

# 1 Clinical grade ACE2 effectively inhibits SARS-CoV-2 Omicron 2 infections

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34 **Abstract**

35 The recent emergence of the SARS-CoV-2 variant Omicron has caused considerable  
36 concern due to reduced vaccine efficacy and escape from neutralizing antibody  
37 therapeutics. Omicron is spreading rapidly around the globe and is suspected to account  
38 for most new COVID-19 cases in several countries, though the severity of Omicron-  
39 mediated disease is still under debate. It is therefore paramount to identify therapeutic  
40 strategies that inhibit the Omicron SARS-CoV-2 variant. Here we report using 3D  
41 structural modelling that Spike of Omicron can still associate with human ACE2. Sera  
42 collected after the second mRNA-vaccination did not exhibit a protective effect against  
43 Omicron while strongly neutralizing infection of VeroE6 cells with the reference Wuhan  
44 strain, confirming recent data by other groups on limited vaccine and convalescent sera  
45 neutralization efficacy against Omicron. Importantly, clinical grade recombinant human  
46 soluble ACE2, a drug candidate currently in clinical development, potently neutralized  
47 Omicron infection of VeroE6 cells with markedly enhanced potency when compared to  
48 reference SARS-CoV-2 isolates. These data show that SARS-CoV-2 variant Omicron can  
49 be readily inhibited by soluble ACE2, providing proof of principle of a viable and effective  
50 therapeutic approach against Omicron infections.

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### 53      **Introduction**

54      The initial step of SARS-CoV-2 infection is binding of the viral Spike protein to Angiotensin  
55      converting enzyme 2 (ACE2) [1-3], followed by proteolytic processing of the trimeric Spike  
56      protein [4, 5]. Blocking the Spike/ACE2 interaction is the fundamental principle for the  
57      activity of neutralizing antibodies induced by all current vaccines [6, 7]. Similarly, all  
58      approved and in development antibodies or nanobodies act by blocking the interaction of  
59      the cell-entry receptor ACE2 and the viral Spike protein [8]. Interfering with binding of  
60      Spike to its surface receptor ACE2 has become a key paradigm of both vaccine design  
61      and multiple therapeutic approaches including ACE2 based therapeutics [9-12]. Vaccines  
62      and antibody therapeutics have had an enormous impact on the COVID-19 pandemic.  
63      However, many variants of SARS-CoV-2 have emerged throughout the pandemic [13,  
64      14], some of which have been designated variants of concern (VOC) by the WHO  
65      because of their increased infectivity and transmissibility.

66      Mutations in the viral Spike protein are of critical importance in viral evolution. These  
67      mutations do not only affect the infectivity and transmissibility of SARS-CoV-2, but also  
68      reduce the potency of vaccines, convalescent sera, and monoclonal antibody  
69      therapeutics [14-20]. The recent emergence of the Omicron variant, which contains 61  
70      nonsynonymous mutations relative to the original Wuhan strain, is a key example [21-23].  
71      More such variants will probably evolve in the future, also in part due to population scale  
72      measurements and thereby mounting evolutionary pressure on the virus strains.  
73      Mathematical modelling to simulate the dynamics of wild-type and variant strains of  
74      SARS-CoV-2 in the context of vaccine rollout and nonpharmaceutical interventions has  
75      shown variants with enhanced transmissibility such as Delta and Omicron frequently  
76      increase epidemic severity, whereas those with partial immune escape either fail to  
77      spread widely or primarily cause reinfections and breakthrough infections [24]. However,  
78      when these phenotypes are combined, a variant can continue spreading even as  
79      immunity builds up in the population, limiting the impact of vaccination and exacerbating  
80      the epidemic. Moreover, based on the experience with HIV therapeutics, it is possible that  
81      SARS-CoV-2 variants will emerge that reduce the efficacy of RNA polymerase and

82 protease inhibitors[25-27]. It is therefore paramount to identify robust and universal  
83 therapeutics for the prevention and treatment of Omicron and future variants of concern.

84 The Spike/ACE2 interaction is the crucial first step of viral infection. There are concerns  
85 that Omicron might also carry mutations that alter its dependency on ACE2 as entry gate,  
86 thereby also changing its infectivity and tissue tropism. Here we report, using molecular  
87 3D modelling, that human ACE2 can associate with the receptor binding domain (RBD)  
88 and full-length Spike proteins of the Omicron SARS-CoV-2 variant. Sera from SARS-CoV-  
89 2 naïve and doubly mRNA vaccinated people fail to neutralize Omicron infections of  
90 VeroE6 cells, in line with other emerging data that Omicron can in part escape humoral  
91 immunity induced by vaccination and in convalescent people [28-30], explaining the  
92 increasing number of breakthrough infections. Most importantly, soluble ACE2/APN01,  
93 already being tested in clinical trials for severe (WHO stage 4-6) COVID-19  
94 (NCT04335136) and in a phase 1 inhalation trial for early intervention, potently and  
95 effectively neutralizes infections of Omicron. In addition to our recent data that ACE2  
96 blocks all other known SARS-CoV-2 variants of concern, these results provide the  
97 blueprint for a universal anti-COVID-19 agent with the potential to alleviate or prevent  
98 infections with Omicron.

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100

## 101 **Results**

102

### 103 **3D modelling of Omicron Spike and Omicron binding to human ACE2.**

104 Many single or compound mutations, especially in the RBD domain of the viral Spike,  
105 have been described and either hypothesized or demonstrated to affect binding to the  
106 cell entry receptor ACE2 (see [14] for a review). For the newly emerged Omicron variant,  
107 it has been proposed, based on clinical presentations and preprint data, that the mutant  
108 Omicron Spike might not or only in an altered manner bind to ACE2, a critical issue for  
109 the understanding of disease pathogenesis and viral tropism and therefore potential  
110 treatment and vaccine designs. Omicron carries 36 mutations in Spike including multiple

111 alterations in the RBD. We first rendered all mutations of the prefusion state Spike of  
112 Omicron in 3D using molecular modelling (Figure 1a). The RBD changes of Omicron and  
113 the location(s) of the respective mutations are further depicted in a 3D model of the viral  
114 Spike RBD domain (Figure 1b).

