Journal Pre-proof

Seasonal human coronavirus antibodies are boosted upon SARS-CoV-2 infection but not associated with protection

Elizabeth M. Anderson, Eileen C. Goodwin, Anurag Verma, Claudia P. Arevalo, Marcus J. Bolton, Madison E. Weirick, Sigrid Gouma, Christopher M. McAllister, Shannon R. Christensen, JoEllen Weaver, Philip Hicks, Tomaz B. Manzoni, Oluwatosin Oniyide, Holly Ramage, Divij Mathew, Amy E. Baxter, Derek A. Oldridge, Allison R. Greenplate, Jennifer E. Wu, Cécile Alanio, Kurt D'Andrea, Oliva Kuthuru, Jeanette Dougherty, Ajinkya Pattekar, Justin Kim, Nicholas Han, Sokratis A. Apostolidis, Alex C. Huang, Laura A. Vella, Leticia Kuri-Cervantes, M. Betina Pampena, The UPenn COVID Processing Unit, Michael R. Betts, E. John Wherry, Nuala J. Meyer, Sara Cherry, Paul Bates, Daniel J. Rader, Scott E. Hensley

in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Pre-Pandemic

■ Individuals with
cross-reactive antibodies

■ Individuals without
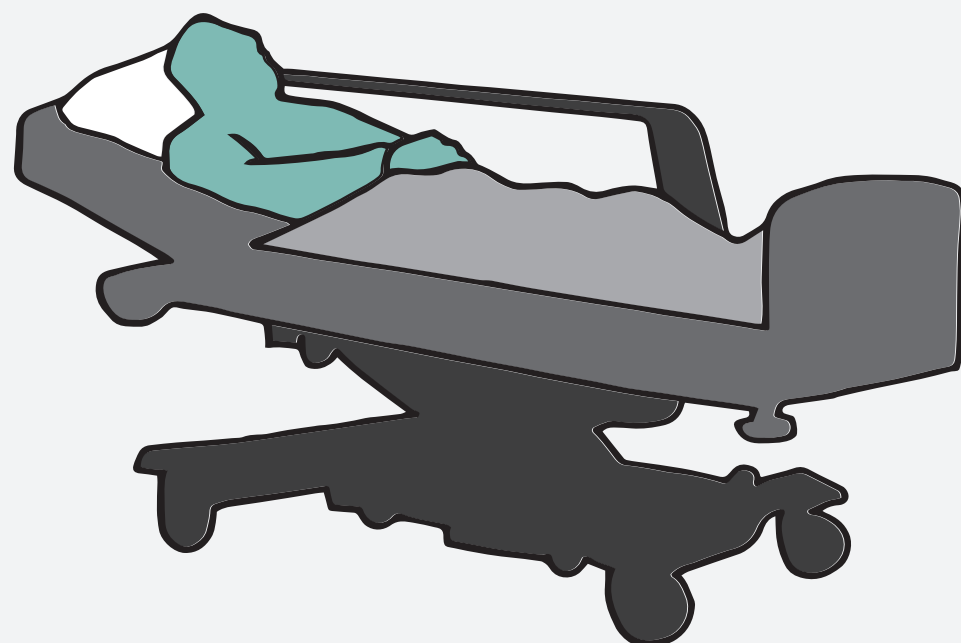cross-reactive antibodies
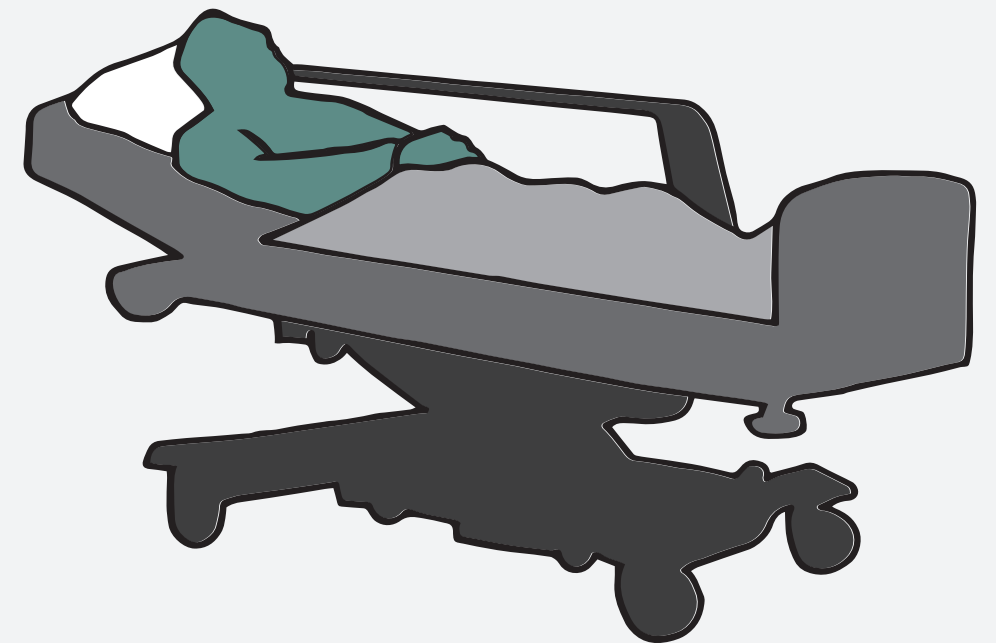
SARS-CoV-2 Susceptibility

Infected

Not Infected

Following
SARS-CoV-2 Infection

Boost of cross-reactive
antibodies

Seasonal human coronavirus antibodies are boosted upon SARS-CoV-2 infection but not associated with protection

Elizabeth M. Anderson[1,13], Eileen C. Goodwin[1,13], Anurag Verma[2,13], Claudia P. Arevalo[1], Marcus J. Bolton[1], Madison E. Weirick[1], Sigrid Gouma[1], Christopher M. McAllister[1], Shannon R. Christensen[1], JoEllen Weaver[2], Philip Hicks[3], Tomaz B. Manzoni[1], Oluwatosin Oniyide[4], Holly Ramage[5,12], Divij Mathew[6,7], Amy E. Baxter[6,7], Derek A. Oldridge[6,7], Allison R. Greenplate[6,7], Jennifer E. Wu[6,7,8], Cécile Alanio[6,7,8], Kurt D'Andrea[6,7], Oliva Kuthuru[6,7], Jeanette Dougherty[6,7], Ajinkya Pattekar[6,7], Justin Kim[6,7], Nicholas Han[6,7], Sokratis A. Apostolidis[6,7], Alex C. Huang[6,7], Laura A. Vella[6,7,9], Leticia Kuri-Cervantes[1], M. Betina Pampena[1], The UPenn COVID Processing Unit[10], Michael R. Betts[1], E. John Wherry[6,7,8], Nuala J. Meyer[4], Sara Cherry[5], Paul Bates[1,11], Daniel J. Rader[2], Scott E. Hensley[1,14*]

[1]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

[2]Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

[3]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

[4]Division of Pulmonary, Allergy, and Critical Care Medicine and Center for Translational Lung Biology, University of Pennsylvania Perelman School of Medicine, Philadelphia PA 19104, USA

[5]Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA

[6]Institute for Immunology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

[7]Department of Systems Pharmacology and Translational Therapeutics, University of Pennsylvania, Philadelphia, PA 19104, USA

[8]Parker Institute for Cancer Immunotherapy, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

[9]Division of Infectious Diseases, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA

[10]The UPenn COVID Processing Unit is a composed of individuals at the University of Pennsylvania who volunteered time and effort to enable the study of COVID-19 patients during the pandemic. Members are listed in the acknowledgement section of this paper.

[11]Penn Center for Research on Coronavirus and Other Emerging Pathogens, University of Pennsylvania, Philadelphia, PA 19104, USA

[12]Current affiliation: Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA 19104, USA

[13]These authors contributed equally

[14]Lead Contact

*Correspondence: hensley@pennmedicine.upenn.edu

1 **SUMMARY**

2 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread within the

3 human population. Although SARS-CoV-2 is a novel coronavirus, most humans had been

4 previously exposed to other antigenically distinct common seasonal human coronaviruses

5 (hCoVs) before the COVID-19 pandemic. Here, we quantified levels of SARS-CoV-2-reactive

6 antibodies and hCoV-reactive antibodies in serum samples collected from 431 humans before the

7 COVID-19 pandemic. We then quantified pre-pandemic antibody levels in serum from a

8 separate cohort of 251 individuals who became PCR-confirmed infected with SARS-CoV-2.

9 Finally, we longitudinally measured hCoV and SARS-CoV-2 antibodies in the serum of

10 hospitalized COVID-19 patients. Our studies indicate that most individuals possessed hCoV-

11 reactive antibodies before the COVID-19 pandemic. We determined that ~20% of these

12 individuals possessed non-neutralizing antibodies that cross-reacted with SARS-CoV-2 spike

13 and nucleocapsid proteins. These antibodies were not associated with protection against SARS-

14 CoV-2 infections or hospitalizations, but they were boosted upon SARS-CoV-2 infection.

15

16

17
18

19   **INTRODUCTION**

20   Coronaviruses commonly infect humans(Dijkman et al., 2012, Friedman et al., 2018, Gaunt et

21   al., 2010, Killerby et al., 2018). The severe acute respiratory syndrome coronavirus 2 (SARS-

22   CoV-2) emerged at the end of 2019 and has rapidly spread among humans, many of whom have

23   been previously exposed to common seasonal human coronaviruses (hCoVs) (Edridge et al.,

24   2020). Common seasonal hCoVs include the betacoronaviruses HKU1 and OC43 and the

25   alphacoronaviruses 229E and NL63(Pfefferle et al., 2009, Pyrc et al., 2006, Vijgen et al., 2006,

26   Woo et al., 2005). SARS-CoV-2 belongs to the betacoronavirus genus and is more closely

27   related to HKU1 and OC43 compared to the alphacoronaviruses 229E and NL63(Jaimes et al.,

28   2020, Okba et al., 2020). A recent study examining electronic medical records suggested that

29   recent hCoV infections are not associated with decreased SARS-CoV-2 infections, but are

30   associated with reducing the severity of Coronavirus Disease 2019 (COVID-19) (Sagar et al.,

31   2020). It is unclear if this apparent cross-protection is mediated by antigen-specific cellular or

32   humoral immunity or if it is due to short-term general cross-protection similar to what has been

33   recently reported with rhinovirus and influenza virus infections (Wu et al., 2020). It is unknown

34   if prior hCoV exposures elicit antibodies that prevent or alter the outcomes of SARS-CoV-2

35   infections. Further, it is unknown if different aged individuals have distinct hCoV immune

36   histories that can affect SARS-CoV-2 susceptibility. To address this, we completed a serological

37   survey using serum samples collected from different aged humans prior to the COVID-19

38   pandemic. We quantified levels of antibodies reactive to viral proteins from hCoVs and

39   determined if these antibodies were associated with SARS-CoV-2 protection. Finally, we

40   completed a series of studies using serum collected from COVID-19 patients to determine if

41   antibodies reactive to hCoVs are boosted upon SARS-CoV-2 infections.

