

**Factors associated with immune responses to SARS-CoV-2 vaccination in autoimmune
disease individuals.**

SUPPLEMENTARY METHODS, FIGURES AND TABLES

SUPPLEMENTARY METHODS

Clinical assessments

Demographic data, concomitant medications, comorbid diseases, and vaccine type were entered into a central Health Insurance Portability and Accountability Act (HIPAA)–compliant REDCap database. Disease activity was categorized as either inactive, mild, moderate, or severe using the Clinical Disease Activity Index (CDAI) for RA (1), SLEDAI-2K for SLE (2), Pemphigus Disease Area Index (PDAI) for pemphigus (3), or physician determination for other diagnoses. Disease flares were defined as an increase in disease activity prompting changes in immunosuppressive medication. Treatment details—including current use of immunosuppressive, immunomodulatory, and biologic medications; history of B cell depletion or cyclophosphamide within the last 12 months; current oral corticosteroid dose; and infusions of > 100 mg of methylprednisolone within the last 6 months—were recorded at the Pre V visit. Changes in medications were recorded at all subsequent visits. Additional medications recorded at post-vaccine visits must have been taken for > 4 weeks, except for cyclophosphamide and B cell–depleting agents, which could be taken for shorter periods. Temporary suspension of immunosuppressives or biologics at the time of vaccination according to or close to American College of Rheumatology (ACR) guidelines was also recorded (4).

T cell activation induced marker (AIM) assays and flow cytometry

Cryopreserved PBMCs were thawed and washed with AIM medium (RPMI) (Fisher, 11875135) supplemented with 5% human AB serum (Fisher, BP2525-100) and 1% penicillin-streptomycin (Fisher, 15140122). Washed PBMCs were cultured at 1×10^6 cells in 200 μ l AIM medium in a 96-well round bottom plate. A separate well for each donor was stimulated in 1 of 4 conditions:

(1) 2% H₂O vehicle control (Gibco, 15230-170), (2) 2 µl washed anti-CD3/CD28 beads (Thermo Scientific, 1132D), (3) 1 µg/mL PepTivator CMV pp65 (Miltenyi Biotec, 130-093-435), or (4) 1 µg/mL PepTivator SARS-CoV-2 Prot_S Complete (Miltenyi Biotec, 130-129-712). Anti-CD3/CD28 beads and CMV stimulations were omitted in samples with a low cell number. Stimulated PBMCs were cultured for 20 to 24 hours at 37°C. Samples were analyzed in groups of 12, with each group containing 1 of 2 deidentified healthy donors ($n = 37$ and $n = 11$).

After incubation, PBMCs were centrifuged and washed with fluorescence-activated cell sorting (FACS) buffer (PBS/2mM EDTA/1% BSA). Cells were stained with the fluorophore conjugated antibodies and viability dye (**Supplementary Table 10**), for 30 min at 4°C. Stained PBMCs were washed twice with FACS buffer and transferred to 5 mL round bottom flow tubes. A Becton Dickinson (BD) LSR Fortessa X-20 cell analyzer was used to acquire data on all samples. Data were analyzed using BD FACS Diva (v9.0) and FlowJo (v10.8.1).

Autoantibody arrays

Antigens (**Supplementary Table 9**) were conjugated to uniquely barcoded, carboxylated magnetic beads (MagPlex-C, Luminex Corp.) as previously described (29). Five µl of bead array was added to each well of a 384-well plate (Greiner BioOne). Forty-five µl of diluted serum or plasma per well was transferred into the 384-well plate containing the bead array. Samples were incubated for 60 minutes on a shaker at room temperature and then left overnight at 4°C. Beads were then washed with 3×60 µl PBS-Tween on a plate washer (EL406, Biotek) and incubated with 50 µl of 1:1000 diluted R-phycoerythrin (R-PE)–conjugated, Fc-γ–specific goat anti-human IgG F(ab')₂ fragment (Jackson ImmunoResearch, 106-116-098) for 30 minutes. The beads were

then washed with $3 \times 60 \mu\text{l}$ PBS-Tween and re-suspended in $50 \mu\text{l}$ PBS-Tween, and the plates were then analyzed with a FlexMap3DTM instrument (Luminex Corp.). Beads were validated on positive control plasma or serum samples with known reactivity patterns derived from subjects with confirmed prior SARS-CoV-2 infection and/or autoimmune diseases (29). Healthy control sera were obtained by the Stanford Biobank prior to the pandemic.

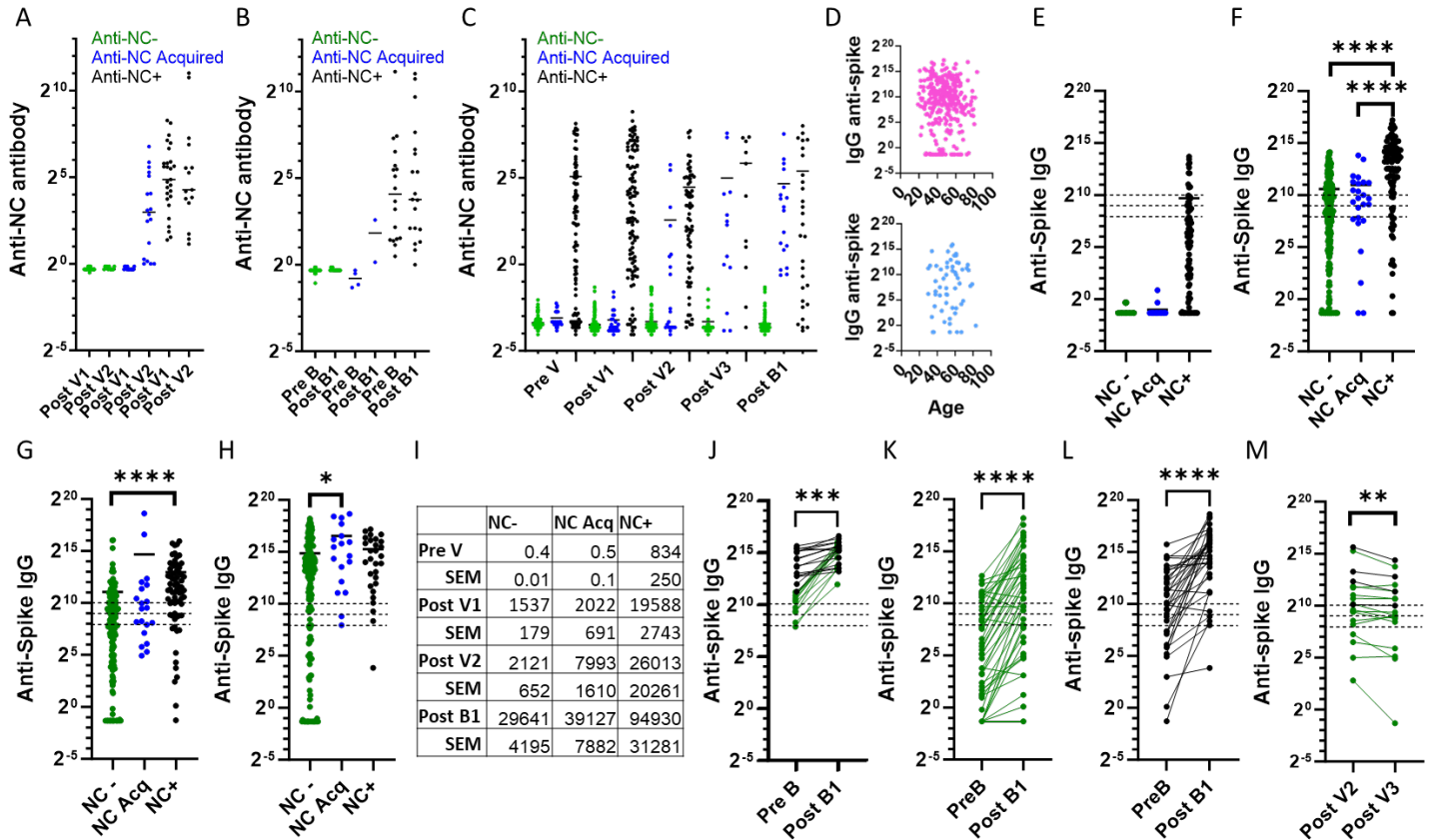
Unbiased predictive models of anti-Spike antibody and T cell response

Linear regression modeling was used to determine correlations of anti-spike IgG values, CD4 T cell % AIM, or CD8 T cell % AIM at the Post V1 visit with other serological and clinical variables.

