

1 **Landscape of infection enhancing antibodies in COVID-19 and healthy donors**

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25

26 **Abstract**

27 To assess the frequency of SARS-CoV-2 infection enhancing antibodies in the general population,
28 we searched over 64 million heavy chain antibody sequences from healthy and COVID-19 patient
29 repertoires for sequences similar to 11 previously reported enhancing antibodies. Although the
30 distribution of sequence identities was similar in COVID-19 and healthy repertoires, the COVID-
31 19 hits were significantly more clonally expanded than healthy hits. Furthermore, among the tested
32 hits, 17 out of 94 from COVID-19, compared with 2 out of 96 from healthy, bound to the enhancing
33 epitope. A total of 6 of the 19 epitope-binding antibodies enhanced ACE2 receptor binding to the
34 spike protein. Together, this study revealed that enhancing antibodies are far more frequent in
35 COVID-19 patients than in healthy donors, but a reservoir of potential enhancing antibodies exists
36 in healthy donors that could potentially mature to actual enhancing antibodies upon infection.

37

38 **Keywords**

39 COVID-19, SARS-CoV-2, Infection enhancing antibodies, Antibody repertoire, InterClone

40

41 **Introduction**

42 Upon virus infection, host B cells that recognize viral antigens undergo affinity maturation and
43 differentiation into antibody-producing cells and memory B cells (Harwood & Batista, 2010;
44 Victora & Nussenzweig, 2012). Together with T cells, antigen-specific neutralizing antibodies
45 resolve infection (Dorner & Radbruch, 2007; Morales-Nunez et al., 2021), while long-lived
46 memory B cells protect against future infections (Akkaya et al., 2020; Cyster & Allen, 2019;

47 Kurosaki et al., 2015; Phan & Tangye, 2017). In the process of producing neutralizing antibodies,
48 infection-enhancing antibodies can also be generated (Bournazos et al., 2020). Antibody-
49 dependent enhancement (ADE) has been observed for multiple virus infections (Guzman et al.,
50 2013; Kapikian et al., 1969; Kim et al., 1969; Polack et al., 2003; Simmons et al., 2012) and
51 represents a challenge for the design of safe and effective vaccines (Arvin et al., 2020; Haynes et
52 al., 2020).

53 In 2021, two groups independently identified antibodies that enhanced SARS-CoV-2 spike protein
54 binding to human ACE2 (Li et al., 2021; Liu et al., 2021). Interestingly, the 11 monoclonal
55 antibodies collectively identified in these two studies were distinct in terms of their amino acid
56 sequences and gene usage yet targeted an overlapping site on the N-terminal domain of the spike
57 protein. Although the molecular mechanism of the observed ACE2-binding and infection
58 enhancement has not been demonstrated conclusively, multiple lines evidence point to a model
59 involving crosslinking of adjacent spike proteins. This evidence includes cell-based assays
60 showing that enhancement did not depend on the Fc domain of the antibody but did require two
61 Fab arms (i.e., full-length IgG or F(ab')₂), as well as molecular modelling that indicated that the
62 two Fab arms could not reach two enhancing epitopes on a single spike.

63 Since the proposed SARS-CoV-2 infection enhancing mechanism appears to be distinct from
64 previously reported ADE models, we sought to quantify the frequency of sequences similar to the
65 known enhancing antibodies in healthy and COVID-19 donors. Based on known structural data,
66 most antibodies recognize their cognate antigens through their complementarity-determining
67 regions (CDRs). Moreover, cryo-EM structural models of 3 out of the 11 enhancing antibodies
68 indicate that most of the physical contacts are mediated by the heavy chain (Li et al., 2021; Liu et
69 al., 2021). We thus reasoned that potential infection-enhancing antibodies could be identified

70 through similarity to heavy chain CDRs. To this end, we utilized a bioinformatics pipeline for
71 identifying antibodies in large BCR repertoire datasets with similar CDR sequences to a set of
72 queries (Figure 1). We also performed antibody expression and binding assays to assess the
73 functional phenotype of these antibodies among our search hits. Although they were less frequent
74 than in COVID-19 patients, we identified potential enhancing antibodies in healthy donors that
75 could lead to the development of actual enhancing antibodies upon infection. This study illustrates
76 that large BCR repertoire data can be used to discover functional human antibodies by sequence
77 similarity.

78

79 **Results**

80 *Diverse antibodies target a common infection-enhancing epitopes on spike protein NTD*

81 Despite targeting overlapping epitopes, the 11 previously reported enhancing antibodies have
82 emerged from different germline genes and possess highly diverse CDRH3 amino acid sequences
83 (Table S1 and Figure S1). Each sequence was expressed as a human IgG1 monoclonal antibody
84 using a mammalian expression system and confirmed to recognize the wildtype spike protein, WT
85 NTD but not to an NTD mutant with known epitope residues substituted with Alanine (W64A,
86 H66A, V213A, and R214A) or to the WT RBD (Figure S2A-D). Binding to the Delta variant of
87 SARS-CoV-2 Spike protein was also confirmed but the known enhancing antibodies lost their
88 binding to the Omicron variant, which has extensive NTD mutations (Figure S2E-F). Moreover,
89 all monoclonal antibodies facilitated ACE2 binding to WT and Delta Spike protein, but not to the
90 Omicron variant (Figure S3).

91

92 *Encoding healthy and COVID-19 antibody repertoires for CDR similarity search*

93 A total of 10 studies of healthy antibody repertoires, 15 studies of COVID-19 repertoires, and 3
94 studies of BNT162b2 vaccinated donor repertoires were collected. The healthy donor RNA
95 sequencing data consisted of 297 donors (Table S2), COVID-19 data consisted of 213 patients
96 (Table S3), and BNT162b2 vaccinated data consisted of 29 donors (Table S4). The data were
97 processed to facilitate an efficient search by CDR similarity. A pseudo-sequence of concatenated
98 CDRH1-3 amino acids was encoded as a MMseqs2 database (Steinegger & Soding, 2017). The
99 resulting databases contained 55,401,329 healthy unvaccinated, 391,201 healthy BNT162b2
100 vaccinated, and 8,490,653 COVID-19 data entries, each linked to a complete variable region amino
101 acid sequence.

102

103 *Sequences similar to enhancing antibodies found in healthy and COVID-19 repertoires*

104 One of our motivations was to understand the relationship between CDRH3 sequence identity and
105 shared epitope. We therefore searched for BCR sequences with rather loose criteria: where both
106 CDRH1 and CDRH2 identity were at least 80% and CDRH3 was at least 60%. This search resulted
107 in 7321 hits from healthy donors, 4679 from COVID-19 patients, and 113 from BNT162b2
108 vaccinated donors (Figure 2A). The distributions of CDR sequence identities among hits were
109 similar for healthy unvaccinated, healthy vaccinated, and COVID-19 donors (Figure 2B). The
110 CDRH3 sequences in COVID-19 hits were slightly more similar than those in healthy
111 unvaccinated or healthy vaccinated donors. However, there were no heavy chain sequences that
112 had the exact same amino acid sequence to a known enhancing antibody. B cells are known to be
113 expanded and to acquire mutations upon antigen exposure to increase their affinity to antigens
114 (Jacob et al., 1991). Indeed, among the hits, antigen exposed donors (COVID-19 and healthy
115 vaccinated donors) had more expanded clones than healthy unvaccinated donors (Figure 2C).

