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**The dual function monoclonal antibodies VIR-7831 and VIR-7832 demonstrate potent in vitro and in vivo activity against SARS-CoV-2**

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23 **ABSTRACT**

24 **VIR-7831 and VIR-7832 are dual action monoclonal antibodies (mAbs) targeting the spike**  
25 **glycoprotein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). VIR-7831 and**  
26 **VIR-7832 were derived from a parent antibody (S309) isolated from memory B cells of a 2003**  
27 **severe acute respiratory syndrome coronavirus (SARS-CoV) survivor. Both mAbs contain an “LS”**  
28 **mutation in the Fc region to prolong serum half-life and potentially enhance distribution to the**  
29 **respiratory mucosa. In addition, VIR-7832 encodes an Fc GAALIE mutation that has been shown**  
30 **previously to evoke CD8<sup>+</sup> T-cells in the context of an in vivo viral respiratory infection. VIR-7831**  
31 **and VIR-7832 potently neutralize wild-type and variant authentic virus in vitro as well as variant**  
32 **pseudotyped viruses. In addition, they retain activity against monoclonal antibody resistance**  
33 **mutations conferring reduced susceptibility to currently authorized mAbs. The VIR-7831/VIR-**  
34 **7832 epitope does not overlap with mutational sites in current variants of concern and continues to**  
35 **be highly conserved among circulating sequences consistent with the high barrier to resistance**  
36 **observed in vitro. Furthermore, both mAbs can recruit effector mechanisms in vitro that may**  
37 **contribute to clinical efficacy via elimination of infected host cells. In vitro studies with these mAbs**  
38 **demonstrated no enhancement of infection. In a Syrian Golden hamster proof-of concept wildtype**  
39 **SARS-CoV-2 infection model, animals treated with VIR-7831 had less weight loss, and significantly**  
40 **decreased total viral load and infectious virus levels in the lung compared to a control mAb. Taken**  
41 **together, these data indicate that VIR-7831 and VIR-7832 are promising new agents in the fight**  
42 **against COVID-19.**

43

44 **INTRODUCTION**

45 The coronavirus disease (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus  
46 2 (SARS-CoV-2) has resulted in more than 191 million confirmed cases and over 4 million deaths

47 worldwide<sup>1</sup>. SARS-CoV-2 infection results in a broad range of disease severity<sup>2</sup>. Infection fatality rates  
48 increase significantly with age, with 28.3% of COVID-19 patients over the age of 85 succumbing to  
49 disease<sup>2</sup>. However, even in mild-to-moderate COVID-19 patients, significant post-infection sequelae can  
50 affect overall health and cause long-term disability<sup>3</sup>. While multiple SARS-CoV-2 vaccines are now  
51 authorized for use, issues of supply, vaccine hesitancy and emergence of variants may prevent rapid  
52 attainment of herd immunity<sup>4-9</sup>. In addition, there may be individuals who remain at risk despite  
53 vaccination due to disease or underlying immunodeficiency. Thus, additional interventions and potential  
54 prophylactic agents are needed to reduce morbidity and mortality due to COVID-19.

55 Several monoclonal antibodies (mAbs) targeting the SARS-CoV-2 spike protein have recently been  
56 authorized for use in early treatment of COVID-19 patients<sup>10-13</sup> and clinical data have been reported to  
57 show promising results in treatment and prophylactic studies<sup>12-15</sup>. However, rapidly spreading variants  
58 exhibit reduced susceptibility in vitro to some currently authorized antibodies that target the receptor  
59 binding motif (RBM) of the viral spike (S) glycoprotein<sup>10,11,16,17</sup>. Therefore, mAbs targeting unique S  
60 epitopes are needed for use alone or in combination with current agents for the treatment and prevention  
61 of COVID-19. Furthermore, in addition to viral neutralization, antibodies possessing potent effector  
62 function to aid in the killing of virally infected cells and the elicitation of T cell immunity could  
63 significantly assist in halting disease progression<sup>18-20</sup>.

64 VIR-7831 and VIR-7832 are dual action mAbs derived from the parent antibody S309 identified from a  
65 2003 SARS-CoV survivor<sup>21</sup>. These mAbs target an epitope containing a glycan (at position N343) that is  
66 highly conserved within the Sarbecovirus subgenus in a region of the S receptor binding domain (RBD)  
67 that does not compete with angiotensin converting enzyme 2 (ACE2) binding<sup>22</sup>. This epitope does not  
68 overlap with mutations observed in current variants of concern<sup>10,11,16,17</sup>. The variable region of VIR-7831  
69 and VIR-7832 have been engineered for enhanced developability. In addition, both antibodies possess an  
70 Fc “LS” mutation that confers extended half-life by binding to the neonatal Fc receptor and potentially  
71 enhances distribution to the respiratory mucosa<sup>23-25</sup>. VIR-7832 is identical to VIR-7831 with the

72 exception of the addition of a 3 amino acid GAALIE (G236A, A330L, I332E) modification to the Fc  
73 domain<sup>26</sup>. The GAALIE modification has previously been shown in vitro to enhance binding to FcγIIa  
74 and FcγIIIa receptors, decrease affinity for FcγIIb compared to typical IgG1 and evoke protective CD8+  
75 T-cells in the context of viral respiratory infection in vivo <sup>27,28</sup>.

76 Here we characterize the antiviral potential of VIR-7831 and VIR-7832. These mAbs effectively  
77 neutralize SARS-CoV-2 live virus in vitro as well as in pseudotyped virus assays against emerging  
78 variants of concern and variants that confer resistance to currently authorized mAbs<sup>29</sup>. In addition to the  
79 neutralizing capacity, both antibodies demonstrate potent effector function and mediate antibody  
80 dependent cellular cytotoxicity (ADCC) and antibody dependent cellular phagocytosis (ADCP) in vitro.  
81 Furthermore, resistance selection experiments and epitope conservation analyses indicate the potential for  
82 a high barrier to resistance. Data derived from the Syrian golden hamster model demonstrates efficacy in  
83 a proof-of-concept in vivo model. Taken together, these data indicate that VIR-7831 and VIR-7832 are  
84 promising key components of the arsenal in the fight against COVID-19.

## 85 RESULTS

### 86 VIR-7831 and VIR-7832 bind SARS-CoV-2 spike and effectively neutralize live virus in vitro.

87 Previously published work showed that S309 bound SARS-CoV-2 recombinant and cell surface-  
88 associated S and neutralized live virus in vitro<sup>21</sup>. We initiated these studies by repeating and extending  
89 these earlier results. To determine the binding activity of VIR-7831 and VIR-7832 to the SARS-CoV-2 S,  
90 enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance (SPR) and flow cytometry  
91 assays were utilized. VIR-7831 and VIR-7832 bound to recombinant S RBD (amino acids 331-541) with  
92 EC<sub>50</sub> values of 20.40 ng/mL and 14.9 ng/mL, respectively, by ELISA (**Figure 1a**). Using SPR, both  
93 antibodies demonstrated potent binding to recombinant S RBD with an equilibrium constant (K<sub>d</sub>) of 0.21  
94 nM (**Figure 1b**). As antibody recognition of cell surface-bound S could mediate killing of virally infected  
95 cells, flow cytometry-based studies using cells transiently transfected with a S-encoding plasmid were

96 used to examine antibody binding to cell surface-expressed S trimer. By this method, both VIR-7831 and  
97 VIR-7832 bound efficiently to surface-expressed S (**Figure 1c**).

98 To examine neutralization capacity, VIR-7831 and VIR-7832 were tested in a VeroE6 cell-based live  
99 SARS-CoV-2 virus system against the Washington 2019 (wild-type) virus as well as against the Alpha  
100 (B.1.1.7), Beta (B.1.351) and Gamma (P.1) variants. Concentration-dependent viral neutralization of the  
101 Washington 2019 strain was observed for both antibodies, with geometric mean  $IC_{50/90}$  values of  
102 100.1/186.3 ng/mL and 78.3/253.1 ng/mL, respectively (**Figure 1d**).  $IC_{50/90}$  values observed for VIR-7831  
103 and VIR-7832 against the Beta and Gamma variant viruses were similar to those against the wild-type  
104 strain. A slight shift in the VIR-7831/VIR-7832  $IC_{50/90}$  compared to wild-type was observed for the Alpha  
105 variant. VIR-7831 showed a 3-fold and 4.1-fold shift in  $IC_{50}$  and  $IC_{90}$ , respectively, against the Alpha  
106 variant compared to wild-type while VIR-7832 had a 3.1-fold shift in  $IC_{50}$  and 3.7-fold shift in  $IC_{90}$  versus  
107 wild-type (**Figure 1d, Table 1**). As variant evolution is a natural part of SARS-CoV-2 biology and  
108 emerging live virus variants are not always readily accessible for testing, a vesicular stomatitis virus  
109 (VSV)-based pseudotyped virus system targeting Vero E6 cells was used to examine VIR-7831 and VIR-  
110 7832 neutralization against emergent variants (**Table 2**). Fold-changes in VIR-7831 and VIR-7832  $IC_{50}$   
111 values compared to wild-type against pseudotyped virus expressing spike from the Alpha, Beta or  
112 Gamma variant were similar to those observed in the authentic virus system. VIR-7831 was tested against  
113 an extended panel of pseudotyped viruses incorporating emerging variants as well as all variants deemed  
114 as Variants of Concern (VOC) or Variants of Interest (VOI) by the World Health Organization (WHO).  
115 VIR-7831 retained activity in vitro against all variants tested including all WHO VOCs and VOIs with  
116 fold changes in  $IC_{50}$ s ranging from 0.4- to 2.3-fold (**Table 2**).

117 **VIR-7831 and VIR-7832 exhibit potent effector function in vitro.** Although direct antiviral  
118 mechanisms are crucial to provide protection, Fc-dependent mechanisms mediated by interaction with Fc  
119 gamma receptors (Fc $\gamma$ Rs) on immune cells or with complement, can contribute to overall potency in vivo.  
120 The potential for VIR-7831 and VIR-7832 to mediate effector functions were assessed in vitro by

121 measuring binding to FcγRs and C1q and in assays designed to demonstrate antibody-dependent cellular  
122 cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP)<sup>31-34</sup>.

123 Antibody binding to the human activating FcγRIIa (low-affinity R131 and high affinity H131 alleles),  
124 FcγRIIIa (low-affinity F158 and high-affinity V158 alleles), and to the inhibitory FcγRIIb were examined  
125 using SPR (**Supplemental figure 1a**). VIR-7831 similarly bound both the H131 and R131 alleles of  
126 FcγRIIa and binds FcγRIIb. VIR-7831 bound both FcγRIIIa alleles, with reduced binding to the F158  
127 allele compared to V158, as is characteristic for human IgG1<sup>35</sup>. Binding of VIR-7831 to C1q was similar  
128 to the parental antibody (S309-LS) (**Supplemental figure 1b**). As previously reported for antibodies  
129 encoding the GAALIE mutation<sup>26,36</sup>, VIR-7832 bound with comparatively higher affinity to activating  
130 FcγRIIa and FcγRIIIa than VIR-7831 (**Supplemental figure 1a**). Conversely, VIR-7832 showed reduced  
131 affinity for FcγIIb and abrogation of binding to C1q (**Supplemental figure 1b**).

132 The antibodies were also assessed for the ability to activate human FcγRIIa, FcγRIIb or FcγRIIIa, using a  
133 Jurkat cell reporter assay<sup>37</sup> (**Figure 2a-d**). S309-GRLR, which contains the effector function-abrogating  
134 G236R, L328R mutations was used as a negative control. Cells stably transfected with the SARS-CoV-2  
135 spike protein (CHO-CoV-2-Spike) served as target cells. Both VIR-7831 and the parental S309-LS  
136 activated signaling of the higher-affinity allele FcγRIIa (H131) but did so less efficiently than the  
137 GAALIE-containing antibody VIR-7832 (**Figure 1a**) while VIR-7831, VIR-7832 and S309-LS induced  
138 similar low-level activation of the inhibitory receptor FcγRIIb (**Figure 1b**). VIR-7831 demonstrated  
139 substantially lower activation of FcγRIIIa F158 versus V158 as expected while VIR-7832 showed  
140 increased activation of both alleles of FcγRIIIa (F158 and V158) (**Figures c,d**).

