

Accelerated Article Preview

Distinguishing features of Long COVID identified through immune profiling

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1 Distinguishing features of Long COVID identified through immune profiling

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45 Summary

46 **Post-acute infection syndromes (PAIS) may develop after acute viral disease¹. Infection with SARS-**
47 **CoV-2 can result in the development of a PAIS known as “Long COVID” (LC). Individuals with**
48 **LC frequently report unremitting fatigue, post-exertional malaise, and a variety of cognitive and**
49 **autonomic dysfunctions²⁻⁴; however, the biological processes associated with the development and**
50 **persistence of these symptoms are unclear. Here, 273 individuals with or without LC were enrolled**
51 **in a cross-sectional study that included multi-dimensional immune phenotyping and unbiased**
52 **machine learning methods to identify biological features associated with LC. Marked differences**
53 **were noted in circulating myeloid and lymphocyte populations relative to matched controls, as well**
54 **as evidence of exaggerated humoral responses directed against SARS-CoV-2 among participants**
55 **with LC. Further, higher antibody responses directed against non-SARS-CoV-2 viral pathogens**
56 **were observed among individuals with LC, particularly Epstein-Barr virus. Levels of soluble**
57 **immune mediators and hormones varied among groups, with cortisol levels being lower among**
58 **participants with LC. Integration of immune phenotyping data into unbiased machine learning**
59 **models identified key features most strongly associated with LC status. Collectively, these findings**
60 **may help guide future studies into the pathobiology of LC and aid in developing relevant**
61 **biomarkers.**

62 Introduction

63
64 Recovery from acute viral infections is heterogeneous and chronic symptoms may linger for months to
65 years in some individuals. Additionally, persistent sequelae may develop after acute infection by a
66 number of viruses from a diverse range of viral families⁵⁻⁹. Post-acute infection syndromes (PAIS)
67 microbial infections have also been described for over a century^{10,11}. Yet despite their ubiquity, the basic
68 biology underlying PAIS development, even for extensively studied PAIS like myalgic
69 encephalomyelitis/chronic fatigue syndrome (ME/CFS), remains unclear^{1,12}.

70
71 SARS-CoV-2 is a betacoronavirus responsible for at least seven million deaths worldwide¹³. Infection
72 causes COVID-19, which can manifest as a severe respiratory disease marked by extensive
73 immunological and multi-organ system dysfunction¹⁴⁻¹⁹. Recovery from COVID-19 is often complete;
74 however, individuals (even those with initially mild disease courses) may have significantly increased
75 risks for adverse clinical events and abnormal clinical findings²⁰⁻²⁵.

76
77 In addition to developing isolated dysfunctions, some convalescent COVID-19 patients may develop a
78 group of new onset or aggravated sequelae known as Long COVID (LC). Clinically, LC presents as a
79 constellation of debilitating symptoms (e.g., unremitting fatigue, post-exertional malaise, cognitive
80 impairment, and autonomic dysfunctions), alongside other less common manifestations²⁻⁴. These
81 persistent sequelae dramatically impair physical and cognitive function and reduce quality of life²⁶.
82 Estimates of LC prevalence vary substantially²⁷, but prospective studies suggest about one in eight
83 individuals with COVID-19 experience persistent somatic symptoms attributable to past SARS-CoV-2
84 infection²⁸. While the underlying pathogenesis of LC remains unclear, current hypotheses include the
85 persistence of virus or viral remnants in tissue reservoirs; development or aggravation of autoimmunity;
86 microbial dysbiosis; reactivation of non-SARS-CoV-2 latent viral infections; and tissue damage caused
87 by chronic inflammation.

88
89 To interrogate the biological underpinnings of LC, a cross-sectional study was designed (Mount Sinai-
90 Yale Long COVID, MY-LC) involving 273 participants comprising five study groups: (1) healthcare
91 workers infected with SARS-CoV-2 before vaccination (HCW); (2) healthy, uninfected, vaccinated
92 controls (healthy controls, HC); (3) previously infected, vaccinated controls without persistent symptoms
93 (convalescent controls, CC); (4) individuals with persistent symptoms after acute infection (Long

94 COVID, LC); and (5) a second group of individuals with persistent symptoms following acute infection
95 from an independent study (External Long COVID, Ext. LC). Among the CC and LC groups, enrolled
96 participants had primarily mild (non-hospitalised) acute COVID-19 and samples for this study were
97 acquired, on average, more than a year after their acute infection. The HC, CC, and LC groups underwent
98 systematic, multi-dimensional immunophenotyping and unbiased machine learning of aggregated data to
99 identify potential LC biomarkers.

100 101 **Results**

102 *Overview of MY-LC cohort*

103 The MY-LC study enrolled 183 participants (101 LC, 42 CC, and 42 HC) at one study site (Mount Sinai
104 Hospital, New York City, New York) and 90 participants at another (Yale New Haven Hospital, New
105 Haven, CT) for a total of 275 participants. After initial enrollment and preliminary review of electronic
106 medical records, two participants were excluded from the LC group (2.0%, for pharmacologic
107 immunosuppression secondary to primary immune deficiency and solid organ transplant); two from HC
108 (4.8%, for pregnancy and misclassification at enrollment); and three from CC (7.1%, for pregnancy,
109 monogenic disorder, and misclassification at enrollment) resulting in a final study size of 268 individuals
110 (**Fig. 1A**). The proportion of participants excluded from the LC group did not significantly differ from
111 those excluded from the other groups (**Extended Data Table 1**).

112 Initial comparison of demographic factors showed the LC and CC groups differed in mean age (46 years,
113 LC; 38 years, CC; Kruskal-Wallis post-hoc, $p = 0.0040$). But these groups did not significantly differ in
114 sex; hospitalisation for acute COVID-19 (**Fig. 1B**); or median elapsed time between initial infection and
115 acute disease (**Fig. 1C**). Most acute infections within the LC group (76%) occurred between
116 epidemiological weeks 7–17 of 2020, when parental SARS-CoV-2 strains (WA-1) drove most new cases.
117 Importantly, the aggregated medical history of individuals with LC did not significantly differ from that
118 of CC individuals in baseline prevalence of anxiety or depression. Complete demographic features and
119 medical histories are reported in **Extended Data Table 1**.

120 Across all surveyed dimensions, participants with LC had significantly higher intensities of reported
121 symptoms and dramatically worsened quality of life (**Extended Data Table 2, Extended Data Fig. 1A**).
122 To address whether LC associated with any pattern of survey responses, responses were aggregated into a
123 single classification metric (Long COVID Propensity Score, LCPS) using a parsimonious logistic
124 regression model (LC vs. Other), which demonstrated significant diagnostic potential (0.94 AUC,
125 bootstrap CI: 0.89–0.97) (**Fig. 1D, Extended Data Fig. 1B, Extended Data Table 3**).

126 Among the self-reported symptoms from the LC group, fatigue (87%), brain fog (78%), memory
127 difficulty (62%), and confusion (55%) were most common (**Fig. 1E**). Postural Orthostatic Tachycardia
128 Syndrome (POTS) was also prevalent; 38% of individuals with LC had formal diagnostic testing and
129 clinical evaluation (**Extended Data Fig. 1C**). Negative impacts on employment status were also reported
130 by half the participants with LC (**Extended Data Fig. 1D**).

131 To find groups of participants with LC with similar sets of self-reported symptoms, an agglomerative
132 hierarchical clustering of binary symptoms was performed (**Extended Data Fig. 1E**). Three LC clusters
133 were identified (bootstrapped mean cluster-wise Jaccard similarity: cluster 1, 0.75 [95% CI: 0.54–1.00];
134 cluster 2, 0.60 [0.47–0.94]; and cluster 3, 0.75 [0.56–1.00]). LC clusters were clearly bifurcated by LCPS:
135 cluster 3 had intermediate propensity scores; clusters 1 and 2, more extreme ones (**Extended Data Fig.**
136 **1F**).

137 *Circulating immune cell differences*

138 Analysis of peripheral blood mononuclear cell (PBMC) populations revealed a significant difference in
139 circulating immune cell populations among MY-LC cohorts. The median level of non-conventional
140 monocytes (CD14^{lo}CD16^{hi}) in the LC group was significantly higher than those in other groups
141 (**Extended Data Fig. 2A, left**). To determine whether LC significantly associated with levels of non-
142 conventional monocytes after accounting for demographic differences across groups, linear models were
143 developed incorporating age, sex, LC status (binary), and body mass index (BMI). By this approach, LC
144 significantly associated with levels of total non-conventional monocytes (**Extended Data Fig. 3J**) and
145 those expressing MHC Class II (HLA-DR) (**Extended Data Fig. 2A, right**). Parallel investigation of
146 absolute cell counts also revealed increased numbers of circulating non-conventional monocytes
147 (**Extended Data Fig. 4A**).

148 Systematic analysis of other immune effector populations revealed significantly lower circulating
149 populations of cDC1s among participants with LC (**Extended Data Fig. 2B, left; Extended Data Fig.**
150 **4B**). Linear models again found LC status and age significantly associated with circulating cDC1 levels
151 (**Extended Data Fig. 2B, right**). Levels of other circulating granulocyte populations (neutrophils,
152 eosinophils, conventional and intermediate monocytes, plasmacytoid dendritic, and cDC2 populations)
153 did not significantly differ among groups, with substantial heterogeneities noted in LC (**Extended Data**
154 **Fig. 3A,B**).

155 The median relative percentage of B lymphocytes was significantly higher in both activated populations
156 (CD86^{hi}HLA-DR^{hi}: 17%, LC; 11%, CC; 12%, HC) and double-negative subsets (IgD⁻/CD27⁻/CD24⁻
157 /CD38⁻: 5%; 2%; 2%) (**Extended Data Fig. 2C**). The absolute count of double-negative B cells also
158 significantly increased in individuals with LC (**Extended Data Fig. 4C**). LC status was again
159 significantly associated with these effector populations in linear modeling (**Extended Data Fig. 3J**).
160 Circulating levels of various B-cell subsets, including naïve B cells, did not significantly differ among
161 groups (**Extended Data Fig. 3C**).

162 Circulating T lymphocyte populations were not strikingly different in effector memory subsets (CD45RA⁻
163 /CD127⁻/CCR7⁻) (**Extended Data Fig. 2D**), although absolute counts of CD4⁺ populations significantly
164 increased (**Extended Data Fig. 4D**). The median relative percentage of circulating CD4⁺ central memory
165 cells (CD45RA⁻/CD127⁺/CCR7⁻) was significantly lower in the LC group (27%, LC; 33%, CC; 32%,
166 HC), although groups did not differ by absolute count (**Extended Data Fig. 4D**). Median percentages of
167 exhausted (PD-1⁺/Tim-3⁺) CD4⁺ subsets (CD4_{Ex}) and exhausted CD8⁺ subsets (CD8_{Ex}) did not significantly
168 differ (**Extended Data Fig. 2D**), but absolute CD4_{Ex} counts were significantly elevated (**Extended Data**
169 **Fig. 4D**). Importantly, neither naïve CD4 nor CD8 T cells significantly differed (**Extended Data Fig.**
170 **3D**).

171 After being stimulated with phorbol myristate acetate and ionomycin, CD4⁺ cells from individuals with
172 LC produced significantly higher median levels of intracellular IL-2 (17%, LC; 14%, CC; 13%, HC); IL-4
173 (11%; 7%; 8%); and IL-6 (1.7%; 1.4%; 1.5%) (**Extended Data Fig. 2E, Extended Data Fig. 4E; top**
174 **row**), as did CD8⁺ T cells (**Extended Data Fig. 2E, Extended Data Fig. 4E, bottom row**). Both age and
175 LC status were significantly associated with intracellular IL-4 and IL-6 production (**Extended Data Fig.**
176 **2K, Extended Data Table 4**). Notably, individuals with LC also had uniquely elevated median levels of
177 IL-4/IL-6 double-positive CD4⁺ T cells (0.3%, LC; 0.2%, CC; 0.2%, HC) and double-positive CD8⁺ T
178 cells (0.5%; 0.2%; 0.2%) (**Extended Data Fig. 2F, Extended Data Fig. 4F**). Levels of IFN- γ and IL-17
179 (in CD4⁺) and TNF- α and GMZB (in CD8⁺) did not significantly differ across groups (**Extended Data**
180 **Fig. 3E-I**). To account for heterogeneous levels of circulating immune cell populations, permutational
181 analysis of variance (PERMANOVA) was performed using effector populations with significant
182 differences between groups at baseline. This multivariate analysis showed that LC status and age
183 significantly predicted levels of circulating immune cell populations (**Extended Data Fig. 2G**).

184 *SARS-CoV-2 specific antibody responses*

185 Initial analysis of anti-SARS-CoV-2 antibody responses was performed only for MY-LC participants who
186 received two doses of vaccine. Anti-S1 IgG levels in the LC group were significantly higher than those in
187 the CC group, and levels of total anti-S and anti-receptor-binding domain (RBD) IgG were elevated in
188 the LC group but did not significantly differ from CC-group levels (**Fig. 2A**). Unvaccinated participants
189 with LC had significantly higher anti-N IgG levels than did historical, unvaccinated controls previously
190 exposed to SARS-CoV-2 (**Extended Data Fig. 5A**).

191 Linear models were constructed to more fully account for baseline differences (demographics, vaccines at
192 blood draw [VAD]) across cohorts (**Fig. 2B, Extended Data Fig. 5B**), which revealed that LC state was a
193 significant, positive predictor of anti-Spike humoral response after accounting for such differences
194 (**Extended Data Table 5**). To gauge whether the elevated responses were to distinct regions of Spike,
195 anti-SARS-CoV-2 IgG responses against linear peptides were profiled among vaccinated participants. LC
196 participant responses were significantly higher than CC responses against a peptide that confers increased
197 neutralization^{29,30}, corresponding to amino acid residues 556–572 (1.3×; Outlier Sum, $p = 0.031$).
198 Responses were also higher (1.4×–1.6×) for peptides corresponding to residues 572–586, 625–638, and
199 682–690 (the furin cleavage site). CC participant responses were higher than LC ones against two S2
200 peptides (residues 1149–1161, 1.5×; 1256–1266, 2.1×) (**Fig. 2C**). Multiple differentially expressed Spike-
201 binding motifs were mapped onto available trimeric-structure models of Spike (PDB: 6VXX). These
202 mapped to highly surface exposed sites in the protein's natural conformational state, near the S1 RBD
203 (RDPQTLE and KFLPFQQ) and the S1/S2 cleavage site (RSVAS, YECDIPIGAGICA, and YMSLG)
204 (**Fig. 2D**), consistent with participants with LC having higher anti-Spike immune responses. By analysing
205 peptide enrichment for Spike motifs corresponding to Protein-based Immunome Wide Association Study
206 (PIWAS)-identified peaks, significantly greater humoral responses against KFLPFQQ (Kruskal-Wallis,
207 $p = 0.023$) (**Fig. 2E**), RDPQTLE ($p = 0.00058$), and LDK[WY]F ($p = 0.0034$) were found (**Extended**
208 **Data Fig. 5C**). Prevalences of antibody reactivities against KFLPFQQ (Fisher's exact, $p = 0.0060$),
209 RDPQTLE ($p = 0.00015$), LDK[WY]F ($p = 0.00066$), and DISGI ($p = 0.0086$) were also significantly
210 higher among participants with LC than among grouped controls (**Extended Data Fig. 5D**). Statistical
211 modeling accounting for baseline differences (demographics, VAD) revealed LC significantly associated
212 with reactivity against KFLPFQQ, RDPQTLE, and DISGI motifs (**Extended Data Fig. 5E**), but not with
213 reactivity against LDK[WY]F (**Extended Data Fig. 5E**), which was elevated in both CC and LC groups
214 (**Extended Data Fig. 5C**).

215 *Cortisol and soluble immune mediators*

216 Parallel multiplex analysis of circulating hormones and immune mediators in plasma samples revealed
217 groups in the MY-LC cohort significantly differed in median levels of cortisol (Kruskal-Wallis,
218 $p < 0.0001$); complement C4b ($p = 0.0001$); CCL19 ($p = 0.00058$); galectin-1 ($p = 0.0015$); CCL20
219 ($p = 0.0032$); CCL4 ($p = 0.0092$); APRIL ($p = 0.013$); LH ($p = 0.022$); and IL-5 ($p = 0.024$). Post-hoc
220 comparisons showed the LC group had significantly increased complement C4b, CCL19, CCL20,
221 galectin-1, CCL4, APRIL, and LH; and marginally but significantly decreased IL-5 (**Extended Data Fig.**
222 **6A–H**). Additional analysis revealed significant correlations with LCPS scores, particularly for cortisol
223 (**Extended Data Fig. 6I**). In the Ext. LC cohort ($n = 53$, excluding an outlier whose level was >8 standard
224 deviations above median), cortisol levels in the LC group were lower than those in the HC and CC groups
225 (**Fig. 2F**). Paired levels of adrenocorticotrophic hormone (ACTH) were evaluated only in the MY-LC
226 cohort; these did not significantly differ across groups (**Fig. 2G**). Median sample collection times
227 significantly differed only between CC and LC groups, and this difference was modest (65 minutes;
228 Dunn's test, $p = 0.027$) (**Fig. 2H**). Subsequent statistical modeling revealed that LC status significantly
229 associated with lower cortisol levels after accounting for individual differences in age, sex, BMI, sample-
230 collection time, and cohort (MY-LC vs. Ext. LC) (**Fig. 2I, Extended Data Table 6**).

231 *Autoantibodies to exoproteome*

232 Next, antibody reactivity against extracellular proteins was assessed in 98 LC and 38 control participants
233 using rapid extracellular antigen profiling (REAP), a method to measure antibody reactivity against
234 >6,000 extracellular and secreted human proteins¹⁶. Although participants with LC had a variety of
235 private reactivities against diverse autoantigens (**Fig. 3A**), the number of autoantibody reactivities per
236 participant did not differ across groups (**Fig. 3B**), nor did the number of reactivities significantly correlate
237 with LC clusters (as assessed by LCPS scores) (**Fig. 3C**). Additionally, the number of autoantibody
238 reactivities correlated with neither double-negative B-cell populations nor days from acute symptom onset
239 (**Extended Data Fig. 7A,B**).

240 Given REAP studies showing functional autoantibodies are elevated in severe acute COVID-19¹⁶,
241 autoantibody reactivities were aggregated into clusters using a manually curated Gene Ontology process
242 list relevant to LC. The magnitudes of reactivity for LC and control groups did not significantly differ in
243 any category (**Extended Data Fig. 5C**). Several reports implicated stereotypical G protein-coupled
244 receptor (GPCR) autoantibodies in LC pathogenesis^{31,32} (e.g., targeting beta adrenergic receptors or the
245 angiotensin II receptor). While several GPCR-directed autoantibodies were detected in this study
246 (**Extended Data Fig. 7D**), the number of GPCR reactivities for participants with LC did not differ from
247 that for controls (**Fig. 3D**). Importantly, individual autoantibody reactivities were not significantly more
248 frequent in either participants with LC or in controls (**Fig. 3E**).

249 *Antibody responses to herpesviruses*

250 Given emerging evidence for the role of latent virus reactivation in LC, three complementary approaches
251 were used to examine anti-viral reactivity patterns in the MY-LC cohorts: REAP, serum epitope
252 repertoire analysis (SERA), and ELISA. Global anti-viral responses were first assessed by REAP, which
253 measures antibody reactivity to 225 viral surface proteins (**Supplementary Table 2**). Reactivities against
254 38 viral conformational epitopes were detected among 98 LC and 38 control participants (**Extended Data**
255 **Fig. 8A**). For SARS-CoV2 reactivities, only participants who received two doses of vaccine were
256 analysed. Reactivities against non-Omicron variant RBDs in the LC cohort were higher than those in the
257 CC controls (**Fig. 4A**); however, as with ELISA, this trend was not significant.

258 Differences in viral reactivities against non-SARS-CoV-2 antigens were striking (**Fig. 4B**). Participants
259 with LC had elevated REAP scores for several herpesvirus antigens, including the Epstein-Barr virus
260 (EBV) minor viral capsid antigen gp23 ($p = 4.62E-3$); the EBV fusion-receptor component gp42
261 ($p = 3.2E-2$); and the VZV glycoprotein E ($p = 1.51E-2$) (**Extended Data Fig. 8B**). Conversely,
262 participants with LC had lower REAP scores for HSV-1 glycoprotein gL ($p = 4.61E-6$) and gD1, although
263 the difference in gD1 reactivity was not significant.

264 Next, the SERA platform (a commercially available random bacterial display library with unlimited
265 multiplex capability) was used to orthogonally analyse non-SARS-CoV-2 antigens. SERA includes
266 epitope panels representing 45 pathogens and disease markers, validated using a database of thousands of
267 controls³³. Importantly, SERA revealed that cohorts significantly differed neither in estimated EBV
268 seroprevalence (**Fig. 4C**) nor for any other tested viral pathogen (**Extended Data Fig. 8C**).

269 First was assessed whether individuals with LC had higher EBV reactivities because of acute EBV
270 infection. Anti-EBV IgM was not elevated in this group (as measured by SERA) (**Extended Data Fig.**
271 **8D**) nor was there evidence of EBV viremia (**Extended Data Fig. 8E,F**), suggesting that the higher
272 reactivity to EBV lytic antigens was more likely caused by recent EBV reactivation than by acute
273 infection. Additionally, these results do not rule out EBV shedding at a local site, such as in saliva³⁴.

274 Next was assessed whether differences in baseline seropositivity affected EBV-antigen reactivity. EBV
275 reactivity was analysed only in EBV-seropositive individuals as identified by SERA and by Identifying
276 Motifs Using Next-generation sequencing Experiments (IMUNE). By REAP, seropositive participants
277 with LC had significantly higher reactivity to EBV p23 (Kruskal-Wallis, $p = 0.00095$, **Fig. 4D**) and gp42
278 (0.0039 , **Fig. 4E**) than did seropositive controls. REAP measurements significantly correlated with
279 ELISA measurements ($R = 0.73$, $p \leq 2.2E-16$), orthogonally validating this finding (**Extended Data Fig.**
280 **8G**). In an orthogonal screen of linear peptides with SERA, the LC cohort had greater reactivity against
281 the gp42 linear peptide (PVXF[ND]K) (Kruskal-Wallis, $p = 0.0031$) (**Fig. 4F**). Mapping of this motif onto
282 available structures of gp42 complexed with EBV gH/gL (PDB: 5T1D) showed these residues are
283 exposed on the surface of EBV virions (**Fig. 4G**, *pink residues*).

284 To investigate lower REAP reactivity to HSV-1 antigens observed in participants with LC, a similar
285 analysis was performed using only HSV-1 seropositive individuals, as identified by SERA. In these
286 individuals, REAP scores for HSV-1 glycoprotein gD1 no longer differed among groups (**Extended Data**
287 **Fig. 8H**). Post-hoc comparisons for HSV-1 gL also showed the groups did not significantly differ
288 (**Extended Data Fig. 8I**). These data suggested that the lower IgG reactivity to gL in REAP (**Fig. 4B**) is
289 probably caused by lower HSV-1 seroprevalence in the LC group. In aggregated initial REAP and SERA
290 results, individuals with LC had elevated IgG reactivity to EBV and VZV surface antigens without
291 evidence of EBV primary infection or acute viremia.

292 Additional analysis revealed LCPS significantly correlated with humoral reactivity against neither gp42
293 PVXF[ND]K nor EBV p23 antigens in EBV-seropositive individuals (**Extended Data Fig. 8J,K**). In
294 contrast, reactivity to gp42 PVXF[ND]K correlated with IL-4/IL-6 producing CD4⁺ T cells in EBV-
295 seropositive individuals with LC ($R = 0.26$, $p = 0.013$) (**Fig. 4H**). This correlation was not observed in
296 control groups. Furthermore, EBV p23 REAP reactivity significantly correlated with terminally
297 differentiated effector memory (T_{EMRA}) CD4⁺ T cells ($R = 0.26$, $p = 0.018$) (**Fig. 4I**), a subset of cells
298 implicated in protection from CMV³⁵. In contrast, anti-SARS-CoV-2 antibody levels did not correlate
299 with IL-4/IL-6 double-positive CD4⁺ T cells (**Extended Data Fig. 8L-O**).

300 *Unique biological markers of Long COVID*

301 To further account for demographic differences among groups that might affect immunophenotypes, each
302 LC participant was explicitly matched to a control participant by using a Gale-Shapley procedure based
303 on participant age, sex, days from acute COVID-19 symptom onset, and vaccination status. Participants
304 with LC did not differ significantly from controls in these criteria (**Extended Data Fig. 9A**), nor in
305 severity of acute COVID-19 disease (whether hospitalisation was required) (**Extended Data Fig. 9B**).
306 Principal component analysis embedding of matched participants with all collected immunological
307 features clearly distinguished individuals with LC from controls (**Fig. 5A**). Consistent with this, k-nearest
308 neighbours classification on the normalised features efficiently discriminated between groups, with an
309 AUC of 0.94 (95% CI: 0.84–1.00) (**Fig. 5B**). Principal components regression of collated immunological
310 data showed that flow cytometry (pseudo- $R^2 = 59\%$) and plasma proteomics and hormones (pseudo-
311 $R^2 = 74\%$) were most informative for separating groups. A final parsimonious LASSO model similarly
312 achieved good fit (pseudo- $R^2 = 82\%$) (**Fig. 5C**). Of the features selected for the final model, several
313 associated positively with LC status (serum galectin-1 concentration, IgG against various EBV epitopes);
314 while others associated negatively (serum cortisol; PD-1⁺ / CD4⁺ T_{em}; cDC1 cells) (**Fig. 5D**). Preliminary
315 external validation in the Ext. LC cohort of selected LASSO-model features revealed similar decreases in
316 cortisol, but galectin-1 and EBV gp42 predicted LC status specifically in the MY-LC cohort (**Extended**
317 **Data Fig. 9C,D**), potentially caused by clinical phenotype differences between the MY-LC and Ext. LC
318 cohorts (**Extended Data Fig. 9E**).

319 Serum cortisol was the most significant predictor of LC status in the model, and cortisol alone achieved
320 an AUC of 0.96 (95% CI: 0.93–0.99) (**Extended Data Fig. 9F, top**). Notably, serum cortisol in the MY-
321 LC cohort was similar in the HC and CC controls, and lower in participants with LC (**Extended Data**
322 **Fig. 9F, bottom**). When used alone, each of the other selected model features predicted status reasonably
323 well (**Extended Data Fig 9G,H**). Last, classification accuracies of LCPS models substantially agreed
324 with machine learning ones (Cohen’s Kappa, 0.79; 95% CI: 0.65–0.93), suggesting that both participant-
325 reported outcomes and immunological features efficiently predict LC status (**Extended Data Table 7**).