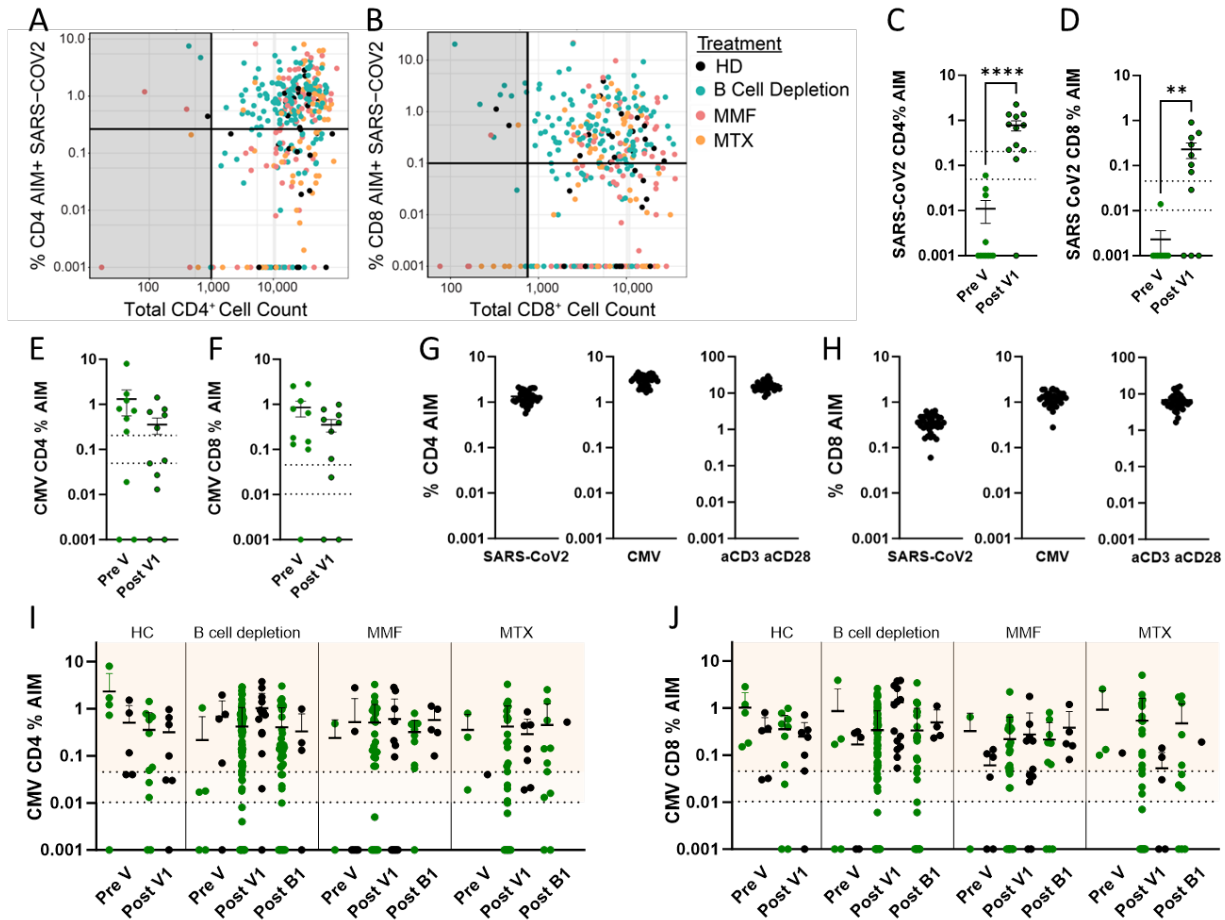
Preprocessing: The variables considered for inclusion in the models were: ethnicity, race, age, sex, autoimmune diagnosis, comorbid disease (diabetes, hypertension, coronary artery disease, multiple comorbidities, other, or none), BMI, disease activity severity (inactive, mild, moderate or severe), medications, months since last B cell depleting agent, total number of doses of B cell depleting agent, COVID exposure, number of weeks since full COVID-19 vaccination, vaccine type, MMF/MPA level, flare in disease activity requiring an increase or change in medication, mean corticosteroid dose since last visit, cytokine levels (IFN α 1,2,6,7, IFN γ , IL-17A, IL-17F, GM-CSF, TNF α , IL-6, CXCL10), anti-NC, anti-spike IgG, CD4 T cell level, and CD8 T cell level. Certain medications were considered together: B cell inhibition (ocrelizumab, obinutuzimab, ofatumumab, rituximab), TNF inhibitors (adalimumab, certolizumab, etanercept, golimumab, infliximab), IL-12/23 and IL-17A inhibitors (ustekinumab, secukinumab), Jak inhibitors and IL-6 receptor antagonist (tocilizumab), and MMF/MPA. Variables with over 50% missing data were excluded from the dataset. The log₂ of the anti-spike IgG and T cell values

were used. KNN imputation was performed on all non-one-hot-encoded data to increase the amount of data points available for training and testing.

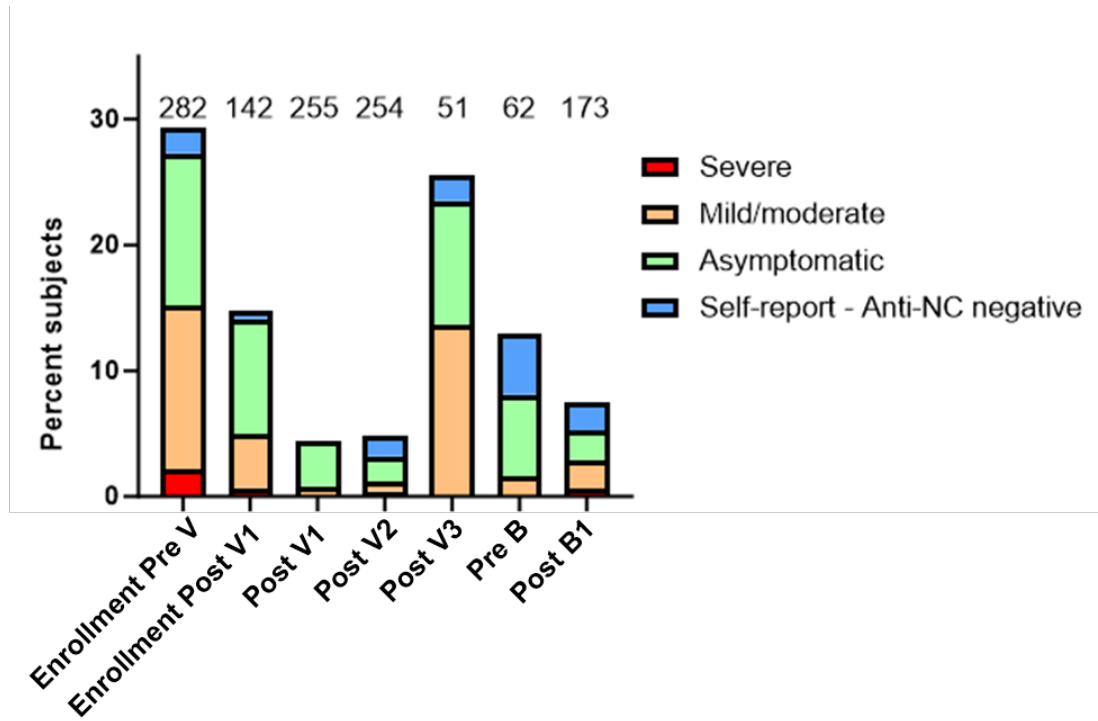
Model building: A linear regression model was used to determine correlations between the serological or clinical variables and the three outcomes of interest (anti-spike IgG values, CD4 T cell % AIM, or CD8 T cell % AIM). 70% of the data was used as a training set, of which 30% was used as a validation set. The remaining 30% of the data was used as a test set. The variables were added into the model sequentially. If including a new variable improved the performance of the linear regression model, as measured by the R^2 value, the new variable was kept in the final model. If including a new variable did not improve the model's performance, it was removed from the dataset. The final performance of the model was evaluated on a validation data set that included thirty percent of the data the testing data that was set aside prior to building the model. The model's accuracy was measured through evaluating the R^2 value. The coefficients of every variable included in the model were also reported.



