

SUPPLEMENTAL MATERIAL

Supplemental methods:

Clinical cohorts:

Adolescents and young adults presenting with chest pain and elevated cardiac troponin T levels after recent SARS-CoV-2 mRNA vaccination, who were admitted to the pediatric wards or the pediatric intensive care units at the Massachusetts General Hospital (MGH) or Boston Children's Hospital (BCH), were approached to enroll in the Institutional Review Board (IRB)-approved Pediatric COVID-19 Biorepository at MGH ¹⁴ (Mass General Brigham IRB #2020P0000955) or the IRB-approved "Taking on COVID-19 Together" Biorepository at BCH (BCH IRB #P00035409). Both biorepositories were established to collect samples from children during the COVID-19 pandemic to understand the impact of SARS-CoV-2 on children.

A convenience sample of healthy controls, who were recruited from local pediatric clinics and referrals, included age-matched adolescents and young adults who received their second SARS-CoV-2 mRNA vaccination within the past three weeks. Healthy controls denied any chest pain and did not require any medical attention following vaccination. Informed consent, and assent when applicable, was obtained from participants and/or parents/guardians prior to enrollment of these individuals in the Pediatric COVID-19 Biorepository at MGH.

Comparison groups also included children with myocarditis (cardiac troponin T values above upper limits of normal) in the setting of MIS-C and healthy SARS-CoV-2 mRNA vaccinated adult controls. Children with MIS-C met CDC criteria with fever following remote SARS-CoV-2 infection, plus evidence of multiorgan damage and inflammation requiring hospitalization ³⁷. Ten children with MIS-C with myocarditis were previously enrolled in the MGH Pediatric COVID-19 Biorepository and had provided banked plasma samples. Healthy SARS-CoV-2 mRNA

vaccinated adult controls included 13 adults, ages 18 years and older, without prior SARS-CoV-2 infection who were vaccinated with the Moderna mRNA-1273 vaccine and did not experience any chest pain requiring medical attention. This adult cohort was part of an IRB-approved specimen collection study conducted at Brigham and Women's Hospital and cohort characteristics were previously published ¹⁶.

Figure 1S provides an overview of the clinical cohorts used in this study.

Sample collection and metadata:

Blood was collected by venipuncture from all participants. Blood was collected from participants hospitalized with post-vaccine myocarditis within 24 hours of presentation, prior to intravenous immunoglobulin therapy. Repeat sampling, coordinated with clinical blood draws while inpatient, was performed. Single time point collections were obtained from healthy controls. Blood was processed and plasma was isolated as previously described ¹⁴. Symptom report, clinical laboratory values, and patient demographic information, including age, sex at birth, race, and ethnicity, were extracted from electronic medical records.

Specifically, we extracted lab values for cardiac and inflammatory tests processed through CLIA-certified laboratories, including cardiac troponin T (MGH: Elecsys Troponin T Gen. 5 STAT Assay, Roche Diagnostics, Indianapolis, Indiana; level of detection [LOD] 6 ng/L; upper limit of normal [ULN] 14 ng/L; coefficient of variation (CV) 2.8%; BCH: Elecsys Troponin T, Roche Diagnostics, Indianapolis, Indiana; LOD 0.01 µg/L; ULN 14 ng/L; CV 10%); Brain natriuretic peptide (BNP) (BCH: Architect BNP, Abbott, Abbott Park, IL; LOD 10 pg/mL; ULN 100 pg/mL; CV 4.0-6.7%); NT-proBNP (MGH: Elecsys ProBNP 2 STAT Immunoassay, Roche Diagnostics, Indianapolis, Indiana; LOD 5 pg/mL, ULN 450 pg/mL, CV 2.5%); and CRP (MGH: Sekisui CRP Ultra Wide Range Reagent Kit: Method is Latex-Enhanced Turbidimetric Assay, measured on

Roche Cobas c502 Analyzer, Sekisui Diagnostics, Charlottetown, Prince Edward Island, Canada, LOD 0.2 mg/L, ULN 8 mg/L, CV 6.97% at 0.47 and 3.34% at 2.18; BCH: Cobas Tinaquant C-Reactive Protein IV, Roche Diagnostics, Indianapolis, Indiana; LOD 0.3 mg/L, ULN 8 mg/L, CV 0.5-0.8%).

Anti-SARS-CoV-2 Antibodies:

A systems serology approach was used to measure antigen-specific antibody titers, Fc receptor binding properties, antibody-dependent complement deposition (ADCD), neutrophil phagocytosis (ADNP), or cellular (THP-1) phagocytosis (ADCP) as described before³⁰. For titer and Fc receptor binding assays, plasma samples were incubated with antigen-coupled Luminex beads and isotype/subclass or Fc receptor binding probed with respective secondary reagents. For the three functional assays (ADCD, ADNP or ADCP) biotinylated antigens were bound to FluoSphere NeutrAvidin beads (Thermo Fisher Scientific; Waltham, MA, USA). To form immune-complexes (ICs), antigen-coated beads were incubated with diluted serum samples. Non-specific antibodies were washed away. For ADCD, ICs were incubated with guinea pig complement factor and C3 deposition on beads were stained with anti-guinea pig C3-FITC antibody (MP Biomedicals; Irvine, California USA, 1:100, polyclonal). For ADNP or ADCP, ICs were incubated with primary neutrophils or THP-1 cells, respectively. Complement beads or cells were fixed with 4% paraformaldehyde and analyzed on an iQue analyzer (Intellicyt; Albuquerque, NM, USA).

For the time course assessment, anti-SARS-CoV-2 IgG, IgA, and IgM antibodies were measured for longitudinal myocarditis plasma samples using the previously described Simoa assays³⁸. Antibody levels are presented as normalized average enzymes per bead (AEB), where the measured AEB value is normalized by calibrators obtained from serially diluting a plasma sample collected from a SARS-CoV-2 positive individual.

