

1 **Preclinical Efficacy of IMM-BCP-01, a Highly Active Patient-Derived Anti-SARS-CoV-2**  
2 **Antibody Cocktail**

3  
4 **One sentence summary:** IMM-BCP-01 cocktail triggers Spike Trimer dissociation, neutralizes  
5 all tested variants *in vitro*, activates a robust effector response and dose-dependently inhibits virus  
6 *in vivo*.

7  
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24

25 **Abstract**

26

27 Using an unbiased interrogation of the memory B cell repertoire of convalescent COVID-19  
28 patients, we identified human antibodies that demonstrated robust antiviral activity *in vitro* and  
29 efficacy *in vivo* against all tested SARS-CoV-2 variants. Here, we describe the pre-clinical  
30 characterization of an antibody cocktail, IMM-BCP-01, that consists of three unique, patient-  
31 derived recombinant neutralizing antibodies directed at non-overlapping surfaces on the SARS-  
32 CoV-2 spike protein. Two antibodies, IMM20184 and IMM20190 directly block spike binding to  
33 the ACE2 receptor. Binding of the third antibody, IMM20253, to its unique epitope on the outer  
34 surface of RBD, alters the conformation of the spike trimer, promoting release of spike monomers.  
35 These antibodies decreased SARS-CoV-2 infection in the lungs of Syrian golden hamsters, and  
36 efficacy *in vivo* efficacy was associated with broad antiviral neutralizing activity against multiple  
37 SARS-CoV-2 variants and robust antiviral effector function response, including phagocytosis,  
38 ADCC, and complement pathway activation. Our pre-clinical data demonstrate that the three  
39 antibody cocktail IMM-BCP-01 shows promising potential for preventing or treating SARS-CoV-  
40 2 infection in susceptible individuals.

41

42

## 43 INTRODUCTION

44

45 With over 472 million cases and more than 6.1 million deaths worldwide ([Johns Hopkins](#)  
46 [University Coronavirus Resource Center](#)), the SARS-CoV-2 pandemic continues to pose  
47 extraordinary health and economic challenges. The scientific community has mitigated this threat  
48 through the discovery and launch of a myriad of vaccines and therapeutics to prevent or treat  
49 infections. While the initial data for the spike (S) protein-directed vaccines have been impressive,  
50 the current rate of protection against variants of concern is decreasing, which was predicted to  
51 occur due to viral escape and patient immunodeficiency or immunosuppression (1–6).

52

53 As such, the discovery and development of effective antibody therapies for passive immunization  
54 with broad range of reactivity is likely to be an important alternative approach to vaccination. The  
55 use of convalescent plasma against SARS-CoV-2 initially yielded mixed results (7, 8), however a  
56 recent retrospective cohort study showed reduced mortality in treated patients (9) outlining the  
57 need for a more robust and more standardized antiviral antibody cocktail. A phase 3 clinical trial  
58 with Lilly’s Bamlanivimab was halted on the basis of data showing no improvement in clinical  
59 outcomes. An early Regeneron trial with a 2-antibody mixture was also paused based on a potential  
60 safety signal and an unfavorable risk/benefit profile (10). Nonetheless, subsequent data  
61 demonstrated that S-protein-directed antibodies can have significant efficacy and safety, and both  
62 the Lilly and Regeneron antibody cocktail candidates received Emergency Use Authorization  
63 (EUA) from the US FDA in November 2020, although the EUA for Bamlanivimab was later  
64 withdrawn and distribution of the Bamlanivimab/Etesevimab cocktail is now limited to areas  
65 where resistant variant frequency is below 5%. Another S-protein specific antibody, Sotrovimab,

66 co-developed by Vir and GlaxoSmithKline, received an EUA in May 2021. As publicly reported,  
67 Vir is currently developing a second-generation antibody aimed for use as a combination with  
68 Sotrovimab. The study published by Regeneron demonstrated that both Regeneron 2-Ab cocktail  
69 and Vir's VIR-7831 antibody generated escape mutants after seven and two passages *in vitro*,  
70 outlining a need for multiple neutralizing antibodies in a cocktail (11). Further, the recent SARS-  
71 CoV-2 variants of concern (VOC), Omicron (BA.1, BA.1.1, and BA.2), was shown to escape  
72 Regeneron and Lilly's antibody cocktails, that led to the FDA's decision to limit the use of  
73 bamlanivimab and etesevimab cocktail and REGEN-COV (casirivimab and imdevimab cocktail)  
74 to patients infected with susceptible variants (that are currently not detected in the US) (12, 13).  
75 The emergence of Omicron variants recently led the FDA to revise the EUA issued for another  
76 combination Evusheld (consists of tixagevimab and cilgavimab), and increase the dose due to loss  
77 of potency to BA.1 and BA.1.1(14). Finally, a new Lilly's antibody bebtelovimab, that received  
78 an EUA in February of 2022, was demonstrated to retain activity against Omicron(15). However,  
79 earlier findings from monoantibody therapies confirm the need for a cocktail treatment to avoid  
80 generation of escape mutants. Therefore, an antibody cocktail with broad reactivity and limited  
81 possibility of escape to current and prospective VOC that consists of several antibodies to block  
82 the generation of escape mutants is an urgent, yet unmet, medical need.

83  
84 Small molecule inhibitors (SMI), which target viral proteins other than S protein, are alternatives  
85 to antibody-based therapies that might not be affected by the current VOC. However, SMI have  
86 additional limitations, such as the requirement to inhibit patient's CYP3A for a viral protease  
87 inhibitor PF-07321332 or a low enough dose to avoid the host DNA mutagenesis for a  
88 ribonucleoside analog molnupiravir(16). In addition, SMIs are associated with toxicity concerns

89 that could limit clinical usefulness for some patient populations. (17, 18). Thus, the collateral  
90 effects and resistance patterns of SMI may need to be considered prior to patient dosing.

91

92 We previously reported the identification of a library of patient-derived antiviral antibodies (19).

93 Based on published reports (20), we hypothesized that interrogation of such patient responses

94 would identify rare immunoglobulins against epitopes that have a synergistic antiviral effect when

95 combined and are resistant to mutational drift. In this report, we describe the pre-clinical efficacy

96 of IMM-BCP-01, that we are planning to move to clinical trials. IMM-BCP-01 consists of three

97 patient-derived antibodies, each selected for its own intrinsic antiviral neutralization and functional

98 effector response activities against current isolates and prospective variants. These antibodies bind

99 to three non-overlapping epitopes on the receptor binding domain (RBD) and, when combined,

100 potently neutralize *in vitro* all tested viral variants, including Alpha, Beta, Gamma, Epsilon,

101 Kappa, Delta, Mu, Omicron, and suppress viral spread in the lungs of infected animals *in vivo*. We

102 observed a drop in viral load in the lungs of animals treated with our antibody cocktail in a dose-

103 dependent manner. Each antibody demonstrates unique binding properties: IMM20190 has a

104 composite epitope involving the ACE2 receptor binding ridge and an area adjacent to the receptor

105 binding loop, IMM20184 binds avidly to two S proteins within the same trimer, and IMM20253

106 binds to a conserved epitope on the outer surface of the RBD. Our experiments show that three

107 antibody cocktail IMM-BCP-01 has potent and broad antiviral activity in animals, which makes it

108 a promising candidate for development for humans to combat infection with emerging SARS-

109 CoV-2 variants.

110

111 **RESULTS**

112 **Three antibody cocktail IMM-BCP-01 binds to conserved non-overlapping epitopes of S**  
113 **protein trimer leading to its re-organization and dissociation into S protein monomers.**

114 Using an unbiased interrogation of a previously described library of patient-derived antiviral  
115 antibodies (19), we identified three monoclonal antibodies (mAbs), IMM20190, IMM20184 and  
116 IMM20253, that had robust additive and synergistic combinatorial antiviral effects. Structural (**Fig**  
117 **1**) and functional (**Fig 2-4**) studies of these antibodies revealed a unique mechanism of action of  
118 the IMM-BCP-01 cocktail (**Fig 5**).

119 Structural analysis of an S protein trimer (Trimer) complexed with bound Fabs of IMM20184,  
120 IMM20190 or IMM20253 (**Fig 1A**) identified binding patterns of IMM-BCP-01 antibodies. A  
121 final 3D reconstruction of cryo-electron microscopy (cryo-EM) micrographs of IMM20184 Fabs  
122 bound to Trimer revealed a 3:1 (Fab:Trimer) complex at 7 Å resolution with a decreased density  
123 in the Trimer core indicating disruption of the Trimer into S protein monomers along with a large  
124 reorganization of the RBD domains (**Fig 1A and Supp. Fig 1A, B**). Cryo-EM micrographs of  
125 IMM20190 Fab complexed with Trimer revealed a 3:1 (Fab:Trimer) complex at 6 Å (**Fig 1A,**  
126 **Supp Fig 1A,B**). While the variable regions of IMM20190 Fabs were clearly resolved, the constant  
127 regions were scattered, suggesting a dynamic binding nature of this antibody. Finally, the cryo-  
128 EM analysis of IMM20253 Fab-Trimer complex was repeated twice with 3:1 and 6:1 molar ratios  
129 (Fab:Trimer), with the same unexpected conclusion. The samples were not aggregated, observed  
130 with good contrast, and clearly converged into two structural families (**Fig 1A and Supp. Figure**  
131 **2A, B**). The first family consisted of 1 Fab:1 Trimer complex that had one S monomer partially  
132 unfolded (revealed by a lower density). The second family included smaller complexes that  
133 converged into a 3D structure of IMM20253 Fab bound to S1 (**Fig 1A, side view, and Supp. Fig**

134 **1A**). The S2 portion of the spike monomer was not visible in the density maps, suggesting it moves  
135 freely in the complex relative to the S1 domain.

136 The Trimer reorganization induced by both IMM20184 and IMM20253 Fabs prompted us to  
137 determine their epitopes at higher resolution. Cryo-EM structures of RBD with simultaneously  
138 bound to Fabs of both IMM20184 and IMM20253 were resolved to  $\sim 3.9$  Å (**Fig 1B, Supp. Fig**  
139 **1D**). To achieve this resolution,  $\sim 1.9 \times 10^6$  particles were subjected to three rounds of 2D  
140 classification analysis,  $\sim 6 \times 10^5$  particles were selected for ab initio reconstruction and  $\sim 1.7 \times 10^5$   
141 particles were used for the final 3D reconstruction at a nominal resolution of 3.87 Å (**Fig 1B** and  
142 **Supp. Fig 1D**). The complex structure demonstrated that both IMM20184 and IMM20253 Fabs  
143 simultaneously bound to RBD protein. Consistent with **Fig 1A**, the epitope of IMM20253 was  
144 located on the outer surface of the RBD, whereas the epitope of IMM20184 faced inward and  
145 sideways, potentially enabling avid binding of IMM20184. Of note, binning of  
146 IMM20184/190/253 antibodies using bio-layer interferometry (BLI) confirmed the cryo-EM data  
147 and showed the antibodies do not compete for binding of S (**Supp. Fig 1H**).

148  
149 The structural data was further confirmed through use of an alanine-scanning shotgun mutagenesis  
150 approach (20) (**Fig 1C**). In brief, we used a validated library of RBD (Wuhan) proteins expressed  
151 on the surface of HEK-293T cells, each containing one amino acid mutation (20). Consistent with  
152 cryo-EM data (**Fig 1A,B and Supp. Fig 1**), mutagenesis identified unique, non-overlapping  
153 epitopes for the three antibodies (**Fig 1C**). IMM20184 bound to a highly conserved region in the  
154 core RBD (**Fig 1**). The binding site lays in close proximity to the previously reported epitopes of  
155 CR3022 and COVA1-16 antibodies that bind to a cryptic epitope on RBD (21, 22). The IMM20184

156 epitope includes residues N370, F374, K378, and SP383-384 that are completely conserved among  
157 all current and previous SARS-CoV-2 VOC (**Fig 1E**), including Omicron and Delta variants.

158 IMM20190 bound to an epitope that included the receptor-binding ridge and an area adjacent to  
159 the receptor-binding loop. The epitope mapping analysis identified 10 residues in the RBD that  
160 interacted with IMM20190. Of these, two residues, K417 and N501 are mutated, either singly  
161 (K417 in Alpha/B.1.1.7) or doubly (K417/N501 in Beta, Gamma, or Omicron) in prior and present  
162 VOC (**Fig 1E**). The Delta variant and WA1/2020 reference strain are conserved at all 10 interaction  
163 residues. The broad epitope may explain the resistance of IMM20190 to the majority of single-  
164 and double-point mutations within the RBD region (**Supp. Table 1**) and the flexible nature of Fab  
165 binding observed by Cryo-EM (**Fig 1A**).

166 Alanine scanning mutagenesis identified only two critical residues for IMM20253 binding to RBD,  
167 K356 and R466, located on the outer surface of the RBD. This complements the cryo-EM data  
168 (**Fig 1A, B**). K356 resides within the surface area buried by the VL, and R466 resides within the  
169 surface area buried by the VH of IMM20253. R466 residue is conserved in all sarbecoviruses or  
170 lineage b betacoronaviruses, whereas K356 is conserved in most ((**Fig 1E**) and (21, 23, 24)). In  
171 summary, IMM20253 binds to a highly conserved epitope on the outer surface of RBD, does not  
172 compete with IMM20184 and IMM20190 mAbs for S binding and induces dissociation of Trimer  
173 complex into monomers.

174 **IMM-BCP-01 cocktail efficiently suppresses the severity of the disease in an *in vivo* model of**  
175 **SARS-CoV-2 infection.**

176 We tested the efficacy of different combinations and doses of these three antibodies in Syrian  
177 Golden hamsters inoculated with SARS-CoV-2 (WA\_CDC-WA1/2020) (**Fig 2A**). When



178 administered to animals 6 hours after viral challenge (treatment paradigm), we observed that  
179 IMM20190 or 2-Ab combinations of IMM20184/IMM20190 or IMM20190/IMM20253 led to  
180 robust viral clearance (**Fig 2B**). However, the greatest clearance was observed with the 3-Ab  
181 cocktail. Five of the six animals in this cohort showed an approximately 2.5- $\log_{10}$  reduction of  
182 viral titer in the lung on day 4 post viral challenge. In a follow-up study, the 3-Ab cocktail  
183 decreased the viral titer in the lungs of animals inoculated with a high-titer ( $3.3 \times 10^5$  TCID<sub>50</sub>) of  
184 WA\_CDC-WA1/2020 (REF variant) by over 100-fold. This efficacy was observed when the  
185 antibodies were administered at either 1:1:1 ( $p=0.0077$ ) or 1:0.5:0.5  
186 (IMM20190:IMM20184:IMM20253;  $p=0.0143$ ) molar ratios (**Fig 2C**). However, when subjected  
187 to an F-test, the variability in clearance level in 1:0.5:0.5 group was higher ( $P < 0.0001$ ) than when  
188 animals were treated with an equimolar ratio cocktail (**Fig 2C**). These studies were performed  
189 using a viral inoculum that was approximately 10-fold higher than what is typically used to  
190 evaluate efficacy of antibody therapies (23, 25). When repeated at a lower inoculating dose ( $3.3 \times$   
191  $10^4$  TCID<sub>50</sub> per animal) (**Fig 2D**), treatment of hamsters with IMM-BCP-01 (0.1 mg each, 0.3 mg  
192 total antibody) resulted in a significant ( $p < 0.0080$ )  $\sim 3.5 \log_{10}$  decrease in viral titer relative to  
193 vehicle-treated controls. Taken together, these data support the IMM-BCP-01 cocktail as  
194 comprising all three antibodies at 1:1:1 ratios to obtain the most consistent level of viral clearance.

