

# MENSA, a Media Enriched with Newly Synthesized Antibodies, to Identify SARS-CoV-2 Persistence and Latent Viral Reactivation in Long-COVID.

Natalie S. Haddad<sup>1,2</sup>, Andrea Morrison-Porter<sup>1,2</sup>, Hannah Quehl<sup>1</sup>, Violeta Capric<sup>1</sup>, Pedro A. Lamothe<sup>1</sup>, Fabliha Anam<sup>3</sup>, Martin C. Runnstrom<sup>1,4</sup>, Alex D. Truong<sup>1</sup>, Adviteeya N. Dixit<sup>1</sup>, Matthew C. Woodruff<sup>3,5</sup>, Anting Chen<sup>1</sup>, Jiwon Park<sup>1</sup>, Doan C. Nguyen<sup>1</sup>, Ian Hentenaar<sup>1</sup>, Caroline Y. Kim<sup>1</sup>, Shuya Kyu<sup>1,6</sup>, Brandon Stewart<sup>1</sup>, Elizabeth Wagman<sup>1</sup>, Hannah Geoffroy<sup>1</sup>, Daniel Sanz, Kevin S. Cashman<sup>3,5</sup>, Richard P. Ramonell<sup>1,7,8</sup>, Monica Cabrera-Mora<sup>1</sup>, David N. Alter<sup>6</sup>, John D. Roback<sup>6</sup>, Michael C. Horwath<sup>6</sup>, James B. O'Keefe<sup>9</sup>, Alexandra W. Dretler<sup>10</sup>, Ria Gripaldo<sup>1</sup>, Samantha M. Yeligar<sup>1,4</sup>, Ted Natoli<sup>11</sup>, Viktoria Betin<sup>11</sup>, Rahul Kumar Patel<sup>1,3</sup>, Kennedy Vela<sup>1,3</sup>, Mindy Rodriguez Hernandez<sup>1,3</sup>, Sabeena Usman<sup>1,3</sup>, John Varghese<sup>1,3</sup>, Anum Jalal<sup>1,3</sup>, Saeyun Lee<sup>1,3</sup>, Sang N. Le<sup>1</sup>, R. Toby Amoss<sup>1,3</sup>, John L. Daiss<sup>2</sup>, Ignacio Sanz<sup>3,5</sup>, F. Eun-Hyung Lee<sup>1,5</sup>

## Affiliations:

<sup>1</sup>Division of Pulmonary, Allergy, Critical Care & Sleep Medicine, Department of Medicine, Emory University, Atlanta, GA, 30322, USA.

<sup>2</sup> MicroB-plex Inc, Atlanta, GA, 30332, USA.

<sup>3</sup>Division of Rheumatology, Department of Medicine, Emory University, Atlanta, GA, 30322, USA.

<sup>4</sup> Department of Medicine, Atlanta Veterans Affairs Health Care System, Decatur, Georgia, 30033, USA

<sup>5</sup>Lowance Center for Human Immunology, Emory University, Atlanta, GA, 30322, USA.

<sup>6</sup>Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA, 30322, USA.

<sup>7</sup> Division of Pulmonary, Allergy, Critical Care, and Sleep Medicine, Department of Medicine, University of Pittsburgh, Pittsburgh, PA, 15213, USA.

<sup>8</sup> Asthma and Environmental Lung Health Institute, University of Pittsburgh Medical Center, Pittsburgh, PA, 15213, USA.

<sup>9</sup> Division of General Internal Medicine, Department of Medicine, Emory University School of Medicine, Atlanta, GA, 30322, USA.

<sup>10</sup> Metro Infectious Disease Consultants, Decatur GA 30033, USA.

<sup>11</sup>ImmunelD, Inc Biotechnology Research, Waltham, MA, 02451, USA.

Disclaimer: This work does not necessarily represent the views of the US Government or Department of Veterans Affairs

Corresponding Author:

F. Eun-Hyung Lee, MD

Division of Pulmonary, Allergy, & Critical Care & Sleep Medicine

Emory University

615 Michael Street, Suite 205

Atlanta, GA 30322

404-712-2970

f.e.lee@emory.edu

**Running Title:**

MENSA identifies viral drivers in PASC.

49 **Author Contributions**

50 FEL, JLD, and IS designed and supervised the study. NSH, FEL, JLD, SMY, AMP, and IS wrote  
51 and/or edited the manuscript. NSH, FEL, and JLD developed and optimized the multiplex  
52 immunoassays and MENSA technology used in this study. NSH, AMP, HQ, FA, VC, AC, and  
53 MCW performed experiments and analyzed the data. AMP, HQ, FA, AC, JP, DCN, IH, CYK, SK,  
54 BS, EW, HG, DS, KSC, MCM, and NSH collected and processed the samples. TN and VB  
55 performed the PhIP-seq experiments. VC, RTA, PAL, MRH, MCR, ADT, AWD, RG, RP, KV, SU,  
56 JV, AJ, SL, SNL, AND, JBO, and RPR recruited and managed patient enrollment. DNA, JDR,  
57 and MCH provided serum samples. All authors contributed to the manuscript revision, read, and  
58 approved the submitted version.

59

60

61 **Acknowledgments**

62 This work was supported by National Institute of Allergy and Infectious Diseases, National  
63 Institutes of Health 3P01AI125180-05S1, R01AI121252, R01AI 172254, P01A1078907,  
64 U01AI045969, U19AI109962, U54CA260563, T32HL116271-07, and NIGMS 2T32GM095442,  
65 NIH Department of Health and Human Services/Public Health Services: 5T32AI74492-14.

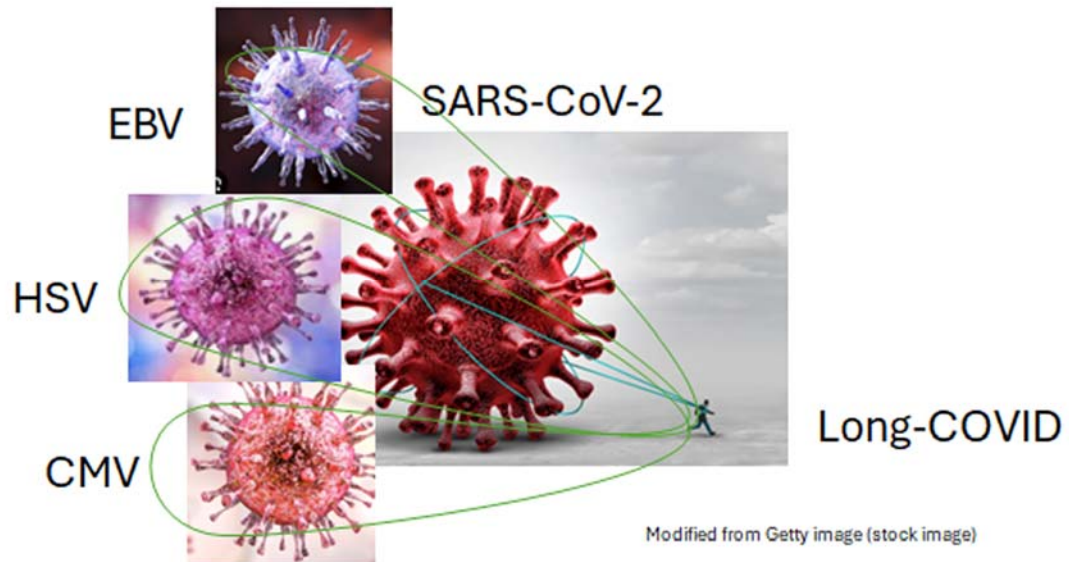
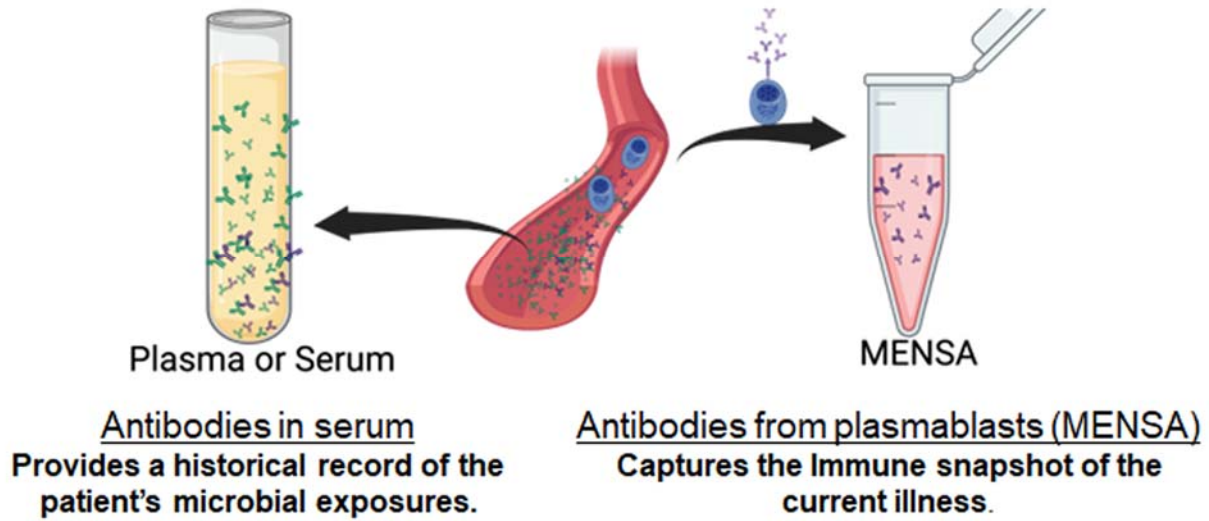
66 **ABSTRACT**

67

68 Post-acute sequelae of SARS-CoV-2 (SARS2) infection (PASC) is a heterogeneous condition,  
69 but the main viral drivers are unknown. Here, we use MENSA, Media Enriched with Newly  
70 Synthesized Antibodies, secreted exclusively from circulating human plasmablasts, to provide  
71 an immune snapshot that defines the underlying viral triggers. We provide proof-of-concept  
72 testing that the MENSA technology can capture the new host immune response to accurately  
73 diagnose acute primary and breakthrough infections when known SARS2 virus or proteins are  
74 present. It is also positive after vaccination when spike proteins elicit an acute immune  
75 response. Applying the same principles for long-COVID patients, MENSA is positive for SARS2  
76 in 40% of PASC vs none of the COVID recovered (CR) patients without any sequelae  
77 demonstrating ongoing SARS2 viral inflammation only in PASC. Additionally, in PASC patients,  
78 MENSAs are also positive for Epstein-Barr Virus (EBV) in 37%, Human Cytomegalovirus (CMV)  
79 in 23%, and herpes simplex virus 2 (HSV2) in 15% compared to 17%, 4%, and 4% in CR  
80 controls respectively. Combined, a total of 60% of PASC patients have a positive MENSA for  
81 SARS2, EBV, CMV, and/or HSV2. MENSA offers a unique antibody snapshot to reveal the  
82 underlying viral drivers in long-COVID thus demonstrating the persistence of SARS2 and  
83 reactivation of viral herpes in 60% of PASC patients.

## Graphical abstract

# Serum vs. MENSA



## 84 INTRODUCTION

85

86 In December 2019, the world was changed when the SARS-CoV-2 (SARS2) virus was identified  
87 in Wuhan, China and rapidly spread throughout the world. The first U.S. case was identified in  
88 January 2020<sup>1</sup>, and by March 2020 the World Health Organization (WHO) had officially declared  
89 COVID-19 to be a global pandemic. By the end of 2020, there had been a total of 79 million  
90 reported cases and over 1.7 million deaths globally<sup>2</sup>. Vaccines and antiviral therapies allowed us  
91 to combat this new global threat and emerge from this devastating pandemic<sup>3,4</sup>. However, after  
92 many in the US received the primary mRNA vaccines, a new Delta virus surged, followed by the  
93 Omicron (B.1.1.529) variant by the end of 2021, which had increased transmissibility<sup>5</sup>.