141 To further elucidate the effector function potential of the antibodies, ADCC and ADCP assays were  
142 performed using donor PBMCs or NK cells as effector cells and CHO cells stably expressing S (CHO-  
143 CoV-2-Spike) as target cells (**Figure 2e-g**). The ability of antibodies to activate NK cell-mediated killing  
144 was measured in vitro using two genotyped donors expressing homozygous low-affinity (F/F158) or high-  
145 affinity (V/V158) (**Figure 2e-f**). Compared to the parental mAb S309-LS, VIR-7831 had slightly

146 increased capacity to induce NK cell-mediated ADCC when using cells from either F/F158 or V/V158  
147 donors. As expected, VIR-7832 induced NK cell-mediated ADCC in cells from donors expressing the  
148 low-affinity F/F158 allele of FcγIIIa more efficiently than VIR-7831. These results were confirmed with  
149 NK cells from a heterozygous donor (F/V 158).

150 The ability of VIR-7831 and VIR-7832 to facilitate ADCP by primary CD14<sup>+</sup> monocytes was measured in  
151 vitro by exposing freshly isolated human PBMCs to CHO-CoV-2-Spike cells that were pre-incubated  
152 with antibody (**Figure 2g**). VIR-7831, VIR-7832 and S309-LS induced similar levels of ADCP by CD14<sup>+</sup>  
153 monocytes. These results indicate that VIR-7831 and VIR-7832 have the potential to trigger ADCC and  
154 ADCP of cells displaying SARS CoV-2 S protein.

155 **Subneutralizing levels of VIR-7831 and VIR-7832 do not enhance virus uptake, replication or**  
156 **cytokine production in vitro.** One potential concern with any antibody therapeutic targeting a viral agent  
157 is the possibility of antibody-dependent enhancement (ADE). ADE is an in vivo phenomenon in which  
158 the presence of an antibody worsens disease. There are several in vitro assays that may provide plausible  
159 correlates for ADE in vivo, though none of these have been proven relevant to COVID-19 as to date ADE  
160 has not been observed in trials of monoclonal antibodies or plasma<sup>13,15,38,39</sup>. ADE can occur by several  
161 potential mechanisms<sup>40</sup>. Poorly neutralizing antibodies or subneutralizing levels of antibody could  
162 theoretically facilitate enhanced virus entry and infection through Fc receptor interactions. A second  
163 theoretical mechanism involves antibody-antigen complex formation leading to enhanced cytokine  
164 production. A third mechanism of ADE has been observed in a porcine model of influenza where the  
165 kinetics of viral fusion to the target cell was enhanced in a Fab-dependent manner by fusion-enhancing  
166 non-neutralizing antibodies<sup>41,42</sup>.

167 To explore whether VIR-7831 and VIR-7832 exhibit in vitro activities that might be related to ADE in  
168 vivo, we evaluated SARS-CoV-2 replication in human cells that express FcγRs: monocyte-derived  
169 dendritic cells (moDCs), peripheral blood mononuclear cells (PBMCs) and the human U937 macrophage  
170 cell line (**Supplemental Figure 2a-b**). Subneutralizing concentrations of VIR-7831 and VIR-7832 were



171 precomplexed with SARS-CoV-2 (MOI =0.01) and added to target cells. Using immunostaining methods,  
172 at 24 hours post-infection no productive entry of SARS-CoV-2 into moDCs, PBMCs, or U937 cells was  
173 observed in the presence or absence of either mAb, while VeroE6 control cells demonstrated  
174 internalization in all conditions evaluated. Reduced internalization of SARS-CoV-2 in VeroE6 cells was  
175 observed at the highest concentration of VIR-7831 and VIR-7832 (p-value <0.05), indicating effective  
176 virus neutralization prevented virus entry. Using a focus forming assay, virus replication and secretion of  
177 infectious virus were detectable by 48 hours post-infection in VeroE6 cells, with comparable levels of  
178 replication in the presence or absence of VIR-7831 or VIR-7832. However, no replication of SARS-CoV-  
179 2 was detected in moDCs, PBMCs or U937 cells regardless of antibody treatment, indicating lack of  
180 productive SARS-CoV-2 infection of these cells, consistent with previously published data<sup>43</sup>.

181 To evaluate the potential for VIR-7831 and VIR-7832 to enhance cytokine release upon SARS-CoV-2  
182 infection in FcγR-expressing cells, cytokines and chemokines were measured in the supernatants from  
183 cells infected with SARS-CoV-2-in the presence of VIR-7831 or VIR-7832 (**Supplemental figure 2c**).  
184 Levels of IFN-γ, IL-10, IL-6, IL 8, IP-10, MCP-1, and TNF-α in the supernatant were quantified by MSD  
185 at 24- and 48-hours post-infection. For all cell types evaluated, cytokine/chemokine production was  
186 similar between all antibody concentrations tested and the no antibody control at both 24- and 48-hours  
187 post-infection. Taken together, these in vitro data indicate that neither VIR-7831 nor VIR-7832 exhibit in  
188 vitro activities that have been proposed to possibly correlate with ADE in vivo.

189 **VIR-7831 and VIR-7832 have a high barrier to resistance in vitro and do not display cross-**  
190 **resistance with other SARS-CoV-2 mAbs.** We next determined whether resistant variants could be  
191 elicited by serial passage of SARS-CoV-2 in the presence of VIR-7832. As VIR-7831 and VIR-7832  
192 differ only in the Fc region of the antibody, resistance selection experiments were conducted with VIR-  
193 7832 as a proxy for both antibodies. SARS-CoV-2 was subjected to 10 passages in the presence of VIR-  
194 7832 at fixed concentrations of ~10x, 20x, 50x or 100x IC<sub>50</sub> (1, 2, 5, or 10 μg/mL) in VeroE6 cells. No  
195 CPE was detected in wells passaged with antibody through 10 passages, while CPE was observed in the

196 no antibody control in all passages. Similarly, no virus was detected by focus forming assay at any  
197 concentration of VIR-7832 through all 10 passages even at the lowest concentration tested.

198 As no viral breakthrough was observed in the fixed concentration resistance selection, a second method  
199 was employed wherein SARS-CoV-2 virus was passaged in sub-IC<sub>50</sub> concentrations of antibody followed  
200 by subsequent passaging in the presence of increasing concentrations of mAb in an attempt to force  
201 resistance emergence (**Supplemental Figure 3**). Passaging was performed in duplicate wells to account  
202 for founder effects, and concentration increases for each well were based on CPE observations. Five  
203 sequential passages were conducted using increasing concentrations of VIR-7832 at 0.5, 1, 2, 5 and ~10x  
204 IC<sub>50</sub> (0.05, 0.1, 0.2, 0.5, 1 µg/mL; **Supplemental Figure 3a**), though no CPE was observed by passages 4  
205 and 5 (0.5 and 1 µg/mL, respectively) indicating that variants originally selected at the lower  
206 concentrations were either unfit or susceptible to the higher concentrations of antibody. To further assess  
207 whether resistance mutations could be generated, selection was restarted using passage 3 virus generated  
208 with ~2x IC<sub>50</sub> (0.2 µg/mL) of VIR-7832 in duplicate wells at ~2x and ~5x IC<sub>50</sub> (0.2, 0.5 µg/mL),  
209 generating two passage lineages (**Supplemental Figure 3b-c**).

210 Supernatants were evaluated for detectable virus at each passage by focus forming assay and cell  
211 supernatants from viral passages containing detectable virus were tested in SARS-CoV-2 neutralization  
212 assays to evaluate IC<sub>50</sub> shifts as a marker of reduced susceptibility (**Supplemental Table 1**). With the  
213 exception of passage 8, modest fold changes were observed, with shifts in IC<sub>50</sub> values ranging from 5.4-  
214 to 6.5-fold compared to the wild-type SARS-CoV-2 stock virus. In lineage 1, the passage 8 virus  
215 displayed a >10-fold shift in IC<sub>50</sub> (greater than highest concentration tested). Sequence analysis detected  
216 an identical 4 amino acid insertion in the N-terminal domain (215-216insKLRS) and 5 amino acid  
217 deletion in correspondence of the furin cleavage site (675-679del) in both lineages at all passages  
218 sequenced, as well as the amino acid substitution E340A in lineage 1, and R682W, and V1128F in  
219 lineage 2. The deletion at amino acids 675-679 has been previously described during passaging of SARS-  
220 CoV-2 in tissue culture suggesting enrichment to be a result of cell culture adaptation<sup>44</sup> while the 215-

221 216insKLRS was detected in the input virus. Neither 215-216insKLRS nor R682W variants were highly  
222 enriched with passaging (**Supplemental Table 1**) and enrichment of 675-679del and V1128F did not  
223 profoundly alter the VIR-7832 IC<sub>50</sub>. However, appearance of the E340A variant at 98.7% did correlate  
224 with a >10-fold shift in IC<sub>50</sub> suggesting this variant may confer resistance.

225 To evaluate whether amino acid variants identified in the resistance selection conferred reduced  
226 susceptibility to VIR-7831 and VIR-7832, neutralization of pseudotyped viruses encoding the S variants  
227 was assessed (**Supplemental Table 2**). VIR-7831 and VIR-7832 neutralized R682W and V1128F SARS-  
228 CoV-2 pseudotyped virus spike variants with IC<sub>50</sub> values similar to wild type (< 2-fold change in IC<sub>50</sub>)  
229 indicating that these variants do not alter susceptibility. In contrast, E340A conferred reduced  
230 susceptibility to VIR-7831 and VIR-7832 (> 100-fold change in IC<sub>50</sub>) indicating that E340A is a VIR-  
231 7831/VIR-7832 monoclonal antibody resistance mutation (MARM).

232 As VIR-7831/VIR-7832 demonstrated a unique in vitro resistance profile, we investigated the potential  
233 for cross-resistance to MARMs that confer reduced susceptibility to the authorized monoclonal antibodies  
234 bamlanivimab, imdevimab and casirivimab<sup>10,11,45-47</sup> using pseudotyped virus. Notably, some of these  
235 mutations are found in highly prevalent variants of concern<sup>17,30,48</sup>. VIR-7831 effectively neutralized  
236 pseudotyped viruses expressing spike MARMs that alter bamlanivimab, casirivimab and/or imdevimab  
237 activity (**Table 3**). Fold changes in IC<sub>50</sub> values compared to wild-type were <3-fold for 18/19 variants  
238 tested. A modest 3.4-fold shift in the VIR-7831 IC<sub>50</sub> was observed for the V445A variant that confers  
239 reduced susceptibility to imdevimab. These data indicate that VIR-7831/VIR-7832 does not display cross-  
240 resistance with currently authorized mAbs and supports the potential combination use of VIR-7831/VIR-  
241 7832 with other mAb therapeutics.

242 **The VIR-7831/VIR-7832 epitope is highly conserved among SARS-CoV-2 sequences.** The parental  
243 antibody of VIR-7831 and VIR-7832 (S309) binds to a highly conserved sarbecovirus epitope that is  
244 potentially intolerant of variation. To investigate the current state of epitope conservation, >1,700,000  
245 spike sequences from SARS-CoV-2 deposited in the GISAID database as of June 4, 2021 were examined

246 for epitope variation. More than 99.8% conservation is seen for those amino acids comprising the epitope  
247 among currently available sequences for all positions including 16/23 amino acid positions that were  
248  $\geq 99.99$  conserved (**Table 4**).

249 VIR-7831 activity against viral mutants carrying single substitutions in the epitope was assessed in  
250 pseudotyped virus assays. VIR-7831 effectively neutralized epitope variants at most amino acid positions  
251 tested (**Table 5**). A moderate shift in activity was observed for the K356T variant (5.9-fold shift in  $IC_{50}$ ).  
252 Variants at two positions, E340 and P337, resulted in significant  $IC_{50}$  shifts indicating reduced  
253 susceptibility to VIR-7831. Moderate shifts in potency were observed for P337H and P337T variants  
254 (7.50- and 5.38-fold, respective) while more significant shifts in potency were observed for P337L/R and  
255 E340A/K/G (27-fold to  $>276$ -fold). Notably, these variants are detected in a low number of sequences  
256 and do not have a pattern that suggest emergence in the GISAID database (93 and 95 variant counts out of  
257  $>1,700,000$  sequences for P337 and E340, respectively). This observation is consistent with the  
258 possibility that variations at these positions come with a fitness cost to the virus.

