Supplementary Material

Sneller MC, Liang CJ, Marques AR, et al. A longitudinal study of COVID-19 sequelae and immunity: baseline findings. Ann Intern Med. 24 May 2022. [Epub ahead of print]. doi:10.7326/M21-4905

Contents

This supplementary material was provided by the authors to give readers further details on their article. The material was not copyedited.

1.0 SUPPLEMENTARY METHODS

1.1 Statistical Analysis

In Tables 2 and 3 in the main text, unadjusted comparisons of continuous variables were made using the t-test, while adjusted comparisons were made using multivariate linear regression by including the adjustment variables as predictors in the multivariate model. Unadjusted comparisons of binary variables were made using Fisher's exact test, while adjusted comparisons were made using multivariate logistic regression by including the adjustment variables as predictors in the multivariate model.

In Figure 1 in the main text, the associations between predictors and presence of PASC at baseline were quantified using Fisher's exact test for binary predictors and univariate logistic regression for continuous predictors.

In Figure 2 in the main text, panels A and B represent the significant analyses from the metacluster and inflammatory biomarker analyses (Fig. 4, 5, and 6C in this supplement), which were done using the Wilcoxon rank-sum test. Panel C was originally planned as an exploratory analysis and was not adjusted for multiplicity but is nonetheless included in the main manuscript due to heightened interest in its results at this point in the pandemic. Comparisons in panel C were made using the t-test.

The Benjamini-Hochberg procedure for controlling the expected false discovery rate at 10% was used to determine which results were significant. The procedure was done separately for three distinct analysis groups defined below. The 46 statistical tests conducted in Tables 2 and 3 in the main text were considered one group of tests; the highest significant p-value was 0.046 and the lowest insignificant p-value was 0.058. The 32 statistical tests

conducted in Figure 1 in the main text were considered a second group of tests; the highest significant p-value was 0.005 and the lowest insignificant p-value was 0.07. The significant predictors were female gender, anxiety, MCS, PCS, and GAD-2. The 28 statistical tests comparing inflammatory biomarkers (Fig. 2A in the main text; Fig. 4 in this supplement), meta-clusters (Fig. 2B in the main text; Fig. 6C in this supplement), and lymphocyte populations (Fig. 5 in this supplement) between survivors and controls were considered a third group of tests; the highest significant p-value was 0.002 and the lowest insignificant p-value was 0.031. Only markers with significant differences were presented as figures in the main text (Fig. 2A and 2B in the main text); comparisons between PASC and no PASC groups were exploratory and were not adjusted for multiplicity. The association between time and binding inhibition percentage was quantified using Spearman's correlation. Exploratory analyses included any analyses not in the three groups defined above, were not adjusted for multiplicity, and should be interpreted cautiously. All p-values are two-sided. Adjustment variables were specified prior to analysis based on a subjective synthesis of literature review and clinical experience. Missing data was minimal, the extent of which was reported in Table legends, and assumed to be missing completely at random. All analyses were performed using R software, version 4.1.1 (R Foundation for Statistical Computing). We used fisher.exact for Fisher's exact test, wilcox.test for the Wilcoxon rank-sum test, t.test for the t-test, cor.test and the bootstrap for Spearman's correlation, glm for multivariate regressions, and p.adjust with method="BH" to calculate FDR-adjusted p-values.

To facilitate interpretability and at the request of a reviewer, we have provided versions of Table 2 and Table 3 in the main text that have replaced odds ratios with differences in proportions (Tables 3 and 4 in this supplement). Additionally, we have provided tables listing all

the raw p-values and corresponding FDR-adjusted p-values that were used in the three separate applications of the Benjamin-Hochberg procedure. As we set the expected false discovery rate at 10%, tests corresponding to adjusted p-values of less than 0.10 are considered significant. The original protocol anticipated an approximately equal number of survivors and controls. Based on this assumption, for a binary outcome with 5% incidence in the control group, using a univariate logistic regression with 200 survivors and 200 controls would allow for detecting a relative risk of 2.6 with 80% power at two-sided 0.05 significance. The table outlining other scenarios that was used in the original protocol is reproduced below.

Relative risk of outcome (e.g. sequelae, symptom, immunological endpoint) that can be detected with 80% power at 0.05 significance (2-sided) when comparing COVID-19 survivor group to control group					
Expected incidence in control group	N=100 per group	N=200 per group	N=300 per group	N=400 per group	N=500 per group
1%	9.0	5.9	4.7	4.1	3.7
2%	5.8	4.0	3.3	2.9	2.7
5%	3.4	2.6	2.3	2.1	1.9
10%	2.4	2.0	1.8	1.7	1.5
20%	1.9	1.6	1.5	1.4	1.4

1.2 Serology, Immunologic, and Autoantibody Testing

Antibody to SARS-CoV-2 nucleocapsid protein were determined using the Bio-Rad Platelia™ assay. The Platelia SARS-CoV-2 Total Ab is a one-step antigen capture format Enzyme-Linked Immunosorbent Assay (ELISA) for qualitative detection of total anti-SARS-CoV-2 nucleocapsid antibodies (IgM/IgA/IgG) in human serum or plasma specimens. The assay uses a recombinant SARS nucleocapsid protein in a one-step antigen capture format assay. Levels of SARS-Co-V2 neutralizing antibody were evaluated using the GenScript™ surrogate virus neutralization assay[1]. A digital immunoassay (Simoa NF-light™) was used for quantitative

determination of neurofilament light chain in plasma[2]. Plasma samples were tested for SARS-CoV-2 nucleocapsid protein using a highly sensitive single molecule array immunoassay (Simoa® SARS-CoV-2 N Protein Antigen Test)[3]. All tests were done according to manufacturer's instructions.

Rheumatoid factor testing was done by an immunoturbidimetric assay at the National Institutes of Health Clinical Laboratory, Bethesda, MD. Anti-nuclear antibody testing was done by enzyme-linked immunoassay using Hep-2 nuclear extract supplemented with purified antigens. Anti-cardiolipin antibody testing, IgM, IgG, was performed using the QUANTA Lite sPS/PT IgM, IgG enzyme-linked immunosorbent assay. Both anti-nuclear antibody and anti-cardiolipin antibody testing was performed at the Mayo Clinic Laboratories, Rochester, MN.

Plasma inflammatory biomarker analysis

Levels of inflammatory biomarkers in plasma were determined using the ELLA platform (ProteinSimple) and performed according to manufacturer's instructions.