3

RESULTS

Identification of SARS-CoV-2-reactive Antibodies in Human Sera Collected Prior to the COVID-19 Pandemic

We completed ELISAs to quantify levels of pre-pandemic SARS-CoV-2-reactive IgG antibodies in 431 human serum samples collected in 2017. We tested serum samples collected from 263 children (age 1-17) at the Children's Hospital of Philadelphia originally collected for lead testing and 168 adults (age 18-90) who had been recruited into the Penn Medicine Biobank. We tested Penn Medicine Biobank samples from individuals who had no medical history of cancer or organ transplantation, pregnancy during the previous 9 months, or an infectious disease within the previous 28 days prior to blood draw. Using these samples, we previously found that different aged individuals possess H3N2 influenza virus antibodies that have different specificities (Gouma et al., 2020).

We found that 4.2% of serum samples collected in 2017 contained IgG antibodies that reacted to the SARS-CoV-2 full length spike (S) protein (**Figure 1A**), 0.93% of samples contained antibodies that reacted to the receptor binding domain (RBD) of the SARS-CoV-2 S protein (**Figure 1B**), and 16.2% of samples contained antibodies that reacted to the SARS-CoV-2 nucleocapsid (N) protein (**Figure 1C**). Several pre-pandemic serum samples contained antibodies that were at similar levels as those in serum from PCR-confirmed COVID-19 recovered donors (**Figure 1A-C**). We found no obvious differences in levels of SARS-CoV-2 cross-reactive antibodies among donors with different birth years (**Figure S1A-C**). We obtained similar results when ELISAs were completed with unpurified serum antibodies and purified IgG (**Figure S2**). Most serum samples with antibodies reactive to the SARS-CoV-2 full length S protein did not have antibodies that reacted to the SARS-CoV-2 S-RBD protein (**Figure 1D**),

65   which is consistent with a recent study showing that some individuals possessed pre-pandemic

66   antibodies against the S2 domain of the SARS-CoV-2 S protein (Nguyen-Contant et al., 2020).

67   There was a poor correlation between N and S antibody titers in pre-pandemic samples (**Figure**

68   **S3**).

69   We completed neutralization assays using a SARS-CoV-2 vesicular stomatitis virus

70   (VSV) pseudotype platform. In contrast to serum antibodies isolated from PCR-confirmed

71   COVID-19 recovered donors, serum antibodies from individuals collected before the pandemic

72   had very low or undetectable levels of SARS-CoV-2 neutralizing antibodies, regardless of

73   whether or not the sample possessed cross-reactive antibodies against SARS-CoV-2 S and N

74   proteins (**Figure 1E** and **Figure S4**). We obtained similar results when we tested pre-pandemic

75   serum samples using a bonafide BSL3-level SARS-CoV-2 neutralization assay (**Figure S5**).

76

77   **Humans with Pre-pandemic SARS-CoV-2-reactive Antibodies Had Elevated Levels of**

78   **Antibodies Against Previously Circulating Betacoronaviruses**

79   We completed ELISAs to quantify levels of pre-pandemic hCoV-reactive IgG antibodies

80   in all 431 human serum samples collected in 2017. Most serum samples possessed antibodies

81   that reacted to the S protein of 229E and NL63 (both alphacoronaviruses), as well as OC43 (a

82   betacoronavirus) (**Figure S1D-F**). There were no major differences in levels of these antibodies

83   among individuals with different birth years, however serum from very young children possessed

84   lower levels of antibodies reactive to the 229E and NL63 S proteins (**Figure S1D-F**). We

85   completed full antibody titrations to directly compared levels of hCoV antibodies in a subset of

86   pre-pandemic samples from individuals who either did (n=17) or did not (n=17) possess cross-

87   reactive SARS-CoV-2 antibodies (**Figure 1F-H**). Pre-pandemic antibody levels against the 229E

88 and NL63 alphacoronavirus S proteins were similar among individuals with and without SARS-

89 CoV-2 reactive antibodies (**Figure 1F-G**). In contrast, antibody levels against the

90 betacoronavirus OC43 S protein were higher in individuals with SARS-CoV-2 reactive

91 antibodies compared to individuals who did not possess pre-pandemic SARS-CoV-2 reactive

92 antibodies (**Figure 1H**). These data suggest that pre-pandemic SARS-CoV-2 reactive antibodies

93 were likely elicited by previously circulating betacoronavirus strains, such as OC43.

94

95 **Pre-existing hCoV Cross-reactive Antibodies Were Not Associated With Protection From**

96 **SARS-CoV-2 Infections**

97 It is unknown if antibodies elicited by prior hCoV infections protect against SARS-CoV-

98 2 infections and/or prevent severe COVID-19. To address this, we measured SARS-CoV-2 IgG

99 antibodies in pre-pandemic serum samples from 251 individuals who subsequently went on to

100 become PCR-confirmed infected with SARS-CoV-2 and in a control group of pre-pandemic

101 samples from 251 matched individuals who did not become infected with SARS-CoV-2. Pre-

102 pandemic samples were collected by the Penn Medicine BioBank from August 2013 to March

103 2020 and PCR-confirmed SARS-CoV-2 infections were identified by nasopharyngeal swab PCR

104 testing results in electronic health records. We found that 2.2% of samples possessed pre-

105 pandemic antibodies reactive to the SARS-CoV-2 full length S protein, 0.6% of samples

106 possessed pre-pandemic antibodies reactive to the SARS-CoV-2 S-RBD, and 23.9% of samples

107 possessed pre-pandemic antibodies reactive to the SARS-CoV-2 N protein. Importantly, we

108 found no differences in SARS-CoV-2-reactive antibodies in serum samples from individuals

109 who did or did not become subsequently infected with SARS-CoV-2 (**Figure 2A**; S protein:

110 p=0.62, S-RBD: p=0.49, N protein: p=0.34 and **Table S1** and **Table S2**). We also measured

111   antibodies reactive to the OC43 S protein and found no differences among samples from

112   individuals who did or did not become infected with SARS-CoV-2 (**Figure 2A**; p=0.90 and

113   **Table S1** and **Table S2**). Among those with PCR-confirmed SARS-CoV-2 infections, we found

114   no relationship between SARS-CoV-2 and OC43 antibody titers and hospitalization or disease

115   severity among hospitalized patients (**Table S1** and **Table S2**). We found no relationship

116   between SARS-CoV-2 and OC43 antibody titers and the need for respiratory support and

117   admittance into the ICU following SARS-CoV-2 infection (**Table S1** and **Table S2**).

118   Previous studies indicated that immunity to hCoV can be short-lived (Huang et al., 2020)

119   and a recent study documented that antibody titers against hCoV can fluctuate over time

120   (Edridge et al., 2020), presumably due to repetitive hCoV exposures. In our study, pre-pandemic

121   serum samples were collected from 2013-2020 and therefore it is possible that antibody levels in

122   some of the samples collected several years prior to 2020 do not accurately reflect antibody

123   levels present during the COVID-19 pandemic. To address this, we compared SARS-CoV-2 and

124   OC43 IgG antibody titers in the serum of individuals in our cohort who had samples collected

125   within one year of the pandemic (between April 2019 and March 2020). Using this smaller

126   cohort (n=39 SARS-CoV-2 cases and n=57 controls), we still found no differences in levels of

127   antibodies reactive to the SARS-CoV-2 S protein, S-RBD protein, N protein, or OC43 S protein

128   (**Figure 2B**). Taken together, our data suggest that a subset of humans possessed non-

129   neutralizing cross-reactive antibodies against SARS-CoV-2 S and N proteins prior to the

130   COVID-19 pandemic, but these antibodies were not associated with protection from SARS-CoV-

131   2 infections or reducing hospitalizations upon SARS-CoV-2 infections.

132

133   **SARS-CoV-2 Boosts Antibodies Reactive to Other Human Betacoronaviruses**

134   Recent studies indicate that COVID-19 recovered donors possess higher levels of

135   antibodies against seasonal betacoronaviruses (Nguyen-Contant et al., 2020). To determine if

136   antibodies against the S protein of hCoVs are boosted upon SARS-CoV-2 infection, we

137   measured 229E, NL63, OC43, and SARS-CoV-2 S IgG antibody levels in sera collected

138   longitudinally from 27 hospitalized COVID-19 patients. Samples from a subset of the

139   hospitalized patients (10 of 27) were tested using an extended respiratory pathogen viral panel to

140   confirm that they were not simultaneously co-infected with SARS-CoV-2 and a different

141   coronavirus. Serum IgG antibodies reactive to the S protein of the 229E and NL63

142   alphacoronaviruses did not change over 7 days of hospitalization (**Figure 3A-B**). Conversely,

143   serum antibodies reactive to the S protein of OC43 and SARS-CoV-2 betacoronaviruses

144   significantly increased over the course of hospitalization (**Figure 3A-B**). We found that boosted

145   antibodies in hospitalized patients primarily targeted the S2 domain, and not the S1 domain, of

146   the OC43 S protein (**Figure S6A-B**) . Overall OC43 IgG antibody titers (**Figure 3C**) and the

147   magnitude of OC43 S antibody boosts (**Figure 3D**) were not associated with outcome of disease.

148   These data indicate that cross-reactive antibodies elicited by previous hCoV infections are not

149   associated with protection from SARS-CoV-2 infections, but are boosted following infection

150   with SARS-CoV-2.