94

95 In addition to new viral variant infections, some patients suffered from sequelae after the initial  
96 acute infection. Long-COVID or post-acute sequelae of SARS-CoV-2 infection (PASC) is a  
97 condition described as ongoing, relapsing, or new symptoms present after the acute phase of  
98 the infection. Incidence of PASC was notable in approximately 10% of patients after acute  
99 infection<sup>6</sup>. Definitions by the CDC used symptoms > 30 days after acute infection whereas the  
100 WHO described continuation or development of new symptoms 3 months after the initial SARS2  
101 infection, with symptoms lasting for at least 2 months with no other explanation<sup>7</sup>. The diversity of  
102 symptoms and differences in plasma proteomics between inflammatory and quiescent PASC  
103 subsets attest to the heterogeneity of long-COVID<sup>8</sup>. Additionally, many multiomic studies have  
104 identified metabolic and inflammatory derangements such as decreased cortisol or serotonin  
105 levels<sup>9-12</sup>, complement dysregulation<sup>13</sup>, and alternations of cytotoxic T cell, atypical B cell, or  
106 neutrophil signatures in PASC patients compared to adults who recovered from SARS2 without  
107 sequelae<sup>9,10</sup>. Some studies suggest that these inflammatory changes may be triggered by viral  
108 persistence of SARS2 and/or reactivation of latent EBV<sup>10,14</sup>. Interestingly, these studies used  
109 viral PCR testing which is known to be less sensitive in the blood or requires ultrasensitive spike  
110 antigen tests. Another study showed higher serum levels of EBV antibodies in PASC patients  
111 (47%) compared to healthy adults (28%) but there was significant overlap between the two  
112 groups<sup>9,15</sup> due to serum antibodies in response to a previous infection, confounding the  
113 observation of new or ongoing immune responses.

114

115 In this study, we use a novel diagnostic platform whereby we capture antibodies secreted from  
116 plasmablasts or newly-minted antibody secreted cells (ASC). This method can identify new or  
117 repeat infections despite elevated serum antibodies since these ASC appear in circulation  
118 shortly after infection or vaccination, then rapidly disappear from the blood<sup>16-20 21,22</sup>. By capturing  
119 antibodies from these special ASC in a new matrix called Media Enriched with Newly  
120 Synthesized Antibodies (MENSA), we provide a signature response from only the new illness.  
121 MENSA antibodies often appear prior to seroconversion and differ from serum antibodies, which  
122 confound results with the patient's entire historical microbial record. In prior studies, we have  
123 successfully used MENSA to diagnose acute Lyme disease, *Clostridioides difficile*,  
124 *Streptococcus pneumoniae*, and deep bone/tissue infections with *Staphylococcus aureus*<sup>19,23-</sup>  
125 <sup>27</sup>. In all, the novel MENSA assay can successfully diagnose repeat bacterial and viral infections  
126 from a single blood sample even when serum antibody titers are extremely high, showing the  
127 assay's exceptional ability to resolve complexity of antibody signals.

128

129 Here, we provide proof-of-concept testing that the MENSA technology can capture the new host  
130 immune response to accurately diagnose acute primary and breakthrough infections when  
131 known SARS2 virus or proteins are present. It is also positive after vaccination when spike  
132 proteins elicit an acute immune response. Applying the same principles for long-COVID  
133 patients, we use MENSA to identify SARS2, EBV, CMV, and/or HSV2 as the underlying viral  
134 drivers in 60% of PASC patients. With a single blood sample, this novel diagnostic assay shows  
135 persistence of SARS2 and/or reactivation of latent herpes viruses in long-COVID patients.

## RESULTS

**Patient enrollment.** For the purpose of measuring serum and MENSA responses against SARS2 during infection and after vaccination, we enrolled a total of 241 adults between 2020 and 2024 at Emory University in Atlanta, GA and collected blood to generate the MENSA matrix and serum samples (**Table 1**). During the first year of the pandemic in 2020, we enrolled 110 adults with PCR- positive nasopharyngeal swabs (NPS) during their primary SARS2 infection. Fifty-four adults were outpatients with mild/moderate (M/M) disease as defined by the NIH criteria<sup>28</sup>, and 56 adults had severe/critical (S/C) illness in the Intensive Care Units. From the 54 M/M adults, we collected 59 blood samples: 16 during acute infection (within 30 days post-symptom onset (DPSO)) and 43 during convalescence (60 DPSO). Five patients provided both acute and convalescent samples. Of the 56 S/C patients, we had 60 samples: 40 provided acute illness time points and 20 at convalescence. Four patients provided samples at both time points. These patients will be referred to as the primary SARS2 infection population. We also enrolled 60 healthy adults who had no known exposure to SARS2 and provided blood samples during the initial lockdown period, between March and June 2020. This group is referred to as the healthy controls without prior SARS2 exposure.

Serum and MENSA samples were collected from 11 vaccinated subjects who had no SARS2 infection prior to their primary two-dose mRNA vaccine series (Pfizer n=5, Moderna n=6). We also enrolled 3 adults with Omicron breakthrough infections from December 2021-June 2022. Finally, we enrolled 61 PASC patients from the Emory Long-COVID clinic from January 2021 to February 2024 and compared against 25 COVID recovered patients without any sequelae (CR). Of note, some of the same subjects were enrolled in different groups. For example, some of the healthy controls were later vaccinated and/or tested positive for breakthrough infections in the 4 years of the study; some patients after acute primary and/or breakthrough COVID infections without any sequelae were also included in the CR groups.

**SARS2 RBD and N antigen selection.** SARS2 antigens were selected for measuring responses to SARS2 infection and vaccination. Fluorescent bead assays were used as previously described for the SARS2 antigens with spike S1, S1 receptor binding domain (RBD), S1 N-terminal domain (NTD), S2, nucleocapsid (N), and ORF-3a<sup>29</sup>. We measured the acute IgG antibody responses against all six antigens in 56 adults with primary acute SARS2 infection between 6-28 DPSO and compared them against 60 healthy control adults with no known SARS2 exposure. MENSA and serum antibody reactivity was significantly higher in the infected groups than in the healthy control group for each of the six antigens (**Supplementary figure 1**). Receiver operating characteristic (ROC) curves yielded Area Under the Curve (AUC) values of  $\geq 0.89$  for the four spike-associated proteins, with MENSA anti-RBD demonstrating the highest value, AUC=1.0 (**Supplementary figure 2**). To distinguish natural infection responses from vaccination responses, we examined the diagnostic potential of two non-spike proteins, N and ORF3a. N yielded much higher signals in the infected groups and a slightly higher AUC value than ORF3a. Therefore, we focus on anti-RBD and anti-N IgG for all subsequent analyses.

**Primary SARS2 infection responses in MENSA and serum.** Primary infected patients were further divided based on the severity of their acute infections. During the acute stage of primary SARS2 infection, we observe a rise in both MENSA and serum anti-RBD IgG for M/M (69%, 88%) and S/C (95%, 95%) patients (**Fig. 1A,B**). We see a similar rise in MENSA and serum anti-N IgG for M/M (69%, 88%) and S/C (80%, 95%) patients as well (**Fig. 1C,D**). During convalescence (60-360 DPSO, 2-12 months after), MENSA levels drop significantly while serum levels rise quickly and remain elevated for months to years especially for anti-RBD IgG (**Fig. 1**). The MENSA levels from S/C were higher than in M/M patients during the acute infection because of higher frequencies of circulating early-minted ASC as previously shown by flow

188 cytometry<sup>30</sup>. Additionally, for S/C compared to MM patients, serum levels rise higher during the  
189 acute illness and remain elevated during convalescence, suggesting more ASC survived in  
190 other tissues such as the spleen, bone marrow, or mucosal sites. During convalescence, 87% of  
191 MENSAs become negative, but are overall slightly higher than pre-pandemic levels. In all,  
192 MENSA rises during acute infection and then rapidly falls to negative whereas serum titers rise  
193 and remain elevated.

194

195 Determining  $C_0$  thresholds for positivity. Since the convalescent baseline MENSA negative  
196 values could be slightly higher than in pre-pandemic controls, we identify a subset of the  
197 convalescent patients from Fig. 1 as COVID Recovered (CR) who had fully recovered with no  
198 sequelae (N=19). CR MENSA samples were used as controls to calculate the MENSA  $C_0$  using  
199 the average Net MFI plus 3 standard deviations. In contrast to MENSA, serum levels rise and  
200 remain high indefinitely in both CR and PASC patients; therefore, the serum  $C_0$  values are  
201 calculated using the average Net MFI plus 5 standard deviations of the 60 healthy controls prior  
202 to SARS2 exposure. These calculated Net MFI  $C_0$  values for MENSA (RBD: 570; N: 441) and  
203 serum (RBD: 1724; N: 682) are used to distinguish positive and negative samples in Figures 1-  
204 3, 5, and Supplementary figures 3, 4.

205

206 MENSA and serum from primary and booster mRNA vaccination. Similar to observations in  
207 primary acute SARS2 infections, adults receiving the COVID-19 vaccination, with no known  
208 prior exposure, have positive MENSA IgG for SARS2 but only to RBD and not specific to N  
209 since only spike proteins are engineered in the mRNA vaccines (**Fig. 2**). This rise in MENSA  
210 IgG to RBD 1-2 weeks after the first dose further increases to a higher peak after the second  
211 dose. MENSA anti-RBD antibodies decline to negative levels prior to the third vaccine dose and  
212 then increase again within a week after the third booster (**Fig. 2A**). Serum anti-RBD antibody  
213 levels increase after dose one and remain elevated throughout months to years (**Fig. 2B**).  
214 Similar to serum titers, MENSA antibody levels to N are also negative providing accurate  
215 responses to only the known proteins in the vaccines (**Fig. 2C, D**).

216

217 Longitudinal time-course of MENSA and serum after SARS2 infection and vaccination. Patients  
218 were recruited during the primary SARS2 infection or prior to the primary mRNA vaccine series.  
219 Serial blood samples were collected for MENSA and serum at the first and each subsequent  
220 SARS2 exposure events (infection and vaccination) to characterize the kinetics of the immune  
221 response during repeated exposure. In **Fig. 3**, we follow a 30-year-old Caucasian male subject  
222 from his initial mild SARS2 infection in 2020, before and after three doses of the Pfizer mRNA  
223 COVID-19 vaccine in 2021, and finally during his breakthrough Omicron infection and recovery  
224 in 2022. This subject's first draw was collected at 9 DPSO from his primary SARS2 infection in  
225 early 2020. MENSA is positive for anti-RBD and anti-N IgG during the acute infection (**Fig.**  
226 **3A,C**). The serum antibody titers are also weakly positive for anti-RBD and anti-N (**Fig. 3B,D**),  
227 as expected in most primary M/M infections. By 80 DPSO, the serum levels have increased  
228 further and remained elevated for several months while the MENSA levels rapidly decrease  
229 back to baseline. Upon three vaccine doses, the MENSA anti-RBD antibody levels rise and fall  
230 as expected whereas the serum antibodies to RBD remain positive from their previous infection  
231 and demonstrate a modest increase. Again, MENSA to the N protein is negative since it is not a  
232 component of the mRNA vaccine (**Fig. 3C**). However, serum anti-N levels remain weakly  
233 positive for several months to years after initial infection and before declining (**Fig. 3D**). In 2022,  
234 two years after the primary SARS2 infection and 8 months since his last vaccine booster  
235 vaccine, this subject had a PCR-confirmed Omicron breakthrough infection. Once again, the  
236 MENSA for anti-RBD and anti-N increases together. As expected, serum anti-N titers rise  
237 rapidly while the serum anti-RBD antibody levels, which were already high, remain elevated  
238 (**Fig. 3**). Unlike serum, the kinetics of MENSA demonstrate a rapid rise after each SARS2  
239 exposure, whether it is due to vaccination or infection, but then decline to baseline negative

240 values. MENSA is also highly discriminatory for spike (RBD) only after vaccination but shows a  
241 combination of spike (RBD) and N antibody levels during acute infections. We present additional  
242 kinetics spanning several years from a 24-year-old Caucasian female through three doses of the  
243 Moderna mRNA COVID-19 vaccine and an Omicron breakthrough infection (**Supplementary**  
244 **figure 3**) and from a 35-year-old Asian male through four doses of the Moderna mRNA COVID-  
245 19 vaccine and an Omicron breakthrough infection (**Supplementary figure 4**). In all, during  
246 SARS2 exposures from infection or vaccination, the MENSA antibody levels rise and then fall  
247 while serum titers rise after the primary exposure and stay elevated.