116 Sequences that were similar to COV2-2210, COV2-2582, and DH1055 dominated the COVID-19
117 and healthy unvaccinated donor hits (Figure 2D). In the healthy vaccinated donors, sequences that
118 were similar to DH1054 were more dominant than sequences similar to COV2-2582. Sequences
119 that were similar to DH1052, which has the longest CDRH3, were very infrequent in all the
120 datasets.

121

122 *NTD-binding antibodies found in healthy donors and COVID-19 repertoire search hits*

123 We next performed random sampling from healthy unvaccinated and COVID-19 search hits at a
124 confidence level of 95% and a margin of error of 10%. Due to the low number of original sequences,
125 the healthy vaccinated donors were excluded from the validation step. This resulted in selection of
126 96 non-redundant heavy chains from healthy unvaccinated donors and 94 from COVID-19 patients.
127 The sampling qualitatively reproduced the original CDR similarity distribution (Figure 3A). The
128 sequences that were similar to known infection-enhancing antibodies were then screened
129 experimentally to observe whether they bound to the enhancing epitope or not. We observed that
130 17 out of 94 antibodies from COVID-19 donors (Figure 3B) and 2 out of 96 from healthy
131 unvaccinated donors (Figure 3C) bound to S NTD, but not to the NTD mutant or the RBD (Figure
132 S4 and S5). The fraction of antibodies from COVID-19 donors that bound to the S NTD or to the
133 enhancing epitope was significantly higher than that in healthy unvaccinated donors (Chi-square
134 test p -value < 0.01) (Figure 3D). Some antibodies exhibited higher binding affinity to the Spike
135 protein from the Delta variant, but most lost their ability to bind to the Omicron variant (Figure
136 S4D-E and S5D-E).

137 Based on the true binders obtained from COVID-19 donors, the probability to find true binders
138 among hits was approximately 30% for CDRH3 amino acid sequence identities above 70% but

139 dropped to below 10% with CDRH3 sequence identities less than 70% (Figure 3E). In general, the
140 binders and non-binders could not be separated by a single sequence identity cutoff. Additional
141 sequence or structural data may allow us to differentiate binders from non-binders more accurately.
142 The current results support the use of loose sequence identity thresholds when searching large
143 repertoire data.

144

145 *A subset of antibodies that bind enhancing epitope enhance ACE2 binding*

146 The previously known enhancing antibodies were able to increase ACE2 binding to Spike protein;
147 therefore, we next assessed ACE2 binding enhancement using soluble ACE2 and Spike protein
148 expressed on Expi293F cells (Liu et al., 2021). Antibodies that bound the enhancing epitope were
149 purified and tested at the same concentration to confirm whether they increased the binding of
150 ACE2 to the Spike protein. Among 19 antibodies tested, we found that 6 were potent ACE2
151 binding enhancers to Spike WT protein (Figure 4A). The ACE2 binding enhancement was
152 increased when we tested these antibodies against Spike Delta variant, but they lost their ability
153 when tested against Omicron variant (Figure 4B-C).

154

155 **Discussion**

156 The observation that a subset of antibodies produced in diverse donors target an overlapping
157 epitope, and that a further subset of the antibodies that binding to this epitope enhance ACE2
158 binding, raises several questions. We first sought to understand the distribution of these antibodies
159 in both antigen-exposed and unexposed donors. Based on CDR sequence-similarity alone, we
160 found highly similar distributions in healthy unvaccinated, healthy vaccinated and COVID-19
161 donors (Figure 2B). However, the clone sizes of these hits were qualitatively different in antigen-

162 exposed and unexposed donors (Figure 2C). Furthermore, based on the experimental results, the
163 frequency of binding to the enhancing epitope was significantly higher for the COVID-19 derived
164 group than the healthy-derived group. From the NTD binding data, we could estimate the
165 frequency of enhancing antibodies within COVID-19 and healthy unvaccinated donors to be less
166 than 100 and 3 per million clones, respectively. Although the proposed enhancing mechanism
167 requires a more detailed study, it may well apply to other coronaviruses that use ACE2 as a host
168 receptor. Given the large reservoir of potential antibodies in the healthy population, this may
169 represent a modest concern for future vaccine design.

170 Ease of access to RNA sequencing technologies, as well as reduction of cost has resulted in a rapid
171 increase in publicly available BCR repertoire sequence data (Marks & Deane, 2020). The approach
172 taken here to search this data is general and will likely aid in the discovery of not only enhancing,
173 but also neutralizing antibodies, autoantibodies, or even T cell receptors. As more data on binders
174 and non-binders accumulates, more sophisticated metrics of similarity can be tested. The methods
175 used here are available as an open-source project with a freely accessible web server
176 (www.sysimm.org/interclone/).

177

178

179 **Acknowledgments**

180 The authors would like to thank RIMD and IFRc Core Experimental Facility for the support in
181 conducting experiments; all Standley Lab member for constructive discussion and comments on
182 the manuscript. This work was supported by Japan Agency for Medical Research and
183 Development (AMED), Platform Project for Supporting Drug Discovery and Life Science

184 Research (Basis for Supporting Innovative Drug Discovery and Life Science Research) under
185 JP21am0101108.

186

187 **Author contributions**

188 H.S.I. performed searching of new enhancing antibodies, binding assay, ACE2 binding
189 enhancement assay, and data analysis. H.S.I. and D.S.S. performed antibodies expression. Z.X.
190 and J.W. developed the backend databases. Z.X., J.W., S.L. and D.M.S. did the bioinformatics
191 pipeline development. H.S.I., D.M.S., D.K.N., Y.H., H.A., M.O. conceptualized and designed the
192 experiments. All authors wrote, reviewed, and edited the manuscript. D.M.S. supervised the
193 overall project.

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195 **Declaration of interests**

196 Authors declare no conflict of interests

197

198 **References**

199 Akkaya, M., Kwak, K., & Pierce, S. K. (2020). B cell memory: building two walls of protection
200 against pathogens. *Nat Rev Immunol*, 20(4), 229-238. [https://doi.org/10.1038/s41577-019-](https://doi.org/10.1038/s41577-019-0244-2)

201 [0244-2](https://doi.org/10.1038/s41577-019-0244-2)

202 Arvin, A. M., Fink, K., Schmid, M. A., Cathcart, A., Spreafico, R., Havenar-Daughton, C.,
203 Lanzavecchia, A., Corti, D., & Virgin, H. W. (2020). A perspective on potential antibody-
204 dependent enhancement of SARS-CoV-2. *Nature*, 584(7821), 353-363.

