medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

1 # Detection, prevalence, and duration of humoral responses to SARS-
2 # CoV-2 under conditions of limited population exposure

3 Tyler J. Ripperger[1,*], Jennifer L. Uhrlaub[1,2*], Makiko Watanabe[1,2*], Rachel Wong[1,3*],
4 Yvonne Castaneda[1,2], Hannah A. Pizzato[1,3], Mallory R. Thompson[4,5], Christine
5 Bradshaw[1,2], Craig C. Weinkauf[4], Christian Bime[6], Heidi L. Erickson[6], Kenneth Knox[6,7],
6 Billie Bixby[6], Sairam Parthasarathy[6], Sachin Chaudhary[6], Bhupinder Natt[6], Elaine
7 Cristan[6], Tammer El Aini[6], Franz Rischard[6], Janet Campion[6], Madhav Chopra[6], Michael
8 Insel[6], Afshin Sam[6], James L. Knepler[6], Andrew P. Capaldi[8,9], Catherine M. Spier[10],
9 Michael D. Dake[11], Taylor Edwards[12], Matthew E. Kaplan[9], Serena Jain Scott[13],
10 Cameron Hypes[6,14], Jarrod Mosier[6,14], David T. Harris[1,15], Bonnie J. LaFleur[16], Ryan
11 Sprissler[12,16], Janko Nikolich-Žugich[1,2,16#], and Deepta Bhattacharya[1,16 #]

12

13 1- Department of Immunobiology, University of Arizona College of Medicine – Tucson,
14 Tucson, AZ, USA; 2- University of Arizona Center on Aging, University of Arizona
15 College of Medicine – Tucson, Tucson, AZ, USA; 3 – Division of Biological and
16 Biomedical Sciences, Washington University, St. Louis, MO, USA; 4 – Department of
17 Surgery, University of Arizona College of Medicine – Tucson, Tucson, AZ, USA; 5 –
18 Department of Cellular and Molecular Medicine, University of Arizona College of
19 Medicine – Tucson, Tucson, AZ, USA; 6 - Division of Pulmonary, Allergy, Critical Care
20 and Sleep Medicine, Department of Medicine, University of Arizona College of Medicine
21 – Tucson, Tucson, AZ, USA; 7- Department of Medicine, University of Arizona-Phoenix,
22 Phoenix, AZ; 8- Department of Molecular and Cellular Biology, University of Arizona –
23 Tucson, Tucson, AZ, USA; 9- Functional Genomics Core, University of Arizona, Tucson,

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

24 AZ; 10-Department of Pathology, University of Arizona College of Medicine – Tucson,
25 Tucson, AZ, USA; 11 – Office of the Senior Vice-President for Health Sciences,
26 University of Arizona, Tucson, USA; 12- University of Arizona Genomics Core, University
27 of Arizona, Tucson, AZ and the Arizona Research Labs, University of Arizona Genetics
28 Core, University of Arizona, Tucson, AZ, USA; 13- Division of Geriatrics, General
29 Medicine and Palliative Care, Department of Medicine, University of Arizona College of
30 Medicine-Tucson, Tucson, USA; 14 – Department of Emergency Medicine, University of
31 Arizona College of Medicine-Tucson, Tucson, USA; 15- University of Arizona Health
32 Sciences Biobank, University of Arizona, Tucson, USA; 16- BIO5 Institute, University of
33 Arizona, Tucson, USA.

34

35

36 * These authors contributed equally and are listed in alphabetical order; # - these
37 authors contributed equally. Address correspondence to Janko Nikolich-Žugich, at
38 nikolich@arizona.edu; or Deepta Bhattacharya, at deeptab@arizona.edu, or by mail at
39 P.O. Box 249221, Department of Immunobiology, University of Arizona College of
40 Medicine-Tucson, 1501 N. Campbell Ave., Tucson, AZ 85724

41

42

43

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

44 **ABSTRACT:**

45 We conducted an extensive serological study to quantify population-level exposure and
46 define correlates of immunity against SARS-CoV-2. We found that relative to mild COVID-19
47 cases, individuals with severe disease exhibited elevated authentic virus-neutralizing titers and
48 antibody levels against nucleocapsid (N) and the receptor binding domain (RBD) and the S2
49 region of spike protein. Unlike disease severity, age and sex played lesser roles in serological
50 responses. All cases, including asymptomatic individuals, seroconverted by 2 weeks post-PCR
51 confirmation. RBD- and S2-specific and neutralizing antibody titers remained elevated and
52 stable for at least 2-3 months post-onset, whereas those against N were more variable with
53 rapid declines in many samples. Testing of 5882 self-recruited members of the local community
54 demonstrated that 1.24% of individuals showed antibody reactivity to RBD. However, 18%
55 (13/73) of these putative seropositive samples failed to neutralize authentic SARS-CoV-2 virus.
56 Each of the neutralizing, but only 1 of the non-neutralizing samples, also displayed potent
57 reactivity to S2. Thus, inclusion of multiple independent assays markedly improved the
58 accuracy of antibody tests in low seroprevalence communities and revealed differences in
59 antibody kinetics depending on the viral antigen. In contrast to other reports, we conclude that
60 immunity is durable for at least several months after SARS-CoV-2 infection.

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

61 **INTRODUCTION:**

62 SARS-CoV-2, the causative agent of COVID-19, has infected over 20 million people
63 worldwide, with over 750,000 dead as of August 13, 2020. Serological testing for SARS-CoV-2
64 antibodies is an important tool for measuring individual exposures, community transmission, and
65 the efficacy of epidemiological countermeasures. While a few epicenters of infection have seen
66 relatively robust spread of the virus (Rosenberg et al., 2020; Stadlbauer et al., 2020), COVID-19
67 prevalence in most of the world has been low. For example, studies in Spain and Switzerland
68 revealed overall seroprevalences of ~5%, with some communities at just 1% antibody positivity
69 (Pollán et al., 2020; Stringhini et al., 2020). The challenges of accurate antibody testing for
70 SARS-CoV-2 in low seroprevalence communities have led to several unexpected conclusions.
71 As an example, a seroprevalence study in Santa Clara county, California suggested higher
72 infection rates than had been anticipated, thereby leading to the interpretation that SARS-CoV-2
73 was much less deadly than originally thought (Bendavid et al., 2020). Yet this conclusion was
74 problematic given that the false positive rates of the administered test approached the true
75 seroprevalence of the community (Bennett and Steyvers, 2020). Thus, it is likely that many
76 positive results were inaccurate, and the overall infection fatality rate was substantially higher
77 than estimated in this study (Bennett and Steyvers, 2020). Reducing this false positive rate is
78 critical for accurate seroprevalence studies. Moreover, serological testing has an additional
79 imperative to guard against false positive results that could entice the subject to falsely assume
80 immunity where none may exist. Indeed, the assumption of immunity associated with a positive
81 test result may be amongst the primary motivations for participation in these serological
82 surveys. Virus neutralization assays are functional correlates of immunity but require Biosafety
83 Level 3 facilities and are difficult to scale and deploy as clinical assays. Yet tests that fail to
84 provide confidence in functional immune status undermine this important epidemiological tool.

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

85 Finally, poor positive predictive values are especially problematic in the context of convalescent
86 plasma donations, where most samples would be ineffective in passive transfer therapies.

87 Serological studies have also been used to estimate the durability of antibody production
88 and immunity after SARS-CoV-2 infections. Here again, several surprising conclusions have
89 been reached regarding the short duration of immunity, with several studies suggesting that in a
90 substantial number of subjects, antibody levels wane to below the limit of detection within a
91 matter of weeks to months (Ibarrondo et al., 2020; Long et al., 2020a; Pollán et al., 2020; Seow
92 et al., 2020). Yet all T-dependent humoral responses, even ones that are exceptionally durable,
93 begin with an initial wave of short-lived plasma cells which decline quickly and are progressively
94 replaced by a smaller number of longer-lived antibody-secreting plasma cells (Amanna, 2007;
95 Manz et al., 1997; Slifka et al., 1998; Sze et al., 2000). Thus, the decay in antibody production
96 after infection or vaccination is not linear and cannot be extrapolated from early timepoints,
97 demonstrating the need for longer-term follow-up studies. Indeed, such short-term antibody
98 production would be without precedent following acute coronavirus infections, which typically
99 induce immunity for at least a year and for SARS-CoV-1, often for much longer (Callow et al.,
100 1990; Guo et al., 2020; Reed, 1984; Tan et al., 2020). Keys to the accurate interpretation of
101 such studies are sensitive assays, PCR confirmation of test cases, and longitudinal tests of
102 seropositive individuals. Authentic virus neutralization assays are also useful as true correlates
103 of immunity (Zinkernagel and Hengartner, 2006). Absent these components, conclusions about
104 the duration of immunity are premature.

105 Here, we successfully employed a strategy using RBD and S2 as antigenically distinct
106 tests to accurately identify seropositive individuals in the community. In doing so, this assay
107 greatly reduced the existing limitations to testing accuracy in low seroprevalence communities
108 and identified individuals for subsequent analysis of the immune response. We found that
109 disease severity, but not age or sex, were correlates of the magnitude of the response. Further,

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

110 use of these two antigens, nucleocapsid protein, and neutralizing antibody titers revealed
111 discordance in the durability of antibody responses depending on the viral protein. In contrast to
112 earlier reports, we demonstrate durable production of functionally important antibodies lasting at
113 least 2-3 months post-disease onset.

114

115 **RESULTS:**

116 Numerous serological tests that have received Food and Drug Administration
117 Emergency Use Authorizations (https://www.fda.gov/medical-devices/coronavirus-disease-
118 2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-
119 performance) rely on reactivity to the SARS-CoV-2 RBD domain of the S protein (Amanat et al.,
120 2020; Premkumar et al., 2020). To begin validation of a serological assay for antibodies to RBD,
121 we tested 43 serum samples from PCR-confirmed COVID-19 patients in the hospital at various
122 stages of disease, 48 convalescent samples, and 23 samples from healthy donors. Serum
123 dilution ELISAs were performed to quantify RBD-reactive antibodies in these samples.
124 Mammalian RBD antigen preparations were selected as targets, as they demonstrated superior
125 signal:noise ratios relative to bacterially-produced protein (**Figure S1A**). Antibody titers were
126 quantified as area under the curve (AUC) and correlated with neutralization of the live USA-
127 WA1/2020 strain of SARS-CoV-2, rather than S-protein pseudotyped virus (Giroglou et al.,
128 2004), due to the poor agreement between these functional assays (**Figure S1B**), and because
129 of the modest sensitivity of some pseudovirus neutralization tests relative to those of authentic
130 virus (Schmidt et al., 2020). The correlation was strong between RBD-reactive IgG and plaque
131 reduction neutralization test (PRNT) titers, which we quantified as the final dilution at which 90%
132 viral neutralization occurred ($PRNT_{90}$) (**Figure 1A**). RBD-reactive IgM antibodies also correlated
133 with $PRNT_{90}$ titers (**Figure S1C**). Because (i) IgM and IgG ratios are not indicative of the timing

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint
(which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

134 of disease onset (Hou et al., 2020; Long et al., 2020b; Qu et al., 2020), (ii) IgA is induced by
135 SARS-CoV-2 (Isho et al., 2020; Iyer et al., 2020; Sterlin et al., 2020), and (iii) both IgG and IgM
136 isotypes correlated with neutralizing titers, we chose to quantify total (all isotypes) antigen-
137 specific antibodies for seroprevalence studies.