High-dimensional analysis of flow cytometry data

High-dimensional flow cytometry was conducted in order to examine differentially expressed immune markers among study groups as previously described[4]. Opt-SNE and FlowSOM analyses were conducted using OMIQ platform (www.omiq.ai). Opt-SNE analysis was performed using equal sampling of 10,000 $CD3^+$ T cells from each FCS file, with 1,000 iterations, a perplexity of 30, and a theta of 0.5. The following markers were used to generate opt-SNE maps: CD4, CD8, CD45RO, CD27, CD25, CD38, and HLA-DR. Resulting opt-SNE maps were used for the FlowSOM algorithm. The self-organizing map (SOM) was generated

using hierarchical consensus clustering and 15 meta-clusters were identified. Heatmap displaying column-scaled z-scores of mean fluorescent intensity (MFI) for individual FlowSOM clusters was generated using OMIQ platform.

1.3 Diagnostic Testing

The pulmonary function tests were collected on Vyaire testing systems (Vyaire Medical, Irvine, CA) in accordance with ATS-ERS standards[5-7]. The GLI reference set was used to determine the percent predicted values[8-10] . Values were considered abnormal if they fell below the lower limits of normal[11]. The 6MWTs were done on a 30-meter indoor course and administered per ATS-ERS standards[12].

Echocardiography was performed and analyzed using commercially available systems and measurements reported in accordance with American Society of Echocardiography guidelines[13] .

1.4 PASC-Specific Symptoms

COVID HISTORY

Date of onset of initial COVID-19 symptoms:	|___|___|/|___|___|/|___|___|___|___| ☐ NA (no symptoms)

COVID Symptoms

Symptom	Yes	No	Not assessed	If yes, Date of onset
Palpitations	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
Tinnitus	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
Heartburn	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
Fatigue	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
Chest pain/discomfort	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
Myalgia (muscle pain)	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
Arthralgia (joint pain)	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
Decreased appetite	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
Headache	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
Concentration impairment	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
Memory impairment	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
Taste alteration	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
Loss of smell	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
Anxiety	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
Insomnia	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
Shortness of breath	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
Alopecia	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|
	☐	☐	☐	☐ same as above or |___|___|/|___|___|/|___|___|___|___|

2.0 SUPPLEMENTARY RESULTS

2.1 Physical Examination Findings

The most common abnormal heart findings in the COVID-19 and control groups respectively were cardiac murmur (1.6% vs. 0.8%) and irregular pulse (1.0% vs. 0.8%), the most common abnormal neurologic finding in the COVID-19 and control groups respectively was unilateral decreased vibratory sensation in a distal extremity (1.0% vs. 0.8%). A single participant in the COVID-19 group had minimal bilateral cervical lymphadenopathy. No participant in either group had abnormal findings on lung auscultation. Three participants had symptoms of palpitations and lightheadedness that worsened with standing. None of these participants exhibited an excessive rise (greater than 30 beats per minute) in resting heart rate or change in systolic blood pressure within 10 minutes of standing.

2.2 Additional Diagnostic Findings

All participants in the COVID-19 group had a chest radiograph at the baseline visit. Twenty-six of the 189 COVID-19 participants had an abnormal chest radiograph. The most common abnormality was small calcified pulmonary nodules (13 participants) consistent with healed granulomatous disease. The remaining 13 participants had mild linear markings suggestive of atelectasis. Of these 13 participants, only 3 had an abnormal pulmonary function test: mild/moderate restriction (2 participants) and mild diffusion defect (1 participant).

All patients with symptomatic fatigue were screened for hypothyroidism with plasma thyroid stimulating hormone (TSH) levels. Only a single participant with fatigue had an elevated TSH. This participant had known hypothyroidism and was receiving inadequate replacement

therapy. Of the 22 participants with headaches, 11 declined brain magnetic resonance imaging (MRI). The remaining 11 participants underwent brain MRI with contrast, which in all cases did not reveal any pathologic findings (such as mass lesions or meningeal enhancement) that would explain their symptoms. Of the 10 participants with palpitations, 7 agreed to undergo 48-hour Holter monitoring. In all 7, the predominant rhythm was sinus, with no pathologic arrythmias noted.

3.0 SUPPLEMENTARY TABLES

Table 1. Selected Laboratory Results		
Test	**Controls (n=120)**	**Total COVID-19 Cohort (n=189)**
no. (%)		
Hemoglobin level below lower limit of normal	7 (6.2)	8 (4.2)
median [IQR]		
Estimated GFR ml/min/1.73 sq.m	104 [90, 120]	93 [84, 106]
Total Lymphocytes (cells/microliter)	1775 [1355, 2133]	1810 [1480, 2150]
IgG (total; mg/dL)	1022 [919, 1199]	1100 [923, 1242]
Albumin (g/dL)	4.3 [4.2, 4.5]	4.4 [4.2, 4.6]
Alkaline Phosphatase (U/L)	69 [55, 82.5]	66.5 [53, 84]
Alanine Aminotransferase (U/L)	18 [14, 27.2]	19 [14, 26.2]
Bilirubin (mg/dL)	0.2 [0.1, 0.3]	0.2 [0.1, 0.2]

Table 2. Pulmonary Function Testing and Echocardiography Results			
Pulmonary Function Testing	**no. (%)**	**Controls (n=120)**	**Total COVID-19 Cohort (n=188)**
Abnormal Pulmonary Function Test		26 (22)	32 (17)
Isolated diffusion defect		16 (13)	15 (8)
Mild		14	15
Moderate		2	0
Isolated restriction		1 (1)	6 (3)
Mild		1	6
Isolated obstruction		6 (5)	4 (2)
Mild		6	2
Moderate		0	2
Other		3 (3)	7 (4)
Mild Restriction; mild/moderate diffusion defect		1	4
Mild obstruction; mild diffusion defect		1	1
Moderate obstruction; mild diffusion defect		1	2
Echocardiogram	**no. (%)**	**n=119**	**n=174**
Abnormal Echocardiogram		22 (18)	30 (17)
Chamber enlargement		14 (12)	21 (12)
Mild		12	15
Moderate		1	5
Severe		1	1
Valvular abnormality		5 (4)	10 (6)
Mild		4	5
Moderate		1	4
Severe		0	1
Decreased Ejection Fraction (mild)		1 (1)	2 (1)
Other		2 (2)	5 (3)
Increased right ventricular pressure		0	1
Septal hypertrophy		0	1
Mild aortic root dilation		2	3

Table 3. Selected Symptoms, Physical Findings, Questionnaires, and Cognitive Testing Results*