259 **VIR-7831 reduces weight loss, total viral load and infectious virus levels in a hamster model of**  
260 **SARS-CoV-2 infection.** To evaluate the efficacy of VIR-7831 in vivo, the hamster model was utilized.  
261 As it was unknown what effect the LS mutation would have in the hamster, a non-LS version of VIR-  
262 7831 (SGHmAb-no-LS) was used for these experiments. Hamsters were administered SGHmAb-no-LS  
263 intraperitoneally at Day -1 (30, 5, 0.5 or 0.05 mg/kg) or Day -2 (15, 5, 0.5 or 0.05 mg/kg) prior to  
264 intranasal SARS-CoV-2 inoculation (**Figure 3a**). Using body weight as a marker of degree of clinical  
265 disease, doses of  $\geq 5$ mg/kg resulted in significantly reduced weight loss at Day 4 compared to controls.  
266 (**Figure 3b-e**). Significant decreases in lung viral load were also observed at  $\geq 5$ mg/kg as measured by  
267 RT-qPCR (**Figures f-g**). Day 4  $TCID_{50}$  measurements indicated that antibody administered at  $\geq 0.5$  mg/kg  
268 resulted in significantly lower levels of infectious virus in lung tissue compared to controls (**Figure 3h-i**).  
269 Notably, across these experiments, no enhancement of disease was observed in animals receiving  
270 SGHmAb-no-LS based on changes in weight, viral RNA in the lungs, or  $TCID_{50}$  infectious virus levels.

271 Collectively, these data indicate that VIR-7831 prevented in a dose-dependent fashion virus replication  
272 and morbidity in SARS-CoV-2 challenged hamsters without signs of ADE at any dose tested.

## 273 **DISCUSSION**

274 Here we show the in vitro and in vivo preclinical characterization of VIR-7831 and VIR-7832, two  
275 monoclonal antibodies being advanced into clinical studies<sup>49–51</sup>. Both antibodies demonstrate high-affinity  
276 binding to S in vitro, including on the surface of cells, and effectively neutralize wildtype SARS-CoV-2  
277 in a live virus assay. VIR-7831 and VIR-7832 retain activity against the Alpha (B.1.1.7), Beta (B.1.351)  
278 and Gamma (P.1) variants in an authentic virus system, consistent with data using pseudotyped viruses.  
279 VIR-7831 bind C1q, activate FcγRs and demonstrate ADCC and ADCP in vitro. Experiments in the  
280 hamster model of SARS-CoV-2 infection show proof-of-concept efficacy in vivo. Notably, in vitro and in  
281 vivo data did not provide any supporting evidence that these antibodies would demonstrate ADE in a  
282 clinical setting.

283 That VIR-7831 and VIR-7832 retain activity against spike variants in authentic virus and pseudotyped  
284 virus assays is a key finding at this stage of the pandemic. With the increased transmissibility and  
285 potential for more severe disease observed with these viruses, the availability of therapeutic or  
286 prophylactic mAbs that remains active against variants is essential. In addition to retaining activity  
287 against all current WHO VOCs and VOIs, in pseudotyped virus experiments VIR-7831 showed no  
288 significant cross-resistance with variants that reduce the activity of authorized mAbs. These data  
289 additionally demonstrate the uniqueness of VIR-7831 and VIR-7832 and further highlight the utility VIR-  
290 7831 and VIR-7832 could have, alone or in combination, as clinical agents.

291 Notably, even at this stage of the pandemic, the VIR-7831/VIR-7832 epitope remains highly conserved  
292 among available sequences of circulating virus with  $\geq 99.8\%$  conservation of epitope amino acids. This is  
293 consistent with the value of the strategy used for isolation of monoclonal antibodies that neutralize both  
294 SARS-CoV and SARS-CoV-2 based on the idea that these two virulent human viruses are

295 phylogenetically divergent within the sarbecovirus subgenus. Furthermore, MARMs identified at  
296 positions P337 and E340 are present at very low levels among current sequences. That amino acids P337  
297 and E340 remain  $\geq 99.99\%$  conserved at this stage of the pandemic indicates that variants at these  
298 positions may confer disadvantageous effects on the virus, consistent with the conservation of this epitope  
299 across the sarbecovirus family<sup>21</sup>.

300 Viral variants of concern for RBM-targeting mAbs are quickly spreading<sup>53</sup>. The vaccines presently being  
301 deployed around the world generate high-titer neutralizing antibodies that target the S protein RBM.  
302 Importantly, the RBM is highly immunodominant for responses to natural infection<sup>54</sup>. Vaccine-induced  
303 and convalescent immunity may therefore potentially put further mutational pressure on the RBM  
304 sequence to evade such antibody responses. In contrast, antibody responses overlapping with the VIR-  
305 7831/VIR-7832 epitope are limited after infection<sup>54</sup>, possibly because of the shielding effect of the highly  
306 conserved N343 glycan. In this regard the epitope may face less vaccine- or infection-generated immune  
307 pressure, potentially preserving this conserved epitope long-term.

308 Recent data have indicated that the cells used to generate live virus stocks and overexpression of  
309 ACE2/TMPRSS2 in target cells used for assays can affect mAb activity in vitro<sup>29,55</sup>. The VIR-7831/VIR-  
310 7832 parental antibody S309 seems particularly sensitive to in vitro methods using ACE2 overexpressing  
311 cells<sup>55</sup>. It is therefore notable that VIR-7831 displays significant efficacy in an in vivo proof-of-concept  
312 SARS-CoV-2 infection experiment using hamsters despite the fact that patterns of engagement of hamster  
313 FcRs by human IgG1 antibodies may not reflect patterns of human IgG1 antibodies with their cognate  
314 human FcRs. These findings argue that in vitro data derived from such ACE2 and/or TMPRSS2  
315 overexpression cell lines do not accurately reflect the in vivo antiviral capacity of tested mAbs.

316 Furthermore, that the significant in vivo effects of VIR-7831 in the hamster model likely occurred in the  
317 absence of full effector functions due to species-specific interactions between antibodies and FcRs, argues  
318 that effects in COVID-19 patients incorporating both the neutralization capacity of the antibody plus the  
319 ability to harness the strength of the immune system could lead to positive clinical outcomes.

320 The clinical potential of VIR-7832, with the inclusion of the GAALIE Fc mutation, is of special interest  
321 in the context of SARS-CoV-2 infection. Previously published data by the Ravetch laboratory comparing  
322 the in vivo efficacy of a hemagglutinin-targeting mAb with and without inclusion of the GAALIE  
323 mutation in a transgenic humanized FcγR mouse model of influenza infection demonstrated superior  
324 efficacy of the GAALIE-containing antibody in both therapeutic and prophylactic experiments<sup>26</sup>. These  
325 effects were mediated by protective CD8<sup>+</sup> T cell responses elicited by the GAALIE antibody. Clinical  
326 data examining the contribution of the adaptive immune response in SARS-CoV-2 infection indicate that  
327 poor T cell induction correlates with severe disease (reviewed in <sup>56</sup>). Thus, the potential for VIR-7832 to  
328 augment the T cell response to SARS-CoV-2 infection could conceivably play a crucial role in limiting  
329 progression to severe COVID-19 disease or in treatment of severe established disease. This latter  
330 possibility is supported by recent publications showing that monoclonal antibodies with effector functions  
331 are especially effective in the therapeutic setting via recruitment of tissue-protective monocyte functions  
332 <sup>19</sup>, and that potency of antibodies in the pre-clinical mouse model does not correlate with in vitro  
333 neutralizing activity of antibodies<sup>18</sup>.

334 Taken together, these data indicate that VIR-7831 and VIR-7832 could play a powerful role in the fight  
335 against COVID-19 through the dual action of broadly neutralizing activity paired with engagement of the  
336 immune system through effector function capabilities.

337

## 338 **METHODS**

339 **Cells.** Vero E6 cells (ATCC) and Lenti-X 293T cells (Takara) were cultured in Dulbecco's Modified  
340 Eagle's medium (DMEM), 10% FBS, 1x Penicillin-Streptomycin at 37°C, 5% CO<sub>2</sub>.

341 **Monoclonal Antibodies.** VIR-7831 and VIR-7832 were produced at WuXi Biologics (China). SGHmAb-  
342 no-LS, S309-LS, and S309-GRLR were produced at Humabs Biomed SA, a subsidiary of Vir

343 Biotechnology (Bellinzona, Switzerland) in expiCHO cells transiently co-transfected with plasmids  
344 expressing the heavy and light chain, as previously described <sup>57</sup>.

345 **Virus.** SARS-CoV-2 isolates USA-WA1/2020, UK/VUI/3/2020, hCoV-19/South Africa/KRISP-  
346 K005325/2020 and hCoV-19/Japan/TY7-503/2021 were obtained from BEI Resources. To propagate  
347 SARS-CoV-2, VeroE6 or VeroE6-TMPRSS2 cells were seeded at  $10 \times 10^6$  cells in T175 flasks in growth  
348 media and infected the next day at a MOI of 0.001 in virus propagation media. Virus was adsorbed for 1  
349 hour at 37°C. Virus inoculum was removed, flasks were washed once with PBS, 25 mL of infection  
350 media was added to the cells and flasks were incubated at 37°C. Supernatants were collected at 48 hours  
351 post-infection once cytopathic effect was visible, centrifuged at 500 x g for 5 minutes, followed by a  
352 second centrifugation at 1000 x g for 5 minutes. Clarified supernatants were then aliquoted and stored at -  
353 80°C. Virus titers were determined using a plaque assay on VeroE6 cells, using standard methods.  
354 Briefly, 10-fold dilutions of virus stock were incubated in 6 well plates with 2.4% colloidal cellulose  
355 overlay for 24 hours. Cells were fixed with 4% PFA for 30 minutes at room temperature (RT),  
356 permeabilized with 0.125% Triton X-100, stained with anti-SARS-CoV-2 nucleocapsid antibody at  
357 1:5000 and goat anti-rabbit IgG HRP at 1:5000. Plaque forming units (PFU) were visualized with  
358 TrueBlue reagent.

359 **In vitro binding ELISA.** For the ELISA assay, 96-well plates were coated with 100 µl/well recombinant  
360 SARS-CoV2 RBD diluted in assay diluent (1% BSA/PBS) at a final concentration of 2 µg/mL and  
361 incubated overnight at 4°C. Plates were washed three times with 300 µl/well wash buffer using an  
362 automated washer. Assay diluent (100 µl/well) was added to block the plates and incubated for 1 hour at  
363 room temperature (RT) with shaking. Assay diluent was removed, and plates washed three times with  
364 wash buffer. Serial 1:3 dilutions of mAb (concentration range from 6 µg/mL to 0.33 ng/mL) in assay  
365 diluent were dispensed at 100 µl/well and incubated 1 hour at RT with shaking, then washed three times  
366 with wash buffer. The HRP-conjugated secondary antibody reagent (1:5,000 dilution in assay diluent)  
367 was added to each well (100 µl/well) and incubated for 1 hour at RT with shaking. After three washes



368 with wash buffer, 100  $\mu$ l/well of 2-component TMB peroxidase substrate solution was dispensed in each  
369 well and developed for 5 minutes at RT. The reaction was stopped with 100  $\mu$ L/well 1M H<sub>2</sub>SO<sub>4</sub> and the  
370 OD was read immediately at 450 nm on a SpectraMax M5 Microplate reader. EC<sub>50</sub> values were calculated  
371 using non-linear regression of log (agonist) versus response in Graph Pad Prism.

372 **Spike binding affinity quantification by SPR.** Antibody was diluted to 2  $\mu$ g/mL (1 mL) in HBS-EP+  
373 buffer and injected at 10  $\mu$ L/min for 30 seconds across one flow cell of a CM5 sensor chip immobilized  
374 with anti-human Fc antibody docked in a Biacore T200. SARS-CoV2-RBD diluted in HBS-EP+ buffer  
375 was then injected at a single concentration, 1:3 dilutions from 100 nM to 3.7 nM, across both the flow cell  
376 containing captured the antibody as well as a reference flow cell containing only anti-human Fc antibody.  
377 Binding was measured with a flow rate of 30  $\mu$ L/min and an injection time of 600 seconds; dissociation  
378 was monitored for 1800 seconds after injection. Data were collected at 10 Hz. After each binding  
379 measurement, regeneration reagent was injected to prepare the surface for a new cycle. Experiments were  
380 performed at 25°C, with the samples held at 15 °C in the instrument prior to injection.