PhiP-Seq (VirScan)

VirScan analysis was performed and analyzed as described before³⁹. In brief, serum samples were incubated with VirScan 3.0 phage library (DNA-barcoded phages expressing overlapping 56mer peptides of more than 1500 different pathogens and IgG bound phages were immunoprecipitated using magnetic Protein A/G beads (Thermo Scientific Pierce). Phages were then lysed and DNA barcodes sequenced on an Illumina NextSeq 500 platform. NGS reads were aligned and respective peptides assigned to NGS counts. These peptides counts were then binned by the sum of four included negative controls. Epitope binding signal (EBS) Z-scores for each sample and peptide were calculated per bin. For the Z-score calculation only the middle 90% (excluded the top and bottom 5%) of the values per bin were used. Peptides were only called 'hit' and further considered if they had an epitope binding signal Z-score of more than 3.5 in both replicates. For peptides with a hit at one timepoint this threshold was lowered to >2 for the other time point for the same peptide. To generate the heatmap, EBS hits were summed by pathogen. Only pathogens with detectable binding in more than three individuals were considered relevant binding. Data was Z-scored to normalize EBS across different strains and to account for gene length difference before heatmap was plotted.

T cell analysis:

SARS-CoV-2 Spike protein-specific T cell responses were measured by multiparameter flowcytometry and intracellular cytokine staining (ICS) assay, as described⁴⁰. Assays were performed with 10^6 PBMC that were incubated for 8 hours at 37°C with R10 media, CD3/CD28 beads (Gibco, ThermoFisher Scientific), or 2 µg/ml Wuhan wild-type SARS-CoV-2 Spike protein peptide pool (PepTivator, Miltenyi Biotec). Cultures contained monensin (GolgiStop; BD Biosciences), brefeldin A (GolgiPlug; BD Biosciences), and 1 µg/ml of a mAb against human CD49d (clone L25) and human CD28 (clone 293). Cells were then stained for viability (Blue

viability dye) and with predetermined titers of mAbs against CD3 (clone UCHT1; BUV395), CD4 (clone RPA-T4; BV605), CD8 (clone HIT8a; FITC), CD137 (clone 4B4-1; APC), CD107a (clone H4A3; PE-Cy7), CD45RA (clone HI100; BV421), CCR7 (clone 4B12; PE-Dazzle) and PD-1 (clone EH12.2H7; BV510); and stained intracellularly with CD154 (clone TRAP1; PE), IFN- γ (clone 4S.B3; APC-Cy7), and TNF- α (clone Mab11; PercCp-Cy5.5). Data and statistical analyses were done in FlowJo version 10 and GraphPad Prism 8.4, unless otherwise stated. Antigen-specific CD4⁺ and CD8⁺ T cells frequencies were derived by subtracting the background (R10 only) from the SARS-CoV-2 peptide pool stimulated cells. Gating strategies are shown in **Figure 8S**.

Cytokines:

Cytokine levels were measured in plasma samples using the CorPlex Cytokine Panel (Quanterix). Plasma samples were diluted 4-fold in sample diluent buffer and assays were performed following the CorPlex manufacturer protocols. Each CorPlex cytokine panel kit was analyzed by the SP-X Imaging and Analysis System (Quanterix).

SARS-CoV-2 antigens:

SARS-CoV-2 S1 and Spike antigens were measured in plasma samples in a similar way to previously described single molecule array (Simoa) assays²⁹. To prepare the reagents, antibodies against S1 (40150-D006, Sino Biological) and S2 (MA5-35946, Invitrogen) were conjugated to carboxylated 2.7 μ m paramagnetic beads (Quanterix). S1 antibodies were conjugated to 488 nm dye-encoded beads and S2 antibodies to 750 nm dye-encoded beads. Each detector antibody was conjugated to EZ-Link NHS-PEG4-Biotin (Thermo Fisher Scientific). The same detector antibody against S1 was used for both the S1 and spike assay (LT-1900, Leinco), therefore full spike was detected by capturing the S2 subunit and detecting the S1 subunit. Although the binding epitopes for the anti-S1 antibodies were proprietary, the anti-S2

antibody, originally developed against SARS-CoV, is known to bind the region containing the heptad repeat domain 2⁴¹. Before analyzing samples, the prepared antibody conjugated beads were diluted in the required volume of Bead Diluent buffer (Quanterix). A total of 500,000 beads were used per single assay for a given sample. Of those 500,000 beads, 125,000 beads were the prepared antibody coated beads against S1 or S2, corresponding to the assays for S1 and spike, respectively, and the remaining 375,000 beads were non-conjugated 647 nm dye-encoded helper beads (Quanterix). Working solutions of the detector antibodies were prepared by diluting each antibody to a final concentration of 0.3 µg/mL in General Detector and Sample Diluent buffer (Quanterix). Solutions of streptavidin conjugated β-galactosidase (SβG) were prepared in a similar manner by diluting a stock solution of SβG in SβG diluent (Quanterix) to a final concentration of 75 pM.

Plasma samples were prepared by first centrifuging the samples at 4°C for 10 minutes at 2000 x g to pellet any cellular debris. To measure free antigen, plasma samples were diluted 8-fold in a sample diluent buffer (Quanterix) with added protease inhibitors (Halt™ Protease Inhibitor Cocktail (100x), Thermo Fisher Scientific) and to measure total antigen concentrations plasma samples were first incubated with 10 mM dithiothreitol (DTT) at 37°C for 15 minutes to denature any bound antibodies.

Calibration curves were prepared by diluting purified S1 and spike in Sample Diluent Buffer at concentrations ranging from 0.64 pg/mL to 10,000 pg/mL. Recombinant S1 (V0591-V08H, Sino Biological) and a stabilized ectodomain of the spike protein, encoding residues 1-1208³⁹ were used as the protein standards for S1 and full spike, respectively.

