ACE-2-like Enzymatic Activity in Anti-SARS-CoV-2 Spike Protein Monoclonal Antibodies

Yufeng Song[1, #], Frances Mehl[1, #], Tom No[1,¶], Lauren Livingston[1], Juan Sebastian Quintero Barbosa[1], Jun Hayashi[2], Ginette Serrero[2], Pamela Schoppee Bortz[3], Jeffrey M Wilson[3], James E Crowe Jr[4], David D Ho[5], Michael T Yin[6], Joshua Tan[7], Steven L Zeichner[1, 8*]

[1] Department of Pediatrics, University of Virginia, Charlottesville, Virginia, USA.

[2] Precision Antibody Service, 9130 Red Branch Rd., Suite X Columbia, Maryland 21045, United States.

[3] Division of Allergy and Clinical Immunology, Department of Medicine, University of Virginia, Charlottesville, Virginia, USA.

[4] Howard Hughes Medical Institute, Seattle, WA 98109, USA; Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN 37232, USA; Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN 37232, USA.

[5] Aaron Diamond AIDS Research Center, Columbia University Vagelos College of Physicians and Surgeons, New York, NY 10032, USA; Division of Infectious Diseases, Department of Medicine, Columbia University Vagelos College of Physicians and Surgeons, New York, NY 10032, USA; Department of Microbiology and Immunology, Columbia University Vagelos College of Physicians and Surgeons, New York, NY 10032, USA.

[6] Columbia University Vagelos College of Physicians and Surgeons, New York, New York, USA.

[7] Antibody Biology Unit, Laboratory of Immunogenetics, National Institute of Allergy and

[8] Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA; Aaron Diamond AIDS Research Center, Columbia University Vagelos College of Physicians and Surgeons, and Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032, USA.

[9] Department of Microbiology, Immunology, and Cancer Biology, University of Virginia, Charlottesville, Virginia, USA.

[#]Contributed equally to this work.

[¶] Present address: Department of Urology, University of California, Irvine, CA, USA.

*Correspondence: zeichner@virginia.edu

Abstract

Many people are affected by post-acute sequelae of COVID-19 (PASC/long COVID, LC). LC has severely affected public health. Features of LC including blood pressure dysregulation, coagulopathies, hyperinflammation, and neuropsychiatric complaints. Mechanisms responsible for LC pathogenesis are not clear. The receptor for SARS-CoV-2 is human angiotensin converting enzyme 2 (ACE2), which binds SARS-CoV-2 spike protein receptor-binding domain (RBD) to initiate infection. We hypothesized that some people produce anti-RBD antibodies that sufficiently resemble ACE2 structure to have ACE2-like catalytic activity. Those antibodies, ACE2-like abzymes, may contribute to LC pathogenesis. We previously showed that ACE2-like activity was associated with immunoglobulin in some people with acute and convalescent COVID-19. ACE2-like catalytic activity correlated with blood pressure changes following moderate exercise challenge in convalescents. We screened human monoclonal antibodies (mAbs) against SARS-CoV-2 spike protein from 4 sources. We identified 4 human mAbs with ACE2-like catalytic activity. The activity was not inhibited by MLN-4760, a compound that inhibits native human ACE2, nor by EDTA, unlike native ACE2, a Zinc metalloprotease, but was inhibited by an overlapping pool of Spike peptides. Enzyme kinetic studies showed that the mAbs had lower Vmax and *Km* values than ACE2. The data suggested that the antibodies cleave angiotensin II via a different mechanism than ACE2. Identification of mAbs with ACE2-like catalytic activity supports the hypothesis that antibodies induced by SARS-CoV-2 infection could help mediate the pathogenesis of COVID-19 and LC, and more generally, the hypothesis that catalytic antibodies induced by infectious agents can contribute to disease pathogenesis.

Introduction

COVID-19 and Post-Acute Sequelae of COVID-19 (PASC, or Long COVID, LC), as reviewed in [1-3], can affect up to 7% of COVID-19 patients[4]. LC patients can have many unusual symptoms such as dizziness, fatigue, malaise, “brain fog”, alterations in smell or taste, abnormal movements, libido changes, and other neuropsychiatric symptoms, GI symptoms, cardiovascular complaints such as palpitations, blood pressure dysregulation, inflammation, coagulopathies, cough, and chest pain[5 6, 7]. While the clinical features, laboratory findings, and epidemiology of LC have been extensively described, clear primary causal etiologies have yet to be determined [8].

Antibodies with catalytic activity, also called “abzymes” have been known for some time [9, 10]. Investigators initially produced abzymes by generating anti-idiotypic antibodies against antibodies targeting enzymes’ active sites[11, 12]. In some instances, the anti-idiotypic antibodies had sufficient resemblance to the original enzyme’s active site, retaining catalytic activity similar to that of the initially targeted enzyme[13, 14]. However, further research showed that the abzymes had decreased substrate specificity and less favorable enzyme constants compared to the targeted enzymes (*e.g.,* *k*cat/*Km* values ~10^2 - 10^4 s^{-1}•M s^{-1} [15] vs. *k*cat/*Km* values of ~10^5 s^{-1}•M s^{-1})[16 12, 13, 17]. These limitations led to a decline in enthusiasm for the use of abzymes in biotechnological applications.

In an attempt to identify potential mechanisms responsible for the unusual features of COVID-19 and LC, we considered that many of these aberrant features could be viewed as consequences of pathologic derangements of proteolytic regulatory cascades, for example, blood pressure regulation (the renin-angiotensin system), inflammation (C’, kallikrein-kinin)[18-20], and coagulation. For LC, the kinetics of symptom development would be consistent with antibody production, rather than the pathology directly produced by viral replication[21, 22]. While the benefits of COVID-19 vaccination vastly outweigh the risks, the fact that a very small, but still non-zero, fraction of patients who received SARS-CoV-2 vaccines had symptoms including hyperinflammation, blood pressure dysregulation, or coagulopathies [23]further suggests that, in

101 some cases, an antibody response could play a role in pathogenesis.

102 Angiotensin Converting Enzyme 2 (ACE2) on the host cell surface serves as the principal
103 receptor for the SARS-CoV-2 Receptor Binding Domain (RBD). Angiotensin Converting Enzyme
104 (ACE) catalyzes the cleavage of angiotensin I to angiotensin II, which acts as vasoconstrictor to
105 increase blood pressure [24]. ACE2 cleaves angiotensin II to produce angiotensin 1-7, which has
106 vasodilatory activity that lowers blood pressure, an activity that is counterregulatory to AC[25]. We
107 hypothesized that the pathogenesis of some of the unusual features of LC might be caused by
108 the induction of antibodies against the SARS-CoV-2 Spike protein (S) that resembled the
109 structure of ACE2 closely enough to have ACE2-like catalytic activity.

110 In our previous studies[26, 27], we found that some patients' plasma with ACE2-like activity did
111 not require divalent cations, unlike native ACE2, a Zinc metalloprotease. This activity co-
112 depleted with immunoglobulin and was inhibited by a tiled overlapping RBD peptide pool. These
113 findings suggested that the ACE2-like activity observed in patients' plasma differed from native
114 ACE2 and was the result of anti-RBD immunoglobulins exhibiting ACE2-like activity.

115 Additionally, ACE-2 like catalytic activity found in plasma collected from convalescent
116 patients correlated with changes in blood pressure following a moderate exercise challenge[26],
117 suggesting that the catalytic antibodies could have physiologic consequences.

118 However, to definitively establish that COVID-19 patients produce antibodies with catalytic
119 activity, it is essential to identify a pure monoclonal antibody derived from COVID-19 patients
120 that exhibits the hypothesized catalytic activity. Here, we survey several sets of monoclonal
121 antibodies (mAbs) from 4 different sources.

122 While we found that most mAbs lacked catalytic activity, two mAbs from one set showed
123 detectable catalytic activity, and two others showed borderline detectable activity. These
124 observations show that some patients develop antibodies with catalytic activity following SARS-
125 CoV-2 infection, with potential clinical consequences.

126

127 ## Materials and Methods

128 **Monoclonal Antibody Collections**

129 We received 129 purified human anti-SARS-CoV-2 RBD mAbs, 1 purified anti-SARS-
130 CoV-2 fusion peptide (FP) mAb, 7 human anti-SARS-CoV-2 RBD mAb hybridoma
131 supernatants, 3 irrelevant mAb hybridoma supernatants, and 1 empty hybridoma culture
132 media as a negative control from 4 different collaborator groups (Table 1). Some of the
133 anti-RBD antibodies were further characterized for their ability to block or enhance
134 ACE2 binding to SARS-CoV-2 spike protein. The hybridoma culture medium is 1/3
135 Medium E (Stem Cell Technologies) and 2/3 DMEM + 10 % FBS.