195 **IMM20184, IMM20190 and IMM20253 antibody combinations demonstrates a dose-**  
196 **dependent inhibition of virus load in lungs of hamsters infected with WA1/2020, Alpha, Beta**  
197 **and Omicron variants of SARS-CoV-2.**

198 IMM-BCP-01 cocktail was designed to recognize and inhibit variants that have and could emerge.  
199 Consistent with that goal, IMM-BCP-01 exhibited a dose-dependent inhibition of all viral variants  
200 tested *in vivo*, including the reference (WA1/2020) variant, Alpha, Beta and Omicron isolates (**Fig**

201 **2E, F**). The 3-Ab cocktail suppressed viral infection in the lungs of hamsters pre-treated with doses  
202 as low as 0.1 mg of each antibody (0.3 mg total dose) 24 hours prior to virus challenge. Higher  
203 doses of IMM-BCP-01 lowered viral loads in hamsters to a greater degree. A 10,000-fold reduction  
204 in viral load in lungs of animals inoculated with WA1/2020, and a 1,000-fold reduction in animals  
205 inoculated with Alpha and Beta isolates were achieved with doses of 0.3 and 0.5 mg each (0.9 mg  
206 and 1.5 mg total for a IMM20190/184/253 cocktail). Animals infected with Omicron isolate  
207 developed a lower viral lung load ( $\sim 4.4 \times 10^4$  PFU/g) comparing to other isolates, that was dose-  
208 dependently reduced by a standalone IMM20253 antibody. 2-Ab combination  
209 IMM20153/IMM20184 (0.5 mg ea or 1 mg total dose) further decreased viral load in lungs to  
210 levels comparable to the lower limit of detection (LOD) for the study. Thus, IMM20184,  
211 IMM20190 and IMM20253 antibody combinations potently suppresses infection of multiple  
212 SARS-CoV-2 variants *in vivo* in a dose-dependent manner.

213

#### 214 **IMM-BCP-01 cocktail exposure and pharmacokinetics.**

215 When administered via i.p. injection, the IMM-BCP-01 cocktail generally followed first-order  
216 absorption and elimination process with a half-life of approximately 100 hours in hamsters  
217 (**Supplemental Fig 2A, B**). Unexpectedly, we observed that some animals treated with IMM-  
218 BCP-01 had lung titers equivalent to non-treated controls. To better understand that lack of effect,  
219 terminal bleeds were assessed for levels of human IgG in the plasma. Those studies demonstrated  
220 that variability in viral clearance correlated directly with systemic distribution of IMM-BCP-01  
221 (**Supplemental Fig 2C**). Animals that exhibited lower viral lung titers were associated with  
222 terminal plasma levels of IMM-BCP-01 greater than 3-5  $\mu\text{g/mL}$ . In contrast, IMM-BCP-01 was  
223 not observed at appreciable levels in the blood of animals that failed to clear virus from the lungs,

224 that rather reflects the difficulties with antibody injection to these animals. Effective levels of IgG  
225 in the blood were achieved with dose levels as low as 0.1 mg each (0.3 mg total dose) in both the  
226 prophylactic and treatment settings when the drug was absorbed and systemic exposure was  
227 achieved (**Suppl. Fig 2C**).

228

229 **IMM-BCP-01 has a combinatorial neutralizing effect against current and prior VOCs of**  
230 **SARS-CoV-2 virus.**

231 IMM-BCP-01 cocktail was evaluated in three live (authentic) virus neutralization assays and one  
232 reporter pseudovirus assay (**Fig 3**) using an array of viral variants. The three independent live virus  
233 neutralization assays provided comparable data, which agreed with pseudovirus neutralization  
234 tests (**Table 1**). The antibody cocktail neutralized all tested VBM and VOC (**Fig 3**). The IMM-  
235 BCP-01 cocktail (IMM20184/190/253), as well as IMM20184/20253 combination, completely  
236 neutralized all pseudovirus variants tested (**Fig 3A,B**). Overall, IMM-BCP-01 potently neutralized  
237 the spectrum of variants tested, with all IC50 values being within 2-log of the reference  
238 pseudovirus encoding a WA1/2020 S protein. The 3-Ab cocktail had a modest, but reproducible,  
239 *increase* in potency against Delta, Lambda (C. 37), and Epsilon (B.1.429) pseudoviruses, which  
240 could be explained by a higher susceptibility of Trimers from these variants to structural  
241 rearrangements. In context of current landscape of antibody therapeutics for COVID-19, IMM-  
242 BCP-01 outperformed S309 against Delta and a WA1/2020 D614G pseudovirus (**Fig 3C**). S309  
243 is the parental clone of VIR-7831, which obtained an EUA and retains activity against some  
244 Omicron variants (25).

245

246 To better understand the 3-Ab cocktail, we performed a series of experiments focusing on the  
247 combinatorial contributions of the component antibodies to the overall neutralizing activity. We  
248 tested 2-Ab mixtures of IMM20190 (1x concentration) with either IMM20184 (1x) or IMM20253  
249 (1x), and a 3-Ab combination of IMM20190 (1x) with IMM20184/IMM202053 (0.5x each) in  
250 pseudovirus neutralization assays (**Supp. Fig 3**). Double and triple antibody combinations dose-  
251 dependently neutralized pseudovirus variants corresponding to three VOC (**Supp. Fig 3A, B**). We  
252 calculated each antibody contribution to the observed neutralizing effect using SynergyFinder 2.0  
253 (26)A score below -10 suggests an antagonistic (competitive) effect; a score between -10 and 10  
254 reflects an additive effect; and a score above 10 suggests a synergistic effect of the combined  
255 treatment. We detected a concentration-dependent synergistic potential of combinations (**Supp.**  
256 **Fig 3C**). In variants that IMM20190 potently neutralized, such as WA1/2020 and Epsilon,  
257 antibody combinations are mainly additive, as IMM20190 neutralization was sufficient and did  
258 not require the two other antibodies. In variants where the potency of IMM20190 was reduced,  
259 such as Beta and Gamma, combinations were also additive. In addition, IMM20184 and  
260 IMM20253 antibodies as a double combination had an additive neutralizing effect against these  
261 variants (**Supp. Fig 3D**). We observed the highest synergistic potential of the 3-Ab combination  
262 IMM20190/184/253 for the Alpha variant, where each of the single antibodies neutralized with  
263 comparable IC<sub>50</sub>'s (Fig 2D). In this variant background, the triple antibody combination  
264 outperformed all three of the individual antibodies. Thus, the 3-Ab cocktail neutralized all tested  
265 variants and was associated with additive or synergistic effects depending on the strain. Combined  
266 with the observed antibody pharmacokinetics data (**Supp. Fig 2A**), these data suggest that  
267 administration of the 3-Ab cocktail (0.5 mg each) reaches serum concentrations in vast excess of

268 the IC50 neutralization concentrations observed for all SARS-CoV-2 variants tested, including the  
269 Alpha, Beta, Gamma, and Delta (**Supp. Fig 2, and Fig 2, 3**).

270  
271 When extended to intact virus isolates, we observed equivalent, or better potency of the IMM-  
272 BCP-01 cocktail against WA1/2020, BavPat (D614G), Alpha, Beta, and Gamma variants  
273 measured in focus (**Fig 3E**) or plaque (**Fig 3F, G**) reduction assays as compared to the  
274 corresponding pseudovirus neutralization assay (**Table 1**).

275  
276 IMM-BCP-01 was evaluated for activity against two different live virus isolates of the Omicron  
277 BA.1 variant, as well as an Omicron variant, BA.1.1, harboring an additional R346K mutation  
278 (**Fig 3F, G; Supp. Fig 4**). Consistently with the data observed *in vivo* (**Fig. 2H**), a standalone  
279 IMM20253 antibody neutralized Omicron (BA.1) authentic virus in plaque reduction assay (Fig  
280 3H) and BA.1 and BA.1.1 in focus reduction neutralization assays (**Supp. Fig 4**). Although no  
281 mutations present in the Omicron isolates mapped to critical binding residues for IMM20184 (**Fig**  
282 **1C**), the antibody lost neutralization potency. A partial loss of IMM20184 activity was observed  
283 in plaque reduction assays with BA.1, but complete loss of neutralizing activity was observed  
284 against BA.1.1 in the context of the FRNT assay (**Fig 3G, Supp. Fig 4**). The IMM20184/253  
285 combination showed an additive effect, compared to the IMM20253 antibody alone, in the plaque  
286 assay (**Table 1**) and (Fig. 3G), that is in agreement with the result observed using Omicron isolate  
287 *in vivo* (**Fig. 2H**).

288  
289 Finally, we observed a higher *in vivo* potency of IMM-BCP-01 cocktail, compared to its activity  
290 by virus neutralization assays *in vitro*. We detected a 100-fold increase in EC50 of IMM-BCP-01

291 cocktail against Beta variant in both pseudovirus (**Fig 3A**) and authentic virus (**Fig 3E**) assays *in*  
292 *vitro* that only resulted in a minor dose increase (from 0.3 mg to 0.5 mg per antibody) *in vivo* (**Fig**  
293 **2F, G and 3A**), outlining the importance of *in vivo* studies for anti-SARS-COV-2 antibodies. We  
294 performed *in vitro* neutralization assays at multiple facilities, including academic and industry  
295 laboratories, and observed a ~10-fold difference in EC50s values for same virus variants (Alpha  
296 variant as an example, **Fig 3H**).

297

298 **IMM20190/184/253 antibody cocktail activates potent effector responses *in vitro*.**

299 A growing body of evidence suggests that intact effector functions are required for optimal viral  
300 clearance in animal models of COVID-19 (27–29). The antibodies comprising IMM-BCP-01  
301 retain intact IgG1 Fc domains and bind to the RBD in a non-competitive manner (**Fig 1, Supp.**  
302 **Fig 1H**). We hypothesized that IMM2019/184/253 might generate an oligoclonal response to S  
303 protein that activates Fc-mediated effector functions including antibody-dependent cellular  
304 cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and classical  
305 complement pathway (CP) (Fig 4). To test this hypothesis, we first measured antibody-induced  
306 phagocytosis of Trimer-coated beads using a published method (30). All three human antibodies  
307 induced phagocytosis of Trimer-coated beads in a dose-dependent manner relative to an IgG1  
308 isotype control (**Fig 4A**). Even a low (~15 pM) concentration of IMM20253 antibody potently  
309 induced phagocytosis. The 3-Ab cocktail (IMM20190/184/253) demonstrated a higher phagocytic  
310 score than a 2-Ab cocktail (IMM20184/253) or each individual antibody (**Fig 3A**). We did not  
311 observe phagocytosis of Trimer-coated beads in the presence of an IgG1 isotype control antibody.  
312 Next, we evaluated activation of the classical CP by IMM20190/184/253 cocktail (Figure 3B). In  
313 brief, we adapted a CP activation assay (31) and measured deposition of the complement

314 component C4 from serum of normal human donors on anti-S antibodies bound to Trimer-coated  
315 surface. IMM20190 and IMM20253 antibodies bound to Trimer promoted detectable levels of C4  
316 deposition. While IMM20184 binding to Trimer alone did not activate CP in this assay, the 2-Ab  
317 cocktail IMM20184/253 induced C4 deposition on antibody-Trimer complexes (**Fig 3B**). The  
318 three antibody cocktail IMM20190/184/253 induced the most robust activation of C4 deposition.  
319 Since all tested antibodies had the same intact heavy chain IgG1 Fc region, we hypothesized that  
320 C4 deposition on Trimer-Ab immune complex might depend on the Fc epitope conformation and  
321 accessibility as previously demonstrated for other antibodies (32, 33). Cryo-EM studies (**Fig 1**,  
322 **Supp. Fig 1**) indeed demonstrated that IMM-BCP-01 cocktail attacks Trimer from different  
323 directions and may indeed create an array of Fc regions that facilitates binding of C1q. Finally,  
324 ADCC assay revealed a similar synergy among IMM20184/190/253 antibodies (Fig 3C). While  
325 each antibody induced a mild (IMM20184, IMM20253) to moderate (IMM20190) activation of  
326 ADCC, the three antibody cocktail IMM20190/184/253 induced the greatest response (**Fig 3C**).

327

### 328 **IMM20184, IMM20190, but not IMM20253, block S interactions with ACE2**

329 The location of IMM20184 and IMM20190 epitopes suggests that these two antibodies likely  
330 block ACE2 binding. To test this hypothesis, we performed a biochemical ELISA-based receptor  
331 inhibition assay. The affinity of a soluble ACE2 protein to Wuhan-1, Alpha, and Beta variant  
332 RBDs coated to an ELISA plate was evaluated in the presence of various concentrations of  
333 antibodies of interest. Consistent with the data from the homogeneous time resolved fluorescence  
334 (hTRF) assay (**Supp. Table 1**), IMM20184 potently inhibited ACE2 binding to all three RBD  
335 variants (**Fig 5A**). IMM20190 blocked ACE2 binding to Wuhan-1 and Alpha variant RBD  
336 proteins, and partially decreased ACE2 binding to the Beta variant RBD. In contrast, IMM20253

337 did not fully block ACE2 interactions with any of the three RBD variants tested (**Fig 5A**). A partial  
338 inhibition of ACE2 binding by IMM20253 (up to 40%, depending on the concentration) was  
339 detected for all three S variants tested. These data are consistent with the location of the IMM20253  
340 epitope relative to the ACE2 binding site and suggest its neutralization occurs through a distinct  
341 mechanism of action (see below). Finally, an equimolar mixture of IMM20190, IMM20185, and  
342 IMM20253 antibodies disrupted ACE2 binding to all three tested RBD variants. The inhibitory  
343 effect of a 3-Ab cocktail was more pronounced than the effect of each individual antibody. Of  
344 note, we detected a minor ACE2 binding preference to Alpha RBD than to Beta RBD variant (**Fig**  
345 **6A**, *left panel*).

346

#### 347 **IMM20190, IMM20184 and IMM20253 bind to soluble RBD and S1 proteins recapitulating** 348 **different SARS-CoV-2 variants**

349 Steady-state binding of the three antibodies across a range of variants was characterized via hTRF  
350 (**Supp. Table 1 and Supp. Fig 6**). Each of the three antibodies was tested for binding against  
351 isolated RBD or S1 protein encompassing over 20 different single and multiple mutations that  
352 correspond to naturally occurring and predicted escape mutations. IMM20184 and IMM20253  
353 retained picomolar EC<sub>50</sub> binding to most of single and multiple point mutations tested, including  
354 those present in VBM and VOC. Furthermore, IMM20253 exhibited some binding to SARS-CoV-  
355 1 Spike. In contrast, IMM20190 binding was reduced, to varying degrees, by K417N and the series  
356 of RBD-localized mutations associated with K417N/E484K/N501Y or K417T/E484K/N501Y  
357 variants. Binding of IMM20190 appeared to be partially restored for two different S variants  
358 containing D614G even in the presence of K417N (**Supp. Table 1**).