248  
249 *MENSA and serum in PASC vs COVID Recovered (CR)*. In addition to the 25 COVID recovered  
250 patients with no subsequent sequelae, we enrolled 61 PASC patients from the Emory Long-  
251 COVID clinic from 2021 to 2024 with self-reported symptom questionnaires at enrollment (**Fig.**  
252 **4**). We reconciled symptoms collected at enrollment and with a physician chart review and  
253 follow-up. The most common self-reported symptom in this PASC cohort was enduring fatigue in  
254 97% of patients, followed by persistent shortness of breath (SOB) in 75% with some who  
255 received new diagnoses of asthma or lung disease following their initial infection. Other common  
256 symptoms included brain fog (67%), dizziness (52%), post-exertional malaise (47%), headache  
257 (42%), chest and muscle pain (38% respectively), chronic cough (37%), depression/anxiety  
258 (37%), palpitations (32%), sleep disturbance (32%) joint pain/arthralgia (32%), and persistent  
259 loss of taste or smell (30%) (**Fig. 4**). In addition to follow-up in the Long-COVID clinic, 35%  
260 were referred for advanced neurology/neurocognitive evaluation and 58% for cardiac issues  
261 with new diagnoses of tachycardia and/or postural orthostatic tachycardia syndrome (POTS)  
262 (31%), arrhythmia/atrial fibrillation (19%), heart failure (11%), ongoing chest pain (11%), venous  
263 reflux/vein compression by doppler ultrasound (11%), dysautonomia (8%) and  
264 pericarditis/myocarditis (8%). Attempting to reconcile the 12 PASC symptom scores to better  
265 predict this long-COVID as recently reported in November 2023<sup>6</sup>, we calculated the average  
266 PASC score of 11.8 at enrollment for the 60 patients. Total scores equal to or greater than 12  
267 correlated with PASC patients at 6 months, while scores less than 12 were more likely to be  
268 PASC indeterminate.

269  
270 For the initial PASC experiment, we tested for only SARS2 antigens in MENSA and serum  
271 samples prepared from the subset of 19 CR subjects mentioned above and 39 PASC patients  
272 recruited during the first year of the Long-COVID clinic at Emory University December 2020-May  
273 2021. For the CR group, fifteen of the samples were taken directly from the Figure 1  
274 convalescent data while four of the patients donated additional follow-up samples. All patients  
275 were enrolled from day 60-279 DPSO after their initial acute infection and prior to any COVID-19  
276 vaccination. Of the 39 PASC patients, 56% had initial M/M acute infections and 44% had initial  
277 S/C acute infections. Of the 19 CR patients, 95% had M/M acute infections and 5% had S/C  
278 acute infections. In the 39 PASC patients, 33% still had positive MENSA for spike RBD after 60  
279 DPSO compared to none in the CR group (**Fig. 5A**). Only 10% of PASC patients and none of  
280 the CR patients had a positive MENSA for N (**Fig. 5C**). In serum, nearly all PASC (97%) and CR  
281 (95%) patients had positive antibodies for RBD and N (**Fig. 5B,D**). One patient from each group  
282 was negative for both antigens in their serum and MENSA.

283  
284 *Human viral scan in MENSA of PASC and CR*. For discovery of other human virus reactivation  
285 in the MENSA samples, we compared MENSAs collected from 10 PASC patients in 2021 and  
286 three CR patients using the human PhIP-seq single-end DNA sequences that were aligned to a  
287 library of reference DNA sequences of 149,259 peptides tiling protein-coding sequences from all  
288 viruses with human hosts<sup>31,32</sup>. Since it was early in the pandemic, the PhIP-seq was not  
289 optimized for SARS2. After quality control, we identified 227 peptides which were positive in  
290 MENSA in greater than three PASC patients and identified three additional major viruses, EBV,



291 CMV, and HSV2 (**Fig. 6**). Thus, a new PASC MENSA assay was developed using viral antigens  
292 from SARS2, EBV, CMV, and HSV2 (see methods).

293  
294 *MENSA for SARS2, EBV, CMV, and HSV2 in PASC and CR.* In a cohort of 60 PASC patients  
295 (39 patients in 2021 and 21 patients from 2022-2024), 21 CR patients (2020-2022), and 16  
296 healthy adult controls (2020), we tested MENSA and serum for a combined SARS2, EBV, CMV,  
297 and HSV2 IgG immunoassay. We had 23 samples from 21 CR patients since two individuals  
298 suffered repeat SARS2 infections in 2020 and 2022. As expected, all healthy controls drawn  
299 prior to SARS2 exposure were negative for SARS2 IgG in both MENSA and serum (**Fig. 7**).  
300 Only 2/16 (13%) were positive in the MENSA for any of the viruses tested (EBV) in the healthy  
301 control group, whereas 14/16 (88%) were positive for EBV, CMV, and/or HSV2 in the serum. In  
302 the PASC vs CR groups, we show positive MENSA for SARS2 in 24/60 (40%) of PASC patients  
303 and none in the CR (**Fig. 7A**). Nearly all PASC and CR patients are positive for the antibodies to  
304 SARS2 in the serum (98% and 96%, respectively) (**Fig. 7B**). Interestingly, the lack of correlation  
305 between MENSA and serum suggests they function as independent variables, although  
306 frequencies of plasmablasts may be linked with rise in serum titers.

307  
308 When examining the latent herpes viruses, MENSA reactivity was greater overall in the PASC  
309 group than in the CR group (**Fig. 7A**). For EBV, more PASC patients had positive MENSA  
310 samples 22/60 (37%) compared to CR subjects 4/23 (17%). A MENSA test was scored positive  
311 if any one of the 3 antigens (EBNA1, VCA, and gB350) was positive. Nearly all individuals in the  
312 general population have been exposed to EBV and our results were consistent with this finding.  
313 Here, EBV serologies are positive for 59/60 (98%) in the PASC group and all 23 CR samples.  
314 MENSAs for CMV are positive in 14/60 (23%) of the PASC patients compared to 1/23 (4%) in  
315 the CR group. Again, a test was scored positive if one of the two CMV antigens (gB or  
316 pentamer) was positive. Similar frequencies of positive CMV serology are notable in the PASC  
317 patients 43/60 (72%) versus CR individuals 18/23 (78%). For HSV2, MENSA samples are  
318 positive in 9/60 (15%) of the PASC patients, and 1/23 (4%) in the CR samples. The serum was  
319 positive in 49/60 (82%) and 12/23 (52%) of the PASC and CR samples, respectively. Overall,  
320 MENSA assays are positive in 47% (28/60) of the PASC patients for EBV, CMV, and/or HSV2  
321 whereas only 17% (4/23) are positive in the CR cohort. In all, we identify a positive MENSA in  
322 36/60 (60%) PASC for any of the 4 viruses (SARS2, EBV, CMV, or HSV2) compared to 4/23  
323 (17%) for the CR group. In conclusion, a positive MENSA for SARS2 in PASC patients  
324 demonstrates ongoing new immune responses consistent with a reservoir for the persistence of  
325 SARS2 virus. Moreover, a positive MENSA for any of the 3 herpes viruses also demonstrates  
326 reactivation of latent EBV, CMV, and HSV2 identifying underlying viral triggers in this condition.

327  
328

## 329 DISCUSSION

330

331 Understanding the main viral drivers of the inflammatory and metabolic changes in patients with  
332 long-COVID has been challenging. Multiomic studies provide a wealth of information but have  
333 not identified the underlying triggers of this chronic condition. Although suggestions of viral  
334 persistence have been raised, it has been difficult to demonstrate ongoing reservoirs or  
335 reactivation of the latent virus by PCR due to the limited sensitivity of the current tests. Thus,  
336 detecting the pathogen has been challenging in patients with normal or even heightened  
337 immune responses. In this paper, we offer MENSA as a novel approach to identify the main viral  
338 drivers of long-COVID. MENSA ascertains unique immune signatures by capturing the  
339 antibodies from the circulating plasmablasts. These antibodies in the MENSA provide an  
340 immune snapshot that reveals the underlying drivers of the current illness. As proof of concept,  
341 we show that with known exposure to SARS2 by infection or vaccination, MENSA from the  
342 blood is positive. Applying these same principles, in a cohort of 60 PASC patients of whom we

343 did not know the underlying cause, we show that 60% have a positive MENSA response against  
344 SARS2, EBV, CMV, and/or HSV2, thereby demonstrating ongoing reservoirs of SARS2 and/or  
345 reactivation of latent herpes viruses.

346  
347 With first time infections, naive B cells are activated and undergo massive expansions through  
348 extrafollicular and germinal center reactions in the lymph nodes to form memory B cells and  
349 newly-minted ASC that produce antibodies. Interestingly, the majority of these ASC die, but a  
350 few successfully migrate to the bone marrow or tissue sites where they can undergo further  
351 maturation to become long-lived plasma cells (LLPC). Nearly all ASC circulating in blood are  
352 newly generated and display markers of recent proliferation such as Ki67<sup>33,34,35</sup>, unlike LLPC  
353 which stop proliferating. Memory B cells persist over a lifetime and differentiate into  
354 plasmablasts when re-encountering the same antigens<sup>35</sup>. During breakthrough or repeat  
355 infections, newly-minted ASC mostly originate from memory B cells and circulate transiently in  
356 the blood<sup>35</sup>. Since MENSA measures antibodies only from these newly-minted ASC and not  
357 from old LLPC, MENSA antibodies provide a unique antibody signature to reveal the cause of  
358 the present-day illness. We show that, during convalescence, the MENSA responses become  
359 negative because memory B cells are no longer differentiating into ASC and released into the  
360 blood. Thus, MENSA offers an immune snapshot to uncover the sources of the patient's  
361 ailment.

362  
363 Despite the high sensitivity of PCR testing in the nasopharyngeal swabs (NPS), blood PCR tests  
364 have limited utility for SARS2 and latent herpes viruses, such as EBV, CMV, and HSV2 due to  
365 strong T cell responses that mediate rapid viral clearance. SARS2 antigen assays have been  
366 shown to identify the spike protein, but quantities are extremely low and require ultrasensitive  
367 assays which carry a high risk of false positives<sup>14</sup>. Autopsies up to 230 days after acute SARS2  
368 infection detected SARS2 RNA in multiple tissues such as the gut, central nervous system  
369 (CNS), muscle, myocardium, and the respiratory tract<sup>36</sup> demonstrating viral reservoirs. Thus,  
370 measuring the MENSA has advantages over pathogen detection by PCR amplification or protein  
371 since MENSA is in the blood, agnostic to viral reservoir locations, and would not require invasive  
372 tissue sampling. Since MENSA culminates from the total newly-minted ASC traveling in the  
373 blood during acute illness, knowledge of the viral reservoir location is not necessary since the  
374 MENSA reveals infections in deep-seated sites similar to a liquid biopsy.

375  
376 Breakthrough or repeat infections can be diagnosed with serum assays, but they typically  
377 require serial blood samples during acute infection and convalescence for comparison. Another  
378 advantage of the MENSA over serum is that only a single blood sample during illness is needed.  
379 The decline of MENSA to negative levels after infection or vaccination demonstrates its clinical  
380 utility in measuring secondary or breakthrough infections.

381  
382 Specificity of MENSA antibodies are also exact in that they can distinguish infection from  
383 vaccination based on spike and the nucleocapsid proteins in some patients. This specificity and  
384 sensitivity along with the kinetics make MENSA an ideal diagnostic platform to reveal viral  
385 triggers that were previously difficult to measure with just serum or PCR tests. The MENSA  
386 diagnostic would be the first of its kind to understand the main viral drivers of this chronic  
387 disease.

388  
389 MENSA antibodies are expected to peak within days after exposure, and then quickly decline  
390 back to baseline within a month after the infection has resolved. Interestingly, CR MENSA does  
391 not revert to pre-pandemic baseline levels and these mechanisms are not clear. Perhaps non-  
392 specific plasma antibody binding to monocytes in the MENSA cultures may be the reason and  
393 will require more studies. Another possibility is low-level bystander responses which have been  
394 suggested<sup>35</sup> to explain the difference between pre- and post-pandemic samples. Interestingly,

395 even when using the post-pandemic MENSA samples as controls, 60% of the PASC patients  
396 have higher SARS2, EBV, CMV, and HSV2 responses in the MENSA.

