

# 1    **Resilience of S309 and AZD7442 monoclonal antibody treatments against**

## 2    **infection by SARS-CoV-2 Omicron lineage strains**

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37 **ABSTRACT**

38 **Omicron variant strains encode large numbers of changes in the spike protein**  
39 **compared to historical SARS-CoV-2 isolates. Although *in vitro* studies have suggested that**  
40 **several monoclonal antibody therapies lose neutralizing activity against Omicron variants<sup>1-</sup>**  
41 **<sup>4</sup>, the effects *in vivo* remain largely unknown. Here, we report on the protective efficacy**  
42 **against three SARS-CoV-2 Omicron lineage strains (BA.1, BA.1.1, and BA.2) of two**  
43 **monoclonal antibody therapeutics (S309 [Vir Biotechnology] monotherapy and AZD7442**  
44 **[AstraZeneca] combination), which correspond to ones used to treat or prevent SARS-**  
45 **CoV-2 infections in humans. Despite losses in neutralization potency in cell culture, S309 or**  
46 **AZD7442 treatments reduced BA.1, BA.1.1, and BA.2 lung infection in susceptible mice**  
47 **that express human ACE2 (K18-hACE2). Correlation analyses between *in vitro***  
48 **neutralizing activity and reductions in viral burden in K18-hACE2 or human Fc $\gamma$ R**  
49 **transgenic mice suggest that S309 and AZD7442 have different mechanisms of protection**  
50 **against Omicron variants, with S309 utilizing Fc effector function interactions and**  
51 **AZD7442 acting principally by direct neutralization. Our data in mice demonstrate the**  
52 **resilience of S309 and AZD7442 mAbs against emerging SARS-CoV-2 variant strains and**  
53 **provide insight into the relationship between loss of antibody neutralization potency and**  
54 **retained protection *in vivo*.**

55

56 **MAIN TEXT**

57 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant strains continue  
58 to emerge and spread globally despite currently employed countermeasures and public health  
59 mandates. Since late 2020, variants of concern (VOC) and interest (VOI) have arisen due to  
60 continued SARS-CoV-2 evolution. Many variants contain substitutions in the N-terminal domain  
61 (NTD) and the receptor binding motif (RBM) of the receptor binding domain (RBD). Omicron  
62 lineage variants containing the largest numbers of spike protein changes described so far have  
63 emerged, spread globally, and become dominant. Moreover, cell-based studies suggest that the  
64 neutralizing activity of many monoclonal antibodies (mAbs) with Emergency Use Authorization  
65 (EUA) status or in advanced clinical development is diminished or abolished against Omicron  
66 lineage strains (BA.1, BA.1.1, and BA.2)<sup>1,2,5,6</sup>. However, the effect of mutations that compromise  
67 antibody neutralization on their efficacy *in vivo* against SARS-CoV-2 remains less clear. Indeed,  
68 for some classes of broadly neutralizing mAbs against influenza<sup>7,8</sup> and Ebola<sup>9,10</sup> viruses, there is  
69 no strict correlation between neutralizing activity *in vitro* and protection in animal models. Here,  
70 using mAbs that are currently in use to prevent or treat SARS-CoV-2 infection, we evaluated  
71 how the antigenic shift in Omicron variants affects neutralization in cells and protection in mice.

72

73 **MAb neutralization against Omicron lineage viruses**

74 We analyzed the substitutions in the RBDs of BA.1 (B.1.1.529), BA.1.1 (B.1.1.529  
75 R346K), and BA.2 strains (**Fig. 1a, Extended Data Fig. 1**) in the context of the structurally-  
76 defined binding epitopes of S309, a cross-reactive SARS-CoV mAb and the parent of  
77 Sotrovimab [VIR-7831], and AZD8895 and AZD1061, two mAbs that together (AZD7442) form  
78 the clinically-used Evusheld combination treatment (**Fig. 1b-e, Extended Data Fig. 1**). S309

79 binds a conserved epitope on the RBD that is spatially distinct from the RBM<sup>11</sup> and the  
80 AZD8895 and AZD1061 antibodies bind non-overlapping RBM epitopes<sup>12</sup>. Across Omicron  
81 lineage strains, substitutions at several antibody contact residues have occurred (**S309**: G339D,  
82 R346K, N440K; **AZD8895**: K417N, S477N, T478K, E484A, Q493R; **AZD1061**: R346K,  
83 N440K, E484A, Q493R).

84 Because of these sequence changes, we assessed the neutralizing activity of S309,  
85 AZD8895, AZD1061, and AZD7442 against BA.1, BA.1.1, and BA.2 viruses in Vero-  
86 TMPRSS2 cells. For these studies, we used mAbs that correspond to the products in clinical use  
87 which have Fc modifications: S309-LS [M428L/N434S], AZD8895-YTE/TM  
88 [M252Y/S254T/T256E and L234F/L235E/P331S], AZD1061-YTE/TM, and AZD7442-  
89 YTE/TM. The LS and YTE Fc substitutions result in extended antibody half-life in humans, and  
90 the TM changes reduce Fc effector functions<sup>13</sup>. Compared to the historical WA1/2020 D614G  
91 strain (hereafter D614G), antibody incubation with BA.1 was associated with 2.5-fold (S309-  
92 LS), 25-fold (AZD7442-YTE/TM), 118-fold (AZD8895-YTE/TM), and 206-fold (AZD1061-  
93 YTE/TM) reductions in neutralization potency (**Fig. 1f-o**), which agree with experiments with  
94 authentic or pseudotyped SARS-CoV-2<sup>1,2,5,6</sup>. Some differences were observed with BA.1.1:  
95 whereas S309-LS and AZD8895-YTE/TM were only slightly less effective against BA.1.1  
96 compared to BA.1, the neutralizing activity of AZD1061-YTE/TM was reduced by almost  
97 1,700-fold. Despite the decrease in activity of the AZD1061-YTE/TM component, the  
98 AZD7442-YTE/TM combination still showed inhibitory activity against BA.1.1 with a 176-fold  
99 reduction compared to D614G. Whereas small (no change to 5-fold) reductions in neutralization  
100 activity were observed with AZD1061-YTE/TM and AZD7442-YTE/TM against BA.2, larger  
101 reductions (32- and 68-fold) were observed for S309-LS and AZD8895-YTE/TM compared to

102 D614G. Overall, these data demonstrate that S309 retains potency against BA.1 and BA.1.1  
103 strains but has less *in vitro* neutralizing activity against BA.2, and the AZD7442 combination  
104 shows reduced yet residual activity against strains from all three Omicron lineages.