359



360 **Analysis of binding kinetics of IMM20184, IMM20190, and IMM20253**

361 Antibody binding kinetics were measured using a multi-cycle kinetics protocol on a Biacore T200  
362 surface plasmon resonance instrument. All three antibodies bound with high affinity to both RBD  
363 and Trimer. (**Fig 5B, C**). All three antibodies bound with rapid on-rates ( $k_a > 2.95 \times 10^4$  1/Ms) to  
364 Trimer, but exhibited even faster on-rates ( $k_a$ ) to RBD. This effect was least pronounced for  
365 IMM20190, suggesting that its epitope is the most accessible in the intact Trimer structure.  
366 IMM20184 and IMM20253 bound to the RBD 17- and 19-fold faster than to the Trimer,  
367 respectively. The dissociation of IMM20184 from Trimer ( $k_d^{\text{Trimer}} = 2.3 \times 10^{-4}$  1/s) was 6-fold slower  
368 than from RBD ( $k_d^{\text{RBD}} = 1.3 \times 10^{-3}$  1/s). This difference in dissociation suggests the antibody/Trimer  
369 complex is stabilized through an avid binding mechanism between the antibody and two subunits  
370 of the Trimer, consistent with the epitope mapping data (**Fig 1**). IMM20253, despite exhibiting the  
371 greatest difference (19-fold) in on-rates between the RBD and Trimer, has the fastest on-rate for  
372 the Trimer of all three antibodies. This result suggests the epitope is readily accessible in the  
373 context of the Trimer structure.

374

375 **IMM20253 binding releases Spike monomers and facilitates protease cleavage.**

376 IMM20253 disruption of Trimer into Spike monomers, detected by cryo-EM (**Fig 1**), suggested  
377 that the antibody might facilitate cleavage of S into S1 and S2. We used a previously published  
378 method to evaluate Trimer sensitivity to protease cleavage in the presence of an antibody (34).  
379 Briefly, Trimer protein was mixed with protease K in the presence of either (1) buffer only, (2)  
380 human recombinant ACE2 protein, (3) IMM20253 or (4) IMM20190, an anti-S antibody that  
381 recognizes ACE2-binding region, and incubated for 0, 15 and 60 minutes. Protease readily cleaved  
382 S incubated with buffer after 60 min. ACE2 or IMM20190 appeared to partially decrease protease

383 cleavage at 60 min, perhaps due to steric hindrance. In contrast, IMM20253 induced S cleavage  
384 after 15 min (**Fig 5D**). In a complementary experiment, we evaluated S samples preincubated with  
385 ACE2, IMM20253 or IMM202190 in the absence of protease. Samples were analyzed using a  
386 standard “premix” protocol by dynamic light scattering (DLS). Incubation of S with ACE2 or  
387 IMM20190 led to the generation of complexes and increased the size of particles, measured as an  
388 increase in hydrodynamic diameter after 2 hours of incubation (Figure 5E). Consistent with cryo-  
389 EM and protease cleavage results, incubation of S with IMM20253 decreased the hydrodynamic  
390 diameter of the resulting complexes, consistent with complex disruption. These data support a  
391 unique mechanism of action for IMM20253 whereby binding disrupts the Trimeric architecture of  
392 the S complex and facilitates cleavage into S1 and S2 in the presence of proteases.

393

394

## 395 **DISCUSSION**

396 We have described three unique antibodies with different mechanisms of action that bind to non-  
397 competing epitopes on S protein, trigger Trimer reorganization into one resembling a post-fusion  
398 confirmation, and induce a potent antiviral response *in vitro* and *in vivo*. Further, we revealed a  
399 unique mechanism of action of IMM20253; this antibody binds to a conserved epitope on Trimer  
400 Spike and induces complex dissociation into monomers and cleavage into S1 and S2 subunits.  
401 When combined with IMM20190 and IMM20184, the 3-Ab cocktail consistently showed a robust  
402 antiviral potency *in vivo* and *in vitro*, neutralized all VOC and VBM tested and induced a potent  
403 multicomponent Fc effector response.

404 We previously reported the generation of several hundred of anti-SARS-CoV-2 antibodies with  
405 diverse antiviral properties (19). Subsequent functional analysis guided us to the selection of three  
406 unique, non-competitive anti-S antibodies with potent yet complementary antiviral effects that  
407 bound to three spatially distinct surfaces of RBD region of Spike protein. While each antibody was  
408 capable of neutralizing many viral variants, the antibody cocktail robustly neutralized all variants  
409 tested with additive or synergistic effect.

410 Each of the three antibodies comprising IMM-BCP-01 appear to elicit viral neutralization through  
411 different mechanisms. IMM20190 and IMM20184 antibodies compete with a cellular receptor  
412 hACE2 for binding to Spike protein. The IMM20190 epitope, identified by Cryo-EM and  
413 confirmed using mutagenesis, extends to two surfaces, including the receptor binding ridge of  
414 RBD and the region around the receptor-binding loop (35). Considering the breadth of this epitope,  
415 IMM20190 was shown to be resistant to small changes in the RBD sequence. The epitope of  
416 IMM20184 antibody is located in N370 – P384 region of the RBD and consists of 5 critical

417 residues, surrounding the RBD core (35), that is conserved in Omicron and all prior VOC of SARS-  
418 CoV-2 and other SARS-related coronaviruses (21, 22). IMM20184 antibody has a higher affinity  
419 to a soluble RBD, yet 5.7-fold slower dissociation from a soluble Trimer, indicating avid binding  
420 to Trimer. The COVA-1 antibody that bound to the area adjacent to IMM20184 epitope has been  
421 demonstrated to have strong cross-neutralizing properties due to its avid binding (21). The  
422 remaining antibody IMM20253 does not directly compete with hACE2, but binds to a conserved  
423 epitope (K356 and R466 residues) that is not a common target of the human immune system for  
424 generating neutralizing antibodies (35). IMM20253 binding leads to a dissociation of Trimer into  
425 S protein monomers, that likely facilitates cleavage into S1 and S2 in the presence of proteases.  
426 Thus, IMM20253 triggers a conformational change of S protein into its post-fusion form and  
427 prevents binding to host cells in ACE2-independent manner. IMM20253 epitope is present in all  
428 human as well as in SARS-related coronaviruses(24) and is, therefore, expected to be retained in  
429 emerging human SARS-related viruses. K356 has been previously shown to participate in a  
430 formation of a hydrophobic pocket in the RBD (36) that may explain its functional importance and  
431 evolutionary conservation. The conserved patch of amino acids around R466 has only rarely  
432 elicited an antibody response (35). R466 is conserved in all SARS-related pangolin and bat  
433 betacoronaviruses, making it an attractive target for the therapeutic intervention and vaccine  
434 design. There are two reported antibodies (a nanobody derived from llama and a mouse-derived  
435 antibody) that recognize larger patches of RBD and appear to have overlapping epitopes with  
436 IMM20253 (34, 37). However, both described antibodies bound to broader epitopes, i.e more  
437 sensitive to mutational drift; both were generated via animal immunizations and need to be tested  
438 for off-target binding to human tissues; and either need to be humanized (mouse-derived) or re-  
439 designed (llama-derived) prior to consideration as therapeutics. These two studies, however,

440 further confirm the importance of the IMM20253 epitope.

441 Importantly, the range of neutralization potency exhibited by IMM-BCP-01, across the breadth of  
442 pseudovirus and live virus tested, translated into *in vivo* efficacy in animal models. This was  
443 illustrated most notably in the setting of Beta and Omicron variants. Despite showing a variable  
444 level of *in vitro* neutralization potency (4.9 – 24 nM) against the Beta isolate depending on the  
445 assay used to measure activity, IMM-BCP-01 exhibited robust *in vivo* efficacy at doses consistent  
446 with those currently being used in the clinic for other SARS-CoV-2 antibodies. While IMM-BCP-  
447 01 appears to neutralize virus comparably to S309, the *in vivo* potency of IMM-BCP-01 exceeds  
448 that of VIR-7831 (18). Consistent with the results obtained for Beta variant, a modest  
449 neutralization of Omicron variant by IMM20253 alone (49.5 nM) or by IMM20184/20253  
450 combination (22 nM) *in vitro* translated into a striking potency of IMM20184/IMM20253  
451 combination against Omicron variant *in vivo*, decreasing the virus lung load in Omicron infected  
452 hamsters ~100 fold to the level comparable to lower limit of detection for the method. We  
453 hypothesize that neutralization potency alone does not account for the overall potency *in vivo* as  
454 compared to what was observed for in the published literature. Published data for REGN10933  
455 and REGN10987, the antibodies comprising REGN-CoV2, suggest they were more potent in *in*  
456 *vitro* neutralization assays (38), yet did not appear to lead to higher levels of viral clearance in  
457 hamster models of COVID-19 (39). We demonstrate that Fc-mediated viral clearance mechanisms  
458 are also enhanced in the context of the IMM-BCP-01 cocktail as compared to any of the three  
459 individual antibodies alone. We argue that the enhanced viral clearance observed *in vivo* may be a  
460 direct result of the oligoclonal nature with which the IMM-BCP-01 antibodies bind to the RBD of  
461 S protein. The ability to neutralize via multiple mechanisms leads to synergy, in conjunction with  
462 the Fc-dependent activation of effector functions, may explain the robust potency detected in the

463 *in vivo* experiments, and is in agreement with previous reports (27–29). Interestingly, no apparent  
464 clinical benefit has been derived by increasing the dose of REGN-CoV2 from 2400 mg to 8000  
465 mg (40). Similarly, viral clearance of WA1/2020 elicited by VIR-7831 in hamster studies  
466 plateaued at approximately 15-fold clearance upon treatment with 5 mg/Kg of antibody; increasing  
467 to 15 mg/kg led to no better clearance (25). In contrast to those findings, administration of IMM-  
468 BCP-01, within the 3 – 9 mg/Kg dose range tested, yielded a dose response of ~300-10,000-fold  
469 clearance of the WA1/2020 virus, with the 10,000-fold decrease being at the limit of detection for  
470 evaluating absolute clearance levels. Importantly, the dose response was not limited to the  
471 WA1/2020 isolate, as a similar dose-response was observed in clearance of Alpha, Beta and  
472 Omicron variants.

473 These three antibodies were identified using the Immunome’s Discovery Platform, based on the  
474 antibody function, target and biochemical properties. Having the structure and mechanistic data  
475 opens up the opportunity for a potential rational design of antibody combinations(41, 42).

476 In summary, we identified and characterized three potent patient-derived antibodies IMM20190,  
477 IMM20184 and IMM20253 with a unique set of antiviral properties. When combined into the  
478 IMM-BCP-01 cocktail, they synergize to neutralize multiple variants of SARS-CoV-2, potently  
479 activate Fc-mediated antiviral effector response, and demonstrate antiviral effects *in vivo*. We  
480 described IMM20253, that belongs to a unique class of human antibodies, that recognizes a rare  
481 epitope and triggers dissociation of Trimer. Based upon the data we have presented, IMM-BCP-  
482 01 is effective across the spectrum of variants known to date and should retain activity against  
483 future variants. Importantly, recent data support the idea that targeting SARS-CoV-2 with several  
484 antibodies should reduce viral escape from IMM-BCP-01 (11). As IMM-BCP-01 antibody cocktail  
485 may be used in both prophylaxis or therapy setting against SARS-CoV-2 variants, clinical trials

486 are warranted.

487

## 488 MATERIAL AND METHODS

### 489 Cells

490 Reporter virus particles (RVP's) were purchased from Integral Molecular, ACE2-293T cells  
491 (Integral Molecular; Cat #C-HA102) were cultured in DMEM containing 10% FBS, 10mM  
492 HEPES, and 0.5 µg/mL Puromycin. Vero E6 cells (BEI resources, NIAID, NIH: VERO C1008  
493 (E6), African green monkey kidney, Working Cell Bank NR-596) were maintained in humidified  
494 incubators at 37°C and 5% CO<sub>2</sub> in DMEM high glucose with GlutaMAX and sodium pyruvate  
495 (Gibco™, cat #10569) and 10% certified US-origin heat-inactivated fetal bovine serum (Gibco™,  
496 cat #10082). African green monkey Vero-TMPRSS2 (43) cells were cultured at 37°C in  
497 Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS),  
498 10 mM HEPES pH 7.3, 1 mM sodium pyruvate, 1× non-essential amino acids, and 100 U/mL of  
499 penicillin–streptomycin with 5 µg/mL of blasticidin.

500

### 501 Animal studies

502 All animal studies described in the manuscript were carried out under Institutional Animal Care  
503 and Use Committee (IACUC)-approved protocols at the respective institutions (BU and  
504 MRIGlobal) and where appropriate were reviewed and approved by Animal Care and Use Review  
505 Office of USAMRDC (ACURO).

506

### 507 Syrian hamster model of SARS-CoV-2 infection

508 Syrian Golden Hamsters (Envigo) were challenged on Study Day 0 with SARS-CoV-2 via  
509 intranasal inoculation using 0.1 mL of either  $1.67 \times 10^5$  or  $1.67 \times 10^6$  TCID<sub>50</sub>/mL material  
510 (WA\_CDC-WA1/2020) (post-exposure treatment experiment), or  $1 \times 10^4$  PFU (WA\_CDC-



511 WA1/2020, Alpha (B.1.1.7) or Beta (B.1.351)) material (pre-exposure treatment experiment).  
512 Hamsters were either treated one day before challenge, or  $6 \pm 1$  hour after challenge via an  
513 intraperitoneal (i.p.) injection. Animals were euthanized on Study Day 4. The lungs were  
514 harvested and homogenized for viral titer determination via TCID<sub>50</sub> or plaque assay. Viral  
515 clearance levels obtained by the various treatments were compared to non-treated controls using  
516 a two-way ANOVA with Tukey's multiple comparisons test. F-tests were performed using an  
517 online calculator (<https://www.statskingdom.com/220VarF2.html>).

518  
519

## 520 **Pseudovirus Production and Neutralization Assay**

521 Neutralization experiments using SARS-CoV-2 luciferase reporter virus particles (RVP's)  
522 (Integral Molecular) were based on the manufacturer's instructions. In brief, RVP's were thawed  
523 for 2-3 minutes in a 37°C water bath. The recommended amount of RVP's was added to the inner  
524 wells of a white opaque 96 well plate (Corning; Cat #3917) or 384 well plate (Greiner Bio-One;  
525 Cat #781080). Media containing the indicated amount of antibody was added to each well,  
526 resulting in a final volume of 100  $\mu$ L per well (96 well plate) or 25  $\mu$ L per well (384 well plate).  
527 The antibody/RVP mixture was pre-incubated for 1 hour in a 37°C incubator containing 5% CO<sub>2</sub>.  
528 ACE2-293T target cells were added to each well ( $2 \times 10^4$  cells in 100  $\mu$ L for a 96 well plate or  $0.9$   
529  $\times 10^4$  cells for a 384 well plate) and incubated for 72 hours. Media was removed from all wells,  
530 equal volumes of PBS and Renilla-Glo Luciferase Assay Reagent (Promega; Cat #E2720) were  
531 added to each well (60  $\mu$ L total for a 96 well plate or 30  $\mu$ L total for a 384 well plate). After 10  
532 minutes, luminescence was measured on the EnSpire Plate Reader (PerkinElmer). Percent  
533 neutralization was calculated with the following equation: [(RLU of Virus + cells) – (RLU of  
534 Experimental Sample)] / [(RLU of Virus + cells) – (RLU of cells only)].

535

### 536 **Epitope Mapping of IMM20190, IMM20184 and IMM20253 antibodies**

537 Shotgun Mutagenesis epitope mapping services were provided by Integral Molecular  
538 (Philadelphia, PA) as described in (44). Briefly, a mutation library of the target protein was created  
539 by high-throughput, site-directed mutagenesis. Each residue was individually mutated to alanine,  
540 with alanine codons mutated to serine. The mutant library was arrayed in 384-well microplates  
541 and transiently transfected into HEK293T cells. Following transfection, cells were incubated with  
542 the indicated antibodies at concentrations pre-determined using an independent  
543 immunofluorescence titration curve on wild type protein. MAb were detected using an Alexa  
544 Fluor 488-conjugated secondary antibody and mean cellular fluorescence was determined using  
545 Intellicyt iQue flow cytometry platform. Mutated residues were identified as being critical to the  
546 MAb epitope if they did not support the reactivity of the test MAb but did support the reactivity  
547 of the reference MAb. This counter-screen strategy facilitates the exclusion of mutants that are  
548 locally misfolded or that have an expression defect.