151

152   **DISCUSSION**

153   Our study demonstrates that ~20% of individuals possessed SARS-CoV-2 cross-reactive

154   serum antibodies prior to the COVID-19 pandemic. Using samples collected in 2017, we found

155   that pre-pandemic cross-reactive antibodies directed against the SARS-CoV-2 N protein were

156   more prevalent compared to those directed against the SARS-CoV-2 S protein (16.2%

157 seropositive versus 4.2% seropositive). We found that most individuals possessed pre-pandemic

158 serum antibodies reactive to the S proteins of 229E, NL63, and OC43 (**Figure S1**); however,

159 pre-pandemic samples with detectable levels of SARS-CoV-2 antibodies had higher levels of

160 antibodies against the OC43 S protein (**Figure 1H**). Although our data suggest that prior

161 infections with seasonal human betacoronaviruses (such as OC43) likely elicit antibodies that

162 cross-react with SARS-CoV-2 proteins, in is unclear why only a subset of OC43 seropositive

163 individuals possessed antibodies reactive to SARS-CoV-2 prior to the pandemic. Further studies

164 will be needed to determine the temporal relationship between seasonal human betacoronavirus

165 infections and the induction of SARS-CoV-2 cross-reactive antibodies. Further studies

166 investigating the relationship of pre-pandemic antibodies against other betacoronaviruses, such

167 as HKU1, with pre-pandemic SARS-CoV-2 cross-reactive antibodies are also needed.

168   Our study is consistent with a recent manuscript demonstrating a lack of SARS-CoV-2

169 neutralizing activity in pre-pandemic sera (Poston et al., 2020). In contrast, a different study

170 reported that pre-pandemic serum from young children possess SARS-CoV-2 neutralizing

171 antibodies (Ng et al., 2020). It is unclear if these differences are due to the specific assays used in

172 each study or other factors such as geographic differences in sampling. For example, the Ng et.

173 al. study (Ng et al., 2020) used a pseudotyped neutralization assay will cells that lack ACE2,

174 which is the cellular receptor for SARS-CoV-2. Our study is unique in that we were able to

175 directly assess whether pre-pandemic antibodies were associated with protection from SARS-

176 CoV-2 infections and hospitalizations. While we found no differences in pre-pandemic antibody

177 levels against SARS-CoV-2 and OC43 among those infected and not infected with SARS-CoV-2

178 (**Figure 2**) and among SARS-CoV-2 infected individuals with different disease severities

179 (**Tables S1-S2**), larger cohorts including individuals with a large range of different clinically-

180   defined disease severities will be required to determine if pre-pandemic levels of antibodies are

181   associated with reducing some aspects of severe COVID-19. Additional studies need to be

182   completed to determine if neutralizing antibodies elicited by SARS-CoV-2 infections protect

183   against subsequent reinfections with SARS-CoV-2.

184   Further studies also need to be completed to determine how immune history affects de

185   novo immune responses following SARS-CoV-2 infection. We find that individuals infected

186   with SARS-CoV-2 produce antibodies reactive to both the SARS-CoV-2 S protein and OC43 S

187   protein (**Figure 3**). In the case of influenza viruses, sequential infections with antigenically

188   distinct strains can elicit antibodies against conserved epitopes between the strains and it is

189   unclear if these cross-reactive antibodies inhibit de novo immune responses or affect disease

190   severity (Cobey and Hensley, 2017). Our studies suggest that SARS-CoV-2 infection boosts

191   antibodies reactive to the S2 domain of the OC43 S protein. Further studies are needed to

192   precisely map the footprints of these antibodies and additional studies need to be completed to

193   determine if these antibodies help resolve infections or if they enhance disease in COVID-19

194   patients.

195   Given that our data suggest that pre-pandemic non-neutralizing antibodies elicited by

196   hCoVs do not provide SARS-CoV-2 protection, special attention should be directed towards

197   evaluating if T cell responses primed against hCoV infections provide partial protection against

198   SARS-CoV-2 infections. Recent studies have clearly shown that some individuals possessed

199   SARS-CoV-2-specific CD4+ and CD8+ T cells prior to the COVID-19 pandemic (Braun et al.,

200   2020, Grifoni et al., 2020, Le Bert et al., 2020, Mateus et al., 2020, Sette and Crotty, 2020,

201   Schulien et al., 2020), and it is possible that pre-existing cellular immunity might play an

10

202 important protective role in the context of pandemic viruses that only share non-neutralizing

203 antibody epitopes with previously circulating viral strains.

204

205 **LIMITATIONS OF THE STUDY**

206   The data presented here show that pre-pandemic serum antibodies that cross-react with

207 SARS-CoV-2 do not correlate with protection against SARS-CoV-2 infections and severity of

208 COVID-19. We generated data using pre-pandemic samples that were collected from individuals

209 who became PCR+ confirmed infected with SARS-CoV-2. We compared antibody levels in

210 these samples to antibody levels in pre-pandemic samples from individuals who did not get

211 infected with SARS-CoV-2. For these studies, we included samples that were collected from

212 August 2013 to March 2020 (**Figure 2A**). Since immunity to hCoVs can be short-lived (Huang

213 et al., 2020) and fluctuate over time (Edridge et al., 2020), we also directly compared antibody

214 titers in samples that were collected within one year of the pandemic (**Figure 2B**). Using both

215 datasets, we found no correlation between pre-pandemic antibody levels and SARS-CoV-2

216 infections and COVID-19 severity. Nonetheless, future studies need to continue exploring the

217 temporal relationship between seasonal coronavirus infections and the induction of SARS-CoV-2

218 cross-reactive antibodies to determine if transient antibody-mediated protection is possible.

219 Future studies should also evaluate the protective potential of pre-pandemic cross-reactive

220 mucosal antibodies. Finally, studies need to address if pre-existing cellular immunity limits

221 COVID-19 severity. Our study only examined serum antibodies and it is possible that rapid

222 engagement of memory B and T cells and long-lived plasma cells provide protection following

223 SARS-CoV-2 exposures of humans with unique immune histories.

224

ACKNOWLEDGEMENTS

AUTHOR CONTRIBUTIONS

Serological Assays, E.M.A., E.C.G., C.P.A., M.J.B., M.E.W., S.G., C.M.M., S.R.C., P.H.,

T.B.M., H.R. and S.C.; Data Analyses, E.M.A., E.C.G., and A.V.; Obtaining and Processing

Samples, O.O., D.M., A.E.B., D.A.O., A.R.G., J.E.W., C.A., K.D., O.K., J.D., A.P., J.K., N.H.,

S.A.A., A.C.H., L.A.V., L.K-C, M.B.P., and N.J.M.; Manuscript Writing, E.M.A., E.C.G., A.V.,

and D.J.R., S.E.H.; Supervision, S.G., J.W., M.R.B., E.J.W., N.J.M., S.C., P.B., D.J.R., and

S.E.H.; Funding Acquisition, P.B., E.J.W., N.J.M., and S.E.H.

248 **DECLARATION OF INTERESTS**

249 A.C.H is a consultant for Immunai. E.J.W. has consulting agreements with and/or is on the

250 scientific advisory board for Merck, Elstar, Janssen, Related Sciences, Synthekine and Surface

251 Oncology. E.J.W. is a founder of Surface Oncology and Arsenal Biosciences. E.J.W. is an

252 inventor on a patent (U.S. patent number 10,370,446) submitted by Emory University that covers

253 the use of PD-1 blockade to treat infections and cancer. D.J.R. is on scientific advisory boards

254 for Alnylam, Metrea, Novartis, Pfizer and Verve, is a consultant for and receives research

255 support from Regeneron for work unrelated to this report, and is a founder of Vascular Strategies

256 and Staten Biotechnologies. S.E.H. has received consultancy fee from Sanofi Pasteur, Lumen,

257 Novavax, and Merck for work unrelated to this report.

258

259 **FIGURE LEGENDS**

260 **Figure 1. Identification of pre-existing cross-reactive SARS-CoV-2 antibodies in human**

261 **serum prior to the pandemic.** ELISAs were completed to quantify levels of serum antibodies

262 binding to the SARS-CoV-2 full-length spike (S) protein (**A**), the receptor binding domain (S-

263 RBD) of S (**B**), and the nucleocapsid (N) protein (**C**); dashed line denotes lower limit of

264 detection (LOD=50), dotted line represents a threshold set 2-fold above LOD (>100). We tested

265 samples collected from 431 individuals in the summer of 2017, prior to the global pandemic. We

266 also tested samples collected from 15 individuals following confirmed SARS-CoV-2 infections.

267 and recovered adults. (**D**) The relationship between antibody titers in donors with detectable IgG

268 against the S-RBD and/or full length S is shown. (**E**) SARS-CoV-2 pseudotype neutralization

269 assays were completed using pre-pandemic serum samples with (n=14) and without (n=29) cross

270 reactive SARS-CoV-2 antibodies, as well as serum samples from individuals following

13

271   confirmed SARS-CoV-2 infections (n=15); one-way ANOVA Tukey's multiple comparisons of

272   log2 transformed antibody titers ****p<0.0001; dotted line denotes lower LOD (=10). (**F**-**H**)

273   ELISAs were completed to quantify levels of serum antibodies binding to the full length S

274   proteins from 229E, NL63, and OC43 using pre-pandemic serum samples with (n=17) and

275   without (n=17). Unpaired t-tests of log2 transformed antibody titers **p=0.002, *p=0.02.

276   Horizontal lines indicate geometric mean and error bars represent standard deviation. See also

277   Figure S1, S2, S3, S4, and S5 .

278

279   **Figure 2. Pre-pandemic SARS-CoV-2 and OC43-reactive antibodies are not associated with**

280   **protection from SARS-CoV-2 infection.** We quantified antibody levels in pre-pandemic serum

281   samples collected from individuals who later became SARS-CoV-2 infected (cases; n=251) and

282   those who did not become SARS-CoV-2 infected (controls; n=251). ELISAs were completed to

283   quantify levels of antibodies reactive to SARS-CoV-2 proteins (S, S-RBD, and N) and the OC43

284   S protein. Shown are data using samples collected from the entire cohort between August 2013

285   and March 2020 (**A**) and samples from a smaller subset of individuals collected between April

286   2019-March 2020 (**B**). Antibody titers between cases and controls were not significantly

287   different as determined by unpaired t-tests of log2 transformed antibody titers. Dashed line

288   denotes lower limit of detection (LOD=50), dotted line represents a threshold set 2-fold above

289   LOD (>100). See also Table S1-S2.

290

291   **Figure 3. SARS-CoV-2 infections boost antibodies that react to OC43 S protein.** We

292   quantified antibody levels in serum collected from 27 individuals 0 and 7 days after

293   hospitalization for COVID-19. ELISAs were completed to quantify levels of antibodies reactive

294 to the S proteins of 229E, NL63, OC43 and SARS-CoV-2. (**A**) IgG titers and (**B**) titer fold

295 change are shown. (**C**) Levels of OC43 S-reactive antibodies and (D) fold change in OC43 S-

296 reactive antibodies were not associated with disease outcome. Paired t-test of log2 transformed

297 antibody titers, ****p<0.0001. One-way ANOVA Tukey's multiple comparisons fold-change in

298 antibody titers, *p<0.04. Horizontal lines indicate the median and error bars show interquartile

299 ranges. See also Figure S6.