Simoa antigen assays are performed in an automated three-step assay format onboard the HD-X Analyzer (Quanterix), as previously described⁴². In the first step, 25 µL of diluted plasma was

incubated with 25 μ L of the prepared bead solution. After a 15-minute incubation, six washes were performed with System Wash Buffer 1 (Quanterix). For the second step, the beads were then resuspended in 100 μ L of the prepared detector antibody solution and incubated for 5.25 minutes. Another wash step was performed with System Wash Buffer 1, and in the third step, the beads were resuspended in 100 μ L of the prepared S β G solution and incubated for 5.25 minutes. After washing the beads, they were then resuspended in 25 μ L of resorufin β -D-galactopyranoside (Quanterix) and loaded into a microwell array, which was subsequently sealed with oil and imaged in the optical channels corresponding to the type of dye-encoded beads used. Average enzyme per bead (AEB) values were calculated by the HD-X Analyzer software and converted to concentration values based on a calibration curve fit with a four-parameter logistic regression. Separately, the limit of detection (LOD) was calculated as the background AEB plus three times the standard deviation and converted to a concentration. LODs were calculated for three separate experiments and the mean LOD was used. Only values above the LOD are reported and values below the LOD were set to the LOD.

Calibration curves, dilution linearity, and spike and recovery for the S1 and spike assays were presented in **Figure 9S** and **Figure 10S**. In addition, spike and recovery experiments were performed in the presence of varying concentrations of an anti-spike neutralizing antibody (40592-R001, Sino Biological) to determine if more S1 and spike could be recovered upon the addition of DTT (**Figure 11S**).

Supplemental Table:

Table 1S: Clinical characteristics of adolescents and young adults presenting with post-vaccine myocarditis. Criteria used to define myocarditis are displayed.

Patient ID	Vaccine Type	Doses Received	Time between vaccine and symptom onset (days)	Symptoms at presentation	Laboratory values				ECG	Echocardiogram			Cardiac MRI			Lake Louise criteria	Free Spike (pg/mL)	Total Spike (pg/mL)
					Peak cardiac troponin T (ng/L) ULN: 14 ng/L	Peak BNP (pg/mL) ULN: 100 pg/mL	Peak NT-proBNP (pg/mL) ULN: 450 pg/mL	Peak CRP (mg/L) ULN: 8 mg/L		LV ejection fraction (%)	Pericardial effusion	Days from symptom onset	Myocardial edema	Late Gadolinium Enhancement	CDC definition myocarditis			
1	BNT162B2	1	4	Chest pain	210	16	n/a	52.4	Early repolarization	58.3	None	10	No	No	Probable	No	26.96	20.4
2	BNT162b2	1	19	Chest pain, right arm numbness, shortness of breath, lightheadedness	2005	n/a	504	41.8	Diffuse ST segment elevation	65	None	5	Yes	Yes	Confirmed	Yes	41.73	46.96
3	BNT162b2	2	2	Chest pain, burning and tightness, chills/rigors, headache, myalgia, fatigue, weakness	910	n/a	n/a	20.7	Diffuse ST segment elevation	58.9	None	4	No	Yes	Probable	No	18.48	not detected
4	BNT162b2	2	2	Chest pain, burning and tightness, fever, headache, cough, sore throat, rhinorrhea, congestion, abdominal pain, nausea, vomiting, diarrhea, loss of appetite, myalgia, fatigue, weakness, altered awareness/confusion delirium, anosmia, swollen and cracked lips, LAD	190	190	n/a	45.1	Non-specific ST wave changes, T-wave inversion	57.3	None	6	No	No	Probable	No	not detected	not detected
5	BNT162b2	2	2	Chest pain, burning and tightness, shortness of breath, chills/rigors, fatigue, weakness	90	73	n/a	76.1	Sinus tachycardia, non-specific ST-T wave changes	55	None	4	No	Yes	Probable	No	38.24	30
6	BNT162b2	2	3	Chest pain	270	46	n/a	74.3	Incomplete right bundle branch block (RSR'), ST-T wave change, non-specific T-wave inversion	43.7	None	2	No	Yes	Probable	No	47.68	22.88
7	BNT162b2	2	5	Chest pain, burning and tightness, fever, headache, shortness of breath, abdominal pain, nausea, loss of appetite, myalgia, arthralgia, weakness, fatigue	250	20	n/a	15.4	Normal ECG	n/a	None	5	No	Yes	Probable	No	114.96	126.4
8	BNT162b2	2	3	Chest pain, burning and tightness, shortness of breath, headache	230	13	n/a	30	Premature atrial beats	60.8	None	2	No	Yes	Probable	No	not detected	not detected
9	BNT162b2	2	3	Chest pain, burning and tightness, fever, chills/rigors, headache, difficulty walking, weakness and fatigue	240	20	n/a	15.9	Non-specific widespread ST-T wave changes	55.1	None	2	No	Yes	Probable	No	34	30.16
10	BNT162b2	2	9	Chest pain, burning and tightness, fever, headache, myalgia, skin rash/ulcers, sleep disturbance	1340	118	n/a	75.2	Non-specific ST-T wave changes in invertebral leads, incomplete RBBB (RSR')	55.2	None	5	No	Yes	Probable	No	not detected	17.76
11	BNT162b2	2	4	Chest pain, burning and tightness, productive cough, wheezing, sore throat, headache, chills/rigors, myalgia, LAD, weakness and fatigue	250	12	n/a	27.4	Normal ECG	61.2	None	4	No	Yes	Probable	No	25.68	23.84
12	BNT162b2	2	1	Chest pain, shortness of breath	184	n/a	73	9.3	Minimal ST segment elevation in inferior and lateral precordial leads	67	None	20	Equivocal	Yes	Probable	No	44	31.12
13	mRNA-1273	2	4	Chest pain, left upper arm pain	1127	n/a	213	11.2	Sinus bradycardia with premature atrial complexes with aberrant conduction, diffuse ST segment elevation	61	None	6	Yes	Yes	Confirmed	Yes	24.38	29.2
14	mRNA-1273	2	2	Chest pain, palpitations	1008	n/a	347	29.5	Sinus rhythm with Premature ventricular complexes or fusion complexes.	58	None	8	Yes	Yes	Confirmed	Yes	32.72	41.92
15	BNT162b2	3	4	Nausea/vomiting, chest pain, left arm numbness, diaphoresis	1074	n/a	606	8	Normal ECG	57	None	14	Yes	Yes	Confirmed	Yes	not detected	26.88
16	BNT162b2	3	2	Chest pain	4040	n/a	115	32.4	ST segment elevation in inferior and lateral precordial leads	61	None	5	Yes	Yes	Confirmed	Yes	32.08	39.36

Supplemental Figures:

Figure 1S: Study design overview. Clinical cohorts, frequency of sample collection and overview of sample analyses are defined.