549

### 550 **Calculation of Synergistic Neutralization by Antibody Combinations**

551 In the context of pseudovirus neutralization, synergy between two or three monoclonal antibodies  
552 in combination is defined as neutralization that is greater than neutralization by the most effective  
553 monoclonal antibody alone. To test whether combinations of antibodies show synergy in  
554 neutralizing SARS-CoV-2, we used an approach similar to one described previously (3).  
555 Pseudovirus neutralization experiments were set up as described above, except that multiple  
556 monoclonal antibodies were tested in combination. Briefly, for combinations of two antibodies,  
557 one test article was titrated in the background of each concentration in a serial dilution of the other

558 test article. Single antibody titrations were included as controls. For combinations of three  
559 antibodies, one test article was titrated in the background of each concentration in a serial dilution  
560 of a 1:1 mixture of the other two test articles. To evaluate antibody synergy in the combinations,  
561 the observed combination response matrix of pseudovirus neutralization was used as input for the  
562 online SynergyFinder platform (4), where quadruplicate data points were input separately. The  
563 highest single agent (HSA) reference model was applied, which quantifies synergy as the excess  
564 over the maximum response of a single drug in the combination. Synergy between antibodies in  
565 each combination is reported as an overall synergy score (the average of observed synergy across  
566 the dose combination matrix) as well as a peak HSA score (the highest synergy score calculated  
567 across the dose combination matrix). Synergy scores of less than -10, between -10 and 10, and  
568 greater than 10 indicate antagonistic, additive, and synergistic antibody combinations,  
569 respectively. While peak HSA scores report on synergy at the most optimal combination  
570 concentrations, the overall synergy score is less affected by outlier data points.

571

## 572 **Phagocytosis assay**

573 Assay was performed with antibodies diluted to 100 ug/mL in PBS + 1% BSA. Antibodies were  
574 subjected to overnight incubation on tube rotator at 4°C in the presence of bead-biotinylated  
575 antigen mixture, followed by 3 washes. THP-1 cells were pelleted, resuspended in serum-free  
576 RPM and then added to wells containing bead-antigen-antibody mixture. The bead-antigen-  
577 antibody-cells mixture was incubated with cells in CO2 incubator for 18 hours. After that, cells  
578 were fixed and immunostained. Flow cytometry was performed on Attune NXT and the resulted  
579 data were analyzed using FlowJo Software.

580

581 **Activation of classical complement pathway**

582 ELISA-based method to evaluate the activation of the classical complement pathway was adapted  
583 from (31, 45). Endotoxin-free ELISA plates were coated with either RBD or Trimer soluble  
584 proteins diluted in endotoxin-free PBS (HyClone) overnight. Plates were blocked with endotoxin-  
585 free 2% gelatin solution (Sigma) and incubated with anti-Spike antibodies of interest for 1 hour at  
586 +4°C. Plates were washed 3 times with endotoxin-free GVB buffer with Ca<sup>2+</sup> and Mg<sup>2+</sup> (GVB++,  
587 Complement Technology) and incubated with normal human serum (Complement Technology)  
588 diluted to 1.25% in GVB++ buffer for 1.5 hours at +37°C on an orbital shaker. Reaction was  
589 stopped by a wash with ice-cold PBS. Cells with deposited complement components were stained  
590 with anti-C4 antisera (Complement Technology) and a secondary anti-goat-HRP antibody  
591 (SouthernBiotech). Plates were incubated with HRP substrate and a stop solution according to  
592 manufacturer's instructions (ThermoFisher). Optical density was measured on EnSpire Plate  
593 Reader (PerkinElmer)

594

595 **Receptor competition assay**

596 ELISA plates were coated with either REF, UK or SA variant of RBD (SinoBio) overnight and  
597 washed with PBS (3x). Single antibody or a 3-Ab cocktail added at equimolar concentrations were  
598 added simultaneously with soluble human ACE2 protein (at a concentration of ~ EC80 of its  
599 normal binding to RBD protein) and incubated at +37°C on an orbital shaker for 1 hour. Plates were  
600 washed (3x) and subsequently probed for ACE2 binding with anti-ACE2 antibody.

601

602 **Authentic virus neutralization assay**

603 Antibody combinations starting at 30 µg/ml per antibody were serially diluted in Dulbecco's  
604 Phosphate Buffered Saline (DPBS)(Gibco™) using half-log dilutions. Dilutions were prepared in  
605 triplicate for each antibody and plated in triplicate. Each dilution was incubated at 37°C and 5%  
606 CO<sub>2</sub> for 1 hour with 10<sup>3</sup> plaque forming units/ml (PFU/ml) of each SARS-CoV-2 variant [isolate  
607 USAWA1/2020, hCoV-19/USA/CA\_CDC\_5574/2020, BEI #NR-54011 (B.1.1.7) and hCoV-  
608 19/South Africa/KRISP-K005325/2020, BEI #NR-54009 (B.1.351)]. Each virus stock was  
609 passaged once from starting material in Vero E6 cells prior to use. Controls included Dulbecco's  
610 Modified Eagle Medium (DMEM) (Gibco™) containing 2% fetal bovine serum (Gibco™) and  
611 antibiotic-antimycotic (Gibco™) only as a negative control and 1000 PFU/ml SARS-CoV-2  
612 incubated with DPBS. Two hundred microliters of each dilution or control were added to confluent  
613 monolayers of NR-596 Vero E6 cells in duplicate and incubated for 1 hour at 37°C and 5% CO<sub>2</sub>.  
614 The plates were gently rocked every 15 minutes to prevent monolayer drying. The monolayers  
615 were then overlaid with a 1:1 solution of 2.5% Avicel® RC-591 microcrystalline cellulose and  
616 carboxymethylcellulose sodium (DuPont Nutrition & Biosciences, Wilmington, DE) and 2X  
617 Modified Eagle Medium (Temin's modification, Gibco™) supplemented with 2X antibiotic-  
618 antimycotic (Gibco™), 2X GlutaMAX (Gibco™) and 10% fetal bovine serum (Gibco™). Plates  
619 were incubated at 37°C and 5% CO<sub>2</sub> for 2 days. The monolayers were fixed with 10% neutral  
620 buffered formalin and stained with 0.2% aqueous Gentian Violet (RICCA Chemicals, Arlington,  
621 TX) in 10% neutral buffered formalin for 30 min, followed by rinsing and plaque counting. The  
622 half maximal inhibitory concentrations (IC<sub>50</sub>) were calculated using GraphPad Prism 8.

623 *Focus reduction neutralization assay.* Serial dilutions of antibodies were incubated with 10<sup>2</sup> focus-  
624 forming units (FFU) of WA1/2020 D614G, BA.1, or BA.1.1. for 1 h at 37°C. Antibody-virus  
625 complexes were added to Vero-TMPRSS2 cell monolayers in 96-well plates and incubated at 37°C

626 for 1 h. Subsequently, cells were overlaid with 1% (w/v) methylcellulose in MEM. Plates were  
627 harvested 30 h (WA1/2020 D614G) or 72 h (BA.1 and BA.1.1) later by removing overlays and  
628 fixed with 4% PFA in PBS for 20 min at room temperature. Plates were washed and sequentially  
629 incubated with an oligoclonal pool (SARS2-02, -08, -09, -10, -11, -13, -14, -17, -20, -26, -27, -28,  
630 -31, -38, -41, -42, -44, -49, , -57, -62, -64, -65, -67, and -71 (37) of anti-S murine antibodies  
631 (including cross-reactive mAbs to SARS-CoV) and HRP-conjugated goat anti-mouse IgG (Sigma  
632 Cat # A8924) in PBS supplemented with 0.1% saponin and 0.1% bovine serum albumin. SARS-  
633 CoV-2-infected cell foci were visualized using TrueBlue peroxidase substrate (KPL) and  
634 quantitated on an ImmunoSpot microanalyzer (Cellular Technologies).  
635

### 636 **Cryo-EM analysis of Trimer-Fab complexes**

637 Cryo-EM analysis was performed at NovAliX (Strasbourg, France). Fabs were mixed with the  
638 SARS-CoV-2 S 6P trimer (6:1 molar ratio Fab per protomer) to a final Fab–S complex  
639 concentration of around 0.8 mg ml<sup>-1</sup> and incubated at room temperature for 1H. Immediately  
640 before deposition of 3.5 µl of complex onto a 200 mesh, 1.2/1.3 C-Flat grid (protochips) that had  
641 been freshly glow-discharged for 30 sec at 3 mA using an ELMO (Cordouan). The sample was  
642 incubated on the grid for 15 s and then blotted with filter paper for 2 s in a temperature and  
643 humidity controlled Vitrobot Mark IV (T = 6 °C, humidity 100%, blot force 2) followed by  
644 vitrification in 100% liquid ethane. Single-particle cryo-EM data were collected on a Glacios  
645 transmission electron microscope (Thermo Fisher) operating at 200 kV. Movies were collected  
646 using EPU software for automated data collection. Data were collected at a nominal underfocus  
647 of -0.6 to -2.8 µm, at magnifications of 120,000× with a pixel size of 1.2 Å. Micrographs were

648 recorded as movie stacks on a Falcon III direct electron detector (Thermo Fisher); each movie  
649 stack was fractionated into 13 frames, for a total exposure of 1.5 s corresponding to an electron  
650 dose of 50 e<sup>-</sup>/Å<sup>2</sup>. Drift and gain correction and dose weighting were performed using  
651 MotionCorr2. A dose-weighted average image of the whole stack was used to determine the  
652 contrast transfer function with the software Gctf. The following workflow was processed using  
653 RELION 4.0. Ab-initio cryo-EM reconstruction was low-pass filtered to 60 Å and used as an initial  
654 reference for 3D classification. The following subclasses depicting high resolution features were  
655 selected for refinement with various number of particles. IMM202190: 3 from 8 subclasses,  
656 173,541 particles; IMM2084L 2 from 6 subclasses, 62,150 particles; IMM20253: 2 from 6  
657 subclasses for Trimer, 86,974 particles, and 1 from 6 for monomers, 40,489 particles. Atomic  
658 models from PDB:7E8C, PDB:6XLU, PDB:6XM5 or PDB:7NOH for the Spike Trimer and  
659 PDB:6TCQ for the Fabs were used as starting point. Models were then rigid body fitted to the  
660 density in Chimera.

### 661 **Cryo-EM analysis of IMM20184/253 Fabs-Spike complex**

662 Cryo-EM analysis was performed at nanoimaging Services (San Diego, USA). Electron  
663 microscopy was performed using an FEI Titan Krios (Hillsboro, Oregon) transmission electron  
664 microscope operated at 300kV and equipped with a Gatan BioQuantum 1967 imaging filter and  
665 Gatan K3 Summit direct detector. Vitreous ice grids were clipped into cartridges, transferred into  
666 a cassette and then into the Krios autoloader, all while maintaining the grids at cryogenic  
667 temperature (below -170C°). Automated data-collection was carried out using Legicon  
668 software(46), where high magnification movies were acquired by selecting targets at a lower  
669 magnification(46). Dose-weighted movie frame alignment was done using MotionCor2 (47) or

670 Full-frame or Patch motion correction in cryoSPARC (48) to account for stage drift and beam-  
671 induced motion. The contrast transfer function was estimated for each micrograph using CTFind4,  
672 gCTF, or Patch CTF in cryoSPARC (49, 50). Individual particles were selected using automated  
673 picking protocols and extracted into particle stacks in either Relion (51, 52) or cryoSPARC. The  
674 particles may then be submitted to reference-free 2D alignment and classification in either Relion  
675 or cryoSPARC. One dataset was collected for sample S Protein RBD + IMM20253 Fab +  
676 IMM20184 Fab, totaling 3,148 high magnification images. About 1.4M particles were selected  
677 from 1,666 manually curated micrographs using cryoSPARC 3.3 live. All subsequent data  
678 processing was carried out in cryoSPARC 3.3. These particles were subjected to three rounds of  
679 2D classification, and about 300k good particles were selected. Second dataset with 30° tilt was  
680 collected for sample S Protein RBD + IMM20253 Fab + IMM20184 Fab, totaling 629 high  
681 magnification images. About 100k particles were selected from 244 manually curated micrographs  
682 using cryoSPARC 3.3 live. Third dataset with 30° tilt was collected for sample S Protein RBD +  
683 IMM20253 Fab + IMM20184 Fab, totaling 1,369 high magnification images. About 420k particles  
684 were selected from 1,055 manually curated micrographs using cryoSPARC 3.3 live. All  
685 subsequent data processing was carried out in cryoSPARC 3.3. These particles were subjected to  
686 one round of 2D classification. All micrographs from three sessions were combined for further  
687 processing in cryoSPARC 3.3. ~1.9M particles were extracted and subjected to three rounds of 2D  
688 classification analysis and about 600k particles were selected for ab initio reconstruction. The  
689 particles were then subjected to three rounds of heterogenous refinement using the good and junk  
690 classes from ab initio reconstruction as reference volume. The good particles were selected and  
691 subjected to homogenous refinement and non-uniform refinement. The final 3D reconstruction  
692 was at a nominal resolution of 3.87 Å using ~170K particles (see Sup. Figure 1E,F). The best 3D



693 classes were submitted to homogeneous 3D refinement that includes dynamic masking. Reported  
694 resolutions were based on the gold standard FSC = 0.143 criterion. Maps were visualized using  
695 Chimera.

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904 **193** (2016), doi:10.1016/j.jsb.2015.11.003.  
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906 52. D. Kimanius, B. O. Forsberg, S. H. W. Scheres, E. Lindahl, Accelerated cryo-EM structure  
907 determination with parallelisation using GPUS in RELION-2. *Elife* **5** (2016),  
908 doi:10.7554/eLife.18722.

911 **ACKNOWLEDGMENTS**

912 This study was funded by the U.S. Department of Defense (DOD) Joint Program Executive  
913 Office for Chemical, Biological, Radiological and Nuclear Defense's (JPEO-CBRND) Joint  
914 Project Manager for Chemical, Biological, Radiological and Nuclear Medical (JPEO-CBRN  
915 Medical), in collaboration with the Defense Health Agency (DHA), under contract  
916 W911QY2090019. The opinions, interpretations, conclusions and recommendations are those of  
917 the authors and are not necessarily endorsed by the U.S. Army.

918  
919 The following reagents were obtained through BEI Resources, NIAID, NIH: VERO C1008 (E6),  
920 Kidney (African green monkey), Working Cell Bank, NR-596; SARS-CoV-2, Isolate hCoV-  
921 19/USA/CA\_CDC\_5574/2020, NR-54011 (deposited by Centers for Disease Control and  
922 Prevention) and SARS-CoV-2, Isolate hCoV-19/South Africa/KRISP-K005325/2020, NR-54009  
923 (contributed by Alex Sigal and Tulio de Oliveira). The SARS-CoV-2 isolate USA-WA1/2020  
924 starting material was provided by the World Reference Center for Emerging Viruses and  
925 Arboviruses (WRCEVA), with Natalie Thornburg (nax3@cdc.gov) as the CDC Principal  
926 Investigator.