300

301 **SUPPLEMENTAL FIGURE LEGENDS**

302 **Figure S1. There are no obvious age-related differences in pre-pandemic SARS-CoV-2 and**

303 **hCoV reactive antibodies, related to Figure 1.** ELISAs were completed to measure levels of

304 serum antibodies binding to the SARS-CoV-2 full-length spike (S) protein (**A**), SARS-CoV-2

305 receptor binding domain (S-RBD) of S (**B**), SARS-CoV-2 nucleocapsid (N) protein (**C**), 229E S

306 protein (**D**), NL63 S protein (**E**), and OC43 S protein (**F**). Serum samples collected from 431

307 individuals in the summer of 2017 were tested. Reciprocal titer from serially-diluted serum

308 samples (**A-C**) and optical densities at 450nm wavelength (OD_{450}) of 1:500 dilution of serum (**D-**

309 **F**) are shown. Dashed line denotes lower limit of detection (LOD=50), dotted line represents a

310 threshold set 2-fold above LOD (>100).

311

312 **Figure S2. Comparison of ELISA data using unpurified and purified serum IgG**

313 **antibodies, related to Figure 1.** IgG was purified from sera samples from individuals without

314 (**A;** n=5) and with (**B;** n=11) pre-pandemic cross-reactive antibodies. IgG was also purified from

315 serum samples from individuals who had recovered from a confirmed SARS-CoV-2 infection

316 (**C;** n=5). ELISAs were completed to quantify levels of serum antibodies binding to SARS-CoV-

317   2 full length S, S-RBD, and N protein with and without IgG magnetic bead purification. The

318   dotted line represents a threshold set 2-fold above the limit of detection (>100).

319

320   **Figure S3. Correlation between N, S, and S-RBD antibody titers in pre-pandemic samples,**

321   **related to Figure 1.**

322   Shown are the relationships between serum IgG antibody titers against the SARS-CoV-2 N

323   protein and S-RBD **(A)** or full length S **(B)** from 431 individuals whose samples were collected

324   prior to the pandemic in the summer of 2017. Dotted line represents a threshold set 2-fold above

325   the limit of detection (>100).

326

327   **Figure S4. SARS-CoV-2 pseudotype neutralization curves, related to Figure 1.** Raw

328   neutralization curves for data from **Figure 1E** are shown, including samples from individuals

329   who did not have pre-pandemic cross reactive SARS-CoV-2 antibodies **(A)**, individuals who

330   possessed pre-pandemic cross reactive SARS-CoV-2 antibodies **(B)**, and individuals following

331   confirmed SARS-CoV-2 infection **(C)**. Mean and error bars are shown for each replicate; dotted

332   line denotes the cut-off for foci reduction neutralization of 50% (FRNT50).

333

334   **Figure S5. Pre-pandemic cross-reactive antibodies do not neutralize SARS-CoV-2 in**

335   **bonafide BSL3-level neutralization assays, related to Figure 1.** Neutralization assays with

336   live SARS-CoV-2 were completed using 9 pre-pandemic samples with cross-reactive SARS-

337   CoV-2 antibodies, 7 pre-pandemic samples without cross-reactive SARS-CoV-2 antibodies, and

338   5 samples from individuals who recovered from a PCR-confirmed SARS-CoV-2 infection. The

339   pre-pandemic samples for these experiments were collected in 2019 and are different from those

340   shown in Figure 1 (which were collected in 2017).

341

342   **Figure S6. Antibodies directed to the S2 region of OC43 spike are boosted during SARS-**

343   **CoV-2 infection, related to Figure 3.** We quantified antibody levels in serum collected from 27

344   individuals 0 and 7 days after hospitalization for COVID-19. ELISAs were completed to

345   measure levels of serum antibodies binding to the OC43 full-length spike (FL) protein and the

346   individual S1 and S2 subunits of the OC43 spike. (**A**) IgG titers and (**B**) titer fold change are

347   shown. Paired t-test of log2 transformed antibody titers, ****$p<0.0001$. One-way ANOVA

348   Tukey's multiple comparisons fold-change in antibody titers, **$p=0.0016$. Horizontal lines

349   indicate the median and error bars show interquartile range.

350

351

352

353 **STAR METHODS**

354 **KEY RESOURCES TABLE**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Goat anti-human IgG-HRP	Jackson ImmunoResearch	109-036-098
mAb CR3022	Expressed for this paper	
mAb 1E9F9	Absolute Antibody	Ab01402-2.0
anti-dsRNA J2	Absolute Antibody	Ab01299-2.0
Goat anti-mouse IgG alexa 488	ThermoFisher Scientific	A-11029
Hoescht 33342	Sigma Aldrich	B2261
Bacterial and Virus Strains		
SARS-CoV-2 VSV pseudotypes	Generated for this paper	N/A
SARS-CoV-2 (WA-1)	BEI	NR-52281
Biological Samples		
Pre-pandemic adult serum samples	Penn Medicine Biobank (PMBB)	N/A
Pre-pandemic children serum samples	Children's Hospital of Philadelphia (CHOP)	N/A
COVID-19 patient serum samples	Hospital of the University of Pennsylvania (HUP	N/A
Chemicals, Peptides, and Recombinant Proteins		
SARS-CoV-2 spike protein	Expressed for this paper	N/A
SARS-CoV-2 RBD protein	Expressed for this paper	N/A
SARS-CoV-2 nucleocapsid protein	Sino Biological	Cat. 40588-V08B
OC43 spike protein	Sino Biological	Cat. 40607-V08B
NL63 spike protein	Sino Biological	Cat. 40604-V08B
229E spike protein	Sino Biological	Cat. 40605-V08B
OC43 S1 subunit protein	Expressed for this paper	N/A
OC43 S2 subunit protein	Expressed for this paper	N/A
Experimental Models: Cell Lines		
293T	ATCC	Cat. CRL-3216, RRID:CVCL_0063
293F	Laboratory of Scott Hensley, University of Pennsylvania, PA	Thermo Fisher cat. R79007
VeroE6/TMPRSS	Laboratory of Stefan Pohlman, German Primate Center, Leibniz Institute for Primate Research	Hoffman et al., 2020
Vero CCL81	ATCC	Cat. CCL-81, RRID:CVCL_0059
Recombinant DNA		
Plasmid: pCAGGS SARS-CoV-2 spike	Laboratory of Florian Krammer, Mt. Sinai, NY	Amanat et al., 2020
Plasmid: pCAGGS SARS-CoV-2 RBD	Laboratory of Florian Krammer, Mt. Sinai, NY	Amanat et al., 2020

Plasmid: pCG1 SARS- 2 S	Laboratory of Stefan Pohlman, German Primate Center, Leibniz Institute for Primate Research	Hoffman et al., 2020
Plasmid: OC43 rS1	Laboratory of Scott Hensley, University of Pennsylvania, PA	This paper
Plasmid: OC43 rS2	Laboratory of Scott Hensley, University of Pennsylvania, PA	This paper
Software and Algorithms		
Prism8	GraphPad Software	www.graphpad.com/scientific-software/prism/
Flouro-X	ImmunoSpot	www.immunospot.com/index-ctl

355

RESOURCES AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be

fulfilled by the Lead Contact, Scott E. Hensley (hensley@pennmedicine.upenn.edu).

Materials Availability

All unique reagents generated in this study will be available from the Lead Contact upon

reasonable request.

Data and Code Availability

All raw data generated in this study have been deposited on Mendeley Data:
http://dx.doi.org/10.17632/ygv2j9psc5.1

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Pre-pandemic Human Serum Samples

Serum samples shown in **Figure 1** were collected before the COVID-19 pandemic between May

and August of 2017 from individuals at the Children's Hospital of Philadelphia (CHOP; n=263,

children age 0-18 years old) and through the Penn Medicine BioBank (n=168, adults $\geq$18 years

old). Samples from CHOP were leftover de-identified blood samples collected for routine lead

testing.

Serum samples shown in **Figure 2** were collected via the Penn Medicine BioBank prior to the

pandemic (n=502, between August 2013 and March 2020). These samples were from adults who

381 subsequently had a reverse transcription quantitative polymerase chain reaction (RT-qPCR)

382 confirmed SARS-CoV-2 infection using nasopharyngeal swabs (cases, n=251), and those who

383 had SARS-CoV-2 PCR negative results (controls, n=251). The RT-qPCR clinical testing results

384 were acquired from Penn Medicine electronic health records and test results between March

385 2020 and August 2020 were included in the analysis. The Penn Medicine BioBank is an

386 established repository that routinely collects blood products from donors visiting the University

387 of Pennsylvania Healthcare system upon written informed consent. All studies were approved by

388 the University of Pennsylvania Institutional Review Board.

389

390 **Human Samples Collected After SARS-CoV-2 Infection**

391 Serum samples were obtained from recovered convalescent donors who had a history of PCR-

392 confirmed SARS-CoV-2 infection (n=15). These samples were used in experiments shown in

393 Figure 1. Additionally, plasma samples were collected from patients admitted to the Hospital at

394 the University of Pennsylvania (HUP) with PCR-confirmed SARS-CoV-2 infections (n=27), as

395 previously described(Mathew et al., 2020). Hospital inpatients were categorized for pneumonia

396 severity using a WHO ordinal scale that was based on the level of oxygen support needed at day

397 0 and day 7. All samples were collected after obtaining informed consent and studies were

398 approved by the University of Pennsylvania Institutional Review Board.

399

400 **Cell lines**

401 293F cells were from Thermo fisher (Thermo Fisher cat. R79007). 293T and Vero CCL81 cells

402 were from ATCC (ATCC cat. CRL-3216, RRID:CVCL_0063 and ATCC cat. CCL-81,

403 RRID:CVCL_0059, respectively).VeroE6/TMPRSS2 cells were a gift from Stefan Pohlman

404 (German Primate Center, Leibniz Institute for Primate Research) as described

405 previously(Hoffmann et al., 2020). All cell lines were cultured using manufacturer's guidelines

406 and used as described in Method Details below.