136

137 **Monoclonal Antibody for Screening**

138 All antibodies were assessed for binding to SARS-CoV-2 RBD (with the exceptions of
139 the negative control mAbs) as described[28-34]. The mAbs purchased from Precision
140 Antibodies targeting the RBD (but not those from other sources) were further screened
141 for interactions with ACE2. This screening used (spike) S and (receptor binding domain)
142 RBD recombinant proteins with HIS tag and an ACE2 human Fc fusion protein. Ni-NTA
143 plates were used to capture both S and RBD proteins. ACE2 binding to both S and RBD
144 proteins was confirmed to ensure that both S and RBD retained the ability to bind
145 ACE2. The S protein captured on Ni-NTA plates was used for the initial screening. All
146 the clones that showed binding to S-protein were subsequently screened for specific
147 RBD binding using RBD-captured Ni-NTA plates. ACE2 was added to the plate to see if

148 any of the anti-RBD antibodies blocked ACE2 binding to the S or RBD proteins captured
149 on the plate. The binding of ACE2 was detected using HRP-anti-human Fc.
150

151 **Angiotensin II Converting Enzyme (ACE2)-like Activity Assays**

152 For the ACE2 activity assay, 25 µg purified antibody or 50 µL hybridoma supernatant
153 was combined with 50 µM MCA-APK(Dnp) fluorogenic ACE2 substrate (AAT Bioquest,
154 Pleasanton CA. Cat. #1355) in assay buffer, 50 mM MES assay buffer (Thermo Fisher,
155 Waltham MA. Cat. # J62231.AK) supplemented with 300 mM NaCl and 10 µM $ZnCl_2$, in
156 a non-binding black bottom plate (Corning, Glendale AZ. Cat. #3991) [35, 36] with total
157 100µL reaction volume[37]. Empty hybridoma culture media was used as a negative
158 control. Fluorescence levels were measured with excitation/emission wavelengths
159 320/405nm and a 325 nm emission filter on the SpectraMax® M5 multi-mode microplate
160 reader (Molecular Devices, San Jose CA.)[36]. Measurements were taken every five
161 minutes for 16 hours, while the plate was kept at a constant temperature of 37°C. 5 mM
162 EDTA was added to the hybridoma culture supernatants to inhibit the ACE2 present in
163 10% FBS medium[38]. Specificity of ACE2 substrate cleavage was determined by parallel
164 comparisons of samples with 25 mM OMNIMMP fluorogenic control substrate (Enzo
165 Life Sciences, Farmingdale, NY. Cat. #MML-P127), MCA-Pro-Leu-OH, according to
166 previous studies [26, 27, 39].
167

168 **ACE2 Substrate Peptide Cleavage Competition Inhibition by SARS-CoV-2 Spike**
169 **RBD Peptide Pools**

170 Twelve peptides (SB-Peptide, SmartBioscience SAS, France) covering the SARS-CoV-
171 2 RBD and RBM were reconstituted in ddH2O or DMSO to a concentration of 10 µg/µL

172 (Table 2) [26, 27]. The peptides were combined in equal amounts. The ACE2 activity assay
173 was set up as described above, and 2.4 µL of the peptide pool was added so that there
174 was 2 µg of each peptide per well. 2.4 µl of peptide diluent (50% ddH_2O and 50%
175 DMSO) was added to other wells as a control.
176
177 **ACE2 Substrate Peptide Cleavage Competition Inhibition by ACE2 Inhibitor**
178 The ACE2 activity assay was run as described above, with the addition of 100 µM MLN-
179 4760 (Bio-Techne, Minneapolis, MN, Cat. #3345), a small-molecule ACE2 active-site
180 inhibitor[37, 40]. This concentration is recommended in commercial ACE2 activity kits
181 (Abcam, Waltham, MA. Cat. #ab273297).
182
183 **Enzyme Kinetics Assay**
184 The ACE2 activity assay was set up as described, but with substrate dilutions raging
185 from 12.5 µM to 200 µM. An MCA standard curve (AAT Bioquest, Pleasanton CA. Cat.
186 #557) was set up with 500, 250, 200, 150, 100, 50, 25, and 0 pmol MCA/well in 100 µL
187 assay buffer. This curve enabled the calculation of pmol of MCA-APK(Dnp) substrate
188 cleaved per minute.
189
190 **RBD-specific Binding Assays by ImmunoCAP**
191 Following our previous studies[26, 27], S-RBD binding was measured by using 50 µL of
192 hybridoma supernatant with a quantitative ImmunoCAP system applying Phadia 250
193 (Thermo Fisher Scientific, Waltham MA. Thermo-Fisher/Phadia). Briefly, biotinylated S-
194 RBD (RayBiotech, Peachtree Corners, GA) was conjugated to streptavidin-coated
195 ImmunoCAPs (Thermo Fisher Scientific, Waltham MA. Thermo-Fisher/Phadia).

196 Unconjugated streptavidin ImmunoCAP was run in parallel with its signal as a
197 background to be subtracted from the singles of other samples.
198
199 **Human IgG ELISA**
200 The total amount of human IgG in hybridoma supernatant was measured by quantitative
201 human IgG ELISA following the kit manufacturer's instructions (Abcam, Waltham, MA.
202 Cat. #ab100547) as described[26, 27].
203
204 **Data Analysis**
205 ACE2 substrate cleavage was monitored by measuring RFU for 16 hours with 5-minute
206 intervals. The RFU values were corrected for background fluorescence by subtracting
207 the minimum RFU value from the remaining RFU values for each well in the assay
208 plate. Linear regression analysis was done on the first 5-120 minutes to determine the
209 increase in RFU over time. This slope (RFU/min) was used as an indicator of ACE2-like
210 activity. A slope of 0.5 RFU/min (the slope of 0.08 ng rhACE2) was determined to be the
211 baseline detection level of ACE2 activity, which is two-fold greater than the lower limit of
212 detection, the activity of 0.04 ng rhACE2[26, 27]. Monoclonal antibodies with a slope
213 greater than 0.5 RFU/min were identified as positive for ACE2-like abzyme activity. For
214 the MCA standard curve, the average RFU over 2 hours was plotted against pmol
215 MCA/well. Linear regression was done to determine the number of RFU per pmol MCA.
216 This value allowed for the calculation of pmols substrate cleaved per minute from the
217 slope data. The supernatant IgG concentration was used to determine the *Kcat* of mAb
218 catalytic activity. All data analysis and visualization were performed with R (version
219 2022-06-23) using R Studio and packages including drc, ggh4x, ggplot2, patchwork,
220 tidyverse, readr, GGally, corrplot and stringr.

Results

We screened 129 purified human anti-RBD mAbs (Table 1) [28, 29, 31, 32, 34, 41] with one purified broadly neutralizing mAb against SARS-CoV-2 fusion peptide[33] as an irrelevant control (Table 1). We also screened 10 human mAb hybridoma culture supernatants purchased from Precision Antibody, including 3 anti-RBD mAbs that blocked ACE2 binding to RBD, 4 anti-RBD mAbs that enhanced ACE2 binding to RBD, and 3 negative control mAbs (Table 1). 5mM EDTA was added to the hybridoma supernatants to inhibit any native ACE2 protein in the media, following previous studies that inhibited ACE2 in 10% FBS media[38]. A concentration of 5 mM EDTA was confirmed to give complete inhibition of ACE2 in the hybridoma culture media serving as our negative control (FIG 1).

We found 4 anti-RBD mAbs (AGRD013-4G9, AGRD016-2B3, AGRD016-4H2, AGRD021-9A10), that had ACE2-like abzyme activity (FIG 2. A). Two of these (AGRD013-4G9 and AGRD016-4H2) had previously been determined to block RBD binding to ACE2, while the other two (AGRD016-2B3 and AGRD021-9A10) had been determined to enhance binding of RBD to ACE2. An additional 9~~97~~ mAbs had catalytic activity higher than the lower limit of detection, but lower than our predetermined threshold for significant ACE2 activity and thus were classified as negative for ACE2-like activity (FIG 2. B). All negative control mAbs had slopes below the lower limit (FIG 2. B).

Antibody concentrations in hybridoma culture supernatants were measured by a total human IgG ELISA[26, 27]. The antibody levels ranged from 16 to 28 μg/ml. Anti-RBD binding activity in those hybridoma culture supernatants was assessed using an ImmunoCAP assay[26, 27], with RBD antibody concentrations ranging from 8 to 13 μg/mL. Significant correlation (R = 0.92, p = 0.0035, Pearson correlation coefficient) was

246 observed between total human IgG concentration and anti-RBD IgG (FIG 3), indicating
247 that ACE2-like activity correlates with anti-RBD antibody, consistent with our previous
248 findings[26, 27].

249 Analysis of the enzymatic activities of those mAbs, ACE2-like activity positive
250 samples, as well as the rhACE2 positive control, was conducted using a Michaelis–
251 Menten analysis[42]. IgG concentrations were determined by human IgG ELISA (FIG 3,
252 Table 3). The ACE2-like abzymes have lower K_ms, and ~4 $\log_{10}$ lower catalytic
253 efficiency (Table 3, FIG 4). The reduced catalytic efficiency (K_{cat}/K_m) is consistent with
254 the data obtained for other abzymes[15, 16]. Our calculated K_{cat}/K_m for rhACE2 at 37°C is
255 0.8 $M^{-1}s^{-1}$ higher than the reported value for rhACE2 at room temperature[43].

256 Substrate specificity of abzyme catalytic activity was determined by comparing
257 the cleavage of ACE2 substrate (MCA-APK(Dnp)) and control substrate (MCA-Pro-Leu-
258 OH), as in prior studies[26, 27, 44]. Abzyme catalytic activities with control substrate had
259 negative slopes (FIG 5), demonstrating that the abzyme activity is ACE2 substrate
260 specific.

