

1    **Title: Protection from Omicron and other VOCs by Bivalent S-Trimer COVID-19 Vaccine**

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10   **Abstract:**

11    The Omicron variant of SARS-COV-2 (GISAID GRA clade [B.1.1.529, BA.1 and BA.2]) is now  
12    the single dominant Variant of Concern (VOC). The high number of mutations in the Omicron  
13    Spike (S) protein promotes humoral immunological escape. Although a third homologous boost  
14    with S, derived from the ancestral strain, was able to increase neutralizing antibody titers and  
15    breadth including to Omicron, the magnitude of virus neutralization could benefit from further  
16    optimization. Moreover, combining SARS-COV-2 strains as additional valences may address the  
17    current antigenicity range occupied by VOCs.

18    Using Trimer-Tag<sup>TM</sup> platform we have previously demonstrated phase 3 efficacy and safety of a  
19    prototypic vaccine SCB-2019 in the SPECTRA trial and have submitted applications for  
20    licensure. Here, we successfully generated a bivalent vaccine candidate including both Ancestor  
21    and Omicron variant S-proteins. Preclinical studies demonstrate this SARS-CoV-2 bivalent S-  
22    Trimer subunit vaccine elicits high titers of neutralizing antibodies against all VOCs, with  
23    markedly enhanced Omicron specific neutralizing antibody responses.

24

25   **Introduction:**

26    Since late 2019, the Severe Acute Respiratory Syndrome Coronavirus -2 (SARS-CoV-2) virus  
27    has led to a global pandemic with over 507 million confirmed infections and 6.2 million deaths,  
28    as reported by the World Health Organization (WHO) in April 2022 (1). Despite historic  
29    achievements in the distribution of SARS-CoV-2 vaccines, significant gaps remain in the  
30    equitable distribution of vaccines with only 15% of people in low income countries having  
31    received at least one immunization out of the 11.5 billion doses distributed globally  
32    (<https://ourworldindata.org/covid-vaccinations>). There is also significant concern that booster  
33    dosing will also result in significant inequity (2). Combined with the current global dominance of  
34    the Omicron VOC (<https://www.gisaid.org/phylogenetics/global/nextstrain/>, 3) ability to escape  
35    humoral immunity (4-6), and the fear of other VOCs yet to emerge due to the pressures of mass  
36    vaccination or infection driven immunity, there is a need for the next generation of more broadly  
37    protective vaccines to be available in sufficient quantities with superior cold-chain requirements  
38    to promote equitable access.

39 Clover has used Trimer-Tag technology to develop a SARS-CoV-2 vaccine (SCB-2019) with a  
40 stabilized prefusion trimeric form of Spike protein (S-Trimer) (7,8). The SCB-2019 vaccine  
41 based on the sequence of the Ancestral strain adjuvanted with CpG 1018/Alum has completed  
42 clinical phase 1 (NCT04405908) and phase 2/3 SPECTRA trials (NCT04672395). The latter  
43 trials enrolled more than 30,000 adult and elderly participants in the Philippines, Colombia,  
44 Brazil, South Africa and Belgium, and demonstrated that the SCB-2019 vaccine has a favorable  
45 safety and tolerability profile, and significant efficacy against VOCs: 81.7% effective against  
46 Delta, 91.8% for Gamma, and 58.6% for Mu against disease of any severity and full protection  
47 against severe disease, hospitalization and deaths (9 and 10). An extended follow-up analysis  
48 confirms earlier findings and show that SCB-2019 elicited high and durable protection in  
49 individuals at approximately six months after the primary vaccination series, including the  
50 elderly (Data presented in World Vaccine Congress 2022).

51  
52 To address Omicron and to drive even broader protection given the potential threat for other  
53 VOC to emerge, using the same Trimer-tag platform technology for SCB-2019, we are  
54 developing vaccine candidates based on trimerized S-proteins to screen their potential in pre-  
55 clinical studies against panels of variants. Based on extensive assessments of immunology and  
56 antigenicity, for which the antigenic distance of VOC can be mapped by comparing  
57 neutralization values for serum / virus pairs; one can hypothesize that breadth can be achieved by  
58 selecting a strain in the centroid range of antigenicity (i.e. the Ancestral strain) and a more distal  
59 variant (e.g. Omicron) (11,12). Here we demonstrate that our bivalent vaccine candidate with  
60 Spike protein derived from the Ancestral strain (our SCB-2019 vaccine) and the Omicron variant  
61 is able to elicit potent cross-protective antibodies against all VOCs, including robust  
62 neutralization of Omicron.

63

## 64 **Materials and Methods:**

### 65 **Animal studies, facilities and ethics statements**

66 Specific pathogen-free (SPF) BALB/c female mice (6-8 weeks old) for immunogenicity studies  
67 were purchased from Charles River Experimental Animals Co., LTD and kept under standard  
68 pathogen-free conditions in the animal care center at Chengdu Hi-tech Incubation Park. All  
69 animals were allowed free access to water and diet and provided with a 12 h light/dark cycle  
70 (temperature: 16-26°C, humidity: 40%-70%). All mouse experiments were conducted according  
71 to international guidelines for animal studies.

72

### 73 **S-Trimer fusion protein expression, purification**

74 S-Trimer fusion proteins SCB-2019 were constructed as previously described (14). Similarly, S-  
75 Trimer fusion proteins SCB-2022B were constructed utilizing a cDNA encoding the ectodomain  
76 of SARS-CoV-2 spike (S) protein from Omicron BA.1 lineage and with a R685A mutation in the  
77 furin site, synthesized using *Cricetulus griseus* (Chinese hamster)-preferred codons by

78 GenScript. The cDNA was subcloned into pTRIMER expression vector (GenHunter  
79 Corporation) at *Hind III* and *Bgl II* sites to allow in-frame fusion of the soluble S protein to  
80 Trimer-Tag (amino acid residue 1156-1406 from human Type I( $\alpha$ ) collagen). The expression  
81 vectors were transiently transfected into HEK-293F cell lines (Clover Biopharma) using PEI  
82 (Polyscience) and grown in OPM-293 CD05 medium (OPM) with OPM-293 proFeed  
83 supplement (OPM). S-Trimer protein was purified to homogeneity from the conditioned medium  
84 using Trimer-Tag specific affinity column (Clover Biopharma).

85

## 86 **SEC-HPLC**

87 The purity of S-Trimer was analyzed by Size-Exclusion Chromatography (SEC-HPLC) using  
88 Agilent 1260 Infinity HPLC with an analytic TSK gel G3000 SW $\times$ L column (Tosoh). Phosphate  
89 Buffered Saline (PBS) was used as the mobile phase with OD280 nm detection over a 20 min  
90 period at a flow rate of 1 ml/min.