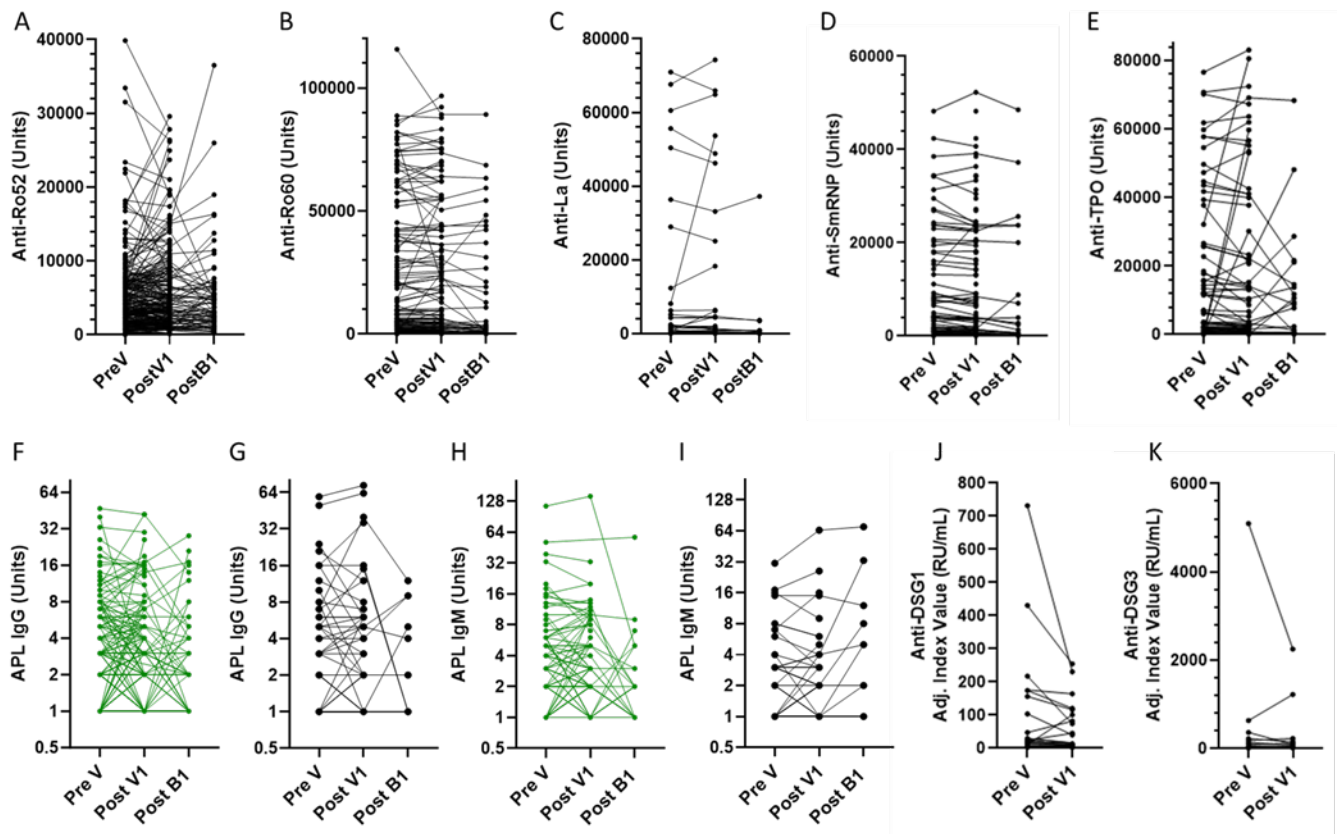
Supplementary Figure 1: SARS-CoV2 Exposure Status in Autoimmune Subjects and Healthy Controls, and Relationship to Serological Response to SARS-CoV2 Vaccination. A, B: Anti-NC values (U/mL) in healthy controls at each visit after the initial (A) and booster (B) vaccination. C: Anti-NC values in autoimmune subjects at each visit before and after the initial vaccination, and after booster vaccination. D: Correlation of anti-spike IgG values (U/mL) with age in female (pink) and male (blue) autoimmune subjects; $p=NS$. E-H: Anti-spike IgG values according to anti-NC status in autoimmune subjects at the visit before the initial vaccine series (E - Pre V), at Post V1 (F), at Post V2 (G) and after booster vaccination (H - Post B1). I: Mean + SEM of anti-spike IgG values in autoimmune subjects according to anti-NC status as shown in D-G. J: Trajectory of anti-spike IgG values and after booster vaccination in anti-NC+ (black) and anti-NC- (green) healthy controls. K-L: Trajectory of anti-spike IgG values after booster vaccination (K) in anti-NC- (green) and anti-NC+ (black) autoimmune subjects. M: Trajectory of anti-spike IgG values between Post V2 and Post V3 (M) in anti-NC- (green) and anti-NC+ (black) autoimmune subjects. E-H, J-M: Dotted lines indicate IgG anti-spike values of 250, 500 and 1000. E-H: Kruskal-Wallis ANOVA with Dunn's correction for multiple comparisons. J-M: Wilcoxon matched pairs rank test; differences between anti-NC+ vs. anti-NC- subjects evaluated using Mann Whitney t test (see text). * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.



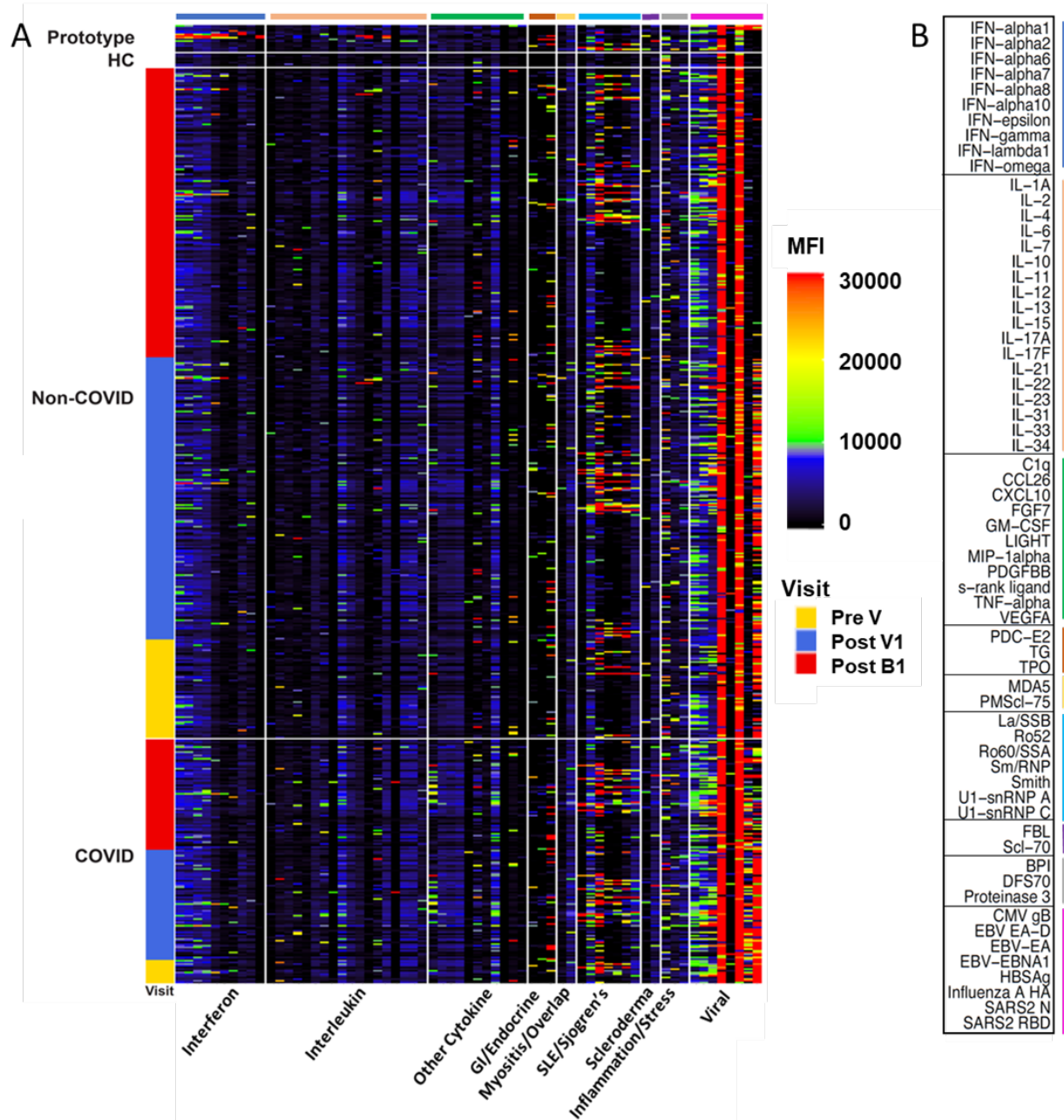
Supplementary Figure 2. AIM Assay Quality Controls. *A-B:* Dot plot colored by treatment group comparing total CD4 (*A*) and CD8 (*B*) counts vs % AIM+ after SARS-CoV-2 peptide pool stimulation. To limit false positives/negatives, samples with fewer than 1,000 CD4 (2.85%) and 750 CD8 T (6.55%) cells were removed from subsequent analysis. *C-F:* CD4 (*C*, *D*) and CD8 (*E*, *F*) AIM+ frequency after SARS-CoV2 (*C*, *D*) or CMV (*E*, *F*) peptide pool stimulation of Pre V and Post V1 samples from anti-NC- healthy donors post vaccination. Positive SARS-CoV2 response thresholds, represented as dotted lines, were calculated using the uninfected Pre V group ($n=11$ for CD4 and $n=10$ for CD8) as follows: mean + 2*SD and mean + 10*SD. Statistical significance was determined by unpaired two-tailed t test. ** $p < 0.01$, **** $p < 0.0001$. *G*, *H:* CD4 (*G*) and CD8 (*H*) AIM frequencies after the indicated stimulation showing consistency of a single deidentified healthy donor included in each run of the AIM assays. Data represent mean $\pm$ SEM ($n = 37$). *I*, *J:* No differences in CD4 (*I*) and CD8 (*J*) AIM frequencies in response to CMV peptide pool stimulation at sequential visits according to SARS-CoV2 exposure and medication. Each data point represents an individual subject.