261 To further confirm the specificity of the catalytic activity, we conducted ACE2
262 substrate cleavage peptide inhibition assays using 12 tiled overlapping peptides that
263 cover the SARS-CoV-2 RBD and RBM amino acid sequences (Table 2). The RBD
264 peptide pools significantly inhibited the ACE2-like activity of two mAbs, AGRD013-4G9
265 and AGRD016-4H2, which block RBD binding to ACE2, but had overall limited inhibition
266 effect on two other mAbs, AGRD016-2B3 and AGRD021-9A10, which enhance RBD
267 binding to ACE2 (FIG 6). The RBD peptide inhibition results show that the mAb's
268 catalytic activity could be inhibited by peptide competition, and that there is a difference
269 in the ability of the peptides to inhibit activity between mAbs which block or enhance
270 receptor binding[26, 27].

271 We also tested the effect of a small molecule ACE2 inhibitor, MLN-4760, a well-
272 documented inhibitor of ACE2 catalytic activity[37, 40]. MLN-4760 completely inhibits
273 rhACE2, consistent with previous findings[37, 40], but does not inhibit ACE2-like abzyme
274 activity (FIG 7). As we previously showed, the divalent cation chelator, EDTA does not
275 affect the mAbs' substrate cleavage activity, but inhibits native ACE2 catalytic activity,
276 since native ACE2 is a Zn^{2+} metalloprotease[26, 27]. These findings suggest that the mAbs
277 with ACE2-like activity employ different catalytic mechanisms than native ACE2.
278
279

280 **Discussion**

281 The COVID-19 pandemic has claimed around 7 million lives, according to the World
282 Health Organization as of January 2026[45], with about 65 million patients experiencing
283 lingering symptoms long after their initial infection[3]. Long COVID, characterized by
284 persistent symptomatology following an initial COVID-19 infection, includes symptoms
285 consistent with dysregulated proteolytic regulatory cascades[18-20] such as the
286 angiotensin-renin system [24].

287

288 Our previous research showed that some patients suffering from acute SARS-CoV-2
289 infection or convalescing from COVID-19 produced antibodies with ACE2-like catalytic
290 activity [26, 27]. In this study, to further explore our hypothesis that some anti-spike
291 antibodies exhibit ACE2-like catalytic activity, we screened 129 anti-SARS-CoV-2
292 mAbs, 7 anti-spike mAb hybridoma supernatants; and 3 irrelevant mAb hybridoma
293 supernatants and an anti-SARS-CoV-2 FP mAb as controls (Table 1). Although most
294 anti-RBD mAbs exhibited no ACE2-like activity, we identified 4 anti-RBD mAb
295 hybridoma supernatants that had ACE2-like activity. This finding is consistent with our
296 hypothesis that only a small fraction of elicited RBD antibodies exhibit ACE2 substrate-
297 cleavage activity after infection [24, 25].

298

299 ACE2-like catalytic activity, whether native ACE2 or other metalloproteases, is inhibited
300 by 5 mM EDTA in the assay buffer. The ACE2-like catalytic activity of the mAbs was not
301 inhibited by 5 mM EDTA (FIG 1), just as we observed directly in samples from research
302 volunteers with both acute COVID-19 and convalescing from COVID-19[26, 27]. ACE2
303 substrate cleavage specificity was further confirmed by conducting assays using either
304 a native substrate or a negative control ACE2 substate widely used to establish

305 specificity in ACE2 assays (FIG 5)[26, 27, 44], and by using a SARS-CoV-2 spike peptide
306 pool in a competition/inhibition assay (FIG 6), which has been used to demonstrate that
307 the specificity of immunoglobulin-associated ACE2-like activity in research subject
308 samples[26, 27.]. The peptide pool inhibited the substrate cleavage of two mAbs
309 (AGRD013-4G9 and AGRD016-4H2), which blocked ACE2 binding to the virus spike
310 protein, suggesting that the catalytic sites of the mAbs have sufficient resemblance to
311 the ACE2 active site to be inhibited by the overlapping spike-derived peptide pool.
312 However, two mAbs (AGRD016-2B3 and AGRD021-9A10) enhance spike protein
313 binding to ACE2, indicating the mAbs have distinct structural and functional features.
314
315 The four mAbs with ACE2-like activity exhibit low catalytic efficiency (Table 3 and FIG
316 4), a known characteristic of other abzymes[16], so the characteristics of the isolated
317 mAbs with ACE2-like catalytic activity fit well within those described for other abzymes.
318 The inability of the native ACE2-specific small-molecule inhibitor, MLN-4760, to inhibit
319 the ACE2-like activity of abzymes (FIG. 7) suggests that the active site structures of the
320 mAbs, and perhaps their catalytic mechanisms, differ from those of native ACE2[12, 13, 17],
321 further defining the unique features of the anti-spike mAbs.
322
323 Our study had several strengths. We screened many mAbs from four laboratories for
324 ACE2-like catalytic activity. Although a large fraction of the tested mAbs did not have
325 ACE2-like catalytic activity, we found that four isolated anti-SARS-CoV-2 spike mAbs
326 have ACE2-like catalytic activity. Our previous studies describing immunoglobulin-
327 associated ACE2-like activity in clinical samples could have had other, unappreciated
328 substances other than Abs responsible for the ACE2-like catalytic activity. The
329 identification of ACE2-like catalytic activity in mAbs uncontaminated by the many

330 substances present in clinical plasma samples strongly supports the existence of Abs
331 with ACE2-like catalytic activity.
332
333 However, our study had certain limitations. By screening pre-established mAb
334 collections, the mAbs were produced without clear linkage to patients with symptoms
335 associated with COVID-19. In particular, the mAbs were not produced from LC patients
336 with some of the most problematic symptoms. This prevents correlation of abzymes
337 characteristics with clinical symptoms. In addition, the motivation behind the production
338 of most of the mAbs was to obtain mAbs capable of inhibiting SARS-CoV-2 infection of
339 cells. Selecting for a mAb that is highly effective at blocking the virus from infecting a
340 cell may bias the mAb production process toward selecting mAbs with catalytic activity,
341 since those mAbs may not be particularly effective at blocking cell infection. In the
342 future, we hope to acquire and screen mAbs isolated from research subjects with
343 defined clinical symptoms and amplify and characterize clones that are not specifically
344 biased toward highly neutralizing mAbs. In patients, highly neutralizing Abs may not
345 dominate the total pool of Abs made in response to SARS-CoV-2 infection[46, 47].
346
347 Identified mAbs with ACE2-like catalytic activity are from hybridoma culture
348 supernatants, of which the mAbs are in a condition close to natural physiological
349 environment, while other mAbs screened in this study are purified IgGs. Nevertheless,
350 the ACE2-like catalytic activity of the hybridoma supernatants did not require divalent
351 cations (was not inhibited by EDTA), so the activity is distinct from native ACE2. The
352 catalytic activities of the identified abzymes are much lower than native ACE2. In future
353 studies, to detect these low catalytic activities it may be important to ensure that the
354 antibodies are maintained in an physiological intact state during isolation, since it is

355 conceivable that binding or infection inhibition may be less fragile properties that
356 catalytic activity[48-51], which could be a conceivable reason why catalytic activity was not
357 detected in the purified mAbs. Nevertheless, the identification of any mAbs with ACE2-
358 like catalytic activity supports the hypothesis underlying this study.
359
360 An essential next step involves elucidating the mechanistic properties of the abzymes'
361 catalytic activity. Structural analyses aimed at delineating these activities could provide
362 critical insight into the mechanisms of ACE2-like catalytic activity. A clear understanding
363 of the catalytic mechanisms of the mAbs with ACE2-like catalytic activity could enable
364 the development of inhibitors against that activity. Alternatively, the cells producing the
365 pathologic abzymes could be targeted.
366
367 Overall, our study further confirms the existence of anti-spike antibodies with ACE2-like
368 activity that can plausibly contribute to the pathogenesis of COVID-19 and LC. While
369 much work remains to be done, including the production and study of new anti-spike
370 mAbs not selected specifically for SARS-CoV-2 neutralizing activity, study of catalytic
371 activities other than ACE2 in Abs made after exposure to SARS-CoV-2 that could
372 potentially mediate the pathogenesis of COVID-19 and LC, studies associating different
373 clinical features with abzymes with particular characteristics, structural and mechanistic
374 studies of the abzymes themselves, and larger epidemiologic survey studies to better
375 determine the rates of abzyme production in persons exposed to SARS-CoV-2. There
376 are also longer-term translational implications. Other viruses, in addition to SARS-CoV-
377 2, use cell surface enzymes as their receptors [52-57]. If pathologic abzymes induced by
378 exposure to SARS-CoV-2 are responsible for some of the pathologic features of

COVID-19 and LC, then abzymes induced by other viruses, including viruses causing subclinical infections, could be responsible for other disorders.

Institutional Review Board Statement: The UVA IRB approved enrollment of all subjects and collection of specimens and related metadata (HSR #HSR13166).

Informed Consent Statement: Informed consent was obtained from the subjects in the study.

Data Availability Statement: Data is presented in the manuscript. Raw data is available upon request.

Acknowledgments and Funding: The study was supported by University of Virginia internal funding, including the Pendleton Laboratory Fund for Pediatric Infectious Disease Research, the Manning Fund for COVID-19 Research at UVA, the Ivy Foundation, University of Virginia Summer Research Internship Program and grants from NIAID, NIH (R01 AI176515).