105

106 **MAb protection *in vivo* against Omicron viruses**

107 Because S309 and AZD7442 mAbs might act *in vivo* by a combination of mechanisms  
108 that are not fully reflected by *in vitro* neutralization potency assays, we evaluated the effects of  
109 the mutations observed in BA.1, BA.1.1 and BA.2 on efficacy in animals. For these studies, we  
110 used S309-LS and a different form of AZD7442, which contained only the TM substitutions and  
111 not the YTE modification. Although the YTE modification promotes antibody recycling to  
112 confer extended antibody half-life in humans and non-human primates, it accelerates antibody  
113 elimination in rodents<sup>14</sup>. To assess the efficacy of S309-LS and AZD7442-TM *in vivo*, we  
114 administered a single 200 µg (~10 mg/kg total) mAb dose to K18-hACE2 transgenic mice by  
115 intraperitoneal injection one day prior to intranasal inoculation with BA.1, BA.1.1, or BA.2  
116 strains. Although Omicron lineage viruses are less pathogenic in mice, they still replicate to high  
117 levels in the lungs of K18-hACE2 mice<sup>15</sup>. Nonetheless, as preliminary studies suggested slightly  
118 different kinetics of replication and spread in mice, we harvested samples at 7 dpi for BA.1 and  
119 BA.1.1 and 6 dpi for BA.2<sup>16</sup>. In BA.1 and BA.1.1-infected mice, S309-LS mAb reduced viral  
120 burden in the lung, nasal turbinates, and nasal washes at 7 dpi compared to isotype mAb-control  
121 treated mice (**Fig. 2a-d**). Nonetheless, control of infection, as judged by viral RNA levels, was  
122 less efficient against BA.1 (182-fold reduction) and BA.1.1 (39-fold reduction) viruses than  
123 against D614G (>500,000-fold reduction). Despite the diminished neutralizing activity against  
124 BA.2 *in vitro*, S309-LS treatment reduced viral RNA levels in the lungs of BA.2-infected mice

125 substantially (742-fold reduction) (**Fig 2a, b**). Protection by S309-LS was not observed in the  
126 nasal turbinates or nasal washes of mice challenged with BA.2 (**Fig. 2c, d**), in part due to the low  
127 and variable levels of infection with this variant. AZD7442-TM treatment differentially reduced  
128 viral burden in the lungs of mice against D614G (>400,000-fold reduction in viral RNA), BA.1  
129 (91-fold reduction in viral RNA), BA.1.1 (4-fold reduction in viral RNA), and BA.2 (>100,000-  
130 fold reduction in viral RNA) (**Fig. 2e, f**). Protection in the upper respiratory tract was less  
131 consistent, as AZD7442-TM treatment lowered viral RNA levels in the nasal washes of D614G  
132 and BA.1-infected mice but not in BA.1.1 or BA.2-infected mice and failed to reduce D614G,  
133 BA.1, BA.1.1, or BA.2 infection in the nasal turbinates (**Fig. 2g, h**).

134 As an independent metric of mAb protection, we measured cytokine and chemokine  
135 levels in the lung homogenates of S309-LS and AZD7442-TM treated animals infected with  
136 Omicron variant strains (**Fig 2i-j, and Extended Data Fig. 2, 3**). All infected K18-hACE2 mice  
137 receiving isotype control mAbs had increased expression levels of several pro-inflammatory  
138 cytokines and chemokines such as G-CSF, GM-CSF, IFN- $\gamma$ , IL-1 $\beta$ , IL-6, CXCL-10, CCL-2, and  
139 CCL-4 when compared to uninfected mice. In contrast, mice treated with AZD7442-TM mAbs  
140 and infected with BA.1 or BA.2 but not BA.1.1. showed reduced levels of pro-inflammatory  
141 cytokines and chemokines, which is consistent with effects on viral burden (**Fig. 2e, f**). In  
142 comparison to the isotype controls, mice treated with S309-LS had lower levels of cytokines and  
143 chemokines in lung homogenates after infection with all three Omicron variants, although the  
144 protection against BA.2-induced inflammation was less than against BA.1. or BA.1.1. Overall,  
145 these experiments suggest that despite losses in neutralizing potency in cell culture, S309-LS or  
146 AZD7442-TM can limit inflammation in the lung caused by Omicron variants.

147 We next evaluated whether the differences in neutralizing activity of S309-LS and  
148 AZD7442-YTE/TM correlated with lung viral burden after infection with the three Omicron  
149 strains. The change in AZD7442-YTE/TM neutralizing activity associated directly with the  
150 differences in lung viral burden of each Omicron variant (**Fig. 2k**). This relationship is consistent  
151 with its likely mechanism of action, virus neutralization and inhibition of entry<sup>17,18</sup>. The  
152 AZD7442-TM version we used, like the clinical drug Evusheld, encodes for modifications in the  
153 constant region of the mAb heavy chains that profoundly decrease binding to Fc-gamma  
154 receptors (Fc $\gamma$ Rs) and complement components (<sup>19</sup> and **Fig 3a**). In comparison, for S309-LS, a  
155 similar direct correlation between changes in neutralization potency *in vitro* and reductions in  
156 viral burden *in vivo* was not observed (**Fig. 2l**), indicating a possible additional protective  
157 mechanism beyond virus neutralization.

158

159 **S309-LS employs Fc effector functions to protect against SARS-CoV-2 variants**

160 To evaluate a potential role for Fc effector functions in S309 mAb-mediated protection  
161 against Omicron strains, we engineered loss-of-function GRLR mutations (G236R, L328R) into  
162 the Fc domain of the human IgG1 heavy chain of S309; these substitutions eliminate antibody  
163 binding to Fc $\gamma$ Rs<sup>13</sup>. Introduction of the GRLR mutations abrogated binding to hFc $\gamma$ RI and  
164 hFc $\gamma$ RIIIa, as expected (**Fig. 3a**) but did not affect neutralization of the SARS-CoV-2 strains  
165 (**Extended Data Fig. 4**). Next, we compared VIR-7831 (the clinical form of S309-LS) and  
166 S309-GRLR in an *in vitro* antibody-dependent cell cytotoxicity (ADCC) assay. When target cells  
167 expressing similar levels of Wuhan-D614, BA.1, or BA.2 spike proteins on the cell surface (**Fig.**  
168 **3b**) were incubated with VIR-7831 mAb, we observed some reductions in binding to Omicron  
169 spike proteins compared to mAb S2X324, an antibody that retains neutralizing activity against

170 BA.1, BA.1.1, and BA.2 and engages a distinct epitope in the RBM<sup>4</sup>. Despite the differences in  
171 binding, target cells expressing Wuhan-D614, BA.1, or BA.2 spike proteins were lysed  
172 efficiently by primary natural killer (NK) cells (antibody-dependent cellular cytotoxicity,  
173 ADCC) isolated from four donors by VIR-7831 but not by S309-GRLR (**Fig. 3c, d, Extended**  
174 **Data Fig. 5**). Similarly, primary CD14<sup>+</sup> monocytes isolated from five donors mediated  
175 comparable antibody-dependent cellular phagocytosis (ADCP) of target cells expressing Wuhan-  
176 D614, BA.1, or BA.2 spike proteins by VIR-7831 but not by S309-GRLR (**Fig. 3e, f, Extended**  
177 **Data Fig. 6, 7**).