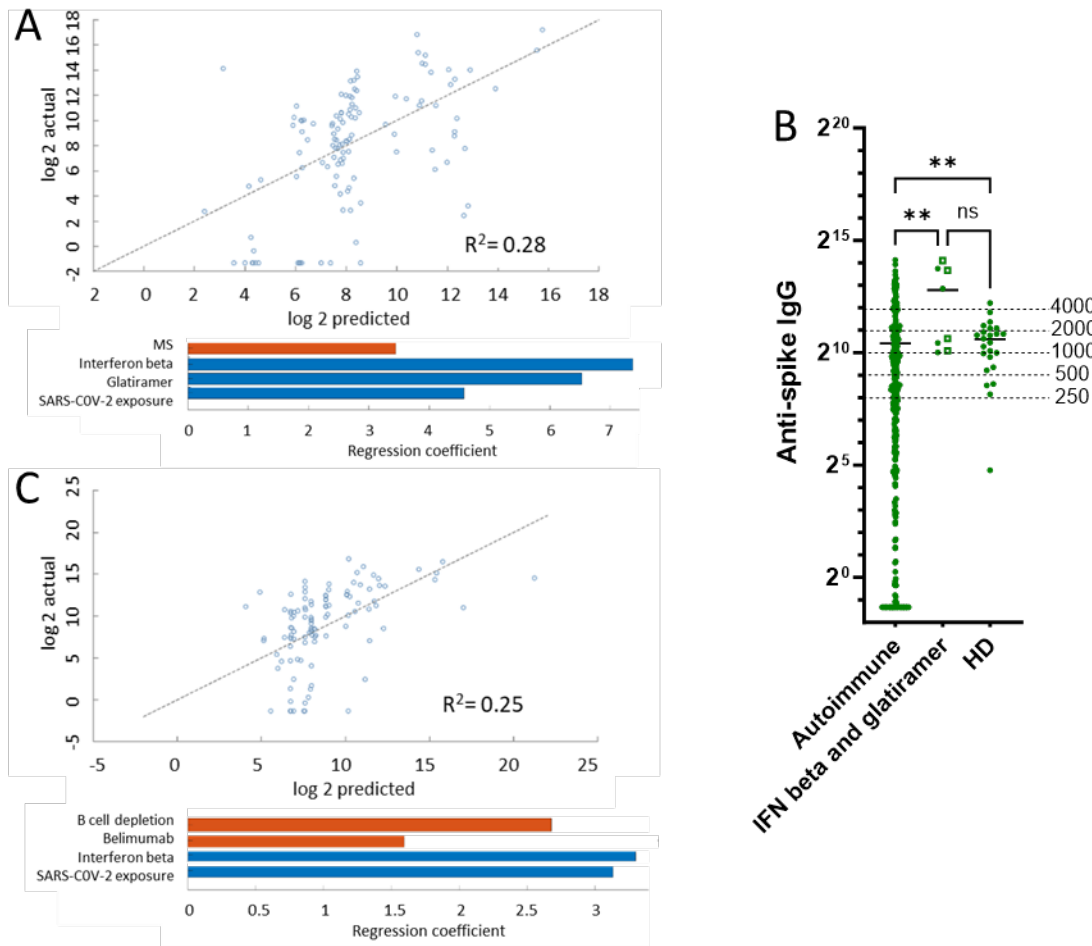
Supplementary Figure 3. Breakthrough infection frequency and severity at each visit. Frequency and severity of infections acquired since the previous visit are shown in each bar as a percentage of subjects with available data at each visit. The subject number for each visit is shown above each bar.



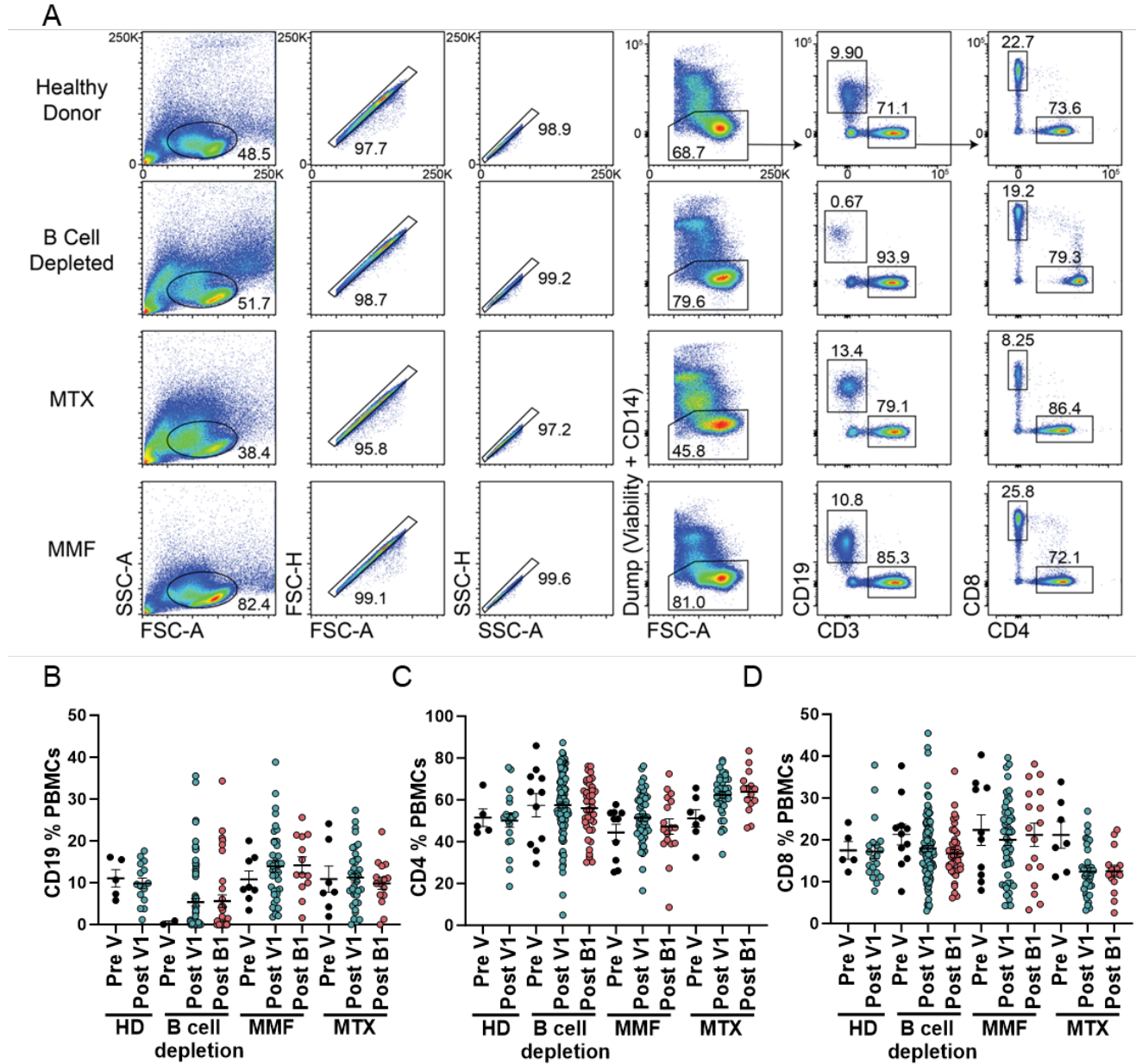
Supplementary Figure 4: Autoantibodies to selected analytes *A-E*: Line plots showing changes in MFI levels for 5 analytes in matched samples from 241 vaccinated subjects with a range of autoimmune diseases. *B-D*: Autoantibodies against Ro60/SSA, La/SSB, and Smith demonstrate stable MFI levels throughout the vaccine series. *E*: Newly detected anti-TPO autoantibodies in a small subset of subjects. *F-I*: Anti-cardiolipin IgG (*F*, *G*) and IgM (*H*, *I*) autoantibodies in matched samples from 186 anti-NC- (green) and 63 anti-NC+ (black) subjects. Values >10 are considered positive. *J*, *K*: Anti-desmoglein1 (*J*) and 3 (*K*) autoantibodies in matched samples from 23 subjects with pemphigus. Each connected set of data points represents 1 subject. $P = NS$ for all comparisons performed using Kruskal-Wallis ANOVA.