397  
398 Autoantigen triggers have also been implicated in PASC patients, and so we tested MENSA  
399 from a limited number of PASC patients for autoantigens using the PhIP-seq human peptidome  
400 library which consists of 605,656 peptides tiling protein-coding sequences, splice variants, non-  
401 coding open reading frames, and endogenous retroviral sequences in the human genome<sup>37,38</sup>.  
402 No differences were observed between the PASC and CR MENSA against the human  
403 peptidome (our unpublished results). However, a larger number of patients using the 3-D  
404 conformational epitopes of the human proteome may be needed to definitively rule out MENSA  
405 responses to autoantigens. Since the original PhIP-seq assays used linear viral peptides, we  
406 may also consider a panel of 3-D conformational epitopes to identify important unique immune  
407 signatures for viruses that infect humans to ensure comprehensive testing for other viruses.

408  
409 There are several limitations of this study. One is that we do not have longitudinal samples from  
410 the PASC patients and thus, it is unclear how consistent the MENSA responses are in the  
411 patients with chronic illness over time. Second, large clinical trials are needed to evaluate  
412 responses to anti-viral therapies in MENSA positive patients identified with SARS2 persistence  
413 and reactivation of EBV, CMV, or HSV2 infections. Finally, the utility of MENSA may be  
414 challenging in immunocompromised patients since the MENSA requires B cell activation to form  
415 new ASC.

416  
417 The real-time immune snapshots provided by MENSA may be leveraged to inform therapeutic  
418 strategies and successful treatment of chronically ill PASC patients. Whether MENSA can also  
419 be useful to identify persistence of viral reservoirs in other chronic illnesses such as multiple  
420 sclerosis, HIV, myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and other  
421 infections with post-sequelae are yet to be determined. For example, EBV was recently  
422 implicated in multiple sclerosis<sup>39</sup>. Interestingly, infection with SARS2 is associated with  
423 increased susceptibility and severity of neurodegenerative disorders, such as Alzheimer's  
424 disease, Parkinson's disease, and dementia but interpreting these correlations has been difficult  
425<sup>40-42</sup>. If MENSA can lead to early diagnosis of these chronic neurodegenerative disorders or help  
426 identify the cause of these disease flares, perhaps treatments may prove more effective in  
427 preventing progression and severity of these pathological conditions.

428  
429 PASC can include symptoms, such as dyspnea, fatigue, and depression<sup>43</sup>, and serious clinical  
430 indications, such as cardiovascular disease or diabetes<sup>44,45</sup>. Recent studies also suggest  
431 increased risk for autoimmune inflammatory rheumatic diseases in PASC and CR patients<sup>46-48</sup>.  
432 Future clinical trials are necessary to perform proof-of-concept studies where MENSA data  
433 could be used to inform treatment modalities for mitigating symptoms associated with SARS2  
434 viral persistence, reactivation of viruses, reactivation of viruses in other chronic illnesses, or  
435 early activation of other chronic illnesses.

436  
437 In summary, MENSA is a novel immune diagnostic which captures unique signatures of the  
438 early-minted ASC in the blood to reveal the cause of illness. In chronic conditions such as PASC  
439 where serum antibody titers are high, MENSA is an independent matrix that identifies  
440 persistence of SARS2 viruses or antigens and can also recognize the reactivation of latent  
441 herpes viruses, such as EBV, CMV, and HSV2 in 60% of patients. This host immune snapshot  
442 reveals the fundamental drivers of viral persistence and reactivation in this chronic disease.

443 **Declaration of Potential Conflicts of Interest**

444 FEL is the founder of MicroB-plex, Inc. and serves on the scientific board of Be Biopharma, is a  
445 recipient of grants from the BMGF and Genentech, Inc., and has served as a consultant for  
446 Astra Zeneca. NSH and AMP were scientists at MicroB-plex, Inc., Atlanta, GA and JLD is a  
447 scientist at MicroB-plex, Inc., Atlanta, GA. IS has consulted for GSK, Pfizer, Kayverna, Johnson  
448 & Johnson, Celgene, Bristol Myer Squibb, and Visterra. FEL, DN, and IS are inventors of the  
449 patents concerning the plasma cell survival media related to this work (issued 9/21/21, US  
450 11,124766 B2 PCT/US2016/036650; and issued 9/21/21, US 11,125757 B2). FEL & JLD are  
451 inventors of MENSA patent U.S. Patent No. 10,247,729. April 2, 2019. FEL, NSH, JLD, & IS are  
452 inventors of the MENSA PASC diagnostic provisional patent, March 28, 2024. All other authors  
453 have declared that no conflict of interest exists.

## 454 METHODS online

455

### 456 Subject Enrollment and Sample Collection

457 Patient enrollment: We enrolled 241 adults between 2020 and 2024 at Emory University in  
458 Atlanta, GA and collected blood to generate the MENSA matrix and serum samples (Table 1). In  
459 2020, we enrolled 110 adults with PCR-positive NPS during their primary SARS2 infection. Fifty-  
460 four adults were outpatients with mild/moderate (M/M) disease as defined by the NIH criteria<sup>28</sup>,  
461 and 56 adults had severe/critical (S/C) illness in the Intensive Care Units. We also enrolled 60  
462 healthy adults with no known exposure to SARS2 early during the pandemic with blood samples  
463 collected between March and June 2020 during the initial lockdown as the healthy adult controls  
464 without prior SARS2 exposure. Eleven vaccinated subjects who had no prior SARS2 infection  
465 were enrolled before and during their primary two-dose mRNA vaccine series (Pfizer n=5,  
466 Moderna n=6) and drawn again before and after their third booster dose. We also enrolled 3  
467 adults experiencing Omicron breakthrough infections between December 2021 and June 2022.

468

469 Finally, we enrolled 61 long-COVID or PASC patients from the Emory Long-COVID Clinic which  
470 was started in January 2021 until February 2024 (Fig. 4). Initially, 40 patients were enrolled in  
471 2021 (Figs. 5, 6, 7) and an additional 21 patients were enrolled in 2022-2024 (Fig. 7). All  
472 patients filled patient-reported symptom questionnaires. Samples from nine of the initial 39  
473 PASC patients from Fig. 5 and one additional PASC patient recruited in 2021 were sent to  
474 Immune ID for PhIP-seq analysis along with samples from three COVID Recovered patients (1/3  
475 CR from initial convalescent cohort in Fig 1; 2/3 new CR patients). All blood samples were  
476 collected under the Emory University Institutional Review Board–approved protocols.

477

478 **MENSA Preparation:** Medium enriched for newly synthesized antibodies (MENSA) was  
479 generated by isolating, washing, and culturing antibody-secreting cells (ASC)-containing  
480 peripheral blood mono-nuclear cells (PBMC) from blood using a modified procedure previously  
481 described<sup>23</sup>. Peripheral blood samples were collected in sodium heparin tubes and PBMC were  
482 isolated by centrifugation (1,000 xg; 10 min) using Lymphocyte Separation Media (Corning) and  
483 Leucosep tubes (Greiner Bio-One). Five washes with RPMI-1640 (Corning) were performed to  
484 remove serum immunoglobulins (800 x g; 5 min) with erythrocyte lysis (3 mL; 3 min), and  
485 harvested PBMCs were cultured at 10<sup>6</sup> cells/mL in R10 Medium (RPMI-1640, 10% Sigma FBS,  
486 1% Gibco Antibiotic/Anti-mycotic) for 24 h at 37° C and 5% CO<sub>2</sub>. After incubation, the cell  
487 suspension was centrifuged (800 xg; 5 min), and the supernatant (MENSA) was separated from  
488 the PBMC pellet, aliquoted, and stored at -80°C for testing.

489

490 **Serum Preparation:** Whole blood was collected and incubated at room temperature for at least  
491 30 minutes. The clot was discarded, and the remaining serum supernatant was centrifuged  
492 (800xg; 10 min), aliquoted and stored at -80 °C for testing.

493

494 **Antigen Selection and Multiplex Immunoassays:** Antigens of interest were selected from  
495 literature, coupled to Luminex MagPlex Microspheres of spectrally distinct regions via  
496 carbodiimide coupling, and tested for antigen specific IgG reactivity against patient samples as  
497 previously described<sup>29</sup>.

498

499 SARS2 antigens: SARS-CoV-2 Spike S1 Receptor Binding Domain (RBD; catalog no. Z03483;  
500 expressed in HEK293 cells) and Nucleocapsid protein (N; catalog no. Z03480; expressed in  
501 Escherichia coli), were purchased from GenScript. S1 (catalog no. S1N-C52H3; HEK293), S2  
502 (catalog no. S2N-C52H5; HEK293) and S1 N-terminal domain (NTD; catalog no. S1D-C52H6;  
503 HEK293) were purchased from ACROBiosystems. The C-terminus sequence of ORF3a  
504 (Accession: QHD43417.1, amino acids 134-275 plus N-terminal His6-Tag) was sent to  
505 Genscript for custom protein expression in E. coli. Each protein was expressed with an N-

506 terminal His6-Tag to facilitate purification, at least 90% pure, and appeared as a predominant  
507 single band on SDS-PAGE analysis.

508

509 *EBV, CMV, and HSV2 antigens:* EBV, CMV, and HSV2 antigens were carefully selected for  
510 antigenicity based on previous reports<sup>49-54</sup>. The following proteins were used: EBV EBNA1  
511 protein from Abcam (produced in E. coli, N-Terminus His Tag, CAT#ab138345); EBV VCA p18  
512 from RayBiotech (produced in E. coli, CAT#227-20127); EBV gp350 protein from  
513 AcroBiosystems (produced in HEK293 cells, His Tag, MALS verified, CAT#GP0-E52H6); CMV  
514 glycoprotein B from AcroBiosystems (strain AD169, expressed from HEK293 cells, His Tag,  
515 MALS verified, CAT#CMB-V52H4); CMV gH pentamer complex, consisting of gH, gL, UL128,  
516 UL130 and UL131A proteins, produced in mammalian HEK293 cells from The Native Antigen  
517 Company (CAT#CMV-PENT); HSV2 envelope glycoprotein D from AcroBiosystems (gD,  
518 expressed HEK293 cells, His Tag, MALS verified, CAT#GLD-V52H4).

519

520 *Serum and MENSA assays for SARS2 and other viruses:* Serum samples were tested at 1:500  
521 dilution in assay buffer (1XPBS, 1% BSA) while MENSA samples were tested neat with no  
522 dilution. Results were analyzed on a Luminex FLEXMAP 3D instrument. Median fluorescent  
523 intensity (MFI) using phycoerythrin-conjugated detection antibodies (Goat Anti-Human IgG-PE,  
524 Southern Biotech cat. #2040-09) was measured for each sample using the Luminex xPONENT  
525 software on Enhanced PMT setting. The background value of assay buffer or R10 media was  
526 subtracted from the serum or MENSA results, respectively, to obtain MFI minus background  
527 (Net MFI). All samples were tested in duplicate and the average of the two results were used for  
528 analysis. In the initial SARS2 assay, all SARS2 protein bound microparticles were run together  
529 as a six-bead SARS2 solution. Serum  $C_0$  values of positivity were calculated as the average  
530 plus five standard deviations of the Healthy Control population (N=60) for each antigen (RBD:  
531 1724; N: 682). MENSA  $C_0$  positivity values were calculated as the average plus three standard  
532 deviations of the Contemporary Controls (COVID Recovered N=19 (CR) group described  
533 above) for each antigen (RBD: 570; N: 441).