Conflicts of Interest: The authors declare no conflict of interest.

413 **Tables**
414
415

416 **Table 1:** Summary of Monoclonal Antibody Characteristics Used in the Study
417

418

Source	Total Number	Form	Characteristics	Number with Abzyme Activity	Reference
Dr. James Crowe, Vanderbilt University Medical Center	2	Purified	Human Anti SARS-CoV-2 RBM	0	28, 29
Dr. Jun Hayashi, Precision Antibody	3	Supernatant	Human Anti SARS-CoV-2 Spike and RBD, block RBD binding to ACE2	2	
	4	Supernatant	Human Anti SARS-CoV-2 Spike and RBD, enhance RBD binding to ACE2	2	
	3	Supernatant	Negative Controls	0	
Dr. David Ho, Columbia University	39	Purified	Human Anti SARS-CoV-2 RBD	0	31, 41, 58

Dr. Joshua Tan at NIH	88	Purified	Human Anti SARS-CoV-2 RBD	0	34
	1	Purified	Human Anti SARS-CoV-2 FP (Negative Control)	0	33

419
420

421 **Table 2:** Synthesized SARS-CoV-2 Spike RBD Peptides for ACE2 Substrate
422 Competition Assay

Cat #	Domain	Sequence (Amino Acid Position)	Concentration (mg/mL)	Reconstitution
SB086-1MG	**RBD**	319-RVQPTESIVRFPNITNL-335	10	**DMSO**
SB087-1MG	**RBD**	336-CPFGEVFNATRF-347	10	**DMSO**
SB088-1MG	**RBD**	348-ASVYAWNRKR-357	10	**ddH_2O**
SB089-1MG	**RBD**	352-AWNRKRISNCVADY-365	10	**ddH_2O**
SB090-1MG	**RBD**	371-SASFSTFKCYGVSPTKLNDLCFTN-394	10	**DMSO**
SB091-1MG	**RBD**	395-VYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFT-430	10	**ddH_2O**
SB092-1MG	**RBM**	438-SNNLDSKVGGNYNYLYRLFRK-458	10	**ddH_2O**
SB093-1MG	**RBM**	450-NYLYRLFRKSNLKPFERDISTEIY-473	10	**ddH_2O**
SB094-1MG	**RBM**	480-CNGVEGFNCYFPLQSYG-496	10	**DMSO**
SB095-1MG	**RBM**	500-TNGVGYQPYR-509	10	**DMSO**
SB096-1MG	**RBD**	513-LSFELLHA-520	10	**DMSO**
SB097-1MG	**RBD**	523-TVCGPKKSTNLVKNKCVNF-541	10	**ddH_2O**

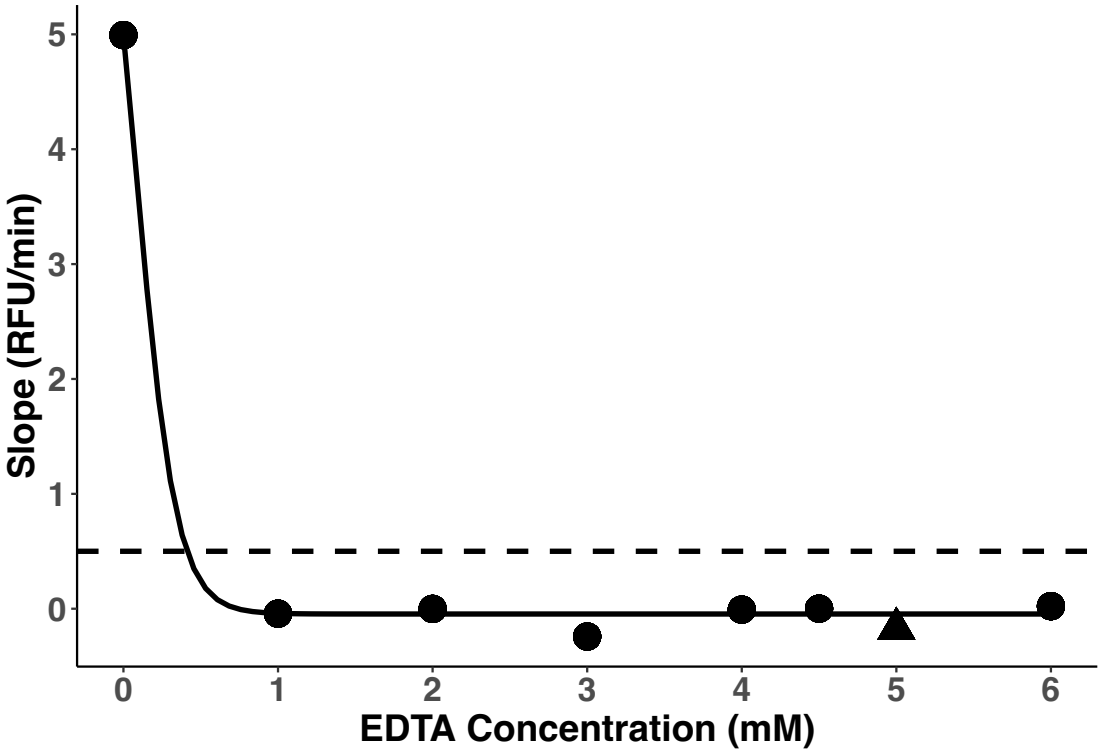
423
424

425
426
427
428
429 **Table 3:** Enzyme Kinetic Studies of ACE2-like Abzymes and Purified Recombinant
430 Human ACE2 Molecule (From FIG 4)
431

Sample	**Assay Conc**	**K_m (µM)**	**V_{max} (pmol/min)**	**V_{max} (mol/s)**	**K_{cat} (s^{-1})**	**K_{cat}/K_m ($M^{-1}s^{-1}$)**
rhACE2[a]	25 ng/mL	43.7	66.9	1.12E-12	3.79E+01	8.68E+05
AGRD013-4G9	5.8 µg/mL	26.9	0.192	3.20E-15	8.27E-04	3.07E+01
AGRD016-2B3	5.6 µg/mL	34.3	0.147	2.45E-15	6.57E-04	1.91E+01
AGRD016-4H2	7.9 µg/mL	38.9	0.295	4.92E-15	9.33E-04	2.40E+01
AGRD021-9A10	5.3 µg/mL	33.9	0.253	4.22E-15	1.19E-03	3.52E+01

432
433 a: Purified recombinant human ACE2 molecule as positive control.
434
435
436

437 **FIGURES**
438



439
440 **FIG 1. ACE2 Background Activity Inhibition by EDTA in Supernatant Control**
441 **Media.** ACE2 background activity in hybridoma supernatant control media (empty
442 culture media without hybridoma) was detected by the ACE2 assay, indicated by the
443 slope calculated from the kinetic curve. ACE2 background activity was effectively
444 inhibited by adding EDTA to the media. The dashed line shows the cutoff, which was
445 the limitation of the ACE2 assay and was determined using a purified recombinant
446 human ACE2 positive control to call a sample positive for ACE2 activity. 5mM EDTA
447 (**Triangle**) were used for following hybridoma culture supernatants screening as
448 reported[38].
449