927

## 928 **AUTHOR CONTRIBUTIONS**

929 PAN, JMD, JPD, NBP, JLBS, BCH, NH, CN, AP, RJH, MN, HS, JPF, TS, NS, LGAM, ELS, RIJ,  
930 SMS, and LEM performed wet laboratory experiments. PAN, JMD, JPD, JLBS, AHN, AG, MSD,  
931 and MKR analyzed and interpreted the data. LFL, AG and DHG consulted and critically discussed  
932 the manuscript. PAN, JDM, PS, MJM and MKR supervised the project. PAN, JDM, JPD, JLBS  
933 and MKR wrote and AG, DHG, PS and MJM edited the manuscript. The manuscript was reviewed  
934 and cleared for publication by DoD's JPEO-CBRN Medical, JPEO-CBRND.

935

936 **COMPETING INTEREST STATEMENT**

937 The described approach, antibodies and cocktail composition have been included in patent  
938 applications. PAN, JMD, JPD, NBP, JLBS, BCH, NH, CN, AP, MN, HS, JPF, LFL, TS, PS, DHG,  
939 MJM and MKR are employees and shareholders of Immunome, Inc. MSD is a consultant for  
940 Inbios, Vir Biotechnology, and Carnival Corporation, and on the Scientific Advisory Boards of  
941 Moderna and Immunome. MSD has stock equity options from Immunome. The Diamond  
942 laboratory has received funding support in sponsored research agreements from Immunome, and  
943 unrelated support from Vir Biotechnology, Moderna, and Emergent BioSolutions.

944

945 **TABLES**

946

947 **Table 1. IC<sub>50</sub> Values (nM) for IMM20184/190/253 antibodies and its combinations against**  
948 **SARS-CoV-2 variants.** Shown values are Means generated using three different methods,  
949 including a live (authentic) virus spot reduction assay, a live (authentic) virus plaque reduction  
950 assay and a pseudovirus reporter neutralization assay.

<b>Spot reduction assay</b>	<b>BavPat</b>	<b>Alpha</b>	<b>Beta</b>	<b>Gamma</b>
	<b>IC<sub>50</sub> (nM)</b>	<b>IC<sub>50</sub> (nM)</b>	<b>IC<sub>50</sub> (nM)</b>	<b>IC<sub>50</sub> (nM)</b>
IMM20184	33.8	43.3	81	18.4
IMM20190	0.4	2.7	>393	>393
IMM20253	39.4	1.4	155.4	13.4
IMM20184/IMM20190	0.4	1	Not Tested	
IMM20184/IMM20253	3.9	5.7		
IMM20190/IMM20253	0.4	0.9		
IMM-BCP-01	0.24	0.4	21.2	4.2

951

<b>Plaque reduction assay</b>	<b>REF (WA1/2020)</b>	<b>Omicron</b>
	<b>IC<sub>50</sub> (nM)</b>	<b>IC<sub>50</sub> (nM)</b>
IMM20184	68.4	>1000
IMM20253	23.9	49.5
IMM20184/IMM20253	19.0	22.2

952

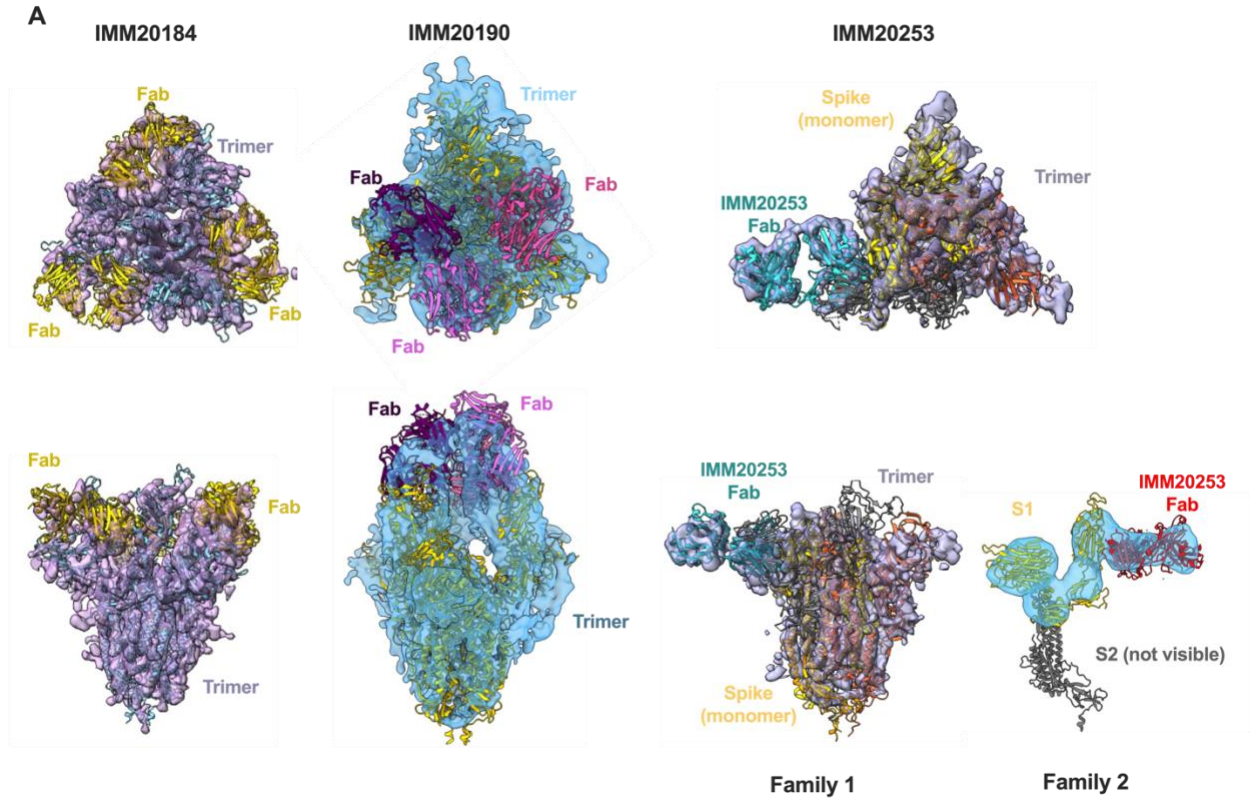
<b>Pseudovirus Spike Neutralization</b>	<b>IC<sub>50</sub> (nM)</b>
REF (WA1/2020)	1.0
D614G	0.6
Alpha	3.0
Beta	13.5
Gamma	24.8
Delta	0.4
Delta plus	3.0
Epsilon	0.6
Kappa (L452R/E484Q)	1.0
Lambda	0.4

953

954 **FIGURE LEGENDS**

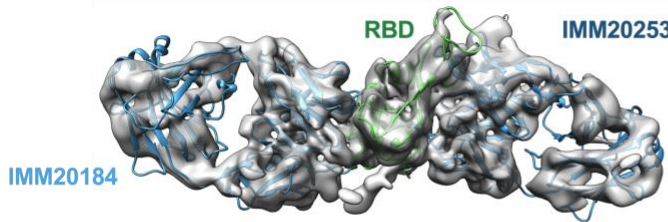
955





956

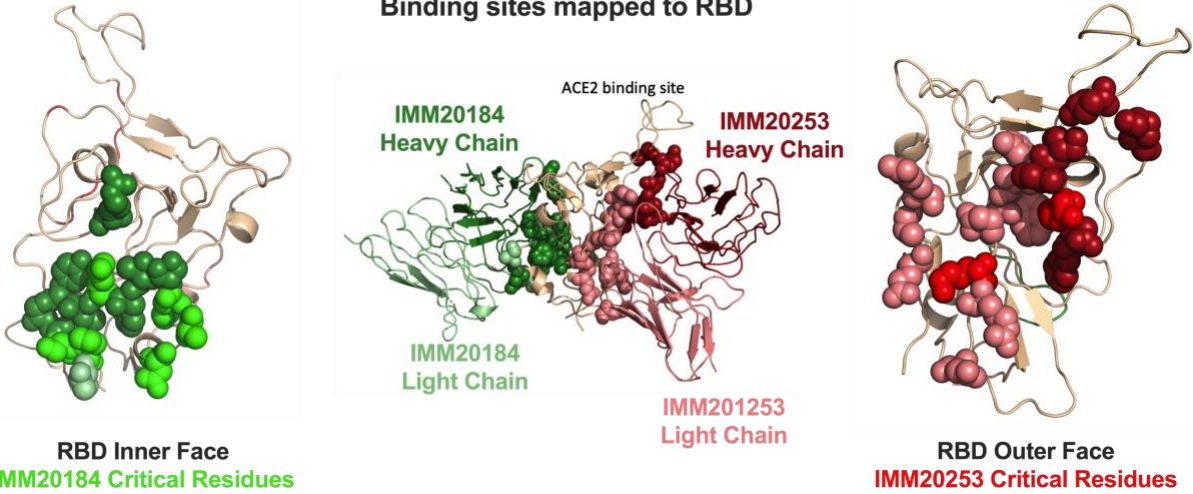
**B IMM20184 and IMM20253 Bound to RBD**



**C Alanine Scanning**

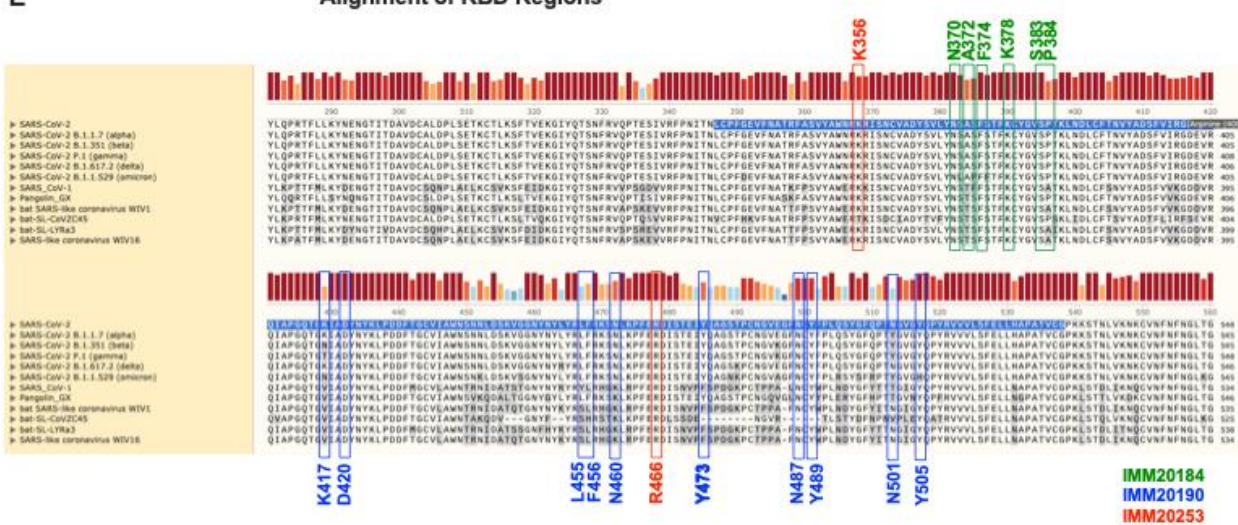
Mutation	Binding Reactivity (% WT)		
	IMM20190 Fab	IMM20184 Fab	IMM20253 Fab HS
K356A	82.2 (6)	61.5 (6)	71.1 (4)
Y365A	75.2 (3)	5.4 (3)	72.0 (3)
Y366A	107.0 (9)	13.2 (2)	14.0 (13)
N370A	63.4 (5)	75.0 (8)	72.4 (4)
A372S	127.3 (42)	3 (2)	99.9 (16)
F374A	64.8 (7)	7.4 (1)	70.8 (8)
K378A	67.9 (3)	67.0 (6)	65.9 (17)
V382A	61.9 (15)	15.6 (11)	49.3 (9)
S383A	110.8 (1)	0.4 (2)	86.3 (2)
F384A	111.5 (1)	6.8 (3)	112 (12)
K417A	15.6 (6)	96.2 (32)	113 (20)
D420A	4.6 (2)	65.5 (14)	69 (6)
L455A	4.5 (7)	170.6 (27)	114 (13)
F456A	2.3 (2)	79.7 (2)	64.8 (4)
N460A	9.4 (6)	76.3 (20)	89 (12)
R466A	70.8 (19)	78.7 (15)	5 (1)
Y472A	1 (1)	83.5 (26)	65.3 (7)
N487A	12.6 (6)	84.5 (7)	67 (3)
Y488A	3.1 (4)	73 (6)	65 (12)
N501A	12.9 (1)	93.4 (5)	80.1 (14)
Y505A	6.3 (4)	130 (9)	108.2 (11)

**D Binding sites mapped to RBD**



957

**E Alignment of RBD Regions**

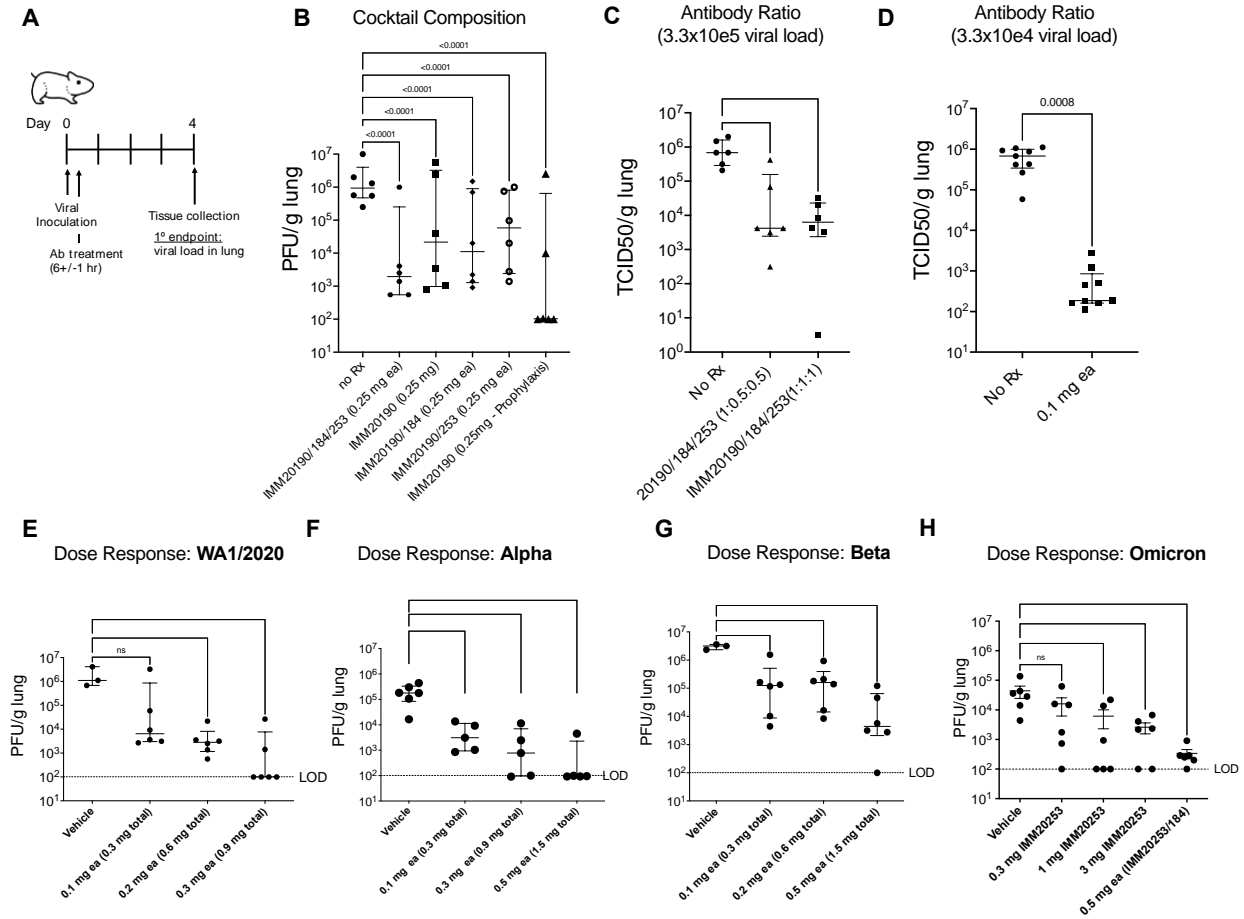


958

959 **Figure 1. IMM20184, IMM190 and IMM20253 antibodies bind to conserved epitopes and**