534

535 Later, the assay was modified to contain only RBD, N, S1, and S2 from SARS2 (NTD and  
536 ORF3a dropped) and also included the addition of all EBV, CMV, and HSV2 antigens, for a  
537 combined multi viral 10-antigen bead assay. All new  $C_0$ s were calculated based on the new  
538 assay data in Figure 7. MENSA  $C_0$ s were calculated as the average plus three standard  
539 deviations of 22/23 CR samples (RBD: 404; N: 266; EBNA1: 475; VCA: 426; gp350: 523; gB:  
540 298; Pentamer: 455; gD: 308). One sample was excluded from the MENSA  $C_0$  calculation due  
541 to multiple antigen reactivity measuring greater than 10 times the median value of the entire  
542 group. SARS2 Serum  $C_0$  was calculated as the average plus three standard deviations of the 16  
543 Healthy Donor samples collected prior to SARS2 exposure. Since the majority of the population  
544 is expected to be positive for EBV, CMV, and/or HSV2 antibodies in their serum, we obtained  
545 de-identified clinically confirmed negative sera from the Emory clinical laboratory. For each  
546 virus, three confirmed negative serum samples were used. The average Net MFI plus three  
547 standard deviations was calculated for each antigen and used as the  $C_0$  threshold for positivity  
548 (RBD: 410; N: 564; EBNA1: 3,578; VCA: 593; gp350: 228; gB: 778; Pentamer: 769; gD: 858).

549

550 *Phage immunoprecipitation sequencing and analysis.* We constructed a custom T7  
551 bacteriophage library consisting of 149,259 peptides tiling all protein-coding sequences from  
552 viruses with human hosts<sup>31,32</sup>. Viral sequences were downloaded from Uniprot, collapsed on  
553 90% identity, and bioinformatically parsed into 90 amino-acid peptide tiles with 45 amino-acid  
554 overlaps between adjacent tiles. Healthy and Covid patients' plasma or serum and matched  
555 MENSA reactivities were profiled using phage immunoprecipitation and sequencing (PhIP-seq).  
556 MENSA samples were profiled in duplicate and plasma/serum samples in triplicate. PhIP-seq  
557 was performed as previously described with some modifications<sup>32</sup>. T7 bacteriophage libraries

558 were aliquoted into 96-well plates and incubated with 20µl each of protein A and G Dynabeads  
559 on a rotator for 4 h at room temperature. Next, plates were placed on a magnet and  
560 supernatants were transferred to a fresh 96-well plate, to which we added patient plasma  
561 containing 2µg of total IgG, and continued with the immunoprecipitation and washing steps, as  
562 previously described. Following the washes, protein A and protein G Dynabeads were  
563 resuspended in PCR master mix, amplified with 16 rounds of PCR, SPRI cleaned to remove  
564 primers, and indexed for sequencing with 8 rounds of PCR with primers containing Illumina p5  
565 and p7 barcodes. NGS libraries were quantified on a TapeStation4200 and normalized for  
566 sequencing on Illumina Nextseq 2000 or Novaseq 6000 instruments. Each sequencing library  
567 received a minimum of 3M reads.

568  
569 PhIP-seq single-end DNA sequences were aligned to a library of reference DNA sequences  
570 (149,259 75bp for viral) with the bowtie2 aligner (v2.0) using end-to-end matching. Read counts  
571 were summarized using samtools (v1.14) and collated into a counts matrix. The raw counts  
572 were converted to counts per million (CPM) using the `cpm` function from the R package edgeR  
573 (v3.36.0). CPM values for healthy controls were summarized by computing the peptide-wise  
574 mean and standard deviation across all healthy control samples. CPM values for each patient  
575 sample were collapsed by computing the peptide-wise minimum across technical replicates.  
576 Peptide-wise z-scores were then computed as:

$$Z_{i,j} = \frac{(C_{i,j} - \mu_j)}{\sigma_j}$$

577 where  $Z_{i,j}$  is the z-score for patient  $i$ , peptide  $j$ ;  $C_{i,j}$  is the minimum CPM for patient  $i$ , peptide  $j$ ;  $\mu_j$   
578 is the mean of peptide  $j$  in the healthy control samples, and  $\sigma_j$  is the standard deviation of  
579 peptide  $j$  in the healthy control samples. For each patient, hits were identified as those peptides  
580 with  $C_{i,j} \geq 10$  AND  $Z_{i,j} \geq 10$ .

### 581 **Data Analysis:**

582 All graphs were designed using GraphPad Prism, Excel, R studio, BioRender, and/or Adobe  
583 Photoshop. Comparisons among and between groups in figures 1,2,5 and supplemental figures  
584 1 and 2 were calculated by Kruskal-Wallis tests, Mann-Whitney tests, and/or receiver-operating  
585 characteristic (ROC) curves using GraphPad Prism. To avoid overrepresentation of negative  
586 values on log scales, all values less than 10 Net MFI were replaced with 10 for figures 1-3 and  
587 5. All calculations and statistics were performed using real values.  
588

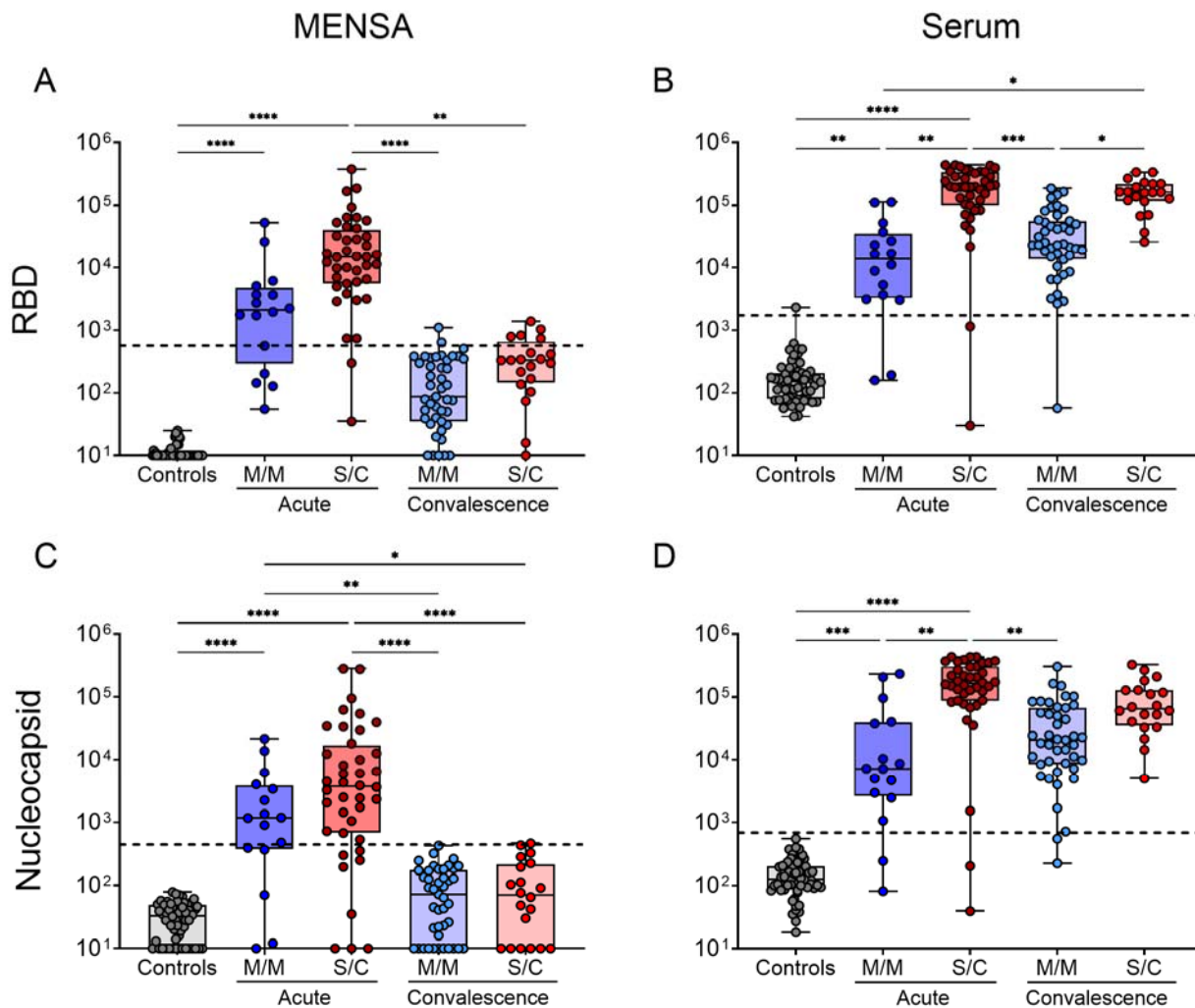
589 **Table 1. Demographics of Controls, COVID-19 patients, and Vaccinated Subjects.**  
590

Subjects	Healthy Adults N = 60	SARS2 Infection		Vaccinated	Post-SARS Infection	
		Mild/Moderate N = 54	Severe/Critical N = 56	Pfizer & Moderna N = 11	PASC N = 61	COVID Recovered N = 25
<b>Age, years</b> (Mean ± SD)	(30 ± 9)	(40 ± 14)	(56 ± 14)	(40 ± 16)	(49 ± 12)	(41 ± 15)
<b>Sex, N (%)</b>						
Males	31 (52)	18 (33)	30 (54)	2 (18)	15 (25)	7 (28)
Females	29 (48)	36 (67)	26 (46)	9 (82)	46 (75)	18 (72)
<b>Race, N (%)</b>						
Caucasian	41 (68)	30 (56)	17 (30)	3 (27)	24 (39)	11 (44)
African American	4 (7)	17 (31)	35 (63)	3 (27)	34 (56)	9 (36)
Asian	10 (17)	4 (7)	4 (7)	5 (45)	2 (3)	3 (12)
Other	5 (8)	3 (6)	0 (0)	0 (0)	1 (2)	2 (8)