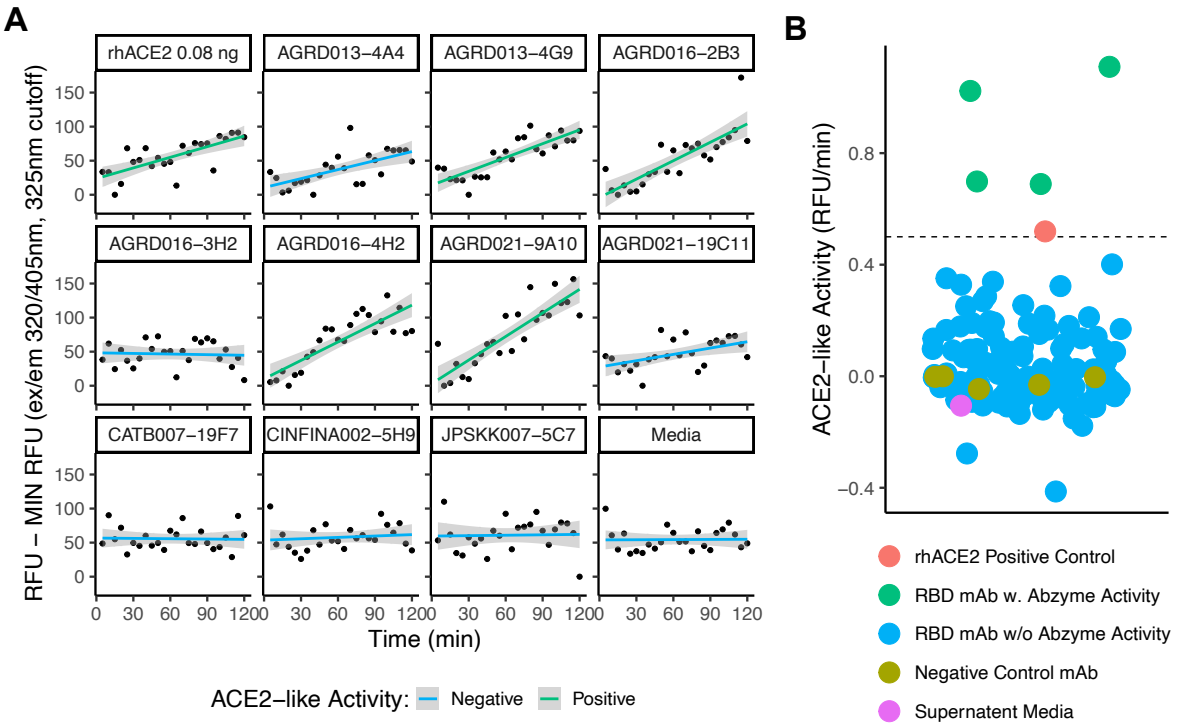


FIG 2. Initial Screening of mAbs for ACE2-like Activity. ACE2 substrate cleavage activity of mAbs. Catalytic activity was determined by calculating the slope of RFU from the initial 2 hours of the kinetics curve. 0.08 ng of purified recombinant human ACE2 served as a positive control and standard to define ACE2-positive activity. mAbs with ACE2-like activity greater than or equal to 0.08ng rhACE2 were identified as having ACE2-like catalytic activity. (A) Representative ACE2 activity screening kinetic curves from the Precision Antibody mAbs and ACE2 activity determination by slope. 0.08 ng rhACE2 served as a positive and standard control, and medium (empty hybridoma culture supernatant) served as a negative control. (B) Summary of average ACE2-like activity of all mAbs screened in this study. Results were from at least 2 rounds of screening. ACE2 substrate cleavage activity was determined by the slope of the RFU from the initial 2 hours of the kinetic curve. The dashed line shows the slope calculated from 0.08 ng of purified recombinant human ACE2, the initial 2-hour kinetic curve.

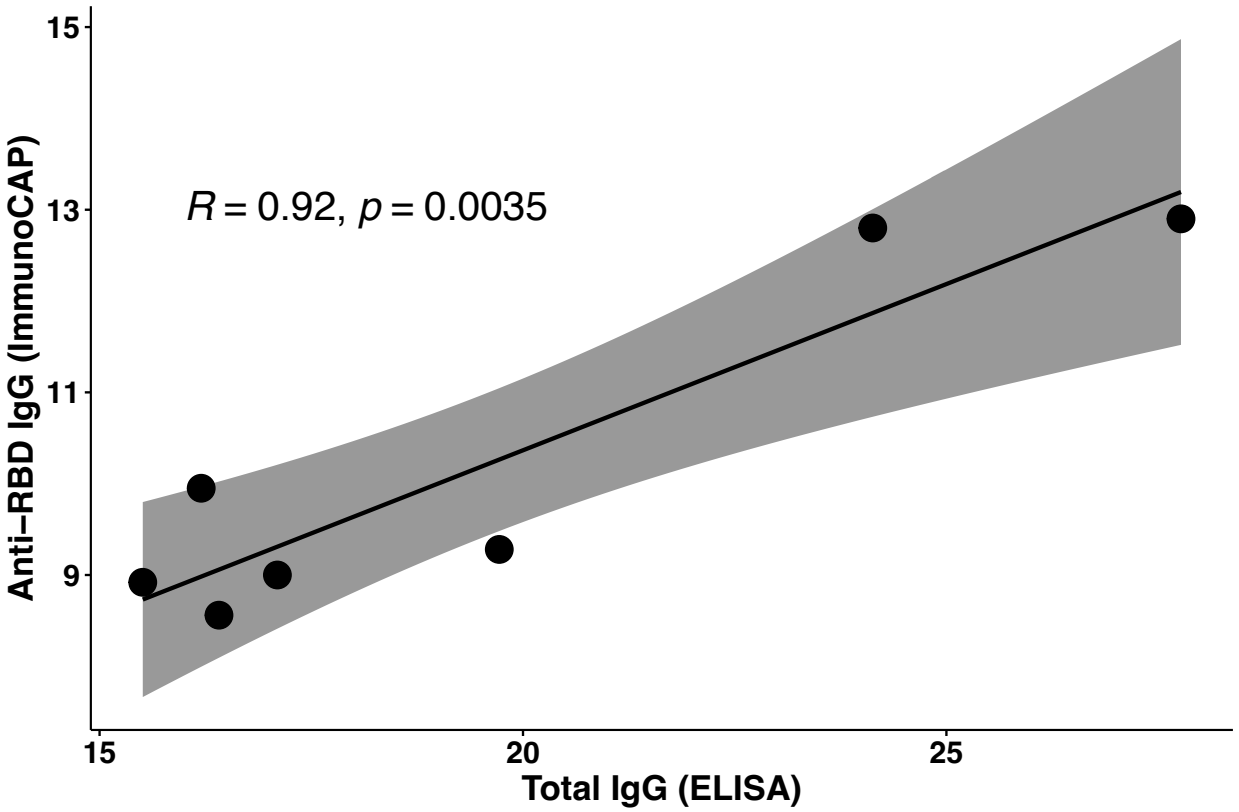
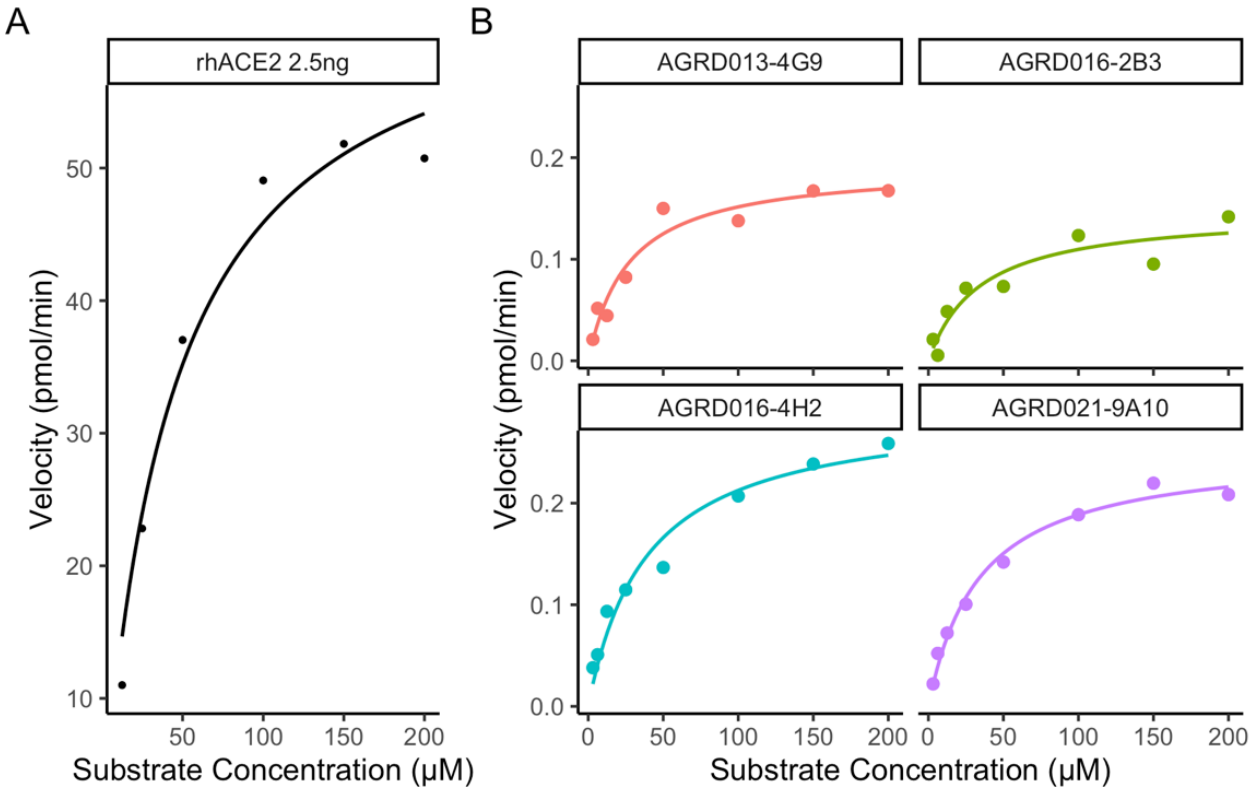


FIG 3. Correlation of total IgG and anti-RBD antibody. Total IgG concentrations in mAb hybridoma culture supernatants were measured by human IgG ELISA. Anti-RBD IgG concentrations of mAb hybridoma culture supernatants were determined by ImmunoCAP antibody-binding assay. (Pearson correlation test).