91

## 92 **Receptor binding studies of S-Trimer to human ACE2**

93 The binding affinity of S-Trimer to ACE2 was assessed by Bio-Layer Interferometry  
94 measurements on ForteBio Octet QKe (Pall). ACE2-Fc (10  $\mu$ g/mL) was immobilized on Protein  
95 A (ProA) biosensors (Pall). Real-time receptor-binding curves were obtained by applying the  
96 sensor in two-fold serial dilutions of S-Trimer (1.125-36  $\mu$ g/mL in PBS). Kinetic parameters  
97 (Kon and Koff) and affinities (KD) were analyzed using Octet software, version 12.0.  
98 Dissociation constants (KD) were determined using steady state analysis, assuming a 1:1 binding  
99 model for a S-Trimer to ACE2-Fc.

100

## 101 **Vaccine preparation**

102 The test vaccine candidates were formulated with alum (Alhydrogel, Croda, Goole, United  
103 Kingdom) plus CpG 1018 (Dynavax Technologies, Emeryville, California).  
104 A total of 36  $\mu$ g of SCB-2019 or SCB-2022B-trimeric protein was mixed first with 900  $\mu$ g of  
105 Alum by gently swirling the mix vial for 30s, then with 1800  $\mu$ g of CpG 1018, in total 600  $\mu$ L  
106 vol. in vial by gentle inversion 30s at room temperature before administration. Then within 8 hr.  
107 50  $\mu$ L of vaccine was injected into the hind leg calf muscle per mouse.  
108 The bivalent vaccine was prepared with mixture of 18  $\mu$ g of SCB-2019 and 18  $\mu$ g of SCB-2022B  
109 S-Trimer in 1:1 ratio, then adjuvanted with 900  $\mu$ g of Alum, inverted gently for 30 seconds and  
110 then 1800  $\mu$ g of CpG 1018 were added, mixed 30 s.

111

## 112 **Animal vaccination**

113 For prime-boost vaccination, Balb/c mice, female (n=10/group) were immunized with SCB-  
114 2019, or SCB-2022B 3  $\mu$ g or Bivalent (1.5  $\mu$ g of SCB-2019 and 1.5  $\mu$ g of SCB-2022B)  
115 adjuvanted with 75  $\mu$ g alum plus 150  $\mu$ g CpG 1018 twice on Day 0 and Day 21. Total 50  $\mu$ L of  
116 vaccine was given each mouse via intramuscular injection. Mice serum was collected on D35.

117 For three dose boost study, Balb/c mice, female (n=10/group) prime and boost with SCB-2019  
118 3  $\mu$ g adjuvanted with 75  $\mu$ g alum plus 150  $\mu$ g CpG 1018 twice on Day 0 and Day 21, then  
119 boosted with 3  $\mu$ g SCB-2019, or SCB-2022B or Bivalent adjuvanted with 75  $\mu$ g alum plus 150  
120  $\mu$ g CpG 1018 on Day 57 via intramuscular injection. Serum was collected on D35 (2 weeks

121 PD2), D56 (Day of 3<sup>rd</sup> dose boost), D85 (1 month post dose 3), D113 (2 months post dose3) and  
122 D141 (3 months post dose 3) for pseudovirus neutralizing antibody test.

123

## 124 **Pseudovirus construction and production**

125 The variants of concern of SARS-CoV-2 spike protein genes were optimized using mammalian  
126 codon and synthesized by Genscript, then cloned into pcDNA3.1(+) eukaryotic expression  
127 vector. Plasmids encoding Ancestor (Wuhan Hu-1), Alpha (B.1.1.7), Beta (B.1.351), Gamma  
128 (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529) SARS-CoV-2 variants S glycoprotein were  
129 constructed (mutations compared to the Ancestor were shown in Table 1). The lentiviral  
130 packaging plasmid psPAX2 and pLVX-AcGFP-N1-Fluc lentiviral reporter plasmid that  
131 expresses GFP and luciferase were obtained from HonorGene (HonorGene, China).  
132 Pseudovirions were produced by co-transfection HEK 293T cells with psPAX2, pLVX-AcGFP-  
133 N1-Fluc, and plasmids encoding various S genes by using Lipofectamine 3000 (Invitrogen,  
134 L3000-015). The supernatants were harvested at  $24 \pm 2$  h post transfection and centrifuged at  
135 1500rpm for 5 min to remove cell debris and then stored at  $-80^{\circ}\text{C}$ . Pseudoviruses stock were  
136 titrated by infecting 293T-ACE2 cells and luciferase activity was determined following a 44-48 h  
137 incubation period at  $37^{\circ}\text{C}$  and 5%  $\text{CO}_2$  by addition Bright-Glo Luciferase Assay System  
138 (Promega, E2650) using a microplate reader (TECAN, Spark). Then  $\text{TCID}_{50}$  of the pseudovirus  
139 was calculated according to the Reed-Muench method (13). The virus stock titers were reported  
140 in table 1.

141

## 142 **Neutralization assay**

143 Aliquots of test serum samples were first heat-inactivated at  $56^{\circ}\text{C}$  for 30 min, then clarified by  
144 centrifugation at 10,000 rcf for 5 min. Samples were serially diluted (3-fold) with assay medium  
145 (in 100  $\mu\text{L}$ ), incubated with 650  $\text{TCID}_{50}$  pseudovirus (in 50  $\mu\text{L}$ ) at  $37^{\circ}\text{C}$  for 1 h, along with  
146 virus-infected untreated control (virus alone) and cell-alone (background control). Then, freshly-  
147 trypsinized 293T-ACE2 cells were added to each well at 20000 cells/well in 100  $\mu\text{L}$ . Following  
148 44-48 h incubation at  $37^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  incubator, the cells were lysed, and luciferase activity  
149 was determined by a Bright-Glo Luciferase Assay System (Promega), according to the  
150 manufacturer's protocol. The  $\text{IC}_{50}$  neutralizing antibody titer of a given serum sample was  
151 defined as the serum dilution where the sample showed the relative light units (RLUs) were  
152 reduced by 50% compared to virus-infected control wells. Details of method were reported  
153 previously (13).

154

## 155 **Human convalescent serum samples**

156 Human convalescent serum samples from recovered COVID-19 patients were obtained from  
157 Public Health Clinical Center of Chengdu in Chengdu, China, under approved guidelines by the  
158 Institutional Review Board (IRB), and all patients had provided written informed consent before  
159 serum sample were collected. These patients were recently discharged from hospital and the

160 serum was collected at 1-5 weeks after they have been diagnosed as COVID19. Details of  
161 sample sourcing and collection are listed in table S1 and certain data previously reported (14).

162

### 163 **Statistical analysis**

164 Data arrangement was performed by Excel and statistical analyses were performed using the  
165 Prism 9.2.0 (GraphPad Software). Two-tailed Mann-Whitney tests were used to compare two  
166 experiment groups. P values < 0.05 were considered significant. \*P < 0.05, \*\*P < 0.01, \*\*\*P <  
167 0.001.