960 **disrupt Spike Trimer. (A) 3D reconstruction of Cryo-EM images of Spike Trimer complex with**

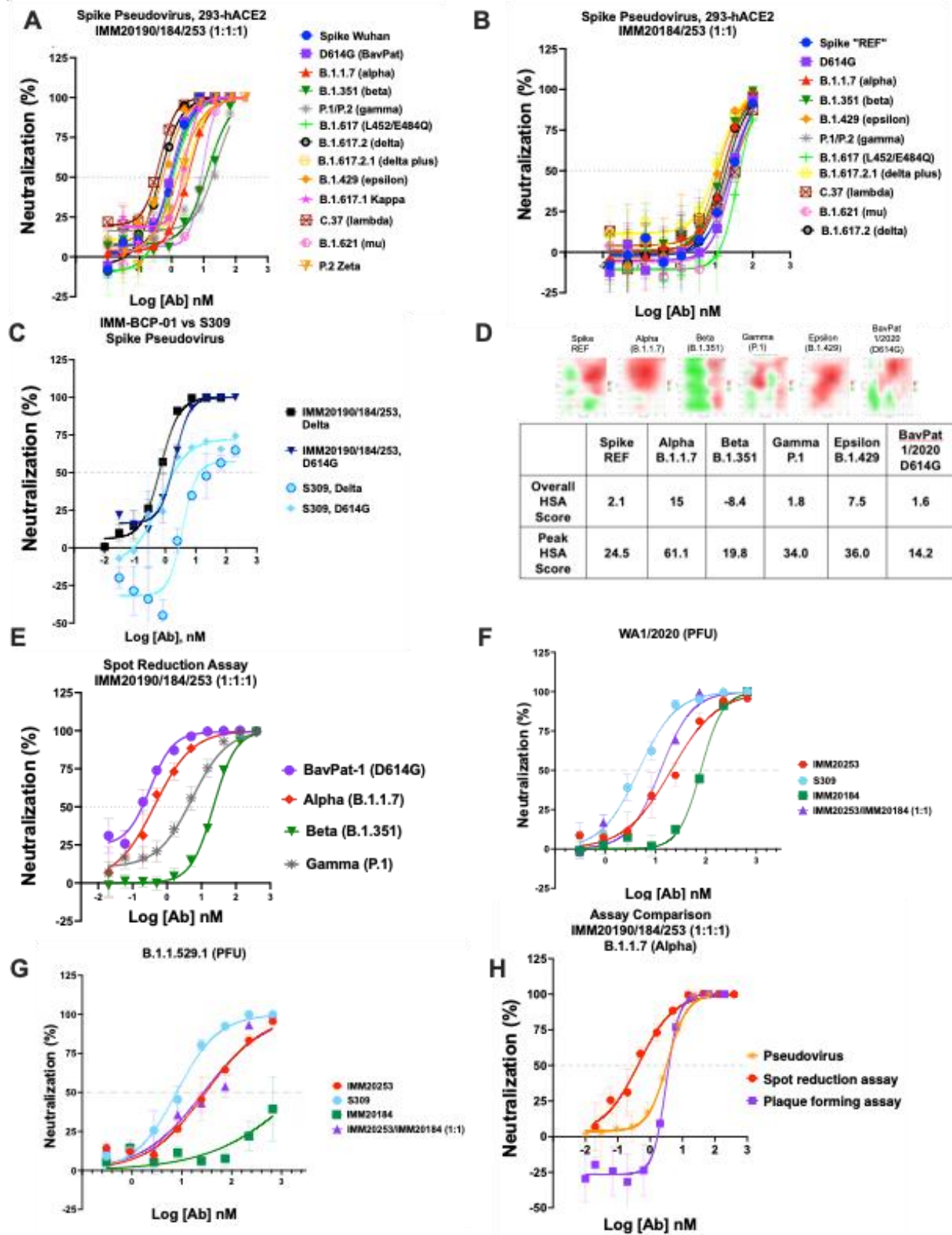
961 either IMM20184 (left), IMM20190 (middle) or IMM20253 (right) Fabs at  $\sim 7\text{\AA}$  resolution. Top-  
962 to bottom view (top) and side view (bottom) are shown. IMM20253 Fab binding to Trimer results  
963 in two families, Family 1 and 2. Models PDB:7E8C, PDB:6XLU, PDB:6XM5 or PDB:7NOH  
964 were fit to density in Chimera for the Spike Trimer and PDB:6TCQ for the Fabs. (B) 3D  
965 reconstruction of Cryo-EM images of a complex of RBD with simultaneously bound Fabs of  
966 IMM20184 and IMM20253 at  $\sim 3.9\text{\AA}$ . Fab model PDB:1M71 was fit to density in Chimera. (C)  
967 Critical residues of antibody epitopes identified as binding pattern to a library of single-point RBD  
968 mutants expressed on the cell surface. (D) Epitopes of IMM20184 and IMM20253 antibodies  
969 mapped on the RBD model PDB: 7A97. Epitopes of IMM20184 (green) and IMM20253 (red)  
970 antibodies and critical residues (bright green and red) are shown. (E) Alignment of Spike protein  
971 sequences from current and prior CDC VOCs, SARS-CoV-1 and closely related coronaviruses.  
972 Critical residues of IMM20190, IMM20184 and IMM20253 epitopes are shown in blue, green and  
973 red. Highlighted sequence indicates RBD.



974

975 **Figure 2. Three antibody combinations IMM20190/184/253 inhibits replication of non-**  
 976 **adapted SARS-CoV-2 in lungs of infected animals.** (A) All studies were carried out in Syrian  
 977 Golden hamsters challenged with an intra-nasal inoculation of SARS-CoV-2 and treated with  
 978 antibodies post-inoculation. Lungs were harvested at Day 4 and viral titers determined by either  
 979 plaque forming or TCID50 assays. (B) Animals were infected with WA\_CDC-WA1/2020 isolate  
 980 and treated with single, double, or triple antibody cocktails, as noted, 6 hours post-inoculation. (C)  
 981 Hamsters were challenged with  $3.3 \times 10^5$  TCID50 viral inoculation and treated with 3-Ab cocktail,  
 982 at two different antibody ratios (1:1:1 or 1:0.5:0.5). (D) Hamsters were challenged with  $3.3 \times 10^4$   
 983 TCID50 viral inoculation and treated with 3-Ab cocktail at 1:1:1 ratio, at 0.1 mg dose each (0.3  
 984 mg total). (E -H) Hamsters were challenged with  $10^4$  PFU of WA1/2020 (E), Alpha (B.1.1.7) (F)

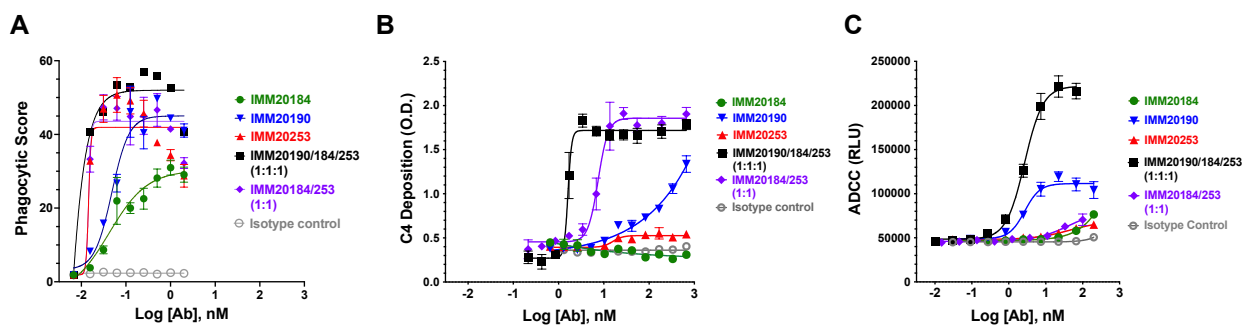
985 Beta (B.1.351) (G), or Omicron (BA.1) (H) SARS-CoV-2 isolates after pre-treatment (Day -1)  
986 with different doses of 3-Ab cocktail IMM20190/184/253 at 1:1:1 ratio. Bar denotes median  
987 values. Error bars denote interquartile range. Statistical analysis in panel B is Two-Way ANOVA  
988 and in panels C-H is One-way ANOVA using Dunnet's multiple comparisons test comparing to  
989 untreated group (No Rx) available in Prizm 9.



990

991 **Figure 3. Immunome antibody combination has a synergistic effect against clinically relevant**

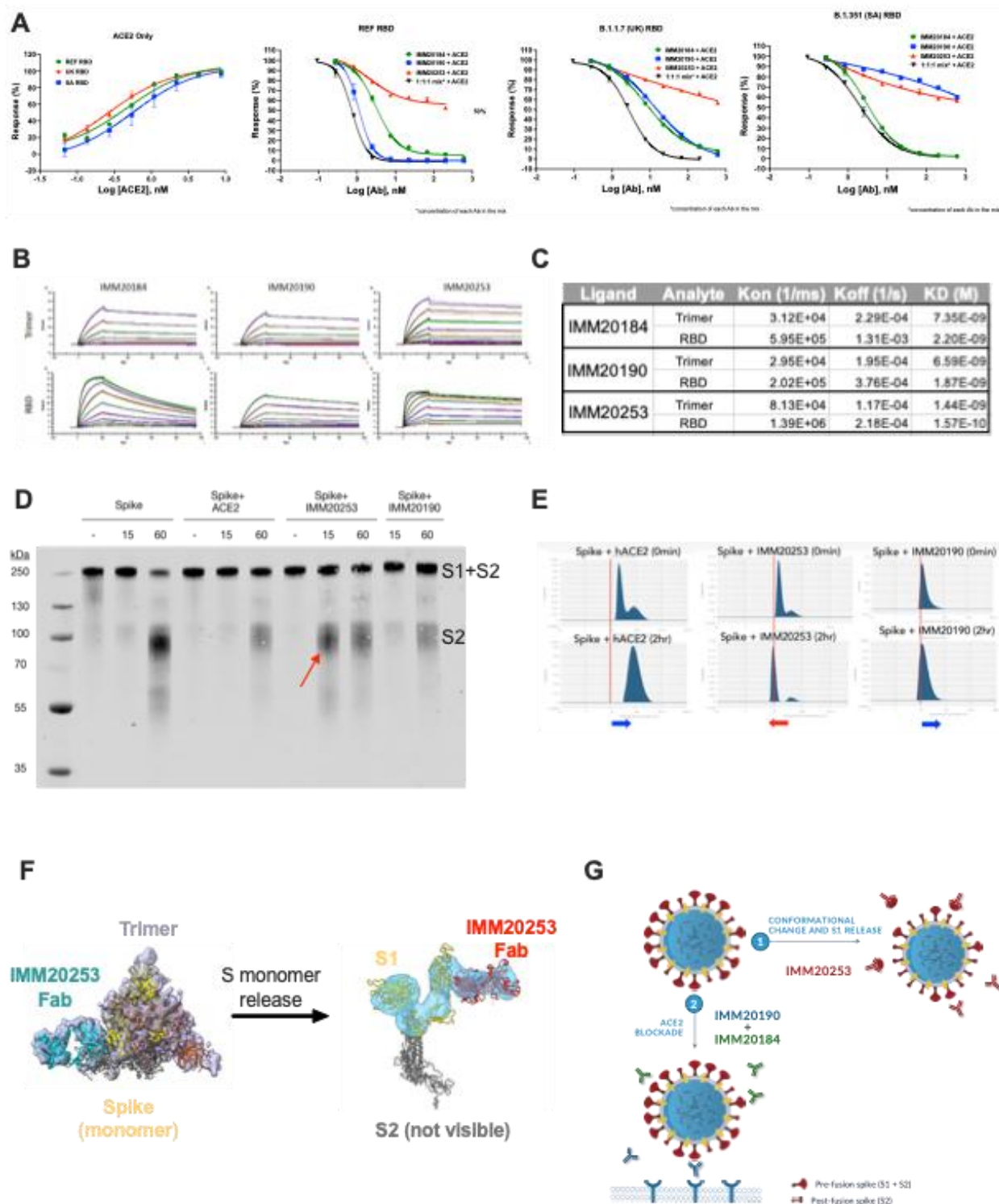
992 **SARS-CoV-2 variants.** (A) IMM20190/184/253 and (B) IMM20185/253 combination neutralizes  
993 Spike pseudoviruses that correspond to CDC VBMs. (C) Neutralization of D614G and Delta Spike  
994 pseudoviruses by three antibody cocktail IMM20190/184/253 and S309 antibodies. Shown data  
995 are representative experiments of two independent repeats. (F) Synergy scores of the 3-Ab cocktail  
996 IMM20190/184/253 against 5 pseudoviruses and one BavPat1/2020 (D614G) live virus isolate  
997 calculated with SynergyFinder 2.0 online tool. HSA, the highest single agent model score,  
998 calculates the excess over the maximum single antibody response. HSA score below -10 indicates  
999 competition; between -10 to 10 shows additive effect; and above 10 demonstrates synergy among  
1000 tested agents. (E) Neutralization of VOI/VOC isolates of SARS-CoV-2 by IMM20190/184/253  
1001 cocktail as measured by ViroSpot assay. Antibodies are mixed at equimolar ratio. (F) Plaque  
1002 reduction assay of REF (WA1/2020) and (E) Omicron (BA.1) virus isolates in the presence of  
1003 IMM20184, IMM20253, IMM20184/253 combination and S309 antibody. (H) Neutralization of  
1004 Alpha (B.1.1.7) variant by IMM20190/184/253 cocktail measured with three different methods,  
1005 including a pseudovirus neutralization, intact virus spot reduction (ViroSpot) and intact virus  
1006 plaque formation assays.



1007  
1008 **Figure 4. Three antibody cocktail activates potent effector function responses *in vitro*.** (A)  
1009 Opsonization with single IMM antibodies and two antibody (IMM20184/20253) and three-  
1010 antibody (IMM190/184/253) combinations induce phagocytosis of Trimer-coated beads. (B)

1011 Deposition of classical complement component C4 on IMM antibodies bound to Trimer-coated  
1012 surface.(C) Activation of antibody-mediated cellular cytotoxicity (ADCC) by IMM antibodies and  
1013 two antibody (IMM20184/20253) and three-antibody (IMM190/184/253) combinations bound to  
1014 S-expressing cells. Denoted points are Mean; error bars are SEM.