472



473

474 **FIG 4. Michaelis-Menten Analysis of mAb Catalytic Activity.** Michaelis-Menten plots

475 for (A) Positive control (2.5ng rhACE2) and (B) mAbs that had ACE2 substrate cleavage

476 activities. Abzyme IgG amount for Michaelis-Menten kinetics was from human IgG

477 ELISA.

478

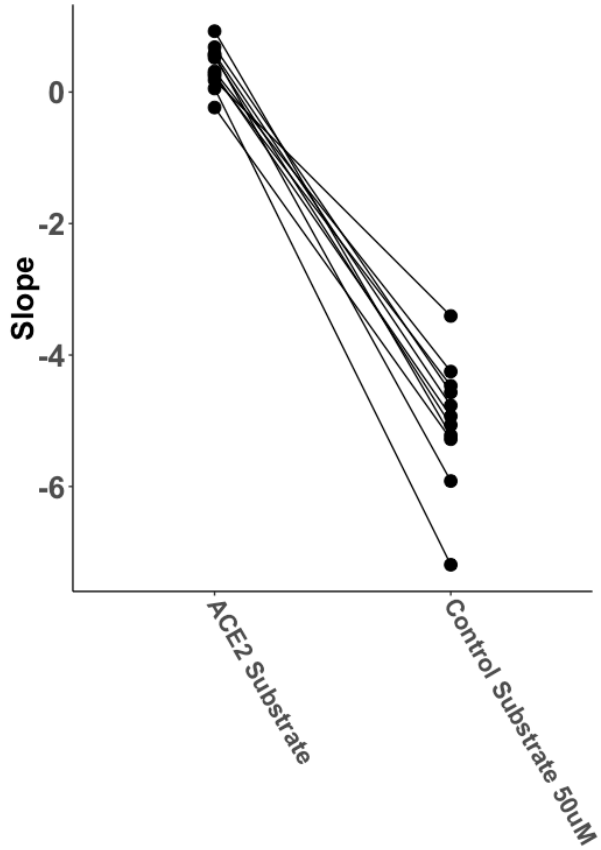
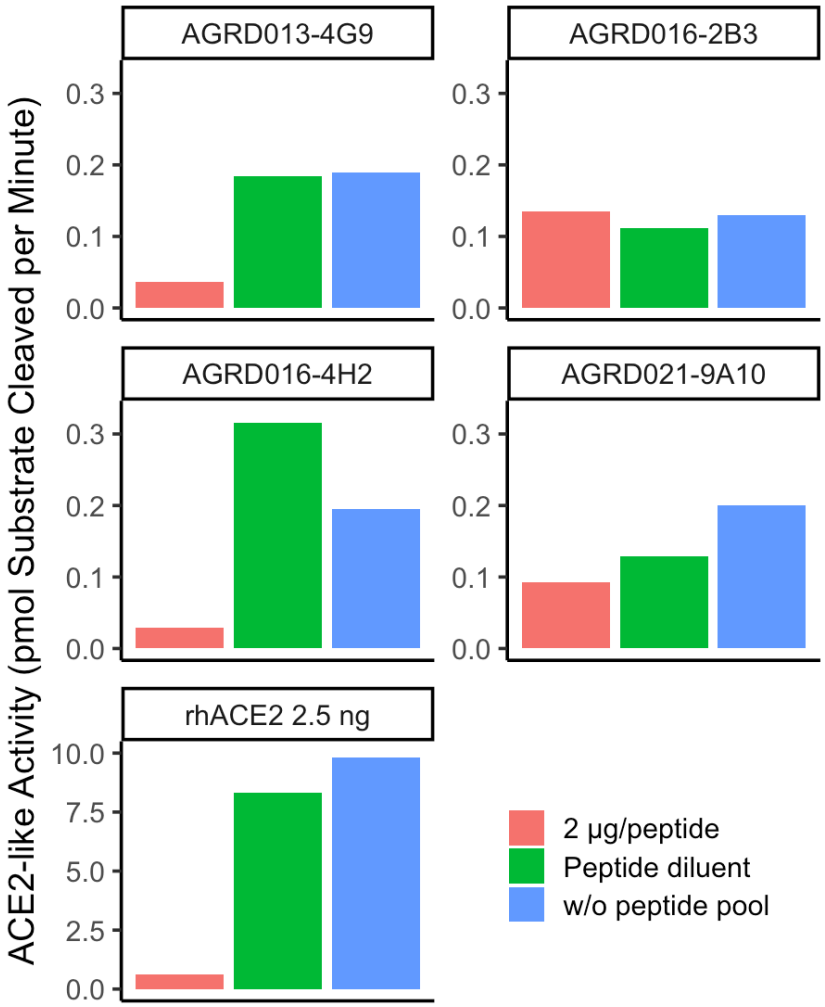


FIG 5. Enzyme Substrate Catalytic Specificity. The substrate cleavage specificity of the mAbs was characterized by comparing the activity of the mAbs using an ACE2 substrate and a control non-cleavable substrate. The slope of the RFU from the initial 2 hours of the kinetic curve was calculated to determine ACE2-like activity. Activity observed with the control substrate was substantially diminished.

487



488

489 **FIG 6. SARS-CoV-2 RBD Peptide Competitive Inhibition of mAb ACE2 Substrate**

490 **Cleavage Activity.** mAbs ACE2-like activity inhibition by RBD peptide competition was

491 performed by adding RBD peptide pools to ACE2 assay reactions containing mAbs

492 defined as ACE2-like activity-positive. Peptide pool diluent, with an equal volume of

493 peptide pool added to the assay reactions, served as a comparison. 2.5 ng purified

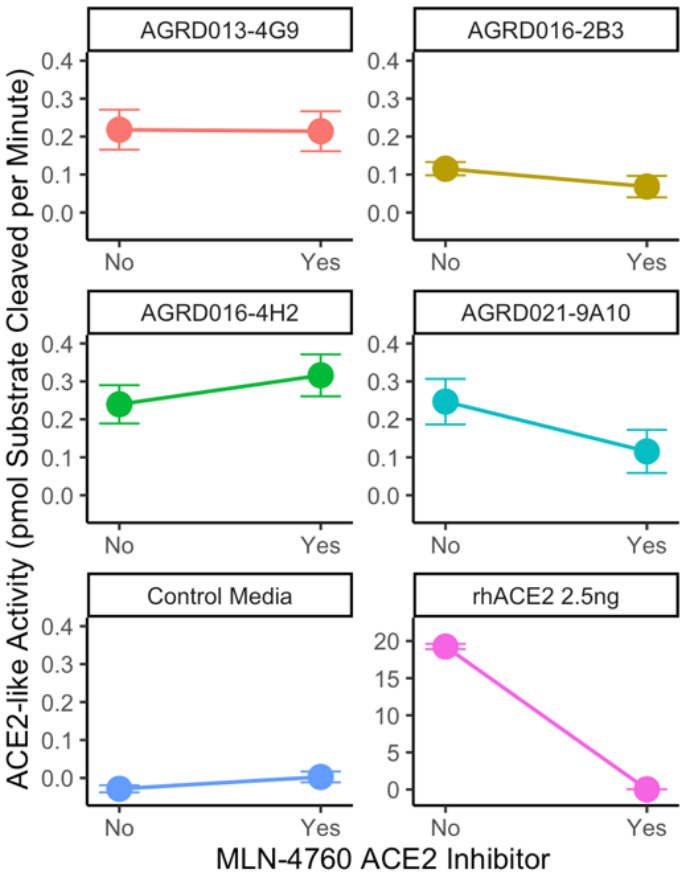
494 recombinant human ACE2 was used as a positive control. ACE2-like activity was

495 determined by calculation of the slope of the RFU from the initial 2 hours of the kinetic

496 curve.

497

498



499

500 **FIG 7. Effect of ACE2 Inhibitor, MLN-4760, on ACE2 Substrate Cleavage Activity of**

501 **mAbs and rhACE2.** 100 μM ACE2 inhibitor, MLN-4760, was added to ACE2 assay

502 reactions for comparison of ACE2 activity with ACE2 assay reactions that had no

503 inhibitor. 2.5 ng purified recombinant human ACE2 was the positive control, and control

504 media (empty hybridoma culture supernatant) was the negative control. ACE2-like

505 activity was determined by calculation of the slope of the RFU from the initial 2 hours of

506 the kinetic curve.

507

508

509

References

1. Altmann DM, Whettlock EM, Liu S, Arachchillage DJ, Boyton RJ. The immunology of long COVID. Nature Reviews Immunology. 2023;23(10):618-34.

2. Perumal R, Shunmugam L, Naidoo K, Abdool Karim SS, Wilkins D, Garzino-Demo A, Brechot C, Parthasarathy S, Vahlne A, Nikolich JŽ. Long COVID: a review and proposed visualization of the complexity of long COVID. Frontiers in immunology. 2023;14:1117464.

3. Davis HE, McCorkell L, Vogel JM, Topol EJ. Long COVID: major findings, mechanisms and recommendations. Nature Reviews Microbiology. 2023;21(3):133-46.

4. Adjaye-Gbewonyo D, Vahratian A, Perrine CG, Bertolli J. Long COVID in Adults: United States, 2022. 26/2023.

5. Marchi M, Grenzi P, Serafini V, Capoccia F, Rossi F, Marrino P, Pingani L, Galeazzi GM, Ferrari S. Psychiatric symptoms in Long-COVID patients: a systematic review. Frontiers in Psychiatry. 2023;14:1138389.

6. Thaweethai T, Jolley SE, Karlson EW, Levitan EB, Levy B, McComsey GA, McCorkell L, Nadkarni GN, Parthasarathy S, Singh U, Walker TA, Selvaggi CA, Shinnick DJ, Schulte CCM, Atchley-Challenner R, Horwitz LI, Foulkes AS, Authors RC, Consortium R. Development of a Definition of Postacute Sequelae of SARS-CoV-2 Infection. JAMA. 2023;329(22):1934-46.