407

408

409 **METHOD DETAILS**

410 **Quantification of serum antibody titers**

411 Serum antibody titers against SARS-CoV-2 and other human coronavirus (hCoV) antigens were

412 quantified by enzyme-linked immunosorbent assays (ELISA) as previously described (Flannery

413 et al., 2020). Plasmids encoding the full-length SARS-CoV-2 spike (S) protein and the receptor

414 binding domain of the S (S-RBD) were provided by Florian Krammer (Icahn School of Medicine

415 at Mt. Sinai, New York City NY)(Amanat et al., 2020). SARS-CoV-2 S-RBD and the SARS-

416 CoV-2 S proteins were purified from 293F transfected cells by Ni-NTA resin. SARS-CoV-2

417 nucleocapsid (N) protein, and full-length hCoV spike antigens (OC43, 229E, and NL63) were

418 purchased (Sino Biological, Wayne PA; cat. 40588-V08B, 40607-V08B, 40604-V08B, and

419 40605-V08B, respectively) and reconstituted in Dulbecco's phosphate buffered saline (DPBS).

420 OC43 subunit proteins were purified by Ni-NTA resin from 293F cells transfected with plasmids

421 encoding the S1 or S2 subunits of the OC43 spike protein. ELISA plates (Thermo Fisher

422 Scientific: cat. 14-245-153) were coated overnight at 4°C with either 2 μg/mL SARS-CoV-2

423 antigen, 1.5 μg/mL hCOV antigen, or DPBS to control for background. Sera was heat-inactivated

424 in a 56°C water bath for 1 hour prior to serial dilutions starting at 1:50 in dilution buffer (DPBS

425 supplemented with 1% milk and 0.1% Tween-20). ELISA plates were blocked with 200μL of

426 blocking buffer (DPBS supplemented with 3% milk and 0.1% Tween-20), washed 3 times with

427 PBS plus 2% Tween (PBS-T), and 50μL of diluted sera was added. After 2 hours of incubation,

428 ELISA plates were washed 3 times with PBS-T and bound antibodies were detected using a

429 1:5000 dilution of goat anti-human IgG conjugated to horseradish peroxidase (Jackson

430 ImmunoResearch Laboratories, West Grove, PA: cat. 109-036-098). ELISA plates were

431 developed with the addition of 50 μL SureBlue 3, 3', 5, 5'-tetramethylbenzidine substrate

432 (SeraCare: material number 5120-0077) and the reactions were stopped by the addition of 25μL

433 of 250mM hydrochloric acid after 5 minutes. Optical densities at 450nm wavelength were

434 obtained on a SpectraMax 190 microplate reader (Molecular Devices, San Jose, CA). Serum

435 antibody titers were expressed as the reciprocal serum dilution at a set OD that was based off of a

436 standard curve from the monoclonal antibody CR3022 (a gift from Ian Wilson, Scripps) starting

437 at 0.5μg/mL (for S-RBD and S ELISAs) or serially diluted pooled serum (for SARS-CoV-2 N

438 ELISAs and hCoV S ELISAs). Standard curves were included on every plate to control for plate-

439 to-plate variation. Antibody titers for each sample were measured in at least two technical

440 replicates performed on separate days.

441

Purification of IgG antibodies

For some experiments, we purified IgG from sera samples before completing ELISAs. IgG was

purified from sera samples using PureProteome Protein G magnetic beads (Millipore, Darmstadt,

Germany: cat. LSKMAGG02) as previously described (Arevalo et al., 2020). Sera samples were

diluted in PBS and incubated with 100 μL of washed magnetic beads for 1 hour at room

temperature with constant mixing. Unbound fractions were removed using the magnetic stand

and beads were washed with PBS. Bound IgG was eluted with the addition of 100 μL of 0.2 M

glycine, pH 2.5 followed by 5 minute incubation at room temperature. The eluant containing

purified IgG was neutralized with 10 μL of 1.0 M Tris, pH 8.8 prior to being run in ELISA.

Generation of SARS-CoV-2 pseudotypes

SARS-CoV-2 pseudotypes were generated with a previously described vesicular stomatitis virus

(VSV) pseudotype platform (Anderson et al., 2020). Briefly, pseudotyped VSV virions with

SARS-CoV-2 Spike were produced through transfection of 293T with 35μg of pCG1 SARS-

CoV-2 S delta18 expression plasmid encoding a codon optimized SARS-CoV-2 S gene with an

18-residue truncation in the cytoplasmic tail (kindly provided by Stefan Pohlmann) (Hoffmann et

al., 2020). 30 hours post transfection, the SARS-CoV-2 spike expressing cells were infected for

2-4 hours with VSV-G pseudotyped VSVΔG-RFP at a multiplicity of infection (MOI) of ~1-3.

Then, the cells were washed twice with media to remove unbound virus. 28-30 hours after

infection, the media containing the VSVΔG-RFP SARS-CoV-2 pseudotypes were harvested and

clarified by centrifugation two times at 6000xg. SARS-CoV-2 pseudotypes were aliquoted and

stored at -80°C until used for antibody neutralization analysis.

Quantification of SARS-CoV-2 pseudotype neutralizing antibody titers

Serum SARS-CoV-2 neutralizing antibodies were measured as previously described (Anderson

et al., 2020). Vero E6 cells stably expressing TMPRSS2 were seeded in 100μl at 2.5×10^4

cells/well in a 96 well collagen coated plate. The next day, heat inactivated serum samples were

serially diluted 2-fold and mixed with 50-200 focus forming units/well of VSVΔG-RFP SARS-

CoV-2 pseudotype virus and 600ng/ml of 1E9F9, a mouse anti-VSV Indiana G (Absolute

Antibody, Oxford, UK: cat. Ab01402-2.0). The serum-virus mixture was incubated for 1 hour at

37□C before being plated on VeroE6 TMPRSS2 cells. 23-24 hours post infection, the cells were

473 washed, fixed with 4% paraformaldehyde, and visualized on an S6 FluoroSpot Analyzer (CTL,

474 Shaker Heights OH) and individual infected foci were enumerated. The focus reduction

475 neutralization titer 50% ($FRNT_{50}$) was measured as the greatest serum dilution at which focus

476 count was reduced by at least 50% relative to control cells that were infected with pseudotype

477 virus in the absence of human serum. $FRNT_{50}$ titers for each sample were measured in at least

478 two technical replicates performed on separate days.

479

480 **BSL-3 SARS-CoV-2 neutralization assays**

481 Vero CCL81 (ATCC: cat. CCL-81) cells were plated in 96 well plates (100µL/well) at a density

482 of 25,000 cells per well. The following day, in the BSL-3, 100 plaque forming units (pfu) of

483 SARS-CoV-2 (WA-1, BEI cat. NR-52281) was diluted into 30 µl DMEM and added to each

484 dilution of serum samples. The serum and virus were incubated together at room temperature for

485 1 hour and transferred to the supernatant of the Vero CCL81 cells. Each sample was prepared

486 independently in duplicate. Cells were incubated under standard cell culture conditions at 37°C

487 and 5% CO2 for 48h. Cells were fixed in 4% formaldehyde/PBS for 15 min at room temperature

488 and then washed three times with PBST. Cells were blocked (2% BSA/PBST) for 60 minutes

489 and incubated in primary antibody (anti-dsRNA J2, Absolute Antibody cat: Ab01299-2.0)

490 overnight at 4C. Cells were washed 3x PBS and incubated in secondary (anti-mouse IgG alexa

491 488 Thermofisher cat. A-11029, and hoescht 33342, Sigma Aldrich cat. B2261) for 2h at room

492 temperature. Cells were washed 3x in PBST and imaged using ImageXpress Micro (Molecular

493 Devices, San Jose, CA) using a 10X objective. Ten sites per well were captured and wells were

494 scored for viral infection.

495

496 **QUANTIFICATION AND STATISTICAL ANALYSIS**

497 Statistical analyses were performed using Prism version 8 (GraphPad Software, San Diego CA).

498 Reciprocal serum dilution antibody titers were log2 transformed for statistical analysis. ELISA

499 antibody titers below the limit of detection (LOD; reciprocal titer <50) were set to a reciprocal

500 titer of 25. Log2 transformed antibody titers were compared with unpaired t-tests and statistical

501 significance was set to p-value <0.05. Linear regressions were also performed using log2

502 transform titers and untransformed data from the other variables. We compared antibody titers in

503 pre-pandemic serum samples from individuals who did and did not have a subsequent PCR-

504   confirmed SARS-CoV-2 infection. For these analyses we selected serum sample from

505   individuals with RT-PCR negative results matching sex, age, and race for each SARS-CoV-2

506   PCR-confirmed case (RT-PCR positive) to define controls for our cohort. In instances we did not

507   find matched controls, we randomly selected patients with RT-PCR negative test results. We also

508   compared antibody titers in pre-pandemic serum samples among SARS-CoV-2 PCR-confirmed

509   individuals in relationship to hospitalization or need for respiratory support due to COVID-19.

510   Multivariate logistic regression was used to compare the antibody differences for these studies.

511   All the models were adjusted by sex, age, race, and analyses were performed in R (R Core Team,

512   2016). We compared Log2 transformed antibody titers in COVID-19 hospitalized patients at day

513   0 and day 7. We also compared the fold change in titer by day 7. We compared the fold change

514   in OC43 titers between patients who survived and patients who died by day 28 of hospitalization.

515

516

REFERENCES

Amanat, F., Stadlbauer, D., Strohmeier, S., Nguyen, T. H. O., Chromikova, V., Mcmahon, M., Jiang, K., Arunkumar, G. A., Jurczyszak, D., Polanco, J., et al. 2020. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat Med*.

Anderson, E. M., Diorio, C., Goodwin, E. C., Mcnerney, K. O., Weirick, M. E., Gouma, S., Bolton, M. J., Arevalo, C. P., Chase, J., Hicks, P., et al. 2020. SARS-CoV-2 antibody responses in children with MIS-C and mild and severe COVID-19. *J Pediatric Infect Dis Soc*.