138 To determine if RBD was capable of distinguishing between SARS-CoV-2 exposed and
139 uninfected individuals and to set preliminary thresholds for positive calls, we initially tested 1:40
140 serum dilutions of samples from 30 PCR+ SARS-CoV-2 infected individuals and 32 samples
141 collected prior to September, 2019, well before the onset of the current pandemic (**Figure S1D**).
142 Using this test data set, we established a preliminary positive cutoff OD_{450} value of 0.12, equal
143 to 3 standard deviations above the mean values of the negative controls. We next used this
144 preliminary threshold to test an expanded cohort of 320 negative control samples collected prior
145 to 2020. **(Figure 1B)**. Reactivity to RBD was clearly distinguishable for the majority of positive
146 samples from negative controls (**Figure 1B**). However, 6.5% of the expanded negative control
147 group displayed RBD reactivity that overlapped with PCR+ individuals (**Figure 1B, blue shade**),
148 some of whom may have been early into disease and had not yet generated high levels of
149 antibodies. To quantify the sensitivity of the assay relative to time of diagnosis, we measured
150 antibody levels to RBD and plotted these values against time following SARS-CoV-2 PCR+
151 confirmation. Whereas the sensitivity was modest within the first two weeks, after 2 weeks, 42 of
152 43 samples showed high ELISA signal (**Figure 1C).** Based on these data, samples were
153 considered seropositive at OD_{450} numbers above 0.39, a value slightly above the highest OD
154 obtained from the 352 subjects in the negative control group (**Figure 1B**). Sera were considered
155 negative at OD_{450} values below 0.12. Finally, we created an indeterminate call at OD_{450} values
156 between 0.12-0.39, as we observed some overlap between negative controls and PCR-
157 confirmed samples in this range (**Figure 1B, blue shade**).

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

158 We next applied this assay to community testing and obtained serum samples from 5882
159 self-recruited volunteers from Pima County. Donors included healthcare workers (~26%), first
160 responders (~27%), University of Arizona students (~5%), and other members of the general
161 public (~42%). Currently febrile or otherwise symptomatic patients were excluded. Sera from 73
162 individuals preliminarily scored as seropositive (**Figure 1D**). These samples, along with another
163 171 samples with OD_{450} values in the indeterminate range were tested for virus neutralization at
164 a serum dilution of 1:20 (**Figure 1E**). Nine samples with RBD OD_{450} values below 0.39 were
165 observed to neutralize SARS-CoV-2 (**Figure 1E**). More problematically, we found that 13 of the
166 73 samples (17.8%) called positive by RBD-reactivity failed to neutralize authentic SARS-CoV-2
167 (**Figure 1E**). If virus neutralization is considered as a measure of 'true' seropositivity, RBD
168 ELISAs alone provided a relatively modest positive predictive value of 82%. These observations
169 indicated a clear need for a secondary screen to accurately quantify seropositivity in a
170 community with low infection rates.

171 To improve the positive predictive value, we considered the use of an orthogonal
172 antigenically distinct test. We first tested nucleocapsid (N) protein, as several other commercial
173 serological tests quantify antibodies to this antigen (Bryan et al., 2020; Burbelo et al., 2020). IgG
174 antibody titers to N protein in our collected sample cohort showed a strong correlation to
175 $PRNT_{90}$ titers (**Figure 2A**). A weaker correlation was observed between N-reactive IgM levels
176 and $PRNT_{90}$ titers (**Figure S2A**). We next assayed reactivity to N antigen using a subset of the
177 pre-2019 validation samples employed for RBD. N protein seroreactivity overlapped
178 substantially between negative and positive controls (**Figure 2B**). Moreover, 5 confirmed
179 COVID-19 samples showed very weak reactivity to N (**Figure 2B**). Because of the relatively
180 poor performance of N protein as an antigen in our hands, we next tested the S2 domain of S
181 protein as another candidate to determine seropositivity. RBD is located on the S1 domain,
182 rendering S2 antigenically distinct (Bosch et al., 2003; Li, 2016; Wrapp et al., 2020). IgG

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

183 antibody titers to S2 correlated well with $PRNT_{90}$ titers (**Figure 2C)**. Assessment of S2 serum
184 reactivity in the pre-2019 cohort revealed that approximately 3.3% of these samples overlapped
185 with signals in PCR-confirmed COVID-19 samples (**Figure 2D)**. We thereafter employed a
186 threshold of OD_{450}>0.35, as our cutoff for S2 positivity, which was 5 standard deviations above
187 the average seroreactivity from the original 32-samples from the negative control cohort.
188 Specificity control testing using 272 negative control sera showed that reactivities of negative
189 samples against RBD and S2 were largely independent of one another, as samples with high
190 signal for one antigen rarely showed similar background for the other (**Figure 2E**). Based on
191 these data, we chose to rely on combined RBD and S2-reactivities as accurate indicators of
192 prior SARS-CoV-2 exposure.

193 With this improved combinatorial RBD and S2 assay to exclude false positives, we re-
194 examined the original samples from the cohort of 5882 subjects that displayed RBD OD_{450}
195 values greater than 0.12 (**Figure 1D-E**). Of the 13 non-neutralizing samples that displayed high
196 (OD_{450} >0.39) RBD reactivity, 12 lacked S2 reactivity (**Figure 2F**). In contrast, the remaining 60
197 RBD+ neutralizing samples all displayed substantial reactivity to S2 (**Figure 2F**). Five of the 9
198 samples that fell below the RBD cutoff, yet still neutralized virus, displayed strong reactivity to
199 S2 (**Figure 2F**). Based on these data, we established a scoring criterion of RBD OD_{450}>0.39, S2
200 OD_{450}>0.35 as seropositive; RBD OD_{450} between 0.12-0.39, S2 OD_{450}>0.35 as indeterminate;
201 and all other samples as seronegative. Applying these criteria to 320 samples obtained prior to
202 2020 would lead to 317 negative, 3 indeterminate, and 0 positive calls. Using these same
203 criteria, we achieved an empirically defined false positive rate of just 0.02%, with only 1 positive
204 sample incapable of neutralizing live SARS-CoV-2 virus. Approximately half the samples called
205 as indeterminate contained neutralizing antibodies. Only 3 samples called as negative
206 possessed neutralizing titers, which were usually low (1:20). To further confirm the sensitivity of
207 the assay, we tested 993 samples at random for neutralizing antibodies. Of these, none of the

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

208 samples called as negative possessed neutralizing activity (data not shown). These data
209 demonstrate that inclusion of S2 as a requisite confirmatory screen markedly improves the
210 positive predictive value of SARS-CoV-2 serological assays, especially in areas with low SARS-
211 CoV-2 seroprevalence.

212 Several recent reports have suggested more robust immune responses in those with
213 severe disease relative to mild cases (Choe et al.; Ko et al., 2020; Long et al., 2020a; Qu et al.,
214 2020). Moreover, the ratios of S and N antibody specificities correlate with disease outcome
215 (Atyeo et al., 2020). We therefore examined our data for these trends. First, in our PCR-
216 confirmed cohort, we plotted IgG titers relative to the time of disease onset, stratified by disease
217 severity. Severe disease (hospital admission) correlated with significantly higher antibody titers
218 against RBD, S2, and N than those with mild disease, who were symptomatic but did not require
219 hospital admission (**Figure 3A-C**). Neutralizing titers were also higher in those with severe
220 disease relative to mild cases (**Figure 3D**). Through campus screening efforts, we also
221 identified 6 PCR+ individuals who either never developed symptoms or had only a brief and mild
222 headache or anosmia. Although previous reports suggested that such individuals may
223 infrequently seroconvert or frequently serorevert (Long et al., 2020a; Sekine et al., 2020), all
224 such individuals in our cohort showed seroreactivity to RBD, S2, and all but one to N (**Figures**
225 **S3A-C**), consistent with other recent studies (Choe et al.; Ko et al., 2020). Given that older
226 adults, as well as those of male sex, exhibit disproportional morbidity and mortality from COVID-
227 19, we also sought to test whether humoral immunity in these subjects may be quantitatively
228 reduced (Liu et al., 2020). Contrary to this expectation, we did not observe any adverse impact
229 of advanced age on humoral immunity (**Figure 3E-H**). Similarly, within our cohort, females and
230 males had similar anti-RBD, N, S2, and neutralizing responses (**Figure S3D-G)**.

231 Individuals with mild disease have been reported to lose SARS-CoV-2-specific
232 antibodies quickly into convalescence (Ibarrondo et al., 2020; Long et al., 2020a; Seow et al.,

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

233 2020). To assess the durability of antibody production in our cohort, we first returned to the
234 community cohort of 5882 individuals. Twenty-nine of the seropositive subjects had reported
235 mild symptoms consistent with COVID-19. These positive samples were thus plotted alongside
236 PCR-confirmed mild disease cases against time post-disease onset to determine if any trends
237 could be observed in declining antibody levels. Across subjects, IgG specific for RBD (**Figure**
238 **4A**) and S2 (**Figure 4B**) appeared to peak near 30 days post-onset and then partially decline
239 before settling to a more stable nadir at later timepoints, as would be expected for all acute viral
240 infections. We considered the possibility that we may have missed subjects that had
241 seroreverted prior to their antibody test, thereby incorrectly raising our estimates of the durability
242 of antibody production. Therefore, to examine the duration of IgG production in more depth, a
243 subset of seropositive individuals with relatively low titers was tested longitudinally up to 122
244 days post-onset. These data again revealed stable RBD and S2 IgG levels at later stages of
245 convalescence (**Figures 4A-B**). However, N-reactive IgG levels were quite variable and
246 approached the lower limit of detection in several subjects at later timepoints (**Figure 4C**). A
247 direct comparison in matched subjects of the changes in RBD, S2, and N IgG titers over time
248 confirmed the variability in N responses and rapid decline in a subset of individuals (**Figure 4D**).
249 Most importantly, neutralizing antibody levels remained high with very little decay as a function
250 of time (**Figure 4E**). These data suggest stable neutralizing, RBD, and S2-specific antibodies,
251 but variable and often declining N-reactive titers during convalescence. Together, these data
252 are consistent with the maintenance of functionally important antibody production for at least
253 several months after infection, and caution against the use of α-N antibodies to estimate
254 immunity or seroprevalence.

255

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

256 **DISCUSSION:**

257 Here, we demonstrated that using two antigenically distinct serological tests can greatly
258 remedy specificity problems that are exacerbated in low SARS-CoV-2 seroprevalence
259 communities. RBD and S2 seroreactivity behaved independently for SARS-CoV-2-unexposed
260 individuals, thereby suggesting that the theoretical false positive rate of the overall assay is the
261 product of the two tests. Using neutralization assays to confirm these results, we found our
262 empirically determined false positive rate to be <0.02% (1/5882), consistent with the
263 independence of the RBD and S2 tests. The tight co-incidence between RBD/S2 positivity and
264 the presence of neutralizing antibodies, even in low seroprevalence populations, is especially
265 valuable for identifying individuals who likely have some degree of immunity and could
266 potentially serve as convalescent plasma donors. Surprisingly, nucleocapsid (N), which is used
267 by several commercial serological tests as an antigen, did not perform as well in our assays,
268 with high false positive and negative rates.