7. Soriano JB, Murthy S, Marshall JC, Relan P, Diaz JV. A clinical case definition of post-COVID-19 condition by a Delphi consensus. Lancet Infect Dis. 2022 Apr;22(4):e102-e7. PMC8691845

8. Castanares-Zapatero D, Chalon P, Kohn L, Dauvrin M, Detollenaere J, Maertens de Noordhout C, Primus-de Jong C, Cleemput I, Van den Heede K. Pathophysiology and mechanism of long COVID: a comprehensive review. Ann Med. 2022 Dec;54(1):1473-87. PMC9132392

9. Tramontano A, Janda KD, Lerner RA. Catalytic antibodies. Science. 1986 Dec 19;234(4783):1566-70.

10. Pollack SJ, Jacobs JW, Schultz PG. Selective chemical catalysis by an antibody. Science. 1986 Dec 19;234(4783):1570-3.

11. Avalle B, Mistro D, Thomas D, Friboulet A. Polyclonal catalytic anti-idiotypic antibodies with a beta-lactamase activity. Ann N Y Acad Sci. 1996 Oct 12;799:172-5.

12. Friboulet A, Izadyar L, Avalle B, Roseto A, Thomas D. Abzyme generation using an anti-idiotypic antibody as the "internal image" of an enzyme active site. Appl Biochem Biotechnol. 1994 May-Jun;47(2-3):229-37; discussion 37-9.

13. Izadyar L, Friboulet A, Remy MH, Roseto A, Thomas D. Monoclonal anti-idiotypic antibodies as functional internal images of enzyme active sites: production of a catalytic antibody with a cholinesterase activity. Proc Natl Acad Sci U S A. 1993 Oct 1;90(19):8876-80. PMC47463

14. Friboulet A, Izadyar L, Avalle B, Roseto A, Thomas D. Antiidiotypic antibodies as functional internal images of enzyme-active sites. Ann N Y Acad Sci. 1995 Mar 31;750:265-70.

15. Hilvert D. Critical analysis of antibody catalysis. Annu Rev Biochem. 2000;69:751-93.

16. Bar-Even A, Noor E, Savir Y, Liebermeister W, Davidi D, Tawfik DS, Milo R. The moderately efficient enzyme: evolutionary and physicochemical trends shaping enzyme parameters. Biochemistry. 2011 May 31;50(21):4402-10.

17. Ponomarenko NA, Pillet D, Paon M, Vorobiev, II, Smirnov IV, Adenier H, Avalle B, Kolesnikov AV, Kozyr AV, Thomas D, Gabibov AG, Friboulet A. Anti-idiotypic antibody mimics proteolytic function of parent antigen. Biochemistry. 2007 Dec 18;46(50):14598-609.

18. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020 Feb 15;395(10223):497-506. PMC7159299

19. Liao D, Zhou F, Luo L, Xu M, Wang H, Xia J, Gao Y, Cai L, Wang Z, Yin P, Wang Y, Tang L, Deng J, Mei H, Hu Y. Haematological characteristics and risk factors in the classification and prognosis evaluation of COVID-19: a retrospective cohort study. Lancet Haematol. 2020 Sep;7(9):e671-e8. PMC7351397

20. Del Valle DM, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, Wang B, Lavin Y, Swartz TH, Madduri D, Stock A, Marron TU, Xie H, Patel M, Tuballes K, Van Oekelen O, Rahman A, Kovatch P, Aberg JA, Schadt E, Jagannath S, Mazumdar M, Charney AW, Firpo-Betancourt A, Mendu DR, Jhang J, Reich D, Sigel K, Cordon-Cardo C, Feldmann M, Parekh S, Merad M, Gnjatic S. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat Med. 2020 Oct;26(10):1636-43. PMC7869028

21. Sun DS, Lien TS, Chang HH. Virus-Induced Pathogenic Antibodies: Lessons from Long COVID and Dengue Hemorrhage Fever. Int J Mol Sci. 2025 Feb 22;26(5). PMC11899886

22. Santos Guedes de Sa K, Silva J, Bayarri-Olmos R, Brinda R, Alec Rath Constable R, Colom Diaz PA, Kwon DI, Rodrigues G, Wenxue L, Baker C, Bhattacharjee B, Wood J, Tabacof L, Liu Y, Putrino D, Horvath TL, Iwasaki A. A causal link between autoantibodies and neurological symptoms in long COVID. medRxiv. 2024 Jun 19. PMC11213106

23. Yousaf AR, Cortese MM, Taylor AW, Broder KR, Oster ME, Wong JM, Guh AY, McCormick DW, Kamidani S, Schlaudecker EP, Edwards KM, Creech CB, Staat MA, Belay ED, Marquez P, Su JR, Salzman MB, Thompson D, Campbell AP. Reported cases of multisystem inflammatory syndrome in children aged 12-20 years in the USA who received a COVID-19 vaccine, December, 2020, through August, 2021: a surveillance investigation. Lancet Child Adolesc Health. 2022 Feb 22. PMC8864018

24. Khurana V, Goswami B. Angiotensin converting enzyme (ACE). Clin Chim Acta. 2022 Jan 1;524:113-22.

25. Turner AJ, Nalivaeva NN. Angiotensin-converting enzyme 2 (ACE2): Two decades of revelations and re-evaluation. Peptides. 2022 May;151:170766. PMC8830188

26. Song Y, Mehl F, Muehling LM, Canderan G, Enfield K, Sun J, Yin MT, Ratcliffe SJ, Wilson JM, Kadl A, Woodfolk JA, Zeichner SL. ACE-2-like enzymatic activity in COVID-19 convalescents with persistent pulmonary symptoms associated with immunoglobulin. mBio. 2025 Jul 22:e0173525.

27. Song Y, Myers R, Mehl F, Murphy L, Brooks B, Wilson JM, Kadl A, Woodfolk J, Zeichner SL. ACE-2-like enzymatic activity is associated with immunoglobulin in COVID-19 patients. mBio. 2024 Apr 10;15(4):e0054124. PMC11005375

28. Zost SJ, Gilchuk P, Case JB, Binshtein E, Chen RE, Nkolola JP, Schäfer A, Reidy JX, Trivette A, Nargi RS, Sutton RE, Suryadevara N, Martinez DR, Williamson LE, Chen EC, Jones T, Day S, Myers L, Hassan AO, Kafai NM, Winkler ES, Fox JM, Shrihari S, Mueller BK, Meiler J, Chandrashekar A, Mercado NB, Steinhardt JJ, Ren K, Loo YM, Kallewaard NL, McCune BT, Keeler SP, Holtzman MJ, Barouch DH, Gralinski LE, Baric RS, Thackray LB, Diamond MS, Carnahan RH, Crowe JE, Jr. Potently neutralizing and protective human antibodies against SARS-CoV-2. Nature. 2020 Aug;584(7821):443-9. PMC7584396

29. Greaney AJ, Starr TN, Gilchuk P, Zost SJ, Binshtein E, Loes AN, Hilton SK, Huddleston J, Eguia R, Crawford KHD, Dingens AS, Nargi RS, Sutton RE, Suryadevara N, Rothlauf PW, Liu Z, Whelan SPJ, Carnahan RH, Crowe JE, Jr., Bloom JD. Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. Cell Host Microbe. 2021 Jan 13;29(1):44-57.e9. PMC7676316

30. Cerutti G, Guo Y, Liu L, Liu L, Zhang Z, Luo Y, Huang Y, Wang HH, Ho DD, Sheng Z, Shapiro L. Cryo-EM structure of the SARS-CoV-2 Omicron spike. Cell Rep. 2022 Mar 1;38(9):110428. PMC8818377

31. Liu L, Iketani S, Guo Y, Reddem ER, Casner RG, Nair MS, Yu J, Chan JF, Wang M, Cerutti G, Li Z, Morano NC, Castagna CD, Corredor L, Chu H, Yuan S, Poon VK, Chan CC, Chen Z, Luo Y, Cunningham M, Chavez A, Yin MT, Perlin DS, Tsuji M, Yuen KY, Kwong PD, Sheng Z, Huang Y, Shapiro L, Ho DD. An antibody class with a common CDRH3 motif broadly neutralizes sarbecoviruses. Sci Transl Med. 2022 May 25;14(646):eabn6859. PMC9017343

32. Liu L, Casner RG, Guo Y, Wang Q, Iketani S, Chan JF, Yu J, Dadonaite B, Nair MS, Mohri H, Reddem ER, Yuan S, Poon VK, Chan CC, Yuen KY, Sheng Z, Huang Y, Bloom JD, Shapiro L, Ho DD. Antibodies targeting a quaternary site on SARS-CoV-2 spike glycoprotein prevent viral receptor engagement by conformational locking. Immunity. 2023 Oct 10;56(10):2442-55.e8. PMC10588992

33. Dacon C, Tucker C, Peng L, Lee CD, Lin TH, Yuan M, Cong Y, Wang L, Purser L, Williams JK, Pyo CW, Kosik I, Hu Z, Zhao M, Mohan D, Cooper A, Peterson M, Skinner J, Dixit S, Kollins E, Huzella L, Perry D, Byrum R, Lembirik S, Zhang Y, Yang ES, Chen M, Leung K, Weinberg RS, Pegu A, Geraghty DE, Davidson E, Douagi I, Moir S, Yewdell JW, Schmaljohn C, Crompton PD, Holbrook MR, Nemazee D, Mascola JR, Wilson IA, Tan J. Broadly neutralizing antibodies target the coronavirus fusion peptide. bioRxiv. 2022 Apr 12:2022.04.11.487879. PMC9016638