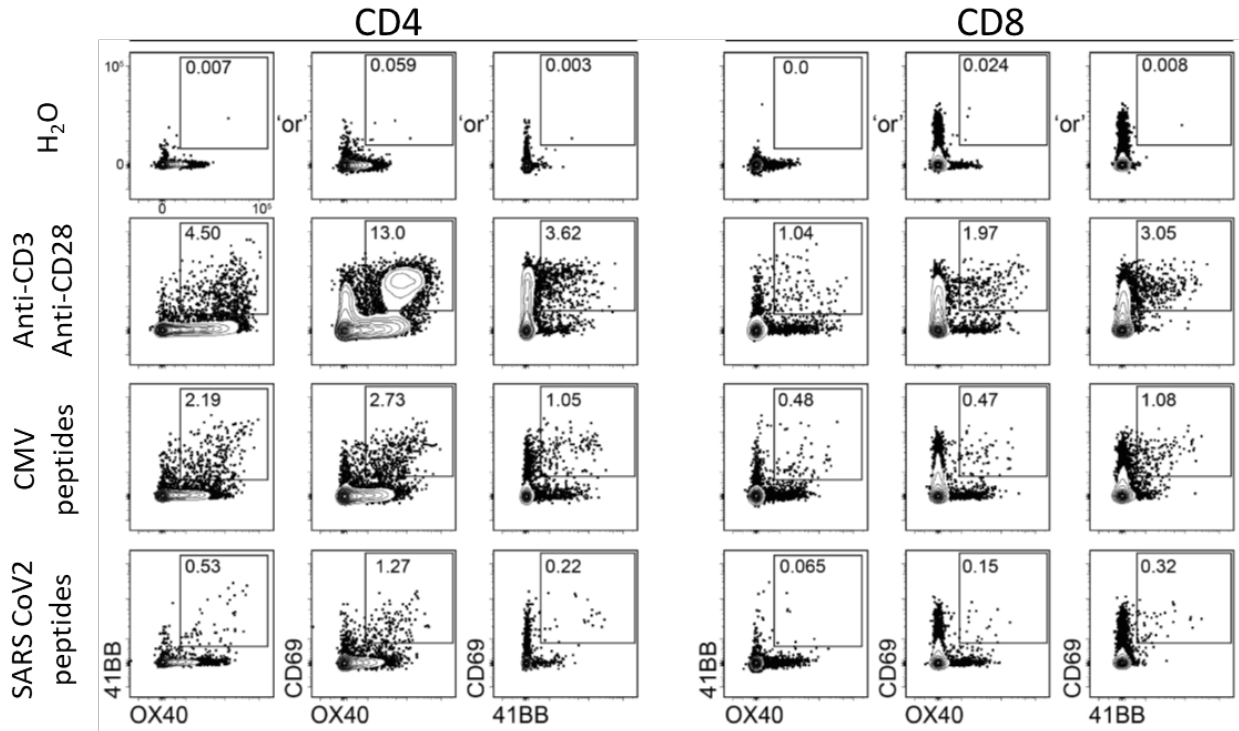
Supplementary Figure 5. Heatmap representing serum IgG detected with an 83-plex array of cytokines and chemokines, traditional autoimmune-associated antigens, and viral antigens. 239 vaccinated autoimmune subjects are represented, grouped by whether they were infected with SARS-CoV-2 by the Post V1 visit. Representative data from 16 prototype samples and 8 healthy control subjects are included. Within each group, samples are clustered and annotated by the visit at which the sample was taken (Pre V, yellow; Post V1, blue; Post B1, red). Analytes that were not cross-reactive and had a value above 5000 MFI are shown. Analytes in each group of antigens are color coded and individual antigens in each group of antigens are shown in B.



Supplementary Figure 6. Unbiased predictive models of anti-spike IgG response. Models including an autoimmune diagnosis (A) or excluding an autoimmune diagnosis (C) are shown together with the top 4 variables contributing to each model. Orange bars indicate variables associated with lower anti-spike IgG values and blue bars indicate variables associated with higher anti-spike IgG values. B: Anti-spike IgG values in subjects treated with IFN beta (circles) or glatiramer (squares) are compared with those from other autoimmune subjects and healthy controls. Kruskal-Wallis ANOVA with Dunn's correction for multiple comparisons. ** $p < 0.01$.



Supplementary Figure 7. Flow Cytometry Gating and Immune Cell Frequencies. *A: Flow gating strategy for classifying CD4⁺ and CD8⁺ cells for each treatment group and timepoint. B-D: Quantified frequency of CD19 (C), CD4 (D) and CD8 (E) populations depicted as frequency of total live lymphocytes for each treatment group. Each data point reflects a single subject. Data represent mean $\pm$ SEM. $p=NS$ for all comparisons performed using Kruskal- Wallis ANOVA.*



Supplementary Figure 8. Boolean OR AIM Gating. Boolean logic OR flow gating strategy implemented to determine the % CD4 (A) and % CD8 (B) AIM+ frequency for each of the indicated stimulation conditions as follows: CD137+OX40+ OR CD137+CD69+ OR OX40+CD69+.

Supplementary Table 1: Response rates to vaccination and booster in autoimmune subjects and healthy controls

	Post V1						Post B1				
*		AI (397)	AI %	HC (67)	HC %	*		AI (211)	AI %	HC (34)	HC %
Anti-NC- (270)	<250	122	45.1	1/24	1.5	(159)	<250	45	28.3	0/13	0
							<4000	67	42.1	0/13	0
Anti-NC acquired (25)	<250	8	32	0/16	0						
Anti- NC+ (102)	<250	16	15.7	0/27	0	(52)	<250	4	7.7	0/21	0
	<200 0	26	25.5	0/27	0		<4000	14	27.5	0/21	0

AI: autoimmune subjects; HC: healthy controls

*Numbers of autoimmune subjects in each group

Supplementary Table 2. Medication Use Associated with Anti-Spike Antibody and Anti-NC Status

	Post V1				Post-Booster			
	Responder		Non-Responder		Responder		Non-Responder	
Medication (Totals for each category in blue)	Anti-NC-	Anti-NC+	Anti-NC-	Anti-NC+	Anti-NC-	Anti-NC+	Anti-NC-	Anti-NC+
B cell depletion alone	24	2	45	6	23	2	36	3
rituximab (RTX)	19 ⁵	2	15	2	15	2	11	2
ocrelizumab (Ocr)	5	-	29	4	7	-	23	1
obinutuzumab	-	-	1	-	1	-	1	-
ofatumumab	-	-	-	-	-	-	1	-
B cell deletion + other drug	2	3	9	5	4	0	0	1
RTX + HCQ	-	1	2	1	1	-	-	-
RTX + MMF	-	-	1	-	-	-	-	-
RTX + MMF + HCQ	2 ²	1 ¹⁸	1 ¹⁹	1	3 ^{ff}	-	-	-
RTX + MMF + other	-	-	-	1 ^q	-	-	-	-
RTX + azathioprine	-	1 ⁶	-	-	-	-	-	-
RTX + azathioprine + HCQ	-	-	-	1	-	-	-	-
RTX + methotrexate	-	-	1	-	-	-	2	-
RTX + other	-	-	4 ^m	1	-	1	4 ⁿⁿ	-
RTX + other + HCQ	-	-	-	-	-	-	-	-
Ocr + methotrexate (MTX)	-	-	-	-	-	-	-	1
Mycophenolic acid or mycophenolate mofetil	23	21	33	0	19	8	8	0
Mycophenolic acid (MPA)								
alone	-	-	1	-	2 ²³	-	-	-
+ HCQ	-	1 ⁴	-	-	-	-	-	-
+ belimumab	-	-	-	-	1 ^{cc}	-	-	-
+ other	-	-	1 ^{ll}	-	1 ^{ll}	-	-	-
Mycophenolate mofetil (MMF)								
alone	5 ¹⁰	6 ¹⁷	6 ¹	-	3	2	2 ²⁵	-
+ HCQ	14 ¹⁶	13 ¹⁵	12 ¹⁴	-	9 ²¹	6 ²⁰	2	-
+ belimumab	-	-	3	-	1 ^{kk}	-	2 ²¹	-
+ belimumab + HCQ	1	-	4	-	-	-	2	-
+ belimumab + methotrexate + HCQ	-	-	1	-	-	-	-	-
+ belimumab + other	-	-	1	-	-	-	-	-
+ methotrexate + HCQ	-		1 ³	-	-	-	-	-
+ other	1 ^a	-	-	-	-	-	-	-
+ other + HCQ	2 ^o	1 ⁱ	3 ^j	-	2 ^{ee}	-	-	-
Methotrexate (MTX)	27	9	13	0	11	4	5	0

alone	13 ⁷	1	6 ⁹	-	5 ²²	2	2	-
+ HCQ	3 ⁸	1	2 ¹³	-	2 ^{dd}	-	1	-
+ TNFi	7 ^d	4 ^h	2 ^r	-	1	1 ^r	2 ^{hh}	-
+ TNFi + HCQ	1 ^r	-	-	-	-	1 ^r	-	-
+ belimumab	-	-	1 ¹²	-	-	-	-	-
+ belimumab + HCQ	1 ¹¹	1	-	-	-	-	-	-
+ ocrelizumab	-	1	-	-	-	-	-	-
+ other	2 ^e	1 ⁱ	2 ^f	-	3 ^{aa}	-	-	-
Belimumab	4	2	7	0	5	0	0	1
alone	1	-	1	-	-	-	-	1 ²⁴
+ HCQ	3	2	5	-	5	-	-	-
+ azathioprine + HCQ	-	-	1	-	-	-	-	-
TNF inhibitors	5	2	3	2	2	1	1	0
alone	4 ^u	2 ^r	1 ^q	1 ^t	2 ^{gg}	1 ^t	-	-
+ HCQ	1 ^w	-	-	-	-	-	-	-
+ other	-	-	1 ^r	-	-	-	1 ^y	-
Azathioprine (AZA)	7	4	5		5	6	0	1
alone	3	2	2	-	2	2	-	-
+ HCQ	4	1	1	-	2	1	-	1 ^{pp}
+ HCQ + other	-	1 ⁱ	2 ^p	-	1 ⁱⁱ	1 ^c	-	-
HCQ	38	19	7	5	11	6	1	1
alone	36	18 [*]	6	5	11 ^{jj}	6	1	1
+ other	2 ^s	1	1 ^y	-	-	-	-	-
Other	18	8	8	3	13	5	15	3
alone	18 ^l	8 ^k	8 ^v	2 ^f	13 ^{bb}	5 ^b	15 ^{oo}	3 ^{mm}
+ abatacept	-	-	-	1 ^z	-	-	-	-
None	14	5	2	0	3	0	1	0
TOTAL	162	75	131	20	96	31	73	10

