# Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA vaccine against SARS-CoV-2 — preliminary report. N Engl J Med. DOI: 10.1056/NEJMoa2022483

Supplementary Appendix to Manuscript Entitled

**A SARS-CoV-2 mRNA Vaccine — Preliminary Report**

## Table of Contents

## mRNA-1273 Study Group

**(listed in pubmed, and ordered alphabetically by institutional affiliation)**

The following study group members were all closely involved with the design, implementation, and oversight of the mRNA-1273 clinical trial.

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## mRNA-1273 Study Team Members

The mRNA-1273 trial was a collective group effort across multiple institutions and locations. Below is a list of sites and staff that significantly contributed to the implementation and conduct of the mRNA-1273 trial (alphabetically by institution).

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Below is a list of sites and staff that contributed serum samples from, and information on, Covid-19 convalescent patients:

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## Supplemental Methods:

*Additional Study Procedure Details*

Safety laboratory evaluations including white blood cell and platelet counts and levels of hemoglobin, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, and lipase were assessed on the day of and 14 days after each vaccination.

*Additional Immunologic Assay Method Details*

SARS-CoV-2 S-2P and RBD ELISA. Briefly, SARS-CoV-2 Spike S-2P antigen (VRC-SARS-CoV-2 S-2P (15-1208)-3C-His8-Strep2x2)[1] was coated onto flat bottom 96-well plates overnight at 4° C at a concentration of 2 mg/mL in DPBS. For SARS-CoV-2 RBD (Ragon-SARS-CoV-2 S-RBD (319-529)-His8-SBP), 4mg/mL were used. Proteins were generously produced, purified and provided by Dr. Dominic Esposito (Frederick National Laboratory for Cancer Research, NCI). After incubation, plates were moved to an integrated Beckman Biomek (Indianapolis, IN, USA) automated system.

Plates were washed and blocked (3% milk TPBS) for 1 hour at room temperature. Duplicate 4-fold serial dilutions of heat-inactivated serum sample diluted in 1% milk in TPBS were added to the wells and incubated at room temperature for 2 hours. Incubation was followed by HRP-labeled goat anti-human antibody detection for 1 hour at room temperature. Detection (Thermo Fisher Catalogue # A1881) was used at a 1:32.000 dilution in TPBS. Plates were washed and 100 uL of TMB (DAKO Catalogue # S1599) substrate was added for 15 minutes at room temperature. Color development was stopped by addition of sulfuric acid and plate absorbance was read within 30 minutes at 450 nm and 650 nm via the Molecular Devices Paradigm (San Jose, CA, USA) plate reader.

Endpoint Titer dilution from raw OD data was interpolated using the plate background OD + 10 STDEV by asymmetric sigmoidal 5-pl curve fit of the test sample. In the rare event the asymmetric sigmoidal 5-pl curve failed to interpolate the endpoint titer, a sigmoidal 4-pl curve was used for the analysis. Area under the curve (AUC) was calculated with baseline anchored by the plate background OD + 10 STDEV.

Pseudovirus neutralization assay (PsVNA). Neutralization activity against SARS-2-CoV was measured in a single-round-of-infection assay with pseudotyped virus particles (pseudoviruses). To produce SARS-CoV-2 pseudoviruses, an expression plasmid bearing codon-optimized SARS-CoV-2 full-length S plasmid (parental sequence Wuhan-1, Genbank #: MN908947.3) was co-transfected into HEK293T/17 cells (ATCC#CRL-11268) cells with packaging plasmid pCMVDR8.2, luciferase reporter plasmid pHR′ CMV-Luc,[2] and a TMPRSS2 plasmid.[3] Pseudoviruses were mixed with serial dilutions of sera or antibodies and then added to monolayers of ACE-2-overexpressing 293T cells (gift of Michael Farzan and Huihui Mu), in triplicate. Three days post infection, cells were lysed, luciferase was activated with the Luciferase Assay System (Promega), and relative light units (RLU) were measured at 570 nm on a Spectramax L luminometer (Molecular Devices). After subtraction of background RLU (uninfected cells), % neutralization was calculated as 100x((virus only)-(virus plus antibody))/(virus only). Dose-response curves were generated with a 5-parameter nonlinear function, and titers reported as the serum dilution or antibody concentration required to achieve 50% (50% inhibitory dilution [ID50]) or 80% (80% inhibitory dilution [ID80]) neutralization.