381 **Measurement of Binding to Human Fc $\gamma$  Receptors by SPR.** Binding of VIR-7831 and VIR-7832 to  
382 human recombinant Fc $\gamma$ Rs was measured by surface plasmon resonance (SPR) on a Biacore T200.  
383 Briefly, Biotin CAPture Reagent (modified streptavidin) was injected across all flow cells of a CAP  
384 sensor chip docked in a Biacore T200. Biotinylated Fc receptors at 1  $\mu$ g/mL were injected across a single  
385 flow cell at 10  $\mu$ L/min for 60 seconds (one receptor per flow cell), with one flow cell reserved as a  
386 reference surface. VIR 7831 or VIR-7832 at 100  $\mu$ g/mL (diluted in HBS-EP+) were injected across all  
387 flow cells for 200 seconds using a flow rate of 30  $\mu$ L/min and association was monitored. Dissociation  
388 was monitored for another 200 seconds after injection. Data was collected at 10 Hz. After each binding  
389 measurement, CAP Regeneration reagent was injected to prepare the surface for a new cycle.  
390 Experiments were performed at 25°C, with the samples held at 15°C in the instrument prior to injection.

391 **Measurement of Binding to Human Complement Protein C1q.** Binding of VIR-7831 and VIR-7832 to  
392 human complement was measured by biolayer interferometry (BLI) using an Octet Red96 instrument

393 (FortéBio). Briefly, anti-human Fab (CH1-specific) sensors were used to capture VIR-7831 and VIR-  
394 7832 at 10 µg/ml for 10 minutes. The IgG-loaded sensors were then exposed to kinetics buffer containing  
395 3 µg/ml of purified human C1q for 4 minutes, followed by a dissociation step in the same buffer for  
396 additional 4 minutes. Association and dissociation profiles were measured in real time as changes in the  
397 interference pattern.

398 **Binding to Cell Surface Expressed SARS-CoV-2 Spike Protein.** The SARS-CoV-2 spike protein  
399 coding sequence (YP\_009724390.1, Wuhan-Hu-1 strain) was cloned into a cell expression plasmid under  
400 the control of the human CMV promoter (pHCMV1) to generate pHCMV1 WT spike. ExpiCHO-S cells  
401 were seeded the day before transfection at  $3 \times 10^6$  cells/mL in ExpiCHO Expression Medium.  
402 Immediately before transfection, the cells were seeded at  $6 \times 10^6$  cells/mL in a volume of 15 mL in  
403 125 mL shake flasks. Six µg of pHCMV1 WT spike plasmid or vector control were diluted in 1.2 mL of  
404 iced OptiPRO SFM., followed by addition of 48 µL of ExpiFectamine CHO Reagent and complexing for  
405 1 minute at RT. The transfection mixture was added dropwise to cells with gentle swirling. Cells were  
406 then incubated at 37°C, 8% CO<sub>2</sub> with shaking for 42 hours. At 42 hours post-transfection, ExpiCHO-S  
407 cells were harvested, washed twice with FACS buffer and resuspended at a concentration of  $1.0 \times 10^6$   
408 cell/mL in PBS. Cells ( $5 \times 10^4$  cells in 50 µL/wells) were dispensed into a 96-well V-bottom plate.  
409 Antibody was serially diluted (1:4, 10 points) starting at a concentration of 10 µg/mL. Cells were pelleted  
410 at 300 x g for 5 minutes and resuspended in 50 µL/well of antibody serial dilutions and plates were  
411 incubated for 45 mins on ice. Cells were washed twice in FACS buffer. Alexa Fluor 647-labelled Goat  
412 Anti-Human IgG secondary Ab was diluted 1:750 in FACS buffer and 50 µL was added to the cell pellet  
413 for 15 min on ice. Cells were washed twice with FACS buffer, resuspended in 1% PFA. Data was  
414 acquired by flow cytometry (CytoFlex LX).

415 **Pseudotyped virus production.** Lenti-X™ 293T cells were seeded in 10-cm dishes for 80% next day  
416 confluency. The next day, cells were transfected with the plasmid pcDNA3.1(+)-spike-D19 (encoding the  
417 SARS-CoV-2 spike protein) or pcDNA3.1(+)-spike-D19 variants using the transfection reagent TransIT-

418 Lenti according to the manufacturer's instructions. One day post-transfection, cells were infected with  
419 VSV-luc (rVSVΔG; Kerafast) at an MOI of 3. The cell supernatant containing SARS-CoV-2 pseudotyped  
420 virus was collected at day 2 post-transfection, centrifuged at 1000 x g for 5 minutes to remove cellular  
421 debris, aliquoted and frozen at -80°C.

422 **In Vitro Neutralization of SARS-CoV-2 Pseudotyped Virus.** VeroE6 cells were seeded into flat  
423 bottom tissue culture 96-well plates at 20,000 cells/well and cultured overnight at 37°C. Twenty-four  
424 hours later, 9-point 1:4 serial dilutions of VIR-7831 were prepared in infection medium and each dilution  
425 was tested in triplicate per plate (range: 20,000 to 0.3 ng/mL final concentration). SARS-CoV-2 virus  
426 stock was diluted in infection media for a final concentration of 2000 plaque forming units per well (MOI  
427 0.1). Antibody dilutions were added to virus and incubated for 30 minutes at 37°C. Media was removed  
428 from the VeroE6 cells, mAb-virus complexes were added, and cells were incubated at 37°C. At 6 hours  
429 post-infection, cells were fixed with 250 µL 4% PFA, incubated for 30 minutes at RT, then washed 3  
430 times with PBS to remove residual PFA. The cells were permeabilized with 50 µL of 0.125% Triton X-  
431 100 in PBS for 30 minutes at RT. The blocking buffer was removed, 50 µL of SARS-CoV-2 nucleocapsid  
432 antibody at 1:2,000 in blocking buffer was added, and plate was incubated for 1 hour at RT. Plates were  
433 washed three times with PBS and then incubated for 1 hour at RT with 50 µL/well of goat anti-rabbit-  
434 Alexa647 secondary antibody at a final dilution of 1:1,000 mixed with 2 µg/mL Hoechst dye in blocking  
435 buffer. After washing 5 times with PBS, 100 µL of fresh PBS was added for imaging. Plates were imaged  
436 on a Cytation5 plate reader. Whole well images were acquired (12 images at 4X magnification per well)  
437 and nucleocapsid-positive cells were counted using the manufacturer's software.

438 **Live virus neutralization.** VeroE6 cells were seeded into flat bottom tissue culture 96-well plates at  
439 20,000 cells/well and cultured overnight at 37°C. Twenty-four hours later, 9-point 1:4 serial dilutions of  
440 VIR-7831 were prepared in infection medium and each dilution was tested in triplicate per plate (range:  
441 20,000 to 0.3 ng/mL final concentration). SARS-CoV-2 virus stock was diluted in infection media for a  
442 final concentration of 2000 plaque forming units per well (MOI 0.1). Antibody dilutions were added to

443 virus and incubated for 30 minutes at 37°C. Media was removed from the VeroE6 cells, mAb-virus  
444 complexes were added, and cells were incubated at 37°C. At 6 hours post-infection, cells were fixed with  
445 250 µL 4% PFA, incubated for 30 minutes at RT, then washed 3 times with PBS to remove residual PFA.  
446 The cells were permeabilized with 50 µL of 0.125% Triton X-100 in PBS for 30 minutes at RT. The  
447 blocking buffer was removed, 50 µL of SARS-CoV-2 nucleocapsid antibody at 1:2,000 in blocking buffer  
448 was added, and plate was incubated for 1 hour at RT. Plates were washed three times with PBS and then  
449 incubated for 1 hour at RT with 50 µL/well of goat anti-rabbit-Alexa647 secondary antibody at a final  
450 dilution of 1:1,000 mixed with 2 µg/mL Hoechst dye in blocking buffer. After washing 5 times with PBS,  
451 100 µL of fresh PBS was added for imaging. Plates were imaged on a Cytation5 plate reader. Whole well  
452 images were acquired (12 images at 4X magnification per well) and nucleocapsid-positive cells were  
453 counted using the manufacturer's software..

454 **Determination of Viral Titer by Focus-Forming Assay.** One day prior to infection,  $1.2 \times 10^4$  VeroE6  
455 cells were plated in black-walled, clear bottomed 96-well plates. Virus samples were diluted 1:5 in  
456 infection media and adsorbed onto VeroE6 cells for one hour at 37°C. The cells were washed once and  
457 overlaid with 1% methylcellulose/serum-containing media. At 24 hours post-infection, the  
458 methylcellulose overlay was removed, and cells were washed with PBS. Cells were fixed with 4% PFA,  
459 incubated for 30 minutes at RT, then washed with PBS to remove residual PFA. The cells were  
460 permeabilized with 50 µL of 0.25% Triton X-100 in PBS for 30 minutes at RT. The Triton X-100 was  
461 removed, cells were washed twice with PBS, and incubated with 50 µL of SARS-CoV-2 nucleocapsid  
462 antibody at 1:2,000 in blocking buffer for one hour at RT. Plates were washed three times with PBS and  
463 then incubated for one hour at RT with 50 µL/well of goat anti-rabbit-Alexa647 secondary antibody at  
464 1:1,000 in blocking buffer. After washing three times with PBS, 50 µL of Hoechst dye at 1:1,000 in PBS  
465 was added for imaging. Plates were imaged on a Cytation5 plate reader. Whole well images were  
466 acquired (12 images at 4X magnification per well) and nucleocapsid-positive foci were counted using the  
467 manufacturer's software and used to determine focus-forming units/mL supernatant (FFU/mL).

468 **Determination of mAb-Dependent Activation of Human FcγRIIa, FcγRIIIa or FcγRIIb.** VIR-7831,  
469 VIR-7832, S309-LS, and a control mAb with abrogated FcγR binding, S309-GRLR, were serially diluted  
470 6-fold in assay buffer from 10,000 ng/ml to 0.006 ng/ml. Nine-point serial dilutions of mAbs were  
471 incubated with 12,500 (for FcγRIIIa and FcγRIIb) or 10,000 (for FcγRIIa) CHO-CoV-2-Spike cells per  
472 96-plate well in a white, flat-bottom plate for 15 minutes at room temperature. Jurkat effector cells  
473 expressing indicated FcγRs and stably transfected with an NFAT-driven luciferase gene were thawed,  
474 diluted in assay buffer, and added to the plate at an effector to target cell ratio of 6:1 for FcγRIIIa and  
475 FcγRIIb or 5:1 for FcγRIIa. Control wells were also included that were used to measure antibody-  
476 independent activation (containing target cells and effector cells but no antibody) and background  
477 luminescence of the plate (wells containing assay buffer only). Plates were incubated for 18 hours at 37°C  
478 with 5% CO<sub>2</sub>. Activation of human FcγRs in this bioassay results in the NFAT-mediated expression of  
479 the luciferase reporter gene. Luminescence was measured with a luminometer after adding the Bio  
480 GloTM Luciferase Assay Reagent according to the manufacturer's instructions. To control for  
481 background, the mean of the relative luminescence units (RLU) values in wells containing only Assay  
482 Buffer was calculated and subtracted from all data points. Data were expressed as the average of RLUs  
483 over the background

484 **Determination of NK-Cell Mediated Antibody-Dependent Cellular Cytotoxicity.** Primary NK cell  
485 activation was tested using freshly isolated cells from two previously genotyped donors expressing  
486 homozygous low affinity (F158) or high affinity (V158) FcγRIIIa. Serial dilutions of mAbs (serially  
487 diluted 10-fold in AIM-V Medium from 40,000 ng/ml to 0.075 ng/ml) were incubated with 7,500 CHO-  
488 CoV-2 Spike cells per well of a 96 well round-bottom plate for 10 minutes. Target cell and antibody  
489 mixtures were then incubated with primary human NK cells as effectors at an effector-to-target ratio of  
490 10:1. ADCC was measured using lactate dehydrogenase (LDH) release as a readout according to the  
491 manufacturer's instructions (Cytotoxicity Detection Kit (LDH), Roche) after 4 hours of incubation at  
492 37°C. In brief, plates were centrifuged for 4 minutes at 400 x g, and 35 μl of supernatant was transferred

493 to a flat 384 well plate. LDH reagent was prepared and 35  $\mu$ l were added to each well. Using a kinetic  
494 protocol, the absorbance at 490 nm and 650 nm was measured once every 2 minutes for 8 minutes, and  
495 the slope of the kinetics curve was used as result. The percent specific lysis was determined by applying  
496 the following formula: (specific release – spontaneous release) / (maximum release - spontaneous release)  
497 x 100.