178 To evaluate the role of effector functions *in vivo* in S309-LS mAb-mediated protection  
179 against Omicron variant strains, we treated K18-hACE2 mice with a single 200 µg (~10 mg/kg  
180 total) dose of S309-GRLR mAb by intraperitoneal injection one day prior to intranasal  
181 inoculation with D614G, BA.1, or BA.2 strains. At 6 (BA.2) or 7 (D614G and BA.1) dpi, viral  
182 RNA levels in the lungs, nasal turbinates, and nasal washes were measured (**Fig. 3g-i**). Although  
183 S309-GRLR treatment reduced viral burden in the lung and nasal turbinates of D614G-infected  
184 mice, it did not limit infection by BA.1 and BA.2 strains in the tissues tested. To corroborate  
185 these findings, we treated human FcγR (hFcγR) transgenic C57BL/6 mice<sup>20</sup> with a single 3  
186 mg/kg dose of S309-LS or S309-GRLR mAbs one day prior to inoculation with a SARS-CoV-2  
187 Beta (B.1.351) isolate; we used the Beta isolate for these studies because Omicron strains  
188 replicate poorly in conventional C57BL/6 mice lacking expression of hACE2<sup>16</sup>. At 2 or 4 dpi,  
189 S309-LS mAb-treated hFcγR mice showed markedly reduced levels of viral RNA (49 to 127-  
190 fold) or infectious virus (56- to 538-fold) in the lung compared to the isotype control-treated  
191 mice, whereas animals administered S309-GRLR showed smaller (2.3- to 13-fold) differences,  
192 most of which did not attain statistical significance (**Fig. 3j, k**). Collectively, these data suggest

193 that the protection mediated by S309-LS mAb *in vivo* is mediated at least in part by Fc effector  
194 functions and engagement of Fc $\gamma$ Rs.

195 **DISCUSSION**

196 Due to the continued emergence of SARS-CoV-2 variants encoding an increasing  
197 number of amino acid changes in the spike protein, antibody countermeasure efficacy requires  
198 continued monitoring. When the BA.1 Omicron virus emerged in late 2021, five mAb therapies  
199 were in late-stage clinical development or had acquired EUA status. *In vitro* assays with  
200 pseudoviruses<sup>5</sup> and authentic viruses<sup>1</sup> established that mAb therapies from Regeneron  
201 (REGN10933 and REGN10987), Lilly (LY-CoV555 and LY-CoV016), and Celltrion (CT-P59)  
202 showed a complete loss in neutralizing activity against BA.1. Subsequent experiments in K18-  
203 hACE2 mice confirmed that the REGN-COV2 mAb cocktail completely lost its efficacy against  
204 the BA.1 variant<sup>21</sup>. More recently, an additional antibody (LY-CoV1404, bebtelovimab), which  
205 shows considerable neutralization activity against a range of SARS-CoV-2 strains, received EUA  
206 status<sup>22</sup>, although protection data *in vivo* against VOC, including Omicron, has not yet been  
207 published.

208 We compared the *in vitro* neutralizing activity and *in vivo* efficacy of S309 (parent mAb  
209 of Sotrovimab) and AZD7442 (Evusheld) that correspond to the clinically-used products. Our  
210 study establishes the utility of S309 and AZD7442 mAbs against highly divergent SARS-CoV-2  
211 variants. Despite losses in neutralization potency against BA.1, BA.1.1, and BA.2 strains, S309-  
212 LS and AZD7442-TM reduced viral burden and pro-inflammatory cytokine levels in the lungs of  
213 K18-hACE2 mice, albeit with some differences in activity and mechanisms of action. Although  
214 AZD7442-TM had a limited protective effect on viral burden in the nasal washes and nasal  
215 turbinates of infected mice, this was not entirely unexpected, as studies with the parental mAbs

216 COV2-2196 and COV2-2130 showed less protection in nasal washes than lungs against multiple  
217 SARS-CoV-2 VOC<sup>23</sup>. Moreover, studies in non-human primates with anti-SARS-CoV-2 human  
218 mAbs showed the concentrations in nasopharyngeal washes are approximately 0.1% of those  
219 found in the serum<sup>24</sup>, which likely explains their diminished benefit in this tissue compartment.

220 We also assessed whether the reductions in mAb neutralization potency against Omicron  
221 variant strains correlated with the observed changes in viral burden. For AZD7442-TM, which  
222 contains L234F/L235E/P331S modifications that abolish Fc receptor engagement<sup>13</sup> and were  
223 introduced to decrease the potential risk of antibody-dependent enhancement of disease<sup>18</sup>,  
224 antibody-mediated reductions in viral titer corresponded directly with neutralization activity  
225 against Omicron variant strains; thus, neutralization is likely a key protective mechanism for  
226 these RBM-specific mAbs. For S309-LS, which only contains half-life extending M428L/N434S  
227 modifications in the human IgG1 Fc domain, and exhibits Fc effector functions including ADCC  
228 and ADCP<sup>11</sup>, changes in neutralization potency did not linearly relate to changes in lung viral  
229 titer. S309-LS mAb treatment still conferred significant protection in the lungs of mice infected  
230 with BA.2 despite a substantial loss in neutralizing activity. Because of these results, we  
231 evaluated the contributions of Fc effector functions in protection in mice using S309-GRLR,  
232 which has G236R/L328R mutations in the Fc domain that abrogate binding to Fc $\gamma$ Rs<sup>13</sup>. We  
233 observed that intact S309-LS but not S309-GRLR mAb protected K18-hACE2 and hFc $\gamma$ R mice  
234 against SARS-CoV-2 variant strains. These results are consistent with prior studies showing a  
235 beneficial role of Fc-effector functions in antibody mediated protection in mice and hamsters<sup>25-</sup>  
236 <sup>29</sup>, and may explain why mAbs with markedly different *in vitro* neutralization potencies against  
237 SARS-CoV-2 strains show similar protective activity in animals  
238 (<https://opendata.ncats.nih.gov/covid19/animal>). Furthermore, they also demonstrate that for

239 some mAbs, Fc effector functions can compensate for losses in neutralization potency against  
240 SARS-CoV-2 variants and act as a protective mechanism *in vivo*. Thus, effector functions can  
241 contribute to resilience of some mAbs against Omicron and other VOC<sup>30,31</sup>. We speculate that  
242 the stoichiometric threshold and antibody occupancy requirements for Fc effector function  
243 activity may be lower than for virion neutralization<sup>32</sup>; if so, this property might clarify how  
244 antibodies with reduced neutralizing potency against VOC that still bind spike protein on the  
245 virion or surface of infected cells retain protective activity *in vivo*.

246 **Limitations of study.** We note several limitations of our study: (a) Female K18-hACE2  
247 mice were used to allow for group caging. Follow-up experiments in male mice to confirm and  
248 extend these results are needed. (b) The BA.1, BA.1.1., and BA.2 viruses are less pathogenic in  
249 mice than the D614G virus<sup>16,33-35</sup>. This could lead to an overestimation of protection compared to  
250 other more virulent strains in mice. (c) We only evaluated the efficacy of S309 or AZD7442 as  
251 prophylaxis. Whereas AZD7442 is authorized only as preventive agent, post-exposure  
252 therapeutic studies with both mAbs and Omicron variants may provide further insight as to  
253 effects on potency. Moreover, the relationship between initial viral dosing and antibody  
254 protection against Omicron variants was not explored. (d) Several experiments were performed  
255 in transgenic mice that over-express human ACE2 receptors. High levels of cellular hACE2 can  
256 diminish the neutralizing activity of mAbs that bind non-RBM sites of the SARS-CoV-2  
257 spike<sup>36,37</sup>. Thus, studies in hACE2-transgenic mice could underestimate the efficacy of mAbs  
258 binding outside of the RBM. Challenge studies in other animal models and ultimately humans  
259 will be required for corroboration, including the contribution of Fc effector functions to mAb  
260 efficacy.