	Controls (n=120)	Total COVID-19 Cohort (n=189)	Mean Difference (95% CI)*†	p-value
Selected symptoms - no. (%)				
Fatigue	0 (0)	50 (26)	0.26 (0.19, 0.33)	<0.001
Dyspnea	0 (0)	35 (19)	0.19 (0.12, 0.25)	<0.001
Anosmia/Parosmia	0 (0)	26 (14)	0.14 (0.08, 0.19)	<0.001
Concentration impairment	0 (0)	23 (12)	0.12 (0.07, 0.18)	<0.001
Headache	0 (0)	22 (12)	0.12 (0.06, 0.17)	<0.001
Memory impairment	0 (0)	18 (10)	0.10 (0.05, 0.14)	0.001
Insomnia	0 (0)	17 (9)	0.09 (0.04, 0.14)	0.002
Chest Pain/Discomfort	0 (0)	16 (8)	0.08 (0.04, 0.13)	0.003
Anxiety	1 (1)	11 (6)	0.05 (0.01, 0.09)	0.056
Myalgia	1 (1)	11 (6)	0.05 (0.01, 0.09)	0.056
Tinnitus	0 (0)	11 (6)	0.06 (0.02, 0.10)	0.017
Palpitations	0 (0)	10 (5)	0.05 (0.01, 0.09)	0.026
Arthralgia	2 (2)	6 (3)	0.02 (-0.03, 0.06)	0.66
Cough	0 (0)	9 (5)	0.05 (0.01, 0.08)	0.038
Taste disorder	0 (0)	9 (5)	0.05 (0.01, 0.08)	0.038
Depression	2 (2)	6 (3)	0.02 (-0.03, 0.06)	0.66
Alopecia	0 (0)	8 (4)	0.04 (0.01, 0.08)	0.055
Dizziness	0 (0)	7 (4)	0.04 (0.00, 0.07)	0.082
Dyspepsia	0 (0)	5 (3)	0.03 (0.00, 0.06)	0.182
Decreased appetite	0 (0)	5 (3)	0.03 (0.00, 0.06)	0.182
Nasal congestion	0 (0)	3 (2)	0.02 (-0.01, 0.04)	0.43
Nausea	0 (0)	3 (2)	0.02 (-0.01, 0.04)	0.43
Visual impairment	0 (0)	2 (1)	0.01 (-0.01, 0.03)	0.69
Paresthesia	0 (0)	2 (1)	0.01 (-0.01, 0.03)	0.69
Selected abnormal physical findings - no. (%)				
Neurologic	2 (2)	2 (1)	-0.01 (-0.04, 0.03)	1
Lung	0 (0)	0 (0)	NA	NA
Musculoskeletal	1 (1)	16 (8)	0.08 (0.03, 0.13)	0.009
Heart	2 (2)	7 (4)	0.02 (-0.02, 0.06)	0.49
Lymphatic	0 (0)	1 (1)	0.01 (-0.01, 0.02)	1
Questionnaires‡				
SF-36 PCS Median [IQR]	58 [55, 60]	52 [45, 58]	-6.9 (-8.7, -5.1)	<0.001
SF-36 MCS Median [IQR]	54 [48, 57]	51 [41, 56]	-3.9 (-6.0, -1.8)	<0.001

GAD-2 Score >= 3: no. (%)	3 (3)	24 (14)	0.12 (0.05, 0.19)	0.003
PHQ-2 Score >= 3: no. (%)	4 (4)	18 (11)	0.07 (0.01, 0.14)	0.053

* CI confidence interval, IQR interquartile range, N/A not applicable.

† All results are compared using mean differences, so comparisons of binary results are differences in proportions. Mean difference greater than 0 indicates higher mean in the COVID-19 cohort.

‡ Short Form-36 version 2 health survey, PCS physical component score, MCS mental component score, GAD-2 generalized anxiety disorder 2 item, PHQ-2 patient health 2 item. 110 Controls and 166 COVID-19 participants had questionnaire scores. The SF-36 scores were compared using the difference in means; GAD-2 and PHQ-2 were compared using odds ratios.

Table 4. Selected Laboratory and Diagnostic Testing Results*					
		Controls (n=120)	Total COVID-19 Cohort (n=189)	Mean Difference (95% CI)	p-value
	no. (%)				
Troponin I ≥ 0.03 mcg/L		1 (0.8)	2 (1)	0.00 (-0.02, 0.03)	0.81
Anti-cardiolipin antibody detected		13 (11)	10 (5)	-0.06 (-0.12, 0.00)	0.071
Anti-nuclear antibody detected		7 (6)	11 (6)	0.00 (-0.05, 0.05)	0.96
Rheumatoid factor detected		7 (6)	7 (4)	-0.02 (-0.07, 0.03)	0.39
	median [IQR]				
Pro-Brain natriuretic peptide pg/ml		34 [18, 59]	33 [17, 65]	7.3 (-9.1, 23.7)	0.39
C-reactive protein mg/L		1 [0.5, 2.8]	1.4 [0.6, 2.9]	-0.08 (-1.01, 0.85)	0.87
Neurofilament light chain pg/ml (plasma)		11.6 [8.1, 16.0]	11.1 [8.4, 15.3]	0.03 (-1.24, 1.30)	0.96
D-dimer mg/L		0.14 [0.14, 0.36]	0.14 [0.14, 0.35]	0.02 (-0.04, 0.08)	0.56
NIH Toolbox Processing Speed: Median [IQR]†		53 [44, 60]	50 [37, 59]	-3.0 (-6.2, 0.1)	0.058
NIH Toolbox Episodic Memory: Median [IQR]†		51 [45, 59]	51 [42, 57]	-0.7 (-3.2, 1.8)	0.57
NIH Toolbox Executive Functioning: Median [IQR]†		52 [44, 61]	52 [43, 62]	-0.3 (-3.0, 2.4)	0.84
Pulmonary Function Testing	no. (%)	(n=120)	(n=188)		
Abnormal Pulmonary Function Test		26 (22)	32 (17)	-0.05 (-0.14, 0.04)	0.28
Meters walked in 6 Minutes: Median [IQR]‡		595 [531, 634]	560 [511, 617]	-24 (-41, -7)	0.006
Echocardiogram	no. (%)	n=119	n=174		
Abnormal Echocardiogram		22 (18)	30 (17)	0.92 (0.50, 1.71)	0.80

* All estimates are adjusted for age and gender; abnormal pulmonary function test also adjusted for preexisting asthma; meters walked in 6 minutes also adjusted for the preexisting conditions of diabetes and hypertension; abnormal echocardiogram also adjusted for the preexisting conditions of diabetes and hypertension. Mean difference greater than 0 indicates higher values in COVID-19 cohort.

† 119 Controls and 188 Survivors had NIH Toolbox Processing Speed and NIH Toolbox Executive Functioning scores; 118 Controls and 188 Survivors had NIH Toolbox Episodic Memory scores. NIH Toolbox scores were compared using the difference in means.