Arevalo, C. P., Le Sage, V., Bolton, M. J., Eilola, T., Jones, J. E., Kormuth, K. A., Nturibi, E., Balmaseda, A., Gordon, A., Lakdawala, S. S., et al. 2020. Original antigenic sin priming of influenza virus hemagglutinin stalk antibodies. *Proc Natl Acad Sci U S A,* 117**,** 17221-17227.

Braun, J., Loyal, L., Frentsch, M., Wendisch, D., Georg, P., Kurth, F., Hippenstiel, S., Dingeldey, M., Kruse, B., Fauchere, F., et al. 2020. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. *Nature*.

Cobey, S. & Hensley, S. E. 2017. Immune history and influenza virus susceptibility. *Curr Opin Virol,* 22**,** 105-111.

Dijkman, R., Jebbink, M. F., Gaunt, E., Rossen, J. W., Templeton, K. E., Kuijpers, T. W. & Van Der Hoek, L. 2012. The dominance of human coronavirus OC43 and NL63 infections in infants. *J Clin Virol,* 53**,** 135-9.

Edridge, A. W. D., Kaczorowska, J., Hoste, A. C. R., Bakker, M., Klein, M., Loens, K., Jebbink, M. F., Matser, A., Kinsella, C. M., Rueda, P., et al. 2020. Seasonal coronavirus protective immunity is short-lasting. *Nat Med*.

Flannery, D. D., Gouma, S., Dhudasia, M. B., Mukhopadhyay, S., Pfeifer, M. R., Woodford, E. C., Gerber, J. S., Arevalo, C. P., Bolton, M. J., Weirick, M. E., et al. 2020. SARS-CoV-2 seroprevalence among parturient women in Philadelphia. *Sci Immunol,* 5.

Friedman, N., Alter, H., Hindiyeh, M., Mendelson, E., Shemer Avni, Y. & Mandelboim, M. 2018. Human Coronavirus Infections in Israel: Epidemiology, Clinical Symptoms and Summer Seasonality of HCoV-HKU1. *Viruses,* 10.

Gaunt, E. R., Hardie, A., Claas, E. C., Simmonds, P. & Templeton, K. E. 2010. Epidemiology and clinical presentations of the four human coronaviruses 229E, HKU1, NL63, and OC43 detected over 3 years using a novel multiplex real-time PCR method. *J Clin Microbiol,* 48**,** 2940-7.

Gouma, S., Kim, K., Weirick, M. E., Gumina, M. E., Branche, A., Topham, D. J., Martin, E. T., Monto, A. S., Cobey, S. & Hensley, S. E. 2020. Middle-aged individuals may be in a perpetual state of H3N2 influenza virus susceptibility. *Nat Commun,* 11**,** 4566.

Grifoni, A., Weiskopf, D., Ramirez, S. I., Mateus, J., Dan, J. M., Moderbacher, C. R., Rawlings, S. A., Sutherland, A., Premkumar, L., Jadi, R. S., et al. 2020. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell,* 181**,** 1489-1501 e15.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Kruger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N. H., Nitsche, A., et al. 2020. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell,* 181**,** 271-280 e8.

Huang, A. T., Garcia-Carreras, B., Hitchings, M. D. T., Yang, B., Katzelnick, L. C., Rattigan, S. M., Borgert, B. A., Moreno, C. A., Solomon, B. D., Trimmer-Smith, L., et al. 2020. A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. *Nat Commun,* 11**,** 4704.

Jaimes, J. A., Andre, N. M., Chappie, J. S., Millet, J. K. & Whittaker, G. R. 2020. Phylogenetic Analysis and Structural Modeling of SARS-CoV-2 Spike Protein Reveals an Evolutionary Distinct and Proteolytically Sensitive Activation Loop. *J Mol Biol,* 432**,** 3309-3325.

Killerby, M. E., Biggs, H. M., Haynes, A., Dahl, R. M., Mustaquim, D., Gerber, S. I. & Watson, J. T. 2018. Human coronavirus circulation in the United States 2014-2017. *J Clin Virol,* 101**,** 52-56.

Le Bert, N., Tan, A. T., Kunasegaran, K., Tham, C. Y. L., Hafezi, M., Chia, A., Chng, M. H. Y., Lin, M., Tan, N., Linster, M., et al. 2020. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature,* 584**,** 457-462.

Mateus, J., Grifoni, A., Tarke, A., Sidney, J., Ramirez, S. I., Dan, J. M., Burger, Z. C., Rawlings, S. A., Smith, D. M., Phillips, E., et al. 2020. Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. *Science,* 370**,** 89-94.

Mathew, D., Giles, J. R., Baxter, A. E., Oldridge, D. A., Greenplate, A. R., Wu, J. E., Alanio, C., Kuri-Cervantes, L., Pampena, M. B., D'andrea, K., et al. 2020. Deep immune profiling of COVID-19 patients reveals distinct immunotypes with therapeutic implications. *Science,* 369.

Ng, K. W., Faulkner, N., Cornish, G. H., Rosa, A., Harvey, R., Hussain, S., Ulferts, R., Earl, C., Wrobel, A. G., Benton, D. J., et al. 2020. Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. *Science*.

Nguyen-Contant, P., Embong, A. K., Kanagaiah, P., Chaves, F. A., Yang, H., Branche, A. R., Topham, D. J. & Sangster, M. Y. 2020. S Protein-Reactive IgG and Memory B Cell Production after Human SARS-CoV-2 Infection Includes Broad Reactivity to the S2 Subunit. *mBio,* 11.

Okba, N. M. A., Muller, M. A., Li, W., Wang, C., Geurtsvankessel, C. H., Corman, V. M., Lamers, M. M., Sikkema, R. S., De Bruin, E., Chandler, F. D., et al. 2020. Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease Patients. *Emerg Infect Dis,* 26**,** 1478-1488.

Pfefferle, S., Oppong, S., Drexler, J. F., Gloza-Rausch, F., Ipsen, A., Seebens, A., Muller, M. A., Annan, A., Vallo, P., Adu-Sarkodie, Y., et al. 2009. Distant relatives of severe acute respiratory syndrome coronavirus and close relatives of human coronavirus 229E in bats, Ghana. *Emerg Infect Dis,* 15**,** 1377-84.

Poston, D., Weisblum, Y., Wise, H., Templeton, K., Jenks, S., Hatziioannou, T. & Bieniasz, P. D. 2020. Absence of SARS-CoV-2 neutralizing activity in pre-pandemic sera from individuals with recent seasonal coronavirus infection. *medRxiv*.

Pyrc, K., Dijkman, R., Deng, L., Jebbink, M. F., Ross, H. A., Berkhout, B. & Van Der Hoek, L. 2006. Mosaic structure of human coronavirus NL63, one thousand years of evolution. *J Mol Biol,* 364**,** 964-73.

R Core Team, 2016. *R: A Language and Environment for Statistical Computing* [Online]. Vienna, Austria. Available: https://www.R-project.org/ [Accessed].

Sagar, M., Reifler, K., Rossi, M., Miller, N. S., Sinha, P., White, L. & Mizgerd, J. P. 2020. Recent endemic coronavirus infection is associated with less severe COVID-19. *J Clin Invest*.

Schulien, I., Kemming, J., Oberhardt, V., Wild, K., Seidel, L. M., Killmer, S., Sagar, Daul, F., Salvat Lago, M., Decker, A., et al. 2020. Characterization of pre-existing and induced SARS-CoV-2-specific CD8+ T cells. *Nature Medicine*.

Sette, A. & Crotty, S. 2020. Pre-existing immunity to SARS-CoV-2: the knowns and unknowns. *Nat Rev Immunol,* 20**,** 457-458.

Vijgen, L., Keyaerts, E., Lemey, P., Maes, P., Van Reeth, K., Nauwynck, H., Pensaert, M. & Van Ranst, M. 2006. Evolutionary history of the closely related group 2 coronaviruses: porcine hemagglutinating encephalomyelitis virus, bovine coronavirus, and human coronavirus OC43. *J Virol,* 80**,** 7270-4.

Woo, P. C., Lau, S. K., Chu, C. M., Chan, K. H., Tsoi, H. W., Huang, Y., Wong, B. H., Poon, R. W., Cai, J. J., Luk, W. K., et al. 2005. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol,* 79**,** 884-95.

Wu, A., Mihaylova, V. T., Landry, M. L. & Foxman, E. F. 2020. Interference between rhinovirus and influenza A virus: a clinical data analysis and experimental infection study. *Lancet Microbe,* 1**,** e254-e262.

27

Highlights
- Some humans possessed cross-reactive SARS-CoV-2 antibodies prior to the pandemic
- Pre-pandemic SARS-CoV-2 reactive antibodies are not associated with protection
- Antibodies to a related betacoronavirus are boosted upon SARS-CoV-2 infection