205 <https://doi.org/10.1038/s41586-020-2538-8>

- 206 Bernardes, J. P., Mishra, N., Tran, F., Bahmer, T., Best, L., Blase, J. I., Bordoni, D., Franzenburg,
207 J., Geisen, U., Josephs-Spaulling, J., Kohler, P., Kunstner, A., Rosati, E., Aschenbrenner,
208 A. C., Bacher, P., Baran, N., Boysen, T., Brandt, B., Bruse, N., . . . Deutsche, C.-O. I.
209 (2020). Longitudinal Multi-omics Analyses Identify Responses of Megakaryocytes,
210 Erythroid Cells, and Plasmablasts as Hallmarks of Severe COVID-19. *Immunity*, 53(6),
211 1296-1314 e1299. <https://doi.org/10.1016/j.immuni.2020.11.017>
- 212 Bournazos, S., Gupta, A., & Ravetch, J. V. (2020). The role of IgG Fc receptors in antibody-
213 dependent enhancement. *Nat Rev Immunol*, 20(10), 633-643.
214 <https://doi.org/10.1038/s41577-020-00410-0>
- 215 Cyster, J. G., & Allen, C. D. C. (2019). B Cell Responses: Cell Interaction Dynamics and Decisions.
216 *Cell*, 177(3), 524-540. <https://doi.org/10.1016/j.cell.2019.03.016>
- 217 Dorner, T., & Radbruch, A. (2007). Antibodies and B cell memory in viral immunity. *Immunity*,
218 27(3), 384-392. <https://doi.org/10.1016/j.immuni.2007.09.002>
- 219 Dunbar, J., & Deane, C. M. (2016). ANARCI: antigen receptor numbering and receptor
220 classification. *Bioinformatics*, 32(2), 298-300.
221 <https://doi.org/10.1093/bioinformatics/btv552>
- 222 Galson, J. D., Schaetzle, S., Bashford-Rogers, R. J. M., Raybould, M. I. J., Kovaltsuk, A.,
223 Kilpatrick, G. J., Minter, R., Finch, D. K., Dias, J., James, L. K., Thomas, G., Lee, W. J.,
224 Betley, J., Cavlan, O., Leech, A., Deane, C. M., Seoane, J., Caldas, C., Pennington, D. J., . . .
225 Osbourn, J. (2020). Deep Sequencing of B Cell Receptor Repertoires From COVID-19
226 Patients Reveals Strong Convergent Immune Signatures. *Front Immunol*, 11, 605170.
227 <https://doi.org/10.3389/fimmu.2020.605170>

- 228 Ghraichy, M., Galson, J. D., Kovaltsuk, A., von Niederhausern, V., Pachlopnik Schmid, J., Recher,
229 M., Jauch, A. J., Miho, E., Kelly, D. F., Deane, C. M., & Truck, J. (2020). Maturation of
230 the Human Immunoglobulin Heavy Chain Repertoire With Age. *Front Immunol*, *11*, 1734.
231 <https://doi.org/10.3389/fimmu.2020.01734>
- 232 Gidoni, M., Snir, O., Peres, A., Polak, P., Lindeman, I., Mikocziova, I., Sarna, V. K., Lundin, K.
233 E. A., Clouser, C., Vigneault, F., Collins, A. M., Sollid, L. M., & Yaari, G. (2019). Mosaic
234 deletion patterns of the human antibody heavy chain gene locus shown by Bayesian
235 haplotyping. *Nat Commun*, *10*(1), 628. <https://doi.org/10.1038/s41467-019-08489-3>
- 236 Goel, R. R., Apostolidis, S. A., Painter, M. M., Mathew, D., Pattekar, A., Kuthuru, O., Gouma, S.,
237 Hicks, P., Meng, W., Rosenfeld, A. M., Dysinger, S., Lundgreen, K. A., Kuri-Cervantes,
238 L., Adamski, S., Hicks, A., Korte, S., Oldridge, D. A., Baxter, A. E., Giles, J. R., . . . Wherry,
239 E. J. (2021). Distinct antibody and memory B cell responses in SARS-CoV-2 naive and
240 recovered individuals following mRNA vaccination. *Sci Immunol*, *6*(58).
241 <https://doi.org/10.1126/sciimmunol.abi6950>
- 242 Goel, R. R., Painter, M. M., Apostolidis, S. A., Mathew, D., Meng, W., Rosenfeld, A. M.,
243 Lundgreen, K. A., Reynaldi, A., Khoury, D. S., Pattekar, A., Gouma, S., Kuri-Cervantes,
244 L., Hicks, P., Dysinger, S., Hicks, A., Sharma, H., Herring, S., Korte, S., Baxter, A. E., . . .
245 Wherry, E. J. (2021). mRNA vaccines induce durable immune memory to SARS-CoV-2
246 and variants of concern. *Science*, *374*(6572), abm0829.
247 <https://doi.org/10.1126/science.abm0829>
- 248 Guzman, M. G., Alvarez, M., & Halstead, S. B. (2013). Secondary infection as a risk factor for
249 dengue hemorrhagic fever/dengue shock syndrome: an historical perspective and role of

- 250 antibody-dependent enhancement of infection. *Arch Virol*, 158(7), 1445-1459.
- 251 <https://doi.org/10.1007/s00705-013-1645-3>
- 252 Harwood, N. E., & Batista, F. D. (2010). Early events in B cell activation. *Annu Rev Immunol*, 28,
- 253 185-210. <https://doi.org/10.1146/annurev-immunol-030409-101216>
- 254 Haynes, B. F., Corey, L., Fernandes, P., Gilbert, P. B., Hotez, P. J., Rao, S., Santos, M. R.,
- 255 Schuitemaker, H., Watson, M., & Arvin, A. (2020). Prospects for a safe COVID-19 vaccine.
- 256 *Sci Transl Med*, 12(568). <https://doi.org/10.1126/scitranslmed.abe0948>
- 257 Jacob, J., Kelsoe, G., Rajewsky, K., & Weiss, U. (1991). Intracloonal generation of antibody
- 258 mutants in germinal centres. *Nature*, 354(6352), 389-392.
- 259 <https://doi.org/10.1038/354389a0>
- 260 Kapikian, A. Z., Mitchell, R. H., Chanock, R. M., Shvedoff, R. A., & Stewart, C. E. (1969). An
- 261 epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus
- 262 infection in children previously vaccinated with an inactivated RS virus vaccine. *Am J*
- 263 *Epidemiol*, 89(4), 405-421. <https://doi.org/10.1093/oxfordjournals.aje.a120954>
- 264 Kim, H. W., Canchola, J. G., Brandt, C. D., Pyles, G., Chanock, R. M., Jensen, K., & Parrott, R.
- 265 H. (1969). Respiratory syncytial virus disease in infants despite prior administration of
- 266 antigenic inactivated vaccine. *Am J Epidemiol*, 89(4), 422-434.
- 267 <https://doi.org/10.1093/oxfordjournals.aje.a120955>
- 268 Kim, S. I., Noh, J., Kim, S., Choi, Y., Yoo, D. K., Lee, Y., Lee, H., Jung, J., Kang, C. K., Song,
- 269 K. H., Choe, P. G., Kim, H. B., Kim, E. S., Kim, N. J., Seong, M. W., Park, W. B., Oh, M.
- 270 D., Kwon, S., & Chung, J. (2021). Stereotypic neutralizing VH antibodies against SARS-
- 271 CoV-2 spike protein receptor binding domain in patients with COVID-19 and healthy
- 272 individuals. *Sci Transl Med*, 13(578). <https://doi.org/10.1126/scitranslmed.abd6990>