‡ Meters walked in 6 minutes was recorded for 119 controls and 187 survivors

Table 5. Raw and FDR-Adjusted P-Values From Tables 2 and 3 in the Main Text		
Selected symptoms	**p-value**	**FDR-adjusted p-value**
Fatigue	<0.001	<0.001
Dyspnea	<0.001	<0.001
Anosmia/Parosmia	<0.001	<0.001
Concentration impairment	<0.001	<0.001
Headache	<0.001	<0.001
Memory impairment	<0.001	0.001
Insomnia	<0.001	0.001
Chest Pain/Discomfort	<0.001	0.002
Anxiety	0.033	0.075
Myalgia	0.033	0.075
Tinnitus	0.008	0.025
Palpitations	0.008	0.025
Arthralgia	0.491	0.66
Cough	0.014	0.037
Taste disorder	0.014	0.037
Depression	0.49	0.66
Alopecia	0.025	0.064
Dizziness	0.046	0.096
Dyspepsia	0.161	0.28
Decreased appetite	0.161	0.28
Nasal congestion	0.29	0.45
Nausea	0.29	0.45
Visual impairment	0.52	0.67
Paresthesia	0.52	0.67
Selected abnormal physical findings		
Neurologic	0.64	0.74
Lung	NA	NA
Musculoskeletal	0.004	0.014
Heart	0.49	0.66
Lymphatic	1.00	1.00
Questionnaires		
SF-36 PCS	<0.001	<0.001
SF-36 MCS	<0.001	0.001
GAD-2 Score >= 3	<0.001	0.004
PHQ-2 Score >= 3	0.040	0.087
Selected Laboratory and Diagnostic Testing Results		
Troponin I > 0.03 mcg/L	0.82	0.90
Anti-cardiolipin antibody detected	0.075	0.144
Anti-nuclear antibody detected	0.94	0.98
Rheumatoid factor detected	0.39	0.57
Pro-Brain natriuretic peptide pg/ml	0.39	0.57
C-reactive protein mg/L	0.62	0.73
Neurofilament light chain pg/ml (plasma)	0.96	0.98
D-dimer mg/L	0.56	0.69
NIH Toolbox Processing Speed	0.058	0.117
NIH Toolbox Episodic Memory	0.57	0.69
NIH Toolbox Executive Functioning	0.84	0.90
Abnormal Pulmonary Function Test	0.27	0.45
Meters walked in 6 Minutes	0.006	0.021
Abnormal Echocardiogram	0.80	0.90

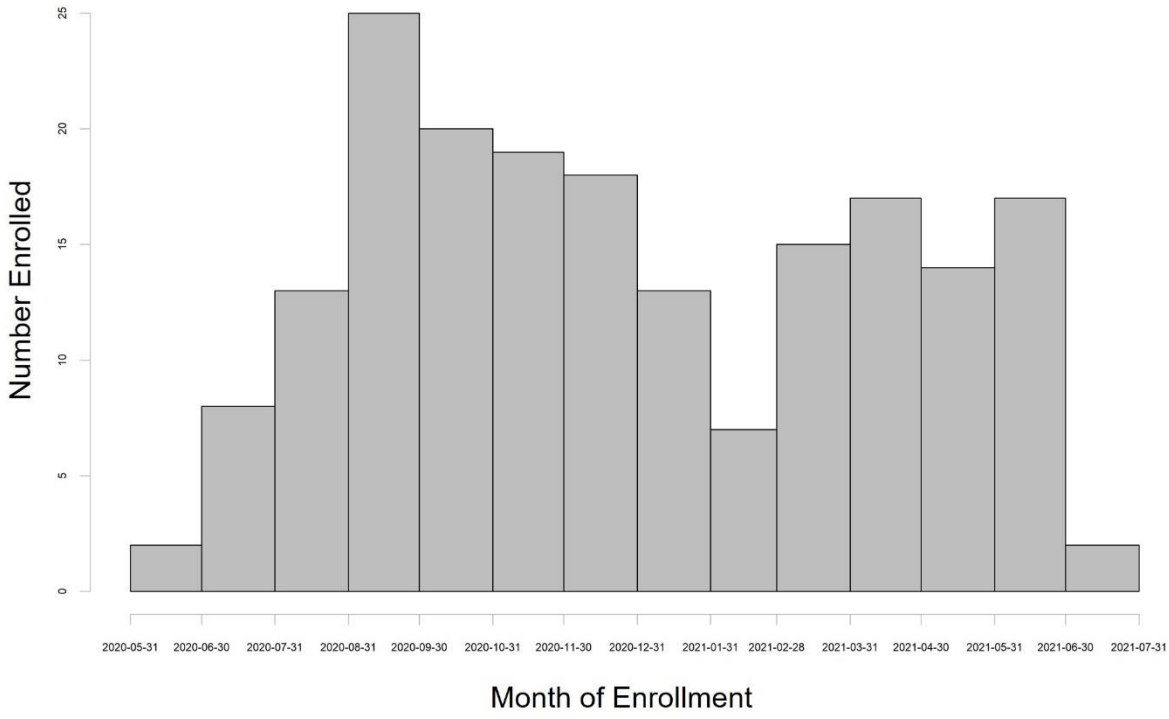
Table 6. Raw and FDR-Adjusted P-Values From Figure 1 in the Main Text		
	p-value	**FDR-adjusted p-value**
Risk Factor		
Age (per 10 yr increase)	0.50	0.67
Female gender	0.005	0.033
Race (ref: White)		
Asian	0.46	0.65
Black	0.45	0.65
Hispanic or Latino	0.085	0.34
Obese	0.071	0.34
Smoking	1.00	1.00
Diabetes	0.115	0.37
Hypertension	0.72	0.84
Asthma	0.27	0.46
Any mental health problem	0.139	0.37
Anxiety disorder	0.003	0.027
Mood disorder	0.22	0.44
Other	0.79	0.84
Any cardiac problem	0.41	0.65
Hospitalized	0.65	0.80
Supplemental O2	0.77	0.84
Diagnostic Testing Result		
PFT abnormal	0.44	0.65
Echocardiogram abnormal	0.076	0.34
NF-L (per pg/mL increase)	0.126	0.37
6 MWT (per 100m decrease)	0.26	0.46
eGFR (per 10 pt decrease	0.76	0.84
Pro BNP (per 2x increase)	0.93	0.96
CRP (per 2x increase)	0.23	0.44
D-dimer (per 2x increase)	0.20	0.44
NIH Toolbox		
Processing Speed (per 5 pt decrease)	0.22	0.44
Episodic Memory (per 5 pt decrease)	0.112	0.37
Executive Function (per 5 pt decrease)	0.65	0.80
Health Survey Score		
MCS (per 5 pt decrease)	<0.001	0.007
PCS (per 5 pt decrease)	<0.001	<0.001
PHQ-2 (>=3)	0.22	0.44
GAD-2 (>=3)	<0.001	0.004