a. voclosporin (1)

b. tacrolimus (1), interferon beta (1), secukinumab (1), tocilizumab (1), fingolimod (1)

c. clinical trial drug (1)

d. adalimumab (5), golimumab (1), etanercept (1)

e. JAK inhibitor (1), ustekinumab (1); 1 subject in which methotrexate was held and ustekinumab was continued (R131); 1 subject in which methotrexate was held and JAK inhibitor continued (R023)

f. abatacept; 1 subject in which both methotrexate and abatacept was held (R011); 1 subject in which abatacept was held (R107)

g. golimumab (1)

h. certolizumab (1), etanercept (3); 1 subject with both methotrexate and etanercept held for vaccine dosing (F701); 1 subject in which methotrexate was stopped but etanercept continued (R001)

i. clinical trial drug (baricitinib) – this subject was non-adherent to MMF (B051)

j. tacrolimus (2), other (1)

k. fingolimod (1), glatiramer acetate (1), interferon beta (1), secukinumab (1), tocilizumab (1), cladribine (1), JAK inhibitor (1), other (1)

l. dimethyl fumarate (1), glatiramer acetate (3), interferon beta (5), natalizumab (1), other (9)

m. cyclophosphamide IVSS (1), other (3)

n. other (1)

o. IVIG (1), other (1); 1 subject who was on "other medication" (colchicine) was non-adherent to MMF (F457)

p. abatacept (1), cyclophosphamide IVSS (1); 1 subject in which abatacept was held and both azathioprine and hydroxychloroquine were continued (R093)

q. golimumab

r. adalimumab; 1 subject in which methotrexate was stopped and adalimumab was held at Post V1 (R008); 1 subject in which methotrexate was held and adalimumab and hydroxychloroquine were continued at Post V1 (F691)

s. dapsone

t. infliximab

u. adalimumab (3), infliximab (1)

v. fingolimod (5), tacrolimus (1), cyclosporine (1), other (1)

w. certolizumab

x. IVIG

y. leflunomide

z. leflunomide + abatacept (abatacept held for vaccine dosing)

aa. abatacept (1), ustekinumab (1), 1 subject in which JAK inhibitor started at Post V2 and adalimumab stopped at Post V2

bb. glatiramer acetate (4), interferon beta (3), mepolizumab (1), leflunomide (2), teriflunomide (1), natalizumab (1); 1 subject for which abatacept was stopped prior to booster dosing

cc. 1 subject in which mycophenolic acid was started at Post V1 and then held for booster dosing

dd. 1 subject in which both methotrexate and HCQ were held for booster dosing

ee. 1 subject non-adherent to MMF, and clinical trial drug started at Post V3; 1 subject non-adherent to MMF and on tacrolimus

ff. 1 subject non-adherent to MMF, and received a mean steroid dose of 400 mg prednisone equivalent between Pre V and Post V1

gg. adalimumab (1), infliximab (1)

hh. adalimumab (2), 1 of these subjects had been on belimumab at Post V1 that was stopped, and adalimumab was started

ii. cyclophosphamide IVSS (1)

jj. 1 subject in which HCQ was held for booster dosing; 1 subject who had also been on belimumab that was stopped at Post V2

kk. 1 subject in which MMF was held for booster dosing, belimumab was started between the Pre B and Post B1 visit, and was on prednisone 60 mg daily at the time of booster dosing

ll. tacrolimus

mm. fingolimod (2), cladribine (1)

nn. eculizumab (1), leflunomide (1); 1 subject with apremilast started at Post V1; 1 subject also received cyclophosphamide IVSS

oo. fingolimod (11), interferon beta (2), glatiramer acetate (1), tocilizumab (1)

pp. subject had also been on belimumab that was stopped at Post V2

1. 1 subject was MMF non-adherent and on an equivalent dose of prednisone ≥ 30 mg; 1 subject in which MMF was held (F694)
2. 1 subject on an equivalent dose of prednisone ≥ 30 mg, and was non-adherent to MMF (F323)
3. 1 subject in which MMF and hydroxychloroquine were stopped, and methotrexate continued, also on an equivalent dose of prednisone ≥ 30 mg (500497)
4. subject on an equivalent dose of prednisone ≥ 30 mg
5. 1 subject on an equivalent dose of prednisone ≥ 30 mg
6. azathioprine held for vaccine dosing
7. 3 subjects in which methotrexate was held (R118, F677, R038)
8. 1 subject in which methotrexate was held and hydroxychloroquine was continued (R105)

9. 1 subject in which methotrexate was held (F717)
10. 2 subjects in which MMF was held (R148, ACE-P-012)
11. 1 subject in which belimumab was held, and methotrexate and hydroxychloroquine were continued (F158)
12. belimumab was stopped and adalimumab started and taken between the 1st and 2nd Pfizer vaccines, while methotrexate was continued (500940)
13. 1 subject in which methotrexate was held and hydroxychloroquine was continued (F355)
14. 1 subject in which MMF was held and hydroxychloroquine was continued (F692)
15. 5 subjects were non-adherent to MMF
16. 3 subjects were non-adherent to MMF
17. 1 subject was non-adherent to MMF (E3588)
18. subject was non-adherent to MMF (B028)
19. 1 subject was non-adherent to MMF (E3702)
20. 2 subjects non-adherent to MMF
21. 1 subject non-adherent to MMF
22. 2 subjects in which MTX was held for booster dosing
23. 1 subject in which MPA held for booster dosing
24. 1 subject on prednisone 30 daily at the time of booster dosing
25. 1 subject in which MMF was held for booster dosing

*1 subject who was ANA positive, without a clear autoimmune diagnosis, and was started on hydroxychloroquine before the Post V1 visit

** 9 subjects who received B cell depletion alone, with the last dose > 60 months prior to vaccination, were excluded from this table

Supplementary Table 3: Medication Use Associated with Anti-Spike Antibody Response in Subjects with both a Post V1 and a Post B1 Visit

	Double Non-Responder (n=36)		Single Non-Responder (n=41)		Responder (n=76)	
Medication (Totals for each category in blue)	Anti-NC-	Anti-NC+	Anti-NC-	Anti-NC+	Anti-NC-	Anti-NC+
B cell depletion alone	18	2	9	1	13	-
rituximab (RTX)	4	1	4	1	11	-
ocrelizumab (Ocr)	13 [†]	1	5	-	2	-
obinutuzumab	1	-	-	-	-	-
B cell depletion + other drug	4	2	1	1	3	0
RTX + MMF + HCQ	-	-	-	-	2 ^{!!!}	-
RTX + belimumab + MMF + HCQ	-	1 [⊥]	1 ^{##}	-	-	-
RTX + cyclophosphamide IVSS	1 [†]	-	-	-	-	-
RTX + eculizumab	1	-	-	-	-	-
RTX + methotrexate	1	-	-	-	-	-
RTX + leflunomide	-	-	-	-	1	-
RTX + leflunomide + apremilast	1	-	-	-	-	-
RTX + IVIG	-	-	-	1 ^{\$\$}	-	-
Ocr + methotrexate (MTX)	-	1	-	-	-	-
Mycophenolic acid or mycophenolate mofetil	4	1	9	2	8	6
Mycophenolic acid (MPA)						
alone	-	-	1	-	-	-
+ tacrolimus	-	-	1	-	-	-
Mycophenolate mofetil (MMF)						
alone	1	-	-	-	4 ⁺	1
+ HCQ	-	-	4 ^{#,1}	1 ^{\$\$}	4 ^{***}	5 ^{**}
+ belimumab	2 [†]	-	-	-	-	-
+ belimumab + HCQ	1	-	2 [@]	-	-	-
+ belimumab + MPA	-	-	1	-	-	-
+ tacrolimus + HCQ	-	-	-	1	-	-
Belimumab	0	1	2	0	3	0
+ HCQ	-	-	2	-	3 ⁺	-
+ azathioprine + HCQ	-	1 ^{!!}	-	-	-	-
Methotrexate (MTX)	0	0	7	1	6	2
alone	-	-	4	-	2 ^{&}	-
+ HCQ	-	-	1 ^{@@}	1 ^{⊥⊥}	1	-
+ abatacept	-	-	1 ^{\$}	-	-	-
+ adalimumab	-	-	-	-	2	1 ^{\$\$}
+ adalimumab + HCQ		-	-	-	-	1 ^{&&}