- 273 Kuri-Cervantes, L., Pampena, M. B., Meng, W., Rosenfeld, A. M., Ittner, C. A. G., Weisman, A.
274 R., Agyekum, R. S., Mathew, D., Baxter, A. E., Vella, L. A., Kuthuru, O., Apostolidis, S.
275 A., Bershaw, L., Dougherty, J., Greenplate, A. R., Pattekar, A., Kim, J., Han, N., Gouma,
276 S., . . . Betts, M. R. (2020). Comprehensive mapping of immune perturbations associated
277 with severe COVID-19. *Sci Immunol*, 5(49). <https://doi.org/10.1126/sciimmunol.abd7114>
- 278 Kurosaki, T., Kometani, K., & Ise, W. (2015). Memory B cells. *Nat Rev Immunol*, 15(3), 149-159.
279 <https://doi.org/10.1038/nri3802>
- 280 Li, D., Edwards, R. J., Manne, K., Martinez, D. R., Schafer, A., Alam, S. M., Wiehe, K., Lu, X.,
281 Parks, R., Sutherland, L. L., Oguin, T. H., 3rd, McDanal, C., Perez, L. G., Mansouri, K.,
282 Gobeil, S. M. C., Janowska, K., Stalls, V., Kopp, M., Cai, F., . . . Saunders, K. O. (2021).
283 In vitro and in vivo functions of SARS-CoV-2 infection-enhancing and neutralizing
284 antibodies. *Cell*, 184(16), 4203-4219 e4232. <https://doi.org/10.1016/j.cell.2021.06.021>
- 285 Liu, Y., Soh, W. T., Kishikawa, J. I., Hirose, M., Nakayama, E. E., Li, S., Sasai, M., Suzuki, T.,
286 Tada, A., Arakawa, A., Matsuoka, S., Akamatsu, K., Matsuda, M., Ono, C., Torii, S.,
287 Kishida, K., Jin, H., Nakai, W., Arase, N., . . . Arase, H. (2021). An infectivity-enhancing
288 site on the SARS-CoV-2 spike protein targeted by antibodies. *Cell*, 184(13), 3452-3466
289 e3418. <https://doi.org/10.1016/j.cell.2021.05.032>
- 290 Marks, C., & Deane, C. M. (2020). How repertoire data are changing antibody science. *J Biol*
291 *Chem*, 295(29), 9823-9837. <https://doi.org/10.1074/jbc.REV120.010181>
- 292 Meng, W., Zhang, B., Schwartz, G. W., Rosenfeld, A. M., Ren, D., Thome, J. J. C., Carpenter, D.
293 J., Matsuoka, N., Lerner, H., Friedman, A. L., Granot, T., Farber, D. L., Shlomchik, M. J.,
294 Hershberg, U., & Luning Prak, E. T. (2017). An atlas of B-cell clonal distribution in the
295 human body. *Nat Biotechnol*, 35(9), 879-884. <https://doi.org/10.1038/nbt.3942>

- 296 Montague, Z., Lv, H., Otwinowski, J., DeWitt, W. S., Isacchini, G., Yip, G. K., Ng, W. W., Tsang,
297 O. T., Yuan, M., Liu, H., Wilson, I. A., Peiris, J. S. M., Wu, N. C., Nourmohammad, A.,
298 & Mok, C. K. P. (2021). Dynamics of B cell repertoires and emergence of cross-reactive
299 responses in patients with different severities of COVID-19. *Cell Rep*, 35(8), 109173.
300 <https://doi.org/10.1016/j.celrep.2021.109173>
- 301 Mor, M., Werbner, M., Alter, J., Safra, M., Chomsky, E., Lee, J. C., Hada-Neeman, S., Polonsky,
302 K., Nowell, C. J., Clark, A. E., Roitburd-Berman, A., Ben-Shalom, N., Navon, M., Rafael,
303 D., Sharim, H., Kiner, E., Griffis, E. R., Gershoni, J. M., Kobiler, O., . . . Freund, N. T.
304 (2021). Multi-clonal SARS-CoV-2 neutralization by antibodies isolated from severe
305 COVID-19 convalescent donors. *PLoS Pathog*, 17(2), e1009165.
306 <https://doi.org/10.1371/journal.ppat.1009165>
- 307 Morales-Nunez, J. J., Munoz-Valle, J. F., Torres-Hernandez, P. C., & Hernandez-Bello, J. (2021).
308 Overview of Neutralizing Antibodies and Their Potential in COVID-19. *Vaccines (Basel)*,
309 9(12). <https://doi.org/10.3390/vaccines9121376>
- 310 Nielsen, S. C. A., Yang, F., Jackson, K. J. L., Hoh, R. A., Roltgen, K., Jean, G. H., Stevens, B. A.,
311 Lee, J. Y., Rustagi, A., Rogers, A. J., Powell, A. E., Hunter, M., Najeeb, J., Otrelo-Cardoso,
312 A. R., Yost, K. E., Daniel, B., Nadeau, K. C., Chang, H. Y., Satpathy, A. T., . . . Boyd, S.
313 D. (2020). Human B Cell Clonal Expansion and Convergent Antibody Responses to
314 SARS-CoV-2. *Cell Host Microbe*, 28(4), 516-525 e515.
315 <https://doi.org/10.1016/j.chom.2020.09.002>
- 316 Niu, X., Li, S., Li, P., Pan, W., Wang, Q., Feng, Y., Mo, X., Yan, Q., Ye, X., Luo, J., Qu, L.,
317 Weber, D., Byrne-Steele, M. L., Wang, Z., Yu, F., Li, F., Myers, R. M., Lotze, M. T.,
318 Zhong, N., . . . Chen, L. (2020). Longitudinal Analysis of T and B Cell Receptor Repertoire