Table 7. Raw and FDR-Adjusted P-Values From Figures 2A and 2B in the Main Text and Figures 4, 5, and 6C in This Supplement

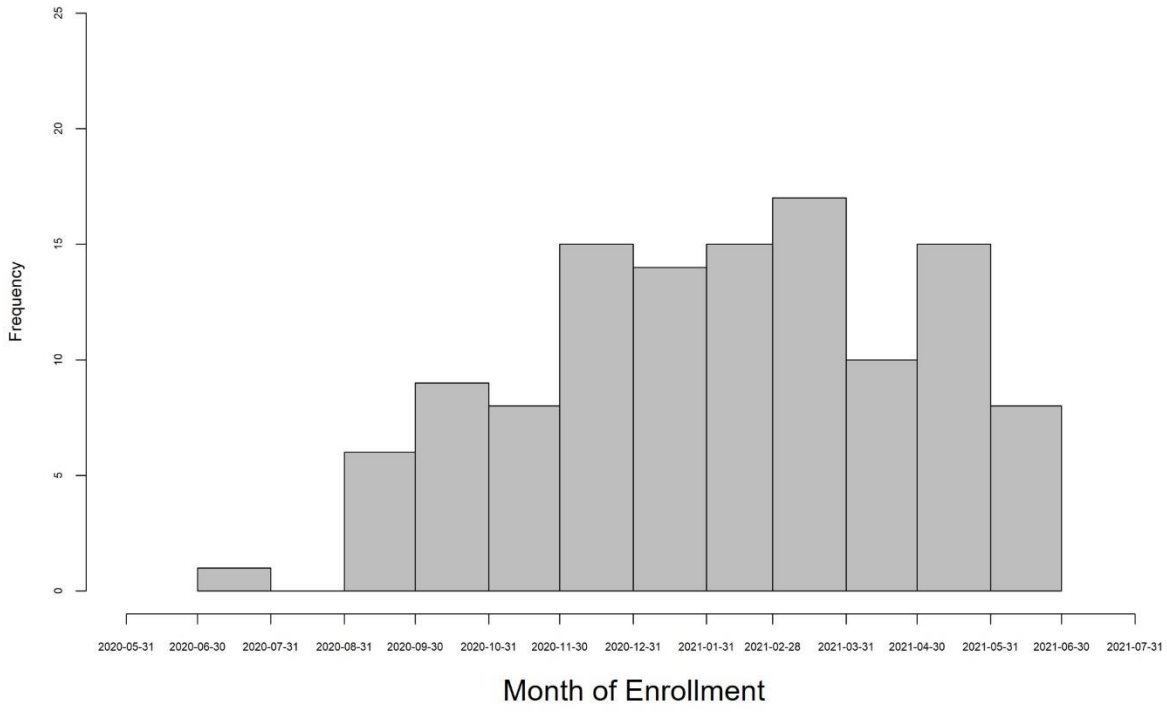
	p-value	FDR-adjusted p-value
Plasma inflammatory markers		
Granzyme B	<0.001	<0.001
IL-1Beta (pg/ml)	0.63	0.90
IL-6 (pg/ml)	0.28	0.79
IP-10 (pg/ml)	0.46	0.86
IL-2Ralpha (pg/ml)	0.64	0.90
IL-8 (pg/ml)	0.46	0.86
PD-L1 (pg/ml)	0.97	1.00
TNF-alpha (pg/ml)	0.064	0.33
CD40 (pg/ml)	1.00	1.00
MIP-1Beta (pg/ml)	0.070	0.33
RANTES (pg/ml)	0.33	0.85
CD4+ and CD8+ T lymphocyte populations		
CD4+ T Cells	0.74	0.99
CD8+ T Cells	0.43	0.86
Phenotypic analysis of T Lymphocytes		
Cluster 1	0.47	0.86
Cluster 2	0.55	0.86
Cluster 3	0.089	0.36
Cluster 4	0.031	0.28
Cluster 5	0.148	0.52
Cluster 6	0.002	0.029
Cluster 7	0.82	1.00
Cluster 8	0.96	1.00
Cluster 9	0.86	1.00
Cluster 10	0.94	1.00
Cluster 11	0.55	0.86
Cluster 12	0.184	0.57
Cluster 13	0.040	0.28
Cluster 14	0.53	0.86
Cluster 15	0.98	1.00

4.0 SUPPLEMENTARY FIGURES

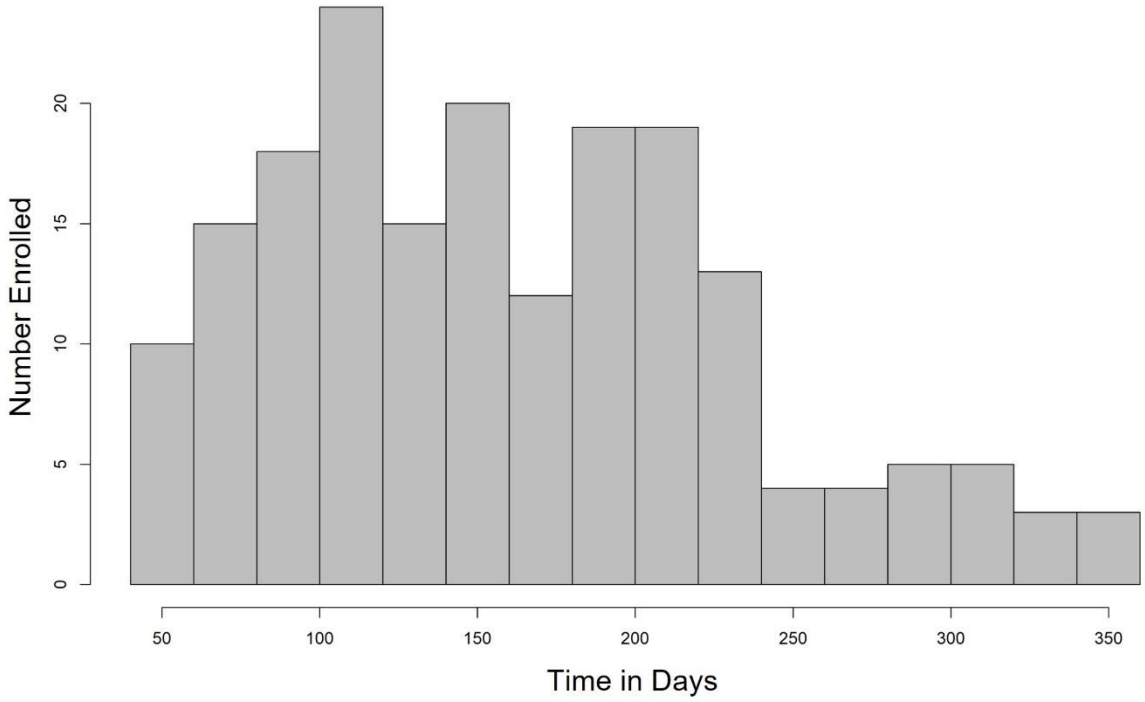
Figure 1. Enrollment over time.