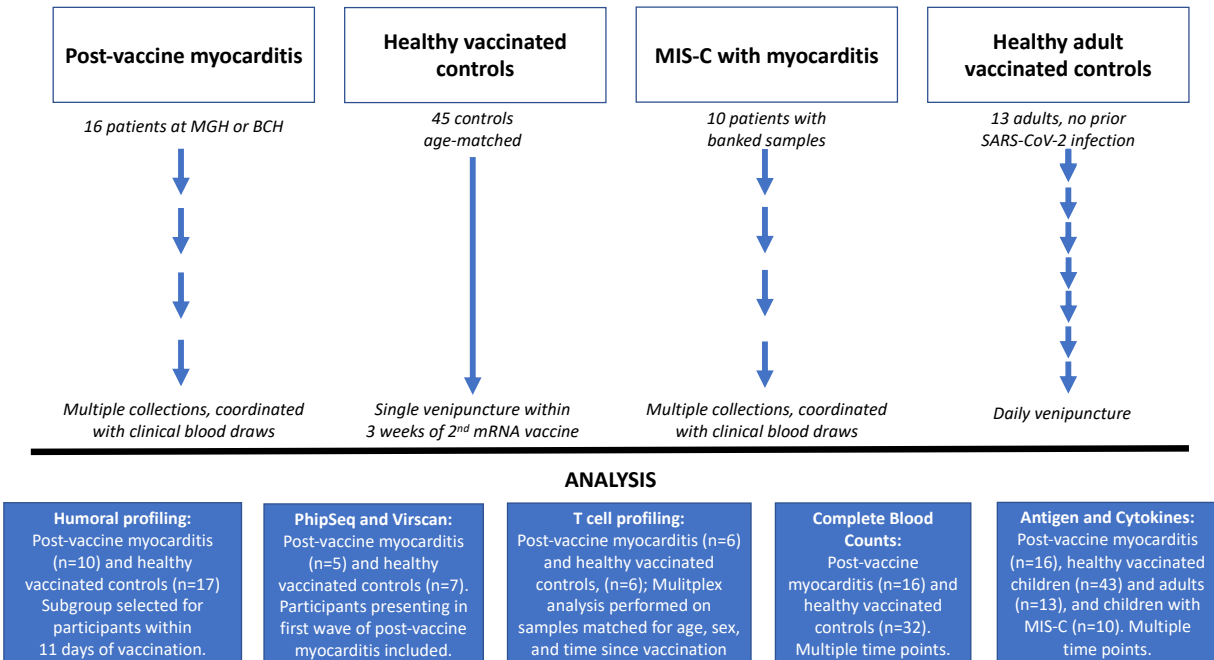


Figure 2S: T cell characteristics in individuals with post-vaccine myocarditis and in

controls. T cells from six individuals with post-vaccine myocarditis compared with healthy, age and sex- matched controls with comparable time from vaccination. Phenotypic distribution of central-memory, naïve, terminally differentiated effector memory cells (RA) and effector-memory cells in bulk CD8+ T cells (**A**) and bulk CD4+ T cells (**B**) as well as SARS-CoV-2 Spike specific CD4+ T cells (**C**). Frequencies of IFN γ -secreting CD8+ T cells (**D**) and CD4+ T cells (**E**) upon stimulation with SARS-CoV-2 Spike peptides. Frequencies of CD107a expressing CD8+ T cells (**F**) and CD4+ T cells (**G**) upon stimulation with SARS-CoV-2 Spike peptides. Frequencies of PD-1 expressing bulk CD8+ T cells (**H**) and CD4+ T cells (**I**). Comparisons with t test; * = $p < 0.05$. ns = not significant.

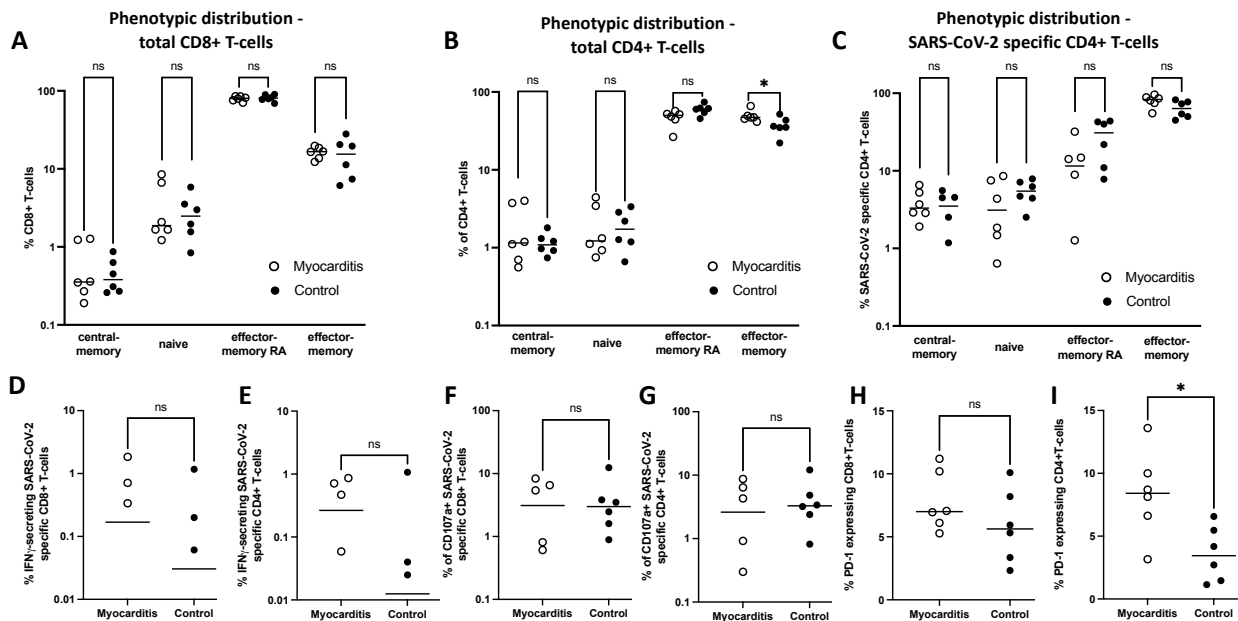


Figure 3S: Hematology profiles of adolescents and young adults with post-vaccine myocarditis as compared to age-matched post-vaccine controls. Leukocyte, neutrophil, platelet, lymphocyte and eosinophil counts shown. Shaded regions indicate normal reference ranges for cell type based on age of cohort. Analysis by unpaired t-test; * $p < 0.05$, ** $p < 0.01$.