1015

1016 **Figure 5. IMM20253 antibody inhibits virus in non-ACE2 dependent manner and facilitates**

1017 **the release of S1 protein. (A) 3-Ab cocktail inhibits RBD binding to its cellular receptor ACE2.**

1018 ELISA-based receptor competition assay. (B). Antibody binding kinetics of IMM20184,  
1019 IMM20190 and IMM20253 antibodies to soluble RBD and Trimer (REF variant) measured using  
1020 Surface Plasmon Resonance (SPR). (C) KD, Kon and Koff values of IMM20184, IMM20190 and  
1021 IMM20253 antibodies measured using a multi-cycle kinetics protocol assuming 1:1 interaction  
1022 model. (D). Western blot analysis of Trimer digested with protease K after 0, 15 and 60 min in the  
1023 presence of either human ACE2, IMM20253 or IMM20190. Anti-S2 staining reveals S monomer  
1024 (S1+S2) and S2 protein. (E) Dynamic light scattering (DLS) analysis of Trimer complex with  
1025 either ACE2, IMM20253 or IMM20190 immediately or after 2 hours incubation. (F) IMM20253  
1026 Fab binding to Trimer triggers complex disruption and release of S monomers. (G) Schematic of  
1027 mechanism of action of IMM20184/190/253 or IMM-BCP-01 cocktail.

1028

## 1029 **SUPPLEMENTARY MATERIALS**

1030

### 1031 **Supplementary Tables**

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1039 **Supplementary Table 1. Breadth of binding of IMM20190/184/253 antibodies to RBD**  
 1040 **proteins bearing mutations found in CDC VOCs. EC50 (pM) relative to respective reference**  
 1041 **proteins measured using hTRF assay.**

Sort	His-tagged protein	Binding region	Variant	IMM20190	IMM20253	IMM20184
1	A352S	RBD		25.4	33.0	27.7
2	A475V	RBD	LY escape-3	70.2	34.6	30.3
3	E406Q	RBD		67.2	44.1	29.2
4	E484K	RBD	South Africa (beta), Brazil (gamma)	39.2	32.7	23.2
5	E484Q	RBD	India (B.1.617)	59.9	22.7	21.0
6	F486S	RBD		41.2	26.3	23.0
7	F490S	RBD	Peru (lambda)	14.7	73.4	60.8
8	K417N	RBD	South Africa (beta), India (delta+)	>500	67.4	44.3
9	K417N, E484K, N501Y	RBD	South Africa (beta)	not detected	25.0	20.7
10	K417T, E484K, N501Y	RBD	Brazil (gamma)	>500	22.6	17.9
11	K444R	RBD		33.9	26.7	20.9
12	L452R	RBD	USA (epsilon), India (all)	40.9	34.3	41.2
13	L452R, T478K	RBD	India (B.1.617.2, delta)	52.8	61.7	111
14	N439K	RBD	Scotland, Europe	76.6	56.5	52.1
15	N440K	RBD		45.6	39.7	32.1
16	N501Y	RBD	UK (alpha), SA (beta), Brazil gamma)	311	59.0	42.2
17	Spike RBD (319-591)	RBD	Wuhan / Washington reference	69.9	53.4	46.2
18	T478I	RBD		16.4	45.5	68.5
19	Y453F	RBD	Denmark (mink)	71.6	65.6	50.1
20	A222S, D614G	S1	Europe, Spain	51.0	45.1	29.9
21	D614G	S1	Multiple	69.9	62.9	44.1
22	K417N, E484K, N501Y, D614G	S1	South Africa (beta)	391	34.7	25.6
23	Spike S1 (WT)	S1	Wuhan / Washington reference	58.8	50.0	34.4
24	SARS-CoV-1	S1	Wild type	not detected	137.0	>500
25	T19R, G142D, E156G, Δ157-158, L452R, T478K, D614G, P681R	S1	India (B.1.617.2, delta)	22.2	40.9	30.6
26	E154K, L452R, E484Q, D614G, P681R	S1	India (B.1.617.1, kappa)	38.2	49.2	22.7
27	ΔHV69/70, Y453F, D614G	S1	Denmark (mink)	48.1	67.1	34.8
28	ΔHV69/70, ΔY144, N501Y, A570D, D614G, P681H	S1	UK (alpha)	>500	63.1	49.1

1042

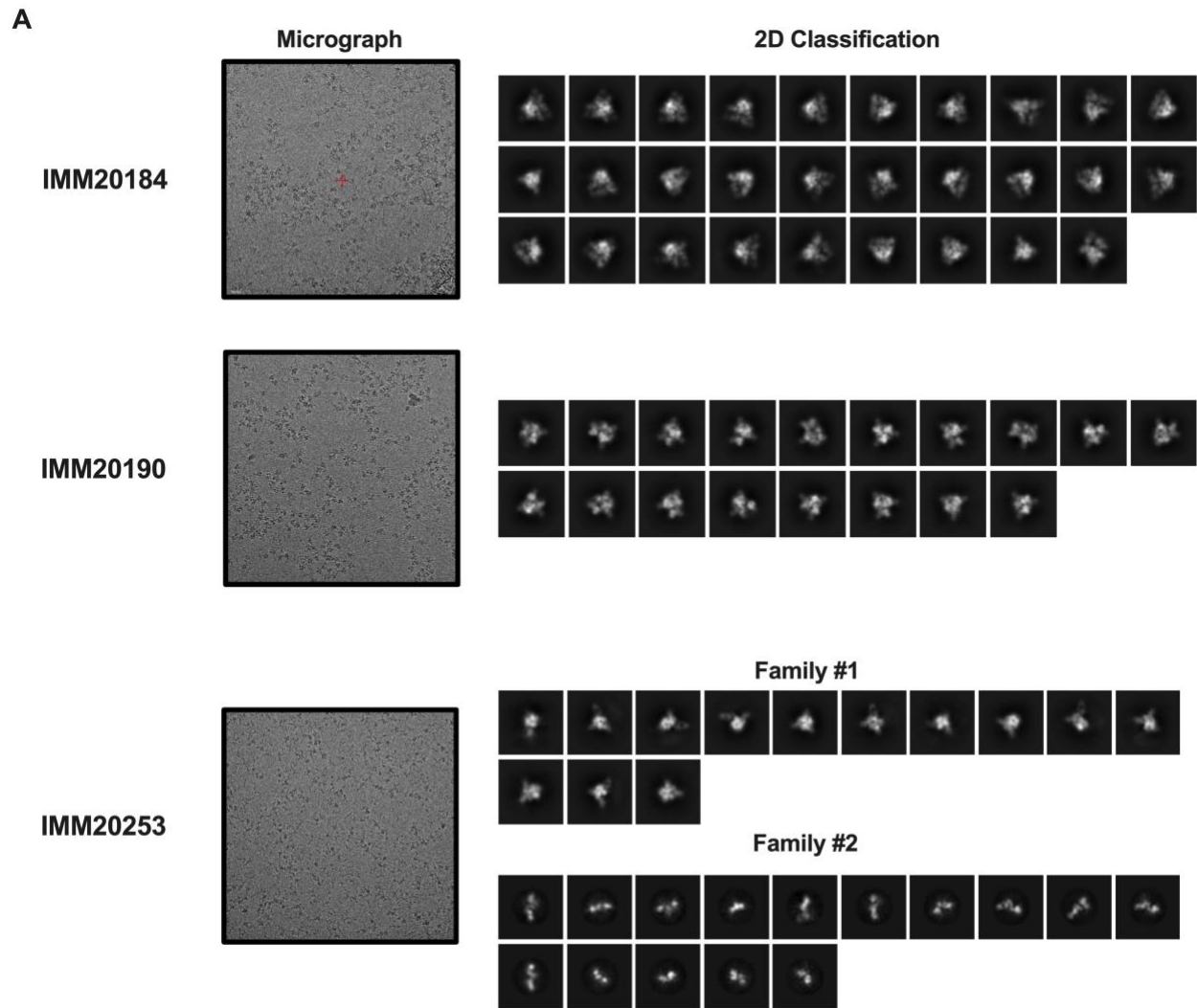
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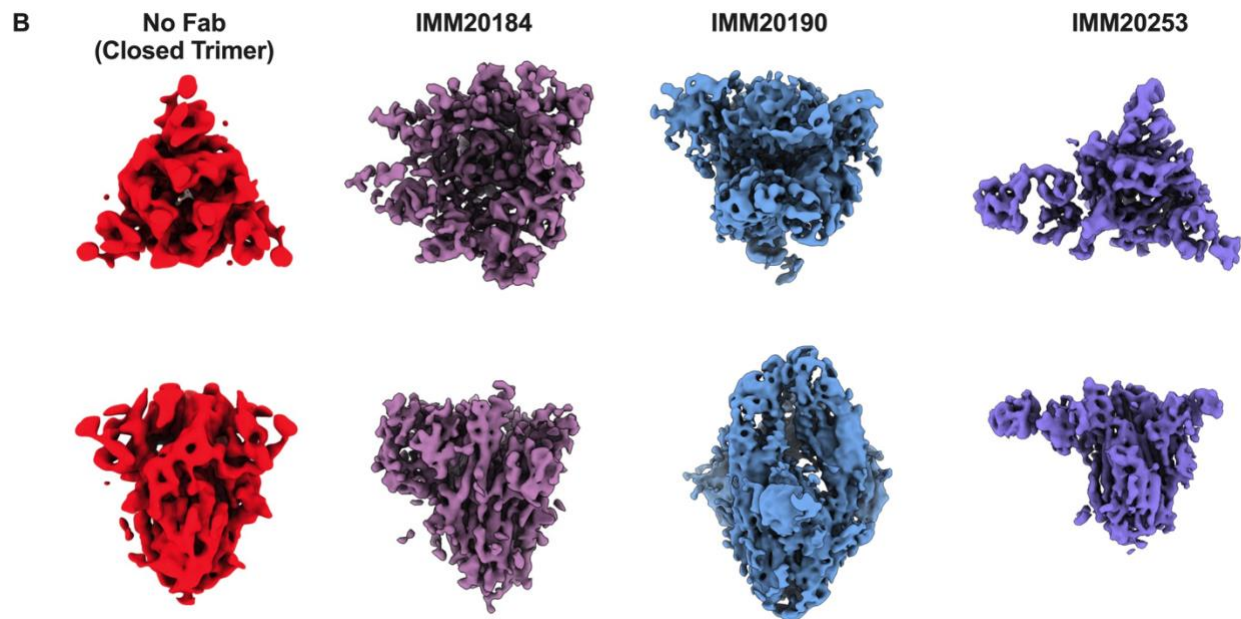
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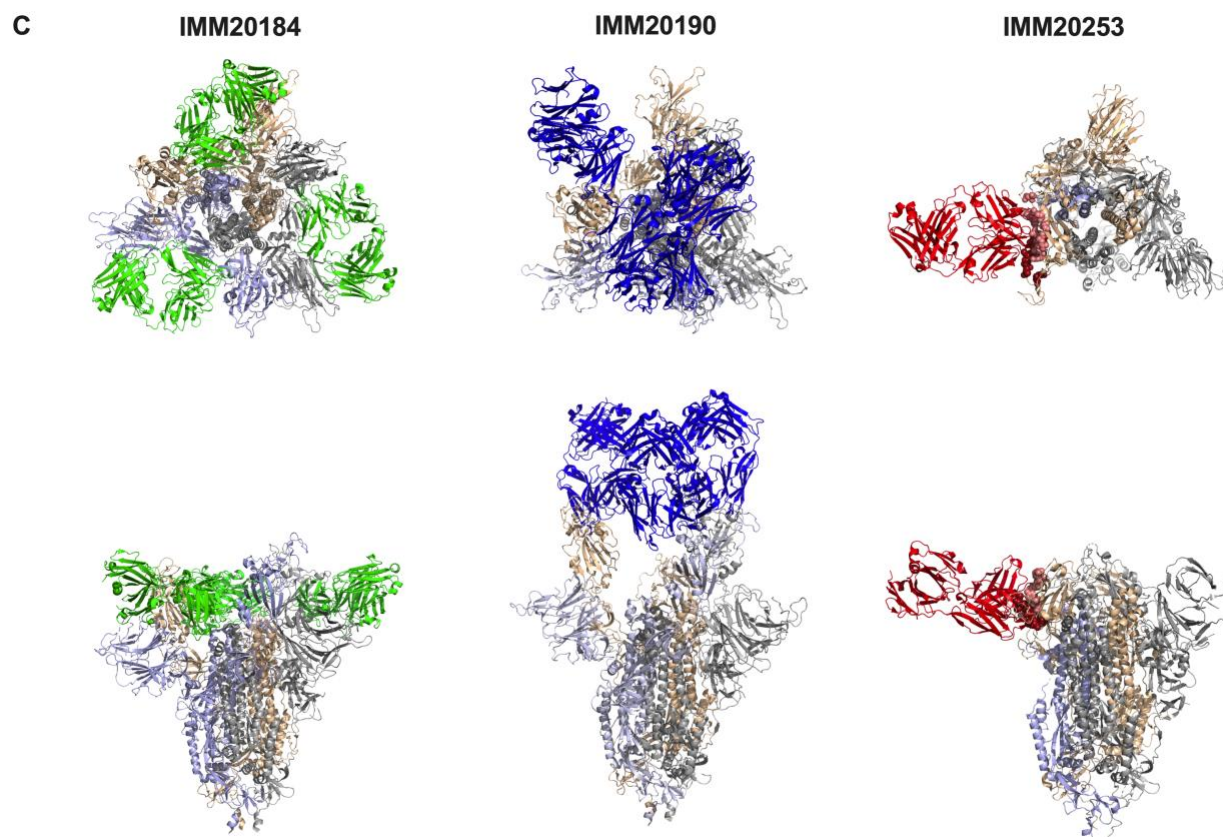
1047 **Supplementary Figures**



1048



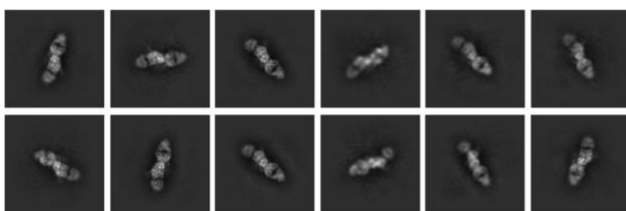
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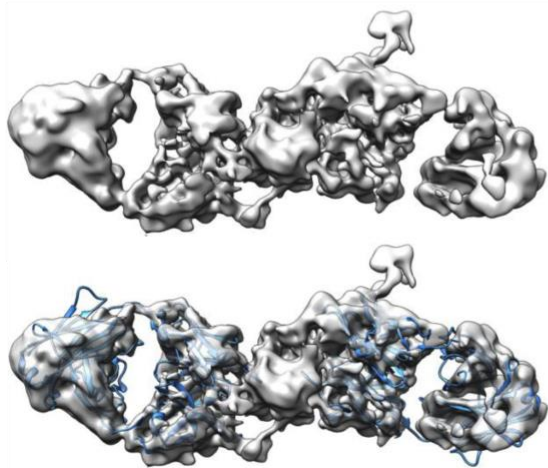
1050

**D RBD-IMM20184/253 Fabs**

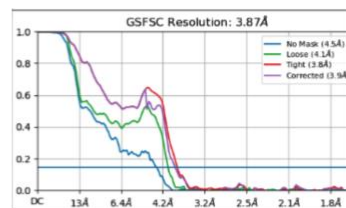
**2D Classification**



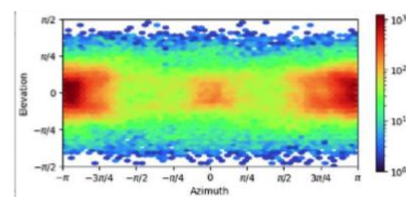
**G Final 3D reconstruction with Fab models (PDB ID:1M71)**