A. Enrollment over time, COVID-19 cohort.

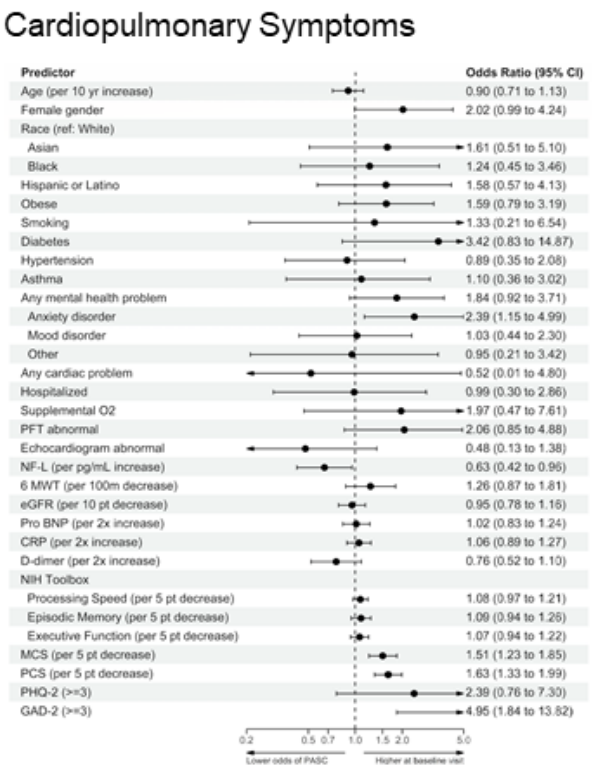


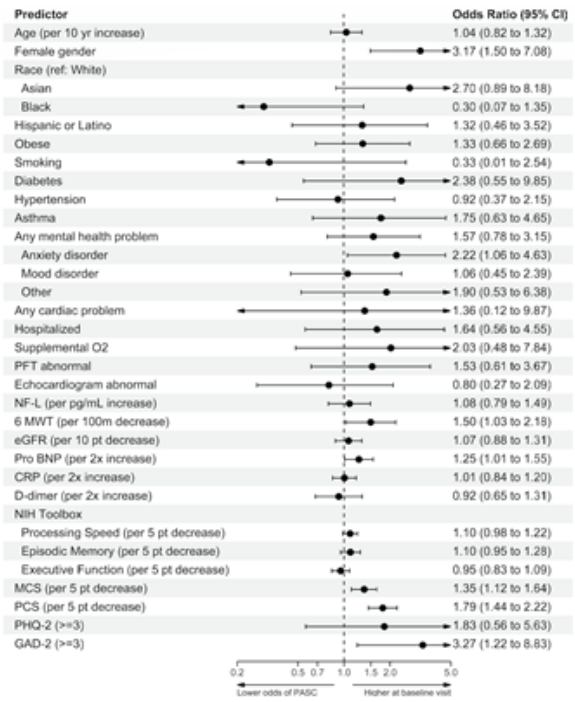
B. Enrollment over time, Control cohort.

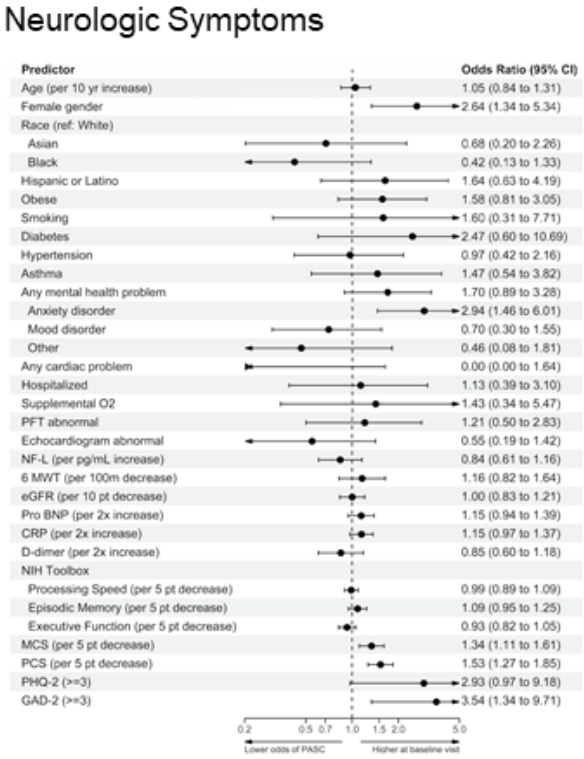


C. Time from onset of COVID-19 symptoms to enrollment visit.

Figure 2. Associations of pre-COVID characteristics, diagnostic testing results and health survey scores with specific groups of PASC symptoms.