34. Cho H, Gonzales-Wartz KK, Huang D, Yuan M, Peterson M, Liang J, Beutler N, Torres JL, Cong Y, Postnikova E, Bangaru S, Talana CA, Shi W, Yang ES, Zhang Y, Leung K, Wang L, Peng L, Skinner J, Li S, Wu NC, Liu H, Dacon C, Moyer T, Cohen M, Zhao M, Lee FE, Weinberg RS, Douagi I, Gross R, Schmaljohn C, Pegu A, Mascola JR, Holbrook M, Nemazee D, Rogers TF, Ward AB, Wilson IA, Crompton PD, Tan J. Bispecific antibodies targeting distinct regions of the spike protein potently neutralize SARS-CoV-2 variants of concern. Sci Transl Med. 2021 Oct 20;13(616):eabj5413. PMC8651051

35. Hifumi E, Ito Y, Tsujita M, Taguchi H, Uda T. Enzymatization of mouse monoclonal antibodies to the corresponding catalytic antibodies. Sci Rep. 2024 May 28;14(1):12184. PMC11133420

36. McConnell SA, Sachithanandham J, Mudrak NJ, Zhu X, Farhang PA, Cordero RJB, Wear MP, Shapiro JR, Park HS, Klein SL, Tobian AAR, Bloch EM, Sullivan DJ, Pekosz A, Casadevall A. Spike-protein proteolytic antibodies in COVID-19 convalescent plasma contribute to SARS-CoV-2 neutralization. Cell Chem Biol. 2023 Jul 20;30(7):726-38.e4. PMC10288624

37. Xiao F, Burns KD. Measurement of Angiotensin Converting Enzyme 2 Activity in Biological Fluid (ACE2). Methods Mol Biol. 2017;1527:101-15. PMC7121061

38. Lubel JS, Herath CB, Velkoska E, Casley DJ, Burrell LM, Angus PW. Angiotensin converting enzyme 2 (ACE2) activity in fetal calf serum: implications for cell culture research. Cytotechnology. 2008 Nov;58(3):119-26. PMC2652558

39. Ferrari M, Mekkaoui L, Ilca FT, Akbar Z, Bughda R, Lamb K, Ward K, Parekh F, Karattil R, Allen C, Wu P, Baldan V, Mattiuzzo G, Bentley EM, Takeuchi Y, Sillibourne J, Datta P, Kinna A, Pule M, Onuoha SC. Characterization of a Novel ACE2-Based Therapeutic with Enhanced Rather than Reduced Activity against SARS-CoV-2 Variants. Journal of Virology. 2021;95(19):10.1128/jvi.00685-21.

40. Svilenov HL, Delhommel F, Siebenmorgen T, Rührnößl F, Popowicz GM, Reiter A, Sattler M, Brockmeyer C, Buchner J. Extrinsic stabilization of antiviral ACE2-Fc

704 fusion proteins targeting SARS-CoV-2. Commun Biol. 2023 Apr 8;6(1):386.
705 PMC10082628
706
707 41. Liu L, Wang P, Nair MS, Yu J, Rapp M, Wang Q, Luo Y, Chan JF, Sahi V,
708 Figueroa A, Guo XV, Cerutti G, Bimela J, Gorman J, Zhou T, Chen Z, Yuen KY, Kwong
709 PD, Sodroski JG, Yin MT, Sheng Z, Huang Y, Shapiro L, Ho DD. Potent neutralizing
710 antibodies against multiple epitopes on SARS-CoV-2 spike. Nature. 2020
711 Aug;584(7821):450-6.
712
713 42. Michaelis L, Menten ML, Johnson KA, Goody RS. The original Michaelis
714 constant: translation of the 1913 Michaelis-Menten paper. Biochemistry. 2011 Oct
715 4;50(39):8264-9. PMC3381512
716
717 43. Rice GI, Thomas DA, Grant PJ, Turner AJ, Hooper NM. Evaluation of
718 angiotensin-converting enzyme (ACE), its homologue ACE2 and neprilysin in
719 angiotensin peptide metabolism. Biochem J. 2004 Oct 1;383(Pt 1):45-51. PMC1134042
720
721 44. Ferrari M, Mekkaoui L, Ilca FT, Akbar Z, Bughda R, Lamb K, Ward K, Parekh F,
722 Karattil R, Allen C, Wu P, Baldan V, Mattiuzzo G, Bentley EM, Takeuchi Y, Sillibourne J,
723 Datta P, Kinna A, Pule M, Onuoha SC. Characterization of a Novel ACE2-Based
724 Therapeutic with Enhanced Rather than Reduced Activity against SARS-CoV-2
725 Variants. J Virol. 2021 Sep 9;95(19):e0068521. PMC8432736
726
727 45. Organization WH. WHO COVID-19 Dashboard. World Health Organization; 2026
728 [cited 2026 14 January 2026]; https://data.who.int/dashboards/covid19/deaths.
729 46. de Souza MO, Madan B, Teng IT, Huang A, Liu L, Fahad AS, Lopez Acevedo
730 SN, Pan X, Sastry M, Gutierrez-Gonzalez M, Yin MT, Zhou T, Ho DD, Kwong PD,
731 DeKosky BJ. Mapping monoclonal anti-SARS-CoV-2 antibody repertoires against
732 diverse coronavirus antigens. Front Immunol. 2022;13:977064. PMC9478573
733
734 47. Amanat F, Thapa M, Lei T, Sayed Ahmed SM, Adelsberg DC, Carreno JM,
735 Strohmeier S, Schmitz AJ, Zafar S, Zhou JQ, Rijnink W, Alshammary H, Borcherding N,
736 Reiche AG, Srivastava K, Sordillo EM, van Bakel H, Turner JS, Bajic G, Simon V,
737 Ellebedy AH, Krammer F. The plasmablast response to SARS-CoV-2 mRNA
738 vaccination is dominated by non-neutralizing antibodies and targets both the NTD and
739 the RBD. medRxiv. 2021 May 1. PMC7987037
740
741 48. Cesaro-Tadic S, Lagos D, Honegger A, Rickard JH, Partridge LJ, Blackburn GM,
742 Plückthun A. Turnover-based in vitro selection and evolution of biocatalysts from a fully
743 synthetic antibody library. Nat Biotechnol. 2003 Jun;21(6):679-85.
744
745 49. Ermakov EA, Kabirova EM, Buneva VN, Nevinsky GA. IgGs-Abzymes from the
746 Sera of Patients with Multiple Sclerosis Recognize and Hydrolyze miRNAs. Int J Mol
747 Sci. 2021 Mar 10;22(6). PMC8000798
748
749 50. Li JW, Xia L, Su Y, Liu H, Xia X, Lu Q, Yang C, Reheman K. Molecular imprint of
750 enzyme active site by camel nanobodies: rapid and efficient approach to produce
751 abzymes with alliinase activity. J Biol Chem. 2012 Apr 20;287(17):13713-21.
752 PMC3340132

51. Richard JP. Protein Flexibility and Stiffness Enable Efficient Enzymatic Catalysis. J Am Chem Soc. 2019 Feb 27;141(8):3320-31. PMC6396832

52. Jin M, Jefferson VA, Zhao Z, Catanzaro NJ, De Sabato L, Keller EL, Menasche BL, Hoffman C, Castelli A, Di Bartolo I, Vaccari G, Starr TN, Seifert SN, Castellanos AA, Han BA, Serebryannyy L, Spaulding AB, Douek DC, Moreno A, Baric RS, Wilen CB, Rini JM, Letko M. Aminopeptidase N is a receptor for hedgehog merbecoviruses. bioRxiv. 2025 Sep 3. PMC12425009

53. Dufloo J, Fernández I, Arbabian A, Haouz A, Temperton N, Gimenez-Lirola LG, Rey FA, Sanjuán R. Dipeptidase 1 is a functional receptor for a porcine coronavirus. Nat Microbiol. 2025 Nov;10(11):2981-96. PMC12578638

54. Dufloo J, Andreu-Moreno I, Moreno-García J, Valero-Rello A, Sanjuán R. Receptor-binding proteins from animal viruses are broadly compatible with human cell entry factors. Nat Microbiol. 2025 Feb;10(2):405-19. PMC11790484

55. Simmons G, Zmora P, Gierer S, Heurich A, Pöhlmann S. Proteolytic activation of the SARS-coronavirus spike protein: cutting enzymes at the cutting edge of antiviral research. Antiviral Res. 2013 Dec;100(3):605-14. PMC3889862

56. López-Cortés GI, Palacios-Pérez M, Hernández-Aguilar MM, Veledíaz HF, José MV. Human Coronavirus Cell Receptors Provide Challenging Therapeutic Targets. Vaccines (Basel). 2023 Jan 13;11(1). PMC9862439

57. Li F. Receptor recognition mechanisms of coronaviruses: a decade of structural studies. J Virol. 2015 Feb;89(4):1954-64. PMC4338876

58. Liu L, Casner RG, Guo Y, Wang Q, Iketani S, Chan JF, Yu J, Dadonaite B, Nair MS, Mohri H, Reddem ER, Yuan S, Poon VK, Chan CC, Yuen KY, Sheng Z, Huang Y, Bloom JD, Shapiro L, Ho DD. Antibodies that neutralize all current SARS-CoV-2 variants of concern by conformational locking. bioRxiv. 2023 Apr 10. PMC10120718