115 Importantly, structural modelling of ACE2 binding revealed that pre-fusion Omicron Spike  
116 could still associate with human ACE2 (Figure 1c). Mutations at residues K417N, E484A,  
117 Q493R, Q498R, N501Y, and Y505H at the RBD directly affect binding of Omicron Spike to  
118 human ACE2, resulting most likely in greater binding affinity [31]. Of note, the real affinity of  
119 the Omicron Spike-ACE2 interaction needs to be determined in direct biochemical  
120 experiments and cannot be deduced faithfully from our modelling. Interestingly, Omicron  
121 carries mutations at Q498 at Q493, both of these mutations have been reported in mouse-  
122 adapted virus strains [32-34], including our recently developed mouse adapted maVie16  
123 SARS-CoV-2 strain that causes severe COVID-19 in mice [35]. Thus, it is likely that  
124 Omicron will infect rodents. We also modelled the 22 N-glycosylation sites of Spike we  
125 and others have previously reported, some of which (N165, N234, N343) directly interact  
126 with ACE2 or its glycans [36, 37]. Intriguingly, despite the unprecedented number of  
127 observed mutations, none of the N-glycosylation sites critical for ACE2 binding are altered  
128 in Omicron Spike (Figure 1c). These molecular modelling data support that pre-fusion  
129 Omicron Spike can still readily associate with human ACE2.

130 **Impaired neutralization of Omicron by RNA vaccine elicited antibodies.**

131 We finally tested whether sera from vaccinated people could also still neutralize Omicron  
132 infections of VeroE6 cells. To this end, we obtained sera from four SARS-CoV-2 naïve  
133 healthcare workers who received an mRNA vaccine (Comirnaty); these sera were  
134 collected following ethical approvals 5-7 weeks after the second vaccination. In all cases  
135 we observed significant inhibition of infection of the reference SARS-CoV-2 strain.  
136 However, at the dilutions used we did not detect any neutralization of Omicron infection  
137 of VeroE6 cells (Figure 2). These results are in line with recently emerging studies [38-  
138 41] that Omicron carries mutations that can in part escape neutralization by peak antibody  
139 levels elicited by the currently standard mRNA vaccines.

140 **ACE2/APN01 effectively neutralizes the Omicron SARS-CoV-2 variant.**

141 We have previously reported that clinical grade soluble recombinant human ACE2  
142 (APN01) can effectively reduce the SARS-CoV-2 viral load in VeroE6 cells in a dose  
143 dependent manner, using a reference virus isolated early during the pandemic[42]. This  
144 virus carried the same Spike sequence as the originally reported virus. Moreover, we and  
145 others have shown that ACE2/APN01 not only binds significantly stronger to RBD or full-  
146 length Spike proteins of all tested variants (alpha, beta, gamma, delta), but also more  
147 potently inhibits viral infection by these strains [30, 31].

148 To test whether APN01 can also neutralize Omicron SARS-CoV-2 isolates, we performed  
149 neutralization assays in VeroE6 cells and compared its inhibitory potency side by side to  
150 our reference strain. Of note, VeroE6 cells are commonly used to assay SARS-CoV-2  
151 infectivity and drug efficacy. The reference virus was previously reported [42, 43] and  
152 carries the Spike amino acid sequence described for the first Wuhan virus isolate. As  
153 reported before [42], APN01 markedly reduced viral replication of the SARS-CoV-2  
154 reference strain in a dose dependent manner (Figure 3a). Importantly, the inhibitory  
155 potency of APN01 was significantly increased towards the Omicron variant of concern  
156 (Figure 3b). These results show that clinical grade soluble human ACE2/APN01 potently  
157 blocks SARS-CoV-2 infections of the recently emerged Omicron VOC.

158

159 **Discussion**

160 Seventeen years after the epidemic of SARS coronavirus, the novel coronavirus SARS-  
161 CoV-2 emerged, resulting in an unprecedented pandemic. Throughout the COVID-19  
162 pandemic, a plethora of genetic SARS-CoV-2 variants have emerged with some strains  
163 displaying increased infectivity and transmissibility, therefore designated Variants of  
164 Concern (VOC; <https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html>  
165 and <https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/> for further  
166 information). Besides the VOC B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma) and  
167 B.1.617.2 (Delta), multiple Variants of Interest (VOI) are also circulating including B.1.526  
168 (Iota), B.1.427, B.1.429, B.1.617.1 (Kappa), B.1.617.3, or B.1.525 (Eta). The last weeks

169 have seen the emergence of the Omicron VOC with an unprecedented number of genetic  
170 alterations, including 36 changes in Spike [44] <https://www.gisaid.org/hcov19-variants/> .  
171 Omicron rapidly spreads and readily re-infects doubly and even triply vaccinated people  
172 as well as patients recovered from previous SARS-CoV-2 infections, sometimes leading  
173 to severe breakthrough COVID-19, as had been previously observed with the Delta  
174 variant [21, 22]. Omicron already has a devastating impact on case numbers in several  
175 countries, causing lockdowns and comparable measures, severely affecting social and  
176 economic life. Besides improving vaccine designs, booster vaccinations, and the  
177 development of adjusted antibodies, it is paramount to identify strategies that might help  
178 prevent and treat infections with all current and potential future variants, with a particular  
179 urgency for Omicron.

180 The SARS-CoV-2 Spike protein interacts with very high affinity with cell membrane bound  
181 ACE2, followed by a subsequent membrane fusion step. Most neutralizing antibodies  
182 from vaccinations and convalescent plasma therapies interfere with the Spike/ACE2  
183 interaction and nearly all therapeutic monoclonal antibodies or nanobodies have been  
184 designed to inhibit the binding of Spike to ACE2 [14]. Conceptually, all SARS-CoV-2  
185 variants and “escape mutants” still bind to ACE2 [45-51], which we have recently  
186 experimentally shown for the alpha, beta, gamma and delta variants [31]. Moreover,  
187 although various other receptors and co-receptors have been proposed, we and others  
188 have recently shown that ACE2 is the essential SARS-CoV-2 receptor for respiratory *in*  
189 *vivo* infections using ACE2 mutant mice as well as various human organoids, namely  
190 human stem cell derived kidney, gastric, and gut organoids as well as stem cell derived  
191 cardiomyocytes [52-54], confirming that ACE2 is of crucial importance for COVID-19  
192 development and the course of the pandemic. ACE2 is a carboxypeptidase which  
193 degrades angiotensin II, des-Arg(9)-bradykinin, and apelin, and thereby is a critical  
194 regulator of cardiovascular physiology and pathology, kidney disease, diabetes,  
195 inflammation, and tissue fibrosis [55]. In addition, the enzymatic activity of ACE2 is  
196 protective against acute respiratory distress syndrome (ARDS) caused by viral and non-  
197 viral pneumonias, aspiration, or sepsis. Upon infection, both SARS-CoV-2 and SARS-  
198 CoV-1 coronaviruses downregulate ACE2 expression, likely associated with the

199 pathogenesis of ARDS[56]. Thus, ACE2 is not only the SARS-CoV-2 receptor but might  
200 also play an important role in multiple aspects of COVID-19 pathogenesis and possibly  
201 post-COVID-19 syndromes, a hypothesis that has now in part been experimentally  
202 confirmed for SARS-CoV-2 induced lung injury using a bacterial orthologue of ACE2  
203 termed B38-CAP [57]. Soluble forms of recombinant human ACE2 are currently utilized  
204 by multiple research groups and companies as a potential pan-variant decoy to neutralize  
205 SARS-CoV-2 and to supplement the ACE2 carboxypeptidase activity. Our data now show  
206 that clinical grade soluble ACE2 can neutralize infections with the SARS-CoV-2 variant  
207 Omicron with more than an order of magnitude increased potency when compared to the  
208 Wuhan reference strain.