261                   Collectively, our data expand on recent *in vitro* findings with BA.1 strains by evaluating  
262                   the level of protection conferred by treatment with two EUA mAbs against the three currently  
263                   dominant Omicron variants. While S309-LS (and by extension Sotrovimab) and AZD7442-TM  
264                   (Evusheld) retained inhibitory activity against several Omicron lineage strains, the impact of  
265                   shifts in neutralization potency *in vitro* may not directly predict dosing in the clinical setting.  
266                   Finally, our studies highlight the potential of both mAb neutralization and Fc effector function  
267                   mechanisms in protecting against SARS-CoV-2-mediated disease and suggest mechanisms of  
268                   action for withstanding mutations in variant strains that reduce but do not abrogate mAb binding  
269                   and neutralization.  
270  
271

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279

280 **AUTHOR CONTRIBUTIONS**

281 J.B.C. performed and analyzed neutralization assays. J.M.E. performed structural analyses  
282 with guidance from D.H.F. J.B.C., S.M., Z.C., and E.A.M. performed mouse experiments and  
283 viral burden analyses. J.B.C. propagated and validated SARS-CoV-2 viruses. B.G. and M.A.S.  
284 designed, performed, and analyzed *in vitro* Fc-mediated effector function studies. K. Rosenthal,  
285 and K. Ren performed antibody analyses. A.J., L.D., and S.A.H. performed deep sequencing  
286 analysis. L.A.P., D.C., Y-M.L., and M.T.E. provided mAbs. P.J.H. and Y.K. provided SARS-  
287 CoV-2 strains. J.E.C. and H.W.V. provided key intellectual contributions to the design of the  
288 study. D.H.F. and M.S.D. obtained funding and supervised the research. J.B.C. and M.S.D. wrote  
289 the initial draft, with the other authors providing editorial comments.

290

291 **COMPETING FINANCIAL INTERESTS**

292 M.S.D. is a consultant for Inbios, Vir Biotechnology, Senda Biosciences, and Carnival  
293 Corporation, and on the Scientific Advisory Boards of Moderna and Immunome. The Diamond  
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300 which J.E.C. is an inventor. B.G., M.A.S, H.W.V., D.C., and L.A.P. are employees of Vir  
301 Biotechnology and may hold equity in Vir Biotechnology. L.A.P. is a former employee and may  
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310

311 **FIGURE LEGENDS**

312 **Figure 1. Neutralization of Omicron lineage strains by mAbs.** **a**, One protomer of the  
313 SARS-CoV-2 spike trimer (PDB: 7C2L) is depicted with BA.2 variant amino acid substitutions  
314 labelled and shown as red spheres. The N-terminal domain (NTD), RBD, RBM, and S2 are  
315 colored in yellow, green, magenta, and blue, respectively. All mutated residues in the BA.2 RBD  
316 relative to WA1/2020 are indicated in **b**, and the BA.2 RBD bound by mAbs S309 (orange,  
317 PDB: 6WPS) (**b**), AZD8895 (green, PDB: 7L7D) (**c**), and AZD1061 (purple, PDB: 7L7E) (**d**) are  
318 shown. BA.2 mutations in the respective epitopes of each mAb are shaded red, whereas those  
319 outside the epitope are shaded green. **e**, Multiple sequence alignment showing the epitope  
320 footprints of each mAb on the SARS-CoV-2 RBD (orange, S309; green, AZD8895; purple,  
321 AZD1061). The WA1/2020 RBD is shown in the last row with relative variant sequence changes  
322 indicated. Red circles below the sequence alignment indicate hACE2 contact residues on the  
323 SARS-CoV-2 RBD<sup>38</sup>. Structural analysis and depictions were generated using UCSF  
324 ChimeraX<sup>39</sup>. **f-i**, Neutralization curves in Vero-TMPRSS2 cells with the indicated SARS-CoV-2  
325 strain and mAb. The average of three to four experiments performed in technical duplicate are  
326 shown. **j-m**, Comparison of EC<sub>50</sub> values for the indicated mAb against D614G, BA.1, BA.1.1,  
327 and BA.2 viruses. Data are the average of three experiments, error bars indicate standard error of  
328 the mean (SEM), and the dashed line indicates the upper limit of detection (one-way ANOVA  
329 with Dunnett's test; ns, not significant, \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P <$   
330 0.0001). **n**, Summary of the EC<sub>50</sub> values for each mAb against the indicated SARS-CoV-2 strain.  
331 **o**, Summary of the fold-change in EC<sub>50</sub> values for each mAb against the indicated Omicron strain  
332 relative to SARS-CoV-2 D614G.

333 **Figure 2. Antibody protection against Omicron variants in K18-hACE2 mice. a-j.**

334 Eight-week-old female K18-hACE2 mice received 200  $\mu$ g (about 10 mg/kg) of the indicated  
335 mAb treatment by intraperitoneal injection one day before intranasal inoculation with  $10^3$  FFU of  
336 the indicated SARS-CoV-2 strain. Tissues were collected at six (BA.2) or seven days (all other  
337 strains) after inoculation. Viral RNA levels in the lungs (**a, e**), nasal turbinates (**c, g**), and nasal  
338 washes (**d, h**) were determined by RT-qPCR, and infectious virus in the lungs (**b, f**) was assayed  
339 by plaque assay (lines indicate median  $\pm$  SEM.; n = 6-8 mice per group, two experiments; Mann-  
340 Whitney test between isotype and mAb treatment; ns, not significant; \*  $P < 0.05$ , \*\*  $P < 0.01$ ,  
341 \*\*\*  $P < 0.001$ ). **i-j**, Heat map of cytokine and chemokine protein expression levels in lung  
342 homogenates from the indicated groups. Data are presented as log<sub>2</sub>-transformed fold-change over  
343 naive mice. Blue, reduction; red, increase. **k-l**, Correlation analysis. The fold-change in EC<sub>50</sub>  
344 value of AZD7442-YTE/TM (**k**) and S309-LS (**l**) for D614G and each Omicron variant strain are  
345 plotted on the x-axis. The fold-change in lung viral RNA titer between the respective isotype or  
346 mAb-treated groups against each Omicron variant strain are plotted on the y-axis. Best-fit lines  
347 were calculated using a simple linear regression. Two-tailed Pearson correlation was used to  
348 calculate the R<sup>2</sup> and P values indicated within each panel.