269 Though we are uncertain why N protein reactivity proved less discriminatory in our
270 hands relative to published work (Bryan et al., 2020; Steensels et al., 2020), as one possible
271 explanation, we observed that in several subjects, N-specific antibodies declined more rapidly
272 than those against RBD or S2. This unexpected finding may in part help explain some
273 discrepancies in the literature. In some reports, SARS-CoV-2-specific N antibodies fell to
274 undetectable levels within 2-3 months in up to 40% of those recovering from mild disease (Long
275 et al., 2020a; Pollán et al., 2020), which would be remarkably transient and very unusual for
276 acute viral infections, even other common coronaviruses (Callow et al., 1990; Reed, 1984).
277 Although most N titers did not fall fully below our detection limits, we also observed such a
278 decline in some subjects. Yet encouragingly, neutralizing antibodies and those against RBD and
279 S2 reached a stable nadir after the initial expected decline, presumably as short-lived plasma
280 cells were replaced with long-lived antibody secreting cells. These data are consistent with

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint
(which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

281 expectations for acute viral infections and with the conclusions of other studies currently on
282 preprint servers (Isho et al., 2020; Iyer et al., 2020; Wajnberg et al., 2020). In this regard, the
283 primary data for S and neutralizing antibody responses seem consistent across several studies
284 (Ibarrondo et al., 2020; Seow et al., 2020), though the interpretations differ. These differences in
285 interpretation are reminiscent of studies on the length of SARS-CoV-1 immunity. Early reports
286 suggested that immunity was transient (Cao et al., 2007), but more recent studies have
287 demonstrated that SARS-CoV-1 neutralizing antibodies can still be detected 12-17 years
288 afterwards (Guo et al., 2020; Tan et al., 2020). Given these lessons, conclusions about the rapid
289 loss of immunity to SARS-CoV-2 are premature and inconsistent with the data we presented
290 here.

291 The reasons for the differences in antibody responses across antigens are difficult to
292 explain, given the identical inflammatory environment in which these responses arose. One
293 possibility is that the avidities of germline precursors differ for N- and S-protein specificities. For
294 both memory and plasma cells, there appears to be a 'sweet spot' of antigen avidity that
295 promotes optimal responses (Abbott et al., 2018). A second possibility is that N-protein
296 responses are driven by cross-reactive memory, rather than naïve B cells. Memory B cells are
297 substantially more diverse than are plasma cells, thereby encoding a hidden repertoire that is
298 not represented in serum antibodies (Lavinder et al., 2014; Purtha et al., 2011; Smith et al.,
299 1997). Consistent with this possibility, N protein is more conserved across coronaviruses than is
300 RBD (Srinivasan et al., 2020). Memory responses, especially by isotype-switched B cells, are
301 directed by fundamentally distinct transcriptional programs than those of naïve cells
302 (Bhattacharya et al., 2007; Jash et al., 2016; Wang et al., 2012; Zuccarino-Catania et al., 2014).
303 For example, the transcription factor ZBTB32 specifically limits the magnitude and duration of
304 memory B cell responses, perhaps to keep chronic infections from overwhelming the system
305 (Jash et al., 2016, 2019). It remains to be established whether such mechanisms may be

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

306 selectively operating on SARS-CoV-2 and other coronavirus N antibody responses due to their
307 antigenic similarity between strains.

308 Taken together, we have reported a highly specific serological assay for SARS-CoV-2
309 exposure that is usable in very low seroprevalence communities, and that returns positive
310 results that are highly co-incident with virus neutralization. Using this assay, we characterize the
311 responses in different subject populations by age, sex and disease severity, we demonstrate
312 that antibody production persists for at least 3 months, and we suggest explanations for some
313 reports that concluded otherwise.

314

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

314 **Limitations of current study:** The above assay allowed us to examine the influence of age,
315 sex, and disease severity on levels of humoral immunity in our tested populations. Similar to
316 other studies (Qu et al., 2020; To et al., 2020), we found that severe disease correlated
317 positively with levels of antibody immunity. While both older adults (>50 and even more >65
318 years of age) and males are more vulnerable to COVID-19 (Klein et al., 2020a, 2020b;
319 Palaiodimos et al., 2020), levels of humoral immunity did not reveal age or sex-related
320 differences that could explain such vulnerability. A caveat here is that our study had a limited
321 longitudinal component and that we could not determine whether there may have been a delay
322 or reduction in humoral immunity at earlier time points of the disease. A second related caveat
323 is that in our community testing cohort we may have missed individuals who were seropositive
324 initially but then seroreverted by the time of the antibody test. Finally, the latest timepoint post-
325 disease onset in our study is 122 days. It remains possible that antibody titers will wane
326 substantially at later times. Additional serial sampling of PCR-confirmed mild cases will be
327 required to test these possibilities.
328
329
330
331
332
333

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY-NC-ND 4.0 International license .

335 **ACKNOWLEDGEMENTS:**

336 The authors are indebted to the nurses in the intensive care units of Banner University Medical
337 Center – Tucson and South Campuses and research coordinators (Cathleen Wilson and Trina
338 Hughes) for facilitating the collection of samples in critically ill hospitalized patients with COVID-
339 19. We thank F. Krammer, V. Simon, M. Rao, and J. Jhang (Mt. Sinai Hospital) and A. Ellebedy
340 and D. Fremont (Washington University in St. Louis) for reagents and protocols. Supported in
341 part by USPHS awards AG020719 and AG057701 and CDC award 75D30120C08379 (J.N-Ž),
342 the contract CTR050053 from the State of Arizona (J.N-Ž and D.B.), R01AI099108 and
343 R01AI129945 (D.B.), by the COVID-19 Rapid Response Grant from the UArizona BIO5 Institute
344 (to C.C.W., D.B. and J.N-Ž), and the Bowman Endowment in Medical Sciences (J. N-Ž).

345

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

343 **AUTHOR CONTRIBUTIONS:**

344 T.J.R., J.L.U., M.W., R.W., R.S., J.N.Z., and D.B. designed the study. T.J.R., J.L.U., M.W.,
345 R.W., H.P., C.B., M.K., and R.S. performed experiments. T.J.R., J.L.U., M.W., R.W., A.C., C.S.,
346 M.K., T.E., R.S., J.N.Z., and D.B. analyzed the data. T.J.R., J.N.Z., and D.B. wrote the paper.
347 All other authors participated in collection of samples and patient care for the study.

348

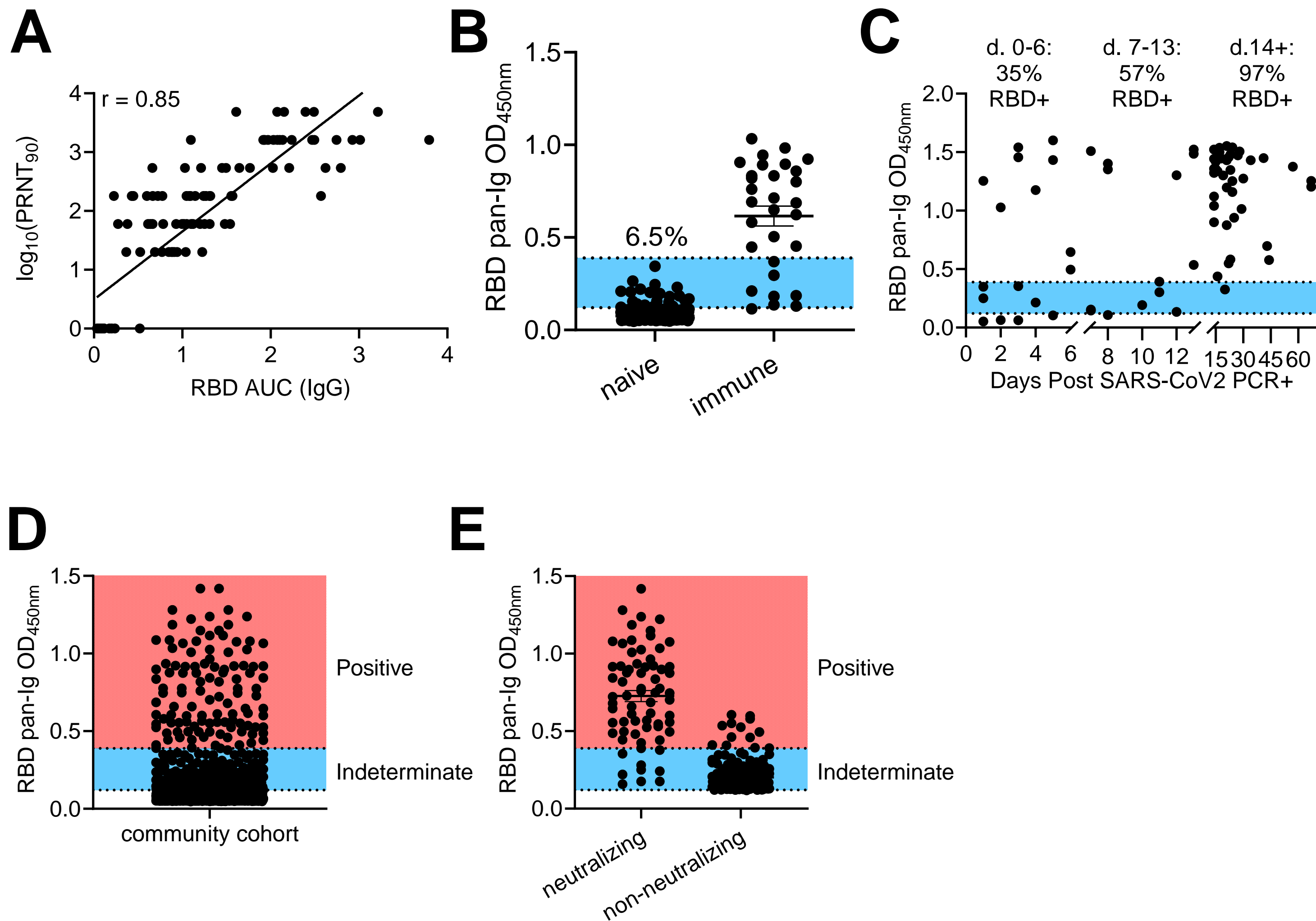
medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

349 **DECLARATION OF INTERESTS:**

350 Unrelated intellectual property of D.B. and Washington University has been licensed by Sana
351 Biotechnology. J.N.Z. is on the scientific advisory board of and receives research funding from
352 Young Blood Inc. R.S. is a founder and chief scientific officer of Geneticure. R.W. is currently an
353 employee of Vir Biotechnology.