Analysis of human serum samples before and after the onset of the COVID-19 pandemic show that antibodies against common seasonal human coronaviruses are cross-reactive against SARS-CoV-2 but do not confer cross-protection against infection or hospitalization.
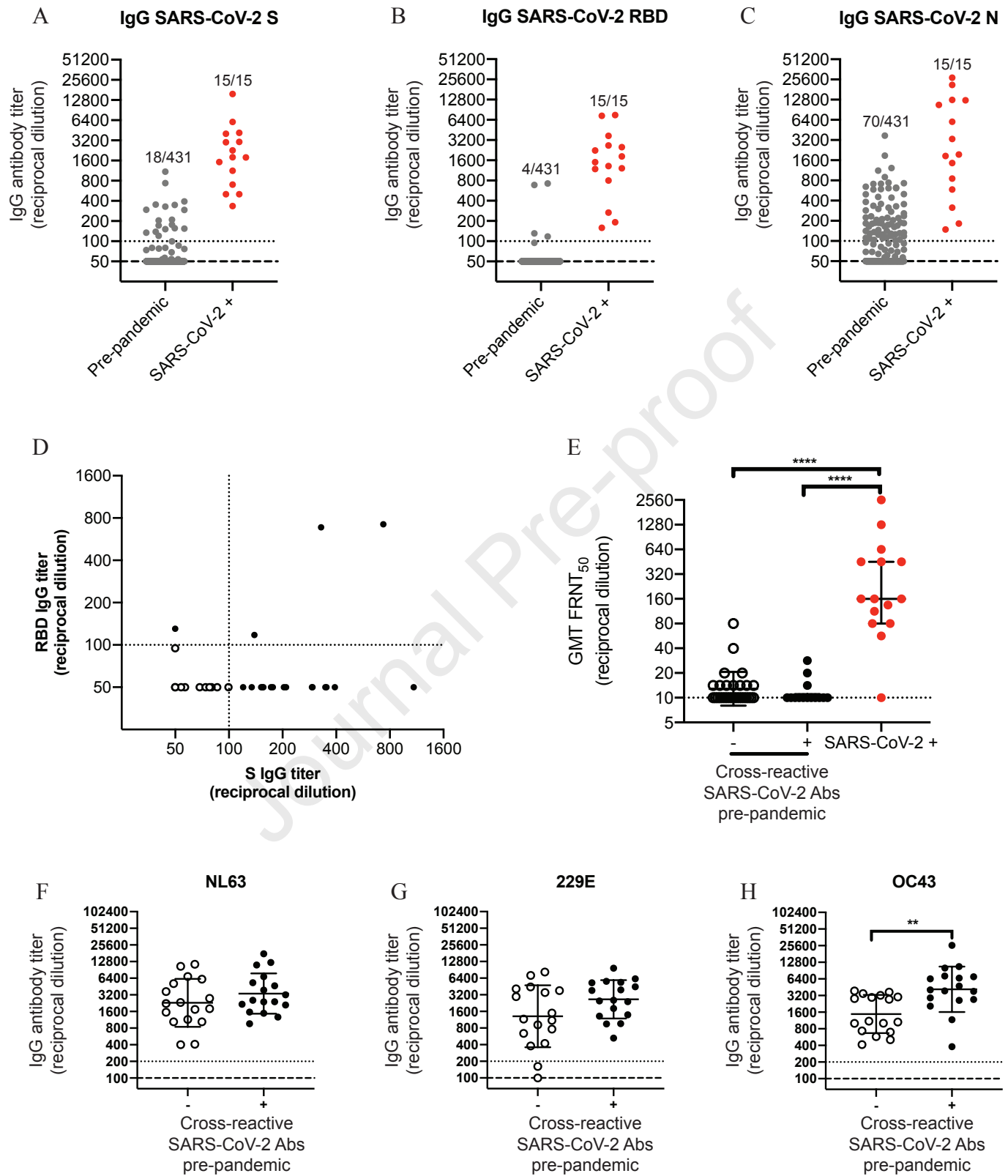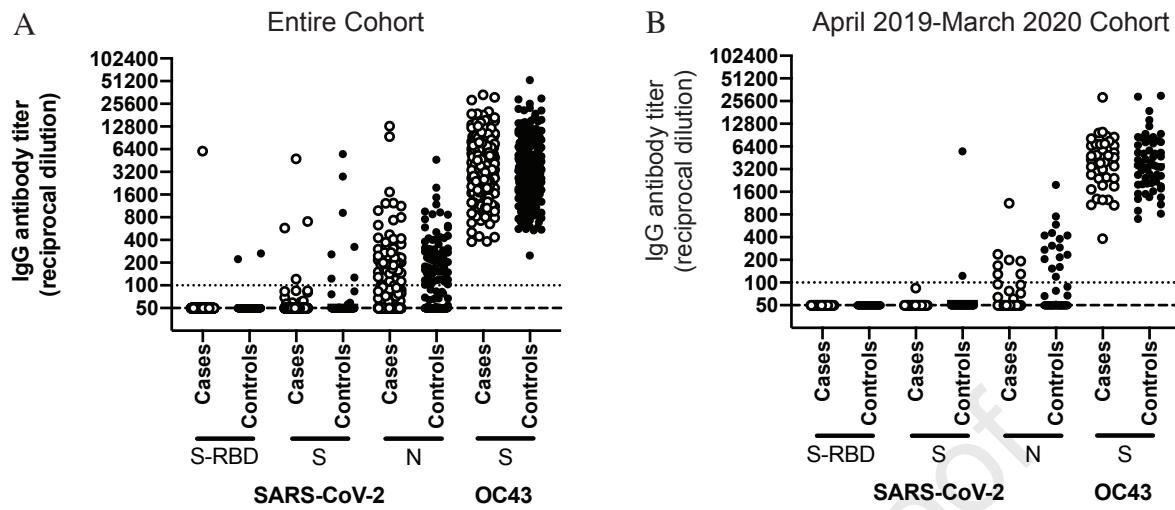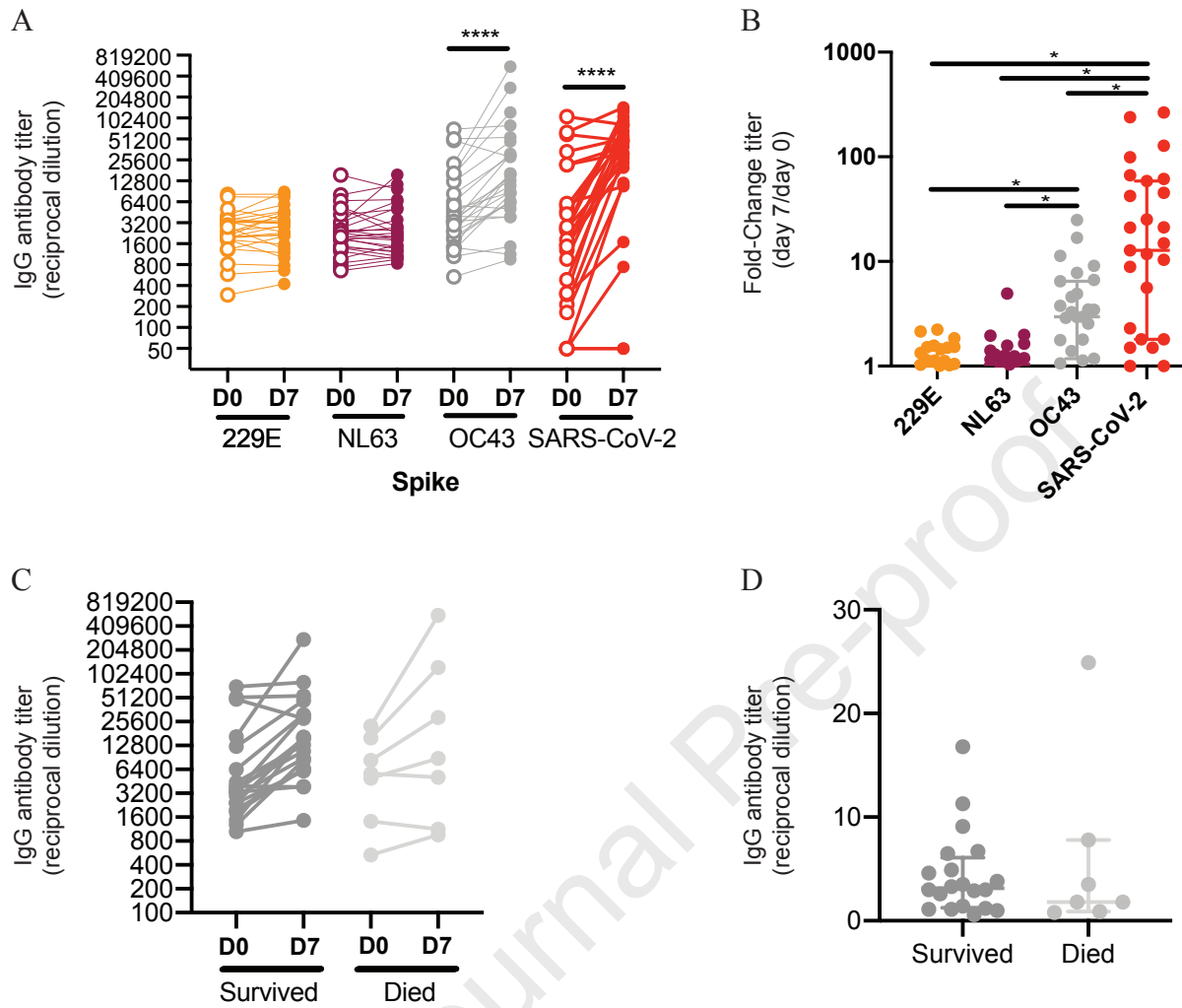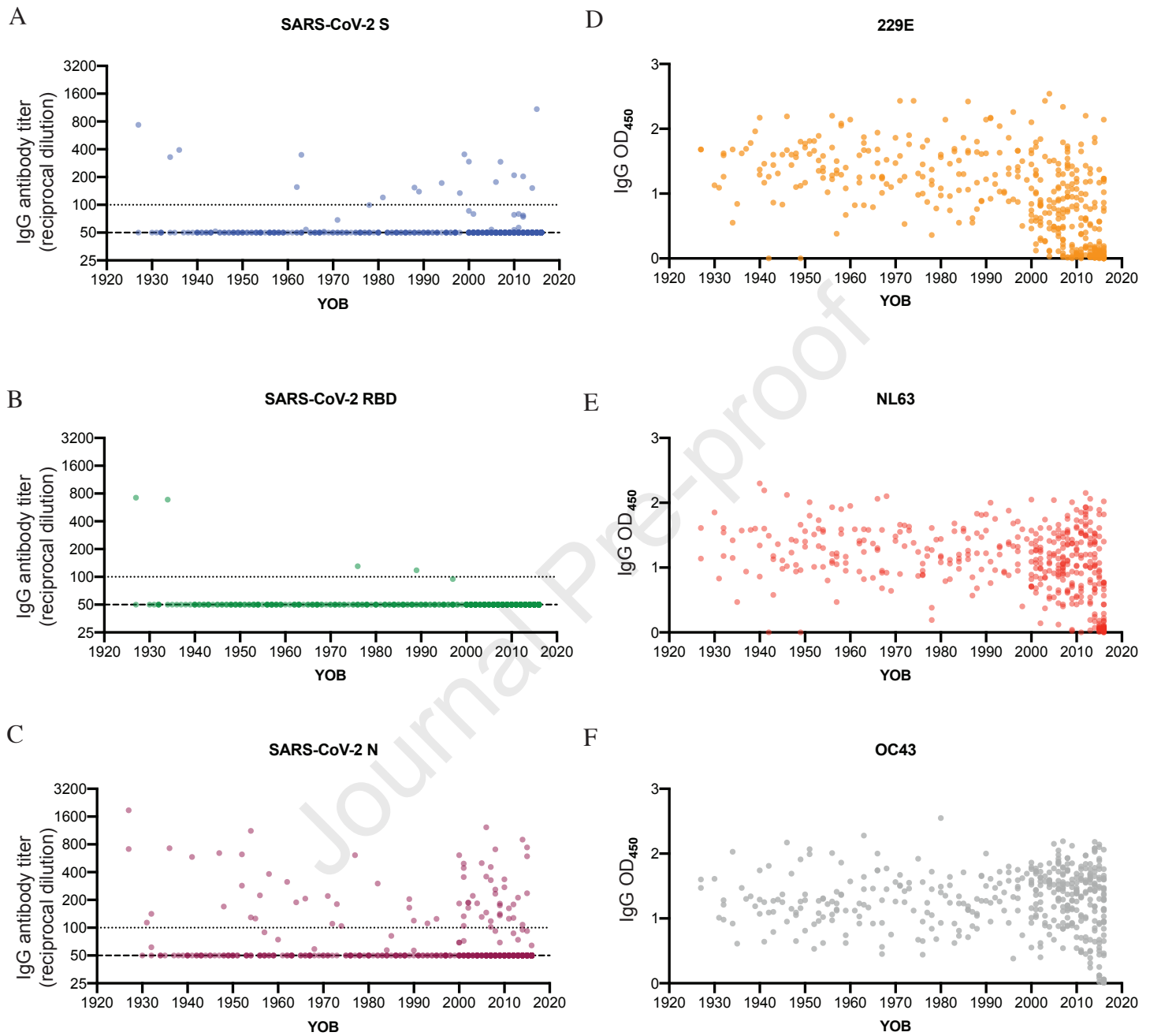
Figure 1