349 **Figure 3. Role of Fc-effector functions in S309 mAb-mediated protection. a**, Binding  
350 of AZD7442-TM, S309-LS, or S309-GRLR mAbs to hFc $\gamma$ RI or hFc $\gamma$ RIIIa (two experiments;  
351 dotted lines indicate the limit of detection). **b**, ExpiCHO-S cells were transiently transfected with  
352 plasmids encoding the indicated SARS-CoV-2 spike protein. 24 to 48 h later, cells were  
353 harvested, washed, and stained with the indicated concentrations of VIR-7831 or S2X324 mAbs  
354 to assess binding to the cell surface. Representative histograms from two-three experiments are  
355 shown. **c**, ExpiCHO-S cells transiently transfected with Wuhan-1 D614, BA.1, or BA.2 spike

356 proteins were incubated with the indicated concentrations of VIR-7831 or S309-GRLR mAb and  
357 mixed with purified NK cells isolated from healthy donors at a ratio of 1:9 (target:effector). Cell  
358 lysis was determined by a lactate dehydrogenase release assay. The error bars indicate standard  
359 deviations (SD). **d**, Area under the curve (AUC) analyses from four NK donors (**Extended Data**  
360 **Fig. 5**). **e**, ExpiCHO-S cells transiently transfected with Wuhan-1 D614, BA.1, or BA.2 spike  
361 proteins and fluorescently labelled with PKH67 were incubated with the indicated concentrations  
362 of VIR-7831 or S309-GRLR mAb and mixed with PBMCs labelled with CellTrace Violet from  
363 healthy donors at a ratio of 1:20 (target:PBMCs). Association of CD14<sup>+</sup> monocytes with spike-  
364 expressing target cells (ADCP) was determined by flow cytometry. The error bars indicate SD. **f**,  
365 AUC analyses of VIR-7831 and S309-GRLR for each Omicron variant for four donors. (**g-i**)  
366 Eight-week-old female K18-hACE2 mice or (**j-k**) 12-week-old male hFcγR mice received a  
367 single 10 mg/kg or 3 mg/kg dose respectively, of S309-LS or S309-GRLR mAb by  
368 intraperitoneal injection one day before intranasal inoculation with 10<sup>3</sup> FFU of D614G, BA.1, or  
369 BA.2 (**g-i**) or 10<sup>5</sup> FFU of Beta (B.1.351) (**j-k**). Tissues were collected at 2 (B.1.351), 4 (B.1.351),  
370 6 (BA.2), or 7 (D614G and BA.1) dpi. Viral RNA levels in the lungs (**g, j-k**), nasal turbinates  
371 (**h**), and nasal washes (**i**) were determined by RT-qPCR, and infectious virus in the lungs (**j-k**)  
372 was measured by plaque assay (**g-k**; lines indicate median ± SEM.; **g-i** and **j-k**; n = 8 and  
373 10 mice per group, respectively; two experiments; **g-i**; (Mann-Whitney test between isotype and  
374 mAb treatment; ns, not significant; \*\* P < 0.01, \*\*\*, P < 0.001); **j-k**; one-way ANOVA with  
375 Tukey's multiple comparisons test; ns, not significant; \* P < 0.05, \*\* P < 0.01, \*\*\*, P < 0.001,  
376 \*\*\*\*, P < 0.0001).

377

378 **EXTENDED DATA FIGURES**

379                   **Extended Data Figure 1. BA.1.1 spike protein substitutions and mAb epitopes.**

380           Mutated residues in the BA.1.1 RBD relative to WA1/2020 are indicated in green in all three  
381           panels. The BA.1.1 RBD bound by mAbs S309 (orange, PDB: 6WPS) (a), AZD8895 (pale  
382           green, PDB: 7L7D) (b), and AZD1061 (purple, PDB:7L7E) (c) are shown. BA.1.1 substitutions  
383           in the respective epitopes of each mAb are shaded red, whereas those outside the epitope are  
384           shaded green. Structural analysis and depictions were generated using UCSF ChimeraX<sup>39</sup>.

385                   **Extended Data Figure 2. Cytokine and chemokine induction after S309-LS**  
386           **treatment and SARS-CoV-2 infection.** Individual graphs of cytokine and chemokine protein  
387           levels in the lungs of S309-LS mAb-treated K18-hACE2 mice at 6 (BA.2) or 7 dpi (all other  
388           strains) with the indicated SARS-CoV-2 strain (line indicates median; n = 3 naive, n = 6-8 for all  
389           other groups (Mann-Whitney test with comparison between the isotype control and mAb: \*, P <  
390           0.05, \*\*, P < 0.01, \*\*\*, P < 0.001).

391                   **Extended Data Figure 3. Cytokine and chemokine induction after AZD7442-TM**  
392           **treatment and SARS-CoV-2 infection.** Individual graphs of cytokine and chemokine protein  
393           levels in the lungs of AZD7442-TM mAb-treated K18-hACE2 mice at 6 (BA.2) or 7 dpi (all  
394           other strains) with the indicated SARS-CoV-2 strain (line indicates median; n = 3 naive, n = 8  
395           for all other groups (Mann-Whitney test with comparison between the isotype control and mAb:  
396           \*, P < 0.05, \*\*, P < 0.01, \*\*\*, P < 0.001).

397                   **Extended Data Figure 4. Neutralization of SARS-CoV-2 variants by S309-LS and**  
398           **S309-GRLR mAbs.** Neutralization curves in Vero-TMPRSS2 cells comparing infection of the  
399           indicated SARS-CoV-2 strain in the presence of each mAb. The average of two experiments  
400           performed in technical duplicate are shown. For D614G, BA.1, and BA.2 strains, the S309-LS  
401           neutralization data from **Fig. 1f** are shown for comparison.

402                   **Extended Data Figure 5. VIR-7831-mediated antibody-dependent cell cytotoxicity**  
403                   **with NK cells.** ExpiCHO-S cells transiently transfected with expression plasmids encoding  
404                    Wuhan D614, BA.1, or BA.2 spike proteins were incubated with the indicated concentrations of  
405                    VIR-7831 or S309-GRLR and mixed with NK cells isolated from healthy donors at a ratio of 1:9  
406                    (target:effector). Target cell lysis was determined by a lactate dehydrogenase release assay. The  
407                    error bars indicate SDs. Each panel is an individual donor. Donors 1 and 3 are heterozygous for  
408                    F158 and V158 Fc $\gamma$ RIIIa, whereas donors 2 and 4 are homozygous for V158.

409                   **Extended Data Figure 6. VIR-7831-mediated antibody-dependent cell phagocytosis**  
410                   **with monocytes.** ExpiCHO-S cells transiently transfected with Wuhan-1 D614, BA.1, or BA.2  
411                    spike proteins and fluorescently labelled with PKH67 were incubated with the indicated  
412                    concentrations of VIR-7831 or S309-GRLR mAb and mixed with PBMCs labelled with  
413                    CellTrace Violet from healthy donors carrying different Fc $\gamma$ RIIA and IIIA genotypes at a ratio of  
414                    1:20 (target:PBMCs). Association of CD14 $^{+}$  monocytes with spike-expressing target cells  
415                    (ADCP) was determined by flow cytometry. The error bars indicate SD. Each panel is an  
416                    individual donor.