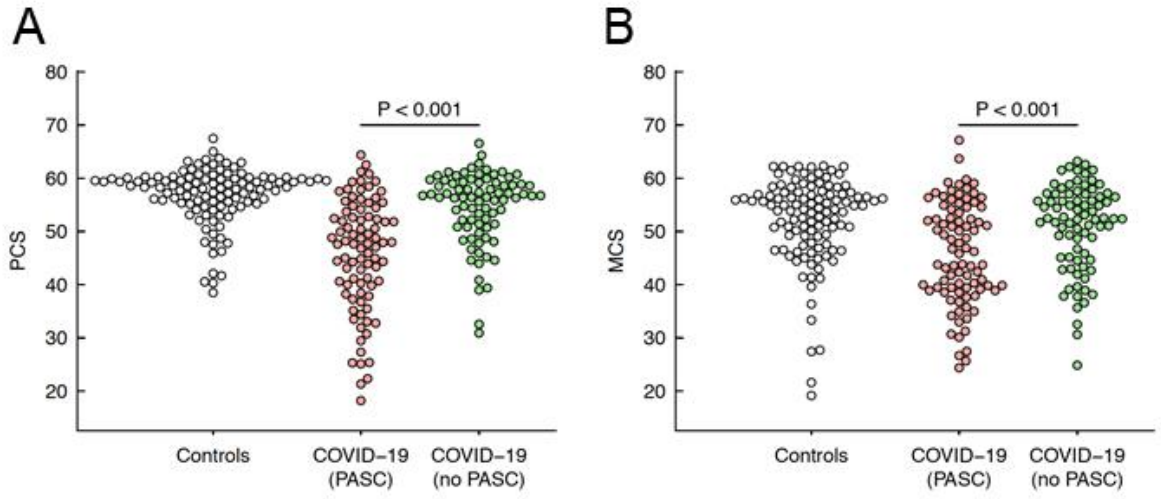






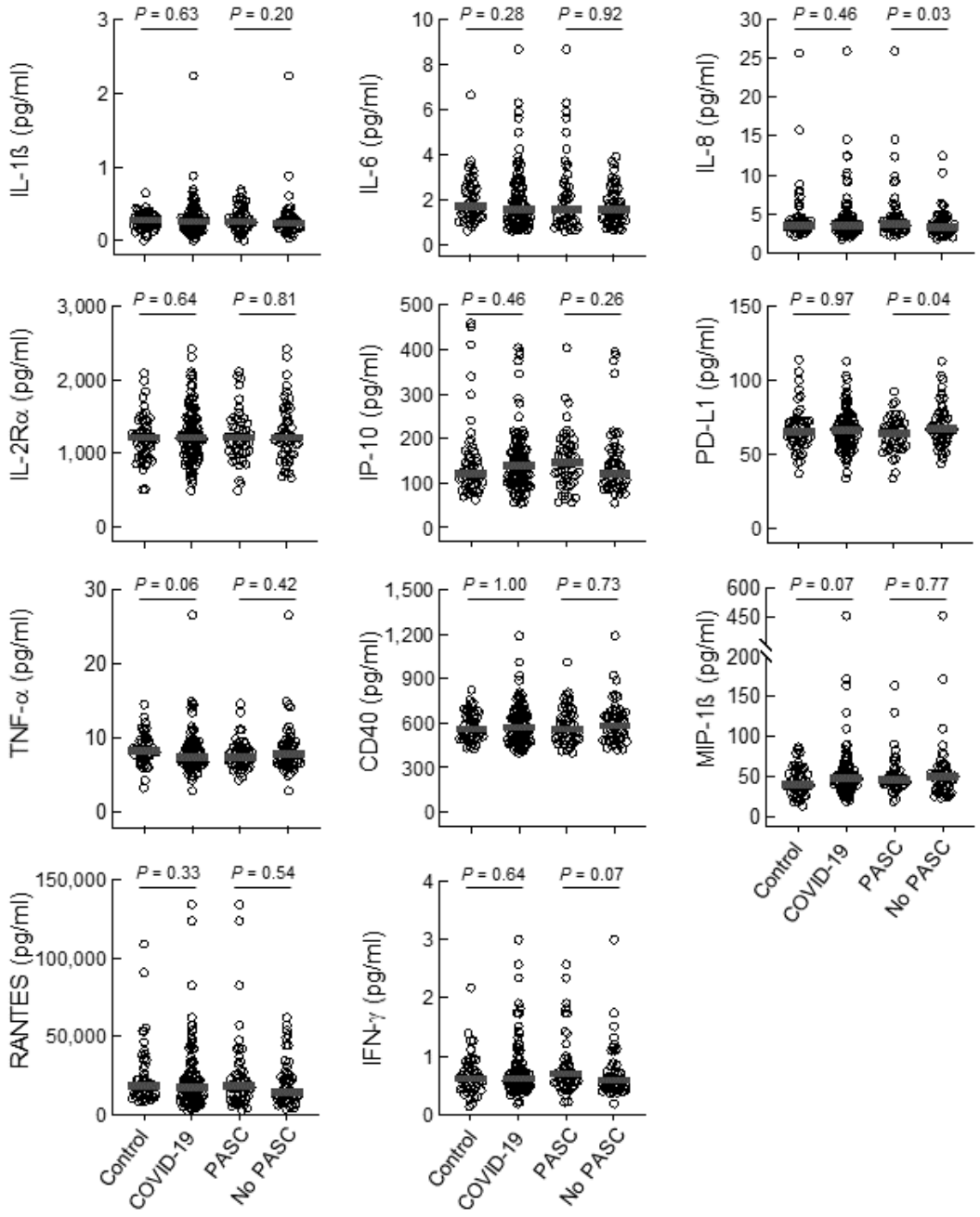
Shown are odds ratios with 95% confidence intervals (CI) quantifying univariate associations between baseline characteristics and measurements and presence of specific groups of symptoms at the baseline visit. Cardiopulmonary symptoms include dyspnea, chest pain, cough, and palpitations. Neurology symptoms include concentration impairment, memory impairment, headache, parosmia, and paresthesia. PFT refers to pulmonary function test, NF-L neurofilament light chain, 6MWT six-minute walk test distance in meters, eGFR estimated glomerular filtration rate, pro-BNP pro-brain natriuretic peptide, CRP c-reactive protein, PCS and MCS physical and mental health component scores (respectively) of the Short Form-36 Health Survey (SF-36, version 2), PHQ-2 Patient Health Questionnaire-2 and GAD-2 Generalized Anxiety Disorder-2.

Figure 3. Short Form-36 Health Survey (SF-36) version 2 scores.



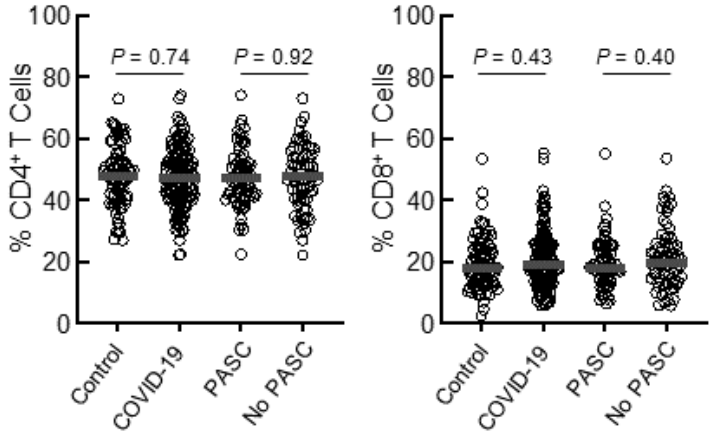
Panel A shows the individual physical health component scores (PCS) in the control, COVID-19 with PACS, and COVID-19 without PASC groups. Panel B shows the individual mental health component scores (MCS) in the same 3 groups. *P* values were determined using the t-test.

Figure 4. Levels of biomarkers in the plasma of study participants.