326 Discussion

327 Studies of individuals with LC reported diverse changes in immune and inflammatory factors^{36,37}. In this
328 study, exploratory analyses identified significant immunological differences between the LC population
329 and demographically matched control populations more than a year from their acute infections.
330 Circulating immune cell populations significantly changed. Populations of non-conventional monocytes,
331 double-negative B cells, and IL-4/IL-6 secreting CD4 T cells increased; and those of conventional DC1
332 and central memory CD4 T cells decreased. In addition, individuals with LC had higher levels of
333 antibodies to SARS-CoV-2, EBV, and VZV antigens. In contrast, levels of individual autoantibodies to
334 human exoproteome did not significantly differ. Marked differences in levels of circulating cytokines and
335 hormones, particularly cortisol, were noted in participants with LC from both MY-LC and Ext. LC
336 cohorts. Unbiased machine learning revealed several core predictive features of LC status within the MY-
337 LC study, identifying potential targets for additional validation and future biomarker development.

338 Multiple hypotheses have been proposed for LC pathogenesis, including persistent virus or viral
339 remnants³⁸, autoimmunity, dysbiosis, latent viral reactivation, and unrepaired tissue damage. The data in
340 this study suggest persistent SARS-CoV-2 viral antigens, reactivation of latent herpesviruses, and chronic
341 inflammation may all contribute to LC. Overall, our data are less consistent with an autoantibody-
342 dominated disease process in LCs. Whether autoreactive T cells play a role in LC pathogenesis was not
343 addressed and requires future investigation.

344 Immune phenotyping of PBMC populations revealed participants with LC had notably higher levels of
345 circulating non-conventional monocytes associated with various chronic inflammatory and autoimmune
346 conditions³⁹. These participants also had significantly lower levels of circulating cDC1 populations,
347 which are responsible for antigen presentation and cytotoxic T cell priming⁴⁰. In addition, the number of
348 CD4⁺ T_{cm} cells was significantly reduced and the absolute number of exhausted CD4⁺ T cells was
349 increased. Cerebral spinal fluid from individuals with LC also have elevated levels of TIGIT⁺ CD8⁺ T
350 cells, consistent with possible immune exhaustion⁴¹. After stimulation, T cells from individuals with LC
351 produced significantly more intracellular IL-2 (CD4, CD8), IL-4 (CD4), and IL-6 (CD8). Notably, subsets
352 of participants with LC also had polyfunctional IL-4/IL-6 co-expressing CD4⁺ T cells, which correlated
353 with reactivity against EBV lytic antigens, but not against SARS-CoV-2 antigens. In aggregate, these
354 findings may be consistent with T_H2-skewed CD4 T cell activation in response to EBV among
355 participants with LC, as suggested for ME/CFS⁴². Levels of IgG against SARS-CoV-2 Spike and S1 in
356 participants with LC were higher than those in vaccination-matched controls, consistent with persistent
357 viral antigens^{43–45}.

358 Participants with LC from two sites had significantly decreased systemic cortisol levels; this remained
359 significant after accounting for variations in demographics and sample-collection times. Interestingly, the
360 decreased cortisol did not associate with a compensatory increase in ACTH levels, suggesting the
361 hypothalamic-pituitary axis response to regulate cortisol may be inappropriately blunted. Importantly,
362 ACTH has an extremely short half-life in plasma, which may impair accurately detecting changes.
363 Dedicated studies must confirm these preliminary findings. Intriguingly, an earlier study of 61 survivors
364 of SARS-CoV infection showed similar evidence of hypocortisolemia and blunted ACTH responses three
365 months after acute disease⁴⁶. Furthermore, decreased cortisol levels during the early phases of COVID-19

366 were associated with development of respiratory LC symptoms⁴⁷. As cortisol is central for a variety of
367 homeostatic and stress responses⁴⁸, the current finding of *persistently* lower cortisol levels in those with
368 LC more than a year after acute infection warrants further investigations.

369 This study also revealed individuals with LC have elevated antibody responses against non-SARS-CoV-2
370 viral antigens, particularly EBV antigens. EBV viremia occurs during acute COVID-19 in hospitalised
371 patients and predicts development of persistent symptoms in the post-acute period⁴⁷. Other studies
372 implicated recent EBV reactivation with LC development^{49,50}. The observation here of elevated IgG
373 against EBV lytic antigens suggests that recent reactivation of latent herpesviruses (EBV, VZV) may be a
374 common feature of LC.

375 Finally, machine learning models designed to accurately classify LC and control populations (after
376 matching individuals to account for potentially confounding features, like sex, age, days from symptom
377 onset, and vaccination status) identified multiple features that significantly predict LC status.
378 Classifications using only immunological data strongly agreed with classifications using survey scores
379 (LCPS; Cohen's Kappa, 0.764), showing the immunological analyses and patient-reported outcomes used
380 here were highly concordant in diagnosing LC.

381 This study has several limitations. Primary among these is that few participants were identified by
382 convenience sampling and that recruitment strategies for cases differed from those for controls. Though
383 broadly covering diverse biological features, this study used far fewer independent observations than
384 traditional machine learning studies use (several thousands) to robustly train and optimise classification
385 models. This study was also restricted to analysing peripheral (circulating) immune factors from
386 participants. As LC often presents with organ system-specific dysfunctions, greater analyses of local
387 immune features would crucially extend these findings. Further, analysis of autoantibodies was restricted
388 to the exoproteome. Whether autoantibodies to intracellular antigens or non-protein antigens participate in
389 LC pathogenesis was not tested.

390 In summary, significant biological differences were identified between participants with LC and
391 demographically and medically matched CC and HC participants, validating extensive reports of
392 persistent symptoms by various individuals with LC and patient advocacy groups. This study provides a
393 basis for future investigations into the immunological underpinnings driving the genesis of LC.

394 References

- 395 1. Choutka, J., Jansari, V., Hornig, M. & Iwasaki, A. Unexplained post-acute infection syndromes. *Nat.*
396 *Med.* **28**, 911–923 (2022).
- 397 2. Thaweethai, T. *et al.* Development of a Definition of Postacute Sequelae of SARS-CoV-2 Infection.
398 *JAMA* (2023) doi:10.1001/jama.2023.8823.
- 399 3. Nalbandian, A. *et al.* Post-acute COVID-19 syndrome. *Nat. Med.* **27**, 601–615 (2021).
- 400 4. Michelen, M. *et al.* Characterising long COVID: a living systematic review. *BMJ Glob. Health* **6**,
401 e005427 (2021).
- 402 5. Wiedemann, A. *et al.* Long-lasting severe immune dysfunction in Ebola virus disease survivors. *Nat.*
403 *Commun.* **11**, 3730 (2020).
- 404 6. Hickie, I. *et al.* Post-infective and chronic fatigue syndromes precipitated by viral and non-viral
405 pathogens: prospective cohort study. *BMJ* **333**, 575 (2006).
- 406 7. Paixão, E. S. *et al.* Chikungunya chronic disease: a systematic review and meta-analysis. *Trans. R.*
407 *Soc. Trop. Med. Hyg.* **112**, 301–316 (2018).
- 408 8. Patel, H., Sander, B. & Nelder, M. P. Long-term sequelae of West Nile virus-related illness: a
409 systematic review. *Lancet Infect. Dis.* **15**, 951–959 (2015).
- 410 9. Trojan, D. A. & Cashman, N. R. Post-poliomyelitis syndrome. *Muscle Nerve* **31**, 6–19 (2005).
- 411 10. Gowers, W. R. A Post-Graduate Lecture on the Nervous Sequelae of Influenza. *The Lancet* **142**,
412 73–76 (1893).

- 413 11. Althus, J. *Influenza : its pathology, symptoms, complications, and sequels its origin and mode of*
414 *spreading and its diagnosis, prognosis, and treatment.* (Longmans, 1892).
- 415 12. Davis, H. E., McCorkell, L., Vogel, J. M. & Topol, E. J. Long COVID: major findings,
416 mechanisms and recommendations. *Nat. Rev. Microbiol.* **21**, 133–146 (2023).
- 417 13. Dong, E., Du, H. & Gardner, L. An interactive web-based dashboard to track COVID-19 in real
418 time. *Lancet Infect. Dis.* **20**, 533–534 (2020).
- 419 14. Lucas, C. *et al.* Longitudinal analyses reveal immunological misfiring in severe COVID-19.
420 *Nature* **584**, 463–469 (2020).
- 421 15. Lucas, C. *et al.* Delayed production of neutralizing antibodies correlates with fatal COVID-19.
422 *Nat. Med.* **27**, 1178–1186 (2021).
- 423 16. Wang, E. Y. *et al.* Diverse functional autoantibodies in patients with COVID-19. *Nature* **595**,
424 283–288 (2021).
- 425 17. Mathew, D. *et al.* Deep immune profiling of COVID-19 patients reveals distinct immunotypes
426 with therapeutic implications. *Science* **369**, eabc8511 (2020).
- 427 18. Su, Y. *et al.* Multi-Omics Resolves a Sharp Disease-State Shift between Mild and Moderate
428 COVID-19. *Cell* **183**, 1479–1495.e20 (2020).
- 429 19. Gupta, A. *et al.* Extrapulmonary manifestations of COVID-19. *Nat. Med.* **26**, 1017–1032 (2020).
- 430 20. Daugherty, S. E. *et al.* Risk of clinical sequelae after the acute phase of SARS-CoV-2 infection:
431 retrospective cohort study. *BMJ* **373**, n1098 (2021).
- 432 21. DeVries, A., Shambhu, S., Sloop, S. & Overhage, J. M. One-Year Adverse Outcomes Among US
433 Adults With Post-COVID-19 Condition vs Those Without COVID-19 in a Large Commercial
434 Insurance Database. *JAMA Health Forum* **4**, e230010 (2023).
- 435 22. Dennis, A. *et al.* Multi-organ impairment and long COVID: a 1-year prospective, longitudinal
436 cohort study. *J. R. Soc. Med.* 1410768231154703 (2023) doi:10.1177/01410768231154703.
- 437 23. Al-Aly, Z., Xie, Y. & Bowe, B. High-dimensional characterization of post-acute sequelae of
438 COVID-19. *Nature* **594**, 259–264 (2021).
- 439 24. Xie, Y., Xu, E., Bowe, B. & Al-Aly, Z. Long-term cardiovascular outcomes of COVID-19. *Nat.*
440 *Med.* **28**, 583–590 (2022).
- 441 25. Xu, E., Xie, Y. & Al-Aly, Z. Long-term neurologic outcomes of COVID-19. *Nat. Med.* **28**, 2406–
442 2415 (2022).
- 443 26. Tabacof, L. *et al.* Post-acute COVID-19 Syndrome Negatively Impacts Physical Function,
444 Cognitive Function, Health-Related Quality of Life, and Participation. *Am. J. Phys. Med. Rehabil.* **101**,
445 48–52 (2022).
- 446 27. Chen, C. *et al.* Global Prevalence of Post-Coronavirus Disease 2019 (COVID-19) Condition or
447 Long COVID: A Meta-Analysis and Systematic Review. *J. Infect. Dis.* **226**, 1593–1607 (2022).
- 448 28. Ballering, A. V., van Zon, S. K. R., Olde Hartman, T. C., Rosmalen, J. G. M., & Lifelines Corona
449 Research Initiative. Persistence of somatic symptoms after COVID-19 in the Netherlands: an
450 observational cohort study. *Lancet Lond. Engl.* **400**, 452–461 (2022).
- 451 29. Poh, C. M. *et al.* Two linear epitopes on the SARS-CoV-2 spike protein that elicit neutralising
452 antibodies in COVID-19 patients. *Nat. Commun.* **11**, 2806 (2020).
- 453 30. Li, Y. *et al.* Linear epitopes of SARS-CoV-2 spike protein elicit neutralizing antibodies in
454 COVID-19 patients. *Cell. Mol. Immunol.* **17**, 1095–1097 (2020).
- 455 31. Wallukat, G. *et al.* Functional autoantibodies against G-protein coupled receptors in patients with
456 persistent Long-COVID-19 symptoms. *J. Transl. Autoimmun.* **4**, 100100 (2021).
- 457 32. Szweczykowski, C. *et al.* Long COVID: Association of Functional Autoantibodies against G-
458 Protein-Coupled Receptors with an Impaired Retinal Microcirculation. *Int. J. Mol. Sci.* **23**, 7209
459 (2022).
- 460 33. Kamath, K. *et al.* Antibody epitope repertoire analysis enables rapid antigen discovery and
461 multiplex serology. *Sci. Rep.* **10**, 5294 (2020).
- 462 34. Fafi-Kremer, S. *et al.* Long-Term Shedding of Infectious Epstein-Barr Virus after Infectious
463 Mononucleosis. *J. Infect. Dis.* **191**, 985–989 (2005).

- 464 35. Gordon, C. L. *et al.* Tissue reservoirs of antiviral T cell immunity in persistent human CMV
 465 infection. *J. Exp. Med.* **214**, 651–667 (2017).
- 466 36. Woodruff, M. C. *et al.* Chronic inflammation, neutrophil activity, and autoreactivity splits long
 467 COVID. *Nat. Commun.* **14**, 4201 (2023).
- 468 37. Altmann, D. M., Whettlock, E. M., Liu, S., Arachchillage, D. J. & Boyton, R. J. The immunology
 469 of long COVID. *Nat. Rev. Immunol.* (2023) doi:10.1038/s41577-023-00904-7.
- 470 38. Peluso, M. J. *et al.* *Multimodal Molecular Imaging Reveals Tissue-Based T Cell Activation and*
 471 *Viral RNA Persistence for Up to 2 Years Following COVID-19.*
 472 <http://medrxiv.org/lookup/doi/10.1101/2023.07.27.23293177> (2023)
 473 doi:10.1101/2023.07.27.23293177.
- 474 39. Narasimhan, P. B., Marcovecchio, P., Hamers, A. A. J. & Hedrick, C. C. Nonclassical Monocytes
 475 in Health and Disease. *Annu. Rev. Immunol.* **37**, 439–456 (2019).
- 476 40. Merad, M., Sathe, P., Helft, J., Miller, J. & Mortha, A. The Dendritic Cell Lineage: Ontogeny and
 477 Function of Dendritic Cells and Their Subsets in the Steady State and the Inflamed Setting. *Annu. Rev.*
 478 *Immunol.* **31**, 563–604 (2013).
- 479 41. Mina, Y. *et al.* Deep Phenotyping of Neurologic Postacute Sequelae of SARS-CoV-2 Infection.
 480 *Neurol. - Neuroimmunol. Neuroinflammation* **10**, e200097 (2023).
- 481 42. Ruiz-Pablos, M., Paiva, B., Montero-Mateo, R., Garcia, N. & Zabaleta, A. Epstein-Barr Virus and
 482 the Origin of Myalgic Encephalomyelitis or Chronic Fatigue Syndrome. *Front. Immunol.* **12**, 656797
 483 (2021).
- 484 43. Swank, Z. *et al.* Persistent Circulating Severe Acute Respiratory Syndrome Coronavirus 2 Spike
 485 Is Associated With Post-acute Coronavirus Disease 2019 Sequelae. *Clin. Infect. Dis.* **76**, e487–e490
 486 (2023).
- 487 44. Stein, S. R. *et al.* SARS-CoV-2 infection and persistence in the human body and brain at autopsy.
 488 *Nature* **612**, 758–763 (2022).
- 489 45. Cheung, C. C. L. *et al.* Residual SARS-CoV-2 viral antigens detected in GI and hepatic tissues
 490 from five recovered patients with COVID-19. *Gut* **71**, 226–229 (2022).
- 491 46. Leow, M. K.-S. *et al.* Hypocortisolism in survivors of severe acute respiratory syndrome (SARS).
 492 *Clin. Endocrinol. (Oxf.)* **63**, 197–202 (2005).
- 493 47. Su, Y. *et al.* Multiple early factors anticipate post-acute COVID-19 sequelae. *Cell* **185**, 881-
 494 895.e20 (2022).
- 495 48. Husebye, E. S., Pearce, S. H., Krone, N. P. & Kämpe, O. Adrenal insufficiency. *The Lancet* **397**,
 496 613–629 (2021).
- 497 49. Peluso, M. J. *et al.* Chronic viral coinfections differentially affect the likelihood of developing
 498 long COVID. *J. Clin. Invest.* **133**, e163669 (2023).
- 499 50. Gold, J. E., Okyay, R. A., Licht, W. E. & Hurley, D. J. Investigation of Long COVID Prevalence
 500 and Its Relationship to Epstein-Barr Virus Reactivation. *Pathog. Basel Switz.* **10**, 763 (2021).

501 Main Figure Legends

503 **Figure 1. Demographic and clinical stratification of participants with Long COVID.** (A) Schematic of
 504 MY-LC study. Numbers indicate participants after exclusion (see ‘Methods’). (B) Select demographics for
 505 LC (top row, purple) and CC (bottom row, yellow) groups. Centre values in ‘Age’ column represent average
 506 group values (n = 40 CC, 99 LC). Statistical significance is reported for relevant post-hoc comparisons
 507 (‘Age’) or Chi-square tests (‘Sex’ and ‘Acute Disease Severity’). Complete statistical results are detailed
 508 in **Extended Data Table 2**. (C) Box plots of days from acute symptom onset between LC and CC groups.
 509 Significance was assessed using a two-tailed Brown-Mood median test with an alpha of 0.05 (n = 39 CC,
 510 99 LC). (D) Box plots of LCPS for each individual (n = 35 HC, 20 CC, 98 LC). Significance was assessed
 511 using Kruskal-Wallis tests corrected for multiple comparisons using Bonferroni’s method. (E) Prevalence
 512 of top 30 self-reported binary symptoms ranked from most prevalent (right) to least prevalent (left).

513 Symptoms are coloured according to common physiological system: Constitutional (Const., light green);
514 Neurological (Neuro., blue); Pulmonary (Pulm., gold); Musculoskeletal (MSK, red); Gastrointestinal (GI,
515 purple); Cardiac (dark green); Endocrine (Endo., pink); Ear, Nose, Throat (ENT, grey); and Sexual
516 Dysfunction (Sex Dys., teal). For all box plots (C,D), central lines indicate group medians; top and bottom
517 lines indicate 75th and 25th percentiles, respectively; whiskers represent 1.5× IQR; and individual data points
518 mark outliers. *Abbreviations: IQR, interquartile range; Long COVID propensity scores, LCPS. For (A),*
519 *clockwise, from top left: HCW, historical, unvaccinated SARS-CoV-2 exposed controls; Ext. LC, external*
520 *participants with Long COVID; CC, convalescent infected individuals without persistent symptoms; LC,*
521 *convalescent infected individuals with persistent symptoms; HC, healthy controls with no prior exposure.*
522 *For (E), top to bottom: EMR, electronic medical record; n.s., not significant; Dif., difficulty; UI, urination;*
523 *Subj., subjective; Alt., altered; Decr., decreased; Abd., abdominal; reg., regulating; temp, body*
524 *temperature; Musc, muscle; Indig., indigestion.*

525 **Figure 2. Exaggerated SARS-CoV-2 specific humoral responses and altered circulating immune**
526 **mediators among Long COVID participants. (A)** SARS-CoV-2 antibody responses-assessed by ELISA
527 (n = 22 HC, 17 CC, 70 LC). Vaccination status for each cohort is indicated by “x2” indicating the number
528 of SARS-CoV-2 vaccine doses at sample collection. Significance for difference in group medians was
529 assessed using Kruskal-Wallis with FDR (Benjamini-Hochberg) for multiple comparison. Central lines
530 indicate group median values; whiskers, 95% CI estimates. **(B)** Coefficients from linear models are reported
531 for various outcomes. Model predictors are indicated on x-axis. Significant predictors ($p \leq 0.05$) are in
532 purple. Detailed model results are reported in **Extended Data Table 5. (C)** PIWAS line profiles of IgG
533 binding within participants with >1 vaccine dose plotted along SARS-CoV-2 Spike amino acid sequence.
534 Various Spike protein domains are indicated by coloured boxes (top). 95th percentile values are arranged
535 by group: LC (purple, n = 80), HC (orange, n = 39) and CC (yellow, n = 38) with peaks ≥ 2.5 PIWAS value
536 annotated by their consensus linear motif sequence (bold) and surrounding residues. Significantly enriched
537 peaks in LC group are marked (*), as calculated by Outlier Sum statistics. **(D)** Three-dimensional mapping
538 of LC-enriched motif sequences onto trimeric Spike protein. (S1, light grey; NTD, light blue; RBD, red;
539 and S2, dark grey, with various LC-enriched motifs annotated.) **(E)** Box plots of z-score enrichments for
540 IgG binding to Spike sequence KFLPFQQ amongst participants who have received ≥ 1 vaccine doses. A z-
541 score >3 indicates significant binding relative to control populations. Box plots of z-score transformed
542 cortisol **(F)** ACTH **(G)**, and sample-collection times **(H)** by group. Participants with potentially
543 confounding medical comorbidities (e.g., pre-existing pituitary adenoma, adrenal insufficiency, oral steroid
544 use) were removed before analysis. **(I)** Coefficients from linear models of cortisol levels. Significant
545 predictors ($p \leq 0.05$) are in purple. Detailed model results are reported in **Extended Data Table 6.** Box
546 plots **(E–H)** are represented as in **Fig. 1.** Significance for difference in group medians was assessed using
547 Kruskal-Wallis with Bonferroni’s correction for multiple comparison. *Abbreviations: ACTH,*
548 *adrenocorticotrophic hormone; NTD, N terminal domain; O.S., Outlier Sum; PIWAS, Protein-based Im-*
549 *munome Wide Association Study; RBD, receptor-binding domain; SP, signal peptide; VAD, vaccines at*
550 *sample draw.*

551
552 **Figure 3. Long COVID participants showed limited but selective autoantibodies against the human**
553 **exoproteome. (A)** Heatmap depicting REAP reactivities across the MY-LC cohort (n = 25 HC, 13 CC, 98
554 LC). Each column is one participant, grouped by cohort (for HC and CC) or by LCPS (for LC). Column
555 clustering within groups was performed by k-means clustering. Each row represents one protein. Proteins
556 were grouped using Human Protein Atlas mRNA expression data for different tissues. Reactivities depicted
557 have at least one participant with a REAP score ≥ 3 . **(B)** The number of autoantibody reactivities per
558 individual by group. Significance assessed by Kruskal-Wallis. Box plots are represented as in **Fig. 1.** **(C)**
559 Correlation plot depicting the relationship between number of autoantibody reactivities per individual and
560 LCPS. Correlation was assessed by Spearman. Black line depicts linear regression with 95% CI shaded.
561 Colours depict LC LCPS group (cluster 1, red; cluster 2, green; cluster 3, blue). Each dot represents one

562 individual. **(D)** Violin plot depicting the number of GPCR autoantibodies per individual. Significance
563 assessed by Kruskal-Wallis. Each dot represents one individual. **(E)** Assessment of the frequency of
564 individual autoantibody reactivities in participants with LC and controls. Significance assessed by Fisher's
565 exact test. Y-axis depicts the negative \log_{10} of unadjusted p-value, with the Bonferroni adjusted significance
566 threshold depicted by the black dashed line. X-axis depicts the difference in the proportion of autoantibody
567 positive individuals in each group. Each dot represents one autoantibody reactivity. *Abbreviations: CNS,*
568 *central nervous system; GPCR, G-protein coupled receptor; Pit., pituitary.*

569 **Figure 4. Long COVID participants demonstrate elevated levels of antibody responses to**
570 **herpesviruses. (A)** Violin plots depicting the REAP score distributions for SARS-CoV2 S1 RBD between
571 LC (n = 69) and CC participants (n = 10) with two doses of mRNA vaccine. Statistical significance assessed
572 by Wilcoxon rank-sum adjusted for multiple comparisons by Benjamini-Hochberg method. **(B)** Violin plots
573 depicting the REAP score distributions for a given viral antigen between LC (n = 25 HC, 13 CC, 98 LC).
574 Statistical significance assessed by Wilcoxon rank-sum adjusted for multiple comparisons by Benjamini-
575 Hochberg method. Only antigens with ≥ 2 LC and ≥ 2 control individuals with reactivity were included. **(C)**
576 Seropositivity as assessed by SERA for EBV amongst LC (n = 99) and control participants (n = 78).
577 Significance assessed by Fisher's exact test adjusted for multiple comparisons by Benjamini-Hochberg
578 method. **(D,E)** REAP scores amongst EBV-seropositive individuals only for EBV p23 **(D)** and gp42 **(E)** by
579 group (n = 25 HC, 13 CC, 98 LC). **(F)** SERA-derived z-scores for gp42 motif PVXF[ND]K amongst EBV-
580 seropositive individuals only plotted by group. Dashed line represents z-score threshold for epitope
581 positivity defined by SERA (n = 39 HC, 38 CC, and 80 LC). **(G)** Three-dimensional mapping of LC-
582 enriched linear peptide sequence PVXF[ND]K (magenta) onto EBV gp42 (purple) in complex with gH
583 (light grey) and gL (dark grey) (PDB: 5T1D). **(H)** Correlation plot depicting the relationship between EBV
584 gp42 PVXF[ND]K z-score and percent IL-4/IL-6 CD4⁺ T cells (of total CD4⁺ T cells) for participants. Only
585 EBV-seropositive individuals were included. Correlation assessed by Spearman. Black line depicts linear
586 regression with 95% CI shaded. (n = 39 HC, 38 CC, and 80 LC). **(I)** Correlation plot depicting the
587 relationship between EBV p23 REAP score and percent CD4⁺ TEMRA (of total CD3⁺ T cells). Only EBV-
588 seropositive individuals were included. Correlation assessed by Spearman. Black line depicts linear
589 regression with 95% CI shaded. Colours depict LCPS Clusters as in **Fig. 3**. Box plots are represented as in
590 **Fig. 1**. Statistical significance of difference in medians determined by Kruskal-Wallis. Post-hoc tests
591 performed using Dunn's test with Holm's method to adjust for multiple comparisons. *Abbreviation: EBV,*
592 *Epstein-Barr virus; REAP, rapid extracellular antigen profiling; SERA, serum epitope repertoire analysis;*
593 *TM, transmembrane domain.*

594 **Figure 5. Biochemical factors differentiate participants with Long COVID from matched controls.**
595 All data shown represent a matched subset of participants (n = 41 HC, 40 CC, 81 LC) selected by a Gale-
596 Shapley procedure on demographic factors (**Extended Data Fig. 9A**). **(A)** PCA projection of participant
597 data comprising cytokine, flow cytometry, and various antibody responses (α -SARS-CoV-2, non-SARS-
598 CoV-2 viral antibodies, and aAb). Marginal histograms display data density along each principal
599 component dimension. **(B)** ROC curve analysis from unsupervised KNN classification. AUC and 95% CI
600 intervals (DeLong's Method) are reported. **(C)** McFadden's pseudo R-squared are reported as bar plot for
601 each data segment. An integrated, parsimonious McFadden's pseudo R-squared is reported for the final
602 classification model ('All'). **(D)** LASSO regression identifies a minimal set of immunologic features
603 differentiating participants with LC from others. Unlabeled dots are significant predictive features not
604 included in the final LASSO regression model. Dots are coloured according to individual data segments:
605 orange, Flow; blue, plasma cytokines; pink, viral epitopes; green, α -SARS-CoV-2; yellow, aAb.
606 *Abbreviations: aAb, autoantibodies; α -SARS-CoV-2, anti-SARS-CoV-2 antibodies; AUC, area under the*
607 *curve; CI, confidence interval; Flow, flow cytometry; FPR, false positive rate; KNN, k-nearest neighbours;*

608 *LC, Long COVID; PCA, principal component analysis; ROC, receiver operating characteristics; TPR, true*
609 *positive rate.*

610 **Methods**

611 **Ethics Statement**

612 This study was approved by the Mount Sinai Program for the Protection of Human Subjects (IRB #20-
613 01758) and Yale Institutional Review Board (IRB #2000029451 for MY-LC; IRB #2000028924 for
614 enrollment of pre-vaccinated Healthy Controls; HIC #2000026109 for External Long COVID). Informed
615 consent was obtained from all enrolled participants.