417                   **Extended Data Figure 7. Gating strategy for CD14 $^{+}$  monocytes used for antibody-**  
418                   **dependent cell phagocytosis assays.** From PBMCs, monocytes were gated as CD3 $^{-}$  CD19 $^{-}$   
419                    CD14 $^{+}$  cells. For ADCP, % FITC $^{+}$  CellTrace Violet $^{+}$  CD14 $^{+}$  monocytes were gated as indicated.  
420                    The gate of positive cells was set based on the no mAb control.

421  
422                   **SUPPLEMENTAL TABLE TITLES**  
423                   **Supplementary Table 1. Omicron variant strain mutations as determined by next-**  
424                   **generation sequencing.**

425

426 **METHODS**

427 **Cells.** Vero-TMPRSS2<sup>40</sup> and Vero-hACE2-TMPRSS2<sup>41</sup> cells were cultured at 37°C in  
428 Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum  
429 (FBS), 10 mM HEPES pH 7.3, 1 mM sodium pyruvate, 1× non-essential amino acids, and  
430 100 U/ml of penicillin–streptomycin. Vero-TMPRSS2 cells were supplemented with 5 µg/mL  
431 of blasticidin. Vero-hACE2-TMPRSS2 cells were supplemented with 10 µg/mL of puromycin.  
432 ExpiCHO-S cells were obtained from Thermo Fisher Scientific. All cells routinely tested  
433 negative for mycoplasma using a PCR-based assay.

434 **Viruses.** The Beta (B.1.351) and Omicron (BA.1 (R346), BA.1.1 (R346K), and BA.2)  
435 strains were obtained from nasopharyngeal isolates. All virus stocks were generated in Vero-  
436 TMPRSS2 cells and subjected to next-generation sequencing as described previously<sup>41</sup> to  
437 confirm the presence and stability of expected substitutions (see **Supplementary Table 1**). All  
438 virus experiments were performed in an approved biosafety level 3 (BSL-3) facility.

439 **Monoclonal antibody purification.** The mAbs studied in this paper, S309, AZD8895,  
440 AZD1061, and the AZD7442 cocktail have been described previously<sup>4,11,18</sup>.

441 S309-LS and S309-GRLR were produced in ExpiCHO-S cells and affinity-purified using  
442 HiTrap Protein A columns (GE Healthcare, HiTrap mAb select Xtra #28-4082-61) followed by  
443 buffer exchange to histidine buffer (20 mM histidine, 8% sucrose, pH 6.0) using HiPrep 26/10  
444 desalting columns. The final products were sterilized after passage through 0.22 µm filters and  
445 stored at 4°C. VIR-7831 (clinical lead variant of S309-LS) was produced at WuXi Biologics.

446 AZD8895 and AZD1061 mAbs were cloned into mammalian expression vectors and  
447 expressed as IgG1 constructs with the TM (L234F/L235E/P331S) Fc modification with or  
448 without a second YTE (M252Y/S254T/T256E) modification to extend half-life in humans.

449 MAbs were expressed in 293F cells after transfection with 293fectin (Thermo Fisher Scientific)  
450 and isolated from supernatants by affinity chromatography using Protein A or Protein G columns  
451 (GE Healthcare). MAbs were eluted with 0.1 M glycine at low pH and dialyzed into PBS.

452 **Mouse experiments.** Animal studies were carried out in accordance with the  
453 recommendations in the Guide for the Care and Use of Laboratory Animals of the National  
454 Institutes of Health. The protocols were approved by the Institutional Animal Care and Use  
455 Committee at the Washington University School of Medicine (assurance number A3381–01).  
456 Virus inoculations were performed under anesthesia that was induced and maintained with  
457 ketamine hydrochloride and xylazine, and all efforts were made to minimize animal suffering.

458 Heterozygous K18-hACE2 C57BL/6J mice (strain: 2B6.Cg-Tg(K18-ACE2)2Prlmn/J)  
459 and wild-type C57BL/6J (strain: 000664) mice were obtained from The Jackson Laboratory.  
460 Human Fc $\gamma$ R transgenic mice<sup>20</sup> (Fc $\gamma$ R $\alpha^{-/-}$   
461 /hFc $\gamma$ RI<sup>+</sup>/hFc $\gamma$ RIIA<sup>R131<sup>+</sup>/hFc $\gamma$ RIIB<sup>+</sup>/hFc $\gamma$ RIIIA<sup>F158<sup>+</sup>/hFc $\gamma$ RIIIB<sup>+</sup>) were a generous gift (J.  
462 Ravetch, Rockefeller University) and bred at Washington University. All animals were housed in  
463 groups and fed standard chow diets. For experiments with K18-hACE2 mice, eight- to ten-week-  
464 old female mice were administered the indicated doses of the respective SARS-CoV-2 strains  
465 (see Figure legends) by intranasal administration. For hFc $\gamma$ R mouse experiments, 12-week-old  
466 male mice were administered 10<sup>5</sup> FFU of a Beta (B.1.351) isolate by intranasal administration. *In*  
467 *vivo* studies were not blinded, and mice were randomly assigned to treatment groups. No sample-  
468 size calculations were performed to power each study. Instead, sample sizes were determined  
469 based on prior *in vivo* virus challenge experiments. Mice were administered the indicated mAb  
470 dose by intraperitoneal injection one day before intranasal inoculation with the indicated SARS-</sup></sup>

471 CoV-2 strain. AZD7442-TM (lacking the YTE modification that accelerates antibody  
472 elimination in rodents) was used in mouse studies.

473 **Focus reduction neutralization test.** Serial dilutions of mAbs were incubated with 10<sup>2</sup>  
474 focus-forming units (FFU) of different strains or variants of SARS-CoV-2 for 1 h at 37°C.  
475 Antibody-virus complexes were added to Vero-TMPRSS2 cell monolayers in 96-well plates and  
476 incubated at 37°C for 1 h. Subsequently, cells were overlaid with 1% (w/v) methylcellulose in  
477 MEM. Plates were harvested 48-72 h later by removing overlays and fixing with 4% PFA in PBS  
478 for 20 min at room temperature. Plates were washed and incubated with an oligoclonal pool of  
479 SARS2-2, SARS2-11, SARS2-16, SARS2-31, SARS2-38, SARS2-57, and SARS2-71<sup>42</sup>. Plates  
480 with Omicron variant strains were additionally incubated with CR3022 and a pool of anti-  
481 SARS-CoV-2 mAbs that cross-react with SARS-CoV<sup>43</sup>. Subsequently, samples were incubated  
482 with HRP-conjugated goat anti-mouse IgG (Sigma, 12-349) and HRP-conjugated goat anti-  
483 human IgG (Sigma, A6029) in PBS supplemented with 0.1% saponin and 0.1% bovine serum  
484 albumin. SARS-CoV-2-infected cell foci were visualized using TrueBlue peroxidase substrate  
485 (KPL) and quantitated on an ImmunoSpot microanalyzer (Cellular Technologies).