498 **Determination of Monocyte-Mediated Antibody-Dependent Cellular Phagocytosis.** ADCP assays  
499 were performed using human PBMCs freshly isolated from whole blood. CHO CoV-2-Spike cells were  
500 used as target cells and were fluorescently labeled with PKH67 Fluorescent Cell Linker Kit (Sigma  
501 Aldrich) prior to incubation with mAbs, according to manufacturer's instructions. Serial dilutions of  
502 mAbs (serially diluted 5-fold from 5,000 ng/ml to 0.32 ng/ml in RPMI-1640 + L-glutamine supplemented  
503 with 10% Hyclone FBS + 2x anti-anti (antibiotic-antimycotic)) were incubated with 10,000 CHO-CoV-2-  
504 Spike cells per well of a 96 well polypropylene plate for 10 minutes. Primary PBMCs were fluorescently  
505 labeled with Cell Trace Violet according to the manufacturer's instructions. Target cell and antibody  
506 mixtures were then incubated with labeled PBMCs at an effector-to-target ratio of 16:1. After an  
507 overnight incubation at 37°C, monocytes were stained with anti-human CD14-APC antibody (BD  
508 Pharmingen). Antibody-mediated phagocytosis was determined by flow cytometry, gating on CD14+  
509 cells that were double positive for cell trace violet and PKH67. Raw data were exported from the flow  
510 cytometer into the flow cytometry analysis software FlowJo v10 (Becton Dickinson) for gating and  
511 determination of the percentage of CD14+ cells that were also double positive for cell trace violet and  
512 PKH67. Cells expressing only cell trace violet or only PKH67 were used to set the positive staining gates.

513 **In vitro resistance selection.** The selection of variants in the presence of increasing concentrations of  
514 VIR-7832 was conducted in VeroE6 cells. The day before infection, 6 x 10<sup>4</sup> VeroE6 cells were seeded in  
515 24 well plates and incubated overnight at 37°C. The next day, 600 focus forming units (FFU) of SARS-  
516 CoV-2 virus (MOI = 0.01) was incubated with 0.5X IC<sub>50</sub> of VIR-7832 (0.05  $\mu$ g/mL) at 37°C for one hour  
517 in infection media. The mAb-virus complexes were adsorbed on VeroE6 cells for one hour at 37°C in

518 duplicate wells. After adsorption, cells were washed with DMEM and overlaid with infection media  
519 containing 0.05 µg/mL VIR-7832. Control wells infected without antibody were included with each  
520 passage. Infected cells were monitored visually for CPE daily. In general, when infected cells exhibited ≥  
521 50% CPE, the culture supernatants were harvested, diluted 1:200, and added to fresh VeroE6 cells in 24-  
522 well plates with equivalent or increasing concentrations of VIR-7832. At each passage, supernatant was  
523 aliquoted and frozen at -80°C for titer and neutralization analyses.

524 **In vitro assessment of potential for ADE.** VeroE6 cells were plated at  $1.25 \times 10^4$  cells/well one day prior  
525 to infection. For each independent experiment, moDCs and PBMCs from five unique moDC donors and  
526 six unique PBMC donors were used, with three unique donors used for each independent experiment.  
527 Cryopreserved monocytes from unique donors were differentiated into moDCs for six days using human  
528 moDC differentiation media according to the manufacturer's protocol. Cryopreserved PBMCs from  
529 unique donors are thawed in the presence 0.3 mg/mL DNase and cultured in media for one day prior to  
530 infection. On the day of infection, moDCs, PBMCs, and U937 cells were counted and plated at  $7.5 \times 10^4$   
531 cells/well.

532 To examine viral entry, 24 hours post-infection, cells were fixed with 4% PFA, incubated for 30 minutes  
533 at RT, then washed with PBS to remove residual PFA. The cells were permeabilized with 50 µL of 0.25%  
534 Triton X-100 in PBS for 30 minutes at RT. The Triton X-100 was removed, cells were washed twice with  
535 PBS, and incubated with 50 µL of SARS-CoV-2 nucleocapsid antibody at 1:2,000 in blocking buffer for  
536 one hour at RT. Plates were washed three times with PBS and then incubated for one hour at RT with 50  
537 µL/well of goat anti-rabbit-Alexa647 secondary antibody at 1:1,000 in blocking buffer. After washing  
538 three times with PBS, 50 µL of Hoechst dye at 1:1,000 in PBS was added for imaging. Plates were  
539 imaged on a Cytation5 plate reader. Whole well images were acquired (12 images at 4X magnification  
540 per well) and nucleocapsid-positive cells were counted using the manufacturer's software. The percent of  
541 nucleocapsid+ cells was quantified using the Gen5 Imager software (Biotek, Vermont) as number of

542 Cy5+ cells, [(nucleocapsid+ cells)/number of Hoechst+ cells (total cells)]x100. Data was analyzed using  
543 Prism v8.00 (GraphPad Software, La Jolla California USA, [www.graphpad.com](http://www.graphpad.com)).

544 In order to quantify chemokines and cytokines from supernatants in a BSL2 laboratory, supernatants were  
545 inactivated by 10 minutes exposure to UVC light at 5,000  $\mu\text{J}/\text{cm}^2$ . Supernatants were diluted 1:5 in  
546 infection media and levels of cytokines/chemokines were quantified using the U-plex 96-well assay  
547 according to the manufacturer's protocol (Meso Scale Diagnostics, Rockville, MD). Quantification of  
548 cytokines and chemokines were determined based on an 8-point standard curve in duplicate, provided by  
549 the manufacturer. Cytokine data was analyzed using the Discovery Workbench v4.0.13 software (Meso  
550 Scale Diagnostics). Data was graphed and statistical analyses were conducted using Prism software.

551 **Sequencing of SARS-CoV-2 Spike Gene.** To isolate nucleic acid from the supernatant of viral passages,  
552 120  $\mu\text{L}$  of cell supernatant was added to 360  $\mu\text{L}$  of Trizol and stored at  $-80^\circ\text{C}$  for further analysis. Trizol  
553 collected samples from viral passages where a shift in neutralization  $> 2$ -fold relative to wild type was  
554 detected were subjected to RNA isolation using PureLink RNA Mini Kit with the incorporation of on-  
555 column PureLink DNase Treatment, following manufacturer's instructions. Reverse transcription  
556 reactions were performed with 6  $\mu\text{L}$  of purified RNA and oligoT primers using the NEB ProtoScript II  
557 First Strand cDNA Synthesis kit, according to manufacturer's instructions. The resulting cDNA was used  
558 as a template for PCR amplification of the spike gene using the KapaBiosystems polymerase (KAPA HiFi  
559 HotStart ReadyMix) with primers 5' aattactcttgcaaacacg-3' and 5' tgaggcttgatcggtatcg-3'.

560 Amplification conditions included an initial 3 minutes at  $95^\circ\text{C}$ , followed by 28 cycles with 20 seconds at  
561  $98^\circ\text{C}$ , 15 seconds at  $62^\circ\text{C}$  and  $72^\circ\text{C}$  for 2 minutes, with a final 4 minutes at  $72^\circ\text{C}$ . PCR products were  
562 purified using AMPure XP beads following manufacturer's instructions. The size of the amplicon was  
563 confirmed by analyzing 2  $\mu\text{L}$  of PCR products using the Agilent D5000 ScreenTape System. Products  
564 were quantified by analyzing 1  $\mu\text{L}$  with the Quant-iT dsDNA High-Sensitivity Assay Kit. Twenty ng of  
565 purified PCR product was used as input for library construction using the NEBNext Ultra II FS DNA  
566 Library Prep kit following manufacturer's instructions. DNA fragmentation was performed for 13



567 minutes. NEBNext Multiplex Oligos for Illumina Dual Index Primer Set 1 was used for library  
568 construction, with a total of 6 PCR cycles. Libraries size was determined using the Agilent D1000  
569 ScreenTape System and quantified with the Quant iT dsDNA High-Sensitivity Assay Kit. Equal amounts  
570 of each library were pooled together for multiplexing and ‘Protocol A: Standard Normalization Method’  
571 of the Illumina library preparation guide was used to prepare 8 pM final multiplexed libraries with 1%  
572 PhiX spike-in for sequencing. The MiSeq Reagent Kit v3 (600-cycle) was used for sequencing the  
573 libraries on the Illumina MiSeq platform, with 300 cycles for Read 1, 300 cycles for Read 2, 8 cycles for  
574 Index 1, and 8 cycles for Index 2.

575 **Bioinformatics Analysis of Conservation.** Available genome sequences for SARS-CoV-2 were  
576 downloaded from Global Initiative on Sharing All Influenza Data (GISAID; <https://www.gisaid.org/>) on  
577 June 4, 2021. Bat and pangolin sequences were removed to yield human-only sequences. The spike open  
578 reading frame was localized by aligning the reference protein sequence (NCBI reference sequence:  
579 YP\_009724390.1) to the genomic sequence of isolates with Exonerate v.2.4.0. Coding nucleotide  
580 sequences were translated in silico using seqkit v.0.12.0. Multiple sequence alignment was performed  
581 using MAFFT v.7.455. Variants were determined by comparison of aligned sequences to the reference  
582 sequence using the R v3.6.3/Bioconductor v.3.10 package Biostrings v.2.54.0.

583 **In vivo studies.** Syrian golden hamster studies were conducted at Lovelace Biomedical (Albuquerque,  
584 NM). Twelve- to sixteen-week-old male hamsters were interperitoneally administered a non-LS version  
585 of VIR-7831 (SGHmAb-no-LS), control antibody or diluent Day -1 or Day -2 prior to virus challenge.  
586 Animals were inoculated intranasally at Day 0 with  $7.4 \times 10^4$  TCID<sub>50</sub> with SARS-CoV-2 (isolate USA-  
587 WA1/2020). Animals were also weighed once daily in the morning beginning on study Day -10 and  
588 continuing until the end of the study. Following euthanasia, RT-qPCR was performed on lung  
589 homogenates using quantitative real-time PCR methods targeting the SARS-CoV-2 N gene and the  
590 median tissue culture infections dose (TCID<sub>50</sub>) was determined per Lovelace internal methodology.

591 **Author Contributions**

592 Conceived studies: A.L.C, C.H-D., F.A.P, D.M., M.S., L.S., A.T., L.A.P., S.H., G.S., H.W.V., D.C., C.M.H.  
593 Designed studies and experiments: A.L.C, C.H-D., F.A.P, D.M., M.S., M.L.A., E. D., B.G., J.D., L.R.,A.C., A.S.,  
594 R.S., J.W., N.C., E.C., S.L., C.C., D.P., C.S., J.N., A.P., A.W., L.S., A.T., L.A.P., S.H., G.S., H.W.V., D.C., C.M.H.  
595 Performed experiments: D.M., M.S., M.L.A., B.G., J.D., E.D., A.S., L.R., H.T., J.D., S.S., D.P., C.S., J.N., B.S.,  
596 S.B., J.W., J.Z., H.K., A.C., M.M-R., A.P., A.W., N.C., E.C. Analyzed and interpreted data: A.L.C., C.H-D., F.A.L.,  
597 D.M., M.S., M.L.A., B.G., J.D., D.P., C.S., J.N., E.L., A.S., R.S., L.R., H.T., B.S., S.B., J.W., J.Z., H.K., A.C., M. M-  
598 R., N.C., E.C., S.L., A.W., C.C., L.S., A.T., S.H., G.S., H.W.G, D.C., C.M.H. Prepared the manuscript with input  
599 from all authors: A.L.C., G.S., A.T., L.P., D.C., H.W.G., C.M.H.

#### 600 **Competing interests**

601 Some authors are current or former employees of Vir Biotechnology or Humabs BioMed SA (a fully-  
602 owned subsidiary of Vir Biotechnology) and may hold shares in Vir Biotechnology. H.W.V. is a founder of  
603 PierianDx and Casma Therapeutics.

604

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628 [emerging-brazilian-variants-harboring-mutations-e484k-and-n501y-in-the-spike-protein/585](https://virological.org/t/phylogenetic-relationship-of-sars-cov-2-sequences-from-amazonas-with-emerging-brazilian-variants-harboring-mutations-e484k-and-n501y-in-the-spike-protein/585)  
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745

## 746 **FIGURE LEGENDS**

747 **Figure 1.** VIR-7831 and VIR-7831 bind S and neutralize SARS-CoV-2 virus and S variants of concern in  
748 vitro. a) Binding of VIR-7831 (black circles) and VIR-7832 (blue squares) to SARS-CoV-2 RBD was  
749 tested by ELISA. Shown is the average of four replicates and SD derived from three independent  
750 experiments. b) Association and dissociation profiles of VIR-7831 to SARS-CoV-2-RBD were measured  
751 using SPR. The double reference subtracted curves (shown for single replicates) are plotted together with  
752 the curve fit in black (obscured by close overlay with the data). Values are from two independent  
753 experiments. c) Binding of VIR-7831 (black circles) and VIR-7832 (blue squares) to cell-surface S  
754 protein was determined by flow cytometry. Data are expressed as the percentage of the positive cells.  
755 Results shown are from one experiment and representative of three independent experiments performed.  
756 d) In vitro neutralization of live SARS-CoV-2 by different concentrations of VIR-7831 (left) and VIR-  
757 7832 (right) measured by nucleocapsid staining 6-hours post-infection. Results shown are from one  
758 experiment and representative of at least three independent experiments performed.