168

### 169 **Results:**

170 To investigate whether S-Trimer COVID19 vaccine candidates can provide cross-protection  
171 against VOCs including Omicron, we have generated a series of SARS-COV-2 pseudoviruses,  
172 using Spike protein sequence from the Ancestor (Wuhan-Hu-1), Alpha (B.1.1.7), Beta (B.1.351),  
173 Gamma (P.1), Delta (B.1.617.2) and Omicron (B.1.1.529) strains (Table 1). Using these  
174 pseudoviruses in neutralization assays, we first tested available serum samples collected from  
175 convalescent patients. A total of 7 human convalescent sera (HCS) samples (4 moderate, 1  
176 severe, 2 unknown) were initially tested for their pseudovirus neutralizing antibodies against  
177 Ancestor, Alpha, Beta, Gamma and Delta; additional human samples (total 35, mild to severe)  
178 were accessible later for Ancestor and Omicron only neutralizing antibody testing (Fig. 1). High  
179 titers of neutralizing antibodies (IC<sub>50</sub> GMT over 3 logs) were detected against multiple  
180 pseudoviruses, including the Ancestor, Alpha and Gamma strains; neutralizing antibodies against  
181 Beta and Delta variants were also maintained at significant levels (IC<sub>50</sub> GMT 2-3 logs, ~5-7-fold  
182 lower compared to Ancestor). However, neutralization was significantly diminished against  
183 Omicron pseudovirus (~155-fold lower compared to the Ancestor), and only 3 samples out of the  
184 total 35 tested were seropositive.

185

186 To generate a more broadly protective next generation vaccine we first designed SCB-2022B  
187 using our Trimer-tag platform based on the Omicron variant full-length Spike protein with an  
188 R685A mutation to avoid cleavage at the S1/S2 boundary by furin protease (Fig. 2A). With this  
189 mutation, SCB-2022B S-protein produced from CHO cells was intact and showed a clear single  
190 band around 250 kDa molecular weight in a reducing SDS-PAGE gel (Fig. 2B) as expected for  
191 the trimerized S protein size. The purity was analyzed by size-exclusion SEC-HPLC showing  
192 82% main peak of SCB-2022B S-Trimer respectively (Fig. 2C). The binding affinity (KD) of  
193 purified Omicron S-Trimers to the human ACE2 receptor using ForteBio BioLayer  
194 interferometry was shown to be 0.8 nM (Fig. 2D). This indicated Omicron S-protein has a high  
195 affinity to ACE2 receptor, as previously reported (6). We next generated the bivalent vaccine  
196 with a mixture of our SCB-2019 vaccine (14) with the new SCB-2022B S-Trimer in a 1:1 ratio,  
197 subsequently formulated with Alum and CpG 1018, the bivalent vaccine contained the same  
198 antigen and adjuvant amount compared to the 1<sup>st</sup> generation vaccine.

199  
200 The immunogenicity of the Omicron monovalent vaccine (SCB-2022B) and bivalent vaccine  
201 were then evaluated in a murine two dose prime/boost immunogenicity study and compared with  
202 SCB-2019 Ancestor vaccine. Balb/c mice (Female, N=10) were immunized intramuscularly (IM)  
203 with total 3 µg of monovalent SCB-2019, or SCB-2022B, or Bivalent constructs all formulated  
204 with CpG (150 µg) plus Alum (75 µg). The vaccines were given at study day 0 and 21, serum  
205 samples were collected at study day 35 (14 days post-dose 2) and used to determinate the  
206 pseudovirus neutralizing antibody responses against VOCs (Fig. 3A). The results indicated two  
207 doses of control SCB-2019 Ancestor vaccine can elicit robust neutralizing antibodies against the  
208 Ancestor, Alpha, Beta, Gamma and Delta pseudoviruses, but diminished responses against  
209 Omicron (Fig. 3B). While SCB-2022B Omicron vaccine immunized mice had significantly  
210 higher neutralizing antibodies against Omicron, cross neutralization of other VOCs were lower.  
211 However, bivalent vaccine immunized mice had high robust neutralizing antibodies against all  
212 VOCs, with significant improvement observed in Omicron specific neutralizing antibodies  
213 (about 70-fold increase in GMT), and non-inferiority to others, compared with SCB-2019 even  
214 contains only half dose of Ancestor vaccine. This suggests that immunization with the bivalent  
215 vaccine can provide enhanced broader protection against VOCs, including the divergent  
216 Omicron strain.

217  
218 Furthermore, to mimic the current situation in humans with many individuals already immunized  
219 with ancestor vaccines, and/or infected, we evaluated the immunogenicity of the bivalent vaccine  
220 candidate in SCB-2019 pre-immunized animals. Balb/c mice (Female, N=10) were primed and  
221 boosted with SCB-2019 formulated with CpG 1018/Alum twice on Day 0 and Day 21, then  
222 boosted with SCB-2019, or SCB-2022B or bivalent vaccine formulated with CpG 1018/Alum on  
223 Day 57. Serum was collected on D35 (14 days PD2), D56 (day of 3<sup>rd</sup> boost), D85 (1 month post  
224 dose 3, 1MPD3), D113 (2-month post dose3, 2MPD3) and D141 (3-month post dose 3, 3MPD3)  
225 for VOCs pseudovirus neutralizing antibody testing (Fig.4A). The results from study day 85  
226 (1MPD3) serum samples indicated, compared with the control group (no 3<sup>rd</sup> boost), that the 3<sup>rd</sup>  
227 dose boost with the bivalent vaccine significantly enhanced the neutralizing antibody responses  
228 against all VOCs, except the Beta variant although such responses were nevertheless robust (Fig.  
229 4B); SCB-2019 monovalent vaccine significantly boosted neutralizing antibodies against Beta,  
230 Gamma and Omicron, while the SCB-2022B monovalent vaccine significantly boosted  
231 responses against Delta and Omicron.

232  
233 The serum neutralizing responses were monitored post-3<sup>rd</sup> dose boost over three months to assess  
234 the durability of protection (Fig.4C). Serum from the control group (no boost) showed robust  
235 neutralizing responses maintained against all VOC except Omicron with a low GMT (95%CI) of  
236 49 (12-1197); SCB-2019 boost significantly improved neutralizing responses against the  
237 Ancestral, Alpha, Beta, Gamma and Delta strains, and raised neutralization levels against  
238 Omicron, albeit less than the other variants with a GMT (95%CI) of 202 (129-2508) against  
239 Omicron. SCB-2022B boost significantly improved neutralizing responses against Omicron with  
240 a GMT (95%CI) of 1349 (1324-2112); with a trend for lower set-point responses against other  
241 VOCs with comparable GMT titers as the control group (no booster). Bivalent vaccine boost also

242 significantly improved neutralizing responses against all VOC with responses trending higher  
243 than the SCB-2022B monovalent boost; with GMT (95% CI) of 799 (762-1973) against Omicron  
244 comparable to those elicited by SCB-2022B. These high Omicron specific titers were maintained  
245 over the extended observation period (Fig. 4C and Table 2).

246

## 247 **Discussion**

248 In this study, we corroborated other reports (15-18) that human convalescent sera have  
249 substantially lower levels of Omicron neutralizing antibodies compared to Ancestral strain,  
250 although the same sera generally maintain broadly cross-reactive neutralizing antibodies against  
251 other VOCs. This verified the utility of our panel of VOCs in our neutralization assay to assess  
252 the consequences of the Omicron S-protein mutations on humoral immunity (19). The evidence  
253 of breakthrough infections in fully vaccinated individuals further emphasizes the importance of  
254 booster doses and potential of next generation vaccines to enhance protection against divergent  
255 VOCs such as the Omicron lineage (4,5).