- 319 Transcripts Reveal Dynamic Immune Response in COVID-19 Patients. *Front Immunol*, *11*,
320 582010. <https://doi.org/10.3389/fimmu.2020.582010>
- 321 Phan, T. G., & Tangye, S. G. (2017). Memory B cells: total recall. *Curr Opin Immunol*, *45*, 132-
322 140. <https://doi.org/10.1016/j.coi.2017.03.005>
- 323 Polack, F. P., Hoffman, S. J., Crujeiras, G., & Griffin, D. E. (2003). A role for nonprotective
324 complement-fixing antibodies with low avidity for measles virus in atypical measles. *Nat*
325 *Med*, *9*(9), 1209-1213. <https://doi.org/10.1038/nm918>
- 326 Roskin, K. M., Jackson, K. J. L., Lee, J. Y., Hoh, R. A., Joshi, S. A., Hwang, K. K., Bonsignori,
327 M., Pedroza-Pacheco, I., Liao, H. X., Moody, M. A., Fire, A. Z., Borrow, P., Haynes, B.
328 F., & Boyd, S. D. (2020). Aberrant B cell repertoire selection associated with HIV
329 neutralizing antibody breadth. *Nat Immunol*, *21*(2), 199-209.
330 <https://doi.org/10.1038/s41590-019-0581-0>
- 331 Schmitz, A. J., Turner, J. S., Liu, Z., Zhou, J. Q., Aziati, I. D., Chen, R. E., Joshi, A., Bricker, T.
332 L., Darling, T. L., Adelsberg, D. C., Altomare, C. G., Alsoussi, W. B., Case, J. B.,
333 VanBlargan, L. A., Lei, T., Thapa, M., Amanat, F., Jeevan, T., Fabrizio, T., . . . Ellebedy,
334 A. H. (2021). A vaccine-induced public antibody protects against SARS-CoV-2 and
335 emerging variants. *Immunity*, *54*(9), 2159-2166 e2156.
336 <https://doi.org/10.1016/j.immuni.2021.08.013>
- 337 Schultheiss, C., Paschold, L., Simnica, D., Mohme, M., Willscher, E., von Wenserski, L., Scholz,
338 R., Wieters, I., Dahlke, C., Tolosa, E., Sedding, D. G., Ciesek, S., Addo, M., & Binder, M.
339 (2020). Next-Generation Sequencing of T and B Cell Receptor Repertoires from COVID-
340 19 Patients Showed Signatures Associated with Severity of Disease. *Immunity*, *53*(2), 442-
341 455 e444. <https://doi.org/10.1016/j.immuni.2020.06.024>

- 342 Setliff, I., McDonnell, W. J., Raju, N., Bombardi, R. G., Murji, A. A., Scheepers, C., Ziki, R.,
343 Mynhardt, C., Shepherd, B. E., Mamchak, A. A., Garrett, N., Karim, S. A., Mallal, S. A.,
344 Crowe, J. E., Jr., Morris, L., & Georgiev, I. S. (2018). Multi-Donor Longitudinal Antibody
345 Repertoire Sequencing Reveals the Existence of Public Antibody Clonotypes in HIV-1
346 Infection. *Cell Host Microbe*, 23(6), 845-854 e846.
347 <https://doi.org/10.1016/j.chom.2018.05.001>
- 348 Simmons, C. P., Farrar, J. J., Nguyen v, V., & Wills, B. (2012). Dengue. *N Engl J Med*, 366(15),
349 1423-1432. <https://doi.org/10.1056/NEJMra1110265>
- 350 Sokal, A., Chappert, P., Barba-Spaeth, G., Roeser, A., Fourati, S., Azzaoui, I., Vandenberghe, A.,
351 Fernandez, I., Meola, A., Bouvier-Alias, M., Crickx, E., Beldi-Ferchiou, A., Hue, S.,
352 Languille, L., Michel, M., Baloul, S., Noizat-Pirenne, F., Luka, M., Megret, J., . . . Mahevas,
353 M. (2021). Maturation and persistence of the anti-SARS-CoV-2 memory B cell response.
354 *Cell*, 184(5), 1201-1213 e1214. <https://doi.org/10.1016/j.cell.2021.01.050>
- 355 Soto, C., Bombardi, R. G., Branchizio, A., Kose, N., Matta, P., Sevy, A. M., Sinkovits, R. S.,
356 Gilchuk, P., Finn, J. A., & Crowe, J. E., Jr. (2019). High frequency of shared clonotypes in
357 human B cell receptor repertoires. *Nature*, 566(7744), 398-402.
358 <https://doi.org/10.1038/s41586-019-0934-8>
- 359 Steinegger, M., & Soding, J. (2017). MMseqs2 enables sensitive protein sequence searching for
360 the analysis of massive data sets. *Nat Biotechnol*, 35(11), 1026-1028.
361 <https://doi.org/10.1038/nbt.3988>
- 362 Turner, J. S., O'Halloran, J. A., Kalaidina, E., Kim, W., Schmitz, A. J., Zhou, J. Q., Lei, T., Thapa,
363 M., Chen, R. E., Case, J. B., Amanat, F., Rauseo, A. M., Haile, A., Xie, X., Klebert, M. K.,
364 Suessen, T., Middleton, W. D., Shi, P. Y., Krammer, F., . . . Ellebedy, A. H. (2021). SARS-

- 365 CoV-2 mRNA vaccines induce persistent human germinal centre responses. *Nature*,
366 596(7870), 109-113. <https://doi.org/10.1038/s41586-021-03738-2>
- 367 Turner, J. S., Zhou, J. Q., Han, J., Schmitz, A. J., Rizk, A. A., Alsoussi, W. B., Lei, T., Amor, M.,
368 McIntire, K. M., Meade, P., Strohmeier, S., Brent, R. I., Richey, S. T., Haile, A., Yang, Y.
369 R., Klebert, M. K., Suessen, T., Teefey, S., Presti, R. M., . . . Ellebedy, A. H. (2020).
370 Human germinal centres engage memory and naive B cells after influenza vaccination.
371 *Nature*, 586(7827), 127-132. <https://doi.org/10.1038/s41586-020-2711-0>
- 372 Victora, G. D., & Nussenzweig, M. C. (2012). Germinal centers. *Annu Rev Immunol*, 30, 429-457.
373 <https://doi.org/10.1146/annurev-immunol-020711-075032>
- 374 Wen, W., Su, W., Tang, H., Le, W., Zhang, X., Zheng, Y., Liu, X., Xie, L., Li, J., Ye, J., Dong, L.,
375 Cui, X., Miao, Y., Wang, D., Dong, J., Xiao, C., Chen, W., & Wang, H. (2020). Immune
376 cell profiling of COVID-19 patients in the recovery stage by single-cell sequencing. *Cell*
377 *Discov*, 6, 31. <https://doi.org/10.1038/s41421-020-0168-9>
- 378 Woodruff, M. C., Ramonell, R. P., Nguyen, D. C., Cashman, K. S., Saini, A. S., Haddad, N. S.,
379 Ley, A. M., Kyu, S., Howell, J. C., Ozturk, T., Lee, S., Suryadevara, N., Case, J. B.,
380 Bugrovsky, R., Chen, W., Estrada, J., Morrison-Porter, A., Derrico, A., Anam, F. A., . . .
381 Sanz, I. (2020). Extrafollicular B cell responses correlate with neutralizing antibodies and
382 morbidity in COVID-19. *Nat Immunol*, 21(12), 1506-1516.
383 <https://doi.org/10.1038/s41590-020-00814-z>
- 384 Zhang, J. Y., Wang, X. M., Xing, X., Xu, Z., Zhang, C., Song, J. W., Fan, X., Xia, P., Fu, J. L.,
385 Wang, S. Y., Xu, R. N., Dai, X. P., Shi, L., Huang, L., Jiang, T. J., Shi, M., Zhang, Y.,
386 Zumla, A., Maeurer, M., . . . Wang, F. S. (2020). Single-cell landscape of immunological