616 **MY-LC Study Design, Enrollment Strategy, and Inclusion / Exclusion Criteria**

617 MY-LC was a cross-sectional, multi-site study comprised of five different groups with differing SARS-
618 COV-2 exposure histories and varied Long COVID status. Participants enrolled in the Long COVID
619 group underwent complete medical evaluations by physicians to rule out alternative medical etiologies for
620 their persistent symptoms before study enrollment.

621
622 Participants with persistent symptoms following acute COVID-19 were recruited from Long COVID
623 clinics within the Mount Sinai Healthcare System and the Center for Post COVID Care at Mount Sinai
624 Hospital. Participants enrolled in healthy and convalescent study arms were recruited via IRB-approved
625 advertisements delivered through email lists, study flyers located in hospital public spaces, and on social
626 media platforms. Informed consent was provided by all participants at the time of enrollment. All
627 participants provided peripheral blood samples and completed symptom surveys the day of sample
628 collection (described below). Self-reported medical histories for all MY-LC participants were also
629 collected at study visits and further reviewed through examination of electronic medical records by
630 collaborating clinicians.

631
632 Inclusion criteria for individuals in the Long COVID group (“LC”) were age ≥ 18 years; previous
633 confirmed or probable COVID-19 infection (according to World Health Organization guidelines⁵¹); and
634 persistent symptoms > 6 weeks following initial COVID-19 infection. Inclusion criteria for enrollment of
635 individuals in the healthy control group (“HC”) were age ≥ 18 years, no prior COVID-19 infection, and
636 completion of a brief, semi-structured verbal screening with research staff confirming no active
637 symptomatology. Inclusion criteria for individuals in the convalescent control group (“CC”) were age \geq
638 18 years; previous confirmed or probable prior COVID-19 infection; and completion of a brief, semi-
639 structured verbal screening with research staff confirming no active symptomatology.

640
641 Pre-specified exclusion criteria for this study were inability to provide informed consent; and any
642 condition preventing a blood test from being performed. Additionally, all participants had electronic
643 health records reviewed by study clinicians following enrollment and were subsequently excluded prior to
644 analyses for the following reasons: (1) current pregnancy, (2) immunosuppression equivalent to or
645 exceeding prednisone 5 mg daily, (3) active malignancy or chemotherapy, and (4) any monogenic
646 disorders. For specific immunological analyses, pre-existing medical conditions were additionally
647 excluded prior to analyses due to high potential for confounding (e.g., participants with hypothyroidism
648 were excluded prior to analysis of circulating T3/T4 levels; participants with pituitary adenomas were
649 excluded prior to analysis of cortisol levels). Specific exclusions are marked by “ Δ ” in figures and
650 detailed in relevant legends.

651
652 The recruitment of individuals in healthcare worker group (HCW) is described at length elsewhere⁵².
653 Individuals in the external Long COVID group (Ext. LC) were identified from The Winchester Center for
654 Lung Disease's Post-COVID-19 Recovery Program at Yale New Haven Hospital by collaborating

655 clinicians. All participants underwent medical evaluation for persistent symptoms following COVID-19.
656 Participants from this group were identified retrospectively for inclusion in the MY-LC study according
657 to the established MY-LC protocol: age ≥ 18 years; previous confirmed or probable COVID-19 infection
658 (according to World Health Organization guidelines³⁹); and persistent symptoms > 6 weeks following
659 initial COVID-19 infection.

661 Participant Surveys

662
663 A comprehensive suite of surveys was administered to MY-LC study participants, combining validated
664 patient-reported outcomes (PROs) with custom, purpose-developed tools by the MY-LC study team.
665 Baseline demographic data collected from surveys included gender, age, body mass index (BMI), race,
666 and medical comorbidities. Additionally, participants in the Long COVID and convalescent groups were
667 asked to provide COVID-19 clinical data including date of symptom onset and acute disease severity
668 (non-hospitalized vs. hospitalized), any SARS-CoV-2 polymerase chain reaction (PCR) diagnostic testing
669 results, and any SARS-CoV-2 antibody testing results. Finally, all participants were asked to report
670 SARS-CoV-2 vaccination status including date of vaccinations and vaccine brand.

671 At the time of blood collection, all participants completed PROs for fatigue (Fatigue Severity Scale (FSS)
672 ⁵³, fatigue visual analogue scale [F-VAS]), post-exertional malaise (DePaul Symptom Questionnaire Post-
673 Exertional Malaise Short Form [DSQ-PEM Short Form])⁵⁴, breathlessness (Medical Research Council
674 [MRC] Breathlessness Scale⁵⁵), cognitive function (Neuro-QOL v2.0 Cognitive Function Short Form⁵⁶),
675 health-related quality of life (HRQoL) (EuroQol EQ-5D-5L⁵⁷), anxiety (GAD-7⁵⁸), depression (PHQ-2⁵⁹),
676 pain (P-VAS), sleep (Single-Item Sleep Quality Scale⁶⁰), as well as pre- and post-COVID-19 employment
677 status (author-developed). Lastly, participants in the MY-LC study were asked to self-report any current
678 persistent symptoms from a study-provided list.

679
680 All survey data were collected and securely stored using REDCap^{61,62} (Research Electronic Data Capture)
681 electronic data capture tools hosted within the Mount Sinai Health System.

682 Long COVID Propensity Score (LCPS)

683
684 Calculation of propensity scores for each participant was achieved through construction of a multivariable
685 logistic regression model generated with Long COVID vs. “Others” (Healthy Controls + Convalescent
686 controls) as the outcome. The model candidate variables included survey responses from the following
687 instruments described previously: FSS, F-VAS, DSQ-PEM Short Form, MRC Breathlessness Scale,
688 Neuro-QOL v2.0 Cognitive Function Short Form, EQ-5D-5L, GAD-7, PHQ-2, P-VAS, Single-Item Sleep
689 Quality Scale. Model selection using Akaike’s Information Criteria (AIC) was used to select the final,
690 parsimonious model. Odds ratios from the final model were normalized by dividing them by their
691 respective standard error (SE) and rounding off to the nearest integer. These integer values were
692 considered the score items for these specific variables and a cumulative prediction score for each subject
693 was calculated by summation (*Equation 1*, below). As the score did not significantly differ between
694 healthy controls and convalescent controls, the two control groups were combined as a single group
695 (“Others”) for final analysis. A ROC curve analysis was performed to identify the optimal cutoff for the
696 LCPS using the maximum value of Youden’s index J for Long COVID vs Others. A 10-fold cross-
697 validation was used for internal validation and to obtain 95% confidence interval (CI) for the area under
698 the curve (AUC). Data were analyzed using Stata version 16 (StataCorp, College Station, Texas).

699
$$\text{Equation 1: LCPS} = 7 * \sum GAD + 1 * \sum MRC + 2 * \sum PHQ2 + 3 \sum EQ5 + 28 * \sum FSS$$

700 Blood Sample Processing

701 Whole blood was collected in sodium-heparin-coated vacutainers (BD 367874, BD Biosciences) from
702 participants at Mount Sinai Hospital in New York City, New York. Following blood draw, all participant
703 samples were assigned unique MY-LC study identifiers and de-identified by clinical staff. Samples were
704 couriered directly to Yale University in New Haven, CT the same day as the sample collection. Blood
705 samples were processed on the same day as collection. Plasma samples were collected after centrifugation
706 of whole blood at 600×g for 10 minutes at room temperature (RT) without brake. Plasma was then
707 transferred to 15-mL polypropylene conical tubes, aliquoted, and stored at –80 °C. The peripheral blood
708 mononuclear cell (PBMC) layer was isolated according to the manufacturer’s instructions using SepMate
709 tubes (StemCell). Cells were washed twice with phosphate-buffered saline (PBS) before counting.
710 Pelleted cells were briefly treated with ACK lysis buffer (ThermoFisher) for 2 minutes and then counted.
711 Viability was estimated using standard Trypan blue staining and a Countess II automated cell counter
712 (ThermoFisher). PBMCs were stored at –80 °C for cryopreservation or plated directly for flow cytometry
713 studies. Plasma samples from the External Long COVID group were obtained using BD Vacutainer CPT
714 tubes (#362753) according to manufacturer’s instructions and stored in aliquots at –80 °C prior to
715 analysis.

716 **Flow cytometry**

717 Freshly isolated PBMCs were plated at 1–2 × 10⁶ cells per well in a 96-well U-bottom plate. Cells were
718 resuspended in Live/Dead Fixable Aqua (ThermoFisher) for 20 min at 4 °C. Cells were washed with PBS
719 and followed by Human TruStain FcX (BioLegend) incubation for 10 min at RT. Cocktails of staining
720 antibodies were added directly to this mixture for 30 minutes at RT. Prior to analysis, cells were washed
721 and resuspended in 100 µl 4% PFA for 30 min at 4 °C. For intracellular cytokine staining following
722 stimulation, the surface marker-stained cells were resuspended in 200 µl cRPMI (RPMI-1640
723 supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin, 1
724 mM sodium pyruvate) and stored at 4 °C overnight. Subsequently, these cells were washed and stimulated
725 with 1× Cell Stimulation Cocktail (eBioscience) in 200 µl cRPMI for 1 h at 37 °C. 50 µl of 5×
726 Stimulation Cocktail in cRPMI (plus protein transport 442 inhibitor, eBioscience) was added for an
727 additional 4 hours of incubation at 37 °C. Following stimulation, cells were washed and resuspended in
728 100 µl 4% paraformaldehyde for 30 min at 4 °C. To quantify intracellular cytokines, cells were
729 permeabilized with 1× permeabilization buffer from the FcX/Transcription Factor Staining Buffer Set
730 (eBioscience) for 10 min at 4 °C. All subsequent staining cocktails were made in this buffer.
731 Permeabilized cells were then washed and resuspended in a cocktail containing Human TruStain FcX
732 (BioLegend) for 10 min at 4 °C. Finally, intracellular staining cocktails were added directly to each
733 sample for 1 h at 4 °C. Following this incubation, cells were washed and prepared for analysis on an
734 Attune NXT (ThermoFisher). Data were analyzed using FlowJo software version 10.8 software (BD).
735 Antibody information can be seen in **Supplementary Table 1**.

736
737 A PERMANOVA test was used to assess the relationship between all circulating immune cell populations
738 presented in **Extended Data Fig. 2** and participant age, sex, Long COVID status, as well as BMI. The
739 PERMANOVA test was run using the “VEGAN” package in R⁶³.

740 741 **SARS-CoV-2 antibody testing by ELISA**

742 ELISAs were performed as previously described¹⁵. Briefly, Triton X-100 and RNase A were added to
743 plasma samples at final concentrations of 0.5% and 0.5 mg/ml, respectively, and incubated at room
744 temperature for 30 minutes prior to use to reduce risk from any potential virus in plasma. MaxiSorp plates
745 (96 wells; 442404, Thermo Scientific) were coated with 50 µl per well of recombinant SARS-CoV-2
746 Total S (SPN-C52H9-100 µg, ACROBiosystems), RBD (SPD-C52H3-100 µg, ACROBiosystems) and the
747 nucleocapsid protein (NUN-C5227-100 µg, ACROBiosystems) at a concentration of 2 µg/ml in PBS and
748 were incubated overnight at 4 °C. The coating was removed, and plates were incubated for 1 hour at room
749 temperature with 200 µl of blocking solution (PBS with 0.1% Tween-20 and 3% milk powder). Plasma

750 was diluted serially at 1:100, 1:200, 1:400 and 1:800 in dilution solution (PBS with 0.1% Tween-20 and
751 1% milk powder), and 100 µl of diluted serum was added for 2 hours at room temperature. Human anti-
752 spike (SARS-CoV-2 human anti-spike [AM006415; 91351, Active Motif] and anti-nucleocapsid SARS-
753 CoV-2 human anti-nucleocapsid (1A6; MA5-35941, Active Motif) were serially diluted to generate a
754 standard curve. Plates were washed three times with PBS-Tween (PBS with 0.1% Tween-20) and 50 µl of
755 HRP anti-human IgG antibody (1:5,000; A00166, GenScript) added to each well in dilution solution.
756 After 1 hour of incubation at room temperature, plates were washed six times with PBS-Tween. Plates
757 were developed with 100 µl of TMB Substrate Reagent Set (555214, BD Biosciences) and the reaction
758 was stopped after 5 min by the addition of 2N sulfuric acid. Plates were then read at an
759 excitation/emission wavelength of 450 nm and 570 nm.

760 **Multiplex proteomic analysis**

761 Participant plasma was isolated and stored at -80 °C as described above. Plasma was shipped to Eve
762 Technologies (Calgary, Alberta, Canada) on dry ice and analytes were measured using the following
763 panels: Human Cytokine/Chemokine 71-plex Discovery Assay (HD71), Steroid/Thyroid 6plex Discovery
764 Assay (STTHD), TGF-Beta 3-plex Discovery Assay (TGFβ1-3), Human Myokine Assay (HMYOMAG-
765 10), Human Neuropeptide Assay (HNPMAG-05), Human Pituitary Assay (HPTP1), Human Cytokine P3
766 Assay (HCYP3-07), Human Cytokine Panel 4 Assay (HCYP4-19), Human Adipokine Panel 2 Assay
767 (HADK2-03), Human Cardiovascular Disease Panel Assay (HDCVD9), Human CVD2 Assay (HCVD2-
768 8), Human Complement Panel Assay (HDCMP1), Human Adipokine Assay (HDADK5). Analysis of
769 plasma proteomics was completed in two batches with internal controls in each shipment to assess for and
770 correct any analyte batch effects (described below)

771 To integrate analytes across batches, two samples from the same representative individuals from each
772 group (2 from LC, 2 from CC, and 2 from HC) were measured in each analysis batch. The median
773 difference between all paired samples between the first and second batch was used as an additive
774 corrective factor to integrate samples across batches. After batch integration, each feature was z-scored
775 using all measurements across both batches. Following z-scoring, features that were found to have
776 persistent batch effects, as defined by a Wilcoxon rank sum test $p < 0.05$ post-correction, were not
777 considered for downstream analysis.

778 **Real-time Taqman assay for detection of EBV DNA**

779 *Nucleic Acid Extraction*

780 Nucleic acid was extracted from 200µL freeze-thawed serum using the MagMAX Viral/Pathogen Nucleic
781 Acid Isolation Kit (ThermoFisher, #A42352), automated on the KingFisher Flex (Thermo Fisher
782 Scientific, Waltham, MA, USA) per manufacturer's protocol. The manufacturer's protocol was
783 additionally modified to reduce salt carry-over by adding a third wash step with 500 µL 80% ethanol and
784 eluting in 50 µL nuclease-free water.

785 *Real-time Taqman PCR*

786 PCR primers for the TaqMan assay were previously validated⁶⁴: EBV p143 forward (5'-
787 GGA.ACC.TGG.TCA.TCC.TTG.C) and EBV p143 reverse (5' ACG.TGC.ATG.GAC.CGG.TTA.AT)
788 (Thermo Fisher Scientific, Waltham, MA, USA). A fluorogenic probe (5'-(FAM)-CGC AGG CAC TCG
789 TAC TGC TCG CT-(MGB)-3') with a FAM reporter molecule attached to the 5' end and an MGB
790 quencher linked at the 3' end was acquired in parallel (Thermo Fisher Scientific). The PCR amplification
791 was performed in a 20-µl volume containing 10 µL 2× Luna Universal Probe One-Step RT-qPCR Kit
792 (New England BioLabs, Ipswich, MA, US), 300 pmol of each primer per µl, 200 pmol of the TaqMan

793 probe, and 5 μ l of isolated DNA. Amplification and detection were performed on a CFX96 Touch
794 instrument (Bio-Rad, Hercules, CA, US). After a 1-minute hold step at 95 °C, the PCR cycling program
795 consisted of 42 two-step cycles of 15 s at 95 °C and 30 s at 60 °C. Real-time measurements were taken,
796 and a threshold cycle (C_t) value for each sample was calculated if fluorescence exceeded a threshold limit.
797 Each specimen was run in duplicate and was considered positive only if both replications were above the
798 threshold limit. Each run contained multiple H₂O controls (no template), and a standard curve containing
799 serial dilutions of quantitative synthetic DNA (described below, ATCC, VR-3247SD). An additional
800 EBV Plasma Control was included as a positive control for each assay plate (Thermo Fisher Scientific,
801 #961231).

802 ***Estimating Genome Copy Number Standards***

803 For standardization of qPCR detection of EBV viral genomes from participant plasma, a commercially
804 available standard containing 5.59×10^8 EBV genome copies per ml (ATCC, VR-3247SD) was used.
805 Serial log dilutions of this standard, ranging from 10^6 to 10^0 copies per ml, were made to establish the
806 sensitivity of the TaqMan RT-PCR and included on each assay plate. The standard curve was created in
807 the usual way by plotting the C_t values against the standard of known concentration. x - y scatter diagrams
808 were drawn, and the correlation coefficient (r^2) was determined. Linear regression analysis was done
809 using GraphPad Prism.

810 **Linear Peptide Profiling**

811 ***SERA serum screening***

812 A detailed description of the SERA assay has been published⁶⁵. For this study, plasma was incubated with
813 a fully random 12-mer bacterial display peptide library (1×10^{10} diversity, 10-fold oversampled) at a 1:25
814 dilution in a 96-well, deep well plate format. Antibody-bound bacterial clones were selected with 50 μ L
815 Protein A/G Sera-Mag SpeedBeads (GE Life Sciences, #17152104010350) (IgG). The selected bacterial
816 pools were resuspended in growth media and incubated at 37 °C shaking overnight at 300 RPM to
817 propagate the bacteria. Plasmid purification, PCR amplification of peptide-encoding DNA and barcoding
818 with well-specific indices was performed as described. Samples were normalized to a final concentration
819 of 4 nM for each pool and run on the Illumina NextSeq500. Every 96-well plate of samples processed for
820 this study contained healthy control run standards to assess and evaluate assay reproducibility and
821 possible batch effects.

822 For IgM isotype screening by SERA, the above IgG protocol was adjusted as follows: 1) after serum
823 incubation with the library, *E. coli* cells were centrifuged, the supernatant removed, and the cells were
824 resuspended in 500 μ L $1 \times$ PBS containing a 1:100 dilution of biotin-SP (long-spacer) conjugated donkey
825 anti-Human IgM secondary antibody (Jackson Immunoresearch, 709-066-073); 2) The plate was
826 incubated for 1 hour at 4 °C with orbital shaking (800 rpm), the cells were again centrifuged, supernatant
827 removed, and cells were resuspended in 700 μ L of $1 \times$ PBS + 100 μ L of Dynabeads MyOne streptavidin
828 T1 coated magnetic beads (ThermoFisher Scientific, 65601); 3) The plate was incubated for 1 hour at 4
829 °C with orbital shaking (800 rpm), after which time the plate was magnetized and the beads-antibody
830 complex along with their bound peptide-bearing cells were captured. All subsequent steps were identical
831 for IgG and IgM screening as described. IgM antibody repertoires were evaluated for Epstein-Barr virus
832 antibodies in two ways; 4) Samples were analyzed on an existing EBV IgM epitope panel that was
833 developed and validated on clinically confirmed mononucleosis patients and EBV IgM negative controls.

834 ***PIWAS analysis***

835 The published PIWAS method⁶⁶ was used to identify antigen and epitope signals against the Uniprot
836 reference SARS-CoV-2 proteome (UP000464024). For each sample, approximately 1–3 million 12-mers
837 were obtained from the SERA assay and these were decomposed into constituent 5- and 6-mers. An
838 enrichment score for each k-mer was calculated by dividing the number of unique 12-mers containing the
839 k-mer divided by the number of expected k-mer reads for the sample, based on amino acid proportions in
840 the sample. A z-score per k-mer was then calculated by comparing the enrichment score with those from a
841 large pre-pandemic cohort (n = 1,500) on a log₁₀ scale. A PIWAS value at each amino acid position
842 along a protein sequence represents an averaged score within a 5 amino acid frame using the tiling z-
843 scores of 5-mers and 6-mers spanning the sequence. 95th quantile bands were calculated based on each
844 population separately.

845 ***Protein-wide identification of epitopes (PIE)***

846 ***Protein-wide identification of epitopes (PIE)***
847
848 PIE methodology for epitope identification was performed to locate regions on a protein sequence that
849 had stronger outlier signals in the case samples relative to control samples from a large pre-pandemic
850 cohort (n = 1,500). The distribution of case sample values relative to the control was analyzed at each
851 amino acid position on the SARS-CoV-2 Spike protein sequence. Specifically, at each position, all case
852 and control sample values were normalized using median absolute deviation based on the distribution of
853 the control sample values. An outlier threshold was defined as $Q_{75} + 1.5 * (Q_{75} - Q_{25})$, where Q_x is the x^{th}
854 percentile of the control values at that specific position⁶⁷. An outlier sum statistic was then calculated as
855 the sum of signal values above the outlier threshold in the case samples⁶⁸. A null distribution for the
856 outlier sum value was calculated by permuting case/control labels and recalculating 1000 times. A p-
857 value was calculated based on a z-score by comparing the observed outlier sum statistic to the null
858 distribution. A significant p-value threshold was set to 0.001 after false discovery rate (FDR) adjustment
859 by the Benjamini–Hochberg procedure and an outlier sum threshold was set to the 99.5th percentile value
860 of all positions with FDR adjusted p-value > 0.001. All sequence positions that exceeded both thresholds
861 were identified, and adjacent positions were merged into regions representing epitopes on the protein.

862 ***IMUNE-based motif discovery***

863 ***IMUNE-based motif discovery***
864 Peptide motifs representing epitopes or mimotopes of SARS CoV-2-specific antibodies were discovered
865 using the IMUNE algorithm⁶⁹. A total of 164 antibody repertoires from 98 hospitalized subjects from the
866 Yale IMPACT study were used for motif discovery. The majority of subjects were confirmed SARS
867 CoV-2 positive by NAT. IMUNE compared ~30 disease repertoires with ~30 pre-pandemic controls and
868 identified peptide patterns that were statistically enriched (p-value ≤ 0.01) in ≥25% of disease and absent
869 from 100% of controls. Multiple assessments were run with different subsets of cases and controls.
870 Peptide patterns identified by IMUNE were clustered using a point accepted mutation 30 (PAM30) matrix
871 and combined into motifs. The output of IMUNE included hundreds of candidate IgG and IgM motifs. A
872 motif was classified as positive in a given sample if the enrichment was ≥3 times the standard deviation
873 above the mean of the training control set. The candidate motifs were further refined based on at least
874 98% specificity. The final set of motifs was validated for sensitivity and specificity on an additional 1,500
875 pre-pandemic controls and 406 unique confirmed COVID-19 cases from four separate cohorts.

876 ***Motif grouping by similarity***

877 ***Motif grouping by similarity***
878
879 For SARS-CoV-2, motifs were grouped if they shared at least 3 of 5 amino acid identities, resulting in 76
880 motifs being assigned into 24 groups. The motif within an epitope group with the greatest sensitivity and
881 mean enrichment was included in the SARS-CoV-2 Infection IgG panel results. In some cases, two motifs
882 were selected from the same group since their combination improved sensitivity. The remaining motifs
883 that did not fall into a group were further down-selected based on a specificity of >99.5%, leaving 24
884 additional motifs.

885
886 **Rapid Extracellular Antigen Profiling (REAP)**

887 ***REAP Library Expansion***

888 The initial yeast library (Exo201) was generated as previously described^{16,70}. In Exo201, only extracellular
889 domains >49 amino acids in length were included in the library. To generate the library used for this
890 study, Exo201 was with all extracellular domains of multi-pass membrane proteins greater than 15 amino
891 acids and 225 extracellular viral antigens. DNA for new antigens was synthesized as either a Gene
892 Fragment (for antigens over 300 nucleotides) or as an Oligo pool by TWIST Bioscience, containing a 5'
893 sequence (CTGTTATTGCTAGCGTTTTAGCA) and 3' sequence (GCGGCCGCTTCTGGTGGC) for
894 PCR amplification. The oligo pool was PCR amplified and transformed into yeast with barcode
895 fragments, followed by barcode-antigen pairing identification as previously described¹². This new yeast
896 library was then pooled with the initial library (Exo201) in the ratio of 1:1 to generate the new version of
897 the library (Exo205) which contained 6,452 unique antigens.

898 ***REAP Protocol***

899 Participant IgG isolation and REAP selections were performed as previously described^{16,70}. Briefly, IgG
900 was purified from participant plasma using protein G magnetic beads followed by adsorption to yeast
901 transformed with the pDD003 empty vector to remove yeast-reactive IgG. The Exo205 yeast library was
902 induced in SGO-Ura medium, and 10⁸ induced yeast cells were washed with PBE and added to wells of a
903 sterile 96-well plate. 10 µg of purified participant IgG was added to the yeast library in duplicate in 100
904 µL PBE and incubated for 1 hour at 4°C. Yeast cells were washed with PBE and incubated with 1:100
905 biotin anti-human IgG Fc antibody (clone QA19A42, Biolegend) for 30 minutes. Yeast cells were washed
906 with PBE and incubated with a 1:20 dilution of Streptavidin MicroBeads (Miltenyi Biotec) for 30
907 minutes. Yeast were resuspended in PBE and IgG-bound yeast were isolated by positive magnetic
908 selection using the MultiMACS M96 Separator (Miltenyi Biotec) according to manufacturer
909 instructions. Selected yeast were resuspended in 1 mL SDO -Ura and incubated at 30 °C for 24 hours and
910 then plasmid DNA was harvested for NGS analysis. Briefly, DNA was extracted from yeast libraries
911 using Zymoprep-96 Yeast Plasmid Miniprep kits or Zymoprep Yeast Plasmid Miniprep II kits (Zymo
912 Research) according to standard manufacturer protocols. A first round of PCR was used to amplify a
913 DNA sequence containing the protein display barcode on the yeast plasmid. A second round of PCR was
914 performed on 1 µL step 1 PCR product using Nextera i5 and i7 dual-index library primers (Illumina).
915 PCR products were pooled, run on a 1% agarose gel, and DNA corresponding to the band at 257 base
916 pairs was cut. DNA (NGS library) was extracted using a QIAquick Gel Extraction Kit (Qiagen) according
917 to standard manufacturer protocols. NGS library was sequenced using an Illumina NextSeq550 and an
918 NextSeq high output sequencing kit with 75 base pair single-end sequencing according to standard
919 manufacturer protocols. Approximately 500,000 reads (on average) per sample was collected and the pre-
920 selection library was sampled at ten times greater read depth than other samples. Samples with less than
921 50,000 reads were classified as a sequencing failure and removed from further analysis.