256

257 Therefore, we designed a bivalent vaccine, to address advanced models that attempt to define the  
258 antigenic range of SARS-CoV-2 (20) by selecting our Ancestor vaccine, SCB-2019, which  
259 occupies a centroid range of antigenicity and has shown high levels of efficacy against VOCs in  
260 a phase 3 trial (10), and the Omicron strain given its divergent antigenicity and its global  
261 prevalence. This approach is supported by the observation that individuals who have received  
262 two doses of Ancestor vaccine and subsequently infected with Omicron have high levels of  
263 broad cross-reactive neutralization against panels of VOCs (21). An Omicron only monovalent  
264 vaccine, while likely to elicit protection against Omicron and related sub-lineages, is however  
265 potentially ineffective against other VOC distal from its antigenic position; a rationale for  
266 bivalency to mitigate this risk. The subsequently derived Omicron component of the bivalent  
267 approach, SCB-2022B, using the same Trimer-Tag technology as SCB-2019, appears trimeric in  
268 nature and binds with high affinity to the ACE-2 receptor. Combined with SCB-2019 at a 1:1  
269 ratio to create the bivalent vaccine, formulated with CpG/alum. The bivalent vaccine keeps the  
270 total same amount of antigen and adjuvant as the 1<sup>st</sup> generation vaccine, therefore not impact the  
271 supply. The murine preclinical studies allowed us to assess the breadth of cross neutralization of  
272 a panel of VOCs including Omicron.

273

274 In a priming two-dose schedule setting, even only contains half dose of Ancestor variant vaccine,  
275 the bivalent vaccine was able to elicit robust cross neutralization of all VOCs (range  $10^3$ - $10^4$   
276 titers, non-inferior to those elicited by monovalent Ancestor vaccine), including high titers  
277 against Omicron ( $IC_{50}$  GMT 2968) in the mice. In animals primed with our SCB-2019 vaccine,  
278 the bivalent vaccine was able to elicit strong booster neutralization responses against all VOCs  
279 tested with substantial levels against Omicron ( $\sim 10^3$  titer range) that were sustained up to 3  
280 months post boosting. The SCB-2019 and SCB-2022B monovalent formulations also boost  
281 neutralization robustly, the latter as expected particularly well against Omicron. In totality, the  
282 bivalent vaccine trended towards incrementally superior titers against the panel of VOCs during

283 the evaluation of humoral kinetics post boosting; however, the third immunization with SCB-  
284 2022B and the bivalent vaccine both elicited Omicron neutralization responses with peak and  
285 set-point titers below the other VOCs. This is suggestive of the original antigenic sin hypothesis,  
286 in which adaptive immunity is partially imprinted against the initial antigens presented to the  
287 naïve immune system (22).

288 In conclusion, despite effectiveness data demonstrating that ancestor vaccines remain highly  
289 effective against severe disease and hospitalization caused by Omicron, there is an opportunity to  
290 improve protection against all cause disease and transmission caused by the currently dominant  
291 Omicron strain. Our murine preclinical priming and booster studies demonstrate the value of a  
292 bivalent formulation, combining our SCB-2019 vaccine with an Omicron specific SCB-2022B  
293 construct, to elicit broad neutralization coverage against a panel of VOCs including Omicron.  
294 Given gaps that remain in equitable vaccination in low-income countries, the ongoing major  
295 outbreaks in China and the threat of future VOCs, a broadly protective bivalent vaccine with the  
296 clinical tolerability and thermal stability profile of our SCB-2019 vaccine, would contribute  
297 towards public health goals.

298

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#### 304 **Author Contribution:**

305 J.G.L, P.L. N.J. and R.X conceived this project, and R.X and D.S. designed the study. D.S.  
306 oversaw mouse studies, cell culture for antigen production and developed in vitro  
307 antibody/neutralizing antibody assays. X.L and C.H. performed expression vector construction  
308 and antibody titer experiments. C.Z conducted protein purification experiments. X.H and Z.M  
309 performed antigen production. Q.W. and W.Q. performed the animal studies. **Competing**  
310 **Interests:** J.G.L. and P.L. have ownership interest in Clover Biopharmaceuticals. All other  
311 authors have no competing interests.