The input dilution of serum is 1:20, thus, 20 is the lower limit of quantification. Samples that do not neutralize at the 50% level are expressed as <20 and plotted at half that dilution, *i.e.,* 10. If duplicate assays return one value above 20 and <20, the result is reported as the geometric mean of 10 and the positive assay; therefore, some values between 10 and 20 are reported.

To monitor for assay quality, the same 6 control samples were included in each assay work session: three negative controls (pre-pandemic human sera collected prior to May 2019) and three sera from SARS-CoV-2 convalescent individuals.

Live SARS-CoV-2 neutralization assay. Sera were incubated at 56°C for 45 min and manually diluted in gelatin saline (0.3% [wt/vol] gelatin in phosphate-buffered saline supplemented with $CaCl_2$ and $MgCl_2$) to generate a 1:4 dilution of the original specimen, which served as a starting concentration for further serial $log_2$ dilutions in gelatin saline using an automated liquid handling system. The terminal serum concentration corresponded to 1/131,072 of the original. Antisera were combined with an equal volume of SARS-CoV-2 clinical isolate, SARS-CoV-2/human/USA/USA-WA1/2020 (GenBank: MN985325.1), in gelatin saline, producing an average final virus concentration of 580 plaque-forming units per ml in each serum dilution ranging from final concentrations of 1/8 to 1/262,144 of the original. Virus/serum mixtures were incubated for 20 min at 37°C, followed by adsorption of 0.1 ml aliquots to each of two confluent Vero E6 cell monolayers in 10-$cm^2$ wells for 30 min at 37°C. Four aliquots of untreated (i.e., no serum) control virus were subjected to identical conditions. Cell monolayers were overlaid with Dulbecco's modified Eagle's medium containing 1% agar and incubated for 3 days at 37°C in humidified 5% CO2. Plaques were enumerated by direct visualization, and the average number of plaques in virus/serum (duplicate) and virus-only (quadruplicate) wells was used to calculate percent neutralization at each serum dilution according to the following formula: 1 - (ratio of mean number of plaques in the presence and absence of serum).

Each specimen was tested in two independent assays performed at different times. Fractional neutralization from duplicate specimens was plotted as a function of $log_2$ serum dilution, and the dose-response relationship was fit to a five-parameter logistic regression model

using the package nplr[4] in R[5]. $PRNT_{80}$ titers, expressed as the reciprocal of the highest serum dilution reducing virus infectivity by 80%, were calculated from resulting curves.

Four dilutions of a COVID-19 convalescent serum control, spanning a 256-fold concentration range, were included with each performance of PRNT for longitudinal monitoring of assay stability. In addition, specimens were retested if duplicate neutralization curves displayed differences that raised concern for irreproducibility, i.e., disproportionate to expected deviations naturally arising from numerous interacting biological as well as technical variables inherent to PRNT. Discrepant results suggestive of technical causation were observed in two sets of duplicate neutralization curves, prompting another assay repeat for the two specimens in question. Suspicious outlier data points were not reproduced upon re-testing. Therefore, the two neutralization curves (of three total) exhibiting closest agreement were used for analysis.

Specimens exhibiting less than 80% inhibitory activity at the lowest dilution tested, 1:8, were assigned a titer of 4.

Antibody Assay Correlation Methods. Spearman correlations were calculated for all post-vaccination time points available. Confidence intervals were calculated using the z-transformation.

Intracellular cytokine stimulation (ICS) assay. An ICS assay was used to evaluate T cell responses elicited by the mRNA-1273 vaccine in clinical samples collected on day 1, day 29, and day 43 post-vaccination. Briefly, frozen peripheral blood mononuclear cells were thawed, counted and rested in R10 culture media ( 90% RPMI 1640 with 10% Fetal Bovine Serum (FBS) and 1% Penicillin Streptomycin and L-Glutamine) overnight at 37°C with 5% $CO_2$. Following the rest period, cells were counted on day 2 and resuspended in R10 cell culture media. 0.5-1.5 x $10^6$ cells were transferred to individual wells of a 96-well V-bottom plate(s) and incubated with

pools of 15-mer peptides overlapping by 10 amino acids covering the N-terminus of SARS-CoV-2 Spike protein up to the furin cleavage site (S1 pool), the C-terminus of the SARS-CoV-2 Spike protein up to the furin cleavage site (S2 pool) for 6 hours at 37°C with 5% $CO_2$. Peptides pools were custom ordered from JPT and were >85% pure. Following stimulation, cells were washed and stained with viability dye for 20 minutes at room temperature, followed by surface stain for 20 minutes at room temperature, cell fixation and permeabilization with BD cytofix/cytoperm kit (catalog # 554714) for 20 minutes at room temperature, and then intracellular stain for 20 minutes at room temperature. Upon completion of staining, cells were collected on a BD FACSymphony Flow Cytometer.