759 **Figure 2.** VIR-7831 and VIR-7832 demonstrate effector function in vitro. In vitro effector function (a-e)  
760 activation profiles of human Fc $\gamma$ RIIa (a), Fc $\gamma$ RIIb (b), Fc $\gamma$ RIIIa low-affinity (F158) (c) or Fc $\gamma$ RIIIa high-  
761 affinity binding allele (V158) (d) using bioreporter assays using S-expressing CHO cells as the target

762 antigen. Data points show means $\pm$  SD of duplicates. NK-cell mediated killing (ADCC) of S-expressing  
763 CHO cells using freshly isolated cells from two donors previously genotyped for homozygous expression  
764 of low-affinity (F/F158) (e) or high-affinity (V/V158) Fc $\gamma$ RIIIa (f). Data points are means of  
765 quadruplicates  $\pm$  SD. g) Antibody-dependent cellular phagocytosis (ADCP) using S-expressing CHO cells  
766 and freshly isolated PBMCs. Data represent the means of duplicates  $\pm$  SD.

767 **Figure 3.** VIR-7831 shows in vivo efficacy in a hamster SARS-CoV-2 model of infection. a) Overview  
768 of hamster in vivo study design. b) and c) Animal weight over time as a percent of starting weight in  
769 animals dosed a Day -1 (b) or Day -2 (c). Medians of at least N=6 animals and interquartile range are  
770 shown. d) and e) Day 4 terminal weights expressed as a percentage of starting weight for animals dosed at  
771 Day -1 (d) or Day -2 (e). Bar denotes median values. f) and g) Day 4 lung viral load in Day -1 (f) or Day -  
772 2 (g) treated animals as assessed by RT-qPCR. Bar denotes median values. h) and i) infectious virus in  
773 lung at Day 4 for Day -1 (h) or Day -2 (i) dosed animals. Bar denotes median values. ns=not significant,  
774 \*\* =  $p < 0.05$ , and \*\*\* =  $< 0.005$  as assessed by the Mann-Whitney U-test.

775 **Table 1.** VIR-7831 and VIR-7832 retain activity against S variants of concern in an authentic virus  
776 system. Average fold change in VIR-7831 and VIR-7832 IC<sub>50</sub> compared to relative wild-type controls for  
777 S variants tested in an authentic virus system. Data shown are averages of at least two independent  
778 experiments.

779 **Table 2.** VIR-7831 and VIR-7832 retain activity against S variants of concern in a pseudotyped virus  
780 system. Average fold change in VIR-7831 and VIR-7832 IC<sub>50</sub> compared to relative wild-type controls for  
781 S variants tested in a VSV/VeroE6 pseudotyped virus system. Data shown are averages of at least two  
782 independent experiments.

783 **Table 3.** VIR-7831 and VIR-7832 retain activity against variants that confer resistance to authorized  
784 mAbs. Activity of VIR-7831 against variants conferring reduced susceptibility to bamlanivimab,  
785 imdevimab or casirivimab in a VSV/VeroE6 pseudotyped virus system. The geometric mean of IC<sub>50</sub>s and



786 average fold-change versus the relative wild-type control from at least two independent experiments are  
787 shown.

788 **Table 4.** The VIR-7831/VIR-7832 epitope is highly conserved. Conservation data comprising >1,700,000  
789 sequences from the GISAID database and variants at each position are shown. Variants in bold were  
790 tested in a pseudotyped virus assay.

791 **Table 5.** Activity of VIR-7831 against epitope variants. VIR-7831/VIR-7832 epitope variants detected in  
792 sequences from the GISAID database were tested in a VSV/VeroE6 pseudotyped virus system. The  
793 geometric mean of IC<sub>50</sub>s and average fold-change versus the relative wild-type control from at least two  
794 independent experiments are shown. Variants marked with “a” indicates that the data shown are from the  
795 parental antibody S309.

796 **Supplemental Figure 1.** Binding of VIR-7831 and VIR-7832 to human FcγRs and C1q as measured by  
797 SPR. Binding of VIR-7831 and VIR-7832 to a) human FcγRIIa (H131 and R131 alleles), FcγRIIIa (F158  
798 and V158 alleles) and FcγRIIb were measured using SPR. Biotinylated purified FcγRs were captured on  
799 the sensor chip surface prior to injection of VIR-7831 or VIR-7832. Association and dissociation profiles  
800 (separated by the vertical dotted line) were measured in real time as change in the SPR signal. b) Binding  
801 of VIR-7831 and VIR-7832 to complement component C1q was measured using BLI on an Octet Red96  
802 instrument. Association and dissociation profiles (separated by the vertical dotted line) were measured in  
803 real time as change in the interference pattern.

804 **Supplemental Figure 2.** Sub-neutralizing concentrations of VIR-7831 and VIR-7832 do not enhance  
805 viral entry, viral replication or cytokine production in vitro. Internalization (a) and replication (b) of  
806 SARS-CoV-2 was evaluated in VeroE6, moDCs or PBMCs at various timepoints. Two independent  
807 experiments with human moDCs and PBMCs from three individual donors were analyzed (5 unique  
808 moDC donors, 6 unique PBMC donors total between two experiments). VeroE6 cells were run in  
809 duplicate for both independent experiments. Data from each replicate well from two independent

810 experiments are plotted as individual points, with horizontal lines representing the median. Mann-  
811 Whitney U-test comparison to no antibody group, \* $p < 0.05$ . c) Supernatant cytokine and chemokine levels  
812 as measured by MSD at the indicated time post infection. Data from two independent experiments (three  
813 replicates each, five unique donors) are plotted as the mean and SD.

814 **Supplemental Figure 3.** Overview of VIR-7832 resistance selection method. All passaging was  
815 conducted in duplicate wells. (a) VIR-7832 concentration was increased during each passage. P3 X  
816 indicates passage 3 virus, after which virus was lost with subsequent increases in concentration. In (b) and  
817 (c), p3X denotes where passage 3 virus from (a) was used to initiate (b) viral lineage 1 and (c) viral  
818 lineage 2. Arrows indicate passages that were subjected to sequence analysis, and \* indicate the passages  
819 in lineage 1 with no detectable virus or CPE. Selection continued for a total of eight passages.

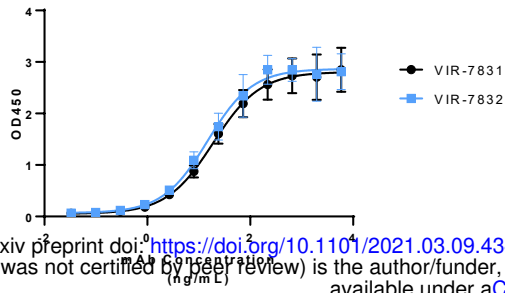
820 **Supplemental Table 1.** Amino acid substitutions identified in the SARS-CoV-2 S upon in vitro selection  
821 with VIR-7832. Spike gene sequences were compared to a SARS-CoV-2 reference sequence (NCBI:  
822 NC\_045512.2) to identify variants. Fold-changes in  $IC_{50}$  were determined compared to the SARS-CoV-2  
823 virus stock.

824 **Supplemental Table 2.** VIR-7831 and VIR-7832 activity against selected S variants. VIR-7831/VIR-  
825 7832 epitope variants observed by in vitro resistance selection were individually tested in a VSV/VeroE6  
826 pseudotyped virus system. The geometric mean of  $IC_{50}$ s and average fold-change versus the relative wild-  
827 type control from at least two independent experiments are shown.

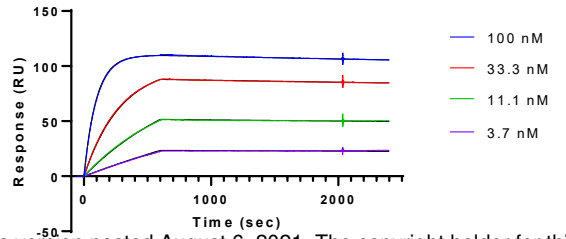
828

# Figure 1

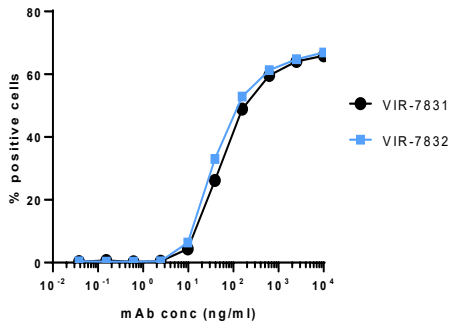
a)



b)



c)



d)

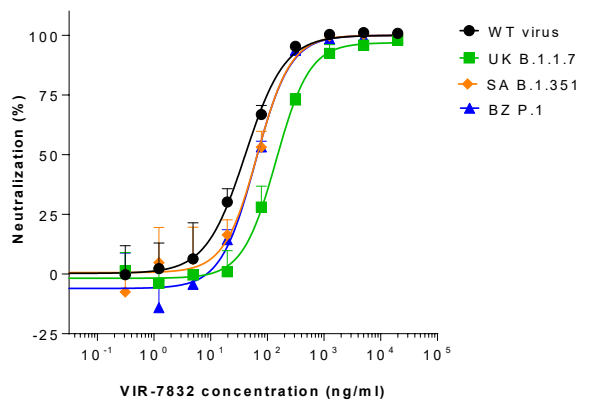
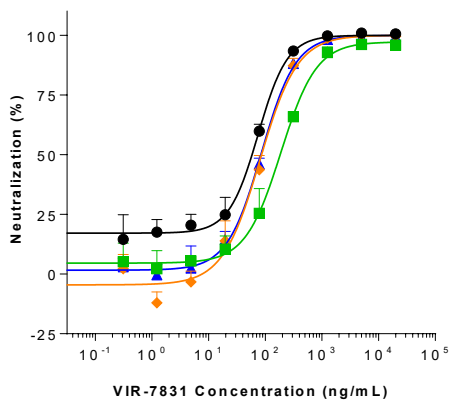