209 Our modelling data predict that soluble ACE2 could readily associate with the RBD and  
210 prefusion trimeric Spike of Omicron, most likely with increased affinity and avidity, which  
211 is in line with our experimental data on the markedly improved efficacy of ACE2/APN01  
212 to block Omicron infections. Moreover ACE2/APN01 has very high affinity to all other  
213 Variants of Concern and markedly enhanced efficacy to block these infections including  
214 the Delta variant, demonstrating that the prediction holds true – clinical grade ACE2 can  
215 effectively block all tested SARS-CoV-2 variants and this inhibition is markedly improved  
216 against all Variants of Concern and Variants of Interest, including Omicron. APN01 has  
217 now undergone phase 2 testing in WHO stage 4-6 COVID-19 patients using intravenous  
218 infusions and, in cooperation with researchers at the NIH, we have developed a  
219 formulation of APN01 that can be inhaled as an aerosol to directly interfere with the  
220 earliest steps of viral infection and COVID-19 development [58]. That this inhalation  
221 approach can indeed protect from SARS-CoV-2 infections has been directly confirmed in  
222 mice infected with our new mouse adapted SARS-CoV-2 variant, that carries two  
223 mutations also found in Omicron [35, 58]. Inhalation of soluble ACE2/APN01 is currently  
224 tested in phase 1 trials to assess its safety and tolerability.

225 The source of the VOC Omicron is currently unclear and multiple hypotheses have been  
226 put forward to explain the high number of mutations, including animal hosts as well as  
227 protracted infections in immunocompromised hosts that could have led to the gradual  
228 evolution of this variant. Additionally, selective pressure by both mass vaccination

229 programs, and antibody and small molecule therapeutics are likely to promote further viral  
230 evolution and drive the emergence of therapeutic-/antibody-resistant variant strains of  
231 SARS-CoV-2. This viral evolution has already led to the emergence of the Delta and now  
232 Omicron VOC that caused devastating global waves of infection and, concerningly, large  
233 numbers of re-infections. Our data now shows that clinical grade ACE2/APN01 blocks  
234 infectivity of Omicron supporting the notion that this therapeutic is inherently resistant to  
235 escape mutations. Our first experimental demonstration that ACE2 inhibits Omicron  
236 infections with high efficacy, studies by other groups working on ACE2 and clinical data  
237 using soluble ACE2/APN01 support the development of this universal and pan-variant  
238 SARS-CoV-2 prevention and therapy. In particular, such an approach should be viable  
239 and effective to prevent and treat Omicron infections.

240 **Limitations of this study.** Our study used VeroE6 cells, the classic cellular model for  
241 SARS and SARS-CoV-2 infection studies. The study should be expanded to additional  
242 cell types as well as human organoids. Moreover, the affinity of Omicron Spike and  
243 Omicron RBD should be determined in direct affinity/avidity measurements as well as the  
244 impact of non-RBD Spike mutations on the infection process. Of note, from all our  
245 previous studies and studies from other groups, the data on soluble ACE2 inhibiting  
246 SARS-CoV-2 infections in VeroE6 cells were always supported by results in all other cell  
247 types tested. Moreover, we used sera collected 4-6 weeks after the second mRNA  
248 vaccination from 4 SARS-CoV-2 naïve healthcare workers, which needs to be expanded  
249 to different vaccine regimens and vaccine types and increased sample numbers, though  
250 multiple studies are now being released also demonstrating impaired vaccine efficacies  
251 to Omicron.

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267  
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269 Apeiron Biologics that is developing soluble ACE2/APN01 for COVID-19 therapy. All other  
270 authors have nothing to disclose.

271

272 **Materials and Methods**

273 **Viral Strains and isolates.** SARS-CoV-2 Wuhan and Omicron strains were isolated from  
274 nasopharyngeal swabs from patients in Sweden. The isolates were sequenced by Next-  
275 Generation Sequencing (Genbank accession number MT093571).

276 **Sera of vaccinees.** Serum was taken 5-7 weeks after the second immunization with the  
277 mRNA vaccine Comirnity (median dose interval 21 days (range 21-24) from four SARS-  
278 CoV-2 naïve healthcare workers (75% female, median age 46 [IQR 37-59]) which took  
279 part in the COMMUNITY study. The COMMUNITY study was approved by the Swedish  
280 Ethical Review Authority (Dnr: 2020-01653).

281

282 **Cell lines and cell culture.** African green monkey kidney epithelial VeroE6 (ATCC) were  
283 grown in Dulbecco's Modified Eagle's Medium (DMEM, ThermoFisher, supplemented with  
284 1% Non-Essential Amino-Acids (ThermoFisher), 10mM HEPES (ThermoFisher) and 10%  
285 FBS) at 37°C, 5% CO<sub>2</sub>. Infection and APN01 mediated viral neutralisation assays were  
286 conducted at the Karolinska Institute and Karolinska University Hospital.

287 **Viral neutralization experiments.** 24h after seeding of VeroE6 cells (5x10<sup>5</sup> per 48 well),  
288 APN01 was mixed with viral particles (MOI of 0.01) of the indicated strains at the given  
289 concentrations in DMEM Medium (ThermoFischer) containing 5% FBS in 100µl per well  
290 and incubated for 30min at 37°C. After the incubation period medium was removed from  
291 VeroE6 cells, cells were washed once with PBS to remove any non-attached cells and  
292 virus/APN01 mixtures. Cells were incubated with virus for 15h, after which cells were  
293 washed 3 times with PBS and lysed with Trizol, subsequently. RNA was extracted using  
294 the direct-zol RNA kit (Zymo Research) and assayed by qRT-PCR as previously  
295 described (Monteil et al, Cell, 2020) [42]. For serum neutralization assays VeroE6 cells  
296 were seeded in 48-well plates as described above 24 hours post-seeding and indicated  
297 dilutions of vaccinated subjects sera were mixed with SARS-CoV-2 Wuhan or Omicron  
298 strains at an MOI of 0.01 in a final volume of 100ml per well in DMEM (0% FBS) at 37°C  
299 under shaking conditions for 30 minutes. The serum dilutions used in this experiment  
300 were determined after a neutralization assay against the Wuhan reference strain. After

301 30 minutes, VeroE6 cells were infected with and Serum/SARS-CoV-2 for 15 hours. 15  
302 hours post-infection, supernatants were removed, cells were washed 3 times with PBS  
303 and then lysed using Trizol (Thermofisher) before analysis by qRT-PCR for viral RNA  
304 detection as previously described (Monteil et al, Cell, 2020) [42].