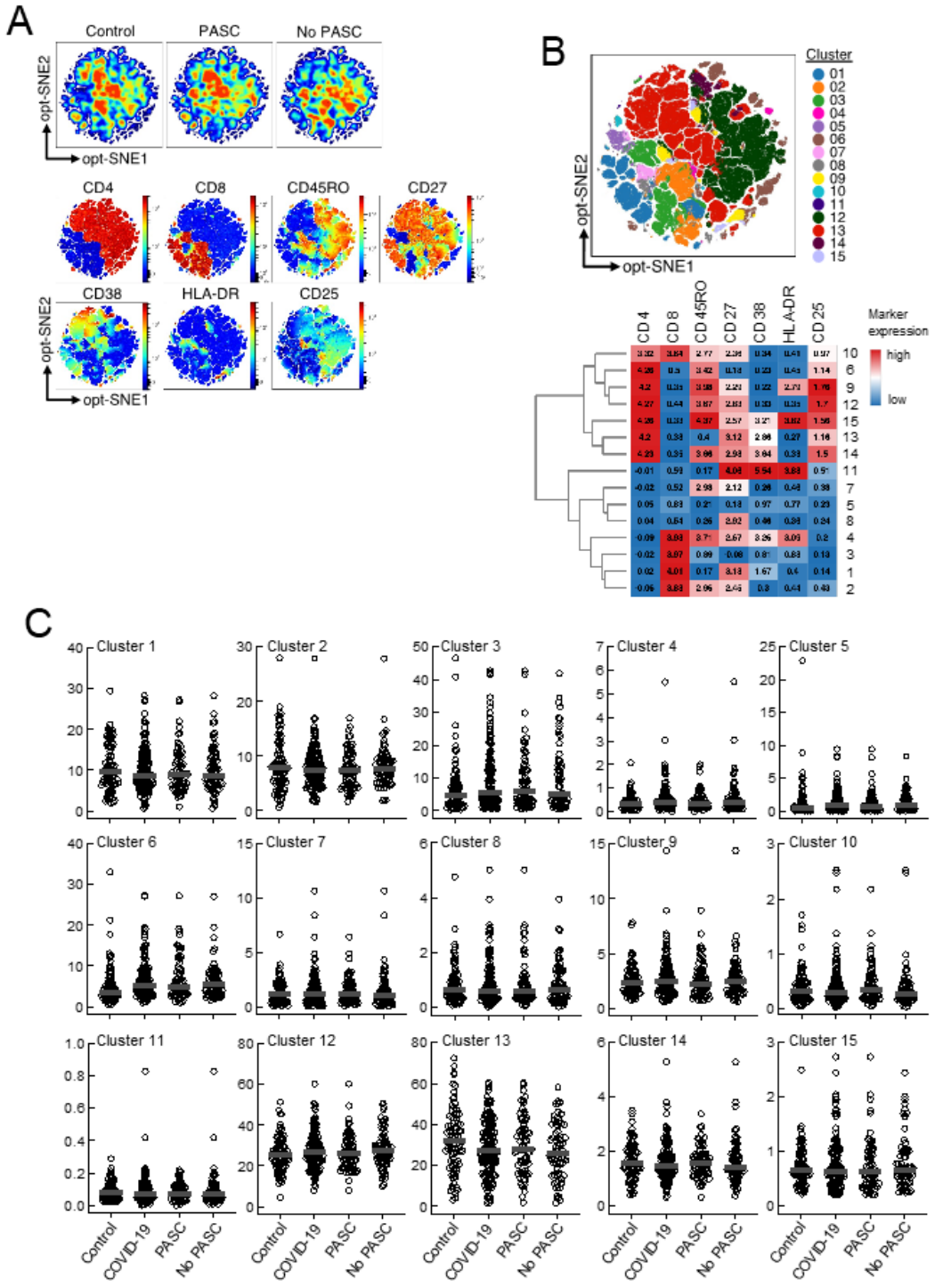
The grey lines indicate median values. *P* values were determined using the Wilcoxon rank-sum test.

Figure 5. CD4$^+$ and CD8$^+$ T cell populations in peripheral blood.



P values were determined using the Wilcoxon rank-sum test.

Figure 6. Phenotypic analysis of T cells.



High-dimensional flow cytometric analyses of peripheral blood mononuclear cells of study participants. Panel A shows global opt-SNE plots of $CD3^+$ T cells of combined data from each group of study participants (upper panel) and Opt-SNE visualization of expression of the indicated markers (lower panel). Panel B shows opt-SNE map of T cell clusters identified by FlowSOM clustering. Each number indicates a distinct cluster. Heatmap shows the level of expression (MFI) within individual clusters. Panel C shows comparison of frequencies of T cells expressing markers associated with indicated clusters.

REFERENCES

1. Tan CW, Chia WN, Qin X, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. Nat Biotechnol 2020;38(9):1073-1078. DOI: 10.1038/s41587-020-0631-z.
2. Hendricks R, Baker D, Brumm J, et al. Establishment of neurofilament light chain Simoa assay in cerebrospinal fluid and blood. Bioanalysis 2019;11(15):1405-1418. DOI: 10.4155/bio-2019-0163.
3. Shan D, Johnson JM, Fernandes SC, et al. N-protein presents early in blood, dried blood and saliva during asymptomatic and symptomatic SARS-CoV-2 infection. Nat Commun 2021;12(1):1931. DOI: 10.1038/s41467-021-22072-9.
4. Blazkova J, Gao F, Marichannegowda MH, et al. Distinct mechanisms of long-term virologic control in two HIV-infected individuals after treatment interruption of anti-retroviral therapy. Nat Med 2021. DOI: 10.1038/s41591-021-01503-6.
5. Graham BL, Brusasco V, Burgos F, et al. 2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung. Eur Respir J 2017;49(1). DOI: 10.1183/13993003.00016-2016.
6. Graham BL, Steenbruggen I, Miller MR, et al. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. Am J Respir Crit Care Med 2019;200(8):e70-e88. DOI: 10.1164/rccm.201908-1590ST.
7. Wanger J, Clausen JL, Coates A, et al. Standardisation of the measurement of lung volumes. Eur Respir J 2005;26(3):511-22. DOI: 10.1183/09031936.05.00035005.
8. Hall GL, Filipow N, Ruppel G, et al. Official ERS technical standard: Global Lung Function Initiative reference values for static lung volumes in individuals of European ancestry. Eur Respir J 2021;57(3). DOI: 10.1183/13993003.00289-2020.
9. Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J 2012;40(6):1324-43. DOI: 10.1183/09031936.00080312.
10. Stanojevic S, Graham BL, Cooper BG, et al. Official ERS technical standards: Global Lung Function Initiative reference values for the carbon monoxide transfer factor for Caucasians. Eur Respir J 2017;50(3). DOI: 10.1183/13993003.00010-2017.

11. Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. Eur Respir J 2005;26(5):948-68. DOI: 10.1183/09031936.05.00035205.
12. Holland AE, Spruit MA, Troosters T, et al. An official European Respiratory Society/American Thoracic Society technical standard: field walking tests in chronic respiratory disease. Eur Respir J 2014;44(6):1428-46. DOI: 10.1183/09031936.00150314.
13. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2015;28(1):1-39 e14. DOI: 10.1016/j.echo.2014.10.003.