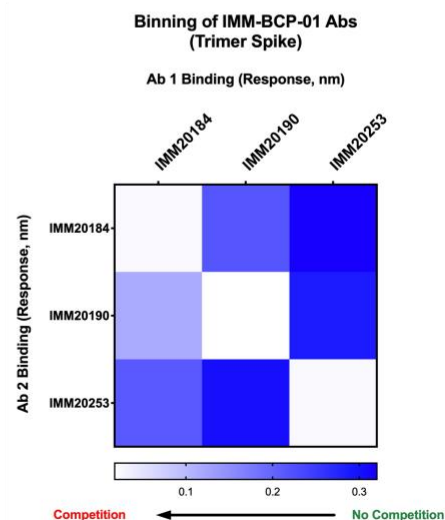
**E**



**F**



**H**



1051

1052

1053 **Supplementary Figure 1. Cryo-EM micrographs reveal sites of IMM20184/20190/20253**

1054 **Fabs binding to Spike protein.** (A) Cryo-EM micrographs and 2D classification of Trimer-Fab

1055 complexes shown in Figure 1A. IMM20253 binding to Trimer generates two families (shown as

1056 Family#1 and 2). (B). Comparison of 3D reconstruction data (density only) for a closed Trimer

1057 conformation, IMM20184 Fab-Trimer, IMM20190 Fab-Trimer and IMM20253 Fab-Trimer

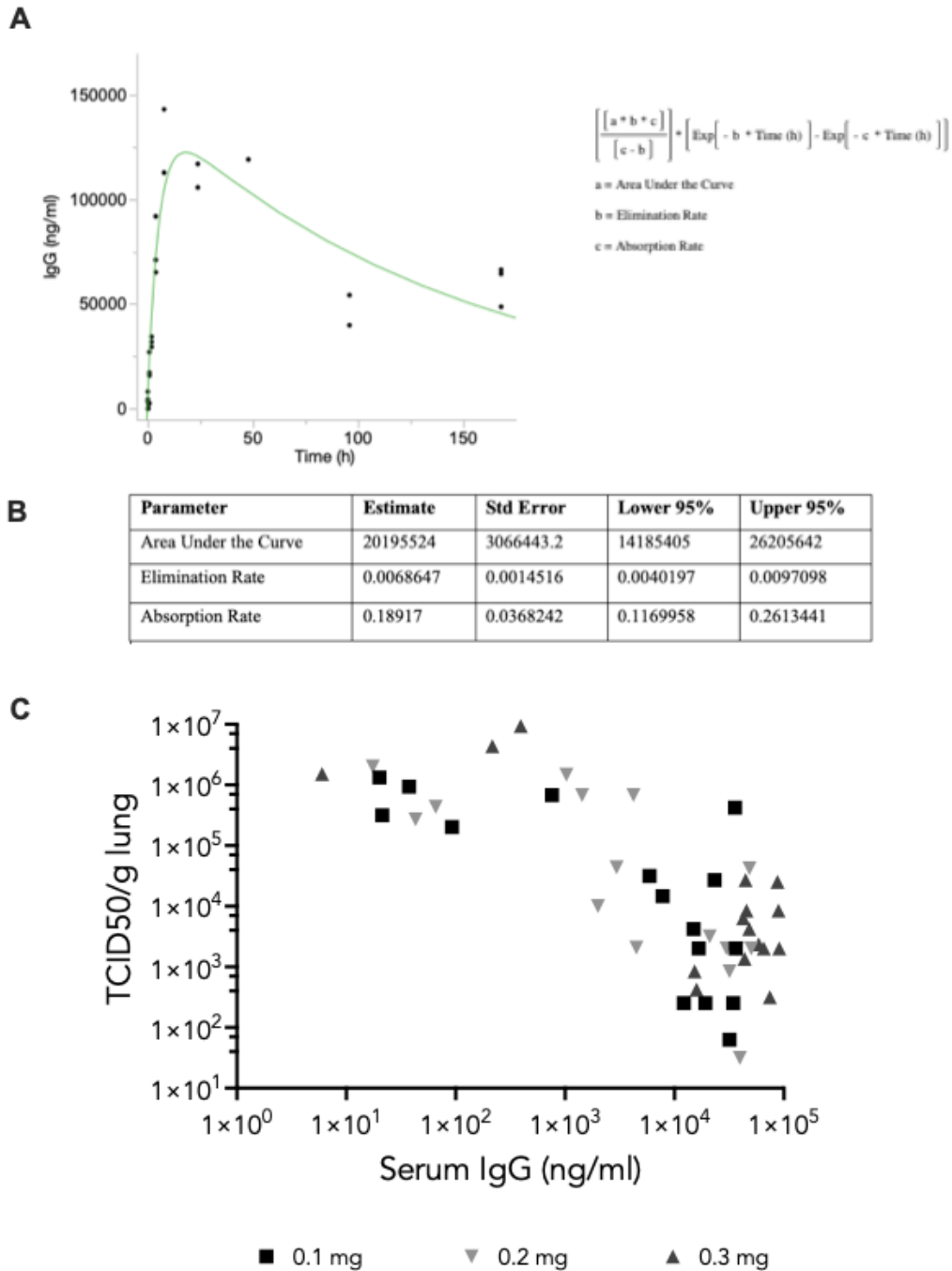
1058 complexes in support of data shown in Figure 1A. (C) Models PDB:7E8C, PDB:6XLU,

1059 PDB:6XM5 or PDB:7NOH for Trimer and PDB:6TCQ for the Fabs demonstrate binding patterns

1060 and attack angles for IMM20184/190/253 antibodies. (D) CryoEM micrographs and 2D

1061 classification of a IMM20184 Fab – RBD – IMM20253 Fab complex. Simultaneous binding of  
1062 both Fabs is clearly visible. (E) Fourier shell correlation (FSC) curves of the final 3D refinement  
1063 of data from panel D in cryoSPARC 3.3 for different types of masks. The resolution of the final  
1064 map was calculated based on a FSC of 0.143. (F). Viewing directional distribution for the final  
1065 refinement run for the complex shown in panels D and E, generated by cryoSPARC 3.3. The  
1066 viewing direction distribution histogram shows the number of images with a particular viewing  
1067 direction at each (elevation, azimuth angle). (G). Final 3D reconstruction of data shown in Supp.  
1068 Figure 1D and Figure 1B. Primary data (density) and modelling using Fab model PDB:1M71 are  
1069 shown. (H). Antibody binning on Octet Qke. IMM20184, IMM20190 and IMM20253 do not  
1070 compete for soluble Trimer and RBD protein binding. Heat map values represent binding of the  
1071 first antibody to Trimer (top), followed by binding of the second antibody (left), measured as  
1072 Response parameter in nm.

1073



1074

1075 **Supplementary Figure 2. Antibody exposure and pharmacokinetics in dosed hamsters.** (A)

1076 Pharmacokinetics of the 3-Ab cocktail in hamsters. The 3-Ab cocktail (0.3 mg each) was

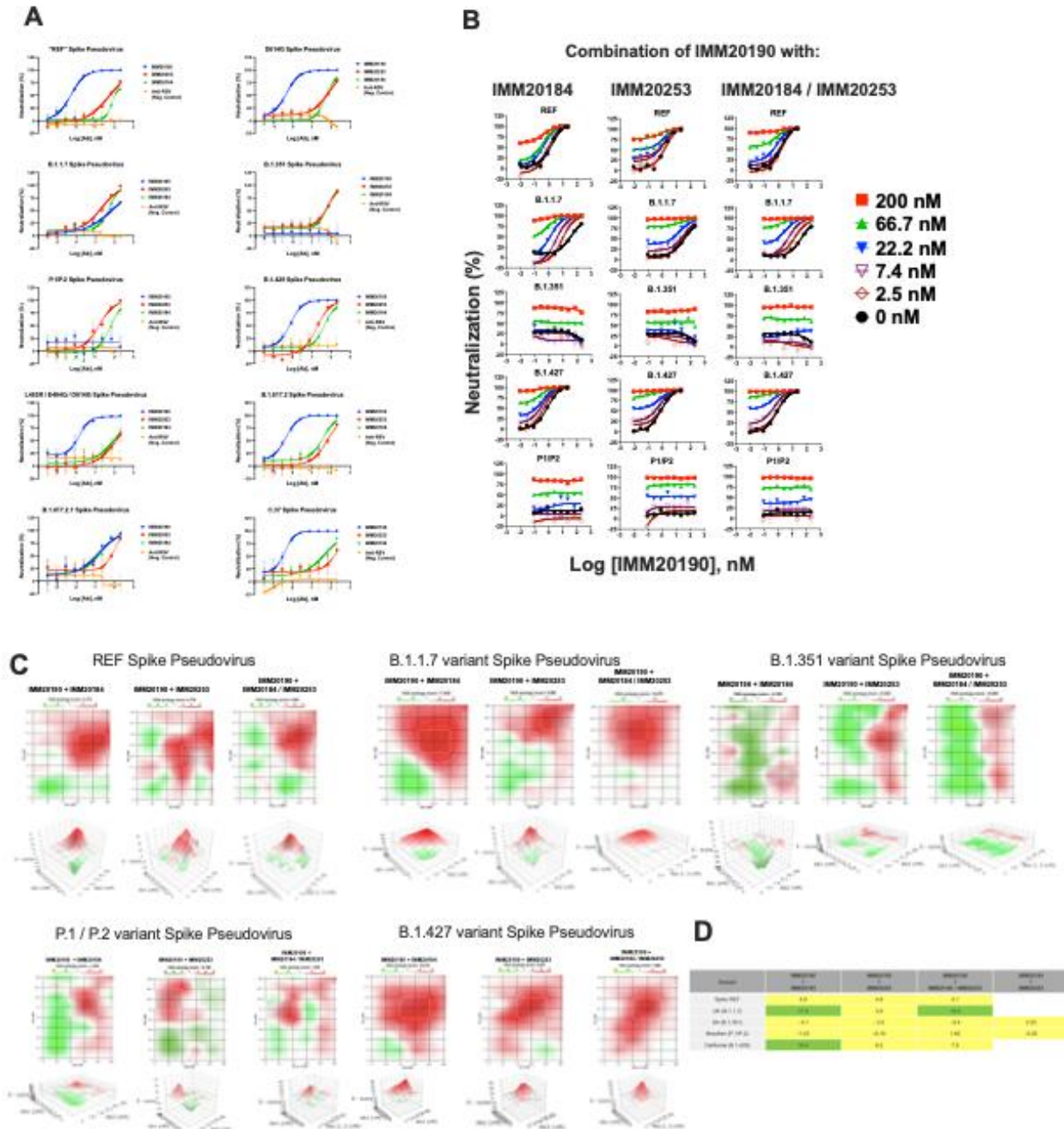
1077 administered i.p. into Syrian Golden hamsters and terminal bleeds (n = 4 per time point) were

1078 taken at 0.25, 0.5, 1, 2, 4, 8, 24, 48, 96, and 168 hours post administration. Total human IgG levels



1079 were determined by anti-human ELISA. Pharmacokinetics in animals exhibiting < 1000 ng/mL  
1080 IgG in serum at timepoints >30 minutes post-injection. Green line is the calculated curve using the  
1081 formula shown on the right. (B) PK parameters of data from panel A. (C) Viral titer in lungs of  
1082 infected hamsters depends upon Ab exposure. Syrian golden hamsters challenged with  $3.3 \times 10^5$   
1083 TCID<sub>50</sub> viral inoculation of a non-adapted WA\_CDC-WA1/2020 SARS-CoV-2 isolate were  
1084 treated with 3-Ab cocktail (IMM20184/IMM20190/IMM20253), at various dose levels, six hours  
1085 post inoculation with virus. Lungs were harvested at day 4 post-treatment and viral titers were  
1086 determined by TCID<sub>50</sub> assay. Terminal levels of IgG in blood were quantified by anti-human  
1087 ELISA.

1088



1089

1090 **Supplementary Figure 3. Three selected antibodies have a synergistic neutralizing effect.**

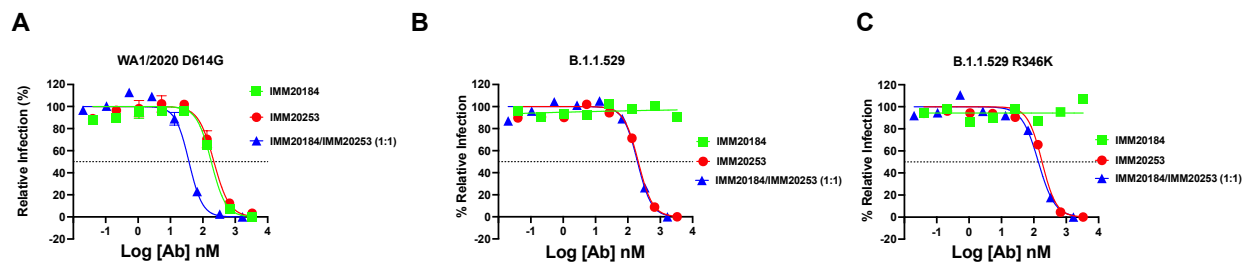
1091 (A) Neutralization properties of standalone IMM20190, IMM20184 and IMM20253 antibodies

1092 against 10 different Spike variant pseudoviruses. (B) REF, B.1.1.7 (alpha), B.1.351 (beta), P1

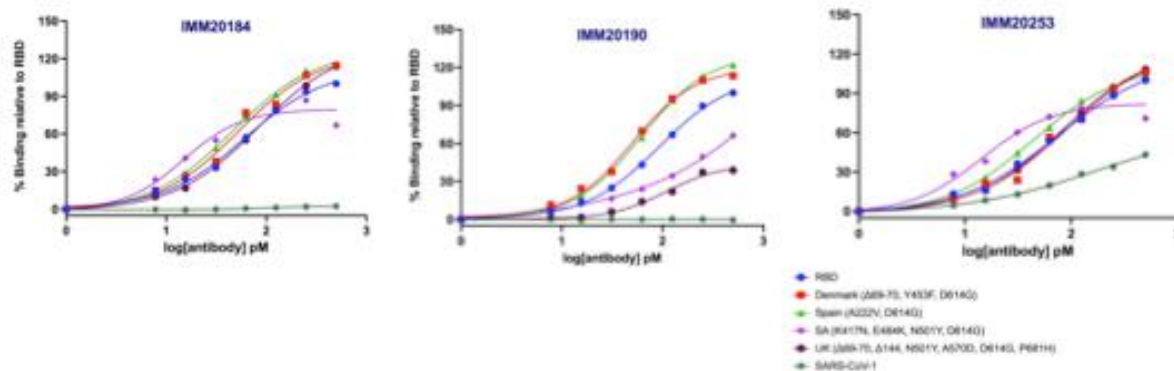
1093 (gamma), and B.1.427 (epsilon) pseudovirus variant neutralization by IMM20190 combination

1094 with either IMM20184, IMM20253 or both. (C) The Highest Single Agent (HSA) scores for 2-  
1095 Ab and 3-Ab combinations. IMM20190 was mixed with IMM20184 and IMM20253 at 1:0.5:0.5  
1096 ratio. (D) HSA scores for two and three antibody cocktail.

1097



1099 **Supplementary Figure 4. Focus reduction neutralization assay (FRNT) of SARS-CoV-2**  
1100 **variants in the presence of IMM20184, IMM20253 and IMM20253/184 combination.** (A)  
1101 Relative infection of WA1/2020 D614G, (B) Omicron (BA.1) and (C) Omicron BA.1.1 virus  
1102 variants in the presence of IMM20184, IMM20253 and IMM20184/253 antibodies. Data are  
1103 representative of three independent experiments performed in duplicate.



1105 **Supplementary Figure 5. Binding of IMM antibodies to soluble RBD proteins from SARS-**  
1106 **CoV-1 and SARS-CoV-2 variants in a steady-state hTRF assay.**