305 **Preparation of recombinant human ACE2.** Clinical grade recombinant human ACE2  
306 (amino acids 18-740) was produced by the contract manufacturer Polymun Scientific  
307 (Klosterneuburg, Austria) from CHO cells according to GMP guidelines under serum free  
308 conditions and formulated as a physiologic aqueous solution, as described previously  
309 (Zoufaly et al, 2021, Lancet Respiratory Medicine).

310 **Visualizations of RBD domains, full-length Spike protein, and Omicron Spike-ACE2  
311 interactions.** Visualizations were rendered with pymol software (the PyMOL Molecular  
312 Graphics System, Version 2.0 Schrödinger, LLC), based on a model of the fully  
313 glycosylated Spike-ACE2 complex described in Capraz et al. [37] and  
314 [https://covid.molssi.org/#spike-protein-in-complex-with-human-ace2-ace2-  
315 spike-binding](https://covid.molssi.org/#spike-protein-in-complex-with-human-ace2-ace2-spike-binding).

316 **Primers.** The following tables lists the primers used in this study:

Name	Sequence	Target	Source
SARS-CoV-2 E gene - fwd	ACAGGTACGTTAATAGTTAATA GCGT	SARS-CoV-2 E gene	Monteil et al, 2020 [42]
SARS-CoV-2 E gene - rev	ATATTGCAGCAGTACGCACAC A	SARS-CoV-2 E gene	Monteil et al, 2020 [42]
SARS-CoV-2 E gene - probe	FAM- ACACTAGCCATCCTACTGCG CTTCG-QSY	SARS-CoV-2 E gene	Monteil et al, 2020 [42]
Human RNase P - fwd	AGATTGGACCTGCGAGCG	Human RNase P	Monteil et al, 2020 [42]
Human RNase P - rev	GAGCGGCTGTCTCCACAAGT	Human RNase P	Monteil et al, 2020 [42]
Human RNase P- probe	FAM-TTCTGACCTGAAGGCTCT GCGCG-MGB	Human RNase P	Monteil et al, 2020 [42]

317

318

319 **Figure Legends**

320

321 **Figure 1. Omicron Spike and RBD mutations and ACE2 interaction.**

322 **(a)** PyMOL rendering of the trimeric full-length SARS-CoV-2 Spike protein of the Wuhan  
323 reference strain. One RBD domain is shown in red. Indicated in green are positions  
324 mutated in the Omicron SARS-CoV-2 variant Spike used in experiments in this study.  
325 Depicted in yellow are the glycan-modifications of the Spike protein. **(b)** PyMOL rendering  
326 depicting the SARS-CoV-2 RBD domain from the Wuhan reference strain with positions  
327 mutated in Omicron RBD highlighted in green. **(c)** PyMOL rendering of trimeric Spike  
328 (RBD in red) with human ACE2 (magenta). Positions mutated in Omicron are highlighted  
329 in green to indicate their position relative to the interaction surface with ACE2. Glycan  
330 modifications are depicted in yellow on both Spike and ACE2 protein.

331

332 **Figure 2. Loss of viral neutralization potency of doubly mRNA vaccinated subjects'**  
333 **sera against Omicron.**

334 **(a)** Diagrams depict the level of infection (left panels) or inhibition of infection (right panels)  
335 with the SARS-CoV-2 Wuhan reference strain isolate in the presence of the indicated  
336 dilutions of four different mRNA vaccinee's sera. Sera were taken 5-7 weeks after the  
337 second mRNA vaccination, i.e. at the peak of the antibody response, **(b)** Diagrams depict  
338 the level of infection with the SARS-CoV-2 Omicron strain isolate in the presence of the  
339 indicated dilutions of sera from (a). Data from Vero E6 cell infections are shown at MOI  
340 0.01. Shown in **(a)** and **(b)** are means of triplicate analyses with standard deviations.  
341 Statistical significance is indicated by asterisks (p-value < 0.01: \*\*; p-value < 0.001: \*\*\*  
342 calculated using Two-way ANOVA).

343

344 **Figure 3. Increased potency of soluble ACE2/APN01 against the Omicron SARS-**  
345 **CoV-2 Variant of Concern.**

346 **(a,b)** Diagrams depict the level of infection **(a)** and level of inhibition of infection **(b)** of  
347 Vero E6 cells with the Wuhan SARS-CoV-2 reference isolate (left panels) and the  
348 Omicron SARS-CoV-2 isolate (right panels) in the presence of the indicated  
349 concentrations of soluble ACE2/APN01 as compared to mock treatment. Vero E6 cells  
350 were infected at MOI 0.01. Shown are means of triplicate analyses with standard  
351 deviations. Statistical significance is indicated by asterisks (p-value < 0.001: \*\*\*; p-value  
352 < 0.0001: \*\*\*\* calculated using Two-way ANOVA).

353

354

## 355 **References**

- 356 1. Shang, J., et al., *Structural basis of receptor recognition by SARS-CoV-2*. Nature, 357 2020. **581**(7807): p. 221-224.
- 358 2. Zhou, P., et al., *A pneumonia outbreak associated with a new coronavirus of* 359 *probable bat origin*. Nature, 2020. **579**(7798): p. 270-273.
- 360 3. Wang, Q., et al., *Structural and Functional Basis of SARS-CoV-2 Entry by Using* 361 *Human ACE2*. Cell, 2020. **181**(4): p. 894-904.e9.
- 362 4. Hoffmann, M., et al., *SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2* 363 *and Is Blocked by a Clinically Proven Protease Inhibitor*. Cell, 2020. **181**(2): p. 364 271-280.e8.
- 365 5. Benton, D.J., et al., *Receptor binding and priming of the spike protein of SARS-* 366 *CoV-2 for membrane fusion*. Nature, 2020. **588**(7837): p. 327-330.
- 367 6. Kyriakidis, N.C., et al., *SARS-CoV-2 vaccines strategies: a comprehensive* 368 *review of phase 3 candidates*. npj Vaccines, 2021. **6**(1): p. 28.
- 369 7. Krammer, F., *SARS-CoV-2 vaccines in development*. Nature, 2020. **586**(7830): 370 p. 516-527.
- 371 8. Chen, J., et al., *Review of COVID-19 Antibody Therapies*. Annual Review of 372 Biophysics, 2021. **50**(1): p. 1-30.
- 373 9. Svilenov, H.L., et al., *Efficient inhibition of SARS-CoV-2 strains by a novel ACE2-* 374 *IgG4-Fc fusion protein with a stabilized hinge region*. bioRxiv, 2020: p. 375 2020.12.06.413443.
- 376 10. Tanaka, S., et al., *An ACE2 Triple Decoy that neutralizes SARS-CoV-2 shows* 377 *enhanced affinity for virus variants*. Scientific Reports, 2021. **11**(1): p. 12740.
- 378 11. Higuchi, Y., et al., *Engineered ACE2 receptor therapy overcomes mutational* 379 *escape of SARS-CoV-2*. Nature Communications, 2021. **12**(1): p. 3802.
- 380 12. Hassler, L., et al., *A novel soluble ACE2 protein totally protects from lethal* 381 *disease caused by SARS-CoV-2 infection*. bioRxiv, 2021: p. 2021.03.12.435191.
- 382 13. Banerjee, A., K. Mossman, and N. Grandvaux, *Molecular Determinants of SARS-* 383 *CoV-2 Variants*. Trends in Microbiology, 2021. **29**(10): p. 871-873.