+ ustekinumab	-	-	-	-	1	-
+ adalimumab + Jak inhibitor	-	-	1 [!]	-	-	-
TNFi	1	0	0	0	2	1
infliximab	-	-	-	-	1	1 ^{\$\$}
+ sulfasalazine	-	-	-	-	1 ^{^^}	-
+ leflunomide	1	-	-	-	-	-
Azathioprine (AZA)	0	0	3	0	2	2
alone	-	-	1	-	1	1
+ HCQ	-	-	1	-	1	-
+ cyclophosphamide IVSS + HCQ	-	-	1 ¹	-	-	-
+ HCQ + other	-	-	-	-	-	1 ^{&&&}
Hydroxychloroquine alone	-	-	1	1	9 ⁺⁺	7
Cladribine	-	-	-	-	-	1
Fingolimod	4	-	1	-	-	-
Glatiramer acetate	-	-	-	-	4	-
Interferon-beta	-	-	-	-	5	1
Mepolizumab	-	-	1	-	-	-
Natalizumab	-	-	-	-	1	-
Tacrolimus alone	-	-	-	1 ^{\$\$}	-	-
TOTAL	31	5	34	7	56	20

1 subject non-adherent to MMF at the Post V1 visit

⊥ 1 subject was on 30 mg of prednisone at the Pre V visit

* 2 subjects on MMF only at Post B1, not Post V1

@ 1 subject only on MMF at Post V1, and only on belimumab at Post B1 (MMF held); this subject was also on 60 mg of prednisone at Post V1

\$ MTX and abatacept held at Post V1

! Jak-inhibitor added at Post V2; adalimumab stopped at Post V2

& 1 subject held MTX at booster

only on belimumab at Post V1; on rituximab and MMF at Post B1; this subject was also on 30 mg of prednisone at the Pre V visit

^ 1 subject on belimumab only at Post V1, not at Post B1 (stopped)

** 2 subjects non-adherent to MMF at the Post V1 visit, 1 of these subjects also had a breakthrough infection

!! belimumab held for booster dosing; HCQ and azathioprine continued; also had breakthrough infection

@@ MTX held at Post V1 and at booster; HCQ continued at Post V1 and held at booster

&& MTX held at Post V1; subject also with breakthrough infection

^^ on adalimumab only at the Post V1 visit

&&& clinical trial drug

+ belimumab stopped at Post V2

++ 1 subject with HCQ held at booster visit

*** 3 subjects non-adherent to MMF at the Post V1 visit

!!! 1 subject non-adherent to MMF at the Post V1 visit; also received a mean of 400 mg prednisone between the Pre V and Post V1 visits

⊥⊥ 1 subject on HCQ alone at the Post V1 visit, and was on HCQ and methotrexate at the Post B1 visit

1. 1 subject with breakthrough infection, anti-NC negative

\$\$ also, with breakthrough infection

Supplementary Table 4: Medications Held/Stopped According to Anti-Spike Antibody and Anti-NC Status

	Post V1				Post-Booster			
	Responder		Non-Responder		Responder		Non-Responder	
	Anti-NC-	Anti-NC+	Anti-NC-	Anti-NC+	Anti-NC-	Anti-NC+	Anti-NC-	Anti-NC+
Rituximab + other drug								
+ azathioprine		AZA held						
Mycophenolic acid (MPA)								
alone					MPA held			
+ belimumab					MPA held			
Mycophenolate mofetil (MMF)								
alone	MMF held (2)		MMF held				MMF held	
+ HCQ			MMF held		MTX and HCQ held			
+ belimumab					MMF held			
+ methotrexate + HCQ			MMF and HCQ stopped					
Methotrexate (MTX)								
alone	MTX held (3)		MTX held		MTX held (2)			
+ HCQ	MTX held (1)		MTX held					
+ TNFi		MTX and etanercept held (1); MTX stopped but etanercept continued				MTX stopped and adalimumab held		
+ TNFi + HCQ						MTX held		
+ belimumab			belimumab stopped					
+ belimumab + HCQ	belimumab held							
+ other	MTX held (2)		MTX and abatacept held					
Azathioprine (AZA)								
+ HCQ + other			abatacept held					
HCQ								
alone					HCQ held			
Other								
alone				abatacept held	abatacept stopped			

+ abatacept				abatacept held				
SUMMARY	MTX held (6); MMF held (2); belimumab held (1)	MTX and etanercept held (1), MTX stopped (1); AZA held (1)	MTX held (2); MTX and abatacept held (1); MMF held (2); MMF and HCQ stopped (1); belimumab stopped (1); abatacept held (1)	abatacept held (2)	MTX held (2), MTX and HCQ held (1); MPA held (2); MMF held (1); HCQ held (1); abatacept stopped (1)	MTX stopped and adalimumab held (1); MTX held (1)	MMF held (1)	none

Supplementary Table 5: Names of other drugs (besides B cell depletion, MMF or MPA, or methotrexate) taken by subjects who had T cell assays performed

Medications	Number of Subjects
azathioprine, cyclophosphamide, hydroxychloroquine	1
azathioprine, belimumab, hydroxychloroquine	1
azathioprine alone	6
azathioprine, hydroxychloroquine	8
belimumab, hydroxychloroquine	9
belimumab alone	1
adalimumab	1
adalimumab	2
fingolimod	1
golimumab	1
infliximab	1
leflunomide	1
mepolizumab	1
hydroxychloroquine alone	2
etanercept	1
interferon-beta	1
glatiramer acetate	1
TOTAL	39

Supplementary Table 6: Vaccine Received, Disease Diagnosis, and Flare Severity

Diagnosis	Visit	Flare Severity	Vaccine Received
Inflammatory Myositis	Post V1	Moderate	Johnson and Johnson
Vasculitis	Post V1	Moderate	Pfizer
Rheumatoid Arthritis	Post V1	Mild	Pfizer
Rheumatoid Arthritis	Post V1	Moderate	Pfizer
Inflammatory Myositis	Post V1	Moderate	Moderna
Scleroderma, Interstitial Lung Disease	Post V1	Severe	Pfizer
Rheumatoid Arthritis	Post V1	Mild	Pfizer
Mixed Connective Tissue Disease	Post V1	Severe	Pfizer
Eosinophilic Granulomatosis with Polyangiitis (EGPA)	Post V1	Mild	Pfizer
Systemic Lupus Erythematosus	Post V1	Mild	Pfizer
Rheumatoid Arthritis	Post V1	Moderate	Pfizer
Rheumatoid Arthritis	Post V1	Moderate	Johnson and Johnson
Pemphigus Foliaceus	Post V1	Mild	Pfizer
Systemic Lupus Erythematosus	Post V1	Moderate	Johnson and Johnson
Systemic Lupus Erythematosus	Post V1	Moderate	Pfizer
Systemic Lupus Erythematosus	Post V1	Moderate	Pfizer
Systemic Lupus Erythematosus	Post B1	Mild	Unknown
Systemic Lupus Erythematosus	Post B1	Mild	Pfizer
Pemphigus Vulgaris	Post B1	Moderate	Moderna
Pemphigus Vulgaris	Post B1	Moderate	Pfizer
Systemic Lupus Erythematosus	Post B1	Severe	Moderna
Systemic Lupus Erythematosus	Post B1	Moderate	Pfizer
Systemic Lupus Erythematosus	Post B1	Moderate	Pfizer
Systemic Lupus Erythematosus	Post B1	Moderate	Pfizer
Systemic Lupus Erythematosus	Post B1	Severe	Moderna
Rheumatoid Arthritis	Post B1	Severe	Moderna
Rheumatoid Arthritis	Post B1	Mild	Pfizer
Rheumatoid Arthritis	Post B1	Moderate	Pfizer
Rheumatoid Arthritis	Post B1	Moderate	Moderna
Psoriatic Arthritis	Post B1	Moderate	Pfizer
Sjogren's Syndrome	Post B1	Mild	Pfizer
Inflammatory Myositis	Post B1	Moderate	Pfizer
Ulcerative Colitis/Enteropathic Colitis	Post B1	Mild	Pfizer
Rheumatoid Arthritis	Post B1	Mild	Moderna
Systemic Lupus Erythematosus	Post B1	Mild	Moderna

Supplementary Table 7: Flares

Post V1 Vaccine Type	Subjects with Flare (n=16)	Subjects without Flare (n=235)	p
Pfizer	12 (75%)	160 (68%)	p=NS
Moderna	1 (6%)	60 (25%)	
Johnson and Johnson	3 (19%)	11 (5%)	
Unknown	0	4 (2%)	
Post B1 Vaccine Type	Subjects with Flare (n=19)	Subjects without Flare (n=187)	p
Pfizer	11 (58%)	105 (56%)	p=NS
Moderna	7 (37%)	66 (35%)	
Johnson and Johnson	0	3 (2%)	
Unknown	1 (5%)	13 (7%)	