387 responses in patients with COVID-19. *Nat Immunol*, 21(9), 1107-1118.
388 <https://doi.org/10.1038/s41590-020-0762-x>

389 Zhou, Y., Zhang, J., Wang, D., Wang, D., Guan, W., Qin, J., Xu, X., Fang, J., Fu, B., Zheng, X.,
390 Wang, D., Zhao, H., Chen, X., Tian, Z., Xu, X., Wang, G., & Wei, H. (2021). Profiling of
391 the immune repertoire in COVID-19 patients with mild, severe, convalescent, or retesting-
392 positive status. *J Autoimmun*, 118, 102596. <https://doi.org/10.1016/j.jaut.2021.102596>

393

394 **Method details**

395 BCR repertoire data mining and processing

396 Datasets were retrieved from publicly available repository such as sequence read archive (SRA)
397 and European nucleotide archive (ENA). Collected study detail can be seen in the Table S2-S4
398 (Bernardes et al., 2020; Galson et al., 2020; Ghraichy et al., 2020; Gidoni et al., 2019; Goel,
399 Apostolidis, et al., 2021; Goel, Painter, et al., 2021; Kim et al., 2021; Kuri-Cervantes et al., 2020;
400 Meng et al., 2017; Montague et al., 2021; Mor et al., 2021; Nielsen et al., 2020; Niu et al., 2020;
401 Roskin et al., 2020; Schmitz et al., 2021; Schultheiss et al., 2020; Setliff et al., 2018; Sokal et al.,
402 2021; Soto et al., 2019; Turner et al., 2021; Turner et al., 2020; Wen et al., 2020; Woodruff et al.,
403 2020; Zhang et al., 2020; Zhou et al., 2021). Raw BCR repertoire sequencing data was formatted
404 into AIRR-formatted files and then CDRs was assigned using ANARCI (Dunbar & Deane, 2016).
405 CDRH1, CDRH3, and CDRH3 that have been assigned then concatenated into single pseudo-
406 sequences for later encoding into MMSeqs2 database format (Steinegger & Soding, 2017). Query
407 sequences (11 enhancing antibodies) were also processed similarly. For each query sequence,
408 database was searched using the minimum sequence identity cutoff. For each hit, pseudo-sequence

409 was separated into CDRH1, CDRH2, and CDRH3 then sequence identity for each CDR was
410 evaluated (CDRH1, CDRH2, and CDRH3 cutoff 80, 80, and 60 %).

411

412 Cell lines

413 Expi293F cells (Thermo) were maintained in Expi293 expression medium (Gibco) supplemented
414 with 100x dilution of 10,000 U/mL penicillin/streptomycin (Gibco) at 37°C incubators under 8%
415 CO₂ and shaking at 125 rpm.

416

417 Production of infection enhancing antibodies from COVID-19 patients and healthy donors 418 sequence database

419 Recombinant antibodies were produced as previously described (Liu et al., 2021). Briefly, the
420 variable regions of sampled heavy chains from the COVID-19 patients and healthy donors were
421 prepared by dsDNA synthesis (IDT) and cloned into pCAGGS vectors containing sequences of
422 human IgG1 constant region. The light chains from known enhancing antibodies were synthesized
423 and cloned into the pCAGGS vector containing the human immunoglobulin kappa constant region.
424 To produce recombinant antibodies, vectors containing heavy chain sequence and light chain
425 sequence from known antibodies were co-transfected into Expi293F cells (Thermo) and the
426 supernatant was collected for further assay.

427

428 Antibody binding assay

429 Antibody binding to SARS-CoV-2 antigen was measured as previously described (Liu et al., 2021)
430 with modification of antigen display cells from HEK293T to Expi293F (Thermo). The pME18S
431 plasmid expressing Spike protein C-terminal retention signal deletion from Wuhan (WT), Delta,

432 and Omicron variant, Flag-NTD-PILR-TM, Flag-RBD-PILR-TM, and Flag-NTD (W64A, H66A,
433 V213A, and R214A)-PILR-TM, were co-transfected with pMx plasmid expressing GFP as the
434 marker to the Expi293F cells (Thermo). The transfectant cells were incubated with supernatant
435 containing expressed antibodies for 30 minutes then followed by incubation with APC-anti-human
436 IgG (H+L) antibody (Jackson ImmunoResearch, USA). Bound antibodies to the GFP-positive
437 cells were then analyzed by flow cytometry (Attune NxT, Thermo).

438

439 ACE2 binding enhancement assay

440 The SARS-CoV-2 S-NTD binders were expressed and purified using protein A spin column
441 (Cosmo Bio) and concentration was measured using ELISA. ACE2 binding enhancement to Spike
442 WT, Delta, and Omicron variants in the presence of enhancing antibodies was measured. Briefly,
443 Expi293F cells (Thermo) that express either Spike WT, Delta, or Omicron variant were incubated
444 by 1 µg/mL antibodies for 30 minutes. Followed by ACE2-biotin at 1 µg/mL (RnD Systems)
445 incubation for 30 minutes and then SA-APC (Biolegend) incubation for 1 hour. The amount of
446 ACE2 that binds to Spike protein was measured using flow cytometry (Attune NxT, Thermo). Fold
447 change was calculated by comparing the amount of bound ACE2 in the presence of antibodies and
448 in the absence of antibodies.

449

450 Quantification and Statistical Analysis

451 Flow cytometry data were analyzed using FlowJo version 10.7 (BD Biosciences, USA). GraphPad
452 Prism version 9 was used for binding assay graph generation. Matplotlib (v. 3.3.4) and Seaborn (v.
453 0.11.0) python packages were used to generate CDRs similarity and ACE2 enhancement graph
454 and violin plot. Scipy (v. 1.7.3) was used to calculate Chi-square test.