312

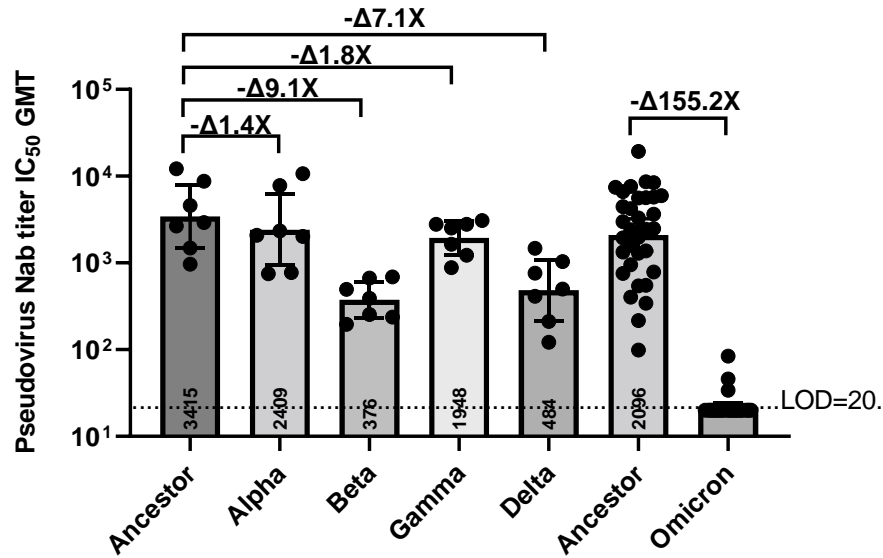


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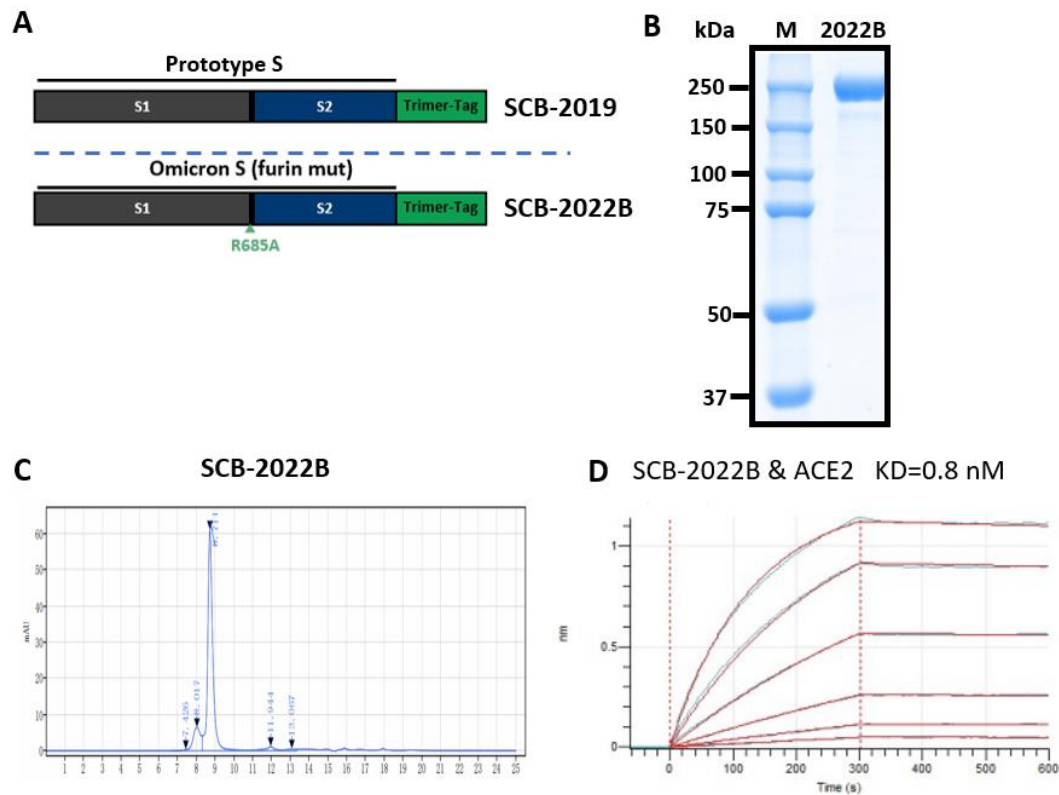


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**Fig.1: VOCs Pseudovirus Neutralizing antibodies (Nab) titer ( $IC_{50}$ ) from human convalescent sera (HCS).**

Seven convalescent serum samples (Sample No.1-7 list in table S1) were initially tested against VOCs including Ancestor Hu-1, Alpha, Beta, Gamma and Delta; an additional 35 (Sample No.1-35 list in table S1) serum samples were later tested against Ancestor Hu-1 or Omicron variants. Limit of detection (LOD) titer ( $IC_{50}$ ) is 20. The numbers marked in each bar are the GMT of each test group. Compared with the Ancestral strain, the Nab titer fold decrease for each VOC is labeled as "x".

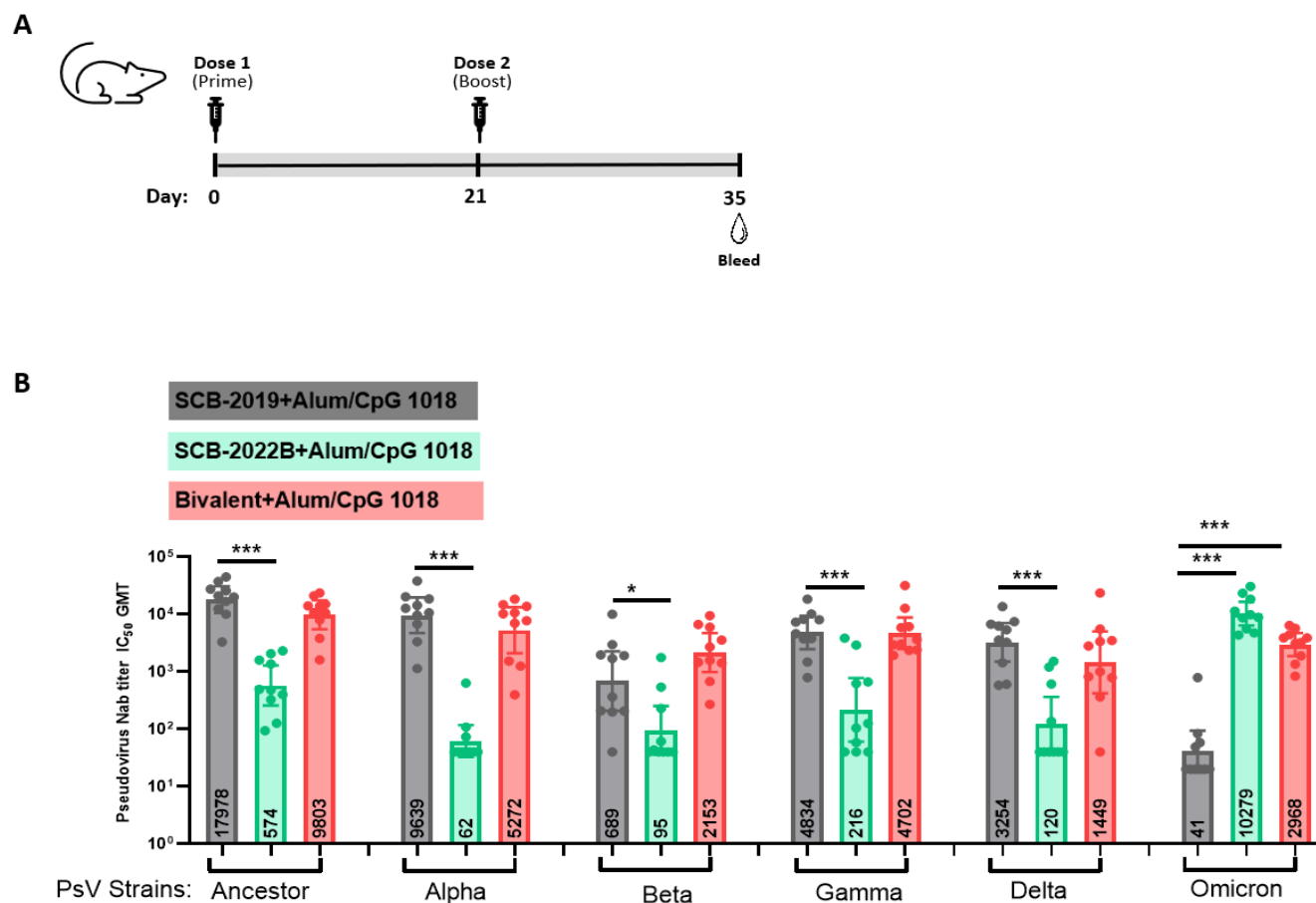
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384 **Fig.2: Omicron S-Trimer construction, protein expression and receptor binding affinity.**

385 A. Structural design of the trimerized SARS-CoV-2 Omicron spike protein. Schematic representation of the  
386 full-length spike protein, SCB-2019: WT ancestor S-Trimer (8). SCB-2022B: a single point mutation R685A at  
387 the S1/S2 cleavage site was introduced in the WT Omicron S-Trimer to generate MT S-Trimer. The  
388 ectodomain of full-length S is fused with a Trimer-Tag derived from the C-terminal domain of human type I  
389 (a) collagen to produce S Trimer. (B) The purified S-Trimer of SCB-2022B was analyzed by Coomassie-  
390 stained reducing SDS-PAGE. (C) SEC-HPLC of the purity of Omicron S-Trimer and a small fraction of  
391 oligomers and cleaved S1 was shown detached from S-Trimer as indicated. (D) ACE2 receptor binding for  
392 SCB-2022B S-Trimer was analyzed by ForteBio BioLayer interferometry indicated.