486 **Measurement of viral RNA levels.** Tissues were weighed and homogenized with  
487 zirconia beads in a MagNA Lyser instrument (Roche Life Science) in 1 mL of DMEM medium  
488 supplemented with 2% heat-inactivated FBS. Tissue homogenates were clarified by  
489 centrifugation at 10,000 rpm for 5 min and stored at -80°C. RNA was extracted using the  
490 MagMax mirVana Total RNA isolation kit (Thermo Fisher Scientific) on the Kingfisher Flex  
491 extraction robot (Thermo Fisher Scientific). RNA was reverse transcribed and amplified using  
492 the TaqMan RNA-to-CT 1-Step Kit (Thermo Fisher Scientific). Reverse transcription was  
493 carried out at 48°C for 15 min followed by 2 min at 95°C. Amplification was accomplished over

494 50 cycles as follows: 95°C for 15 s and 60°C for 1 min. Copies of SARS-CoV-2 *N* gene RNA in  
495 samples were determined using a previously published assay<sup>44</sup>. Briefly, a TaqMan assay was  
496 designed to target a highly conserved region of the *N* gene (Forward primer:  
497 ATGCTGCAATCGTGCTACAA; Reverse primer: GACTGCCGCCTCTGCTC; Probe: /56-  
498 FAM/TCAAGGAAC/ZEN/AACATTGCCAA/3IABkFQ/). This region was included in an RNA  
499 standard to allow for copy number determination down to 10 copies per reaction. The reaction  
500 mixture contained final concentrations of primers and probe of 500 and 100 nM, respectively.

501 **Viral plaque assay.** Vero-TMPRSS2-hACE2 cells were seeded at a density of  $1 \times 10^5$   
502 cells per well in 24-well tissue culture plates. The following day, medium was removed and  
503 replaced with 200  $\mu$ L of material to be titrated diluted serially in DMEM supplemented with 2%  
504 FBS. One hour later, 1 mL of methylcellulose overlay was added. Plates were incubated for 72 h,  
505 then fixed with 4% paraformaldehyde (final concentration) in PBS for 20 min. Plates were  
506 stained with 0.05% (w/v) crystal violet in 20% methanol and washed twice with distilled,  
507 deionized water.

508 **Transient expression of recombinant SARS-CoV-2 protein and flow cytometry.**  
509 ExpiCHO-S cells were seeded at  $6 \times 10^6$  cells/mL in a volume of 5 mL in a 50 mL bioreactor.  
510 The following day, cells were transfected with SARS-CoV-2 spike glycoprotein-encoding  
511 pcDNA3.1(+) plasmids (BetaCoV/Wuhan-Hu-1/2019, accession number MN908947, Wuhan  
512 D614; Omicron BA.1 and BA.2 generated by overlap PCR mutagenesis of the Wuhan D614  
513 plasmid) harboring the  $\Delta$ 19 C-terminal truncation<sup>26</sup>. Spike encoding plasmids were diluted in  
514 cold OptiPRO SFM (Life Technologies, 12309-050), mixed with ExpiFectamine CHO Reagent  
515 (Life Technologies, A29130) and added to cells. Transfected cells were then incubated at 37°C  
516 with 8% CO<sub>2</sub> with an orbital shaking speed of 250 RPM (orbital diameter of 25 mm) for 24 to 48

517 h. Transiently transfected ExpiCHO-S cells were harvested and washed twice in wash buffer  
518 (PBS 2% FBS, 2 mM EDTA). Cells were counted and distributed into round bottom 96-well  
519 plates (Corning, 3799) and incubated with serial dilutions of mAb starting at 10 µg/mL. Alexa  
520 Fluor647-labelled Goat Anti-human IgG secondary Ab (Jackson Immunoresearch, 109-606-098)  
521 was prepared at 2 µg/mL and added onto cells after two washing steps. Cells were then washed  
522 twice and resuspended in wash buffer for data acquisition at ZE5 cytometer (BioRad).

523 **Fc-mediated effector functions.** Primary cells were collected from healthy human  
524 donors with informed consent and authorization via the *Comitato Etico Canton Ticino*  
525 (Switzerland). ADCC assays were performed using ExpiCHO-S cells transiently transfected with  
526 SARS-CoV-2 spike glycoproteins (Wuhan D614, BA.1 or BA.2) as targets. NK cells were  
527 isolated from fresh blood of healthy donors using the MACSxpress NK Isolation Kit (Miltenyi  
528 Biotec, cat. no. 130-098-185). Target cells were incubated with titrated concentrations of mAbs  
529 for 10 min and then with primary human NK cells at an effector:target ratio of 9:1. ADCC was  
530 measured using LDH release assay (Cytotoxicity Detection Kit (LDH) (Roche; cat. no.  
531 11644793001) after 4 h incubation at 37°C.

532 ADCP assays were performed using ExpiCHO-S cells transiently transfected with SARS-  
533 CoV-2 spike glycoproteins (Wuhan D614, BA.1, or BA.2) and labelled with PKH67 (Sigma  
534 Aldrich) as targets. PMBCs from healthy donors were labelled with CellTrace Violet  
535 (Invitrogen) and used as source of phagocytic effector cells. Target cells (10,000 per well) were  
536 incubated with titrated concentrations of mAbs for 10 min and then mixed with PBMCs (200,000  
537 per well). The next day, cells were stained with APC-labelled anti-CD14 mAb (BD Pharmingen),  
538 BV605-labelled anti-CD16 mAb (Biolegend), BV711-labelled anti-CD19 mAb (Biolegend),  
539 PerCP/Cy5.5-labelled anti-CD3 mAb (Biolegend), APC/Cy7-labelled anti-CD56 mAb

540 (Biolegend) for the identification of CD14<sup>+</sup> monocytes. After 20 min, cells were washed and  
541 fixed with 4% paraformaldehyde before acquisition on a ZE5 Cell Analyzer (Biorad). Data were  
542 analyzed using FlowJo software. The % ADCP was calculated as % of monocytes (CD3<sup>-</sup> CD19<sup>-</sup>  
543 CD14<sup>+</sup> cells) positive for PKH67.

544 **Data availability.** All data supporting the findings of this study are available within the  
545 paper and are available from the corresponding author upon request.

546 **Statistical analysis.** All statistical tests were performed as described in the indicated  
547 figure legends using Prism 8.0. Statistical significance was determined using a one-way ANOVA  
548 when comparing three or more groups. When comparing two groups, a Mann-Whitney test was  
549 performed. The number of independent experiments performed are indicated in the relevant  
550 figure legends. For correlation analyses, best-fit lines were calculated using a simple linear  
551 regression. Two-tailed Pearson correlation was used to calculate the R<sup>2</sup> and P values indicated  
552 within each panel.