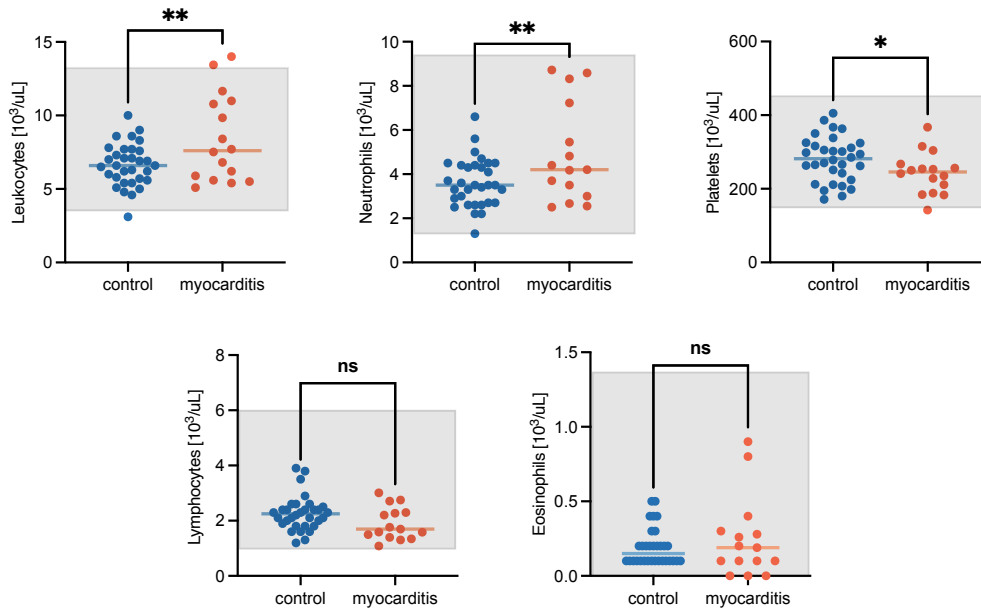


Figure 4S. Adult vaccinated controls. (A) Cohort characteristics. **(B)** Total antigen levels measured in the plasma of adult healthy controls versus time post vaccination.

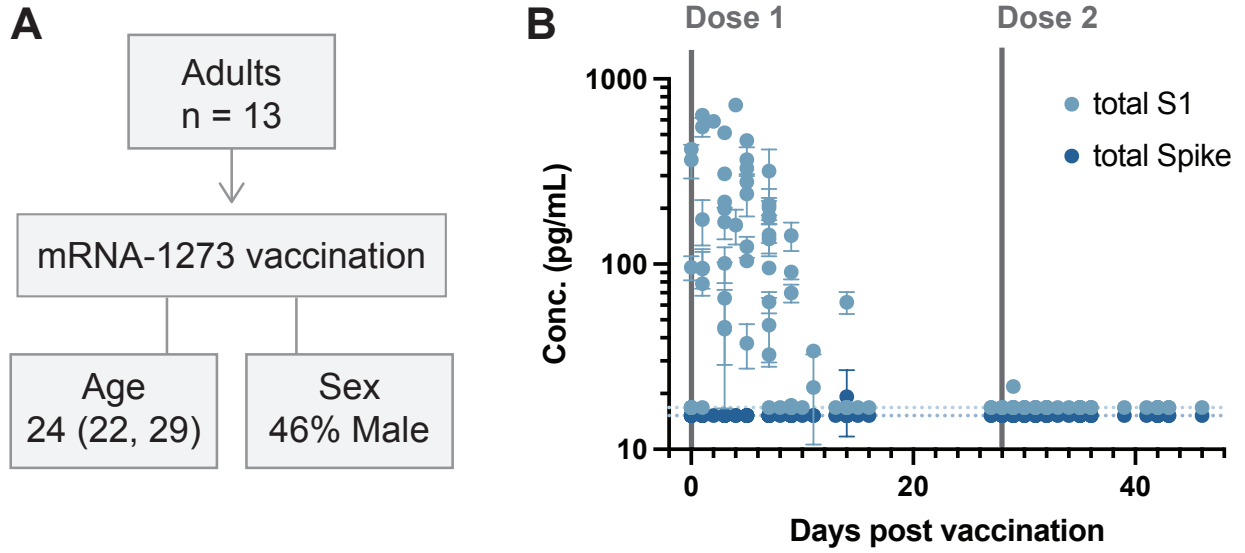


Figure 5S. Circulating SARS-CoV-2 antigen by sex. Free and total S1 (A) and Spike (B) levels measured in the plasma of the vaccinated control and myocarditis cohorts, grouped by female and male sex of healthy vaccinated control or patient with post-vaccine myocarditis.