591



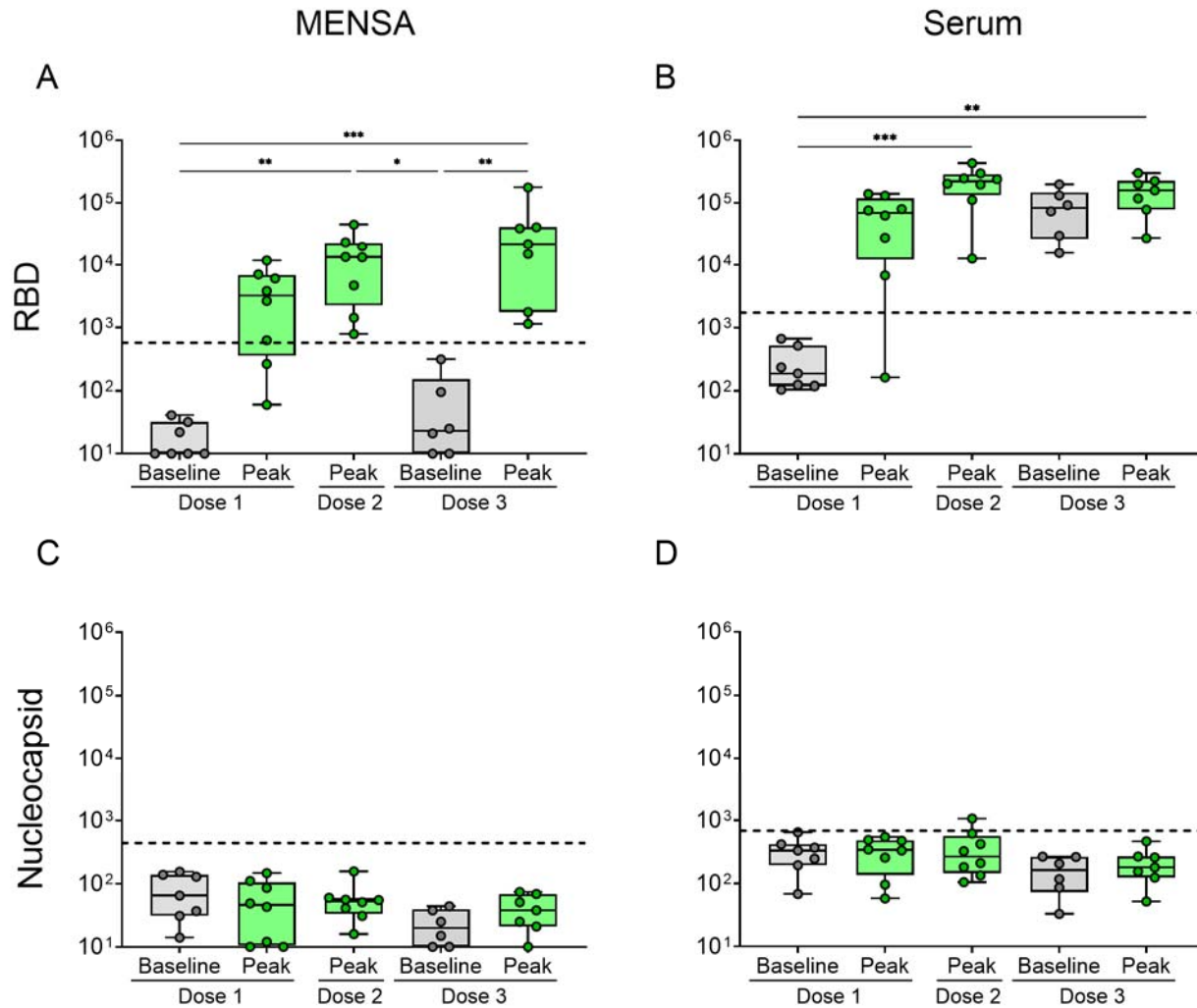
592 **Figures:**



593

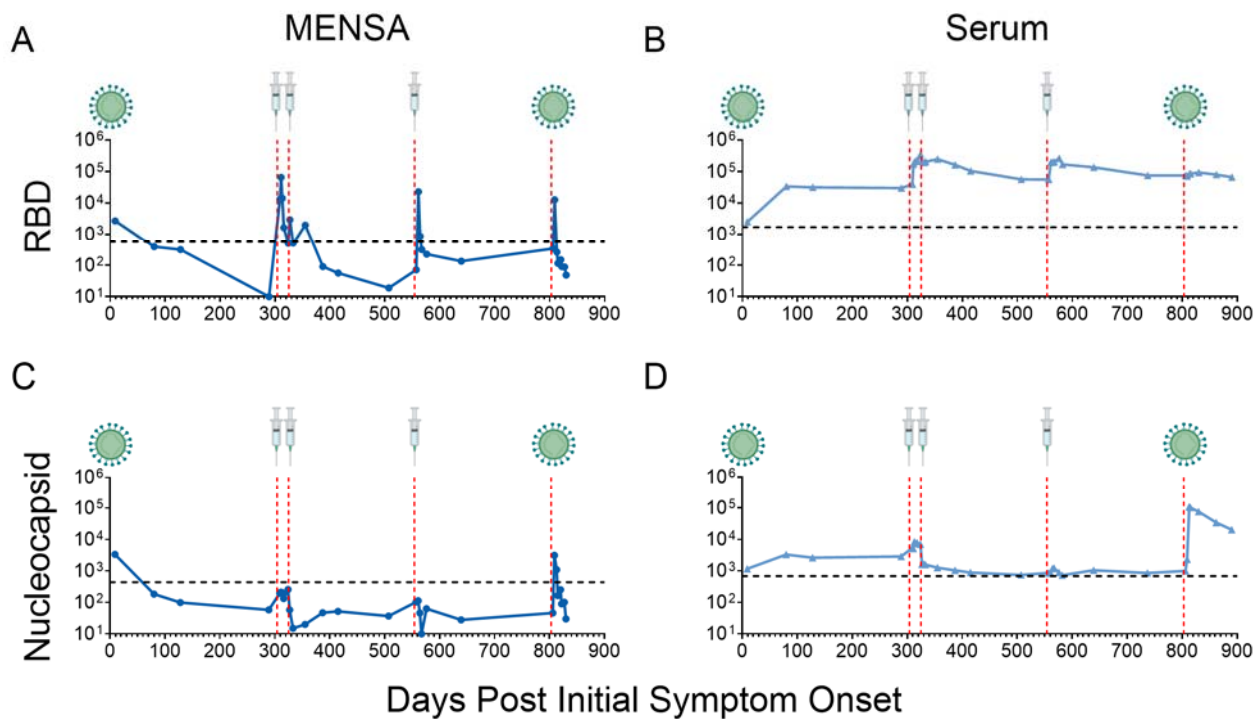
594

595 **Figure 1. Primary wild type COVID-19 infection responses in MENSEA and serum.** Dot plots  
 596 show IgG antibody reactivity against S1-RBD in the MENSEA (A) and serum (B) of patients  
 597 experiencing Wild Type SAR-CoV-2 infections in 2020. Similar results are also shown for anti-  
 598 Nucleocapsid IgG in the MENSEA (C) and serum (D). Samples from patients with acute  
 599 Mild/Moderate (59 samples from 54 patients; blue dots) and Severe/Critical (60 samples from 56  
 600 patients; red dots) infections were collected less than 30 DPSO (acute) and/or after 60 DPSO  
 601 (convalescence). Early pandemic healthy controls, with no prior exposure to SARS2 (n=60), are  
 602 shown as black dots on the left of each panel. All units are represented as Median Fluorescent  
 603 Intensity minus background (Net MFI). Dashed lines indicate the C<sub>0</sub> threshold of positivity for  
 604 each sample type and antigen. Serum C<sub>0</sub>s were calculated as the average Net MFI plus five  
 605 standard deviations of the 60 healthy controls (RBD: 1724; N: 682). For MENSEA C<sub>0</sub>s, a subset  
 606 of the convalescent patients was identified as a confirmed COVID Recovered (CR) population  
 607 (no sequelae; N=19) and was used as a contemporary control group to calculate the average  
 608 Net MFI plus 3 standard deviations (RBD: 570; N: 441). Pair-wise comparisons were performed  
 609 using the Kruskal-Wallis test in GraphPad Prism (unpaired, nonparametric test; ns p > 0.05, \* p  
 610 ≤ 0.05, \*\* p ≤ 0.01, \*\*\* p ≤ 0.001, \*\*\*\* p ≤ 0.0001).



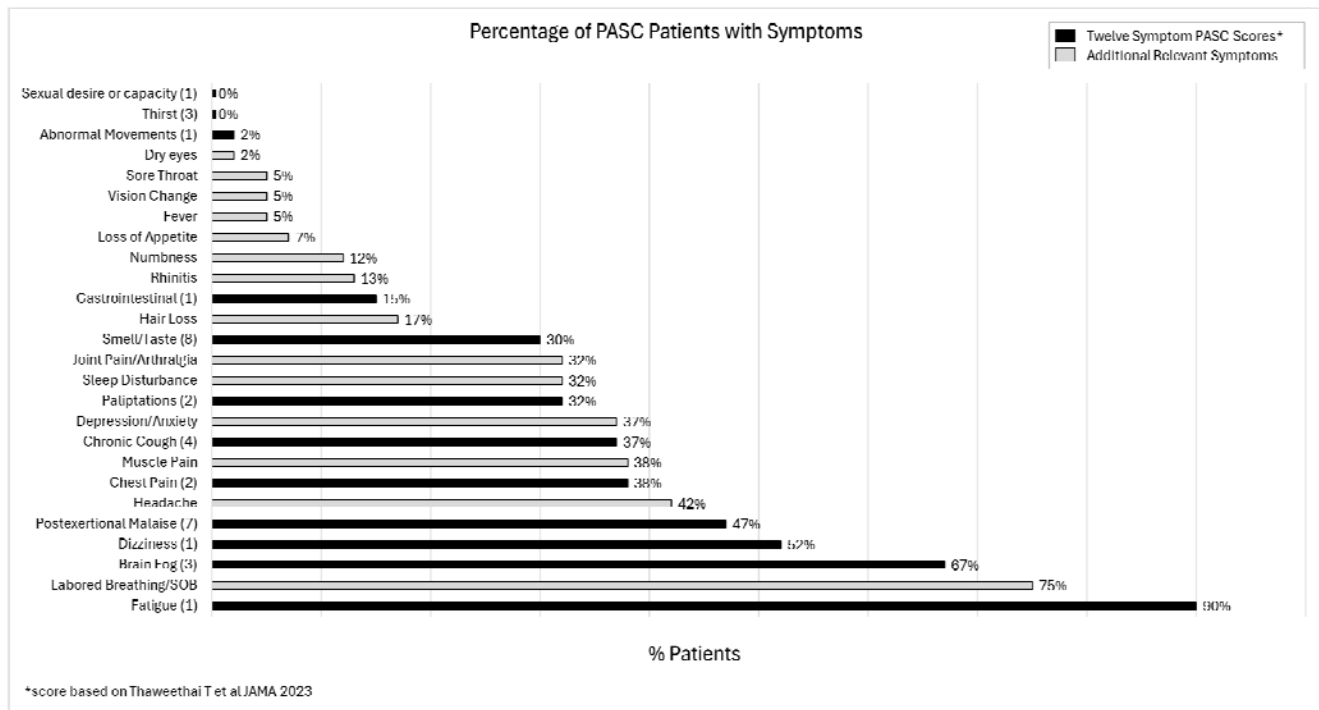
611  
612  
613  
614  
615  
616  
617  
618  
619  
620  
621  
622

**Figure 2. Primary vaccination responses in MENSAs and serum.** Dot plots show IgG antibody reactivity against S1-RBD in the MENSAs (A) and serum (B) of subjects receiving their primary COVID-19 mRNA vaccination, with no prior infection. Similar results are also shown for anti-nucleocapsid IgG in the MENSAs (C) and serum (D). Samples were collected from 11 healthy adults prior to vaccination (Baseline; n=7), after Dose 1 Peak (9-20 DPV; n=8), Dose 2 Peak (6-12 DPV; n=8), Dose 3 Baseline (>80 DPV dose 2 through 0 DPV dose 3; n=6), and Dose 3 Peak (4-12 DPV dose 3; n=7). All values are reported as average Net MFI (Median Fluorescent Intensity – Background). Dashed lines indicate the  $C_0$  threshold of positivity for each sample type and antigen as determined in Figure 1. Pair-wise comparisons were performed using the Kruskal-Wallis test in GraphPad Prism (unpaired, nonparametric test; ns p > 0.05, \* p ≤ 0.05, \*\* p ≤ 0.01, \*\*\* p ≤ 0.001, \*\*\*\* p ≤ 0.0001).



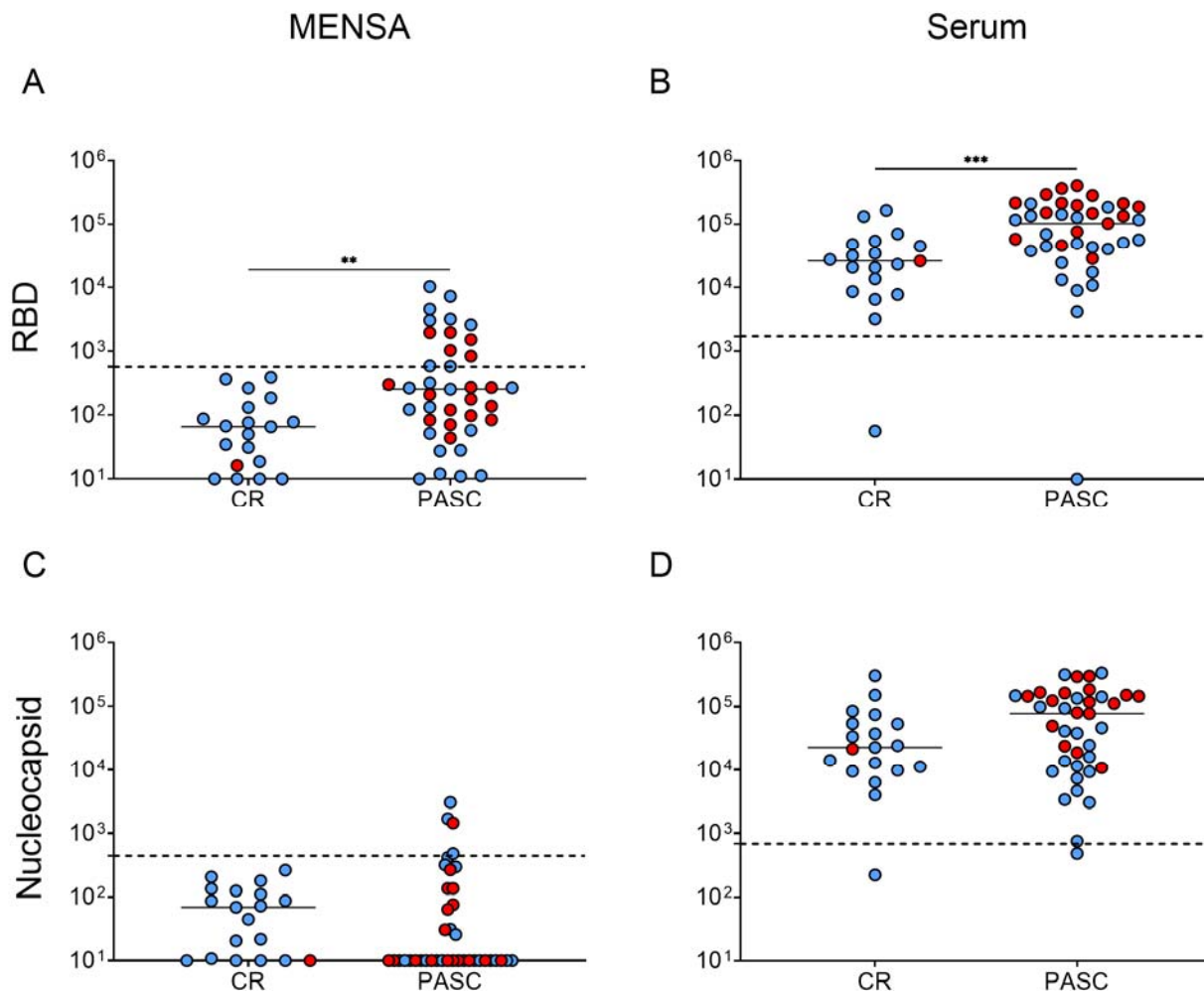
623  
624  
625  
626  
627  
628  
629  
630  
631  
632