354

Figure 1



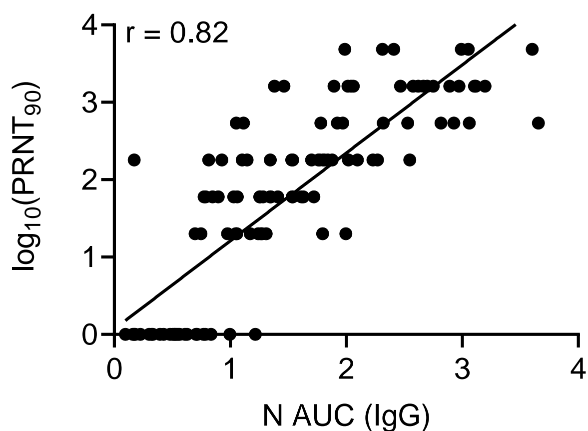
medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

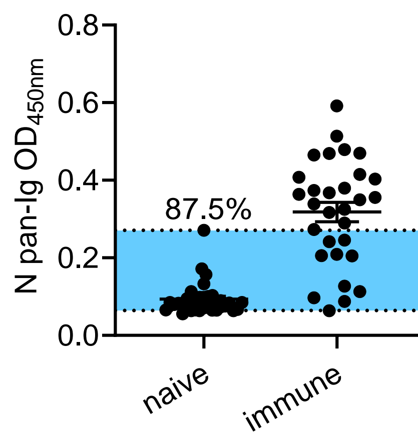
355 **FIGURE LEGENDS:**

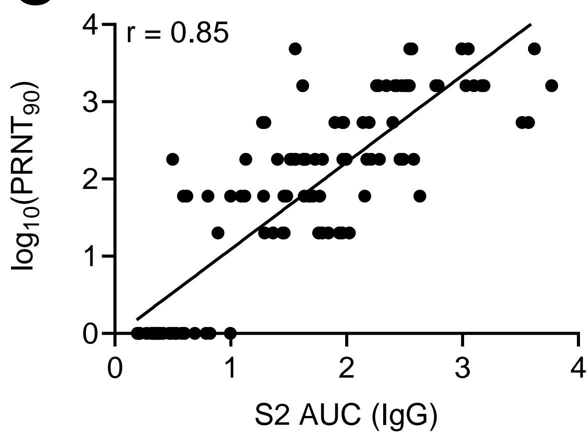
356 **Figure 1: Assessment of RBD-based sensitivity and specificity in serological testing.** (**A**)
357 Serum samples from healthy controls and confirmed COVID-19 cases were assessed for RBD
358 reactivity by ELISA and neutralization of live SARS-CoV-2. $PRNT_{90}$ values were determined as the
359 last dilution by which 90% neutralization occurred. Antibody titers were quantified for RBD by
360 quantifying area under the curve (AUC) across a serial dilution curve. r values were calculated by
361 Pearson's Correlation Test. (**B**) Pre-2020 negative control samples (352) and 30 samples from
362 SARS-CoV-2 exposed individuals were screened by ELISA at a single 1:40 dilution against RBD.
363 The blue region indicates overlap of OD values between negative and positive control samples. %
364 indicates frequency of negative control values in this range. Experiments were repeated 3 times. (**C**)
365 RBD seroreactivity was quantified based upon time elapsed from PCR+ confirmation of SARS-CoV-
366 2 infection. (**D**) Individuals recruited from the community (5882) were screened for seroreactivity to
367 RBD. (**E**) $PRNT_{90}$ analysis from community drawn samples that displayed indeterminate or positive
368 RBD seroreactivity. Samples that neutralized 90% of virions at least at a 1:20 dilution were
369 considered positive. Experiments were repeated at least once.

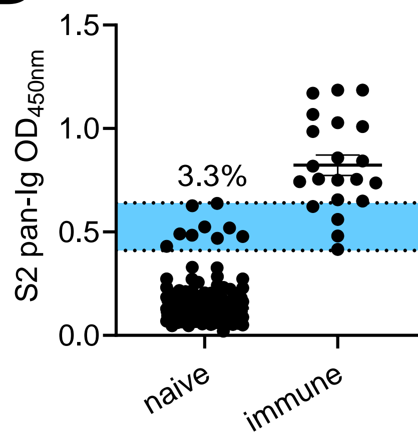
370

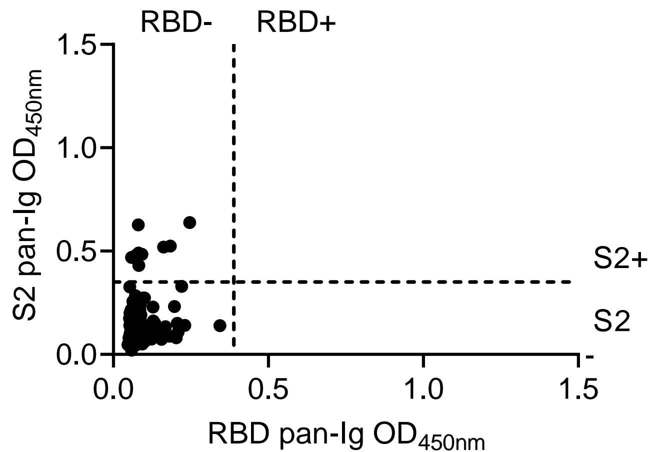
Figure 2

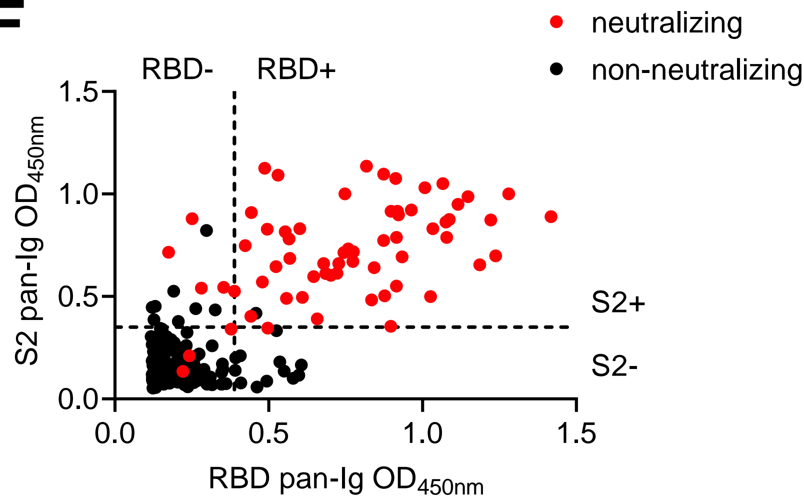












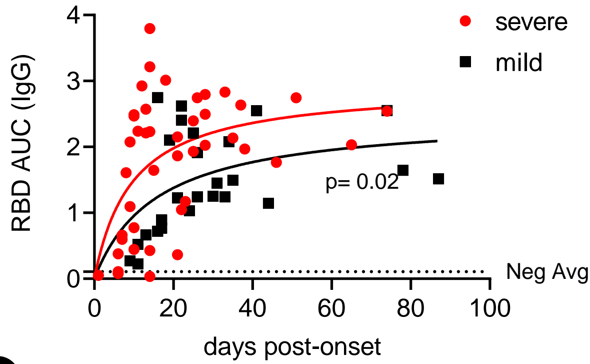
medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

371 **Figure 2: Assessment of S2 and N antibodies as secondary confirmations of seropositivity.**
372 (**A**) Correlations of neutralization and N-specific IgG ELISA titers across 115 serum samples from
373 healthy controls and COVID-19 cases. (**B**) A sample set of 32 pre-pandemic controls and 30 PCR+
374 SARS-CoV-2 samples were assayed for seroreactivity to N protein. Blue shaded region indicates
375 overlap between negative and positive controls. Frequency of negative controls in this range is
376 shown. (**C**) Correlations of neutralization and S2-specific IgG ELISA titers across 114 serum
377 samples from healthy controls and COVID-19 cases. (**D**) Pre-pandemic negative control samples
378 (272) were screened for seroreactivity against S2 and compared to 30 PCR-confirmed SARS-CoV-
379 2-exposed sera. (**E**) Comparison of RBD and S2 seroreactivity across 272 pre-pandemic serum
380 samples. (**F**) ELISA results from indeterminate and putative seropositive samples from community
381 testing. Thresholds for seropositivity were defined as in (**E**). Red circles indicate samples that have
382 $PRNT_{90}$ titers of at least 1:20. Experiments were repeated at least once.

