1,155 (109)
R-work	0.171 (0.270)	0.230 (0.360)
R-free	0.243 (0.329)	0.271 (0.458)
CC (work)	0.971 (0.821)	0.899 (0.834)
CC (free)	0.962 (0.771)	0.604 (0.753)
Number of non-hydrogen atoms	10,482	4,912
macromolecules	9,884	4,884
ligands	111	28
solvent	487	0
Protein residues (no.)	1,278	633
RMS (bonds) (Å)	0.008	0.009
RMS (angles) (°)	0.98	1.15
Ramachandran favored (%)	97.5	96.3
Ramachandran allowed (%)	2.5	3.7
Ramachandran outliers (%)	0	0
Rotamer outliers (%)	0.91	0
Clashscore	2.6	6.11
Average B-factor (Å^2)	47	73.0
macromolecules	46.7	72.7
ligands	85.0	118.3
solvent	44.3	-
PDB ID	7UZC	7UZD

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1381 **Table S3. X-ray crystallography data collection, processing, and refinement**

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