Samples were analyzed using FlowJo 10.6.2. Anomalous “bad” events were separated from “good” events using FlowAI.[6] “Good events” were used to determine cytokine responses. Cytokine positive cells were determined by gating on singlets, lymphocytes, viability dye$^-$CD3$^+$, followed by CD4$^+$ or CD8$\alpha^+$. Individual cytokines were plotted on the Y-axis vs CD69 on the X-axis and only the CD69$^+$cytokine$^+$ events were used to determine positive responses. Positive cytokine gates were determined using unstimulated samples during qualification testing. A template of gating was created during assay qualification and was applied to all vaccine samples without manipulation. “Any responses” are any combination of the indicated individual cytokines by a population of CD4 or CD8 T cells and were calculated using Boolean combination gates. All antigen-specific cytokine frequencies are reported after background subtraction of identical gates from the same sample incubated with the negative control stimulation (DMSO).

*Additional Convalescent Sera Description*

Convalescent sera were collected from a total of 41 individuals with confirmed Covid-19 diagnosis, 23-60 days after onset of symptoms. Thirty-eight samples were collected under IRB-

approved protocols at the National Institutes of Health, Bethesda MD (NCT00067054); Aaron Diamond AIDS Research Center, Columbia University, New York NY (NCT04342195); and the University of Washington, Seattle WA (HAARVI study and STUDY00000959). These samples were included in convalescent sera panels and tested along with the vaccine trial participant samples as comparators for the ELISA and PsVNA vaccine-induced responses and to establish correlations across the ELISA (S-2P and RBD) and the PsVNA. The Covid-19 illness severity was known for these 38 individuals and was classified as mild in 63%, moderate in 22%, and severe (hospitalization requiring intensive care and/or ventilation) in 15%.

Three additional convalescent sera collected by Vanderbilt University Medical Center, Nashville, TN (NCT04362176 and IRB VUMC protocol # 070258) were included in graphical representations in the distribution of values for the panels of convalescent specimens for ELISA and PsVNA and were the only convalescent sera used as comparators for the PRNT.

**Supplemental Study Results:**

*Report of adverse events post-second vaccination in a participant in the 250 mcg dose group*

A participant in the 250 mcg dose group had severe fever, onset the evening of the second vaccination, along with severe chills and mild fatigue, myalgia, and headache. In the early morning of the day after vaccination the participant developed recurrent severe fever, chills, fatigue, and headache, moderate myalgia and nausea, and mild arthralgia. The participant was evaluated in an urgent care center and received symptomatic treatment prior to discharge. A nasal swab specimen was negative for SARS-CoV-2 by polymerase chain reaction and positive for adenovirus by a fluorescent antibody assay. After sleeping for several hours at home, upon standing the participant was lightheaded and nauseous, vomited, and then fainted. Lightheadedness persisted for several hours. Other systemic symptoms improved over the course of the day. Mild headache was present the next day and mild fatigue was reported through post vaccination day 6.

The unsolicited adverse events of lightheadedness and fainting (syncope) were judged as severe and related to vaccination and were classified as MedDRA® system organ class nervous system disorders (Table S3).

*Safety laboratory grade 2 or higher adverse events*

A total of five grade 2 safety laboratory adverse events, defined by a standard toxicity grading scale,[7] were identified; no grade 3 events were identified. Of the five grade 2 events, one was an elevated total bilirubin value (1.75 x upper limit of normal [ULN]) on the day of the second vaccination (obtained prior to vaccination) in the 25 mcg dose group that was judged to be not clinically significant and not related to vaccine, with an alternate etiology of suspected Gilbert's syndrome. One was an elevated lipase (1.9 x ULN) in the 250 mcg dose group obtained seven days after the second vaccination that was judged to be not clinically significant

and to be related to the vaccine. Three were grade 2 hemoglobin value adverse events, all in the 250 mcg dose group, for all those events the hemoglobin value was within the normal range but was lower than the Day 1 value, by a magnitude that defined a grade 2 event. All three events were defined as not clinically significant, two were from specimens obtained seven days after the second vaccination, one of which was defined as related and one as not related (alternate etiology specified as normal variation). The third value, obtained at seven days after first vaccination, was defined as not related (alternate etiology specified as study procedure).