922
923 ***REAP data analysis***

924
925 REAP scores were calculated as previously described^{16,70}. Briefly, barcode counts were extracted from raw
926 NGS data using custom codes and counts from technical replicates were summed. Next, aggregate and
927 clonal enrichment was calculated using edgeR⁷¹ and custom computer scripts. Aggregate enrichment is the
928 log₂ fold change of all barcodes associated with a particular protein summed in the post-library relative to
929 the pre-library, with zeroes in the place of negative fold changes. Log₂ fold change values for clonal
930 enrichment were calculated in an identical manner, but barcode counts across all unique barcodes

931 associated with a given protein were not summed. Clonal enrichment for a given reactivity was defined as
932 the fraction of clones out of total clones that were enriched (\log_2 fold change ≥ 2). Aggregate (E_a) and
933 clonal enrichment (E_c) for a given protein, a scaling factor (β_u) based on the number of unique yeast
934 clones (yeast that have a unique DNA barcode) displaying a given protein, and a scaling factor (β_f) based
935 on the overall frequency of yeast in the library displaying a given protein were used as inputs to calculate
936 the REAP score, which is defined as follows:

937 Equation 2: REAP score = $E_a \times (E_c)^2 \times \beta_u \times \beta_f$

938 β_u and β_f are logarithmic scaling factors that progressively penalize the REAP score of proteins with low
939 numbers of unique barcodes or low frequencies in the library, and are described in detail in previous
940 publications^{16,70}.

941 Antigens with an average REAP score greater than 0.5 across all samples were defined as non-specific
942 and excluded from further analysis. Autoantibody reactivities were defined as antigens with REAP score
943 greater than or equal to 1.

944 ***REAP Antigen ELISA Validation***

945 96-well MaxiSorp plates (Thermo Scientific #442404) were coated with 200 ng per well of recombinant
946 EBV p23 protein (ProSpec #ebv-274) in PBS and incubated overnight at 4 °C. Plates were dumped out
947 and incubated with 3% Omniblock non-fat dry milk (American Bioanalytical #AB10109-00100) in PBS
948 for 2 hours at RT. Plates were washed 3× with 200 ul wash buffer (PBS 0.05% Tween). Samples were
949 diluted in 1% Omniblock non-fat dry milk in PBS and added to the plate to incubate 2 hours at RT. Plates
950 were washed 6× with wash buffer. Goat anti-human IgG Fc HRP (Sigma Aldrich, #AP112P) diluted
951 1:10000 in 1% Omniblock non-fat dry milk in PBS was added to the plates and incubated 1 hour at RT.
952 Plates were washed 6×. Plates were developed with 100 µl of TMB Substrate Reagent Set (BD
953 Biosciences #555214) and the reaction was stopped after 5 min by the addition of 2 N sulfuric acid. Plates
954 were then read at a wavelength of 450 nm.

955 **Machine Learning**

956 ***Data Preprocessing.***

957 All collected data for immune profiling were collated. Features containing redundant information were
958 manually removed from the dataset (e.g., nested flow cytometry populations include only the extant
959 population).

960 All features were linearly scaled to unit variance and zero-centered using the R programming language
961 base libraries^{72,73}. Median absolute deviation was calculated for each feature across all samples, with
962 missing values removed. Features with a median absolute deviation equal to zero or features where data
963 was not available in at least half the samples were not included in downstream analysis. Prior to
964 visualization of the data using principal component analysis, features were additionally quantile-
965 normalized using the “normalize.quantiles” function of the “preprocessCore” package in R⁷⁴.

966 ***Gale-Shapley matching of participants by demographics.***

967 To ensure that immunologic features of participants in the LC cohort would be compared against the most
968 similar set of controls in the CC and HC cohorts, a Gale-Shapley matching procedure was employed⁷⁵.
969 Participants in the LC cohort were first matched against participants in the CC cohort. Unmatched

970 participants in the LC cohort were subsequently matched against participants in the HC cohort. Preference
971 lists required by the Gale-Shapley algorithm were determined using an affinity function calculated as the
972 cosine similarity of participants in a unit scaled and zero centered demographics matrix containing age,
973 sex, vaccination status, and days from the initial onset of acute COVID-19. The matching was performed
974 by the “galeShapley.marriageMarket” function of the “matchingR” package in R⁷². To evaluate matching
975 efficacy, differences between groups in age, sex, vaccination status, acute COVID-19 hospitalization
976 status, and days from initial onset of acute COVID-19 were assessed using a Chi-square test. For age,
977 participants were segmented into groups as either less than 32 years of age, between 33 and 51 years of
978 age, or greater than 52 years of age. For days from symptom onset, participants were segmented into
979 groups as either 1–2 months from acute infection, 2–5 months from acute infection, 6–8 months from
980 acute infection, or ≥ 9 months from acute infection. An alpha of 0.05 was used throughout.

981 *Unsupervised Analysis.*

982 Principal component analysis was performed on the set of normalized features for all matched participants
983 ⁷⁶. To assess how well participants were grouped by all features, a k-nearest neighbor classifier with k=10
984 was applied separating participants with Long COVID from those without (either convalescent
985 participants or healthy controls). A k of 10 was chosen by heuristic as approximately equal to the square
986 root of the number of samples included⁷⁷. A range of values for k from 5-15 were tested and found to give
987 similar results. Area under the receiver operating characteristic curve (AUC) and 95% confidence
988 intervals were calculated using DeLong's method; p-values were calculated using the Mann-Whitney U
989 statistic^{78,79}.

990 *Supervised Analysis.*

991 Principal components regression was applied to each of a predefined set of data segments: autoantibodies,
992 SARS-CoV-2 antibodies, non-SARS-CoV-2 viral antibodies, plasma proteomics, and flow cytometry
993 readouts. The precise definitions of these data segments are provided as metadata. The first n principal
994 components based on explained variance (see below for selection method) were selected from the
995 normalized feature set and used to fit a logistic regression model (implemented as a binomial generalized
996 linear regression with a logit link) for classification of participants with Long COVID as compared to
997 matched convalescent participants without long term symptoms and uninfected controls.

998 To determine the optimal value for n (number of principal components), values were scanned, and seven-
999 fold cross validation was performed on the data set. The average mean squared error was calculated for
1000 each cross-validation iteration at a particular value of n . For the binomial regression run using a logit link
1001 function, McFadden's pseudo- R^2 was calculated and averaged across each of the cross-validation folds.

1002 Plots of explained variance and mean squared error across all scanned values for n were generated and
1003 visually inspected to choose an optimal value for n that maximized explained variance while minimizing
1004 overfitting as identified by increasing average mean squared error. This procedure was performed on each
1005 of the segments, and an optimal n was chosen for each of the following: autoantibodies ($n = 5$), SARS-
1006 CoV-2 antibodies ($n = 3$), non-SARS-CoV-2 viral antibodies ($n = 33$), plasma proteomics ($n = 20$), and
1007 flow cytometry ($n = 21$).

1008 A model fitted on the first n principal components (or any linear transformation) was related to each of
1009 the original features as follows. Each principal component may be considered as a weighted linear
1010 combination of the original features. The principal component loading vectors were used to project the
1011 fitted beta values from the logistic regression model using the linearity of expectation, $E(X + Y) = E(X) +$
1012 $E(Y)$, such that the estimated parameter for each variable was the weighted sum of the parameter

1013 estimates for the principal components to which it contributed. The variance of fit for each of the original
1014 features was similarly projected from the fitted principal components as the variance of a sum of random
1015 variables $\text{Var}(X + Y) = \text{Var}(X) + \text{Var}(Y) + 2\text{Cov}(X, Y)$. P-values were calculated for each variable in the
1016 original feature space using z-scores.

1017 Following per-segment model construction and evaluation, features with a Bonferroni-corrected p-value
1018 of less than 0.05 were selected for inclusion in a final principal components regression. These selected
1019 features were considered as a separate integrated data segment and processed in the same way as each
1020 individual data segment with a selected ($n = 9$) number of included principal components. A least absolute
1021 shrinkage and selection operator (LASSO) regression was employed to select a subset of the features with
1022 p-values less than 0.05 as a minimal model, and McFadden's pseudo- R^2 was calculated.

1023 An implementation has been made publicly accessible as an R library on GitHub at
1024 (<https://github.com/rahuldhodapkar/puddlr>).

1025 ***Symptom Bi-clustering.***

1026 Participants with Long COVID were clustered based on binary self-reporting of Long COVID symptoms.
1027 Hamming distance was used with complete linkage clustering as an agglomeration method. Visualization
1028 of the bi-clustering was performed using the '*ComplexHeatmap*' package in R⁸⁰. Cluster stability was
1029 assessed by bootstrapped resampling with 100 iterations using the '*fpc*' package in R⁸¹.

1030 **General Statistical Analysis**

1031 Study sample sizes were not pre-determined through formal power analysis. Specific statistical
1032 methodology can be found in relevant figure legends and manuscript text. Generally, comparison of
1033 immunophenotypic features including systemic cytokine levels and antibody concentrations between
1034 study cohorts was performed using estimates of group medians, primarily with non-parametric Kruskal-
1035 Wallis tests. All statistical tests were two sided.

1036 The difference in median between the days from the symptom onset (DFSO) of acute COVID-19 in the
1037 LC and CC groups was assessed using a two-tailed Brown-Mood median test with an alpha of 0.05. The
1038 test was performed using the '*coin*' package in R⁸². Flow cytometry populations were assessed using
1039 estimates of group means with permutational testing using PERMANOVA to control for within-group
1040 heterogeneity (described above).

1041 In cases where Kruskal-Wallis testing indicated significant differences, post-hoc testing using Dunn's test
1042 was performed. Correction for multiple comparisons was performed using Bonferroni or Bonferroni-
1043 Holm method as indicated. All statistical tests were performed using R, PRISM, and MATLAB software.

1044 **Data availability**

1045 All of the raw fcs files for the flow cytometry analysis are available at the FlowRepository platform
1046 (<http://flowrepository.org/>) under Repository ID: FR-FCM-Z6KL. Protein structures were visualized
1047 using UniProt repository under the following accession numbers: trimeric Spike (PDB: 6VXX) and EBV
1048 gH/gL (PDB: 5T1D). Raw data are included in Supplementary Table 3.

1049 **Code availability**

1050 Computer codes are available as indicated (e.g., <https://github.com/rahuldhodapkar/puddlr>) or otherwise
1051 available upon request.

1052 Additional References

- 1053 51. World Health Organization. Public health surveillance for COVID-19: interim guidance. (2022).
1054 52. Lucas, C. *et al.* Impact of circulating SARS-CoV-2 variants on mRNA vaccine-induced
1055 immunity. *Nature* **600**, 523–529 (2021).
1056 53. Krupp, L. B., LaRocca, N. G., Muir-Nash, J. & Steinberg, A. D. The fatigue severity scale.
1057 Application to patients with multiple sclerosis and systemic lupus erythematosus. *Arch. Neurol.* **46**,
1058 1121–1123 (1989).
1059 54. Cotler, J., Holtzman, C., Dudun, C. & Jason, L. A. A Brief Questionnaire to Assess Post-
1060 Exertional Malaise. *Diagn. Basel Switz.* **8**, 66 (2018).
1061 55. Stenton, C. The MRC breathlessness scale. *Occup. Med. Oxf. Engl.* **58**, 226–227 (2008).
1062 56. Iverson, G. L., Connors, E. J., Marsh, J. & Terry, D. P. Examining Normative Reference Values
1063 and Item-Level Symptom Endorsement for the Quality of Life in Neurological Disorders (Neuro-
1064 QoL™) v2.0 Cognitive Function-Short Form. *Arch. Clin. Neuropsychol. Off. J. Natl. Acad.*
1065 *Neuropsychol.* **36**, 126–134 (2021).
1066 57. Herdman, M. *et al.* Development and preliminary testing of the new five-level version of EQ-5D
1067 (EQ-5D-5L). *Qual. Life Res. Int. J. Qual. Life Asp. Treat. Care Rehabil.* **20**, 1727–1736 (2011).
1068 58. Spitzer, R. L., Kroenke, K., Williams, J. B. W. & Löwe, B. A brief measure for assessing
1069 generalized anxiety disorder: the GAD-7. *Arch. Intern. Med.* **166**, 1092–1097 (2006).
1070 59. Kroenke, K., Spitzer, R. L. & Williams, J. B. W. The Patient Health Questionnaire-2: validity of a
1071 two-item depression screener. *Med. Care* **41**, 1284–1292 (2003).
1072 60. Snyder, E., Cai, B., DeMuro, C., Morrison, M. F. & Ball, W. A New Single-Item Sleep Quality
1073 Scale: Results of Psychometric Evaluation in Patients With Chronic Primary Insomnia and Depression.
1074 *J. Clin. Sleep Med. JCSM Off. Publ. Am. Acad. Sleep Med.* **14**, 1849–1857 (2018).
1075 61. Harris, P. A. *et al.* The REDCap consortium: Building an international community of software
1076 platform partners. *J. Biomed. Inform.* **95**, 103208 (2019).
1077 62. Harris, P. A. *et al.* Research electronic data capture (REDCap)--a metadata-driven methodology
1078 and workflow process for providing translational research informatics support. *J. Biomed. Inform.* **42**,
1079 377–381 (2009).
1080 63. Jari Oksanen [aut, cre], Gavin L. Simpson [aut], F. Guillaume Blanchet [aut], Roeland Kindt
1081 [aut], Pierre Legendre [aut], Peter R. Minchin [aut], R.B. O'Hara [aut], Peter Solymos [aut], M. Henry
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1087 Smith [aut], Adrian Stier [aut], Cajo J.F. Ter Braak [aut], James Weedon [aut]. *vegan: Community
1088 Ecology Package.*
1089 64. Niesters, H. G. *et al.* Development of a real-time quantitative assay for detection of Epstein-Barr
1090 virus. *J. Clin. Microbiol.* **38**, 712–715 (2000).
1091 65. Kamath, K. *et al.* Antibody epitope repertoire analysis enables rapid antigen discovery and
1092 multiplex serology. *Sci. Rep.* **10**, 5294 (2020).
1093 66. Haynes, W. A., Kamath, K., Waitz, R., Daugherty, P. S. & Shon, J. C. Protein-Based Immunome
1094 Wide Association Studies (PIWAS) for the Discovery of Significant Disease-Associated Antigens.
1095 *Front. Immunol.* **12**, 625311 (2021).
1096 67. Tukey, J. W. *Exploratory data analysis.* (Addison-Wesley Pub. Co, 1977).
1097 68. Tibshirani, R. & Hastie, T. Outlier sums for differential gene expression analysis. *Biostatistics* **8**,
1098 2–8 (2007).

- 1099 69. Pantazes, R. J. *et al.* Identification of disease-specific motifs in the antibody specificity repertoire
1100 via next-generation sequencing. *Sci. Rep.* **6**, 30312 (2016).
- 1101 70. Wang, E. Y. *et al.* High-throughput identification of autoantibodies that target the human
1102 exoproteome. *Cell Rep. Methods* **2**, 100172 (2022).
- 1103 71. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR : a Bioconductor package for
1104 differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139–140 (2010).
- 1105 72. R Core Team, R. R: A language and environment for statistical computing. (2013).
- 1106 73. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*. (Springer New York, 2009).
1107 doi:10.1007/978-0-387-98141-3.
- 1108 74. Bolstad, B. M., Irizarry, R. A., Åstrand, M. & Speed, T. P. A comparison of normalization
1109 methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* **19**,
1110 185–193 (2003).
- 1111 75. Gale, D. & Shapley, L. S. College Admissions and the Stability of Marriage. *Am. Math. Mon.* **69**,
1112 9–15 (1962).
- 1113 76. Becht, E. *et al.* Dimensionality reduction for visualizing single-cell data using UMAP. *Nat.*
1114 *Biotechnol.* **37**, 38–44 (2019).
- 1115 77. Zhang, Z. Introduction to machine learning: k-nearest neighbors. *Ann. Transl. Med.* **4**, 218–218
1116 (2016).
- 1117 78. Robin, X. *et al.* pROC: an open-source package for R and S+ to analyze and compare ROC
1118 curves. *BMC Bioinformatics* **12**, 77 (2011).
- 1119 79. DeLong, E. R., DeLong, D. M. & Clarke-Pearson, D. L. Comparing the areas under two or more
1120 correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* **44**, 837–845
1121 (1988).
- 1122 80. Gu, Z., Eils, R. & Schlesner, M. Complex heatmaps reveal patterns and correlations in
1123 multidimensional genomic data. *Bioinforma. Oxf. Engl.* **32**, 2847–2849 (2016).
- 1124 81. Christian Hennig. Flexible Procedures for Clustering.
- 1125 82. Hothorn, T., Hornik, K., van de Wiel, M. A. & Zeileis, A. Implementing a Class of Permutation
1126 Tests: The coin Package. *J. Stat. Softw.* **28**, 1–23 (2008).

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1138 Author contributions

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1149 **Declaration of Interests**

1150 In the past three years, H.K. received expenses and/or personal fees from UnitedHealth, Element Science,
1151 Eyedentifeye, and F-Prime. He is a co-founder of Refactor Health and HugoHealth, and is associated with
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1155 Astra Zeneca, RohBar, Veracyte, Galapagos, Fibrogen, and Thyron over the last 3 years, reports equity in
1156 Pliant and Thyron, and acknowledges grants from Veracyte, Boehringer Ingelheim, BMS. A. I. co-
1157 founded and consults for RIGImmune, Xanadu Bio and PanV, consults for Paratus Sciences, InvisiShield
1158 Technologies, and is a member of the Board of Directors of Roche Holding Ltd. A.M.R. and Y.D. are
1159 inventors of a patent describing the REAP technology. A.M.R. is the founder and director of Seranova
1160 Bio; and A.M.R. and Y.D. hold equity in Seranova Bio. All other authors have no conflict of interest to
1161 declare.

1162 **Additional Information**

1163 **Supplementary information** is included within this manuscript as Supplementary Tables 1-3.

1164 **Correspondence and requests for materials** should be addressed to: A.I., D.P., A.M.R., and D.V.D.

1165 **Extended Data Figure Legends**

1166 **Extended Data Figure 1. Additional demographic and clinical analysis of Long COVID cohort. (A)**
1167 Box plots of Min-Max normalized survey responses (n = 35 HC, 20 CC, 98 LC). Individual survey
1168 instruments are arranged in columns with corresponding health dimensions below. Surveys in red were
1169 aggregated to generate Long COVID Propensity Scores (LCPS). Significance was assessed using Kruskal-
1170 Wallis tests corrected for multiple comparisons using Bonferroni's method. **(B)** Receiver-Operator Curve
1171 (ROC) analysis of LCPS scores. Area under the curve (AUC) is reported with Bootstrap Bias-corrected
1172 95% confidence intervals (CI) of AUC. **(C)** Ring plots of prevalence of Postural Orthostatic Tachycardia
1173 Syndrome (POTS) among Long COVID cohort. "No diagnosis" is represented by grey regions, "positive
1174 diagnosis" is represented by shaded purple regions. Purple regions are further stratified by diagnostic
1175 modality: clinical = diagnosed through clinical evaluation (light purple); Tilt-table = diagnosed by Tilt-
1176 table (middle purple); Stand / Lean = diagnosed by Stand / LEAN test (dark purple). **(D)** Ring plots of
1177 prevalence of self-reported negative impacts on employments status among individuals with Long COVID.
1178 Negative responses are represented by grey region, positive responses are indicated by purple region. **(E)**
1179 Heatmap of self-reported binary symptoms clustered by Hamming distances (rows and columns) and
1180 colored according to physiological system as previous. Columns are annotated by LCPS scores with
1181 bootstrapped cluster reproducibility scores reported in parentheses (bootstrapped Jaccard similarity) **(F)**
1182 Boxplots of Long Covid Propensity Score (LCPS) plotted by group (HC = healthy control; CC =
1183 convalescent control; LC = Long COVID) and cluster. Central lines represent group medians, bottom and
1184 top lines represent 25th and 75th percentiles, respectively. Whiskers represent 1.5× inter-quartile range
1185 (IQR). Significance for difference in median LCPS was assessed using Kruskal-Wallis with correction for
1186 multiple comparisons using Bonferroni-Holm.

1187 **Extended Data Figure 2. Immunological differences in myeloid and lymphocyte effectors among**
1188 **participants with Long COVID. (A-B)** Violin plots of myeloid peripheral blood mononuclear populations
1189 (PBMcs) plotted by group as percentages of respective parent populations (gating schemes detailed in

1190 **Extended Data Fig. 10. (B, right)** Coefficients from linear model are shown. Model predictors are
1191 indicated on x-axis. Significant predictors ($p \leq 0.05$) are plotted in purple. Detailed model results are
1192 reported in **Extended Data Table 4. (C)** Violin plots of B lymphocyte subsets from PBMCs plotted as
1193 percentages of respective parent populations (gating schemes detailed in **Extended Data Fig. 10. (D,E)**
1194 Violin plots of various CD4 (top row) and CD8 (bottom row) populations. **(F)** Violin plots of IL-4 and IL-
1195 6 double-positive CD4⁺ (left) and CD8⁺ (right) T cells plotted as percentages of total CD4⁺ or CD8⁺ T cells.
1196 **(G)** A PERMANOVA test of the association between all cell populations shown and participant age, sex,
1197 LC status, and body mass index (BMI). For all violin plots **(A–F)**, significance was assessed using Kruskal-
1198 Wallis corrected for multiple comparisons using Bonferroni-Holm. Each dot represents a single patient
1199 ($n = 40$ HC, 33 CC, 99 LC). Central bars indicate the median value of each group. Only significant
1200 differences between group medians are shown.

1201 **Extended Data Figure 3. Circulating myeloid, B cell, and cytokine producing immune cell**
1202 **populations among MY-LC participants. (A–I)** Violin plots of various myeloid, B, and T cell PBMC
1203 populations stratified by healthy (HC), convalescent (CC), and Long COVID (LC) groups. Significance for
1204 differences in group medians was assessed using Kruskal-Wallis tests with correction for multiple
1205 comparisons using Bonferroni-Holm. Each dot represents a single patient ($n = 40$ HC, 33 CC, 99 LC) **(J–**
1206 **K)** Coefficients from linear models for various PBMC populations. Bars in purple indicate significant
1207 predictors of specific PBMC populations ($p \leq 0.05$).

1208 **Extended Data Figure 4. Absolute Counts of in myeloid and lymphocyte effectors among participants**
1209 **with Long COVID. (A–B)** Violin plots of myeloid peripheral blood mononuclear populations (PBMCs)
1210 plotted by group (HC, healthy control; CC, convalescent control; LC, Long COVID) as absolute cell counts
1211 (gating schemes detailed in **Extended Data Figure 10A**). Significance for differences in group medians
1212 was assessed using Kruskal-Wallis tests with correction for multiple comparisons using Bonferroni-Holm.
1213 **(C)** Violin plots of B lymphocyte subsets from peripheral blood mononuclear populations (PBMCs) plotted
1214 as absolute cell counts (gating schemes detailed in **Extended Data Figure 10D**). Significance was assessed
1215 using Kruskal-Wallis with correction for multiple comparison using Bonferroni-Holm. **(D, E)** Violin plots
1216 of various CD4 (top row) and CD8 (bottom row) populations. Significance was assessed using Kruskal-
1217 Wallis with correction for multiple comparison using Bonferroni-Holm. **(F)** Violin plots of IL-4 and IL-6
1218 double positive CD4⁺ (left) and CD8⁺ (right) T cells plotted as absolute cell counts. Significance was
1219 assessed using Kruskal-Wallis with correction for multiple comparison using Bonferroni-Holm. For all
1220 plots **(A–F)**, central bar in the violin plot indicated the median value of each group. Each dot represents a
1221 single patient ($n = 37$ HC, 28 CC, 94 LC).

1222 **Extended Data Figure 5. Humoral Analysis of SARS-CoV-2 specific antibodies. (A)** Dot plots of IgG
1223 concentrations from historical, unvaccinated SARS-CoV-2 exposed controls (HCW+) and unvaccinated
1224 Long COVID participants. Central lines indicate median group values with bars representing 95% CI
1225 estimates. Vaccination status for each cohort is indicated by the form “x0” where the digit indicates the
1226 number of SARS-CoV-2 vaccine doses. Significance for differences in group medians were assessed using
1227 Kruskal-Wallis with correction for multiple comparison using FDR (Benjamini-Hochberg). Each dot
1228 represents a single patient ($n = 19$ HCW, 19 LC). **(B)** Coefficients from linear models are reported for anti-
1229 RBD antibody responses. Model predictors are reported along the x-axis and included age, sex (categorical),
1230 Long COVID status (categorical), body mass index (BMI), and number of vaccinations at blood draw.
1231 Significant predictors ($p \leq 0.05$) are plotted in purple. Detailed model results are reported in **Extended Data**
1232 **Table 5. (C)** Boxplots of antibody binding to various SARS-CoV-2 linear peptide sequences plotted by
1233 group (HC = healthy control; CC = convalescent control; LC = Long COVID) amongst participants who
1234 have received 1 or more vaccine doses. Each dot represents one individual. Central bars represent groups
1235 medians, with bottom and top bars representing 25th and 75th percentiles, respectively. Dashed line

1236 represents z-score threshold for epitope positivity defined by SERA. Statistical significance determined by
1237 Kruskal-Wallis with correction for multiple comparisons using Bonferroni-Holm. Each dot represents an
1238 individual patient: LC (purple, n = 80), HC (orange, n = 39) and CC (yellow, n = 38). **(D)** Proportion of each
1239 group amongst participants who have received 1 or more vaccine doses (LC: n = 80, control: n = 77) that is
1240 seropositive ($z\text{-score} \geq 3$) for each of 7 linear Spike motifs mapping to outlier peaks. Motifs with
1241 significantly different seropositivity between groups are highlighted in red, as determined by Fisher's exact
1242 test corrected for multiple comparisons by FDR (Benjamini-Hochberg). **(E)** Coefficients from linear models
1243 are reported for anti-RBD antibody responses. Model predictors are reported along the x-axis and included
1244 age, sex (categorical), Long COVID status (categorical), body mass index (BMI), and number of
1245 vaccinations at blood draw. Significant predictors ($p \leq 0.05$) are plotted in purple. Detailed model results
1246 are reported in **Extended Data Table 5**. *Abbreviation: HCW+, previously SARS-CoV-2 infected healthcare*
1247 *worker.*

1248 **Extended Data Figure 6. Significantly different soluble plasma factors across MY-LC cohorts. (A–**
1249 **H)** Violin plots of various z-score transformed circulating plasma factors across healthy (HC), convalescent
1250 (CC), and Long COVID (LC) cohorts. Significance of difference in group medians was assessed using
1251 Kruskal-Wallis corrected for multiple comparisons using Bonferroni's method. P-values from multiple
1252 Kruskal-Wallis testing were adjusted using the Benjamini-Hochberg procedure. **(I)** Negative Log_{10}
1253 transformed p-values from Kruskal-Wallis tests plotted against Spearman correlations with LCPS for
1254 various plasma factors. Reported p-values are adjusted for multiple comparisons using FDR (Benjamini-
1255 Hochberg). Horizontal line represents significance threshold for a difference in group medians. Vertical
1256 lines represent the minimum correlation values for plasma factors significantly correlating with LCPS
1257 scores. Red depicts factors with significant differences in group medians and significant correlations with
1258 LCPS.