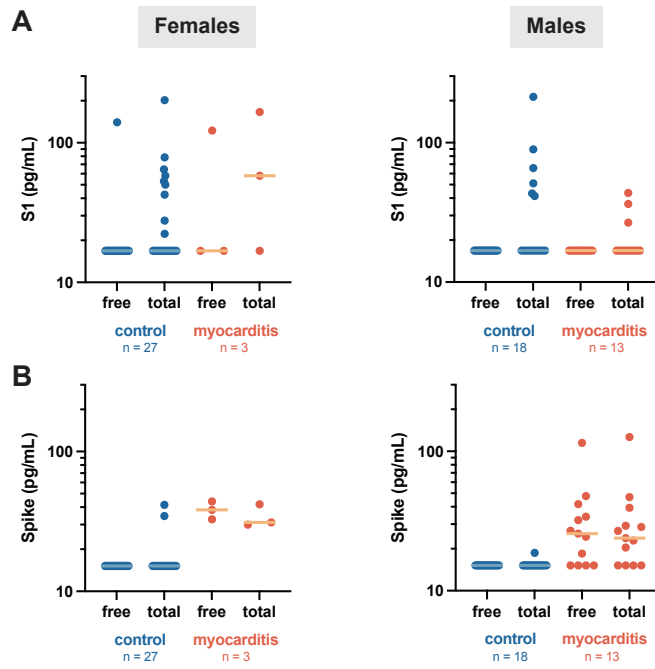


Figure 6S. Antibody neutralization capacity (NT50) values for healthy vaccinated controls and myocarditis patients.

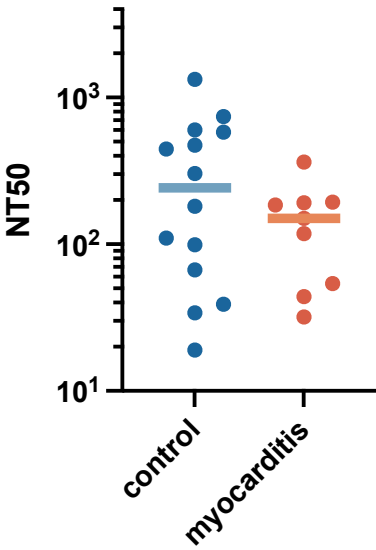


Figure 7S. Longitudinal patient data. Cardiac troponin T, SARS-CoV-2 antigen, anti-SARS-CoV-2 IgG, IgA, IgM, and cytokine levels displayed over time for four individuals with post-vaccination myocarditis. Patients 1-3 developed myocarditis following the second dose and patient 4 developed myocarditis after the first dose. Dashed lines indicate the assay LODs.

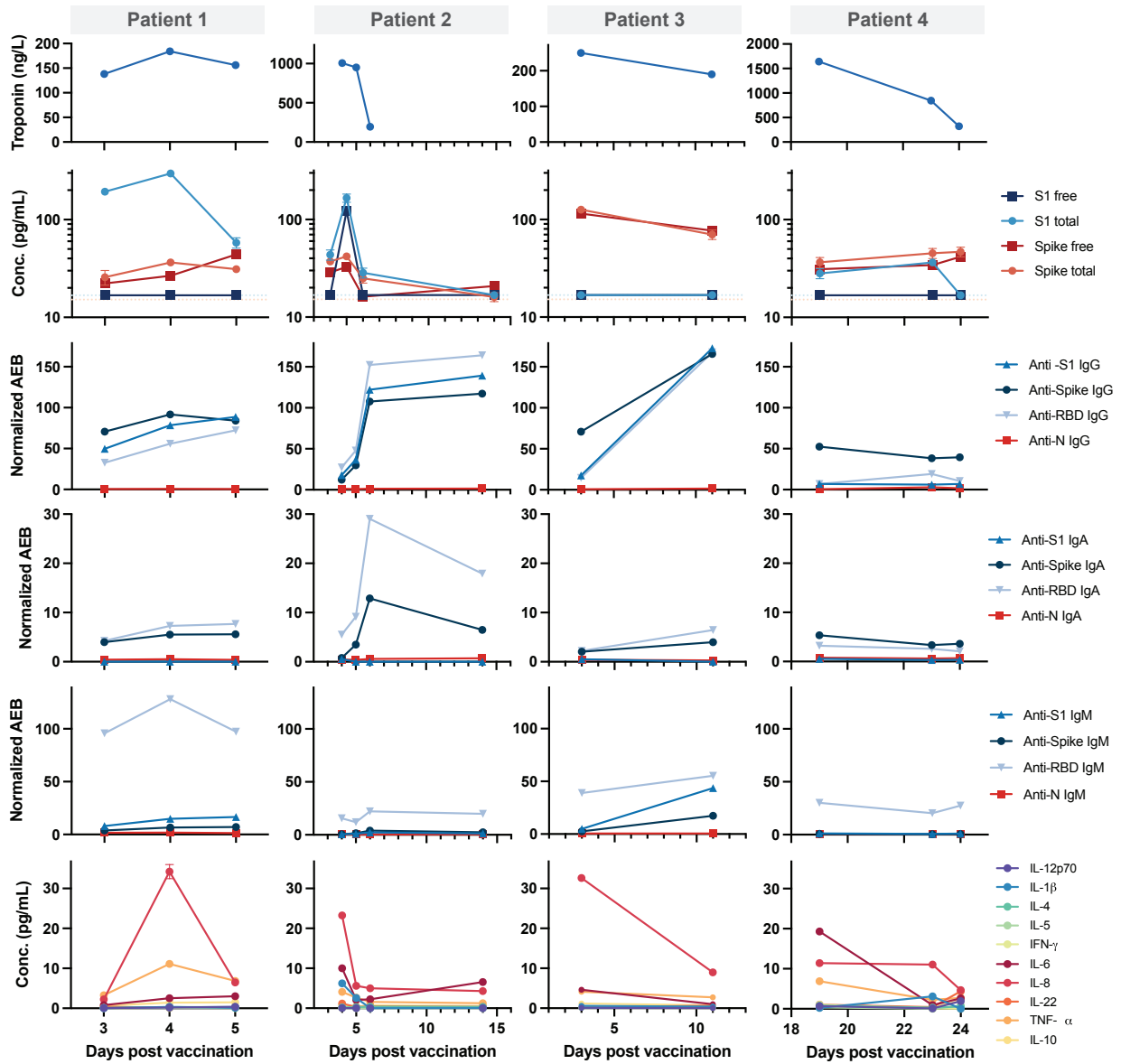


Figure 8S: Flow cytometry gating strategy used to define **(A)** CD8+ T-cell and CD4+ T-cell subpopulations (Lymphocytes -> singlets -> live CD3+ T-cells -> CD8+ or CD4+ T-cells -> phenotyping), defined by CD45RA and CCR7 expression (CD45RA+CCR7+: naïve, CD45RA-CCR7+: Central memory, CD45RA-CCR7-: effector-memory, CD45RA+CCR7-: effector-memory RA); **(B)** CD4+T-cells expressing CD154 and secreting TNF- α following SARS-CoV-2 peptide stimulation and applying similar phenotyping as before; **(C)** unstimulated and SARS-CoV-2 peptide stimulated CD4+ T-cells as measured by IFN- γ , TNF- α secretion, CD107a upregulation and PD-1 expression.