553

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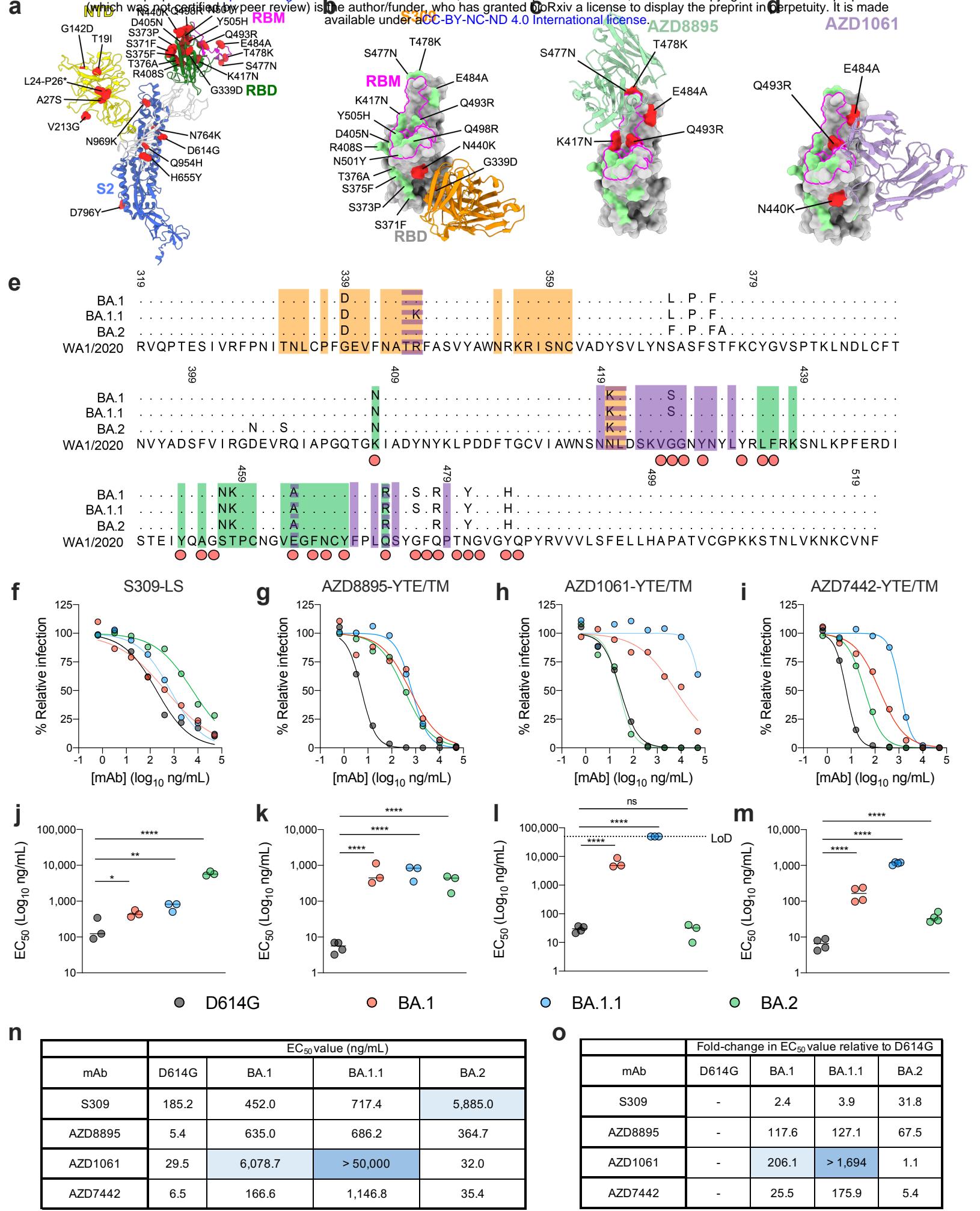


Figure 1

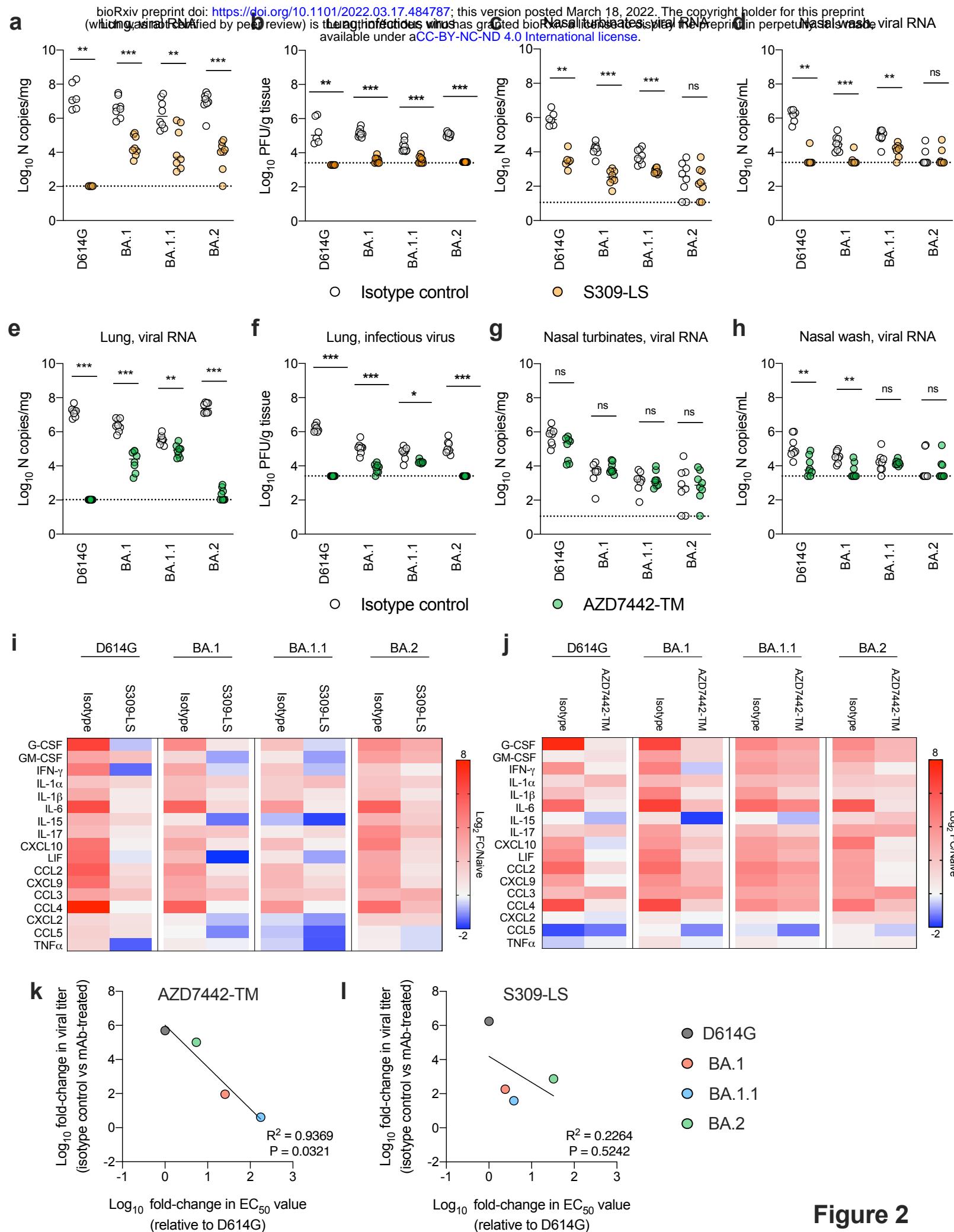


Figure 2