1259 **Extended Data Figure 7. Analysis of private autoantibodies within the MY-LC cohort. (A–B)**
1260 Correlation plots depicting relationships between number of autoantibody reactivities and %DN of B cells
1261 **(A)** or days from symptom onset (DFSO) and number of autoantibody reactivities **(B)**. For all panels,
1262 correlation was assessed using Spearman's method. Black line depicts linear regression with 95% CI
1263 shaded. Colors depict Long COVID cluster (cluster 3, blue; cluster 2, green; cluster 1, red). Each dot
1264 represents one individual. **(C)** Grouped box plot depicting reactivity magnitude per individual in the listed
1265 GO Process domain. Reactivity magnitude is calculated as the sum of REAP scores for all reactivities per
1266 individual in a given GO Process domain. Statistical significance assessed by Kruskal-Wallis and adjusted
1267 for multiple comparisons using FDR (Benjamini-Hochberg) correction. Boxplot colored box depicts 25th
1268 to 75th percentile of the data, with the middle line representing the median, and outliers depicted as points.
1269 **(D)** Heatmap depicting autoantibody reactivity for GPCRs included in the REAP library. Each column is
1270 one participant, grouped by control or LCPS cluster. HC = healthy control, CC = convalescent control, LC
1271 = Long COVID. *Abbreviations: GPCR = G-protein coupled receptor.*

1272 **Extended Data Figure 8. Non-SARS-CoV-2 humoral responses among participants with Long**
1273 **COVID. (A)** Heatmap depicting REAP reactivities to viral antigens across the MY-LC cohort. Each
1274 column is one participant, grouped by control or LCPS cluster. Column clustering within groups performed
1275 by K-means clustering. Each row is one viral protein. Reactivities depicted have at least one participant
1276 with a REAP score ≥ 2 . **(B)** REAP scores for VZV gE by group (HC = healthy control; CC = convalescent
1277 control; LC = Long COVID). Statistical significance determined by Kruskal Wallis with correction for
1278 multiple comparison using Bonferroni-Holm. Each dot represents one individual (n = 25 HC, 13 CC, 98
1279 LC). Bottom and top lines depict 25th to 75th percentile of the data, with the middle line representing the
1280 median. Whiskers represent 1.5x the inter-quartile range (IQR). **(C)** Proportion of each group (LC: n = 99,
1281 control: n = 78) seropositive for each of 30 common pathogen panels as determined by SERA, grouped by
1282 pathogen-type (LC = Long COVID). Statistical significance determined by Fisher's exact test corrected

1283 with FDR (Benjamini Hochberg). **(D)** Sum of SERA-derived z-scores for IgM reactivity to EBV antigens
1284 plotted by group. Statistical significance determined by Kruskal-Wallis with correction for multiple
1285 comparison using Bonferroni-Holm. Each dot represents one individual (n = 22 Mono-control, 40 HC, 38
1286 CC, 98 LC). Boxplot colored box depicts 25th to 75th percentile of the data, with the middle line representing
1287 the median. Whiskers represent 1.5× the inter-quartile range (IQR). **(E)** Standard curve for Taqman PCR
1288 of EBV *BNRF1*. Serial dilutions of EBV standard ranging from 1 to 10⁶ copies per 200 uL input material
1289 were made. C_t values are plotted against standard copy number, demonstrating ability to detect 1 genome
1290 copy. **(F)** Copies of EBV genome detected in participant serum by Taqman PCR for EBV *BNRF1* plotted
1291 by group. All samples were below the limit of detection. **(G)** Correlation plot depicting the relationship
1292 between EBV p23 REAP score and EBV p23 ELISA O.D. 450 nm. Correlation assessed by Spearman.
1293 Black line depicts linear regression with 95% CI shaded. Colors depict group (purple, LC; yellow, CC;
1294 orange, HC). Each dot represents one individual. **(H,I)** REAP scores for HSV1 gD1 (H) and HSV1 gL (I)
1295 amongst HSV1 seropositive individuals only, separated by group (HC = healthy control; CC = convalescent
1296 control; LC = Long COVID). Statistical significance determined by Kruskal Wallis with correction for
1297 multiple comparison using Bonferroni-Holm. Each dot represents one individual. Boxplot colored box
1298 depicts 25th to 75th percentile of the data, with the middle line representing the median. Whiskers represent
1299 1.5× the inter-quartile range (IQR). Each dot represents one individual. **(J,K)** Correlation plot depicting the
1300 relationship between Long COVID Propensity Score (LCPS) and EBV gp42 PVXF[ND]K (J) or EBV p23
1301 REAP score (K). Correlation assessed by Spearman. Each dot represents one individual. Colors depict Long
1302 COVID cluster (cluster 1, blue; cluster 2, green; cluster 3, red). Black line depicts the linear regression,
1303 with the 95% CI shaded. **(L-O)** Linear regressions of various SARS-CoV-2 antigens and IL-4/IL-6 double
1304 positive CD4 T cells. Spearman's correlation were calculated for each pair of variables, with corresponding
1305 p-values reported. Black lines depict linear regressions with the shaded area representing 95% confidence
1306 boundaries.

1307 **Extended Data Figure 9. Gale-Shapley matching of Long COVID group and controls harmonizes**
1308 **samples by disease and demographics characteristics.** **(A)** Features used in the preference list
1309 construction for Gale-Shapley matching are shown. Individual paired samples are shown for participant age
1310 and days from initial acute COVID-19 infection (dfso). Paired plots for sex and vaccination status are
1311 shown. **(B)** Additionally, differences between populations in the severity of initial acute COVID-19
1312 infection are shown. No differences between groups are significant by a Chi-square test. **(C,D)** Box plots
1313 of selected features assessed in the Ext. LC group. Center lines represent median values with error bars
1314 representing 1.5 standard deviation. **(E)** Distribution of respiratory symptoms (“dyspnea” or “shortness of
1315 breath”) between individuals with Long COVID in the MY-LC study and the Ext. LC group. Significance
1316 was assessed using Fisher's exact test. **(F-H)** ROC curve analysis using cortisol, cDC1, and galectin-1
1317 levels as an individual classifier of Long COVID status. AUC and 95% CI intervals (DeLong's Method)
1318 for each feature are displayed (top). Kernel-density smoothed histograms for HC, CC and LC cohorts for
1319 selected model predictors. Vertical lines depict threshold values for each feature with maximal
1320 discriminatory accuracy (bottom).

1321 **Extended Data Figure 10. Flow Cytometry gating schematics. (A-D).** Various gating strategies for
1322 granulocyte and myeloid populations **(A)**, T lymphocytes **(B)**, intracellular cytokine staining **(C)**, and B
1323 lymphocytes **(D)**.

1324 **Extended Data Table Legends**

1325 **Extended Data Table 1. Clinical Demographics of MY-LC Cohort.** Summary demographic and clinical
1326 characteristics for the MY-LC Study. Participants were stratified into three study arms at enrollment: (1)
1327 Long COVID (prior SARS-CoV-2 infection with persistent, unexplained symptoms); (2) healthy study site
1328 cohort (no prior SARS-CoV-2 infection); or (3) convalescent COVID-19 cohort (prior SARS-CoV-2

1329 infection without persistent symptoms). Various demographic features and clinical characteristics are
1330 reported by row for each cohort (row measurement units are specified in parentheses). Within each cell,
1331 counts or clinical feature averages are reported, with sample standard deviations, relative cohort
1332 percentages, and participant numbers reported where pertinent. Results from statistical tests are reported as
1333 p-values and accompanying test statistics: † Chi-square test p-value (Chi-square test statistic, degrees of
1334 freedom (df)); †† Kruskal-Wallis ANOVA p-value; ††† Fisher's exact test p-value (Odd's Ratio: [95%
1335 Confidence Interval (Baptista-Pike)]); ‡ Mann-Whitney U test p-value. Post-hoc comparisons were
1336 conducted using Dunn's test with Tukey's correction for multiple comparison (column comparison order
1337 left-right: 1-2, 1-3, 2-3). Participant medical histories were collected and collated from binary self-reports
1338 of prior medical history and review of electronic medical records by study staff (positive responses in either
1339 participant self-report or EMR review were considered an overall binary positive response). Abbreviations:
1340 *n*, number; *M*, male; *F*, female; *BMI*, body mass index; +PCR, positive result from SARS-CoV-2 nucleic
1341 acid test; +Ab, positive result from SARS-CoV-2 antibody test; *Y*, Yes; *N*, No.

1342
1343 **Extended Data Table 2. Normalized survey responses across MY-LC cohorts.** Survey responses for
1344 participants are organized by individual instruments (columns) and MY-LC cohorts (rows). Participant
1345 responses for each survey instrument were summed and normalized using standard min-max normalization
1346 procedures such that a value of 1 equals the maximum possible aggregate score and 0 equals the minimum
1347 possible aggregate score. Additionally, individual survey elements were oriented through inversion such
1348 that higher normalized scores on each instrument indicate a higher intensity or degree of agreement with
1349 survey prompts. For each cohort, median values are displayed.

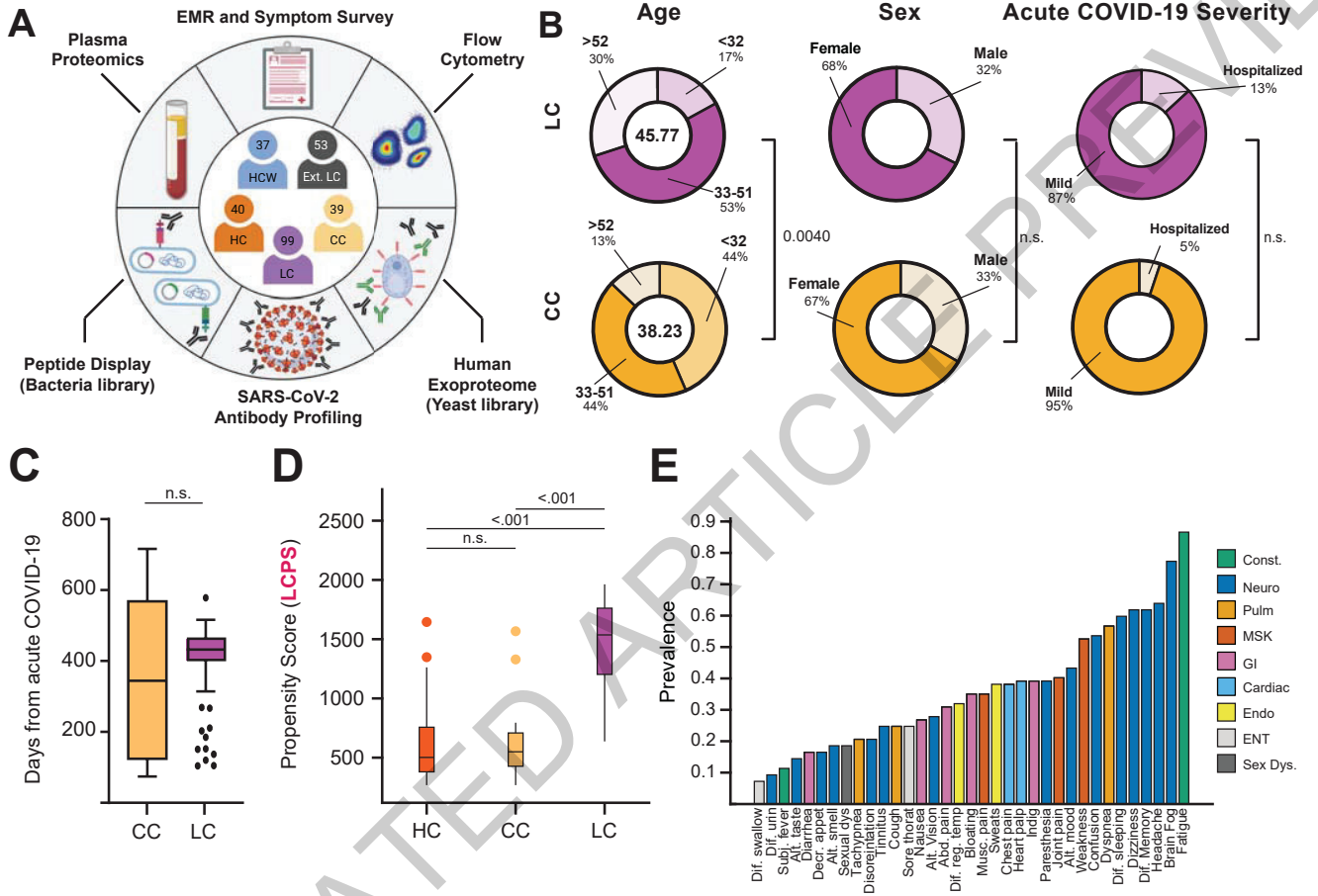
1350 **Extended Data Table 3. Determinations of optimal LCPS threshold.** Classification metrics across
1351 different LCPS thresholds ('Cut-offs') (*Upper table*). Summary area-under the curve (AUC) statistics and
1352 bootstrap confidence intervals for Receiver-Operator curve analysis (ROC) (*lower table*)

1353 **Extended Data Table 4. Modeling of select flow cytometry populations. (A-L)** Detailed linear modeling
1354 results are reported for various cytokine producing T cell populations analyzed by flow cytometry.

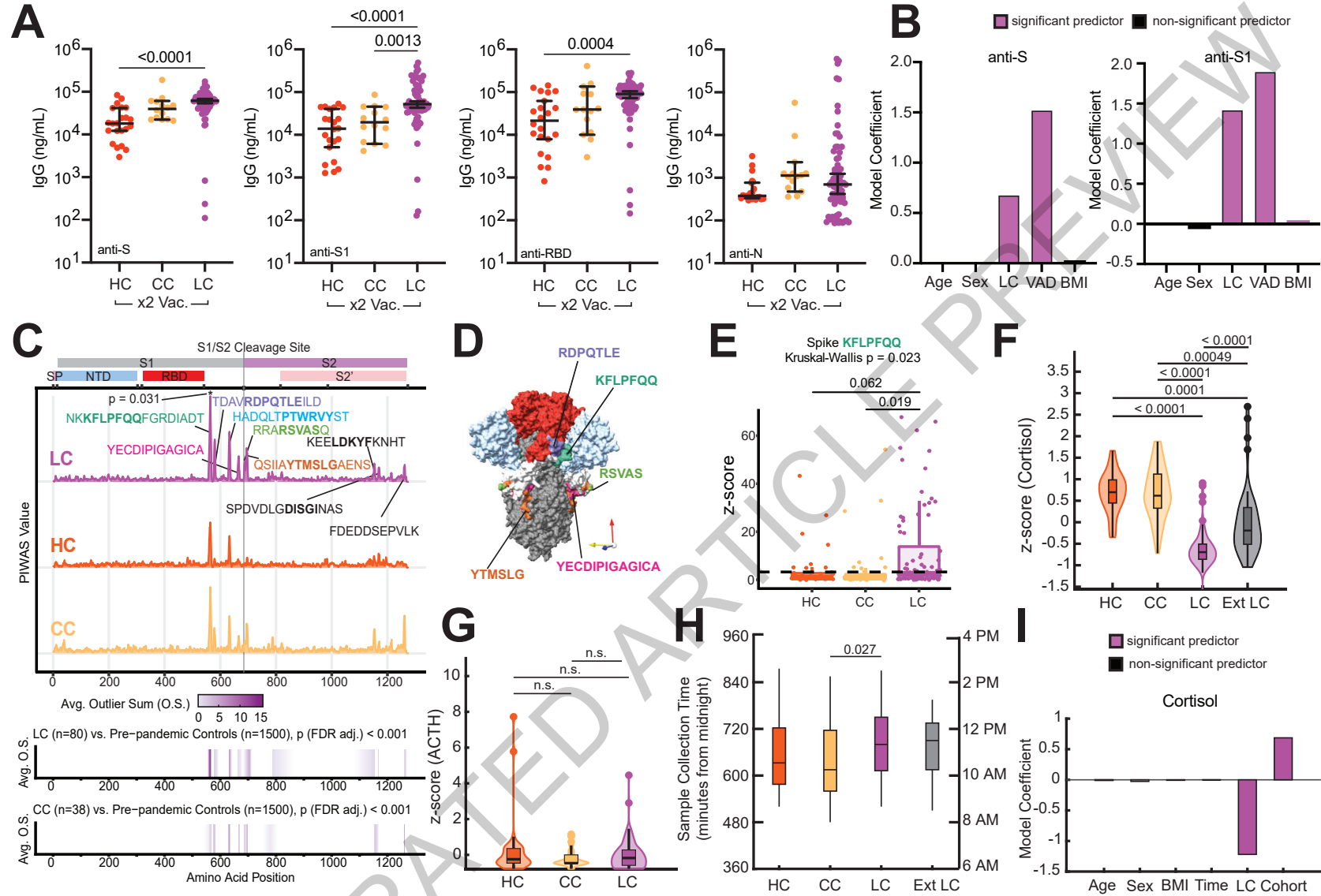
1355 **Extended Data Table 5. Modeling of anti-SARS-CoV-2 antibody and linear motif responses. (A-E)**
1356 Detailed linear modeling results are reported for SARS-CoV-2 specific antibody responses and peptide
1357 motifs with corresponding model formulations.

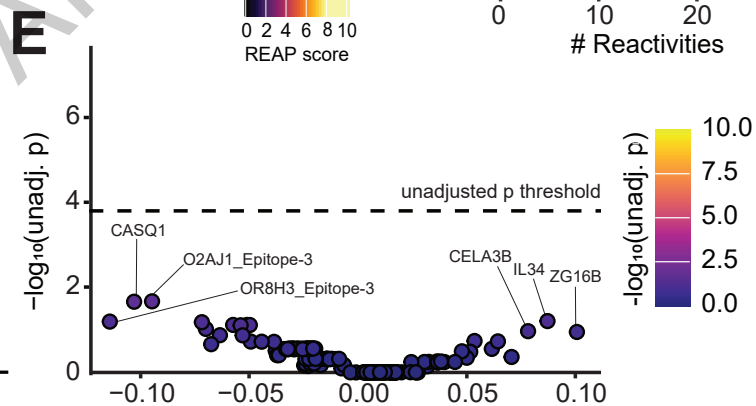
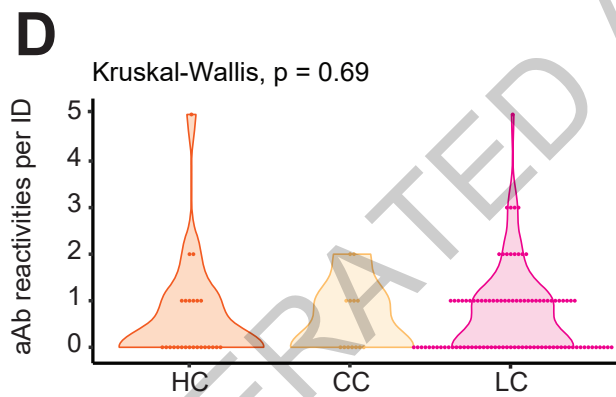
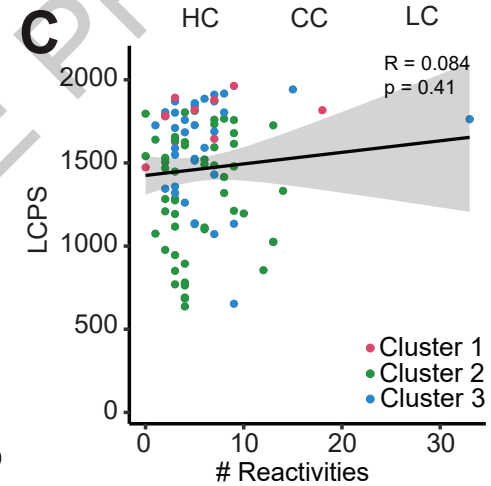
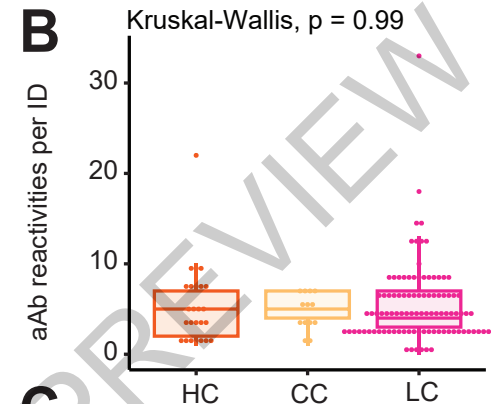
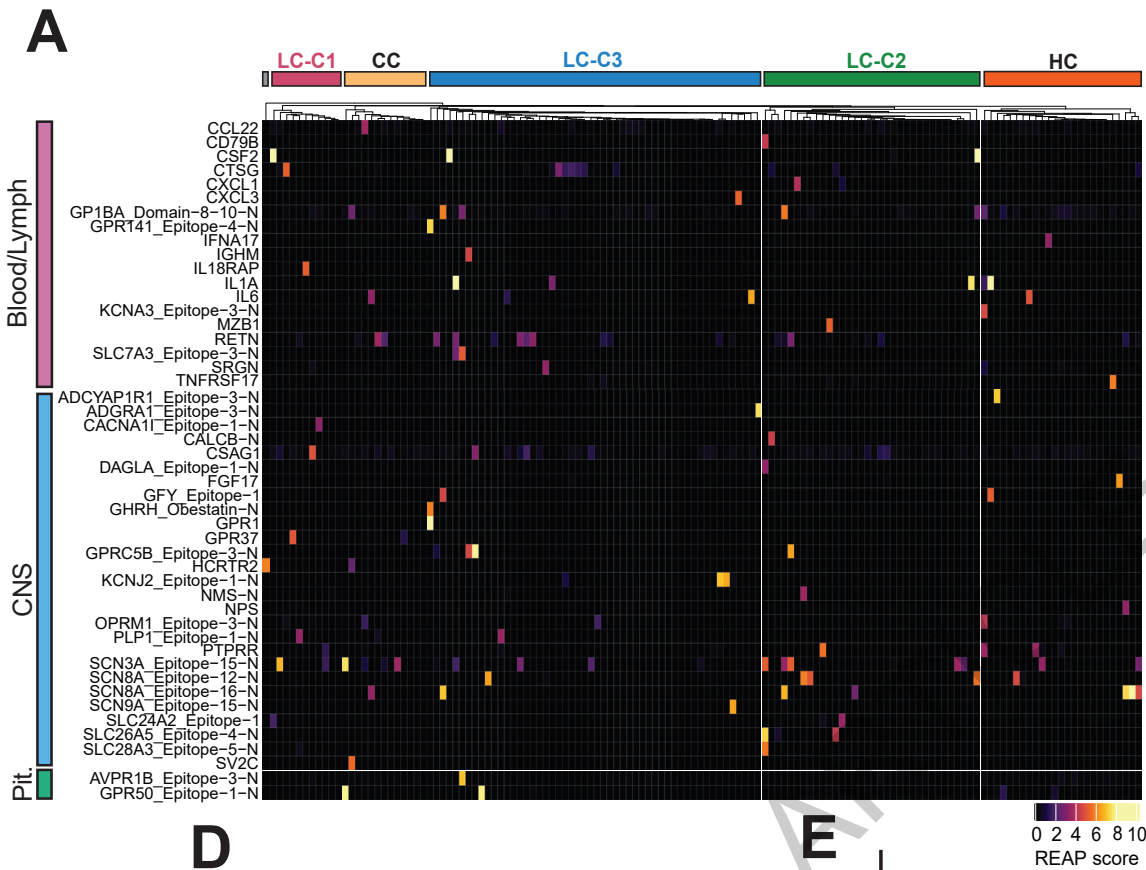
1358 **Extended Data Table 6. Modeling of cortisol levels.** Detailed linear modeling results are reported for
1359 cortisol levels across groups with corresponding model formulation.

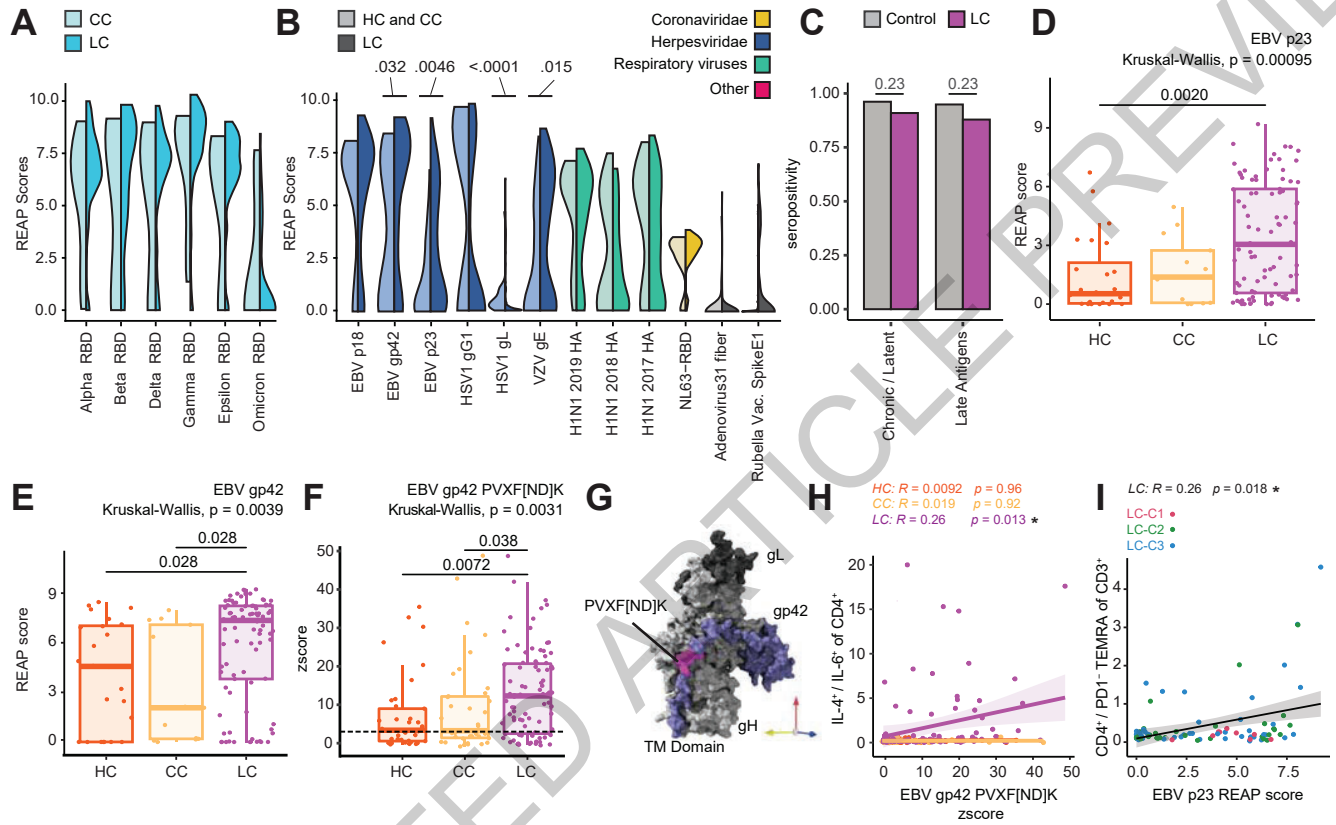
1360 **Extended Data Table 7. Inter-model Long COVID classification comparison.** Cohen's Kappa analysis
1361 of agreement between LCPS and Integrated immunological classification of Long COVID status.