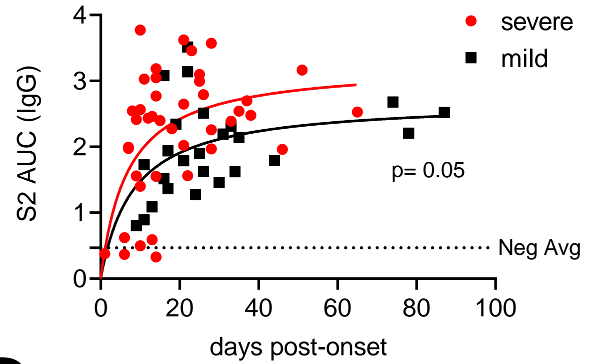
383

Figure 3

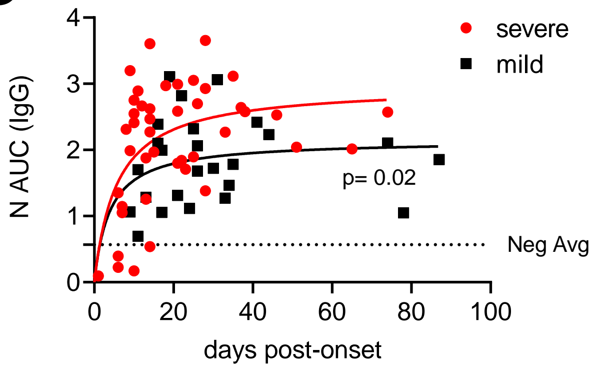
A



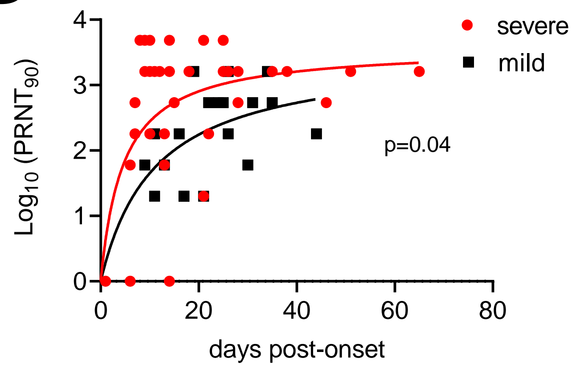
B



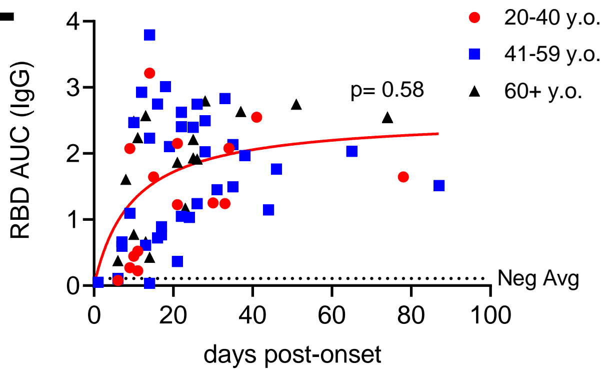
C



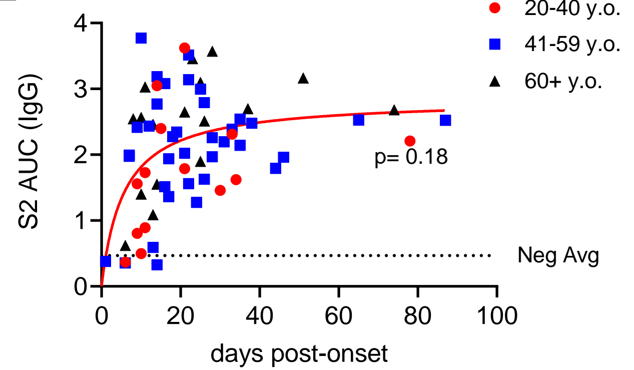
D



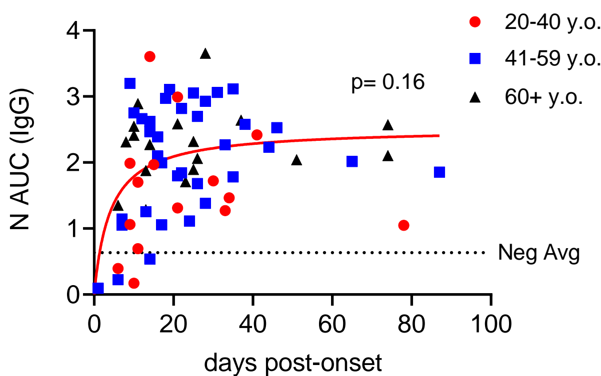
E



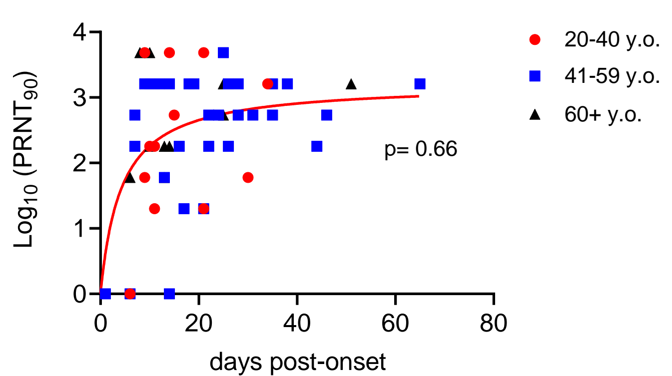
F



G



H



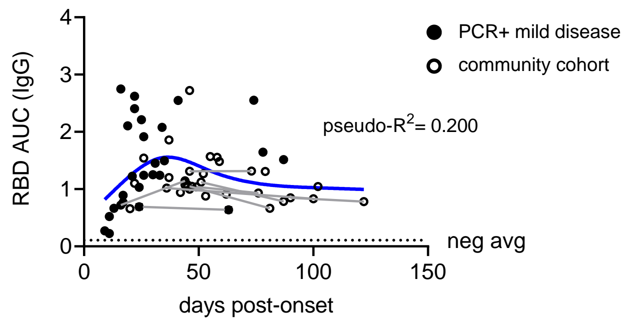
medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

384 **Figure 3: Antibody responses to SARS-Cov2 as a function of disease severity and age. (A-C)**
385 Antibody titers to RBD (A), S2 (B), and N (C), over time post-onset of SARS-CoV-2 infection
386 symptom grouped by case severity. The negative control average was determined by calculating the
387 average AUC value of negative control (n=25) samples. P values represent comparison of fit in non-
388 linear regression model between displayed groups. **(D)** $PRNT_{90}$ values over time post-onset of
389 SARS-CoV-2 infection symptoms. P values were calculated as in (A). **(E-H)** Antibody titers over time
390 post-onset of SARS-CoV-2 infection symptoms from PCR+ confirmed patients or seropositive
391 individuals from community wide cohort for RBD (E), N (F), and S2 (G), grouped by patient age. **(H)**
392 $PRNT_{90}$ values over time post-onset of SARS-CoV-2 infection symptoms grouped by patient age.

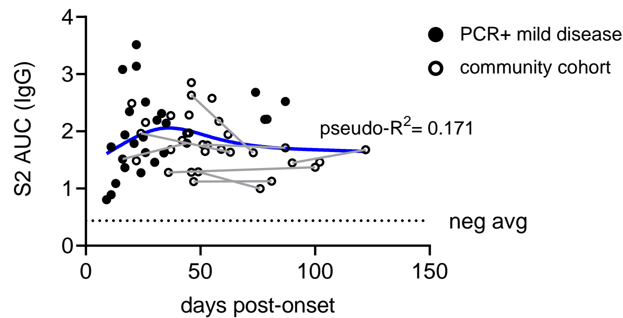
393

Figure 4

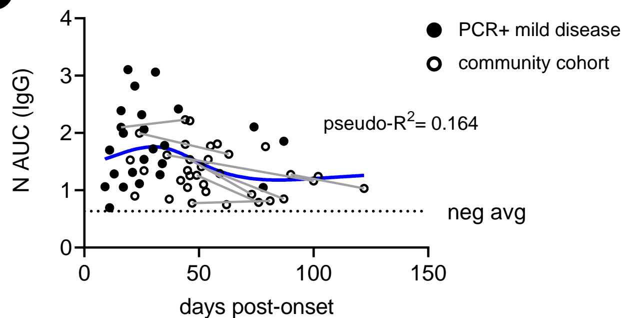
A



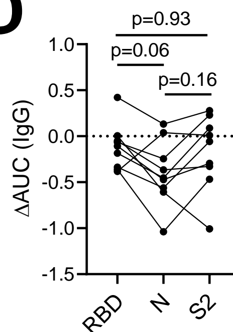
B



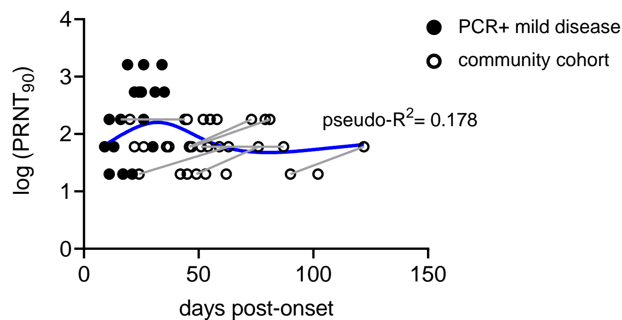
C



D



E



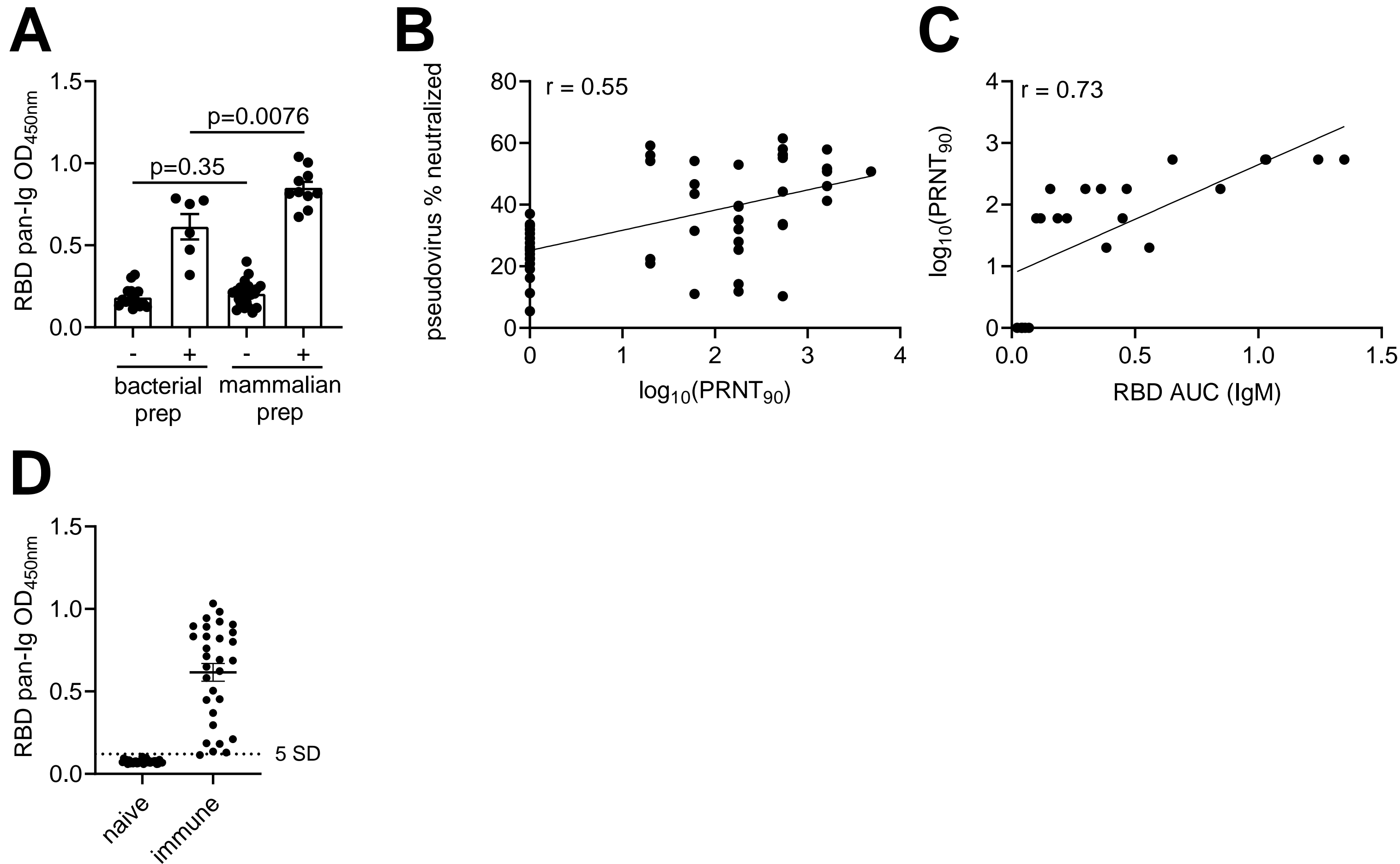
medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

394 **Figure 4: Antibody Responses to Spike Glycoprotein are more stable than responses to**
395 **Nucleocapsid: (A-C)** Antibody titers for mild infections over time to RBD (A), S2 (B), and N (C)
396 for PCR-confirmed subjects and seropositive samples from community serological testing. Solid
397 lines connect data from individuals sampled serially over time. Blue line depicts smoothing
398 splines curve fit with 4 knots. Dashed line depicts mean values from seronegative controls. **(D)**
399 Subjects sampled serially were assessed for changes in antibody titers to RBD, S2, and N from the
400 first draw to the last draw collected. Only subjects in which the last draw occurred >6 weeks from
401 onset are shown. P values were calculated by paired 1-way ANOVA. **(E)** Neutralizing titers were
402 measured for longitudinal subjects over time post-onset. Solid lines connect data from individuals
403 sampled serially over time. Curve (blue line) was generated in using smoothing splines with 4
404 knots.

405

406

Figure S1 related to Figure 1



medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

407 **Figure S1 related to Figure 1: Optimization of RBD-based ELISAs and neutralization assays.**

408 (**A**) RBD derived from mammalian and bacterial expression system were compared by ELISA using

409 SARS-CoV-2-neutralizing sera and controls. Statistical differences were determined by students'

410 unpaired 2-tailed t-test. (**B**) Correlation of pseudovirus neutralization with $PRNT_{90}$ values of live

411 SARS-CoV-2 determined as in Figure 1A. Pseudovirus neutralization titers were determined by %

412 neutralization at a dilution of 1:20. (**C**) Neutralizing titers correlate with IgM antibody titers for RBD.