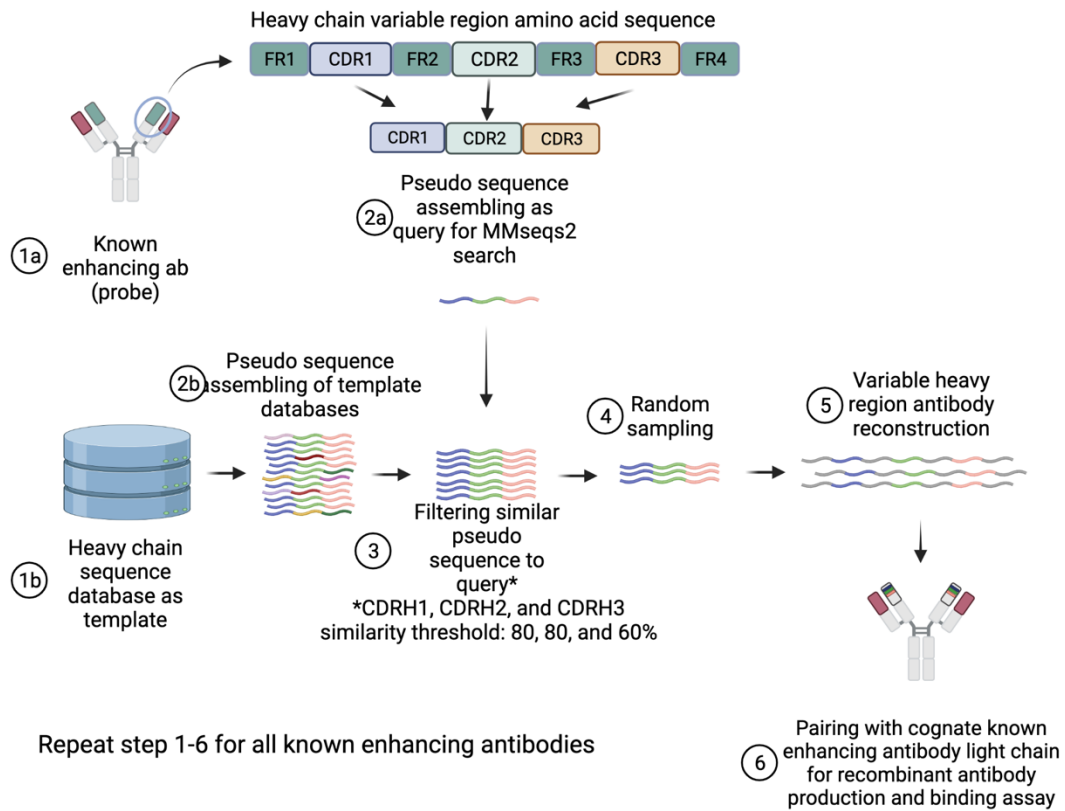


Figure 1. Schematic illustration of bioinformatics pipeline for finding functionally similar antibodies

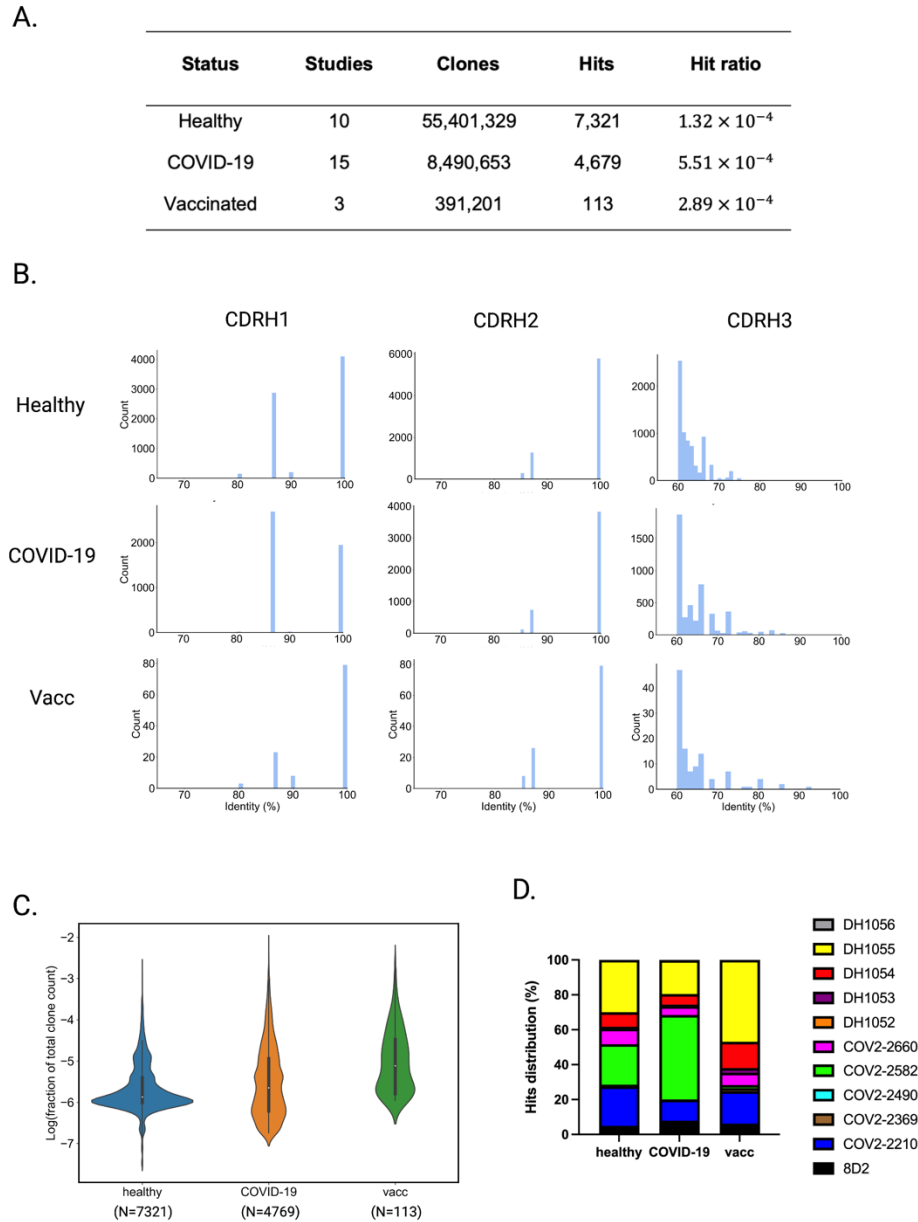


Figure 2. Finding enhancing antibodies from BCR sequencing data

(A) Enhancing antibodies hits from BCR sequencing data of healthy unvaccinated, COVID-19, and healthy vaccinated donors. (B) Hits CDRH1-3 identity distribution. (C) Fraction of total clone count of hits. The violin plot shows the distribution of fraction of total clone count with the higher fraction represent clonal expansion. (D) Distribution of enhancing antibodies hits based on the similarity to known enhancing antibodies.