Supplementary Table 8: Breakthrough infections

Visit	Medication 1	Medication 2	Diagnosis	Infection	Anti-NC	Anti-Spike IgG at prior visit
Post V2	ocrelizumab		MS	Symptomatic	Neg	152
Post V2	MTX	abatacept	RA	Symptomatic	Neg	46.8
Post V2	RTX	CTX	ANCA+ Vasculitis	Symptomatic	Neg	1.24
Post V2	MMF	HCQ	SLE	Asymptomatic	Pos	158
Post V2	HCQ		SLE	Symptomatic	Pos	2205
Post V2	MTX		RA	Asymptomatic	Pos	445
Post V2	HCQ	prednisone 30mg	SLE	Symptomatic	Pos	839
Post V2	RTX		RA	Hospitalized	Pos	0.4
Post V2	RTX	IVIg	CVID	Asymptomatic	Pos	188
Post V2	azathioprine	belimumab	SLE	Symptomatic	Pos	0.4
Post V2	IVIg		SLE	Asymptomatic	Pos	3680
Post V2	RTX	MMF	RA	Symptomatic	Pos	2.2
Post V3	MMF	HCQ	SLE	Symptomatic	Neg	343
Post V3	RTX		Pemphigus	Symptomatic	Pos	39.7
Post V3	MMF	HCQ	SLE	Asymptomatic	Pos	5220
Post V3	HCQ		SLE	Symptomatic	Pos	241
Post V3	MMF	voclosporin	SLE	Asymptomatic	Pos	4150
Post V3	Dapsone	HCQ	SLE	Symptomatic	Pos	162
Post V3	adalimumab		PsA	Asymptomatic	Pos	824
Post V3	MTX		RA	Asymptomatic	Pos	1410
Post V3	azathioprine		Scleroderma/ILD	Symptomatic	Pos	652
Post V3	belimumab		SLE	Symptomatic	Pos	2655
Post V3	None		SLE	Asymptomatic	Pos	1030
Post V3	MMF*		SLE	Symptomatic	Pos	11930
Post V3	None		SLE	Symptomatic	Pos	*N/A
Pre B	ocrelizumab		MS	Symptomatic	Neg	N/A
Pre B	RTX		MS	Symptomatic	Neg	N/A
Pre B	RTX		Pemphigus	Symptomatic	Neg	N/A
Pre B	HCQ		SLE	Asymptomatic	Pos	553
Pre B	azathioprine		SLE	Symptomatic	Pos	N/A
Pre B	fingolimod		MS	Asymptomatic	Pos	N/A
Pre B	RTX		Pemphigus	Asymptomatic	Pos	N/A
Pre B	HCQ		SLE	Asymptomatic	Pos	N/A
Post B1	ocrelizumab		MS	Symptomatic	Neg	18.3
Post B1	azathioprine (previous CTX)	HCQ	SLE	Symptomatic	Neg	26.3

Post B1	MMF	belimumab	SLE	Symptomatic	Neg	1.09
Post B1	MMF		SLE	Symptomatic	Neg	96.4
Post B1	RTX	leflunomide	RA	Hospitalized	Neg	0.45
Post B1	HCQ		SLE	Asymptomatic	Pos	744
Post B1	azathioprine	HCQ	SLE	Symptomatic	Pos	301
Post B1	MTX	adalimumab	RA	Symptomatic	Pos	4070
Post B1	MTX	adalimumab	RA	Asymptomatic	Pos	1120
Post B1	MTX		RA	Asymptomatic	Pos	1830
Post B1	azathioprine		SLE	Asymptomatic	Pos	55.3
Post B1	tacrolimus		SLE	Symptomatic	Pos	139
Post B1	MMF		Inflammatory Myositis	Symptomatic	Pos	696
Post B1	MTX		PsA	Asymptomatic	Pos	522
Post B1	infliximab		IBD	Symptomatic	Pos	553
Post B1	MMF	HCQ	SLE	Symptomatic	Pos	N/A
Post B1	MMF*		SLE	Symptomatic	Pos	8780
Post B1	MMF		MCTD	Symptomatic	Pos	N/A
Post B1	HCQ		SLE	Symptomatic	Pos	109

* MMF level = undetectable at Post V1

**N/A Not available

Supplementary Table 9: Analytes used on autoantibody arrays

Bead ID	Antigen	Vendor	Catalog #
Control Analytes			
1	Bare Bead		
2	Human IgG from serum	Sigma	I4506
4	Anti-Human IgG Fc fragment specific	Jackson	109-005-008
7	Anti-Human IgG (H+L)	Jackson	109-005-003
8	Anti-Human IgG F(ab') fragment specific	Jackson	109-005-006
Cytokines			
9	IFN-alpha1	Prospec	CYT-291
12	IFN-alpha2	R&D	11101-2
13	IFN-alpha6	Origene	TP760329
14	IFN-alpha7	Prospec	CYT-196
15	IFN-alpha8	Sino Biological	10347-H08H
18	IFN-alpha10	Sino Biological	10349-H08H
19	IFN-epsilon	R&D	9667-ME-025/CF
20	IFN-gamma	Peprtech	300-02
21	IFN-lambda1	Peprtech	300-02L
22	IFN-lambda2	Peprtech	300-02K
25	IFN-omega	Peprtech	11395-1
26	IL-10	Peprtech	200-10
28	IL-11	Prospec	CYT-214
29	IL-12	Sigma	SRP3073-10UG
30	IL-13	Peprtech	200-13
31	C3a	R&D	3677-C3-025
32	IL-15	Peprtech	200-15
33	IL-17A	Peprtech	200-17

34	IL-17F	Peprotech	200-25
35	IL-1A	Peprotech	200-01A
36	IL-1RA	Peprotech	200-01RA
38	IL-2	Peprotech	200-02
39	IL-4	Peprotech	200-04
42	IL-6	Peprotech	200-06
43	IL-7	Peprotech	200-07
44	IL-21	Peprotech	200-21
45	IL-22	Peprotech	200-22
46	IL-23	Peprotech	200-23
47	IL-31	Prospec	CYT-625
48	IL33	Peprotech	200-33
49	IL-34	Prospec	CYT-1069
51	ACE2	Sino Biological	10108-H05H
52	CCL26	Peprotech	300-48
54	CXCL10	Peprotech	300-12
55	CXCL9	Peprotech	300-26
56	FGF7	Peprotech	100-19
57	GM-CSF	Peprotech	300-03
58	Leptin	Peprotech	300-27
59	LIF	Peprotech	300-05
60	LIGHT	Peprotech	310-09B
61	MIP-1alpha	Peprotech	300-08
62	OPN	Peprotech	120-35
63	Orexin	MyBioSource	161400
64	Platelet derived growth factor-BB (PDGFBB)	Peprotech	100-14B
65	sIL2R	Peprotech	200-02RC
66	s-rank ligand	Peprotech	310-01C

67	TMEM149	Prospec	PRO-1432
68	TNF-alpha	Peprtech	300-01A
69	VEGFA	Peprtech	100-20A
Traditional Autoimmune-Associated Antigens			
70	PMScl-75	Surmodics	A17001
76	C1q	Biodesign	A90150H
78	Bactericidal/permeability-increasing protein (BPI)	Sigma	SRP6307
79	MDA5	Surmodics	A30001
80	Thyroglobulin (TG)	Surmodics	A12201
81	Proteinase 3	Surmodics	A18601
82	CENPA	Surmodics	A16901
88	Fibrillarin (FBL)	Prospec	ENZ-566
89	Scl 70	Surmodics	A12401
94	Islet antigen-2 (IA2)	Novus	H00005798-Q01
96	U1-snRNP C	Surmodics	A13201
99	U1-snRNP A	Surmodics	A13101
100	Pyruvate dehydrogenase (PDC-E2)	Surmodics	A17901
125	EJ synthetase	Surmodics	A11101
127	Thyroid peroxidase (TPO)	Surmodics	A12101
204	Dense fine speckles-70 (DFS70)	Diarect	A30300
228	Ro52	Diarect	A12701
235	Ro60/SSA	Diarect	A17401
284	La/SSB	Diarect	A12801
285	Sm/RNP	Immunovision	SRC-3000
309	Smith	Immunovision	SMA-3000
Viral Antigens			
37	EBV Early Antigen	Prospec	EBV-272

40	SARS-CoV-2 Receptor binding domain (RBD)	Taia Wang	Stanford University, Stanford, CA
41	SARS-CoV-2 Nucleocapsid		
50	EBV-EBNA1	Prospec	EBV-276
71	EBV Early Antigen-D	MyBioSource	MBS319448
77	CMV glycoprotein-B	Prospec	CMV-211
84	Influenza A hemagglutinin	Abcam	ab124596
91	HBSAg	My BioSource	MBS142509

Supplementary Table 10 - Flow Cytometry Antibodies for AIM assays

Epitope	Clone	Catalog Number	Company	Fluorochrome	Concentration
CD19	H1B19	740287	BD Biosciences	BUV395	1:25
CD3	UCHT1	75-0038	Tonbo Biosciences	V450	1:25
CD14	M5E2	561392	BD Biosciences	V500	1:25
CD38	HIT2	303531	Biolegend	BV605	1:25
HLA-DR	G46-6	563696	BD Biosciences	BV711	1:25
CD8	SK1	35-0087	Tonbo Biosciences	FITC	1:25
CD137	4B4-1	309803	Biolegend	PE	1:25
OX40	Ber-ACT35	350012	Biolegend	PE-Cy7	1:25
CD4	OKT4	20-0048	Tonbo Biosciences	APC	1:25
CD69	FN50	560912	BD Biosciences	APC-Cy7	1:25
Viability Dye	-	50-105-2992	Tonbo Biosciences	V510	1:100

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