Figure 2

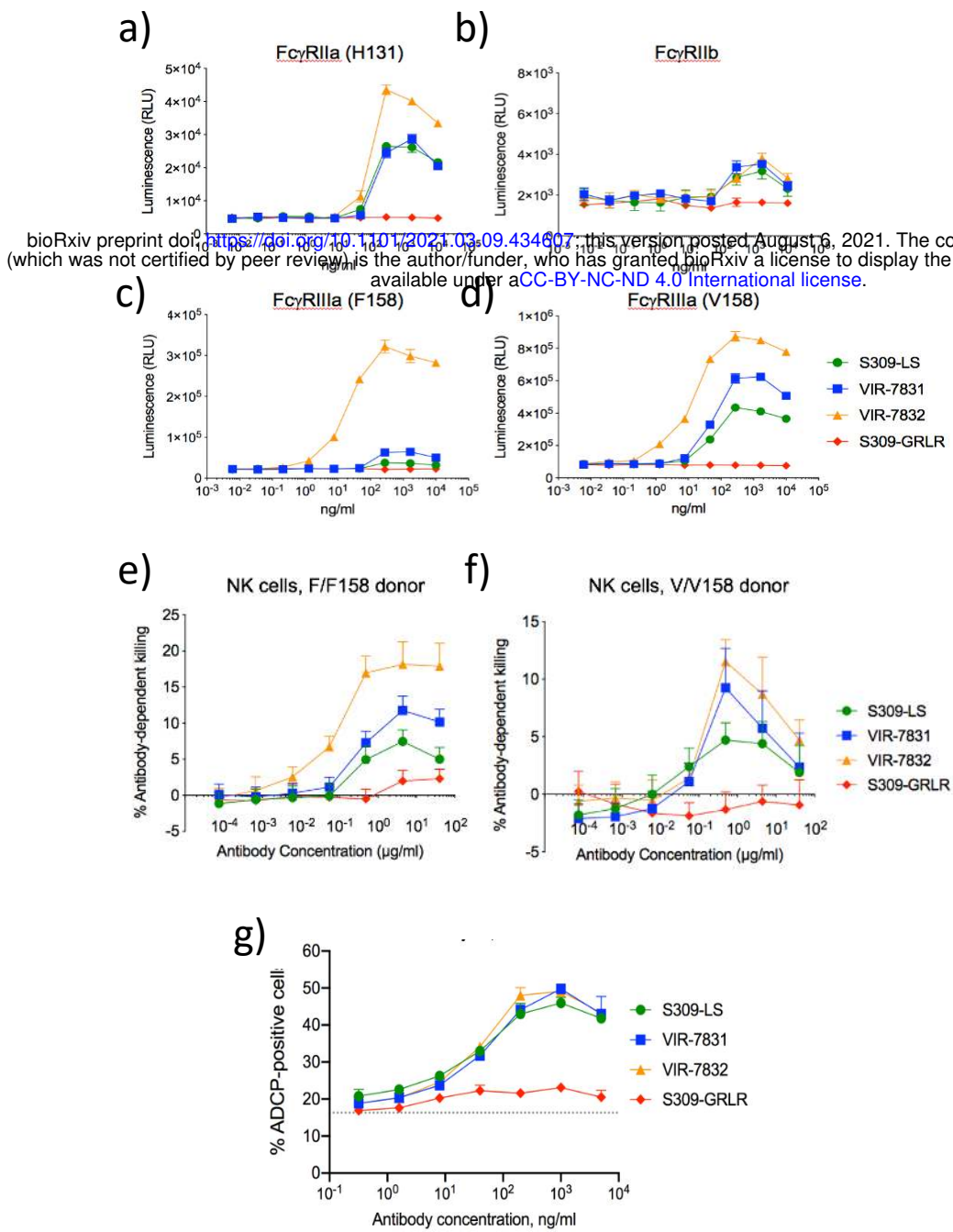


Figure 3

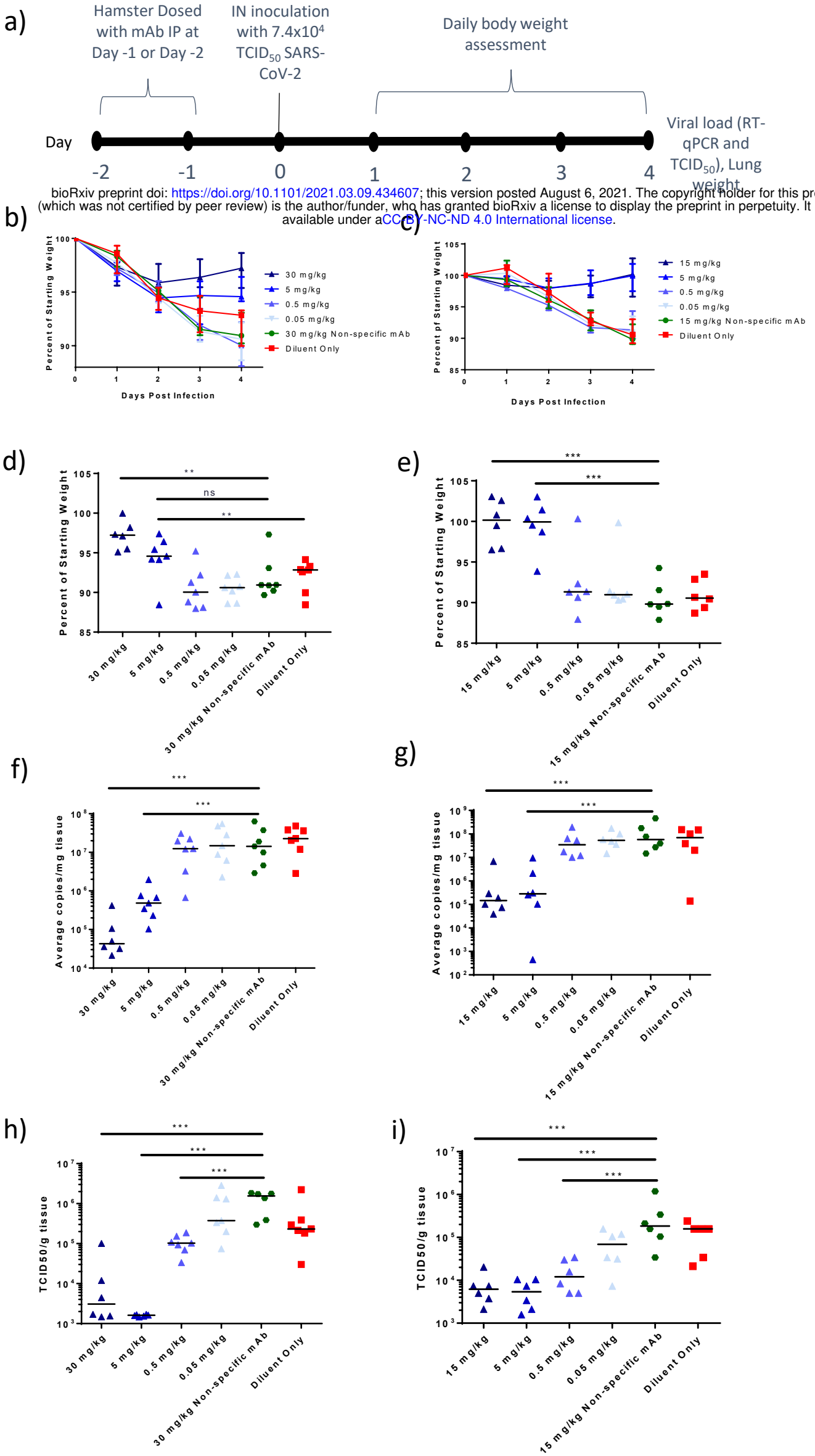


Table 1

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<b>SARS-CoV-2 Variant</b>	<b>Geometric Mean VIR-7831 IC<sub>50</sub> (ng/ml) (Average Fold Change IC<sub>50</sub> vs. Wild-Type)</b>	<b>Geometric Mean VIR-7831 IC<sub>90</sub> (ng/ml) (Average Fold Change IC<sub>90</sub> vs. Wild-Type)</b>	<b>Geometric Mean VIR-7832 IC<sub>50</sub> (ng/ml) (Average Fold Change IC<sub>50</sub> vs. Wild-Type)</b>	<b>Geometric Mean VIR-7832 IC<sub>90</sub> (ng/ml) (Average Fold Change IC<sub>90</sub> vs. Wild-Type)</b>
UK (B.1.1.7)	187.15 (3.0)	1246.86 (4.1)	181.12 (3.1)	1222.95 (3.7)
South Africa (B.1.351)	71.89 (1.2)	385.01 (1.3)	59.67 (1.1)	309.84 (0.9)
Brazil (P.1)	73.11 (1.6)	335.79 (1.4)	48.94 (1.2)	217.02 (0.9)

Table 2

Variant	Spike Mutations	Fold-Change in VIR-7831 IC <sub>50</sub> vs. Wild-type	Fold-Change in VIR-7832 IC <sub>50</sub> vs. Wild-type
Alpha (B.1.1.7)	H69-, V70-, Y144-, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H	2.3	2.5
Beta (B.1.351)	L18F, D80A, D215G, R246I, K417N, E484K, N501Y, D614G, A701V	0.6	0.7
Gamma (P.1)	D138Y, D614G, E484K, H655Y, K417T, L18F, N501Y, P26S, R190S, T1027I, T20N, V1176F	0.4	0.4
Delta (B.1.617.2)	T19R, G142D, E156G, F157-, R158-, L452R, T478K, D614G, P681R, D950N	1	NT
Epsilon (B.1.427/B.1.429)	S13I, W152C, L452R, D614G	0.7	NT
Eta (B.1.525)	Q52R, A67V, H69-, V70-, Y144-, E484K, D614G, Q677H, F888L	0.9	NT
Iota (B.1.526)	L5F, T95I, D253G, E484K, D614G, A701V	0.6	NT
Kappa (B.1.617.1)	T95I, G142D, E154K, L452R, E484Q, D614G, P681R, Q1071H	0.7	NT
Lambda (C.37)	G75V, T76I, del246-252, L452Q, F490S, T859N	1.5	NT
Delta Plus (AY.1)	T19R, T95I, G142D, E156G, F157-, R158-, W258L, K417N, L452R, T478K, D614G, P681R, D950N	1.1	NT
Delta Plus (AY.2)	T19R, V70F, G142D, E156G, F157-, R158-, A222V, K417N, L452R, T478K, D614G, P681R, D950N	1.3	NT
Mexico/Swiss (B.1.1.519)	T478K, D614G, P681H, T732A	0.8	NT
Scotland (B.1.258)	H69-, V70-, N439K, D614G	0.9	NT
US (R.2)	E484K, D614G, Q677H, T732S, E1202Q	0.8	NT
Liverpool (A.23.1)	R102I, F157L, V367F, E484K, Q613H, P681R	1.1	NT
Cameroon (B.1.619)	I210T, N440K, E484K, D614G, D936N, S939F, T1027I	1.3	NT
Bristol (B.1.1.7+E484K)	H69-, V70-, Y144-, E484K, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H	1.7	NT

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Table 3

Amino Acid position	Substitution / Deletion	mAb with Reduced Susceptibility	Variants in Tested Spike Sequence	VIR-7831 EC <sub>50</sub> (ng/ml)	Average Fold Change in EC <sub>50</sub> Compared to Relative Wild-Type
E406	W	casirivimab, imdevimab		3.66	0.74
K417	E	casirivimab	K417E	67.71	0.89
N439	K	imdevimab	N439K, D614G	17.05	0.86
N440	D	imdevimab	N440D	80.47	1.29
N440	K	imdevimab	N440K, D614G	19.99	0.48
K444	Q	imdevimab	K444Q	79.68	1.11
V445	A	imdevimab	V445A	41.74	3.38
G446	V/I	imdevimab	G446V, D614G	18.41	1.50
Y453	F	casirivimab	G261D, Y453F	27.28	2.19
L455	F	casirivimab	L455F, D614G	21.65	0.56
G476	S	casirivimab	G476S	36.97	2.94
E484	K	bamlanivimab	E484K, D614G	12.91	0.33
F486	V/I	casirivimab	F486V	82.24	1.10
Y489	H	casirivimab	Y489H	92.29	1.48
F490	S	bamlanivimab	F490S	33.10	0.85
Q493	K	casirivimab, bamlanivimab	Q483K	69.79	0.98
S494	P	casirivimab, bamlanivimab	S494P, D614G	29.10	2.50

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Table 4

Amino Acid Position	Reference Amino Acid <sup>a</sup>	Variants Identified in order of Prevalence <sup>b</sup>	Percent Reference AA Conservation
332	I	V, T	>99.99
333	T	I, K, A	>99.99
334	N	K, I, S, D, Y	99.99
335	L	F, S, V, M	99.99
336	C	S	>99.99
337	P	S, T, L, H, R, K	>99.99
339	G	D, S, V, C, F, N	99.98
340	E	D, K, G, A, Q	>99.99
341	V	I, A, F, P, S	>99.99
343	N	Y, K, S	>99.99
344	A	S, V, T, D, F, P	99.98
345	T	S, I, N	>99.99
346	R	S, K, I, T, G, F	99.89
354	N	K, D, S, H, I, T, Y, G	99.96
356	K	R, M, N, T, E, G, Q, W, Y, I	99.99
357	R	K, I, G, T, C, L	99.93
358	I	V, T, F, L, A, E	>99.99
359	S	T, N, G, R, C, F, I	99.98
360	N	Y, S, F, A, L, T	>99.99
361	C	T, G, F, R, N, S	>99.99
440	N	K, S, Y, T, D, I, H, F, R	99.83
441	L	R, F, I, V, Y, H, M, S, E, N	>99.99
509	R	K, T, I, S	>99.99