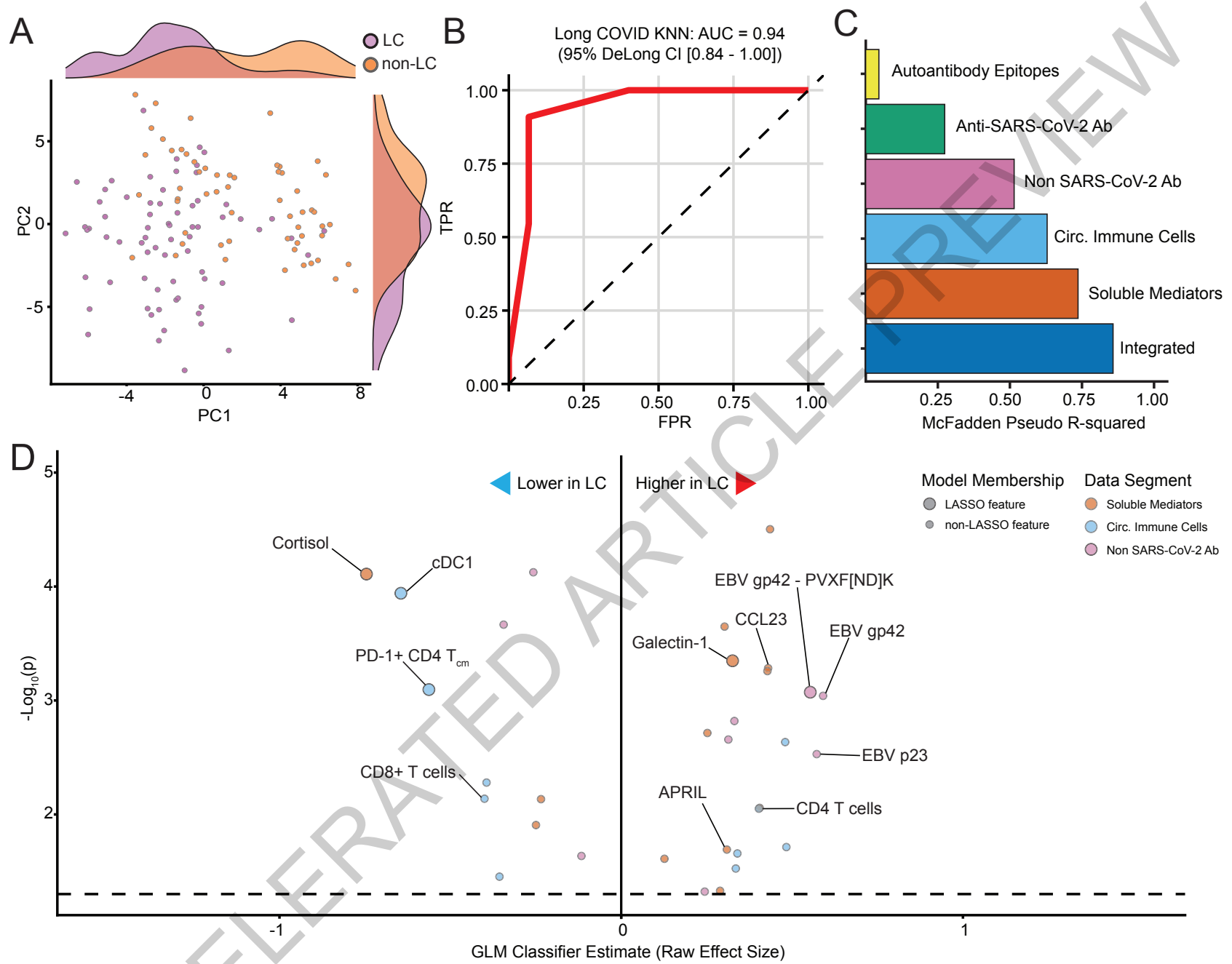
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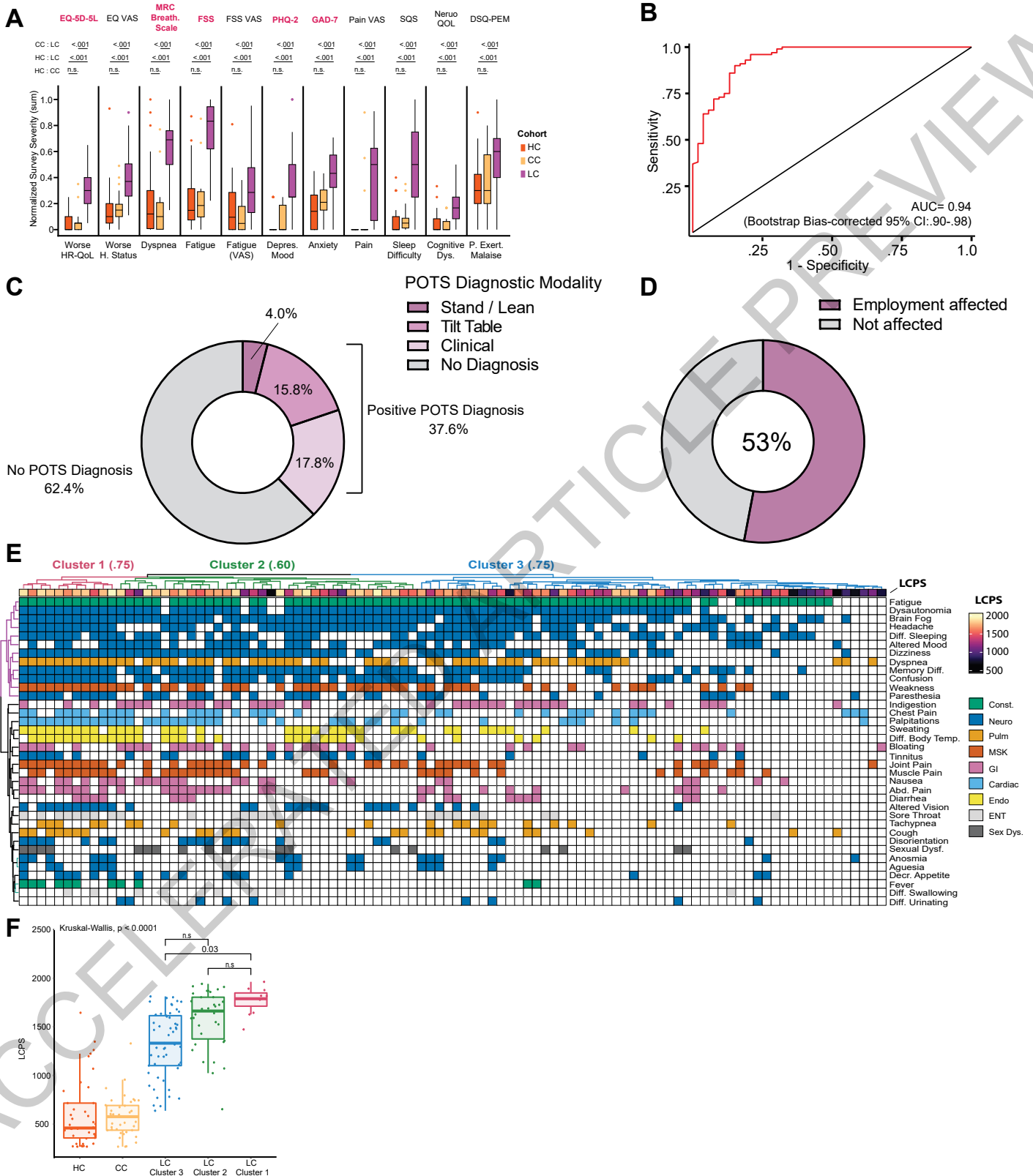




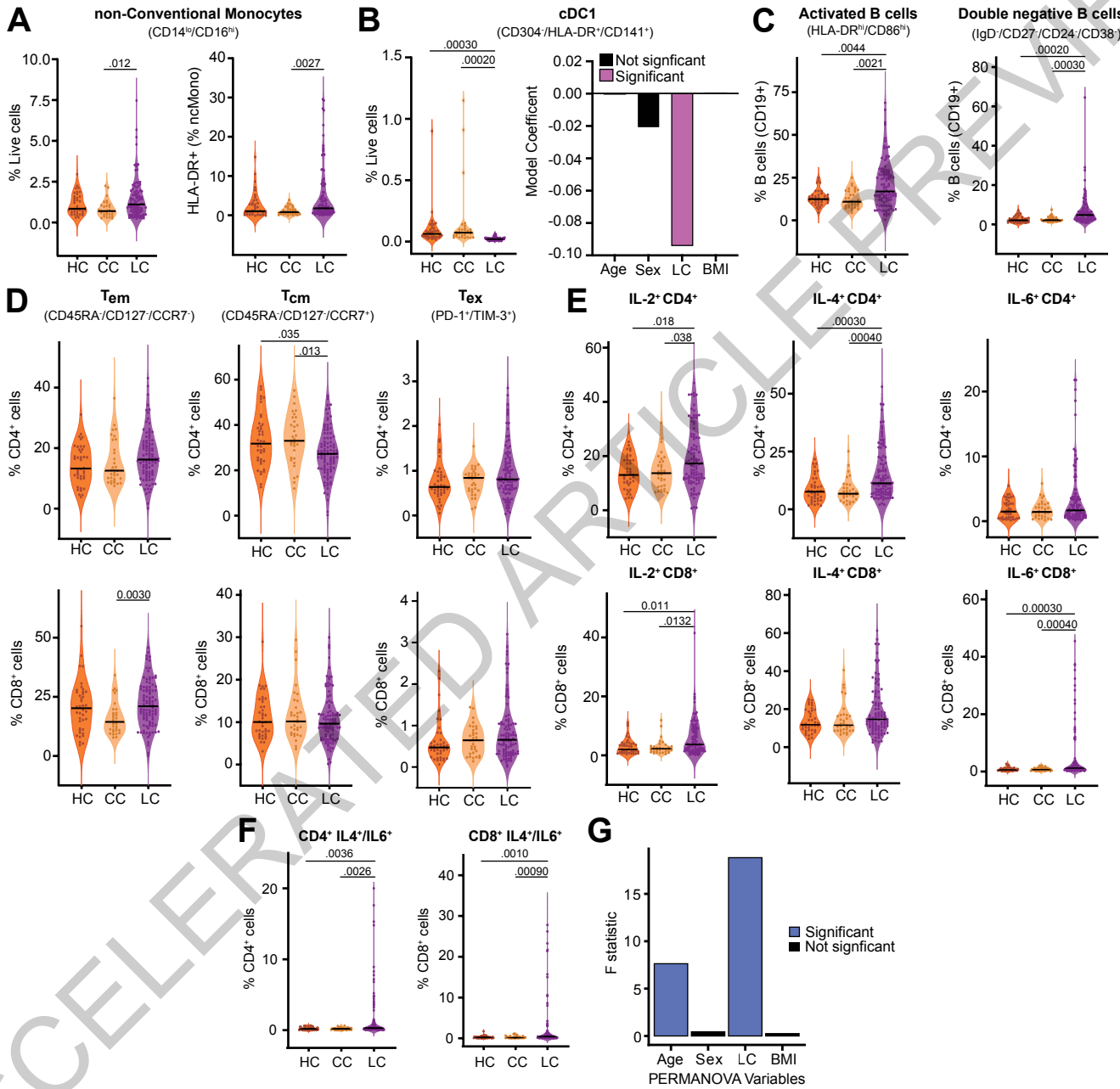


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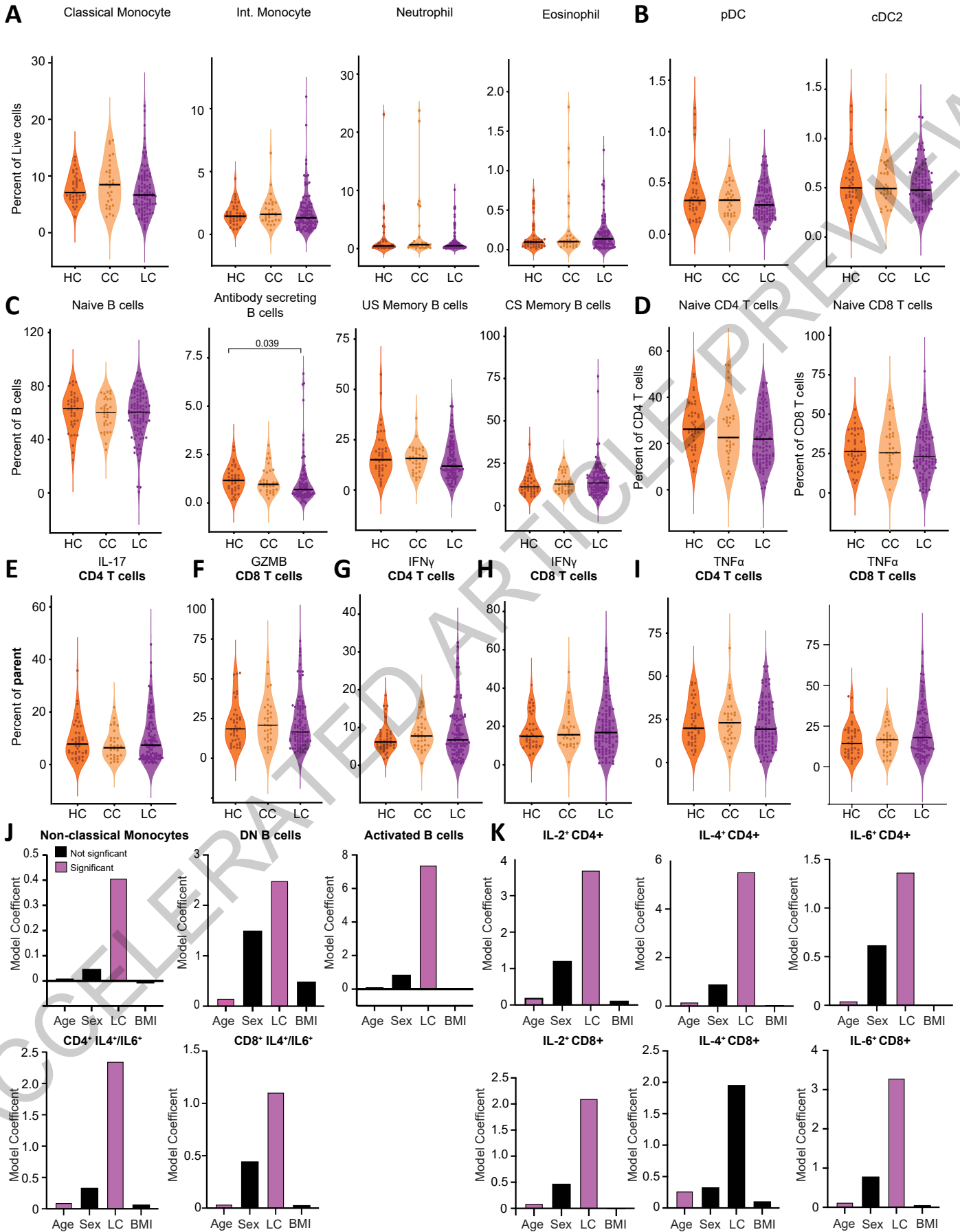




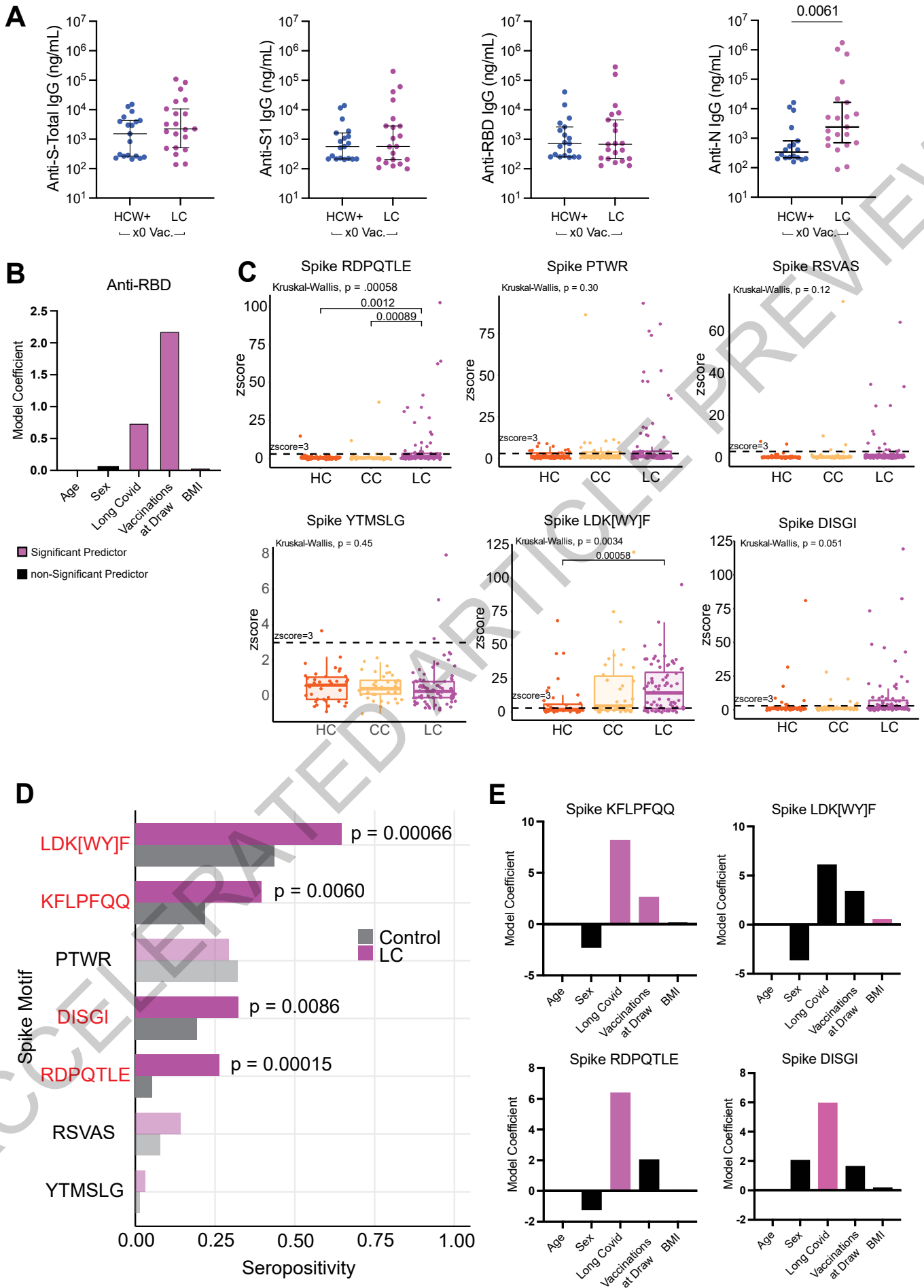
Extended Data Fig. 1



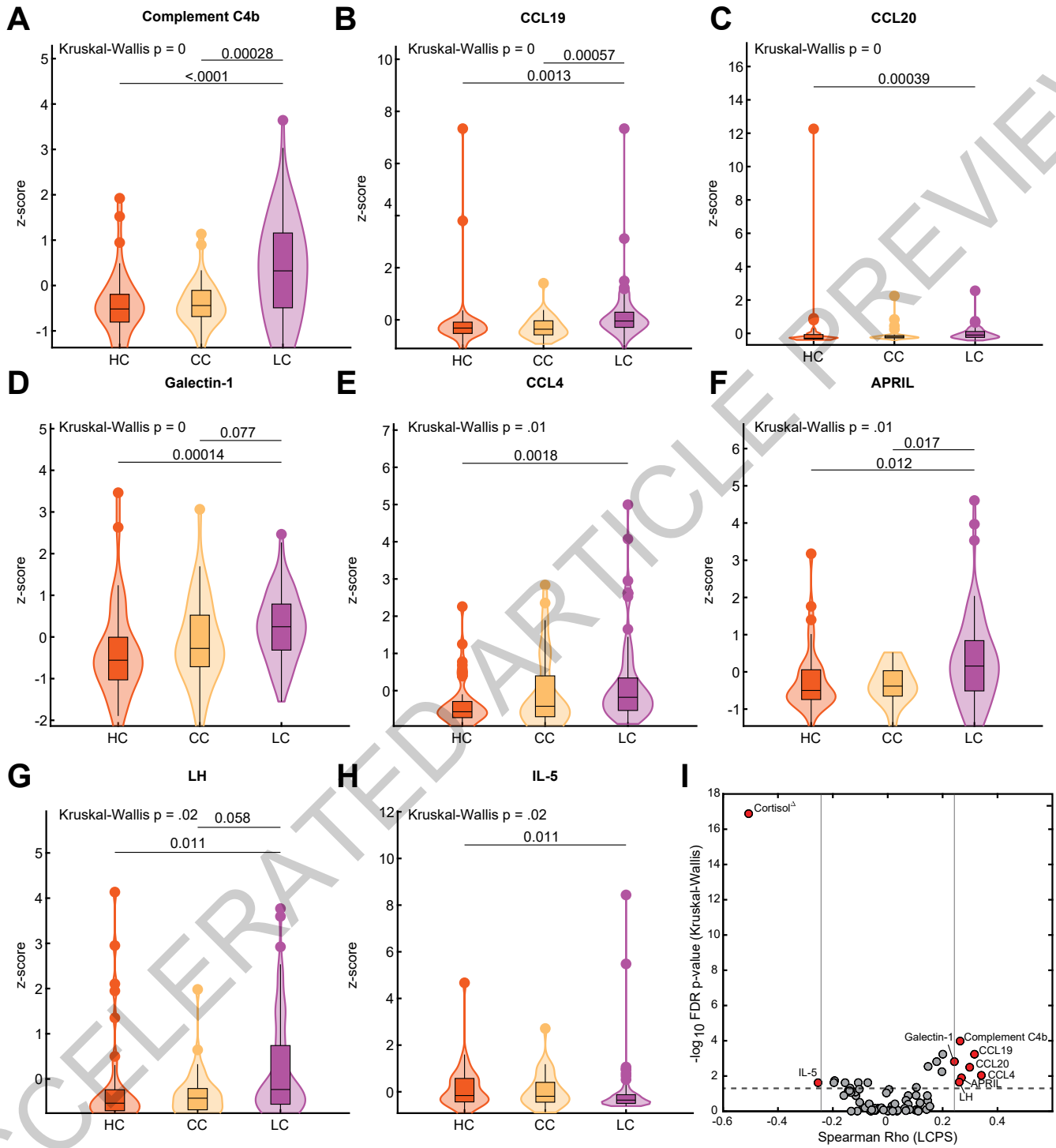
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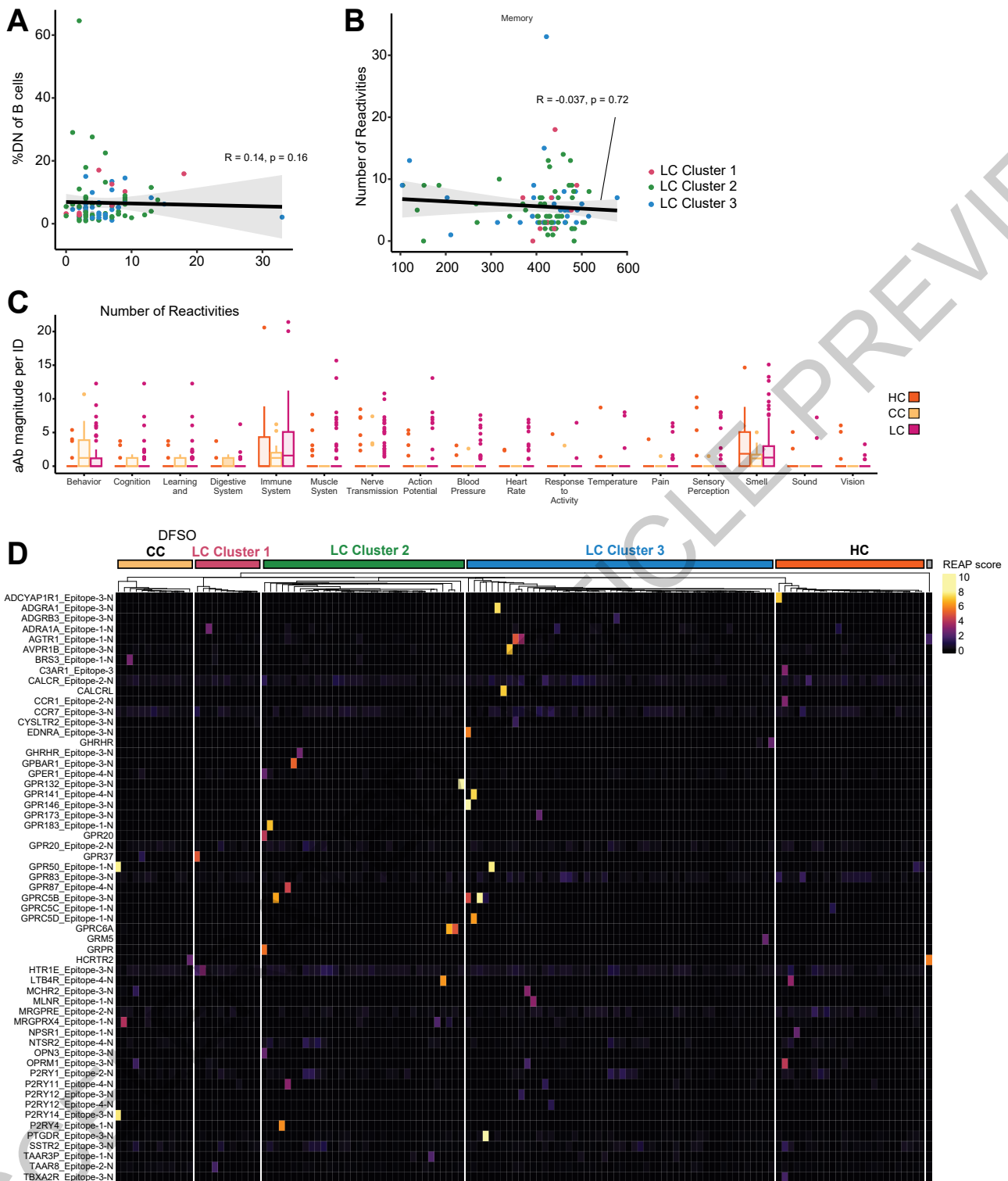
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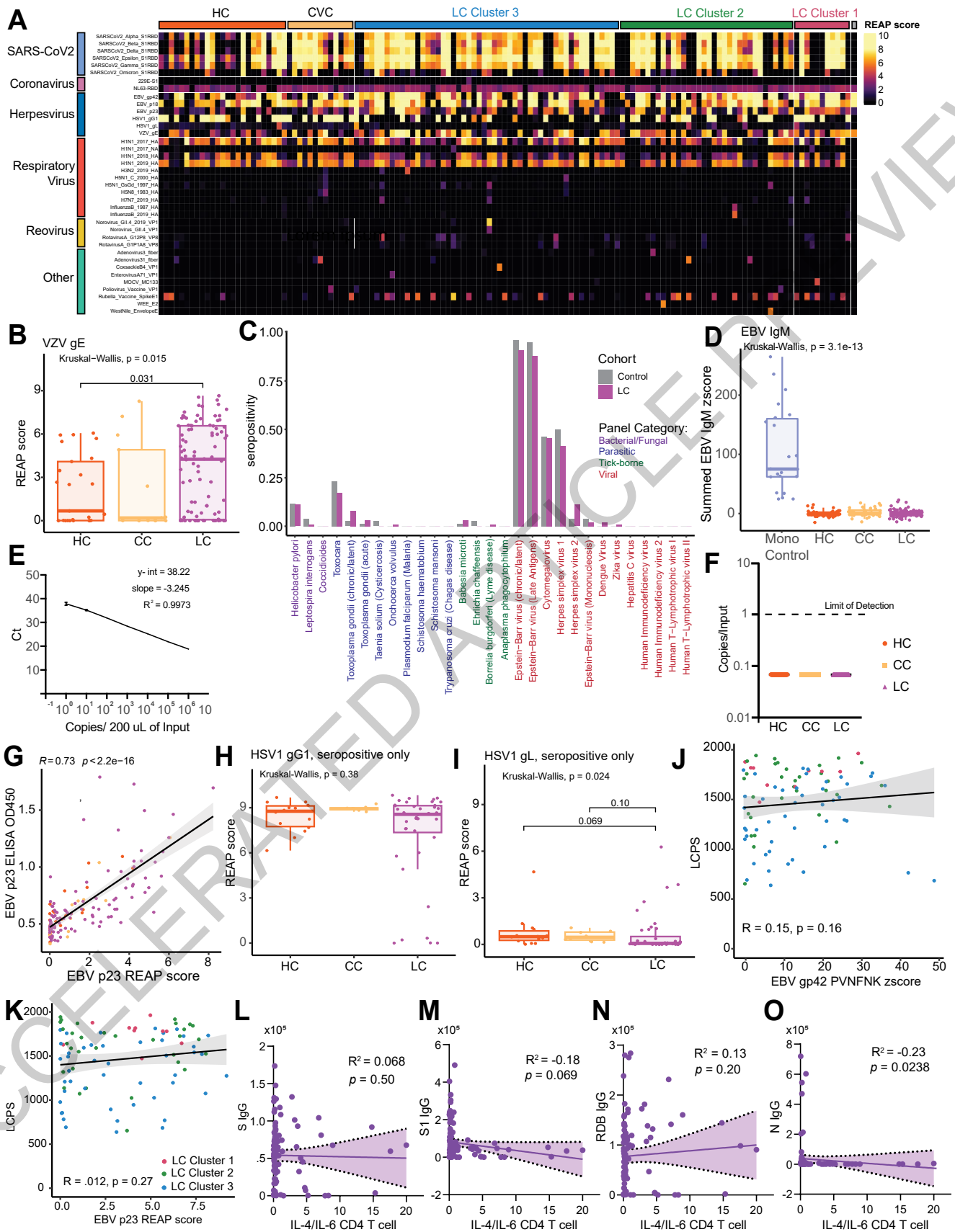
Extended Data Fig. 5