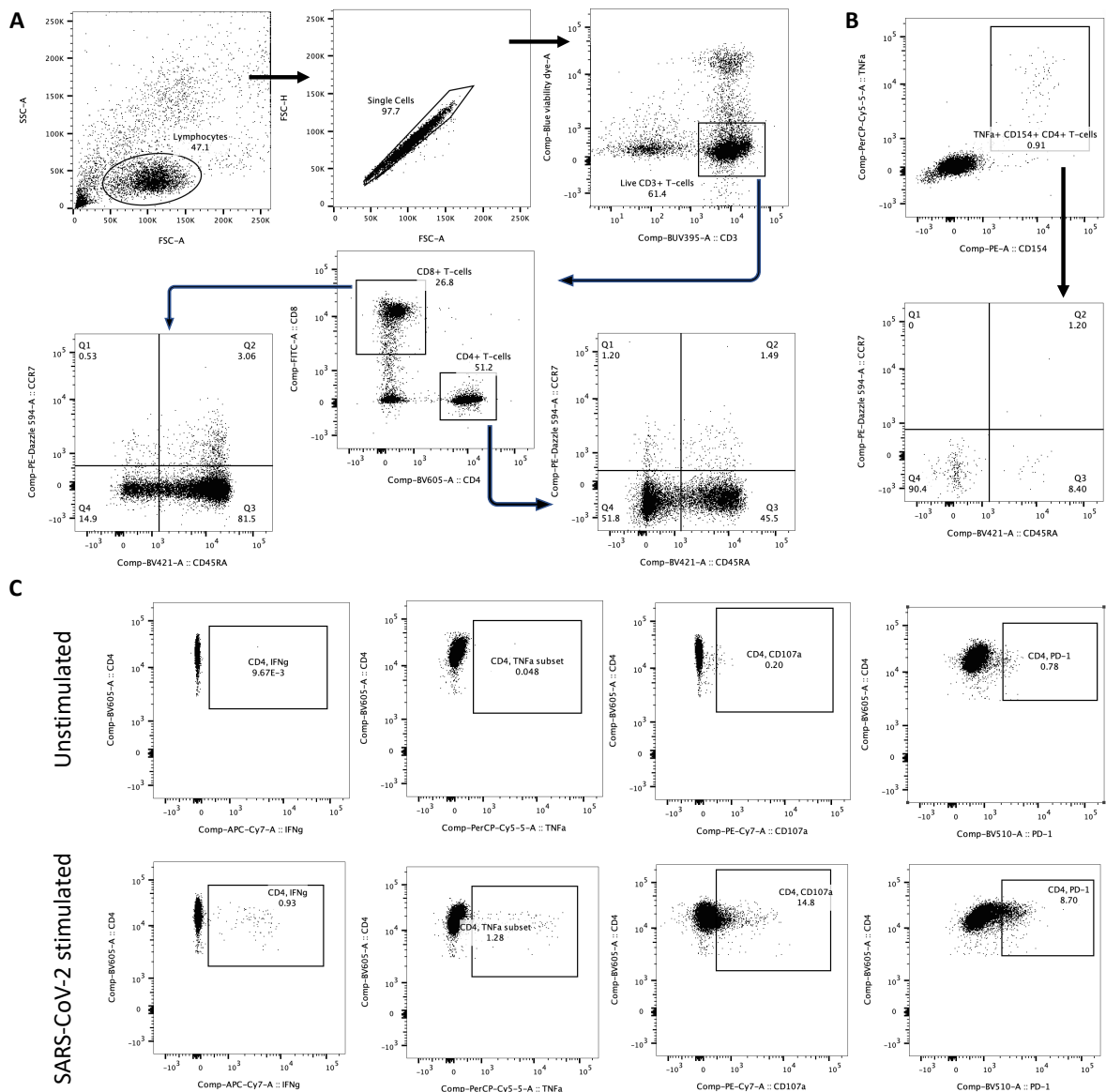
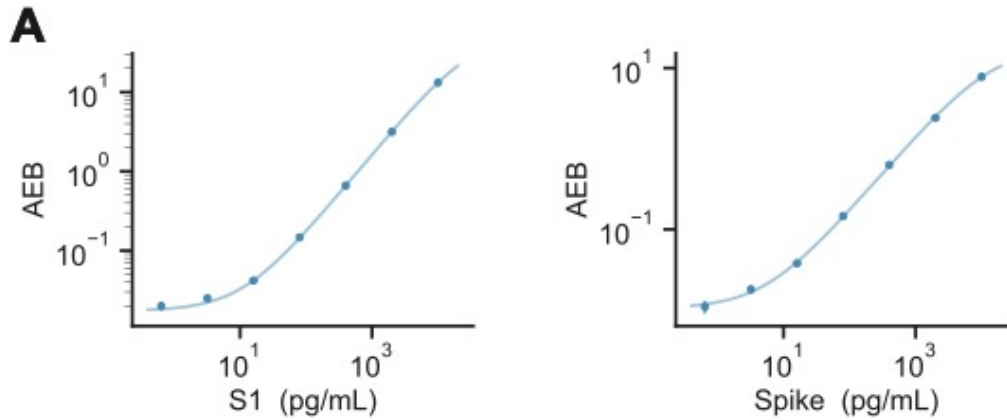


Figure 9S. Calibration curves for SARS-CoV-2 antigen assays. (A) Known concentration of S1 or Spike versus measured average enzyme per bead (AEB) values. **(B)** Measured mean AEB (n = 2) and standard deviation (SD) values for each assay. Data was fit with a four-parameter logistic regression and the determined parameters are listed.