384 14. Harvey, W.T., et al., *SARS-CoV-2 variants, spike mutations and immune escape*.  
385 *Nature Reviews Microbiology*, 2021. **19**(7): p. 409-424.

386 15. Planas, D., et al., *Reduced sensitivity of SARS-CoV-2 variant Delta to antibody*  
387 *neutralization*. *Nature*, 2021. **596**(7871): p. 276-280.

388 16. Garcia-Beltran, W.F., et al., *Multiple SARS-CoV-2 variants escape neutralization*  
389 *by vaccine-induced humoral immunity*. *Cell*, 2021. **184**(9): p. 2372-2383.e9.

390 17. Cele, S., et al., *Escape of SARS-CoV-2 501Y.V2 from neutralization by*  
391 *convalescent plasma*. *Nature*, 2021. **593**(7857): p. 142-146.

392 18. Greaney, A.J., et al., *Mapping mutations to the SARS-CoV-2 RBD that escape*  
393 *binding by different classes of antibodies*. *Nature Communications*, 2021. **12**(1):  
394 p. 4196.

395 19. Lopez Bernal, J., et al., *Effectiveness of Covid-19 Vaccines against the B.1.617.2*  
396 *(Delta) Variant*. *New England Journal of Medicine*, 2021. **385**(7): p. 585-594.

397 20. Jangra, S., et al., *SARS-CoV-2 spike E484K mutation reduces antibody*  
398 *neutralisation*. *The Lancet Microbe*, 2021. **2**(7): p. e283-e284.

399 21. Farinholt, T., et al., *Transmission event of SARS-CoV-2 Delta variant reveals*  
400 *multiple vaccine breakthrough infections*. *medRxiv*, 2021: p.  
401 2021.06.28.21258780.

402 22. Christensen, P.A., et al., *Delta Variants of SARS-CoV-2 Cause Significantly*  
403 *Increased Vaccine Breakthrough COVID-19 Cases in Houston, Texas*. *The*  
404 *American Journal of Pathology*, 2021.

405 23. Wolter, N., et al., *Early assessment of the clinical severity of the SARS-CoV-2*  
406 *Omicron variant in South Africa*. *medRxiv*, 2021: p. 2021.12.21.21268116.

407 24. Bushman, M., et al., *Population impact of SARS-CoV-2 variants with enhanced*  
408 *transmissibility and/or partial immune escape*. *Cell*, 2021. **184**(26): p. 6229-  
409 6242.e18.

410 25. Eckerle Lance, D., et al., *High Fidelity of Murine Hepatitis Virus Replication Is*  
411 *Decreased in nsp14 Exoribonuclease Mutants*. *Journal of Virology*, 2007. **81**(22):  
412 p. 12135-12144.

413 26. Eckerle, L.D., et al., *Infidelity of SARS-CoV Nsp14-Exonuclease Mutant Virus*  
414 *Replication Is Revealed by Complete Genome Sequencing*. *PLOS Pathogens*,  
415 2010. **6**(5): p. e1000896.

416 27. Kabinger, F., et al., *Mechanism of molnupiravir-induced SARS-CoV-2*  
417 *mutagenesis*. *Nature Structural & Molecular Biology*, 2021. **28**(9): p. 740-746.

418 28. Wilhelm, A., et al., *Reduced Neutralization of SARS-CoV-2 Omicron Variant by*  
419 *Vaccine Sera and Monoclonal Antibodies*. *medRxiv*, 2021: p.  
420 2021.12.07.21267432.

421 29. Schmidt, F., et al., *Plasma neutralization properties of the SARS-CoV-2 Omicron*  
422 *variant*. *medRxiv*, 2021: p. 2021.12.21267646.

423 30. Zhang, X., et al., *SARS-CoV-2 Omicron strain exhibits potent capabilities for*  
424 *immune evasion and viral entrance*. *Signal Transduction and Targeted Therapy*,  
425 2021. **6**(1): p. 430.

426 31. Wirnsberger, G., et al., *Clinical grade ACE2 as a universal agent to block SARS-*  
427 *CoV-2 variants*. *bioRxiv*, 2021: p. 2021.09.10.459744.

428 32. Gu, H., et al., *Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine*  
429 *efficacy*. *Science*, 2020. **369**(6511): p. 1603-1607.

430 33. Dinnon, K.H., 3rd, et al., *A mouse-adapted model of SARS-CoV-2 to test COVID-19 countermeasures*. Nature, 2020. **586**(7830): p. 560-566.

431 34. Huang, K., et al., *Q493K and Q498H substitutions in Spike promote adaptation of SARS-CoV-2 in mice*. EBioMedicine, 2021. **67**: p. 103381.

432 35. Gawish, R., et al., *ACE2 is the critical &lt;em&gt;in vivo&lt;/em&gt; receptor for SARS-CoV-2 in a novel COVID-19 mouse model with TNF- and IFNy-driven immunopathology*. bioRxiv, 2021: p. 2021.08.09.455606.

433 36. Hoffmann, D., et al., *Identification of lectin receptors for conserved SARS-CoV-2 glycosylation sites*. The EMBO Journal, 2021. **40**(19): p. e108375.

434 37. Capraz, T., et al., *Structure-guided glyco-engineering of ACE2 for improved potency as soluble SARS-CoV-2 decoy receptor*. bioRxiv, 2021: p. 2021.08.31.458325.

435 38. Aggarwal, A., et al., *SARS-CoV-2 Omicron: evasion of potent humoral responses and resistance to clinical immunotherapeutics relative to viral variants of concern*. medRxiv, 2021: p. 2021.12.14.21267772.