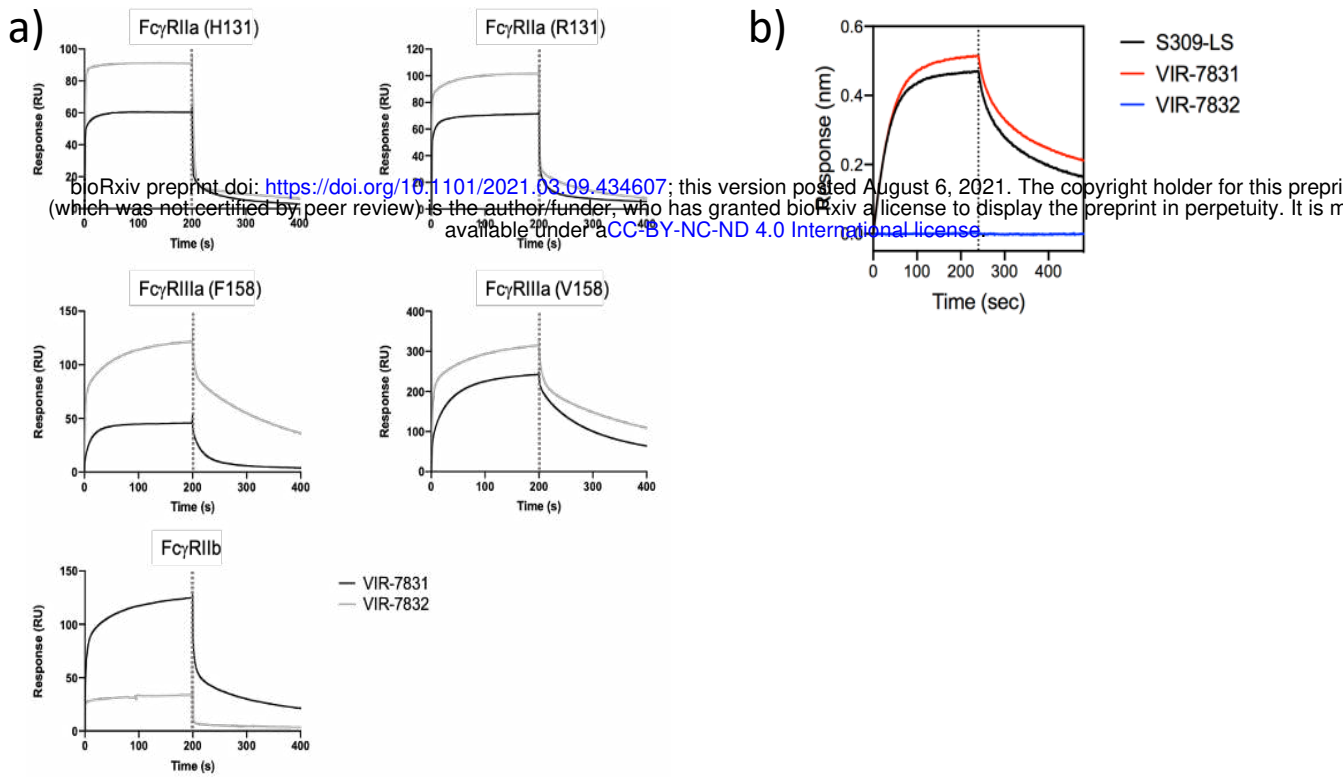
bioRxiv preprint doi: <https://doi.org/10.1101/2021.03.09.434607>; this version posted August 6, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Table 5

Epitope Reference Amino Acid	Amino Acid Changes in Spike protein	Geomean Neutralization EC <sub>50</sub> (ng/mL)	Average Fold-Change EC <sub>50</sub> Relative to Wild-Type
I332	I332V, D614G	53.43	1.48
N334	N334H, D614G	30.74	0.87
	N334Y, D614G	32.89	0.96
L335	L335S, D614G	29.80	0.85
P337	P337L, D614G	>10000	>192
	P337H, D614G	185.29	5.13
	P337T, D614G	383.27	10.62
	P337R, D614G	>10000	>192
G339	G339V, D614G	78.32	1.26
E340	E340Q, D614G	>2500	>50
	E340G, D614G	640.07	18.21
N343	N343S, D614G	16.87	0.48
A344	A344V, D614G	53.87	1.14
T345	T345S, D614G	42.92	0.69
	T345N, D614G	20.29	0.57
R346	R346G, D614G	43.73	0.85
N354	N354I, D614G	37.09	1.05
K356	K356M, D614G	64.09	1.03
	K356T, D614G	281.13	5.90
I358	I358F, D614G	42.69	1.19
S359	S359T, D614G	39.98	0.84
N360	N360S, D614G	34.08	0.72
	N360Y, D614G	29.67	0.61
N440	N440T, D614G	50.81	0.82
L441	L441R, D614G	42.30	0.87
	L441V, D614G	48.24	0.98

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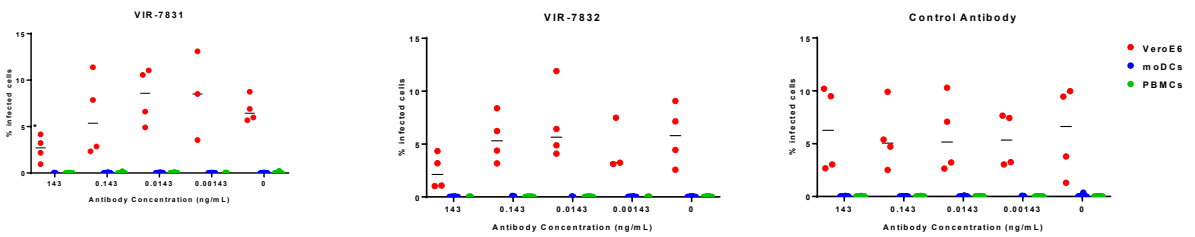
# Supplemental Figure 1



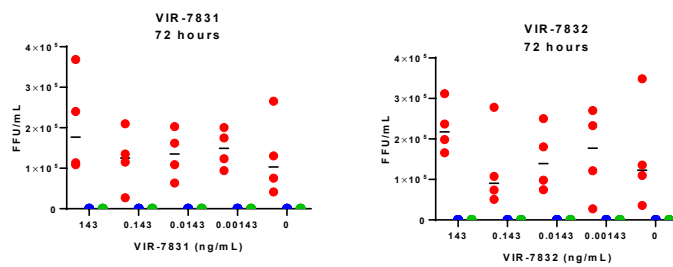
# Supplemental Figure 2

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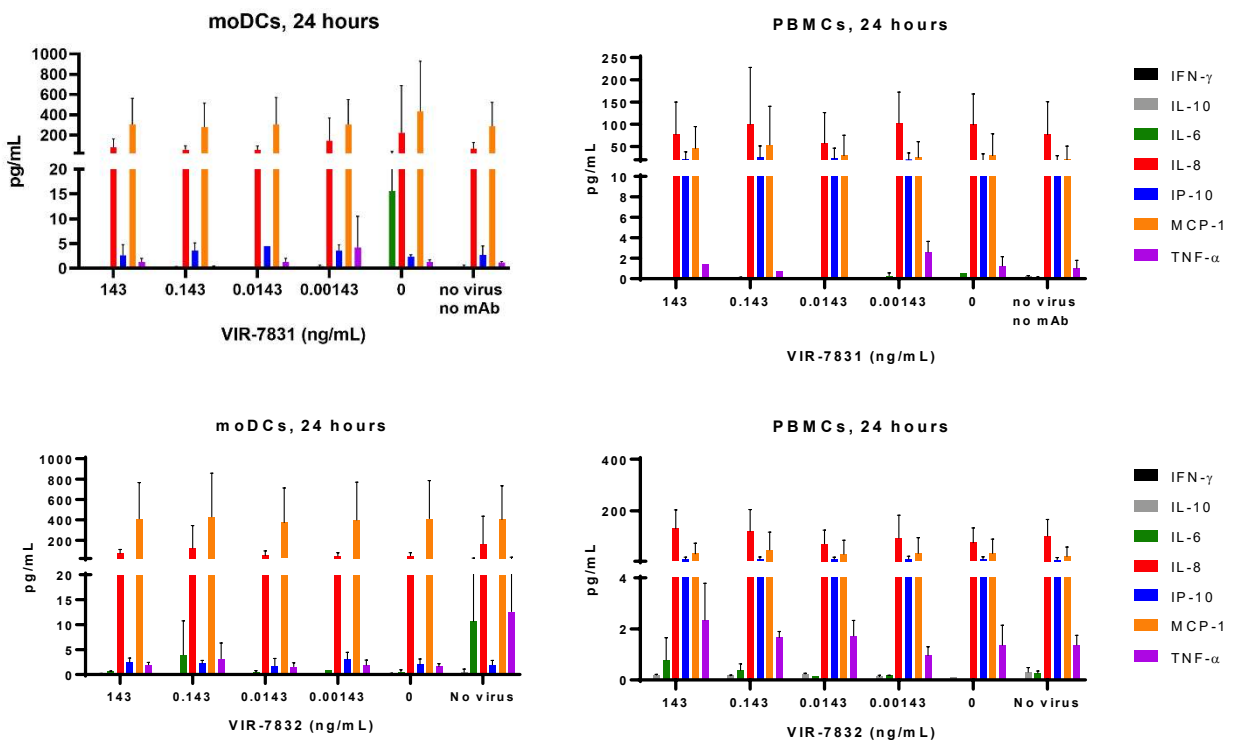
a)



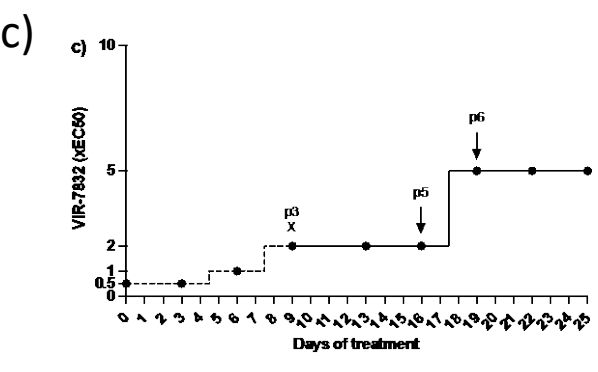
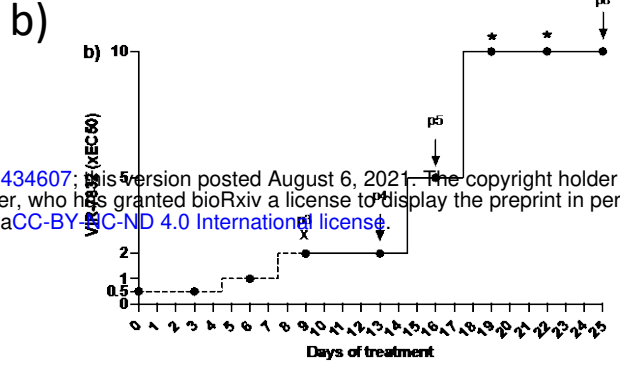
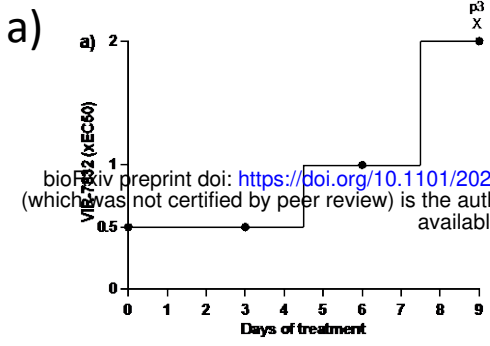
b)



c)



# Supplemental Figure 3



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# Supplemental Table 1

Passage	Spike Gene Amino Acid Substitution (Freq) <sup>a,b</sup>	EC <sub>50</sub> (µg/mL)	Fold Change in EC <sub>50</sub> to WT <sup>c</sup>
SARS-CoV-2 virus stock <sup>c</sup> bioRxiv preprint doi: <a href="https://doi.org/10.1101/2021.03.09.434607">https://doi.org/10.1101/2021.03.09.434607</a> ; this version posted August 6, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.	H66R (5.7%) S74P (12.6%) T76I (5.6%) 215-216insKLRS (60.9%) H655Y (3.1%)	0.06	NA
VIR-7832 Lineage 1, passage 4	215-216insKLRS (74.5%) 675-679 del (20.6%)	0.34	5.64
VIR-7832 Lineage 1, passage 5	215-216insKLRS (74.6%) 675-679del (66.0%)	0.35	5.93
VIR-7832 Lineage 1, passage 8	215-216insKLRS (74.7%) E340A (98.7%) 675-679del (84.5%)	ND	>10
VIR-7832 Lineage 2, passage 5	215-216insKLRS (73.9%) 675-679del (47.3%) R682W (4.9%) V1128F (3.5%)	0.32	5.40
VIR-7832 Lineage 2, passage 6	215-216insKLRS (75.3%) 675-679del (74.2%) R682W (4.9%) V1128F (30.9%)	0.39	6.54

## Supplemental Table 2

Amino Acid Changes in Spike protein	VIR-7831		VIR-7832	
	Geomean Neutralization EC <sub>50</sub> (ng/mL)	Fold Change Relative to Wild-Type	Geomean Neutralization EC <sub>50</sub> (ng/mL)	Fold Change Relative to Wild-Type
Wild Type	104.46	NA	100.79	NA
E340A	> 10,000	> 107	> 10,000	> 107
R682W	53.96	0.52	47.78	0.49
V1128F	50.65	0.53	49.69	0.60

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