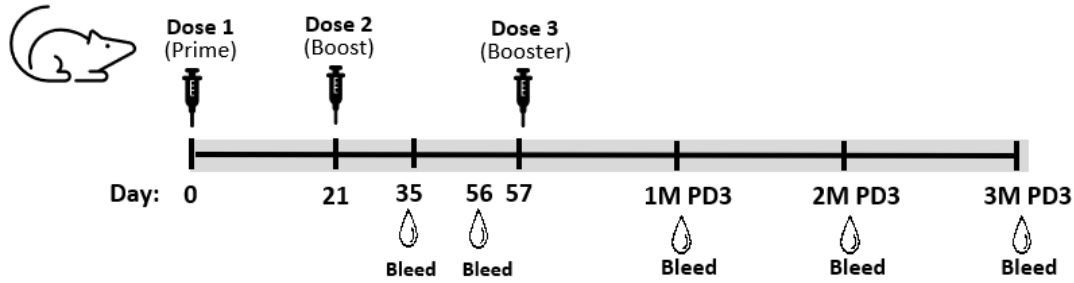


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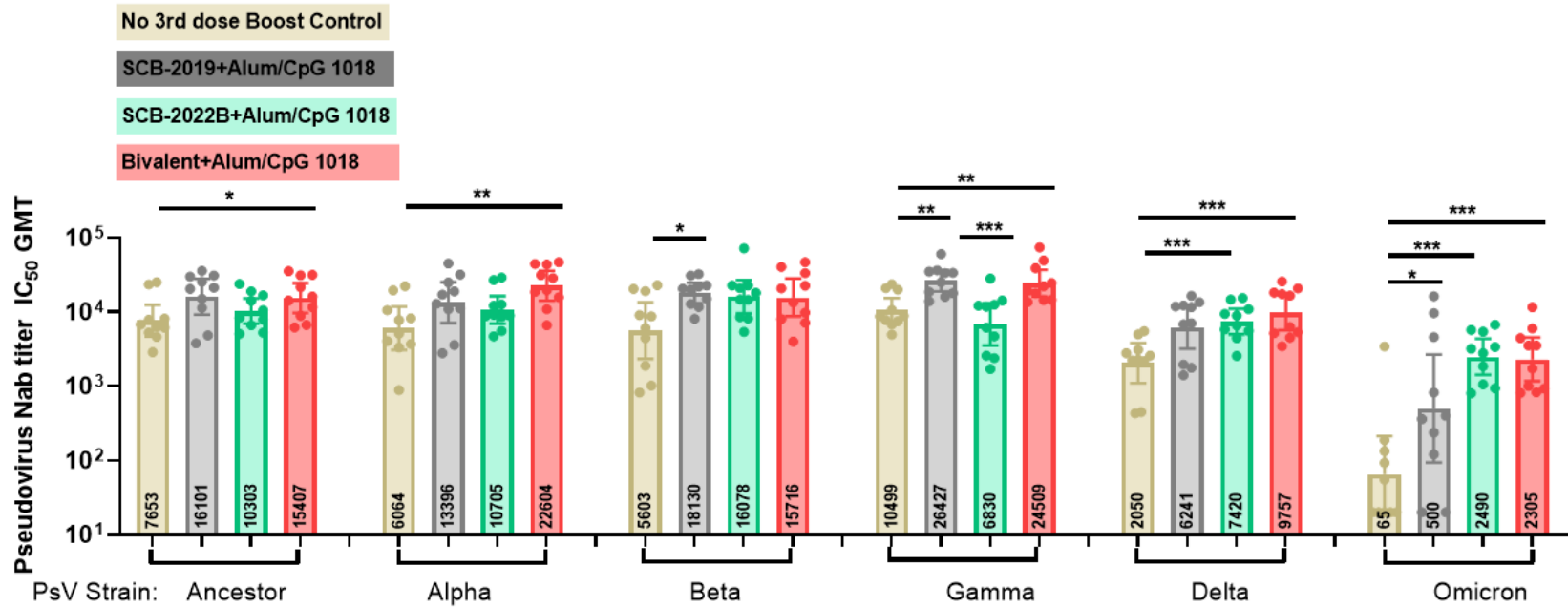
**Fig.3: Broadly neutralizing antibody coverage elicited in Bivalent vaccine immunized mice.**

A. Balb/c mice (n=10/group) were immunized with SCB-2019 (3  $\mu$ g), SCB-2022B (3  $\mu$ g) or Bivalent (1.5  $\mu$ g of SCB-2019 + 1.5  $\mu$ g of SCB2022B) constructs formulated with 150  $\mu$ g CpG 1018 plus 75  $\mu$ g alum twice on Day 0 and Day 21. Serum was collected on D35 for pseudovirus neutralizing antibody testing. B. The study day 35 serum samples were analyzed against VOCs in the pseudovirus neutralization assay (PsVN). Data points represent the pseudovirus neutralizing antibody titer ( $IC_{50}$ ) of the individual animals; Bar horizontal lines indicate geometric mean titers (GMT) for each group  $\pm$ SEM. The grey bars represent the samples from SCB-2019 immunized mice; Green bars represent the samples from SCB-2022B immunized mice; Red bars represent the samples from SCB-2019 and 2022B bivalent immunized mice. Limit of detection (LOD) titer ( $IC_{50}$ ) is 20. The numbers marked in each bar are the GMT of each test group. For statistical analysis, the comparisons were conducted with Two-tailed Mann-Whitney tests. P values < 0.05 were considered significant. \*:P < 0.05, \*\*\*:P < 0.001.