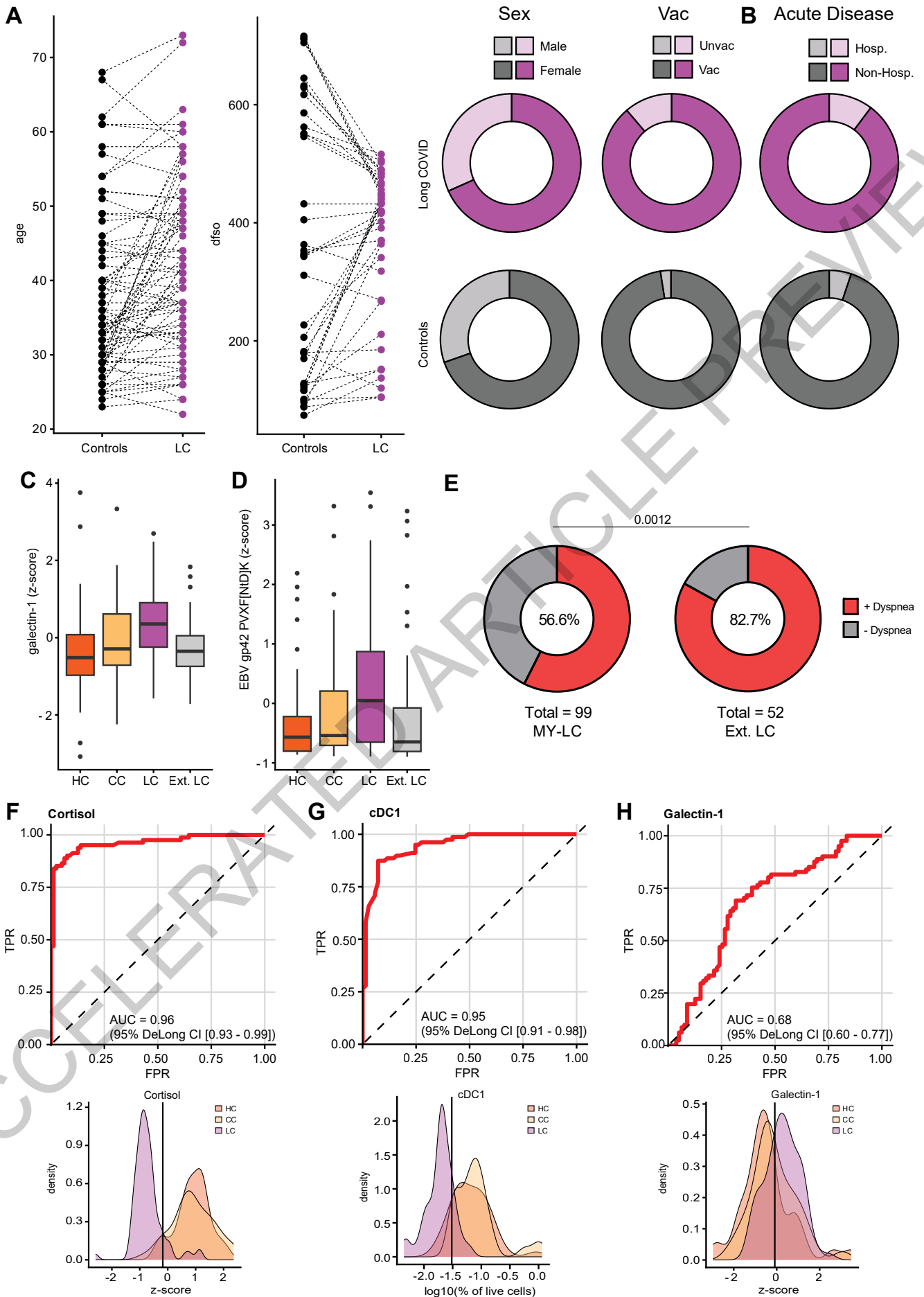
Extended Data Fig.6



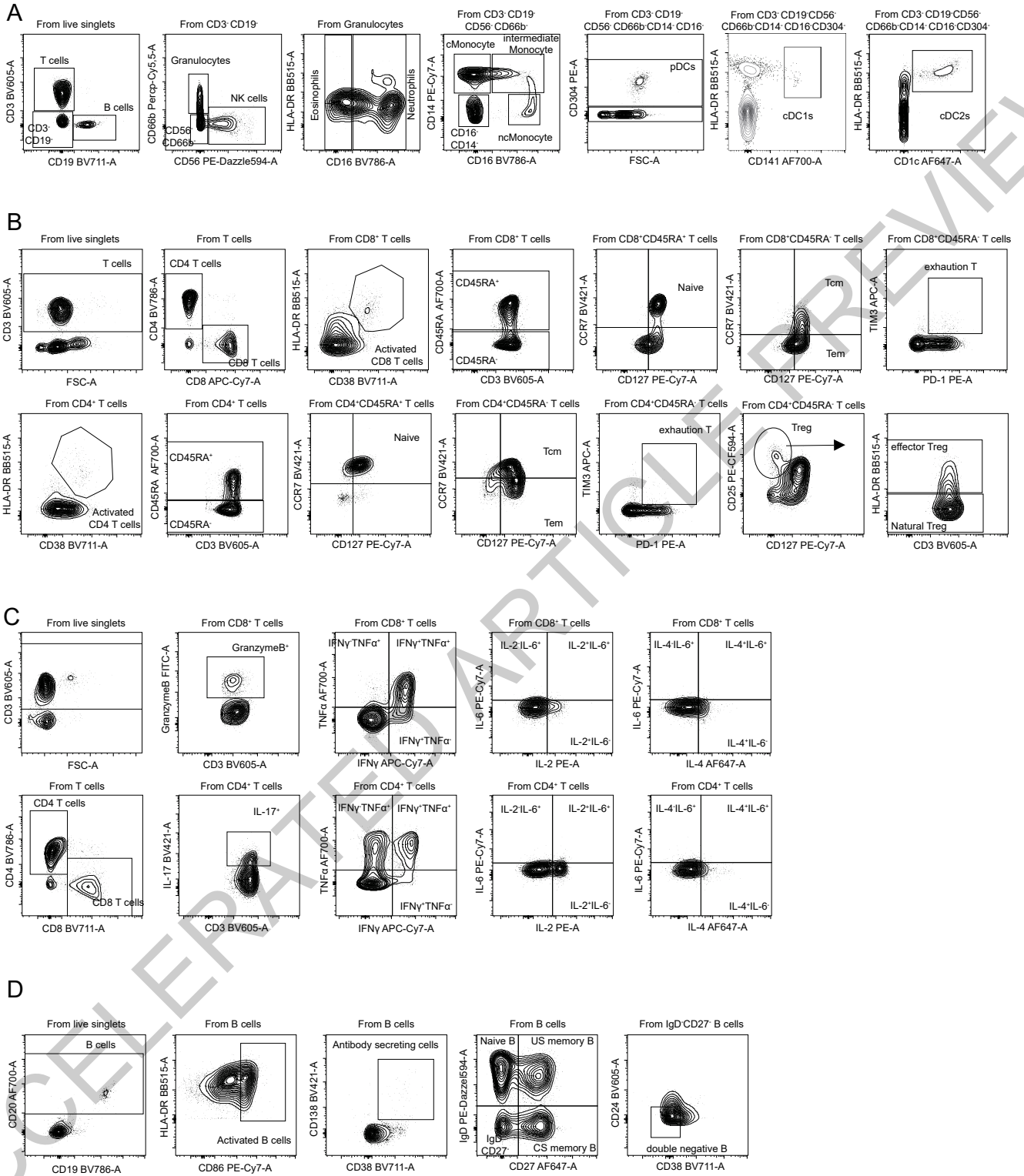
Extended Data Fig. 7



Extended Data Fig. 8



Extended Data Fig. 9



Extended Data Fig. 10

Demographics	Long COVID	Healthy Site Controls	Convalescent COVID-19 Controls	p-value (test statistics)	post-hoc (1-3, 1-3, 2-3)	Total
Enrolled Participants (n)	101	42	42	Long COVID vs Not Long COVID	-	185
Excluded Participants (n)	2 (1.98%)	2 (4.76%)	3 (7.14%)	p = 0.41 (OR: 0.4040 [0.07569-1.779]) [†]	-	7
Cohort Size (n)	99.00	40.00	39.00	-	-	178
age (years)	45.77 ± 13.18 (n = 99)	36.73 ± 10.17 (n = 40)	38.23 ± 11.67 (n = 39)	p < 0.0001 ^{††}	0.0006, 0.0040, >0.9999	42.08 ± 12.87 (n = 178)
sex (M F)	32 67 (32.32% 67.68%) (n = 99)	12 28 (30% 70%) (n = 40)	13 26 (33.33% 66.67%) (n = 39)	p = 0.9465 (1101, 2) ^{††††}	-	57 121 (32.02% 67.98%) (n = 178)
BMI	26.04 ± 7.02 (n = 99)	24.86 ± 6.78 (n = 39)	24.56 ± 3.41 (n = 38)	p = 0.32 ^{††}	-	25.46 ± 6.36 (n = 176)
Race						
Asian	5 (5.05%)	4 (10%)	3 (7.69%)	-	-	12 (6.74%)
Black	7 (7.07%)	1 (2.5%)	2 (5.13%)	-	-	10 (5.62%)
American Indian / Alaskan Native	0 (0%)	1 (2.5%)	0 (0%)	-	-	1 (0.56%)
Native Hawaiian and Other Pacific Islander	1 (1.01%)	0 (0%)	0 (0%)	-	-	1 (0.56%)
White	74 (74.75%)	27 (67.5%)	27 (69.23%)	-	-	128 (71.91%)
Other	5 (5.05%)	7 (17.5%)	6 (15.38%)	-	-	18 (10.11%)
Unknown	7 (7.07%)	0 (0%)	0 (0%)	-	-	7 (3.93%)
Ethnicity						
Hispanic	8 (8.08%)	13 (32.5%)	9 (23.08%)	-	-	30 (16.85%)
COVID-19 Clinical Testing				Positive test vs. No Positive Test		
Clinically Confirmed COVID-19 (+PCR) (+ab)	70 (70.71%)	-	33 (84.62%)	p = .2276 (OR: 4.889 [1.707 - 1.115]) [†]	-	103 (69.13%)
Probable COVID-19 (Negative Diagnostic)	26 (26.26%)	-	1 (2.56%)	-	-	27 (18.12%)
Probable COVID-19 (No Diagnostic)	3 (3.03%)	-	5 (12.82%)	-	-	8 (6.56%)
SARS-CoV-2 Exposure						
Acute COVID-19 Hospitalized (Y N)	13 (13.13%)	-	2 (5.13%)	p = .2234 (OR: 2.797 [0.551 - 12.90]) [†]	-	15 (9.55%)
Vaccination Status						
Unvaccinated	19 (19.19%)	1 (2.5%)	0 (0%)	-	-	20 (11.6%)
1 vaccine dose (Y N)	11 (11.11%)	0 (0%)	0 (0%)	-	-	11 (6.1%)
2 vaccine doses (Y N)	69 (69.7%)	21 (52.5%)	15 (38.5%)	-	-	105 (58.3%)
3 vaccine doses (Y N)	0 (0%)	17 (42.5%)	24 (61.5%)	-	-	41 (23.1%)
Past Medical History						
Most recent Thyroid Stimulating Hormone (TSH)	1.82 (0.378-6.54) (n = 57)	-	-	-	-	-
Most recent Hemoglobin (Hb)	13.9 (10.2-17.7) (n = 86)	-	-	-	-	-
Self-Reported and EMR Aggregated Diagnosis				Long COVID vs. Other		
Hypertension	16 (15.84%)	6 (14.29%)	1 (2.38%)	p = 0.1805 (OR: 1.983 [0.725 - 5.040]) [†]	-	23 (12.71%)
Diabetes Mellitus Type I and II	4 (3.96%)	1 (2.38%)	0 (0%)	p = 0.3839 (OR: 3.284 [0.247 - 40.64]) [†]	-	5 (2.76%)
Kidney Dysfunction (e.g. Chronic)	4 (3.96%)	0 (0%)	0 (0%)	p = 0.1303 (OR: n.c.) [†]	-	4 (2.21%)
Liver Dysfunction (e.g. Fatty)	2 (1.98%)	1 (2.38%)	0 (0%)	p = >0.9999 (OR: 1.608 [0.1839 - 23.57]) [†]	-	3 (1.66%)
Asthma	26 (25.74%)	2 (4.76%)	4 (9.52%)	p = 0.0014 (OR: 4.333 [1.671 - 10.67]) [†]	-	32 (17.68%)
COPD	2 (1.98%)	0 (0%)	0 (0%)	p = 0.5035 (OR: n.c.) [†]	-	2 (1.1%)
Other Lung Dysfunction (e.g. Chronic)	1 (0.99%)	0 (0%)	0 (0%)	p = >0.9999 (OR: n.c.) [†]	-	1 (0.55%)
Stroke	1 (0.99%)	0 (0%)	0 (0%)	p = >0.9999 (OR: n.c.) [†]	-	1 (0.55%)
Spinal Cord Injury	1 (0.99%)	1 (2.38%)	0 (0%)	p = >0.9999 (OR: 0.7959 [0.0151 - 15.30]) [†]	-	2 (1.1%)
Neurological Dysfunction (e.g. Parkinson's, Epilepsy, Dementia)	1 (0.99%)	0 (0%)	1 (2.38%)	p = >0.9999 (OR: 0.7959 [0.0151 - 15.30]) [†]	-	2 (1.1%)
Obesity	8 (7.92%)	0 (0%)	0 (0%)	p = >0.9999 (OR: 1.070 [0.422 - 3.283]) [†]	-	14 (7.73%)
Immunological Dysfunction (e.g. Autoimmune)	3 (2.97%)	1 (2.38%)	3 (7.14%)	p = 0.7014 (OR: 0.5859 [0.1446 - 2.244]) [†]	-	7 (3.87%)
Cancer	6 (5.94%)	0 (0%)	1 (2.38%)	p = 0.1342 (OR: 5.032 [0.7916 - 58.44]) [†]	-	7 (3.87%)
Anxiety	15 (14.75%)	7 (16.67%)	10 (23.81%)	p = 0.3978 (OR: 1.232 [0.6171 - 2.425]) [†]	-	32 (17.68%)
Depression	16 (15.84%)	2 (4.76%)	9 (21.43%)	p = 0.3239 (OR: 1.192 [0.5994 - 2.407]) [†]	-	27 (14.92%)
Other Psychological Diagnoses	2 (1.98%)	0 (0%)	1 (2.38%)	p = >0.9999 (OR: 1.608 [0.1839 - 23.57]) [†]	-	3 (1.66%)
Eating Disorder	2 (1.98%)	0 (0%)	2 (4.76%)	p = >0.9999 (OR: 0.7928 [0.1123 - 5.164]) [†]	-	4 (2.21%)
Irritable bowel syndrome	15 (14.85%)	0 (0%)	3 (7.14%)	p = 0.0129 (OR: 4.524 [1.295 - 15.09]) [†]	-	18 (9.94%)
Other	0 (0%)	0 (0%)	0 (0%)	-	-	0 (0%)
None	22 (21.78%)	29 (69.05%)	28 (66.67%)	p = <0.0001 (OR: 0.1103 [0.05787 - 0.2181]) [†]	-	79 (43.65%)
Prior Autoimmune Diagnosis (Yes No)	18 81 (18.18% 81.82%) (n = 99)	2 38 (5% 95%) (n = 40)	2 32 (8.57% 91.43%) (n = 35)	p = .0765 (5.140, 2) ^{†††}	-	23 151 (12.22% 86.78%) (n = 174)
Hypothyroidism	9 (9.09%)	0 (0%)	1 (5.56%)	-	-	10 (6.7%)
Crohn's Disease	1 (1.01%)	0 (0%)	0 (0%)	-	-	1 (0.67%)
Hyperthyroidism	1 (1.01%)	0 (0%)	1 (5.56%)	-	-	2 (1.33%)
Inclusion Body Myositis	1 (1.01%)	0 (0%)	0 (0%)	-	-	1 (0.67%)
Microscopic colitis	1 (1.01%)	0 (0%)	0 (0%)	-	-	1 (0.67%)
Pernicious anemia	1 (1.01%)	0 (0%)	0 (0%)	-	-	1 (0.67%)
Polymyositis	1 (1.01%)	0 (0%)	0 (0%)	-	-	1 (0.67%)
Polyarthralgia	1 (1.01%)	0 (0%)	0 (0%)	-	-	1 (0.67%)
Primary Biliary Cholangitis	1 (1.01%)	0 (0%)	0 (0%)	-	-	1 (0.67%)
Rheumatoid Arthritis	1 (1.01%)	1 (3.03%)	0 (0%)	-	-	2 (1.33%)
Sicca	1 (1.01%)	0 (0%)	0 (0%)	-	-	1 (0.67%)
Systemic Lupus Erythematosus	1 (1.01%)	1 (3.03%)	0 (0%)	-	-	2 (1.33%)
Ulcerative Colitis	1 (1.01%)	0 (0%)	0 (0%)	-	-	1 (0.67%)
Multiple Sclerosis (remission)	0 (0%)	0 (0%)	1 (5.56%)	-	-	1 (0.67%)

Extended Data Table 1

Cohort	eq5	eq5vas	mrc	fss_tot	fatigue_vas	phq2total	gadtotal	pain_vas	prom_sleep	neuroqol_t	pem
HC	0	0.1	0	0.148148148	0.12	0	0.095238095	0	0.3	0.140569395	0
CC	0.1	0.13	0	0.166666667	0.1	0	0.071428571	0	0.3	0.209964413	0.1
LC	0.3	0.35	0.3	0.833333333	0.69	0.166667	0.285714286	0.5	0.5	0.432384342	0.5