436 39. Cameroni, E., et al., *Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron antigenic shift*. bioRxiv, 2021: p. 2021.12.12.472269.

437 40. Cao, Y.R., et al., *Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies*. bioRxiv, 2021: p. 2021.12.07.470392.

438 41. Planas, D., et al., *Considerable escape of SARS-CoV-2 variant Omicron to antibody neutralization*. bioRxiv, 2021: p. 2021.12.14.472630.

439 42. Monteil, V., et al., *Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2*. Cell, 2020. **181**(4): p. 905-913.e7.

440 43. Monteil, V., et al., *Human soluble ACE2 improves the effect of remdesivir in SARS-CoV-2 infection*. EMBO Molecular Medicine, 2021. **13**(1): p. e13426.

441 44. Karim, S.S.A. and Q.A. Karim, *Omicron SARS-CoV-2 variant: a new chapter in the COVID-19 pandemic*. The Lancet, 2021. **398**(10317): p. 2126-2128.

442 45. Gobeil Sophie, M.C., et al., *Effect of natural mutations of SARS-CoV-2 on spike structure, conformation, and antigenicity*. Science. **373**(6555): p. eabi6226.

443 46. Tchesnokova, V., et al., *Acquisition of the L452R Mutation in the ACE2-Binding Interface of Spike Protein Triggers Recent Massive Expansion of SARS-CoV-2 Variants*. Journal of Clinical Microbiology. **59**(11): p. e00921-21.

444 47. Tian, F., et al., *N501Y mutation of spike protein in SARS-CoV-2 strengthens its binding to receptor ACE2*. eLife, 2021. **10**: p. e69091.

445 48. Zhou, D., et al., *Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera*. Cell, 2021. **184**(9): p. 2348-2361.e6.

446 49. Ou, J., et al., *V367F Mutation in SARS-CoV-2 Spike RBD Emerging during the Early Transmission Phase Enhances Viral Infectivity through Increased Human ACE2 Receptor Binding Affinity*. Journal of Virology. **95**(16): p. e00617-21.

447 50. Motozono, C., et al., *SARS-CoV-2 spike L452R variant evades cellular immunity and increases infectivity*. Cell Host & Microbe, 2021. **29**(7): p. 1124-1136.e11.

448 51. Cai, Y., et al., *Structural basis for enhanced infectivity and immune evasion of SARS-CoV-2 variants*. Science, 2021. **373**(6555): p. 642-648.

474 52. Garreta, E., et al., *A diabetic &lt;em&gt;milieu&lt;/em&gt; increases cellular*  
475 *susceptibility to SARS-CoV-2 infections in engineered human kidney organoids*  
476 *and diabetic patients.* bioRxiv, 2021: p. 2021.08.13.456228.

477 53. Beumer, J., et al., *A CRISPR/Cas9 genetically engineered organoid biobank*  
478 *reveals essential host factors for coronaviruses.* Nature Communications, 2021.  
479 **12**(1): p. 5498.

480 54. Iwanski, J., et al., *Antihypertensive drug treatment and susceptibility to SARS-*  
481 *CoV-2 infection in human PSC-derived cardiomyocytes and primary endothelial*  
482 *cells.* Stem Cell Reports, 2021. **16**(10): p. 2459-2472.

483 55. Kuba, K., et al., *Trilogy of ACE2: A peptidase in the renin–angiotensin system, a*  
484 *SARS receptor, and a partner for amino acid transporters.* Pharmacology &  
485 Therapeutics, 2010. **128**(1): p. 119-128.

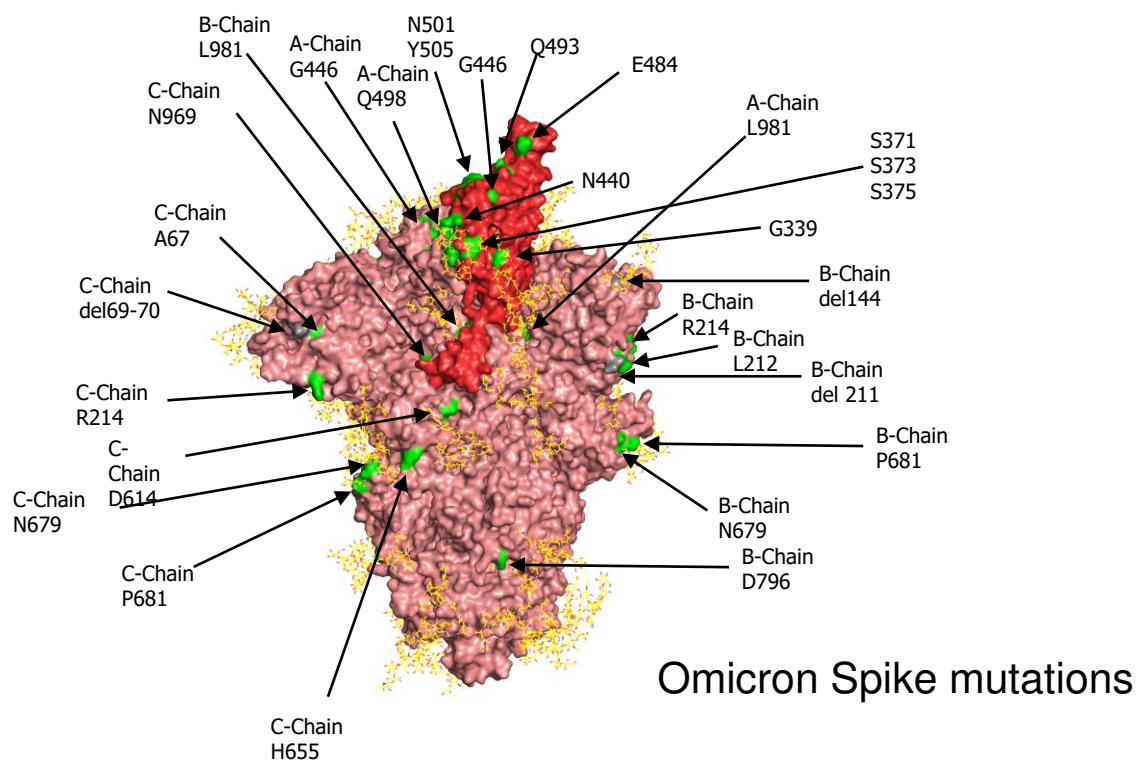
486 56. Kuba, K., T. Yamaguchi, and J.M. Penninger, *Angiotensin-Converting Enzyme 2*  
487 *(ACE2) in the Pathogenesis of ARDS in COVID-19.* Frontiers in Immunology,  
488 2021. **12**(5468).