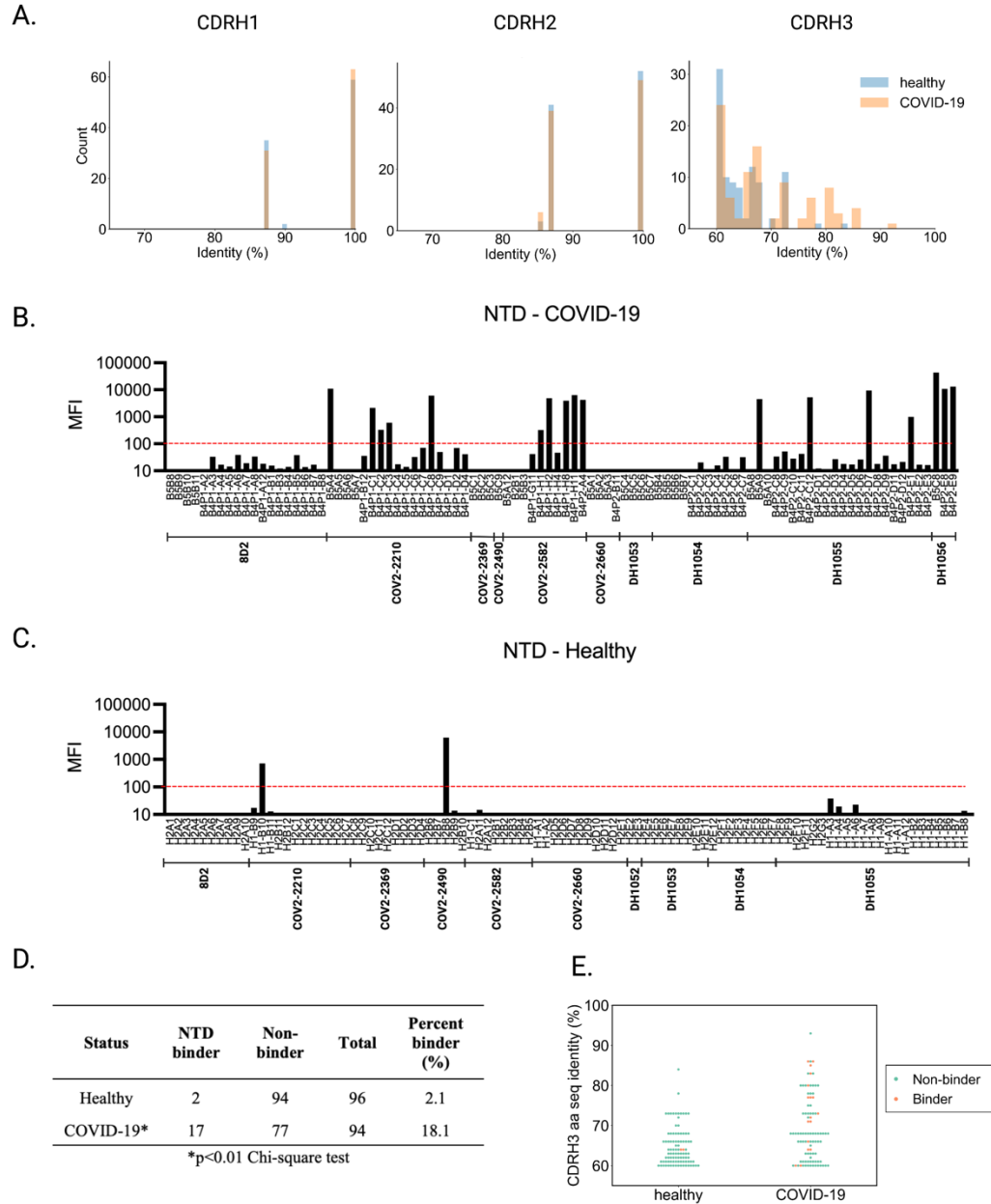


Figure 3. Sampling and testing the binding of hits to NTD

(A) CDRH1-3 distribution of sampled heavy chains. (B) NTD binding of produced antibodies from COVID-19 patients. Bars above the red dashed line are considered to be NTD binders. (C) NTD binding of produced antibodies from healthy unvaccinated donors. (D) NTD binders found from sampled antibodies and binder true positive rate for healthy unvaccinated and COVID-19. (E) CDRH3 distribution of NTD binders and non-binders.

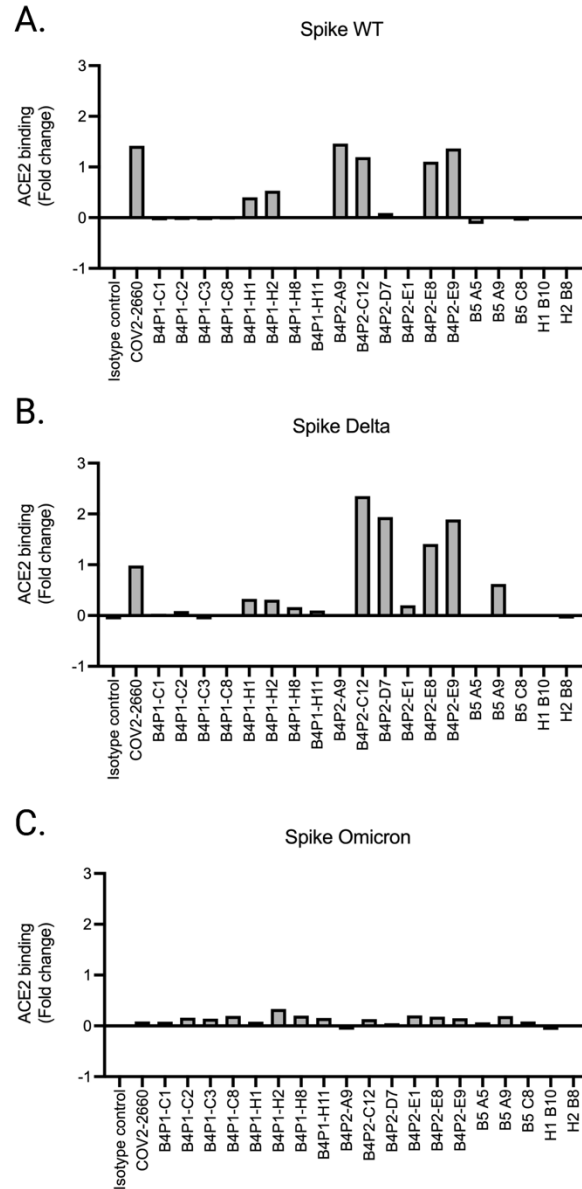


Figure 4. Enhancement of ACE2 binding to Spike protein in the presence of antibodies

ACE2 binding to Spike WT (A), Delta (B), Omicron variant (C) enhancement are observed in the presence of sampled antibodies or isotype control (hIgG1).