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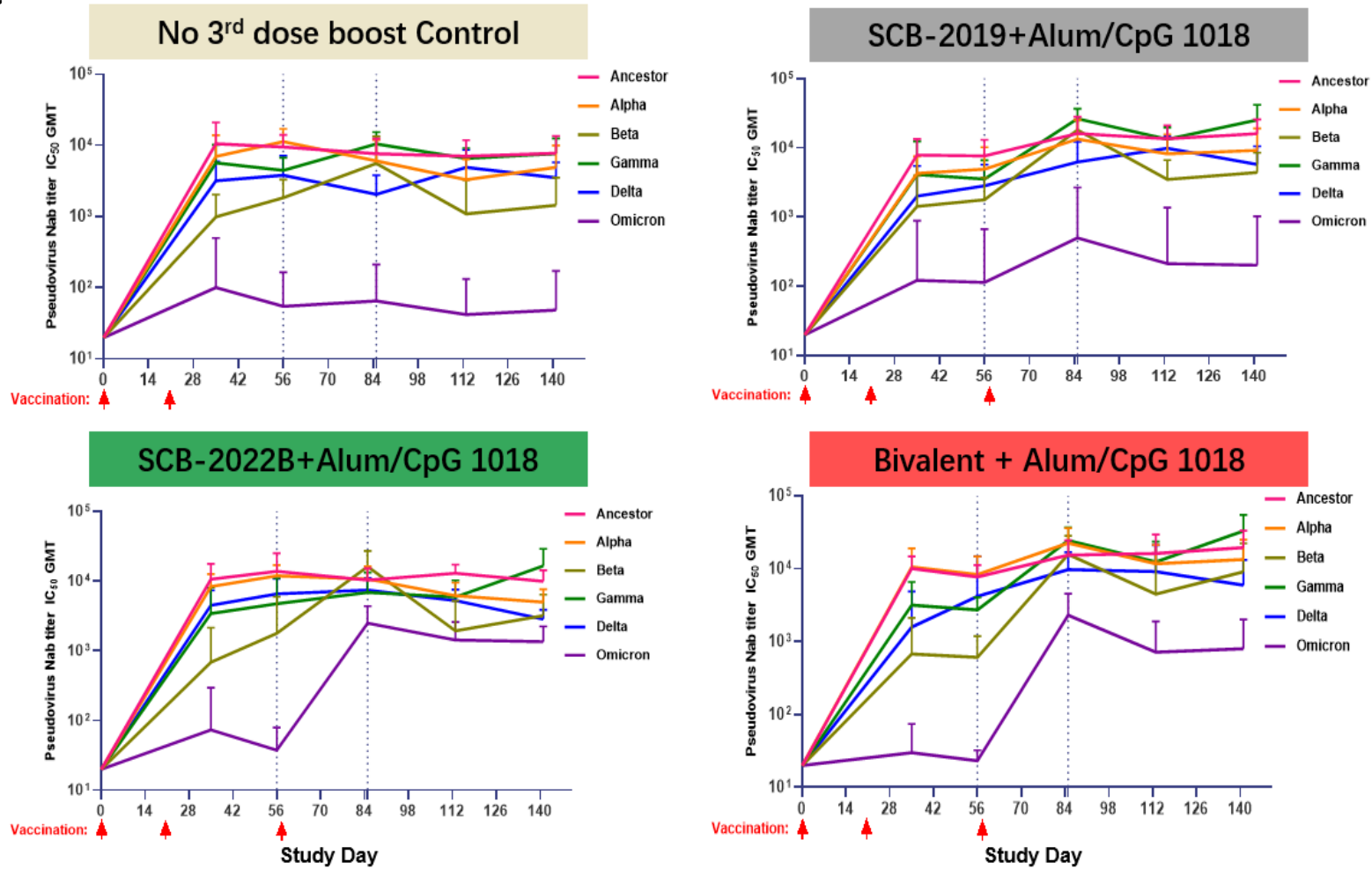


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**Fig.4: Persistent Broad neutralizing antibody elicited with SCB-2019 (ancestor), SCB-2022B (Omicron) and Bivalent vaccine (SCB-2019+SCB-2022B) as 3<sup>rd</sup> booster in SCB-2019 prime/boost immunized mice.**

414 A. Balb/c mice (n=10/group) were primed and boosted with 3 µg SCB-2019 formulated with 150 µg CpG 1018 plus 75 µg alum on Day 0 and Day 21,  
415 then left as control or boosted with SCB2019 (3 µg), SCB-2022B (3 µg) or Bivalent (1.5 µg of SCB-2019 + 1.5 µg of SCB2022B) formulated with 150 µg  
416 CpG 1018 plus 75 µg alum twice on Day 57. B. The study day 85 serum samples were analyzed against VOCs via PsV neutralizing assay. Data points  
417 represent the pseudovirus neutralizing antibody titer (IC<sub>50</sub>) of the individual animals; Bar horizontal lines indicate geometric mean titers (GMT) for each  
418 group ±SEM. Limit of detection (LOD) titer (IC<sub>50</sub>) is 20. The numbers marked in each bar are the GMT of each test group. C. The serum from D0, D35,  
419 D56, D85(1M post dose 3), D113 (2M post dose3) and D141 (3M post dose 3) were analyzed with 6 indicated pseudovirus neutralizing for antibody  
420 kinetics. The light-yellow bars/box represent the samples from control mice who received no further immunization; Grey bars/box represent the samples  
421 from SCB-2019 immunized mice; Green bars/box represent the samples from SCB-2022B immunized mice. Pink bars/box represent the samples from the  
422 Bivalent immunized mice. For statistical analysis, the comparisons were conducted with Two-tailed Mann–Whitney tests. P values < 0.05 were  
423 considered significant. \*:P < 0.05, \*\*:P < 0.01, \*\*\*:P < 0.001.

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428 **Table 1. Information of the pseudovirus.** Characteristics and information of 7 pseudovirus, including TCID<sub>50</sub> and mutation on the envelop S protein.

Table 1: Production of variants of concern of SARS-COV-2 virus pseudotype virus stock.			
PsV Name	Sub-lineage	Stock Titer (TCID <sub>50</sub> /mL)	Mutations On S
Ancestor	Wuhan-HU-1	1.26x10 <sup>5</sup>	/
Alpha	B.1.1.7	3.79x10 <sup>5</sup>	H69-, V70-, Y144-, N501Y, D614G, A570D, P681H, T716I, S982A, D1118H
Beta	B.1.351	2.19x10 <sup>5</sup>	L18F, D80A, D215G, L242-,A243-, L244-, R246I,K417N, E484K, N501Y, D614G, A701V
Gamma	P.1	2.88x10 <sup>5</sup>	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I
Delta	B.1.617.2	5.85x10 <sup>4</sup>	T19R, G142D, E156-, F157-, R158G, L452R, T478K, D614G, P681R, D950N
Mu	B.1.621	4.21x10 <sup>4</sup>	T95I, Y144T, Y145S, Ins146N, R346K, E484K, N501Y, P681H
Omicron	B.1.1.529	3.79x10 <sup>5</sup>	A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, G339D, R346K, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F

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Table 2. Statistical analysis of the 3<sup>rd</sup> boost pseudovirus antibody titer.