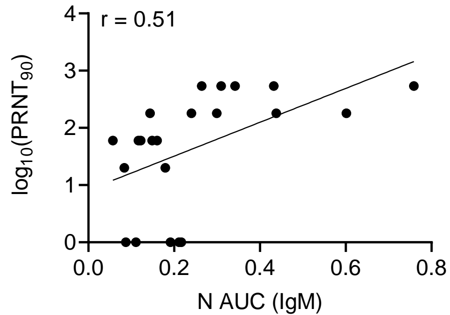
413 For (B) and (C), r value was determined by Pearson correlation. (**D**) ELISA of pre-2020 sera (32)

414 and COVID-19 samples (30) for RBD reactivity.

415

Figure S2 related to Figure 2

A



B

SARS-CoV2 positive
SARS-CoV2 indeterminate
SARS-CoV2 negative

47%
35%
18%

Serum collection 1-7 days
post PCR+, n=17

SARS CoV2 positive
SARS CoV2 indeterminate
SARS CoV2 negative

21.5%
21.5%
57%

serum collection 7-13 days
post PCR+, n=14

2.5%

SARS CoV2 positive
SARS CoV2 indeterminate

97.5%

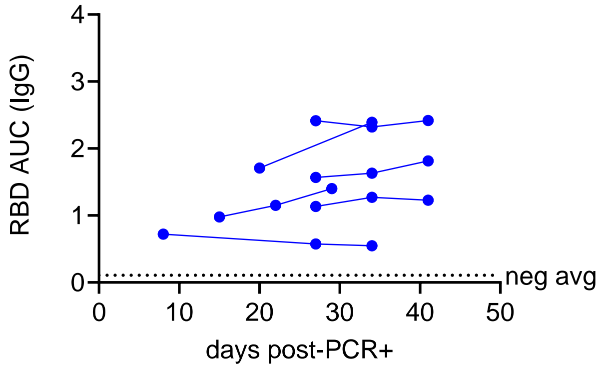
serum collection ≥14 days
post PCR+, n=40

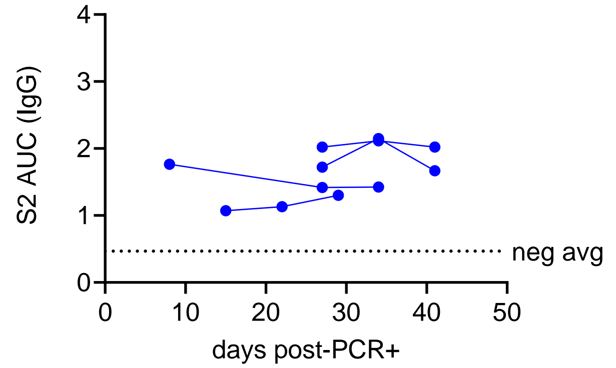
medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

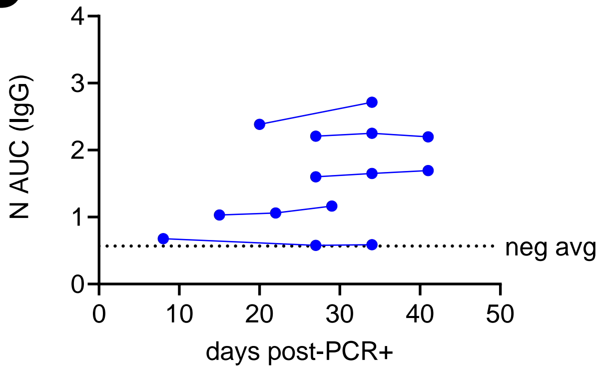
416 **Figure S2 related to Figure 2: Optimization of secondary screens for S2 and N.** (**A**) N-reactive
417 IgM antibody titers were correlated to $PRNT_{90}$ neutralization titers. R value was determined by
418 Pearson's Correlation. (**B**) Following inclusion of S2 as secondary screen, seropositivity results are
419 shown for individuals collected 1-7 days, 7-13 days, or >14 days post-PCR confirmation. Samples
420 match those used in Figure 1C.

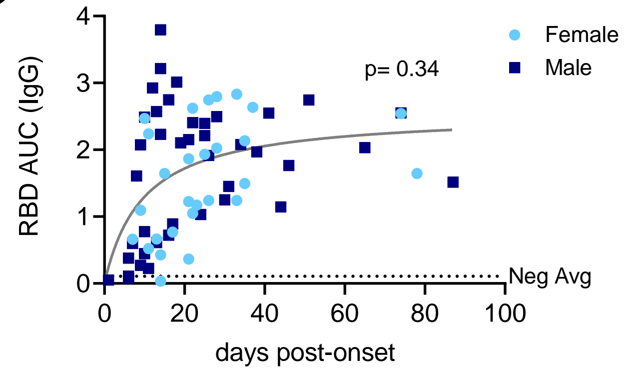
421

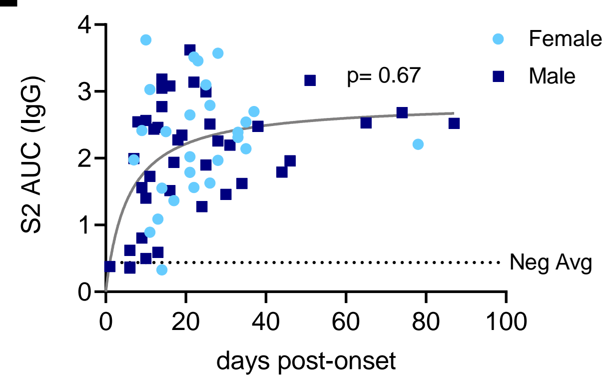
Figure S3 related to Figure 3

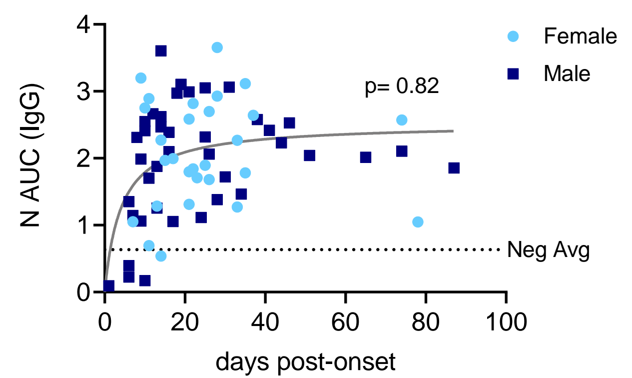


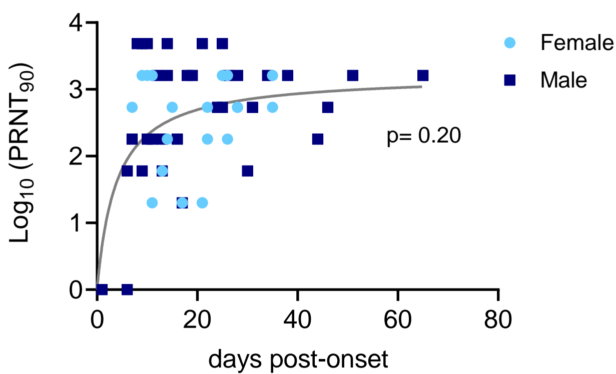












medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

423 **Figure S3 related to Figure 3: Antibody responses to SARS-Cov2 in asymptomatic**
424 **individuals and in females and males. (A-C)** Antibody titers over time post-PCR confirmation of
425 asymptomatic subjects for RBD (A), S2 (B), and N (C). **(D-F)** Antibody titers over time post-onset of
426 SARS-CoV-2 infection symptoms from PCR+ confirmed patients or ELISA seropositive $PRNT_{90}$+
427 individuals from community wide cohort for RBD (A), S2 (B), and N (C), grouped by patient sex. **(G)**
428 $PRNT_{90}$ values over time post-onset of SARS-CoV-2 infection symptoms grouped by patient sex, p
429 value calculated as described previously.

430

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

430 **Table S1. Demographics and essential clinical characteristics of subjects analyzed in this**
431 **study.** Recruitment started in early April, 2020, and included the groups described above and in
432 Methods. Banner-UMC group was restricted to hospitalized subjects; Targeted Community included
433 subjects recruited via fliers and word-of-mouth within faculty, staff and contacts of University of
434 Arizona and Banner-UMC. Self-enrolled community were enrolled via website into the UArizona
435 Antibody Testing Study, supported by the State of Arizona contract; the testing was open to
436 community, first responders and health care workers in Tucson, AZ. *1 intersex participant.

437

COHORT	Number of Subjects	Age Range (Median age)	Sex Female/Male (% F/M)	PCR+ # (%)	Required O2/ intubation	Deceased # (%)
Banner-UMC Hospital	57	22-83 (59)	25/32 (43.9/56.1)	57 (100)	44 (77.2)	15 (26.3)
Targeted Community	32	22-80 (47)	14/18 (43.8/56.3)	32 (100)	N/A	N/A
Self-enrolled Community	5882	18-85 (40)	3082/2799* (52.4/47.6)	6 out of 148 tested (4.1)	N/A	N/A

438
439

440

441

442

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

443 **METHODS:**

444 **Human subjects:** All human subject work was approved by the University of Arizona
445 IRB and was conducted in accordance with all federal, state and local regulations and
446 guidelines under the protocols # 1510182734 and 1410545697A048. Human subject group
447 characteristics are described in Supplementary Table 1, as well as below in the text. Subjects
448 were recruited in three ways. First, targeted recruitment was used to recruit confirmed positive
449 COVID-19 PCR test subjects with severe COVID-19, defined as one that needed hospitalization
450 into the Banner-University Medical Center. Second, targeted recruitment was used to recruit
451 subjects with confirmed positive COVID-19 PCR test who did not require hospitalization
452 (mild/moderate COVID-19 cases). Finally, the vast majority of subjects were recruited via public
453 announcement and website registration as part of the University of Arizona Antibody Testing
454 Pilot. Following website registration, subjects were consented and bled. Blood was centrifuged
455 at six sites across Tucson, AZ, between April 30 and May 7th. For all subjects, venous blood
456 was obtained by venipuncture into SST Vacutainer tubes (Becton-Dickinson, Sunnyvale, CA,
457 cat. #367988), serum separated by centrifugation at 1,200 rpm and sent to the central
458 processing laboratory within 4 h. For both hospitalized and non-hospitalized targeted
459 recruitment groups, following aliquoting, serum was used for the ELISA assay with or without
460 freezing and thawing as described below. Finally, sera from 352 subjects recruited into the
461 above two IRB protocols prior to September, 2019, served as negative controls for assay
462 development. Based on local and general prevalence, it would be expected that 96-98% of
463 these subjects have previously encountered seasonal coronaviruses (Gorse et al., 2010).
464 Freezing and thawing had no effect on levels of antibodies detected by ELISA or PRNT.

465 **Virus:** SARS-Related Coronavirus 2, Isolate USA-WA1/2020 (BEI NR-52281) was
466 passaged once on Vero (ATCC #CCL-81) cells at a MOI of 0.01 for 72 hours. Supernatant and
467 cell lysate were combined, subjected to a single freeze-thaw, and then centrifuged at 3000RPM
468 for 10 minutes to remove cell debris.