489 57. Yamaguchi, T., et al., *ACE2-like carboxypeptidase B38-CAP protects from*  
490 *SARS-CoV-2-induced lung injury.* Nature Communications, 2021. **12**(1): p. 6791.

491 58. Shoemaker, R.H., et al., *Development of a novel, pan-variant aerosol*  
492 *intervention for COVID-19.* bioRxiv, 2021: p. 2021.09.14.459961.

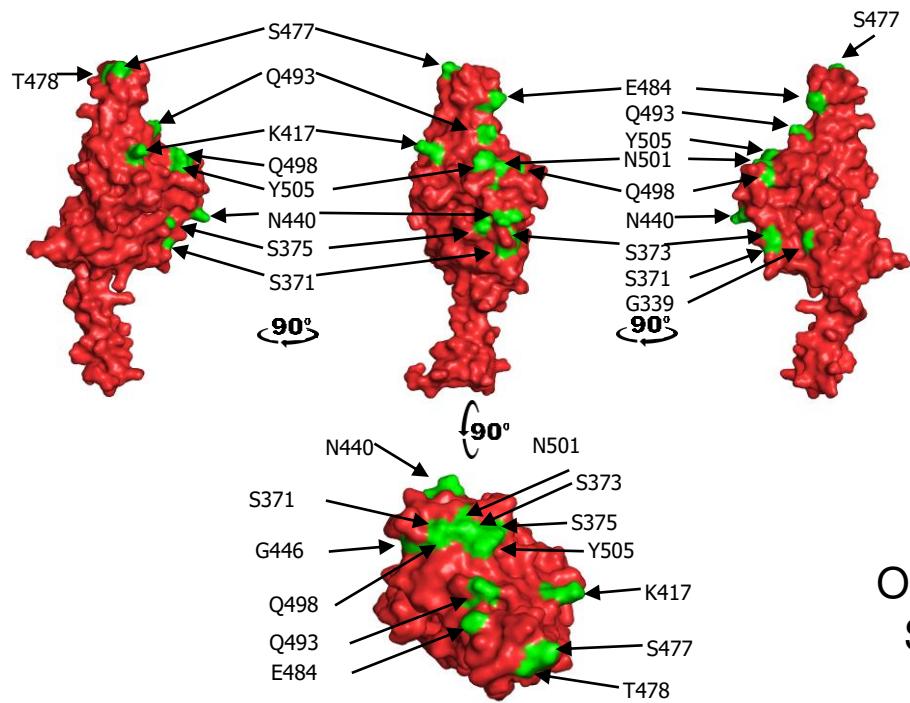
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**a**

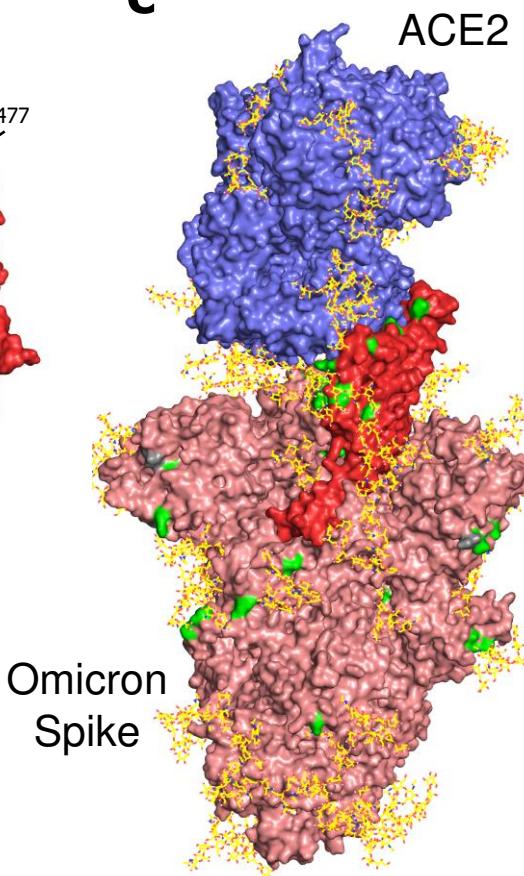


**b**

### Omicron RBD mutations



**c**

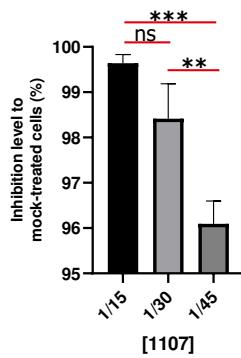
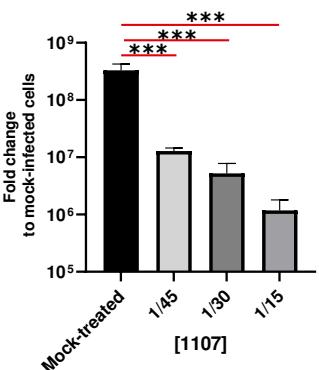


**Figure 1**

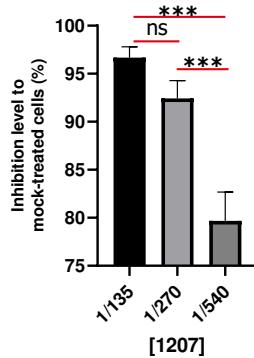
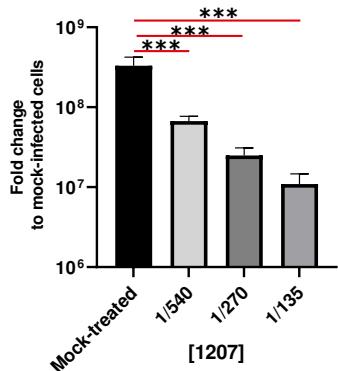
**a**