**Figure 3. Kinetics of MENSAs and serum after SARS2 infection and multiple vaccine doses.** Line graphs show MENSAs (dark blue) and serum (light blue) IgG antibody responses to S1-RBD (A,B) and Nucleocapsid (C,D) over time for a single patient starting with an initial primary SARS2 infection in 2020, through three doses of Pfizer COVID-19 mRNA vaccination in 2021, and a breakthrough Omicron infection in 2022. All values are reported as average Net MFI (Median Fluorescent Intensity – Background). Red vertical dashed lines represent a new exposure event. The primary and breakthrough infection events are symbolized as virions. Each vaccination dose event is symbolized as a syringe. Horizontal dashed black lines represent the  $C_0$  threshold of positivity for each sample and antigen combination as determined from Figure 1.



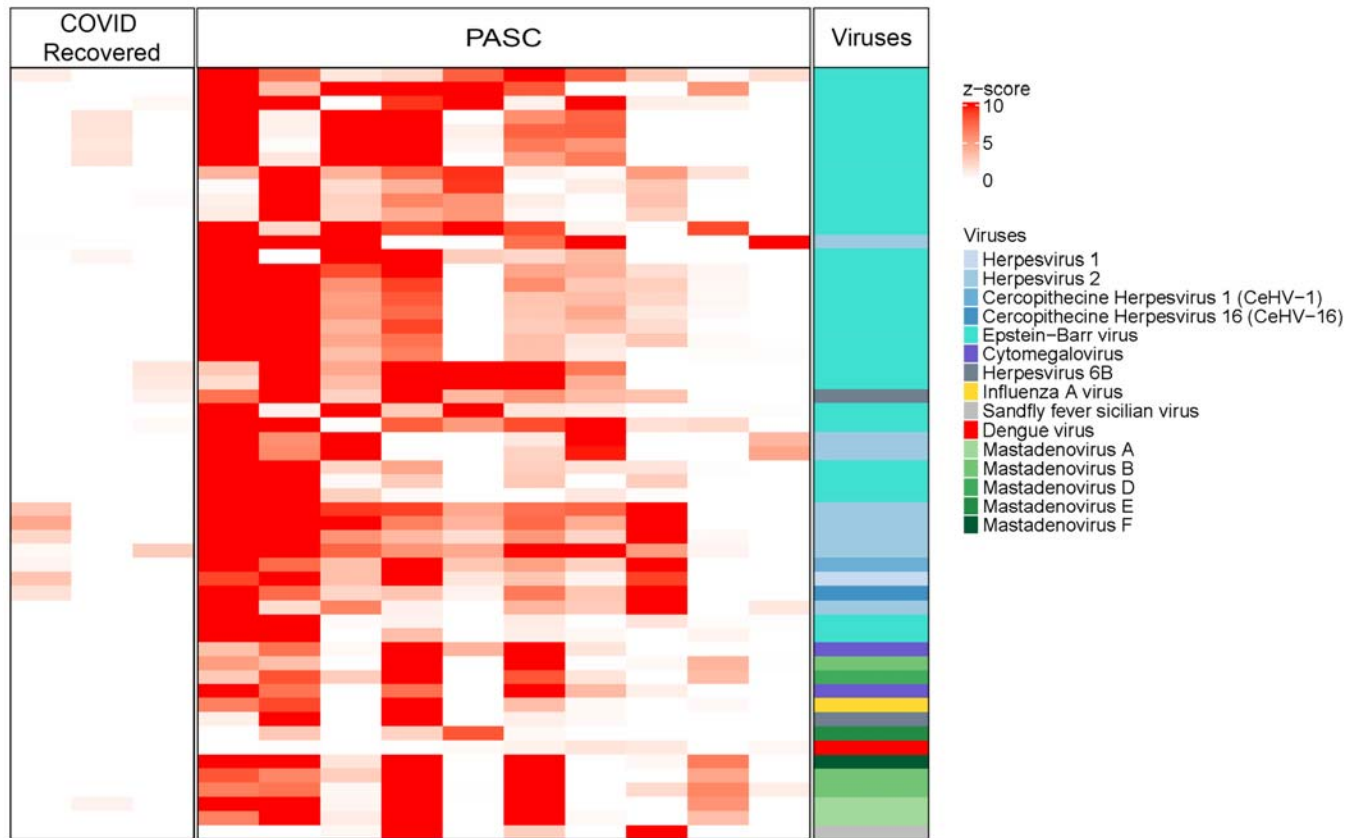
633  
634  
635  
636  
637  
638

**Figure 4. Percentage of PASC patients with symptoms.** Sixty PASC patients recruited in the Emory Long-COVID clinic with self-reported symptom questionnaires at enrollment. Percentages of each symptom is shown to the right of each bar. The twelve-symptom PASC scores based on Thaweethai et al JAMA 2023 are shown in black with point scores in parentheses after each symptom.



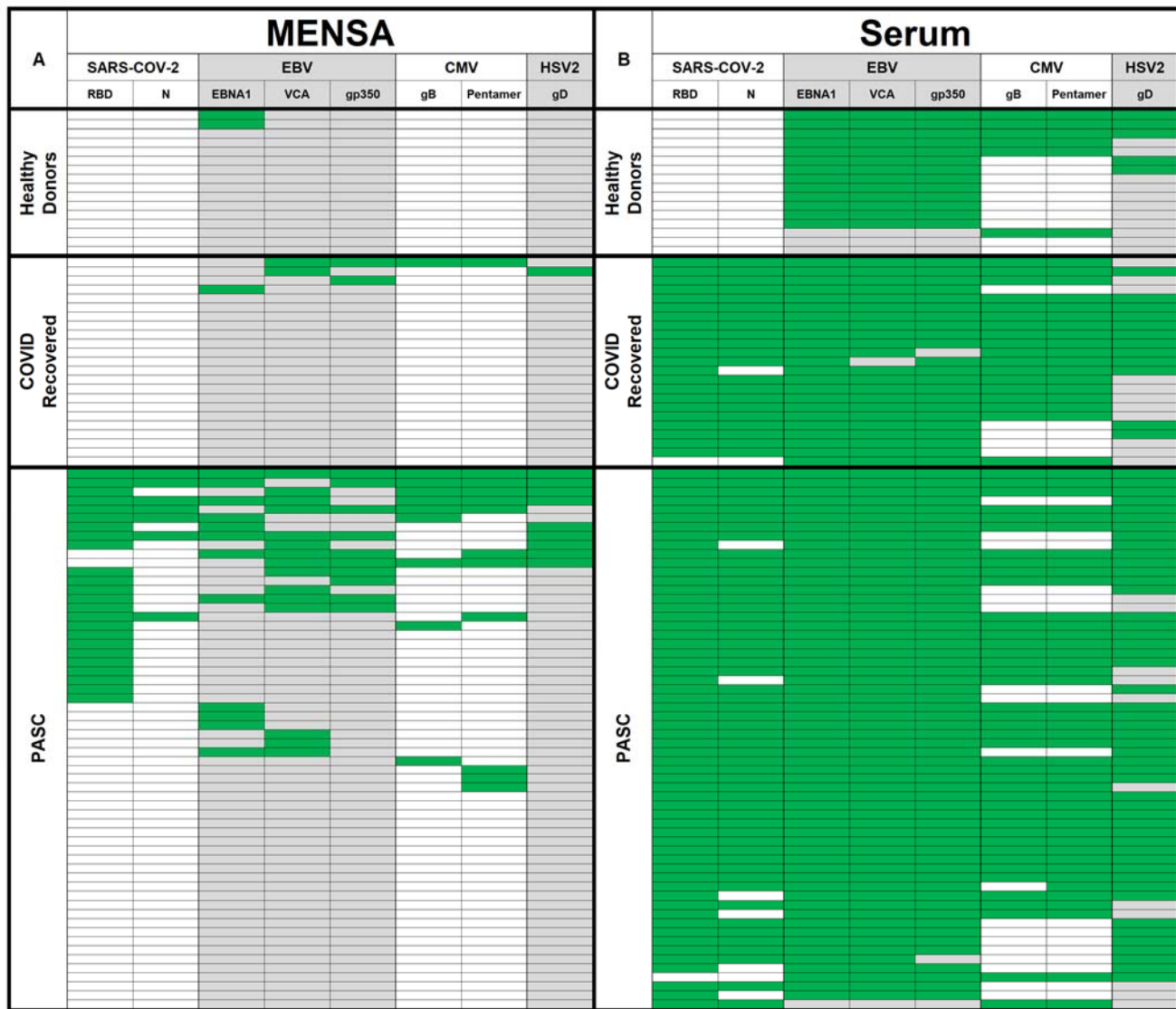
639  
640  
641  
642  
643  
644  
645  
646  
647  
648  
649

**Figure 5. Prolonged, elevated MENSEA IgG responses for SARS2 in a subset of PASC patients.** Dot plots show MENSEA and serum IgG antibody responses to S1-RBD (A,B) and Nucleocapsid (C,D) in samples collected between 60-279 DPO since initial COVID-19 Wild Type infection from patients who completely recovered from their acute illness (CR; n=19) and patients who suffer PASC (n=39). Blue dots represent a Mild/Moderate acute disease severity. Red dots represent a Severe/Critical acute disease severity. All values are reported as average Net MFI (Median Fluorescent Intensity – Background). Dashed lines indicate the C<sub>0</sub> threshold of positivity for each sample type and antigen combination as determined from Figure 1. Pair-wise comparisons were performed using the Mann-Whitney test in GraphPad Prism (unpaired, nonparametric test; ns p > 0.05, \* p ≤ 0.05, \*\* p ≤ 0.01, \*\*\* p ≤ 0.001, \*\*\*\* p ≤ 0.0001).



650  
651  
652  
653  
654  
655  
656  
657

**Figure 6. Phage immunoprecipitation sequencing (PhIP-Seq) analysis of MENSEA of PASC and CR patients for discovery.** PhIP-seq analysis determines the level of binding of antibodies to 149,259 peptides tiling all protein-coding sequences from viruses with human hosts in MENSEA samples from three CR (left column) and 10 PASC patients (middle column). Data are presented as z-scores of the anti-viral antibodies detected. Each row represents a linear peptide of viruses that were differentially bound in PASC and CR groups. Color code identifies virus species (right column).