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

469 **Antigens and Antiviral antibody assay**: The bacterially-produced recombinant
470 receptor-binding domain (RBD) of the spike (S) glycoprotein was a gift of Dr Daved Fremont
471 (Washington University, St. Louis, MO). Mammalian RBD was purchased from Genscript
472 (catalog # Z03483). SARS-CoV-2 N (nucleocapsid) protein was purchased from Genscript
473 (catalog # Z03488), and S2 subdomain of the SARS-CoV-2 S glycoprotein was purchased from
474 Sino Biological (catalog # 40590-V08B).

475 Enzyme-linked immunosorbent assay (ELISA) was performed as described (Amanat et
476 al., 2020) with several minor modifications. To obtain titers, antigens were immobilized on high-
477 adsorbency 96-well plates at 5 ng/ml. Plates were blocked with 1% non-fat dehydrated milk
478 extract (Santa Cruz Biotechnology #sc-2325) in sterile PBS (Fisher Scientific Hyclone PBS
479 #SH2035,) for 1 hour, washed with PBS containing 0.05% Tween-20, and overlaid with serial
480 dilutions of the serum or plasma for 60 min. Plates were then washed and incubated for 1hr in
481 1% PBS and milk containing an anti-human IgG-HRP conjugated antibody (Jackson
482 Immunoresearch catalog 709-035-149) at a concentration of 1:2000 for 1 hour. For IgM
483 detection an anti-human IgM-HRP conjugated antibody (Jackson Immunoresearch catalog 709-
484 035-073) was used at a concentration of 1:5000 and incubated for 1 hour. Plates were washed
485 with PBS-Tween solution followed by PBS wash. To develop, plates were incubated in
486 tetramethylbenzidine prior to quenching with 2N H_2SO_4. Plates were then read for 450nm
487 absorbance.

488 ELISAs on community-wide samples were performed at the University of Arizona
489 Genomics Core. A 384 well format was applied for high throughput screening, with protocol
490 conditions remaining identical except for the substitution of anti-human Pan-Ig HRP conjugated
491 antibody (Jackson Immunoresearch catalog 109-035-064). Plates were read for 450nm
492 absorbance on CLARIOstar Plus from BMG Labtech. Samples with OD_{630} values greater than
493 0.05 were re-run. Every plate contained at least 32 seronegative controls and either CR3022 or

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

494 HM3128 (Creative Diagnostics) monoclonal antibodies as a positive control for RBD or S2,
495 respectively.
496
497 **Plaque reduction neutralization test:** A plaque reduction neutralization test (PRNT) for
498 SARS-CoV-2 was developed based on our prior work (Uhrlaub et al., 2011). Briefly, Vero cells
499 (ATCC # CCL-81) were plated in 96 well tissue culture plates and grown overnight. Serial
500 dilutions of plasma/serum samples were incubated with 100 plaque forming units of SARS-CoV-
501 2 for 1 hour at 37° C. Plasma/serum dilutions plus virus were transferred to the cell plates and
502 incubated for 2 hours at 37°C, 5% CO_2 then overlayed with 1% methylcellulose. After 72
503 hours, plates were fixed with 10% Neutral Buffered Formalin for 30 minutes and stained with 1%
504 crystal violet. Plaques were imaged using an ImmunoSpot Versa (Cellular Technology Limited,
505 Cleveland, OH) plate reader. The serum/plasma dilution that contained 10 or less plaques was
506 designated as the NT90 titer.
507 **Statistical analysis:** Statistical analyses are described in the corresponding Figure
508 Legends.
509
510
511
512
513

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

514 **REFERENCES:**

515 Abbott, R.K., Lee, J.H., Menis, S., Skog, P., Rossi, M., Ota, T., Kulp, D.W., Bhullar, D.,
516 Kalyuzhniy, O., Havenar-Daughton, C., et al. (2018). Precursor Frequency and Affinity
517 Determine B Cell Competitive Fitness in Germinal Centers, Tested with Germline-Targeting HIV
518 Vaccine Immunogens. Immunity *48*, 133-146.e6.

519 Amanat, F., Stadlbauer, D., Strohmeier, S., Nguyen, T.H.O., Chromikova, V., McMahon, M.,
520 Jiang, K., Arunkumar, G.A., Jurczyszak, D., Polanco, J., et al. (2020). A serological assay to
521 detect SARS-CoV-2 seroconversion in humans. Nature Medicine 1–4.

522 Amanna, I.J. (2007). Duration of Humoral Immunity to Common Viral and Vaccine Antigens.
523 The New England Journal of Medicine *357*, 1903–1915.

524 Atyeo, C., Fischinger, S., Zohar, T., Slein, M.D., Burke, J., Loos, C., McCulloch, D.J., Newman,
525 K.L., Wolf, C., Yu, J., et al. (2020). Distinct Early Serological Signatures Track with SARS-CoV-
526 2 Survival. Immunity *0*.

527 Bendavid, E., Mulaney, B., Sood, N., Shah, S., Ling, E., Bromley-Dulfano, R., Lai, C.,
528 Weissberg, Z., Saavedra-Walker, R., Tedrow, J., et al. (2020). COVID-19 Antibody
529 Seroprevalence in Santa Clara County, California. MedRxiv 2020.04.14.20062463.

530 Bennett, S.T., and Steyvers, M. (2020). Estimating COVID-19 Antibody Seroprevalence in
531 Santa Clara County, California. A re-analysis of Bendavid et al. MedRxiv 2020.04.24.20078824.

532 Bhattacharya, D., Cheah, M.T., Franco, C.B., Hosen, N., Pin, C.L., Sha, W.C., and Weissman,
533 I.L. (2007). Transcriptional Profiling of Antigen-Dependent Murine B Cell Differentiation and
534 Memory Formation. The Journal of Immunology *179*, 6808–6819.

535 Bosch, B.J., van der Zee, R., de Haan, C.A.M., and Rottier, P.J.M. (2003). The Coronavirus
536 Spike Protein Is a Class I Virus Fusion Protein: Structural and Functional Characterization of the
537 Fusion Core Complex. J. Virol. *77*, 8801.

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

538 Bryan, A., Pepper, G., Wener, M.H., Fink, S.L., Morishima, C., Chaudhary, A., Jerome, K.R.,
539 Mathias, P.C., and Greninger, A.L. (2020). Performance Characteristics of the Abbott Architect
540 SARS-CoV-2 IgG Assay and Seroprevalence in Boise, Idaho. Journal of Clinical Microbiology.

541 Burbelo, P.D., Riedo, F.X., Morishima, C., Rawlings, S., Smith, D., Das, S., Strich, J.R.,
542 Chertow, D.S., Davey, R.T., and Cohen, J.I. (2020). Detection of Nucleocapsid Antibody to
543 SARS-CoV-2 is More Sensitive than Antibody to Spike Protein in COVID-19 Patients. MedRxiv
544 2020.04.20.20071423.

545 Callow, K.A., Parry, H.F., Sergeant, M., and Tyrrell, D. a. J. (1990). The time course of the
546 immune response to experimental coronavirus infection of man. Epidemiology & Infection *105*,
547 435–446.

548 Cao, W.-C., Liu, W., Zhang, P.-H., Zhang, F., and Richardus, J.H. (2007). Disappearance of
549 antibodies to SARS-associated coronavirus after recovery. N. Engl. J. Med. *357*, 1162–1163.

550 Choe, P.G., Kang, C.K., Suh, H.J., Jung, J., Kang, E., Lee, S.Y., Song, K.-H., Kim, H.B., Kim,
551 N.J., Park, W.B., et al. Early Release - Antibody Responses to SARS-CoV-2 at 8 Weeks
552 Postinfection in Asymptomatic Patients - Volume 26, Number 10—October 2020 - Emerging
553 Infectious Diseases journal - CDC.

554 Giroglou, T., Cinatl, J., Rabenau, H., Drosten, C., Schwalbe, H., Doerr, H.W., and Laer, D. von
555 (2004). Retroviral Vectors Pseudotyped with Severe Acute Respiratory Syndrome Coronavirus
556 S Protein. Journal of Virology *78*, 9007–9015.

557 Gorse, G.J., Patel, G.B., Vitale, J.N., and O'Connor, T.Z. (2010). Prevalence of Antibodies to
558 Four Human Coronaviruses Is Lower in Nasal Secretions than in Serum. Clin. Vaccine Immunol.
559 *17*, 1875–1880.

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

560 Guo, X., Guo, Z., Duan, C., Chen, Z., Wang, G., Lu, Y., Li, M., and Lu, J. (2020). Long-Term
561 Persistence of IgG Antibodies in SARS-CoV Infected Healthcare Workers. MedRxiv
562 2020.02.12.20021386.

563 Hou, H., Wang, T., Zhang, B., Luo, Y., Mao, L., Wang, F., Wu, S., and Sun, Z. (2020). Detection
564 of IgM and IgG antibodies in patients with coronavirus disease 2019. Clinical and Translational
565 Immunology.

566 Ibarrondo, F.J., Fulcher, J.A., Goodman-Meza, D., Elliott, J., Hofmann, C., Hausner, M.A.,
567 Ferbas, K.G., Tobin, N.H., Aldrovandi, G.M., and Yang, O.O. (2020). Rapid Decay of Anti–
568 SARS-CoV-2 Antibodies in Persons with Mild Covid-19. N Engl J Med.

569 Isho, B., Abe, K.T., Zuo, M., Jamal, A.J., Rathod, B., Wang, J.H., Li, Z., Chao, G., Rojas, O.L.,
570 Bang, Y.M., et al. (2020). Evidence for sustained mucosal and systemic antibody responses to
571 SARS-CoV-2 antigens in COVID-19 patients. MedRxiv 2020.08.01.20166553.

572 Iyer, A.S., Jones, F.K., Nodoushania, A., Kelly, M., Becker, M., Slater, D., Mills, R., Teng, E.,
573 Kamruzzaman, M., Garcia-Beltran, W.F., et al. (2020). Dynamics and significance of the
574 antibody response to SARS-CoV-2 infection. MedRxiv 2020.07.18.20155374.

575 Jash, A., Wang, Y., Weisel, F.J., Scharer, C.D., Boss, J.M., Shlomchik, M.J., and Bhattacharya,
576 D. (2016). ZBTB32 Restricts the Duration of Memory B Cell Recall Responses. J. Immunol. *197*,
577 1159–1168.

578 Jash, A., Zhou, Y.W., Gerardo, D.K., Ripperger, T.J., Parikh, B.A., Piersma, S., Jamwal, D.R.,
579 Kiela, P.R., Boon, A.C.M., Yokoyama, W.M., et al. (2019). ZBTB32 restrains antibody
580 responses to murine cytomegalovirus infections, but not other repetitive challenges. Sci Rep *9*,
581 15257.

582 Klein, S.L., Dhakal, S., Ursin, R.L., Deshpande, S., Sandberg, K., and Mauvais-Jarvis, F.
583 (2020a). Biological sex impacts COVID-19 outcomes. PLOS Pathogens *16*, e1008570.