A — IgG SARS-CoV-2 S

B — IgG SARS-CoV-2 RBD

C — IgG SARS-CoV-2 N

D

E

F — NL63

G — 229E

H — OC43

Figure 2

A   Entire Cohort   B   April 2019-March 2020 Cohort

Figure 3

A

B

C

D

Figure S1

A

SARS-CoV-2 S

B

SARS-CoV-2 RBD

C

SARS-CoV-2 N

D

229E

E

NL63

F

OC43

Figure S2

A Cross-reactive SARS-CoV-2 Abs pre-pandemic -

B Cross-reactive SARS-CoV-2 Abs pre-pandemic +

C SARS-CoV-2 +

Figure S3

A. Pre-pandemic not cross-reactive

B. Pre-pandemic cross-reactive

C. COVID-19+

Figure S5

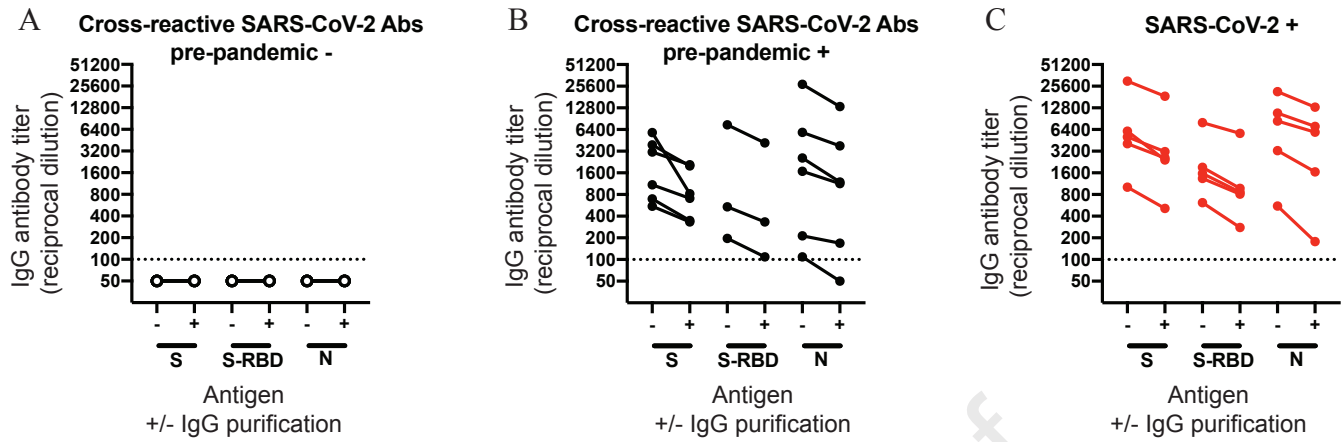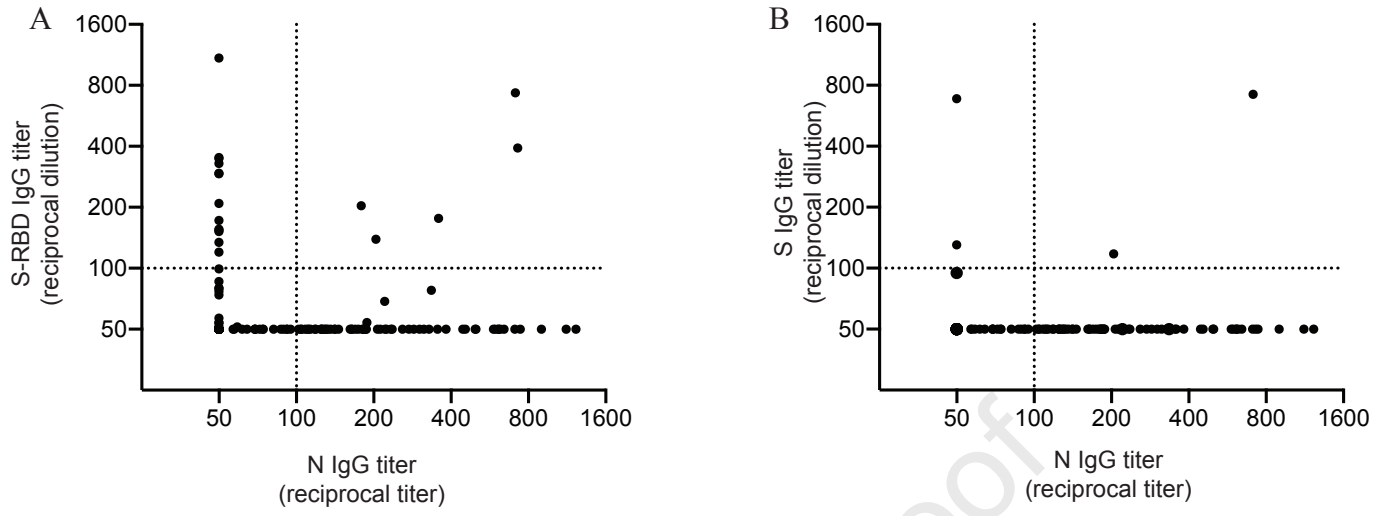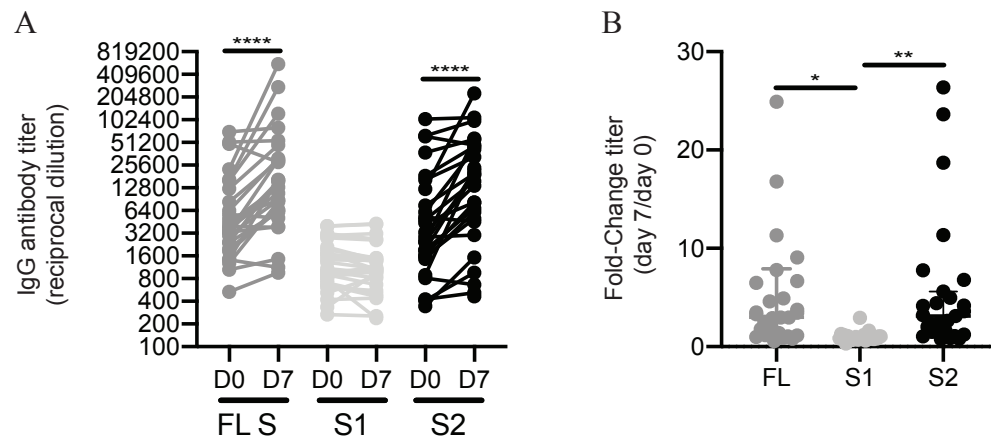assay using live virus in BSL-3

Figure S6

A

B

Table S1: Comparison between antibody titers and COVID-19 phenotypes, related to Figure 2.

Phenotype Name	Antibody Titers	Beta	SE	P	Cases	Controls
SARS-CoV-2 Susceptibility	N titer	9E-05	1E-04	0.47	251	251
SARS-CoV-2 Susceptibility	Spike-FL Titer	-1E-04	3E-04	0.65	251	251
SARS-CoV-2 Susceptibility	Spike-RBD Titer	5E-04	9E-04	0.53	251	251
SARS-CoV-2 Susceptibility	OC43 Spike Titer	1E-06	2E-05	0.93	251	251
COVID-19 Hospitalization	N Titer	1E-04	1E-04	0.40	80	171
COVID-19 Hospitalization	Spike-FL Titer	-5E-04	1E-03	0.61	80	171
COVID-19 Hospitalization	Spike-RBD Titer	-2E-03	1E-01	0.99	80	171
COVID-19 Hospitalization	OC43 Spike Titer	1E-05	3E-05	0.62	80	171
COVID-19 Severe Hospitalization	N Titer	-5E-04	1E-03	0.70	24	171
COVID-19 Severe Hospitalization	Spike-FL Titer	-1E-04	8E-04	0.88	24	171
COVID-19 Severe Hospitalization	Spike-RBD Titer	-2E-03	2E-01	0.99	24	171
COVID-19 Severe Hospitalization	OC43 Spike Titer	2E-05	5E-05	0.74	24	171

Table S2: Phenotype definitions related to Table S1, related to Figure 2.

Phenotype Name	Case Definition	Control Definition	Case	Controls
SARS-CoV-2 Susceptibility	RT-PCR confirmed *positive* test for SARS-CoV2 infection	RT-PCR confirmed *negative* test for SARS-CoV2 infection	251	251
COVID-19 Hospitalization	RT-PCR confirmed positive test for SARS-CoV2 infection and *hospitalized* due to COVID-19	RT-PCR confirmed positive test for SARS-CoV2 infection and *not hospitalized* due to COVID-19	80	171
COVID-19 Severe Hospitalization	RT-PCR confirmed positive test for SARS-CoV2 infection and *required respiratory support or had ICU stay* due to COVID-19	RT-PCR confirmed positive test for SARS-CoV2 infection and *not hospitalized* due to COVID-19	24	171