658  
659  
660  
661  
662  
663  
664  
665  
666  
667  
668  
669  
670

**Figure 7. MENSA and serum in PASC, CR, and healthy adults for SARS2, EBV, CMV, and HSV2.** MENSA (A) and serum (B) samples were collected from healthy donors prior to SARS2 exposure (top), COVID recovered (middle), and PASC (bottom) patients, tested for IgG reactivity against SARS2, EBV, CMV, and HSV2 antigens, and presented in a heat map. Green cells represent Net MFI values  $\geq$  the  $C_0$  thresholds calculated for each sample type and antigen combination, while white or grey cells represent values below the  $C_0$ . In MENSA, a  $C_0$  was calculated as the average Net MFI of 22/23 CR samples plus three standard deviations for each antigen. Each SARS2 Serum  $C_0$  was calculated as the average plus three standard deviations of the 16 Healthy Donor samples collected prior to SARS2 exposure. For each of the remaining viruses, three clinically confirmed negative serum samples were obtained as virus specific negative controls and the average Net MFI plus three standard deviations were used to calculate  $C_0$  for each antigen.

671 **REFERENCES**

672

- 673 1. Omer, S.B., Malani, P. & Del Rio, C. The COVID-19 Pandemic in the US: A Clinical Update. *JAMA* **323**, 1767-  
674 1768 (2020).
- 675 2. Organization, W.H. COVID-19 weekly epidemiological update, 29 December 2020. (2020).
- 676 3. El Sahly, H.M., *et al.* Efficacy of the mRNA-1273 SARS-CoV-2 Vaccine at Completion of Blinded Phase. *N*  
677 *Engl J Med* **385**, 1774-1785 (2021).
- 678 4. Walsh, E.E., *et al.* Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. *The New*  
679 *England journal of medicine* **383**, 2439-2450 (2020).
- 680 5. Kumar, S., Thambiraja, T.S., Karuppanan, K. & Subramaniam, G. Omicron and Delta variant of SARS-CoV-2:  
681 A comparative computational study of spike protein. *J Med Virol* **94**, 1641-1649 (2022).
- 682 6. Thaweethai, T., *et al.* Development of a Definition of Postacute Sequelae of SARS-CoV-2 Infection. *JAMA*  
683 **329**, 1934-1946 (2023).
- 684 7. Post COVID-19 condition (Long COVID).
- 685 8. Woodruff, M.C., *et al.* Chronic inflammation, neutrophil activity, and autoreactivity splits long COVID. *Nat*  
686 *Commun* **14**, 4201 (2023).
- 687 9. Klein, J., *et al.* Distinguishing features of long COVID identified through immune profiling. *Nature* **623**, 139-  
688 148 (2023).
- 689 10. Su, Y., *et al.* Multiple early factors anticipate post-acute COVID-19 sequelae. *Cell* **185**, 881-895 e820  
690 (2022).
- 691 11. Wong, A.C., *et al.* Serotonin reduction in post-acute sequelae of viral infection. *Cell* **186**, 4851-4867 e4820  
692 (2023).
- 693 12. Liew, F., *et al.* Large-scale phenotyping of patients with long COVID post-hospitalization reveals  
694 mechanistic subtypes of disease. *Nat Immunol* **25**, 607-621 (2024).
- 695 13. Cervia-Hasler, C., *et al.* Persistent complement dysregulation with signs of thromboinflammation in active  
696 Long Covid. *Science* **383**, eadg7942 (2024).
- 697 14. Swank, Z., *et al.* Persistent Circulating Severe Acute Respiratory Syndrome Coronavirus 2 Spike Is  
698 Associated With Post-acute Coronavirus Disease 2019 Sequelae. *Clin Infect Dis* **76**, e487-e490 (2023).
- 699 15. Peluso, M.J., *et al.* Chronic viral coinfections differentially affect the likelihood of developing long COVID. *J*  
700 *Clin Invest* **133**(2023).
- 701 16. Carter, M.J., Mitchell, R.M., Meyer Sauteur, P.M., Kelly, D.F. & Truck, J. The Antibody-Secreting Cell  
702 Response to Infection: Kinetics and Clinical Applications. *Front Immunol* **8**, 630 (2017).
- 703 17. Halliley, J.L., *et al.* Long-Lived Plasma Cells Are Contained within the CD19(-)CD38(hi)CD138(+) Subset in  
704 Human Bone Marrow. *Immunity* **43**, 132-145 (2015).
- 705 18. Lee, F.E., *et al.* Circulating human antibody-secreting cells during vaccinations and respiratory viral  
706 infections are characterized by high specificity and lack of bystander effect. *J Immunol* **186**, 5514-5521  
707 (2011).
- 708 19. Oh, I., *et al.* Tracking Anti-Staphylococcus aureus Antibodies Produced In Vivo and Ex Vivo during Foot  
709 Salvage Therapy for Diabetic Foot Infections Reveals Prognostic Insights and Evidence of Diversified  
710 Humoral Immunity. *Infect Immun* **86**(2018).
- 711 20. Lee, F.E., Falsey, A.R., Halliley, J.L., Sanz, I. & Walsh, E.E. Circulating antibody-secreting cells during acute  
712 respiratory syncytial virus infection in adults. *J Infect Dis* **202**, 1659-1666 (2010).
- 713 21. Wrarmert, J., *et al.* Broadly cross-reactive antibodies dominate the human B cell response against 2009  
714 pandemic H1N1 influenza virus infection. *The Journal of experimental medicine* **208**, 181-193 (2011).
- 715 22. Wrarmert, J., *et al.* Rapid cloning of high-affinity human monoclonal antibodies against influenza virus.  
716 *Nature* **453**, 667-671 (2008).
- 717 23. Haddad, N.S., *et al.* Novel immunoassay for diagnosis of ongoing Clostridioides difficile infections using  
718 serum and medium enriched for newly synthesized antibodies (MENSA). *J Immunol Methods* **492**, 112932  
719 (2021).
- 720 24. Haddad, N.S., *et al.* Detection of Newly Secreted Antibodies Predicts Non-recurrence in Primary  
721 Clostridioides difficile Infection. *J Clin Microbiol*, jcm0220121 (2022).
- 722 25. Kyu, S., *et al.* Diagnosis of Streptococcus pneumoniae infection using circulating antibody secreting cells.  
723 *PLoS One* **16**, e0259644 (2021).



- 724 26. Muthukrishnan, G., *et al.* A Bioinformatic Approach to Utilize a Patient's Antibody-Secreting Cells against  
725 Staphylococcus aureus to Detect Challenging Musculoskeletal Infections. *Immunohorizons* **4**, 339-351  
726 (2020).
- 727 27. Haddad, N.S., *et al.* Circulating antibody-secreting cells are a biomarker for early diagnosis in patients with  
728 Lyme disease. *PLoS one* **18**, e0293203 (2023).
- 729 28. Panel, C.-T.G. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. (National Institutes of  
730 Health, 2020).
- 731 29. Haddad, N.S., *et al.* One-Stop Serum Assay Identifies COVID-19 Disease Severity and Vaccination  
732 Responses. *Immunohorizons* **5**, 322-335 (2021).
- 733 30. Woodruff, M., *et al.* Critically ill SARS-CoV-2 patients display lupus-like hallmarks of extrafollicular B cell  
734 activation. *medRxiv* (2020).
- 735 31. Nakagawa, S. & Takahashi, M.U. gEVE: a genome-based endogenous viral element database provides  
736 comprehensive viral protein-coding sequences in mammalian genomes. *Database (Oxford)* **2016**(2016).
- 737 32. Xu, G.J., *et al.* Viral immunology. Comprehensive serological profiling of human populations using a  
738 synthetic human virome. *Science* **348**, aaa0698 (2015).
- 739 33. Nguyen, D.C., *et al.* Majority of human circulating IgG plasmablasts stop blasting in a cell-free pro-survival  
740 culture. *Scientific reports* **14**, 3616 (2024).
- 741 34. Magri, G., *et al.* Human Secretory IgM Emerges from Plasma Cells Clonally Related to Gut Memory B Cells  
742 and Targets Highly Diverse Commensals. *Immunity* **47**, 118-134 e118 (2017).
- 743 35. Phad, G.E., *et al.* Clonal structure, stability and dynamics of human memory B cells and circulating  
744 plasmablasts. *Nat Immunol* **23**, 1076-1085 (2022).
- 745 36. Stein, S.R., *et al.* SARS-CoV-2 infection and persistence in the human body and brain at autopsy. *Nature*  
746 **612**, 758-763 (2022).
- 747 37. Koren, I., *et al.* The Eukaryotic Proteome Is Shaped by E3 Ubiquitin Ligases Targeting C-Terminal Degrons.  
748 *Cell* **173**, 1622-1635 e1614 (2018).
- 749 38. Larman, H.B., *et al.* Autoantigen discovery with a synthetic human peptidome. *Nat Biotechnol* **29**, 535-541  
750 (2011).
- 751 39. Lanz, T.V., *et al.* Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GialCAM. *Nature* **603**,  
752 321-327 (2022).
- 753 40. Bedran, D., Bedran, G. & Kote, S. A Comprehensive Review of Neurodegenerative Manifestations of SARS-  
754 CoV-2. *Vaccines (Basel)* **12**(2024).
- 755 41. Grant, R.A., *et al.* Prolonged exposure to lung-derived cytokines is associated with activation of microglia  
756 in patients with COVID-19. *JCI Insight* **9**(2024).
- 757 42. Rippee-Brooks, M.D., *et al.* Viral Infections, Are They a Trigger and Risk Factor of Alzheimer's Disease?  
758 *Pathogens* **13**(2024).
- 759 43. Mizrahi, B., *et al.* Long covid outcomes at one year after mild SARS-CoV-2 infection: nationwide cohort  
760 study. *BMJ* **380**, e072529 (2023).
- 761 44. Xie, Y. & Al-Aly, Z. Risks and burdens of incident diabetes in long COVID: a cohort study. *Lancet Diabetes*  
762 *Endocrinol* **10**, 311-321 (2022).
- 763 45. Xie, Y., Xu, E., Bowe, B. & Al-Aly, Z. Long-term cardiovascular outcomes of COVID-19. *Nat Med* **28**, 583-590  
764 (2022).
- 765 46. Chang, R., *et al.* Risk of autoimmune diseases in patients with COVID-19: A retrospective cohort study.  
766 *EClinicalMedicine* **56**, 101783 (2023).
- 767 47. Syed, U., *et al.* Incidence of immune-mediated inflammatory diseases following COVID-19: a matched  
768 cohort study in UK primary care. *BMC Med* **21**, 363 (2023).
- 769 48. Tesch, F., *et al.* Incident autoimmune diseases in association with SARS-CoV-2 infection: a matched cohort  
770 study. *Clin Rheumatol* **42**, 2905-2914 (2023).
- 771 49. Middeldorp, J.M. Epstein-Barr Virus-Specific Humoral Immune Responses in Health and Disease. *Current*  
772 *topics in microbiology and immunology* **391**, 289-323 (2015).
- 773 50. Karsten, C.B., *et al.* Evolution of functional antibodies following acute Epstein-Barr virus infection. *PLoS*  
774 *pathogens* **18**, e1010738 (2022).

- 775 51. Wiegers, A.K., Sticht, H., Winkler, T.H., Britt, W.J. & Mach, M. Identification of a neutralizing epitope  
776 within antigenic domain 5 of glycoprotein B of human cytomegalovirus. *Journal of virology* **89**, 361-372  
777 (2015).
- 778 52. Lilleri, D., *et al.* Fetal human cytomegalovirus transmission correlates with delayed maternal antibodies to  
779 gH/gL/pUL128-130-131 complex during primary infection. *PLoS one* **8**, e59863 (2013).
- 780 53. Macagno, A., *et al.* Isolation of human monoclonal antibodies that potently neutralize human  
781 cytomegalovirus infection by targeting different epitopes on the gH/gL/UL128-131A complex. *Journal of*  
782 *virology* **84**, 1005-1013 (2010).
- 783 54. Belshe, R.B., *et al.* Correlate of immune protection against HSV-1 genital disease in vaccinated women.  
784 *The Journal of infectious diseases* **209**, 828-836 (2014).