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint
(which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

584 Klein, S.L., Pekosz, A., Park, H.-S., Ursin, R.L., Shapiro, J.R., Benner, S.E., Littlefield, K.,
585 Kumar, S., Naik, H.M., Betenbaugh, M., et al. (2020b). Sex, age, and hospitalization drive
586 antibody responses in a COVID-19 convalescent plasma donor population. J Clin Invest *online*
587 *ahead of print*.

588 Ko, J.-H., Joo, E.-J., Park, S.-J., Baek, J.Y., Kim, W.D., Jee, J., Kim, C.J., Jeong, C., Kim, Y.-J.,
589 Shon, H.J., et al. (2020). Neutralizing Antibody Production in Asymptomatic and Mild COVID-19
590 Patients, in Comparison with Pneumonic COVID-19 Patients. Journal of Clinical Medicine *9*,
591 2268.

592 Lavinder, J.J., Wine, Y., Giesecke, C., Ippolito, G.C., Horton, A.P., Lungu, O.I., Hoi, K.H.,
593 DeKosky, B.J., Murrin, E.M., Wirth, M.M., et al. (2014). Identification and characterization of the
594 constituent human serum antibodies elicited by vaccination. Proc Natl Acad Sci USA *111*, 2259.

595 Li, F. (2016). Structure, Function, and Evolution of Coronavirus Spike Proteins. Annu. Rev.
596 Virol. *3*, 237–261.

597 Liu, K., Chen, Y., Lin, R., and Han, K. (2020). Clinical features of COVID-19 in elderly patients:
598 A comparison with young and middle-aged patients. Journal of Infection *80*, e14–e18.

599 Long, Q.-X., Tang, X.-J., Shi, Q.-L., Li, Q., Deng, H.-J., Yuan, J., Hu, J.-L., Xu, W., Zhang, Y.,
600 Lv, F.-J., et al. (2020a). Clinical and immunological assessment of asymptomatic SARS-CoV-2
601 infections. Nat. Med. *online ahead of print*.

602 Long, Q.-X., Liu, B.-Z., Deng, H.-J., Wu, G.-C., Deng, K., Chen, Y.-K., Liao, P., Qiu, J.-F., Lin,
603 Y., Cai, X.-F., et al. (2020b). Antibody responses to SARS-CoV-2 in patients with COVID-19.
604 Nat Med *26*, 845–848.

605 Manz, R.A., Thiel, A., and Radbruch, A. (1997). Lifetime of plasma cells in the bone marrow.
606 Nature *388*, 133–134.

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

607 Palaiodimos, L., Kokkinidis, D.G., Li, W., Karamanis, D., Ognibene, J., Arora, S., Southern,
608 W.N., and Mantzoros, C.S. (2020). Severe obesity, increasing age and male sex are
609 independently associated with worse in-hospital outcomes, and higher in-hospital mortality, in a
610 cohort of patients with COVID-19 in the Bronx, New York. Metabolism *108*, 154262.

611 Pollán, M., Pérez-Gómez, B., Pastor-Barriuso, R., Oteo, J., Hernán, M.A., Pérez-Olmeda, M.,
612 Sanmartín, J.L., Fernández-García, A., Cruz, I., Larrea, N.F. de, et al. (2020). Prevalence of
613 SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological
614 study. The Lancet *online ahead of print*.

615 Premkumar, L., Segovia-Chumbez, B., Jadi, R., Martinez, D.R., Raut, R., Markmann, A.J.,
616 Cornaby, C., Bartelt, L., Weiss, S., Park, Y., et al. (2020). The receptor-binding domain of the
617 viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-
618 2 patients. Science Immunology *5*, eabc8413.

619 Purtha, W.E., Tedder, T.F., Johnson, S., Bhattacharya, D., and Diamond, M.S. (2011). Memory
620 B cells, but not long-lived plasma cells, possess antigen specificities for viral escape mutants.
621 The Journal of Experimental Medicine *208*, 2599–2606.

622 Qu, J., Wu, C., Li, X., Zhang, G., Jiang, Z., Li, X., Zhu, Q., and Liu, L. (2020). Profile of
623 Immunoglobulin G and IgM Antibodies Against Severe Acute Respiratory Syndrome
624 Coronavirus 2 (SARS-CoV-2). Clinical Infectious Diseases.

625 Reed, S.E. (1984). The behaviour of recent isolates of human respiratory coronavirus in vitro
626 and in volunteers: Evidence of heterogeneity among 229E-related strains. Journal of Medical
627 Virology *13*, 179–192.

628 Rosenberg, E.S., Tesoriero, J.M., Rosenthal, E.M., Chung, R., Barranco, M.A., Styer, L.M.,
629 Parker, M.M., John Leung, S.-Y., Morne, J.E., Greene, D., et al. (2020). Cumulative incidence
630 and diagnosis of SARS-CoV-2 infection in New York. Annals of Epidemiology.

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

631 Schmidt, F., Weisblum, Y., Muecksch, F., Hoffmann, H.-H., Michailidis, E., Lorenzi, J.C.C.,
632 Mendoza, P., Rutkowska, M., Bednarski, E., Gaebler, C., et al. (2020). Measuring SARS-CoV-2
633 neutralizing antibody activity using pseudotyped and chimeric viruses. J Exp Med *217*.

634 Sekine, T., Perez-Potti, A., Rivera-Ballesteros, O., Strålin, K., Gorin, J.-B., Olsson, A., Llewellyn-
635 Lacey, S., Kamal, H., Bogdanovic, G., Muschiol, S., et al. (2020). Robust T cell immunity in
636 convalescent individuals with asymptomatic or mild COVID-19. BioRxiv 2020.06.29.174888.

637 Seow, J., Graham, C., Merrick, B., Acors, S., Steel, K.J.A., Hemmings, O., O'Bryne, A.,
638 Kouphou, N., Pickering, S., Galao, R., et al. (2020). Longitudinal evaluation and decline of
639 antibody responses in SARS-CoV-2 infection. MedRxiv 2020.07.09.20148429.

640 Slifka, M.K., Antia, R., Whitmire, J.K., and Ahmed, R. (1998). Humoral Immunity Due to Long-
641 Lived Plasma Cells. Immunity *8*, 363–372.

642 Smith, K.G.C., Light, A., Nossal, G.J.V., and Tarlinton, D.M. (1997). The extent of affinity
643 maturation differs between the memory and antibody-forming cell compartments in the primary
644 immune response. The EMBO Journal *16*, 2996–3006.

645 Srinivasan, S., Cui, H., Gao, Z., Liu, M., Lu, S., Mkandawire, W., Narykov, O., Sun, M., and
646 Korkin, D. (2020). Structural Genomics of SARS-CoV-2 Indicates Evolutionary Conserved
647 Functional Regions of Viral Proteins. Viruses *12*, 360.

648 Stadlbauer, D., Tan, J., Jiang, K., Hernandez, M., Fabre, S., Amanat, F., Teo, C., Arunkumar,
649 G.A., McMahon, M., Jhang, J., et al. (2020). Seroconversion of a city: Longitudinal monitoring of
650 SARS-CoV-2 seroprevalence in New York City. MedRxiv 2020.06.28.20142190.

651 Steensels, D., Oris, E., Coninx, L., Nuyens, D., Delforge, M.-L., Vermeersch, P., and Heylen, L.
652 (2020). Hospital-Wide SARS-CoV-2 Antibody Screening in 3056 Staff in a Tertiary Center in
653 Belgium. JAMA *324*, 195–197.

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint
(which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

654 Sterlin, D., Mathian, A., Miyara, M., Mohr, A., Anna, F., Claer, L., Quentric, P., Fadlallah, J.,
655 Ghillani, P., Gunn, C., et al. (2020). IgA dominates the early neutralizing antibody response to
656 SARS-CoV-2. MedRxiv 2020.06.10.20126532.

657 Stringhini, S., Wisniak, A., Piumatti, G., Azman, A.S., Lauer, S.A., Baysson, H., Ridder, D.D.,
658 Petrovic, D., Schrempft, S., Marcus, K., et al. (2020). Seroprevalence of anti-SARS-CoV-2 IgG
659 antibodies in Geneva, Switzerland (SEROCoV-POP): a population-based study. The Lancet
660 *online ahead of print*.

661 Sze, D.M.-Y., Toellner, K.-M., de Vinuesa, C.G., Taylor, D.R., and MacLennan, I.C.M. (2000).
662 Intrinsic Constraint on Plasmablast Growth and Extrinsic Limits of Plasma Cell Survival. J Exp
663 Med *192*, 813–822.

664 Tan, C.W., Chia, W.N., Qin, X., Liu, P., Chen, M.I.-C., Tiu, C., Hu, Z., Chen, V.C.-W., Young,
665 B.E., Sia, W.R., et al. (2020). A SARS-CoV-2 surrogate virus neutralization test based on
666 antibody-mediated blockage of ACE2-spike protein-protein interaction. Nat. Biotechnol. *online*
667 *ahead of print*.

668 To, K.K.-W., Tsang, O.T.-Y., Leung, W.-S., Tam, A.R., Wu, T.-C., Lung, D.C., Yip, C.C.-Y., Cai,
669 J.-P., Chan, J.M.-C., Chik, T.S.-H., et al. (2020). Temporal profiles of viral load in posterior
670 oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2:
671 an observational cohort study. The Lancet Infectious Diseases *20*, 565–574.

672 Uhrlaub, J.L., Brien, J.D., Widman, D.G., Mason, P.W., and Nikolich-Žugich, J. (2011).
673 Repeated In Vivo Stimulation of T and B Cell Responses in Old Mice Generates Protective
674 Immunity against Lethal West Nile Virus Encephalitis. The Journal of Immunology *186*, 3882–
675 3891.

medRxiv preprint doi: https://doi.org/10.1101/2020.08.14.20174490.this version posted August 16, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

676 Wajnberg, A., Amanat, F., Firpo, A., Altman, D., Bailey, M., Mansour, M., McMahon, M., Meade,
677 P., Mendu, D.R., Muellers, K., et al. (2020). SARS-CoV-2 infection induces robust, neutralizing
678 antibody responses that are stable for at least three months. MedRxiv 2020.07.14.20151126.

679 Wang, N.S., McHeyzer-Williams, L.J., Okitsu, S.L., Burris, T.P., Reiner, S.L., and McHeyzer-
680 Williams, M.G. (2012). Divergent transcriptional programming of class-specific B cell memory by
681 T-bet and RORα. Nature Immunology *13*, 604–611.

682 Wrapp, D., Wang, N., Corbett, K.S., Goldsmith, J.A., Hsieh, C.-L., Abiona, O., Graham, B.S.,
683 and McLellan, J.S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion
684 conformation. Science *367*, 1260.

685 Zinkernagel, R.M., and Hengartner, H. (2006). Protective 'immunity' by pre-existent neutralizing
686 antibody titers and preactivated T cells but not by so-called 'immunological memory.'
687 Immunological Reviews *211*, 310–319.

688 Zuccarino-Catania, G.V., Sadanand, S., Weisel, F.J., Tomayko, M.M., Meng, H., Kleinstein,
689 S.H., Good-Jacobson, K.L., and Shlomchik, M.J. (2014). CD80 and PD-L2 define functionally
690 distinct memory B cell subsets that are independent of antibody isotype. Nature Immunology *15*,
691 631–637.

692