Wuhan

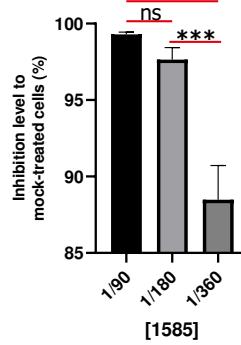
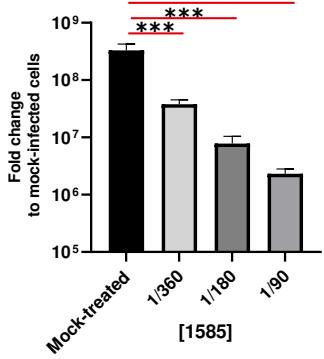
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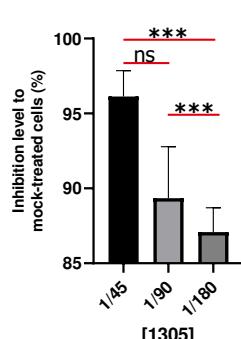
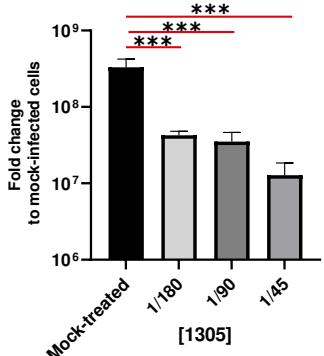
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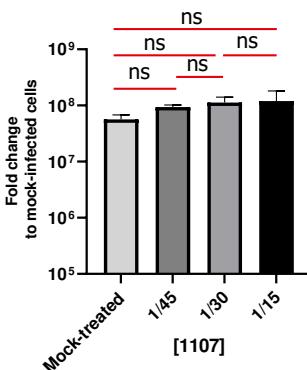
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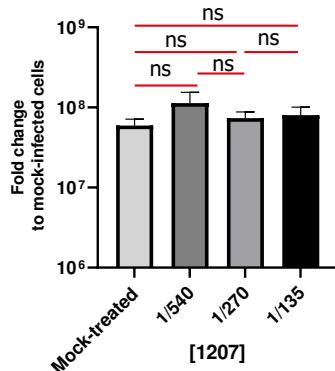
**b**

Omicron

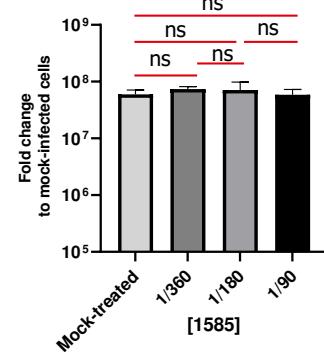
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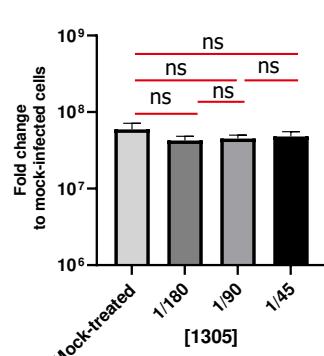
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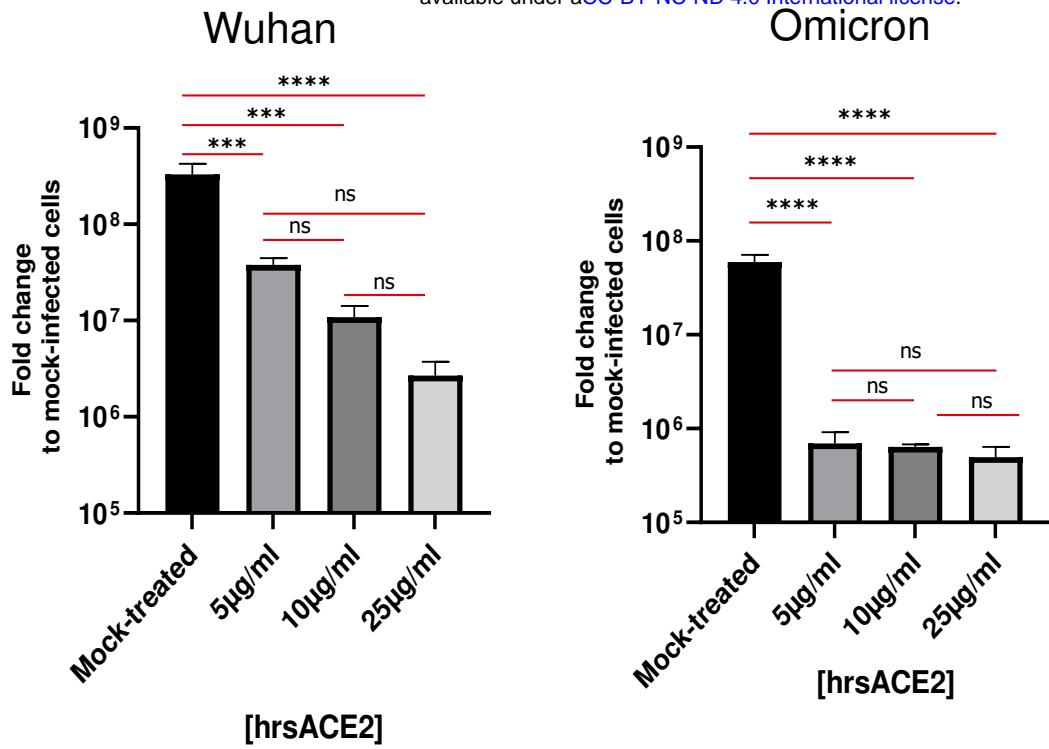


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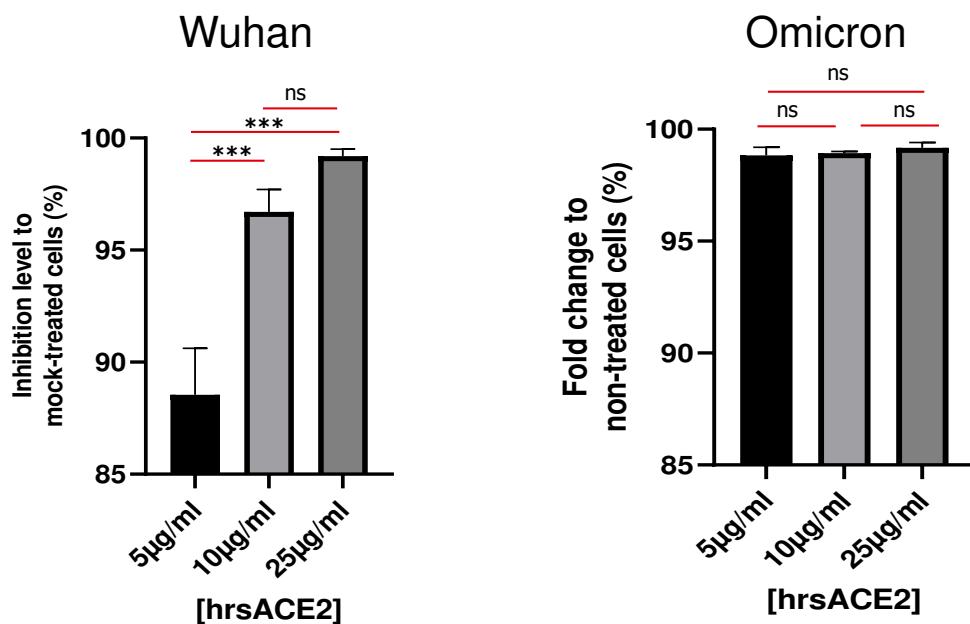


**Figure 2**

**a**



**b**



**Figure 3**