B

S1

Spike

Conc. (pg/mL)	AEB mean	AEB SD
0	0.017	0.001
0.64	0.020	0.002
3.2	0.025	0.002
16	0.042	0.002
80	0.147	0.003
400	0.662	0.008
2000	3.158	0.147
10000	13.225	0.118

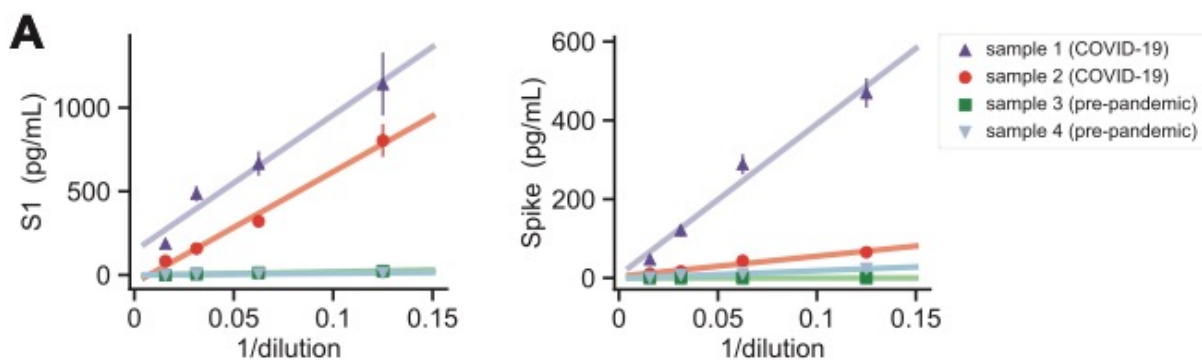
Conc. (pg/mL)	AEB mean	AEB SD
0	0.010	0.001
0.64	0.011	0.002
3.2	0.018	0.002
16	0.038	0.003
80	0.146	0.002
400	0.630	0.009
2000	2.414	0.095
10000	7.790	0.527

$$y(x) = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^B}$$

	S1	Spike
A	0.0173	0.0104
B	1.015	0.952
C	32343.4	13883.8
D	56.642	18.248

Figure 10S. Dilution linearity and spike and recovery for SARS-CoV-2 antigen assays. (A)

S1 and spike concentrations measured in four plasma samples (two samples from COVID-19 patients and two samples collected from individuals before 2019) diluted 8-fold down to 64-fold. Linear fits are shown for each sample. **(B)** Recombinant S1 and spike were added at known concentrations to pre-pandemic plasma samples diluted 8-fold in sample diluent buffer and the measured concentrations (mean values, n = 2), standard deviations (SD), and percent recoveries are shown for two plasma samples.

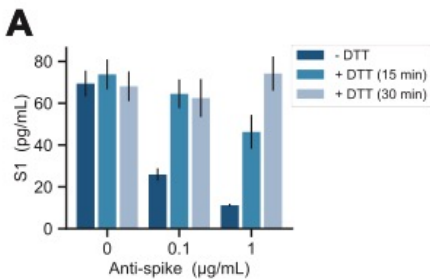


		S1				Spike			
		Spiked conc. (pg/mL)	Measured conc. (pg/mL)	SD (pg/mL)	Percent recovery	Spiked conc. (pg/mL)	Measured conc. (pg/mL)	SD (pg/mL)	Percent recovery
Sample 1		2000	2131.4	153.9	107%	2000	2359.6	106.8	118%
		400	409.3	67.2	102%	400	411.2	53.8	103%
		80	68.3	8.26	85%	80	83.5	9.0	104%
		0	0	0		0	0	0.3	
Sample 2		2000	2133.9	150.7	106%	2000	2435.3	106.8	121%
		400	444.9	40.0	111%	400	461.3	47.6	115%
		80	79.7	16.4	99%	80	66.2	6.9	82%
		0	0.2	0		0	1.4	0.3	

Figure 11S. Recovery of S1 and spike with added neutralizing antibodies.

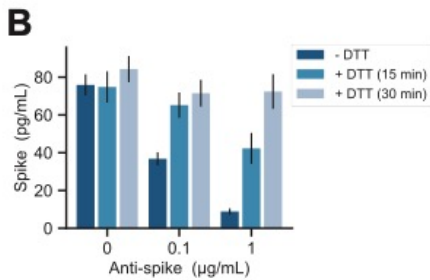
Concentration of S1 **(A)** and Spike **(B)** recovered after adding 0, 0.1, and 1 $\mu\text{g/mL}$ of neutralizing anti-Spike antibody without and with DTT pre-treatment for 15 or 30 minutes.

Percent recovery values are shown in the tables to the right. In all cases, S1 and Spike were spiked to a final concentration of 80 pg/mL in pre-pandemic plasma samples. **(C)** S1 concentration measured in three samples collected from COVID-19 patients without and with DTT pre-treatment for 15 minutes. All samples were collected four days after diagnosis by nasopharyngeal swab RT-PCR. The fold increase of S1 after DTT pre-treatment for each sample is shown in the table.



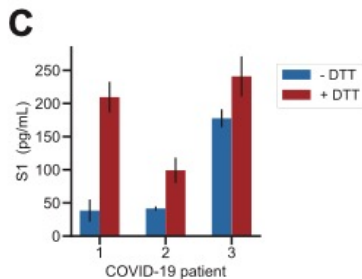
Percent recovery

Anti-spike ($\mu\text{g/mL}$)	- DTT	+ DTT (15 min)	+ DTT (30 min)
0	86.7%	92.3%	85.1%
0.1	32.4%	80.6%	78.1%
1	13.9%	57.8%	92.7%



Percent recovery

Anti-spike ($\mu\text{g/mL}$)	- DTT	+ DTT (15 min)	+ DTT (30 min)
0	94.8%	93.5%	105.4%
0.1	46.1%	81.5%	89.4%
1	11.2%	52.7%	90.5%



Fold increase after DTT

Patient	Fold increase
1	5.5
2	2.3
3	1.4