PsV Type	Group	Neutralizing antibody titers GMT (95%CI) N=10					
		Day 0	Day 35	Day 56	Day 86	Day 113	Day 141
Ancestor	Group 1: No 3rd dose boost Control	20 (/-/)	10577 (10170-23287)	9532 (9391-13962)	7653 (7497-12535)	7056 (6872-12816)	7771 (7560-14348)
	Group 2: SCB-2019 CpG 1018/Alum	20 (/-/)	7865 (7706-12851)	7628 (7473-12468)	16101 (15881-22958)	13559 (13395-18698)	15982 (15787-22064)
	Group 3: SCB-2022B CpG 1018/Alum	20 (/-/)	10641 (10431-17212)	13759 (13265-29200)	10303 (10176-14281)	12932 (12820-16410)	9936 (9819-13601)
	Group 4: Bivalent CpG 1018/Alum	20 (/-/)	10089 (9984-13368)	7731 (7607-11621)	15407 (15188-22248)	16230 (15752-31169)	19525 (19209-29412)
Alpha	Group 1: No 3rd dose boost Control	20 (/-/)	7025 (6749-15664)	11336 (11223-14886)	6064 (5923-10488)	3305 (3242-5291)	4929 (4792-9196)
	Group 2: SCB-2019 CpG 1018/Alum	20 (/-/)	4293 (4222-6495)	4920 (4800-8663)	13396 (13136-21507)	8168 (8046-11979)	9177 (9009-14405)
	Group 3: SCB-2022B CpG 1018/Alum	20 (/-/)	8325 (8196-12338)	11872 (11702-17170)	10705 (10537-15958)	6183 (6078-9469)	4980 (4894-7684)
	Group 4: Bivalent CpG 1018/Alum	20 (/-/)	10541 (10364-16067)	8323 (8074-16084)	22604 (22314-31663)	11756 (11518-19189)	13356 (13001-24437)
Beta	Group 1: No 3rd dose boost Control	20 (/-/)	991 (965-1825)	1838 (1801-2978)	5603 (5436-10819)	1097 (994-4297)	1441 (1333-4830)
	Group 2: SCB-2019 CpG 1018/Alum	20 (/-/)	1427 (1391-2543)	1777 (1746-2747)	18130 (17972-23089)	3498 (3431-5593)	4416 (4316-7520)
	Group 3: SCB-2022B CpG 1018/Alum	20 (/-/)	686 (647-1913)	1781 (1701-4268)	16078 (15700-27906)	1926 (1818-5306)	3190 (3096-6134)
	Group 4: Bivalent CpG 1018/Alum	20 (/-/)	676 (639-1840)	608 (597-971)	15716 (15418-25050)	4482 (4142-15086)	9113 (8688-22403)
Gamma	Group 1: No 3rd dose boost Control	20 (/-/)	5692 (5583-9123)	4474 (4423-6070)	10499 (10363-14731)	6585 (6517-8705)	7699 (7555-12186)
	Group 2: SCB-2019 CpG 1018/Alum	20 (/-/)	4125 (4022-7372)	3534 (3477-5342)	26427 (26150-35086)	13288 (13140-17895)	25355 (24863-40755)
	Group 3: SCB-2022B CpG 1018/Alum	20 (/-/)	3414 (3270-7914)	4722 (4501-11630)	6830 (6669-11845)	5903 (5779-9773)	16461 (16103-27663)
	Group 4: Bivalent CpG 1018/Alum	20 (/-/)	3165 (3083-5738)	2727 (2698-3661)	24509 (24116-36787)	12403 (12005-24844)	33037 (32450-51404)

<b>Delta</b>	Group 1: No 3rd dose boost Control	20 (/-/)	3196 (3113-5800)	3824 (3768-5554)	2050 (2018-3066)	4914 (4840-7237)	3521 (3473-5027)
	Group 2: SCB-2019 CpG 1018/Alum	20 (/-/)	2009 (1957-3648)	2832 (2787-4219)	6241 (6133-9618)	9879 (9787-12759)	5768 (5694-8085)
	Group 3: SCB-2022B CpG 1018/Alum	20 (/-/)	4482 (4406-6861)	6537 (6422-10127)	7420 (7335-10074)	5244 (5188-6977)	2839 (2810-3754)
	Group 4: Bivalent CpG 1018/Alum	20 (/-/)	1581 (1518-3547)	4195 (4070-8119)	9757 (9594-14854)	9162 (8852-18854)	5961 (5749-12589)
<b>Omicron</b>	Group 1: No 3rd dose boost Control	20 (/-/)	101 (58-1440)	55 (39-547)	65 (44-727)	42 (20-740)	49 (12-1197)
	Group 2: SCB-2019 CpG 1018/Alum	20 (/-/)	122 (-47-5392)	114 (51-2083)	500 (390-3929)	213 (123-3023)	202 (129-2508)
	Group 3: SCB-2022B CpG 1018/Alum	20 (/-/)	73 (42-1049)	37 (34-147)	2490 (2448-3815)	1425 (1402-2134)	1349 (1324-2112)
	Group 4: Bivalent CpG 1018/Alum	20 (/-/)	30 (23-245)	23 (23-36)	2305 (2237-4403)	716 (683-1751)	799 (762-1973)

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**Table S1. Human Convalescent Sera(HCS) Sample Information**

Table S1: Human Convalescent Sera (HCS) Sample Information							
Sample No.	Patient ID.	Sample collection Time	Patient Age	Gender	Mild/Moderate/Severe	Admission Date	Signed ICF or not
1	38	03/11/2020	37	M	Severe	01/29/2020	Yes
2	56	03/11/2020	55	M	Moderate	02/08/2020	Yes
3	K57	03/04/2020	54	F	Moderate	02/08/2020	Yes
4	K62	Unknown	Unknown	Unknown	Unknown	Unknown	Yes
5	K85	Unknown	Unknown	Unknown	Unknown	Unknown	Yes
6	K88	03/04/2020	47	M	Moderate	02/12/2020	Yes
7	110	03/15/2020	23	M	Moderate	02/22/2020	Yes
8	94s	02/16/2020	55	F	Moderate	02/12/2020	Yes
9	1s	02/06/2020	63	F	Mild	01/23/2020	Yes
10	10s	02/05/2020	47	M	Severe	01/26/2020	Yes
11	21s	Unknown	34	M	Mild	01/25/2020	Yes
12	31s	unknown	65	M	Mild	01/30/2020	Yes
13	36s	02/05/2020	62	F	Mild	01/29/2020	Yes
14	37s	Unknown	67	F	Mild	01/29/2020	Yes
15	39s	Unknown	25	M	Mild	01/29/2020	Yes
16	60s	02/15/2020	41	M	Mild	02/08/2020	Yes
17	61s	02/15/2020	27	F	Mild	02/09/2020	Yes
18	93s	02/15/2020	62	F	Moderate	02/12/2020	Yes
19	95s	02/17/2020	35	M	Moderate	02/12/2020	Yes
20	3	03/11/2020	60	M	Severe	01/26/2020	Yes
21	81	03/11/2020	59	M	Moderate	02/12/2020	Yes
22	83	03/15/2020	50	M	Moderate	02/12/2020	Yes
23	108	03/11/2020	57	F	Moderate	02/21/2020	Yes
24	109	Unknown	36	F	Moderate	02/21/2020	Yes

25	117	03/15/2020	74	F	Moderate	02/28/2020	Yes
26	130	03/15/2020	47	F	Moderate	03/05/2020	Yes
27	K27	03/04/2020	66	M	Mild	01/25/2020	Yes
28	K80	unknow	unknown	unknown	unknown	unknown	Yes
29	K87	03/04/2020	Unknown	F	Moderate	02/12/2020	Yes
30	K92	03/04/2020	59	F	Moderate	02/12/2020	Yes
31	K111	Unknown	Unknown	Unknown	Unknown	Unknown	Yes
32	K114	Unknown	Unknown	Unknown	Unknown	Unknown	Yes
33	K120	03/04/2020	Unknown	F	Mild	02/29/2020	Yes
34	K121	Unknown	Unknown	Unknown	Unknown	Unknown	Yes
35	K122	Unknown	Unknown	Unknown	Unknown	Unknown	Yes