Extended Data Table 2

Detailed report of sensitivity and specificity

Cutpoint	Correctly		Lb	Lb
	Sensitivity	Specificity		
(=268)	100.00%	0.00%	55.32%	1.0000
(=271)	100.00%	4.94%	57.46%	1.0519
(=275)	100.00%	8.64%	59.12%	1.0946
(=281)	100.00%	11.11%	60.22%	1.1350
(=299)	100.00%	13.58%	61.33%	1.1571
(=310)	100.00%	16.05%	62.43%	1.1921
(=322)	100.00%	17.28%	62.98%	1.2090
(=358)	100.00%	18.52%	63.54%	1.2273
(=359)	100.00%	19.75%	64.09%	1.2462
(=369)	100.00%	20.99%	64.64%	1.2656
(=380)	100.00%	22.22%	65.19%	1.2851
(=397)	100.00%	25.93%	66.85%	1.3500
(=408)	100.00%	28.40%	67.96%	1.3868
(=414)	100.00%	29.63%	68.51%	1.4211
(=418)	100.00%	30.86%	69.06%	1.4464
(=436)	100.00%	32.10%	69.61%	1.4727
(=437)	100.00%	33.33%	70.17%	1.5000
(=443)	100.00%	35.80%	71.27%	1.5571
(=446)	100.00%	37.04%	71.82%	1.5882
(=452)	100.00%	38.27%	72.38%	1.6200
(=453)	100.00%	39.51%	72.93%	1.6531
(=454)	100.00%	40.74%	73.48%	1.6875
(=463)	100.00%	41.98%	74.03%	1.7224
(=467)	100.00%	43.21%	74.59%	1.7600
(=478)	100.00%	44.44%	75.14%	1.8000
(=482)	100.00%	45.68%	75.69%	1.8420
(=502)	100.00%	48.15%	76.80%	1.9288
(=506)	100.00%	49.38%	77.35%	1.9766
(=514)	100.00%	50.62%	77.90%	2.0250
(=530)	100.00%	51.85%	78.45%	2.0749
(=523)	100.00%	53.09%	79.01%	2.1164
(=554)	100.00%	54.32%	79.56%	2.1892
(=576)	100.00%	55.56%	80.11%	2.2500
(=583)	100.00%	56.79%	80.66%	2.3143
(=587)	100.00%	58.02%	81.22%	2.3824
(=594)	100.00%	59.26%	81.77%	2.4545
(=596)	100.00%	60.49%	82.32%	2.5113
(=600)	100.00%	61.73%	82.87%	2.5720
(=604)	100.00%	62.96%	83.43%	2.7000
(=620)	100.00%	64.20%	83.98%	2.7921
(=627)	100.00%	65.43%	84.53%	2.8929
(=635)	100.00%	66.67%	85.08%	3.0000
(=637)	100.00%	67.90%	85.64%	3.1154
(=651)	99.00%	69.14%	85.64%	3.2076
(=653)	99.00%	70.37%	85.19%	3.3412
(=674)	98.00%	70.37%	85.64%	3.3075
(=684)	98.00%	71.60%	85.19%	3.4513
(=689)	97.00%	71.60%	85.64%	3.4161
(=691)	97.00%	72.84%	86.19%	3.5714
(=694)	96.00%	72.84%	85.64%	3.5345
(=716)	96.00%	74.07%	86.19%	3.7029
(=726)	96.00%	75.31%	87.29%	4.0006
(=744)	96.00%	77.78%	87.85%	4.3200
(=755)	96.00%	81.48%	89.50%	5.1840
(=763)	96.00%	82.72%	90.06%	5.5441
(=770)	95.00%	82.72%	89.50%	5.4964
(=781)	94.00%	82.72%	88.95%	5.4386
(=795)	93.00%	82.72%	88.40%	5.3807
(=840)	93.00%	83.95%	88.95%	5.7946
(=851)	93.00%	85.19%	89.50%	6.2775
(=855)	92.00%	85.19%	88.95%	6.2100
(=869)	91.00%	85.19%	88.40%	6.1425
(=879)	91.00%	86.42%	88.95%	6.7009
(=894)	91.00%	87.65%	89.50%	7.3716
(=930)	90.00%	87.65%	88.95%	7.2900
(=946)	90.00%	88.89%	89.50%	8.1000
(=957)	89.00%	88.89%	88.95%	8.0137
(=977)	89.00%	90.12%	89.50%	9.0133
(=1024)	88.00%	90.12%	88.95%	8.9300
(=1026)	87.00%	90.12%	88.40%	8.8088
(=1069)	86.00%	90.12%	87.85%	8.7075
(=1072)	86.00%	91.36%	88.40%	9.5152
(=1075)	85.00%	91.36%	87.85%	9.4337
(=1101)	84.00%	91.36%	87.30%	9.7000
(=1112)	83.00%	91.36%	86.74%	9.6043
(=1117)	82.00%	91.36%	86.19%	9.4688
(=1133)	81.00%	91.36%	85.64%	9.3729
(=1134)	80.00%	91.36%	85.08%	9.2571
(=1137)	79.00%	91.36%	84.53%	9.1414
(=1146)	78.00%	91.36%	83.98%	9.0257
(=1153)	77.00%	91.36%	83.43%	8.9100
(=1186)	76.00%	91.36%	82.87%	8.7943
(=1197)	75.00%	91.36%	82.32%	8.6786
(=1209)	75.00%	92.59%	82.87%	10.2250
(=1212)	74.00%	92.59%	82.32%	9.9900
(=1244)	73.00%	92.59%	81.77%	9.6500
(=1261)	73.00%	93.83%	82.32%	11.8260
(=1262)	72.00%	93.83%	81.77%	11.6840
(=1275)	72.00%	95.06%	82.32%	14.5800
(=1283)	71.00%	95.06%	81.77%	14.3775
(=1285)	70.00%	95.06%	81.22%	14.1750
(=1295)	69.00%	95.06%	80.66%	13.9725
(=1319)	68.00%	95.06%	80.11%	13.7700
(=1329)	66.00%	95.06%	79.01%	13.3650
(=1332)	66.00%	96.30%	79.56%	17.8200
(=1341)	65.00%	96.30%	79.01%	17.5500
(=1348)	64.00%	96.30%	78.45%	17.2800
(=1357)	64.00%	97.53%	79.01%	20.3000
(=1415)	63.00%	97.53%	78.45%	21.5100
(=1430)	62.00%	97.53%	77.90%	21.1100
(=1448)	61.00%	97.53%	77.35%	20.7000
(=1468)	60.00%	97.53%	76.80%	20.3000
(=1473)	59.00%	97.53%	76.24%	20.8900
(=1479)	58.00%	97.53%	75.69%	21.4800
(=1485)	57.00%	97.53%	75.14%	22.0700
(=1492)	56.00%	97.53%	74.59%	22.6800
(=1505)	55.00%	97.53%	74.03%	22.2750
(=1514)	54.00%	97.53%	73.48%	21.8700
(=1521)	53.00%	97.53%	72.93%	21.4650
(=1535)	52.00%	97.53%	72.38%	21.0600
(=1531)	51.00%	97.53%	71.82%	20.6550
(=1541)	50.00%	97.53%	71.27%	20.2500
(=1569)	49.00%	97.53%	70.72%	19.8450
(=1567)	48.00%	97.53%	70.17%	19.4400
(=1588)	48.00%	98.77%	70.72%	28.8001
(=1591)	47.00%	98.77%	70.17%	28.0701
(=1594)	46.00%	98.77%	69.61%	27.2601
(=1606)	45.00%	98.77%	69.06%	26.4501
(=1615)	44.00%	98.77%	68.51%	25.6401
(=1625)	43.00%	98.77%	67.96%	24.8301
(=1628)	42.00%	98.77%	67.40%	24.0201
(=1635)	40.00%	98.77%	66.30%	23.4001
(=1640)	39.00%	98.77%	65.75%	22.5901
(=1641)	38.00%	98.77%	65.19%	21.7801
(=1645)	37.00%	98.77%	64.64%	20.9701
(=1656)	36.00%	100.00%	64.64%	0.8480
(=1679)	35.00%	100.00%	64.64%	0.6500
(=1684)	34.00%	100.00%	63.54%	0.6600
(=1689)	33.00%	100.00%	62.98%	0.6700
(=1710)	32.00%	100.00%	62.43%	0.6800
(=1726)	31.00%	100.00%	61.88%	0.6900
(=1737)	29.00%	100.00%	60.77%	0.7100
(=1733)	28.00%	100.00%	60.22%	0.7200
(=1758)	27.00%	100.00%	59.67%	0.7300
(=1760)	25.00%	100.00%	58.66%	0.7500
(=1763)	24.00%	100.00%	58.01%	0.7600
(=1865)	23.00%	100.00%	57.46%	0.7700
(=1764)	22.00%	100.00%	56.91%	0.7800
(=1767)	21.00%	100.00%	56.36%	0.7900
(=1782)	20.00%	100.00%	55.80%	0.8000
(=1789)	19.00%	100.00%	55.25%	0.8100
(=1794)	18.00%	100.00%	54.70%	0.8200
(=1804)	17.00%	100.00%	54.14%	0.8300
(=1805)	16.00%	100.00%	53.59%	0.8500
(=1818)	14.00%	100.00%	52.49%	0.8600
(=1817)	13.00%	100.00%	51.93%	0.8700
(=1819)	12.00%	100.00%	51.38%	0.8800
(=1843)	11.00%	100.00%	50.83%	0.8900
(=1859)	10.00%	100.00%	50.28%	0.9000
(=1870)	9.00%	100.00%	49.72%	0.9100
(=1878)	7.00%	100.00%	48.22%	0.9300
(=1886)	6.00%	100.00%	48.07%	0.9400
(=1892)	5.00%	100.00%	47.51%	0.9500
(=1910)	4.00%	100.00%	46.96%	0.9600
(=1917)	3.00%	100.00%	46.41%	0.9700
(=1942)	2.00%	100.00%	45.86%	0.9800
(=1963)	1.00%	100.00%	45.30%	0.9900
(=1963)	0.00%	100.00%	44.75%	1.0000

ROC - Binomial Exact -
Obs Area Std Err [95% Conf Interval]
181 0.9542 0.0144 0.91477 0.98873

Anti-S

Deviance Residuals:

Min	1Q	Median	3Q	Max
-6.0875	-0.359	0.1374	0.5489	3.6782

Coefficients:

	Estimate	Std.	Error	t	value	Pr(> t)
(Intercept)	6.355241	0.712105	8.925	6.33E-16	***	
age	0.003949	0.007573	0.521	0.60272		
sex	0.003601	0.198135	0.018	0.98552		
LC_Status	0.681862	0.230898	2.953	0.00358	**	
VAD	1.525492	0.128148	11.904	<	2.00E-16	***
BMI	0.025414	0.014638	1.736	0.08433	.	

Anti-S1

Deviance Residuals:

Min	1Q	Median	3Q	Max
-6.1029	-0.6179	0.0336	0.794	3.7732

Coefficients:

	Estimate	Std.	Error	t	value	Pr(> t)
(Intercept)	4.754091	0.808882	5.877	2.09E-08	***	
age	0.000207	0.008602	0.024	0.9808		
sex	-0.05942	0.225062	-0.264	0.7921		
LC_Status	1.425864	0.262278	5.436	1.83E-07	***	
VAD	1.903577	0.145563	13.077	<	2.00E-16	***
BMI	0.040236	0.016628	2.42	0.0166	*	

Anti-RBD

Residuals:

Min	1Q	Median	3Q	Max
-6.0942	-0.6904	0.133	0.82	5.5916

Coefficients:

	Estimate	Std.	Error	t	value	Pr(> t)
(Intercept)	4.702146	0.897883	5.237	4.70E-07	***	
age	0.007358	0.009549	0.771	0.442		
sex	0.059682	0.249826	0.239	0.8115		
LC_Status	0.742004	0.291136	2.549	0.0117	*	
VAD	2.182805	0.161579	13.509	<	2.00E-16	***
BMI	0.039673	0.018457	2.149	0.033	*	

Seropositive Spike Motif KFLPFQQ

Residuals:

Min	1Q	Median	3Q	Max
-14.263	-6.93	-3.938	-0.66	55.635

Coefficients:

	Estimate	Std.	Error	t	value	Pr(> t)
(Intercept)	0.37259	7.96592	0.047	0.96275		
age	-0.07079	0.08471	-0.836	0.40452		
sex	-2.26258	2.21642	-1.021	0.30876		
LC_Status	8.16153	2.58293	3.16	0.00186	**	
VAD	2.61206	1.43352	1.822	0.07016	.	
BMI	0.15053	0.16375	0.919	0.35924		

Seropositive Spike Motif LDKWYF

Residuals:

Min	1Q	Median	3Q	Max
-24.663	-12.466	-6.899	5.72	101.332

Coefficients:

	Estimate	Std.	Error	t	value	Pr(> t)
(Intercept)	-0.54018	11.641	-0.046	0.963		
age	-0.04612	0.1238	-0.373	0.7099		
sex	-3.58417	3.23897	-1.107	0.27		
LC_Status	6.0783	3.77456	1.61	0.1091		
VAD	3.36872	2.09487	1.608	0.1096		
BMI	0.52293	0.2393	2.185	0.0302	*	

Seropositive Spike Motif RDPQILE

Residuals:

Min	1Q	Median	3Q	Max
-8.71	-4.904	-2.189	-0.169	94.84

Coefficients:

	Estimate	Std.	Error	t	value	Pr(> t)
(Intercept)	-3.84559	6.872183	-0.56	0.57648		
age	0.008607	0.073083	0.118	0.90639		
sex	-1.20714	1.912105	-0.631	0.52867		
LC_Status	6.383147	2.228285	2.865	0.00469	**	
VAD	2.028998	1.236691	1.641	0.10268		
BMI	0.073859	0.141268	0.523	0.60176		

Seropositive Spike Motif DISGI

Residuals:

Min	1Q	Median	3Q	Max
-12.264	-6.206	-4.179	-1.252	110.718

Coefficients:

	Estimate	Std.	Error	t	value	Pr(> t)
(Intercept)	-7.55943	9.121243	-0.829	0.4084		
age	-0.00614	0.097001	-0.063	0.9496		
sex	2.036719	2.53788	0.803	0.4233		
LC_Status	5.940301	2.957536	2.009	0.0461	*	
VAD	1.630025	1.641423	0.993	0.3221		
BMI	0.160742	0.187501	0.857	0.3925		

Extended Data Table 5

Generalized linear regression model

zscore_Cortisol ~ 1 + x0_Demographics_Age + x0_Demographics_Sex + x0_Demographics_BMI + x0_Sample_Time_Min + x0_LC_Status + x0_Study_Cohort
Distribution = Normal

Estimated	Coefficients:				
	Estimate	SE	tStat	pValue	
	(Intercept)	1.6342	0.32856	4.9737	1.33E-06
	x0_Demographics_Age	-0.00847	0.002902	-2.9186	0.003882
	x0_Demographics_Sex_2	-0.02619	0.081514	-0.32131	0.74828
	x0_Demographics_BMI	-0.00774	0.005611	-1.3798	0.16905
	x0_Sample_Time_Min	-0.00065	0.000431	-1.5003	0.13498
	x0_LC_Status_1	-1.2198	0.089809	-13.582	6.92E-31
	x0_Study_Cohort_2	0.68657	0.1	6.8658	6.73E-11

226 observations, 219 error degrees of freedom

Estimated Dispersion: 0.304

F-statistic vs. constant model: 44.1, p-value = 4.04E-35

Extended Data Table 6

kappa	kappa_lower_ci	kappa_upper_ci
0.786407767	0.645964174	0.92685136

Extended Data Table 7

ACCELERATED ARTICLE PREVIEW

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | n/a | Confirmed |
|-------------------------------------|--|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of all covariates tested |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

- | | |
|-----------------|--|
| Data collection | All participant survey data were collected and securely stored using REDCap 13.4 (Research Electronic Data Capture) electronic data capture tools hosted within the Mount Sinai Health System. All other de-identified research data were stored securely in password protected internal electronic repositories. All Flow Cytometry data was collected and analyzed using FlowJo software version 10.8 software (BD). |
| Data analysis | All data analysis was performed using MATLAB (2023b), R, and GraphPad Prism (9.8.1). A repository of computer code used for analysis can be found at: https://github.com/rahuldhodapkar/puddlr |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All research data for study participants used in this manuscript are included in Supplementary Table 3. All of the raw fcs files for the flow cytometry analysis are

available at the FlowRepository platform (<http://flowrepository.org/>) under Repository ID: FR-FCM-Z6KL. Accession numbers for protein structure are used UniProt and are as follows: trimeric Spike (PDB: 6VXX) and EBV gH/gL (PDB: 5T1D).

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender	Sex was determined through self-report and review of electronic medical records. No sex disaggregated analysis was performed. Study demographics, including proportion sex by individual study group, are included in Extended Table 1.
Population characteristics	All relevant population demographics are described in Extended Table 1.
Recruitment	Participants with persistent symptoms following acute COVID-19 were recruited from Long COVID clinics within the Mount Sinai Healthcare System and the Center for Post COVID Care at Mount Sinai Hospital. Participants enrolled in healthy and convalescent study arms were recruited via IRB-approved advertisements delivered through email lists, study flyers located in hospital public spaces, and on social media platforms. Informed consent was provided by all participants at the time of enrollment. Individuals in the external Long COVID group ("Ext. LC") were identified from The Winchester Center for Lung Disease's Post-COVID-19 Recovery Program at Yale New Haven Hospital by collaborating clinicians. Recruitment from treatment clinics predisposes this study to a degree of self-selection bias among participants, which was accounted for through demographic matching procedures.
Ethics oversight	This study was approved by the Mount Sinai Program for the Protection of Human Subjects (IRB #20-01758) and Yale Institutional Review Board (IRB #2000029451 for MY-LC; IRB #2000028924 for enrollment of pre-vaccinated Healthy Controls; HIC #2000026109 for External Long COVID). Informed consent was obtained from all enrolled participants.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was not predetermined prior to enrollment of study participants. Samples sizes were chosen based on prior experience with multiplexed immune phenotyping assays and available study resources.
Data exclusions	<p>Data exclusions are stated explicitly in Methods under the heading "MY-LC Study Design, Enrollment Strategy, and Inclusion / Exclusion Criteria " and are reproduced here for convenience: "Inclusion criteria for individuals in the Long COVID group ("LC") were age \geq 18 years; previous confirmed or probable COVID-19 infection (according to World Health Organization guidelines¹); and persistent symptoms > 6 weeks following initial COVID-19 infection. Inclusion criteria for enrollment of individuals in the healthy control group ("HC") were age \geq 18 years, no prior COVID-19 infection, and completion of a brief, semi-structured verbal screening with research staff confirming no active symptomatology. Inclusion criteria for individuals in the convalescent control group ("CC") were age \geq 18 years; previous confirmed or probable prior COVID-19 infection; and completion of a brief, semi-structured verbal screening with research staff confirming no active symptomatology.</p> <p>Pre-specified exclusion criteria for this study were inability to provide informed consent; and any condition preventing a blood test from being performed. Additionally, all participants had electronic health records reviewed by study clinicians following enrollment and were subsequently excluded prior to analyses for the following reasons: (1) current pregnancy, (2) immunosuppression equivalent to or exceeding prednisone 5 mg daily, (3) active malignancy or chemotherapy, and (4) any monogenic disorders. For specific immunological analyses, pre-existing medical conditions were additionally excluded prior to analyses due to high potential for confounding (e.g., participants with hypothyroidism were excluded prior to analysis of circulating T3/T4 levels; participants with pituitary adenomas were excluded prior to analysis of cortisol levels). Specific exclusions are marked by "Δ" in figures and detailed in relevant legends."</p>
Replication	Each participant plasma and PBMC sample was partitioned into aliquots for use in various assays. Technical replicates were performed on patient samples where sample volume limitations permitted. When performed (e.g. ELISA, qPCR), technical replicates were successful.
Randomization	Randomization was not applicable to this study as it is a cross-sectional, observational human research study of a pre-existing medical condition.
Blinding	Blinding of study investigators was not performed due to pre-existing intrinsic knowledge of clinical condition / study groups by both participants and investigators, as well as necessary logistical accommodations for scheduling of sample draws by study participants.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	<i>Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).</i>
Research sample	<i>State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.</i>
Sampling strategy	<i>Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.</i>
Data collection	<i>Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.</i>
Timing	<i>Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.</i>
Data exclusions	<i>If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.</i>
Non-participation	<i>State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.</i>
Randomization	<i>If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.</i>

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	<i>Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.</i>
Research sample	<i>Describe the research sample (e.g. a group of tagged <i>Passer domesticus</i>, all <i>Stenocereus thurberi</i> within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.</i>
Sampling strategy	<i>Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.</i>
Data collection	<i>Describe the data collection procedure, including who recorded the data and how.</i>
Timing and spatial scale	<i>Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken</i>
Data exclusions	<i>If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.</i>
Reproducibility	<i>Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.</i>
Randomization	<i>Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.</i>
Blinding	<i>Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.</i>

Did the study involve field work? Yes No

Field work, collection and transport

Field conditions	<i>Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).</i>
Location	<i>State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).</i>
Access & import/export	<i>Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).</i>
Disturbance	<i>Describe any disturbance caused by the study and how it was minimized.</i>

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Included in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

Methods

n/a	Included in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	All antibodies, dilutions, and catalog numbers are used in this manuscript are detailed in Supplementary Table 1.
Validation	All antibodies used in this study are commercially available, and all have been validated by the manufacturers and used by other publications. Likewise, we titrated these antibodies according to our own staining conditions. The following were validated in the following species: BB515 anti-hHLA-DR (G46-6) (BD Biosciences) (Human, Rhesus, Cynomolgus, Baboon), BV785 anti-hCD16 (3G8) (BioLegend) (Human, African Green, Baboon, Capuchin Monkey, Chimpanzee, Cynomolgus, Marmoset, Pigtailed Macaque, Rhesus, Sooty Mangabey, Squirrel Monkey), PE-Cy7 anti-hCD14 (HCD14) (BioLegend) (Human), BV605 anti-hCD3 (UCHT1) (BioLegend) (Human, Chimpanzee), BV711 anti-hCD19 (SJ25C1) (BD Biosciences) (Human), AlexaFluor647 anti-hCD1c (L161) (BioLegend) (Human, African Green, Baboon, Cynomolgus, Rhesus), Biotin anti-hCD141 (M80) (BioLegend) (Human, African Green, Baboon), PE-Dazzle594 anti-hCD56 (HCD56) (BioLegend) (Human, African Green, Baboon, Cynomolgus, Rhesus), PE anti-hCD304 (12C2) (BioLegend) (Human), APCFire750 anti-hCD11b (ICRF44) (BioLegend) (Human, African Green, Baboon, Chimpanzee, Common Marmoset, Cynomolgus, Rhesus, Swine), PerCP/Cy5.5 anti-hCD66b (G10F5) (BD Biosciences) (Human), BV421 anti-CD15 (W6D3) (BioLegend) (Human), BV785 anti-hCD4 (SK3) (BioLegend) (Human), APCFire750 or BV711 anti-hCD8 (SK1) (BioLegend) (Human, Cross-Reactivity: African Green, Chimpanzee, Cynomolgus, Pigtailed Macaque, Rhesus, Sooty Mangabey), BV421 anti-hCCR7 (G043H7) (BioLegend) (Human, African Green, Baboon, Cynomolgus, Rhesus), AlexaFluor 700 anti-hCD45RA (HI100) (BD Biosciences) (Human), PE anti-hPD1 (EH12.2H7) (BioLegend) (Human, African Green, Baboon, Chimpanzee, Common Marmoset, Cynomolgus, Rhesus, Squirrel Monkey), APC anti-hTIM3 (F38-2E2) (BioLegend) (Human), BV711 anti-hCD38 (HIT2) (BioLegend) (Human, Chimpanzee, Horse), BB700 anti-hCXCR5 (RF8B2) (BD Biosciences) (Human), PE-Cy7 anti-hCD127 (HIL-7R-M21) (BioLegend) (Human), PE-CF594 anti-hCD25 (BC96) (BD Biosciences) (Human, Rhesus, Cynomolgus, Baboon), BV421 anti-hIL-17a (N49-653) (BD Biosciences) (Human), AlexaFluor 700 anti-hTNFa (MAB11) (BioLegend) (Human, Cat, Cross-Reactivity: Chimpanzee, Baboon, Cynomolgus, Rhesus, Pigtailed Macaque, Sooty Mangabey, Swine), APC/Fire750 anti-hIFNy (4S.B3) (BioLegend) (Human, Cross-Reactivity: Chimpanzee, Baboon, Cynomolgus, Rhesus), FITC anti-hGranzymeB (GB11) (BioLegend) (Human, Mouse, Cross-Reactivity: Rat), AlexaFluor 647 anti-hIL-4 (8D4-8) (BioLegend) (Human, Cross-Reactivity: Chimpanzee, Baboon, Cynomolgus, Rhesus), BB700 anti-hCD183/CXCR3 (1C6/CXCR3) (BD Biosciences) (Human, Rhesus, Cynomolgus, Baboon), PE-Cy7 anti-IL-6 (MQ2-13A5) (BioLegend) (Human), PE anti-hIL-2 (5344.111) (BD Biosciences) (Human), BV785 anti-hCD19 (SJ25C1) (BioLegend) (Human), BV421 anti-hCD138 (MI15) (BioLegend) (Human), AlexaFluor700 anti-hCD20 (2H7) (BioLegend) (Human, Baboon, Capuchin Monkey, Chimpanzee, Cynomolgus, Pigtailed Macaque, Rhesus, Squirrel Monkey), AlexaFluor 647 anti-hCD27 (M-T271) (BioLegend) (Human, Cross-Reactivity: Baboon, Cynomolgus, Rhesus), PE/Dazzle594 anti-hIgD (IA6-2) (BioLegend) (Human), PE-Cy7 anti-hCD86 (IT2.2) (BioLegend) (Human, African Green, Baboon, Capuchin Monkey, Common Marmoset, Cotton-topped Tamarin, Chimpanzee, Cynomolgus, Rhesus), APC/Fire750 anti-hIgM (MHM-88) (BioLegend) (Human, African Green, Baboon, Cynomolgus, Rhesus), BV605 anti-hCD24 (ML5) (BioLegend) (Human, Cross-Reactivity: Chimpanzee), AlexaFluor 700 Streptavidin (1:300) (ThermoFisher).

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.
Authentication	Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.
Mycoplasma contamination	Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Palaeontology and Archaeology

Specimen provenance	Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.
Specimen deposition	Indicate where the specimens have been deposited to permit free access by other researchers.
Dating methods	If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.
<input type="checkbox"/>	Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.
Ethics oversight	Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other research organisms

Policy information about [studies involving animals; ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	For laboratory animals, report species, strain and age OR state that the study did not involve laboratory animals.
Wild animals	Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.
Reporting on sex	Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.
Field-collected samples	For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.
Ethics oversight	Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.
Study protocol	Note where the full trial protocol can be accessed OR if not available, explain why.
Data collection	Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

- | No | Yes |
|-------------------------------------|---|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Public health |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> National security |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Crops and/or livestock |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Ecosystems |
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Experiments of concern

Does the work involve any of these experiments of concern:

- | No | Yes |
|-------------------------------------|--|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Demonstrate how to render a vaccine ineffective |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Confer resistance to therapeutically useful antibiotics or antiviral agents |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Enhance the virulence of a pathogen or render a nonpathogen virulent |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Increase transmissibility of a pathogen |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Alter the host range of a pathogen |
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| <input checked="" type="checkbox"/> | <input type="checkbox"/> Enable the weaponization of a biological agent or toxin |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Any other potentially harmful combination of experiments and agents |

ChIP-seq

Data deposition

- Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission

Provide a list of all files available in the database submission.

Genome browser session (e.g. [UCSC](#))

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates

Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth

Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters

Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.

Data quality

Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software

Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Freshly isolated PBMCs were plated at $1-2 \times 10^6$ cells per well in a 96-well U-bottom plate. Cells were resuspended in Live/Dead Fixable Aqua (ThermoFisher) for 20 min at 4°C. Cells were washed with PBS and followed by Human TruStain FcX (BioLegend) incubation for 10 min at RT. Cocktails of staining antibodies were added directly to this mixture for 30 minutes at RT. Prior to analysis, cells were washed and resuspended in 100 μ l 4% PFA for 30 min at 4°C. For intracellular cytokine staining following stimulation, the surface marker-stained cells were resuspended in 200 μ l cRPMI (RPMI-1640 supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin, 1 mM sodium pyruvate) and stored at 4°C overnight. Subsequently, these cells were washed and stimulated with 1 \times Cell Stimulation Cocktail (eBioscience) in 200 μ l cRPMI for 1 h at 37°C. Fifty μ l of 5 \times Stimulation Cocktail in cRPMI (plus protein transport 442 inhibitor, eBioscience) was added for an additional 4 hours of incubation at 37°C. Following stimulation, cells were washed and resuspended in 100 μ l 4% paraformaldehyde for 30 min at 4°C. To quantify intracellular cytokines, cells were permeabilized with 1 \times permeabilization buffer from the FOXP3/Transcription Factor Staining Buffer Set (eBioscience) for 10 min at 4°C. All subsequent staining cocktails were made in this buffer. Permeabilized cells were then washed and resuspended in a cocktail containing Human TruStain FcX (BioLegend) for 10 min at 4°C. Finally, intracellular staining cocktails were added directly to each sample for 1 h at 4°C. Following this incubation, cells were washed and prepared for analysis on an Attune NXT (ThermoFisher).

Instrument

Attune NXT (ThermoFisher)

Software

Data were analyzed using FlowJo software version 10.8 software (BD).

Cell population abundance

No sorting of PBMC fractions was performed in this study.

Gating strategy

Gating Strategy is described in Extended Figure S10

- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI

Used

Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction,

Preprocessing software	<i>segmentation, smoothing kernel size, etc.).</i>
Normalization	<i>If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.</i>
Normalization template	<i>Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.</i>
Noise and artifact removal	<i>Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).</i>
Volume censoring	<i>Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.</i>

Statistical modeling & inference

Model type and settings	<i>Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).</i>
Effect(s) tested	<i>Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.</i>
Specify type of analysis:	<input type="checkbox"/> Whole brain <input type="checkbox"/> ROI-based <input type="checkbox"/> Both
Statistic type for inference (See Eklund et al. 2016)	<i>Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.</i>
Correction	<i>Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).</i>

Models & analysis

n/a	Involvement in the study
<input type="checkbox"/>	<input type="checkbox"/> Functional and/or effective connectivity
<input type="checkbox"/>	<input type="checkbox"/> Graph analysis
<input type="checkbox"/>	<input type="checkbox"/> Multivariate modeling or predictive analysis
Functional and/or effective connectivity	<i>Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).</i>
Graph analysis	<i>Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).</i>
Multivariate modeling and predictive analysis	<i>Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.</i>