*Unsolicited adverse events*

There were 90 unsolicited adverse events report, none met the definition of a serious adverse event (Table S3). Of those, 69 were mild in severity, of which 21 (35%) were judged related to vaccination, 19 were moderate in severity, of which 12 (63%) were judged related to vaccination, and 2 were severe events judged related to vaccination. There was no obvious clustering of events by MedDRA® System Organ Class.

## Supplemental Figures and Tables:

### Figure S1. Consort Flow Diagram.

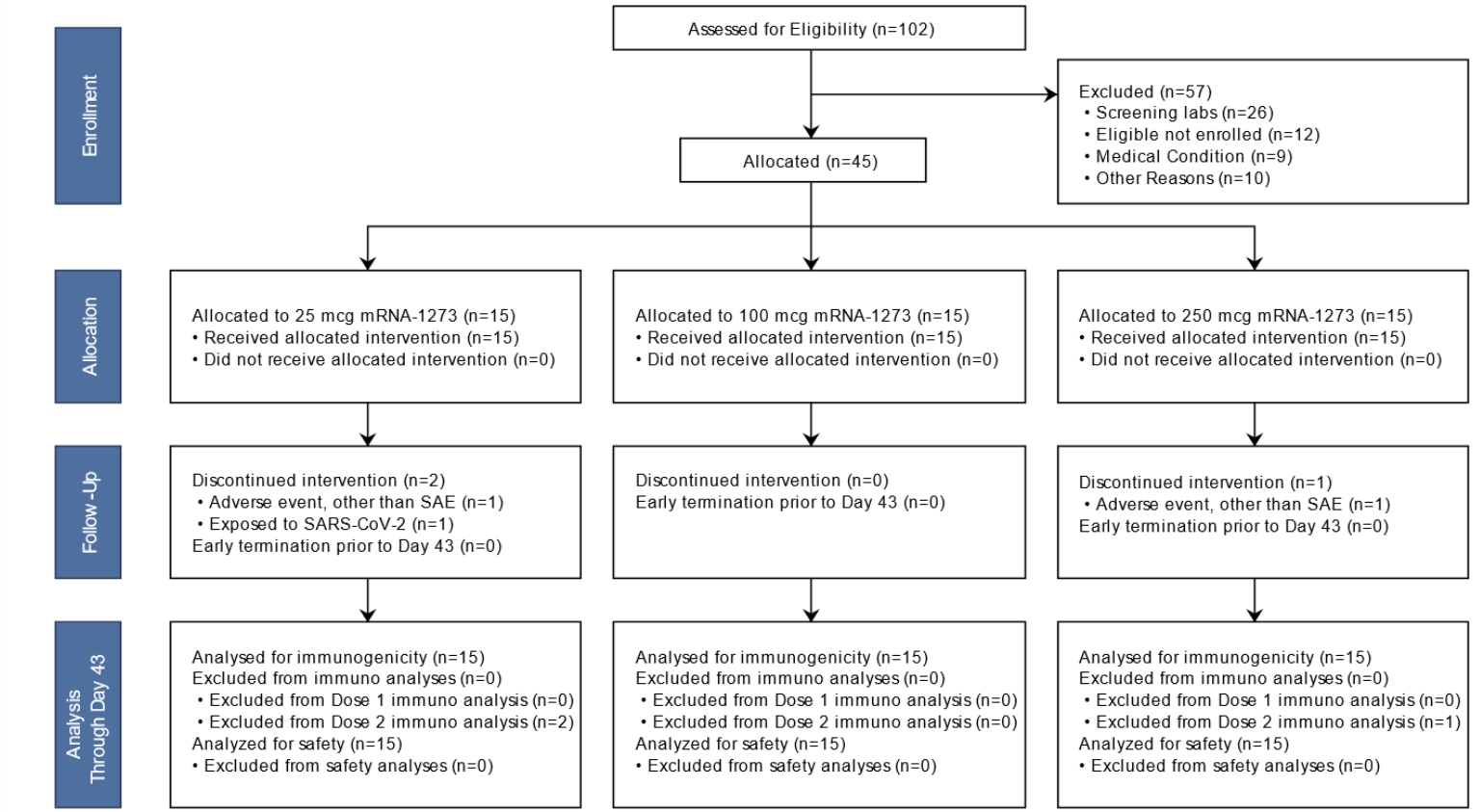
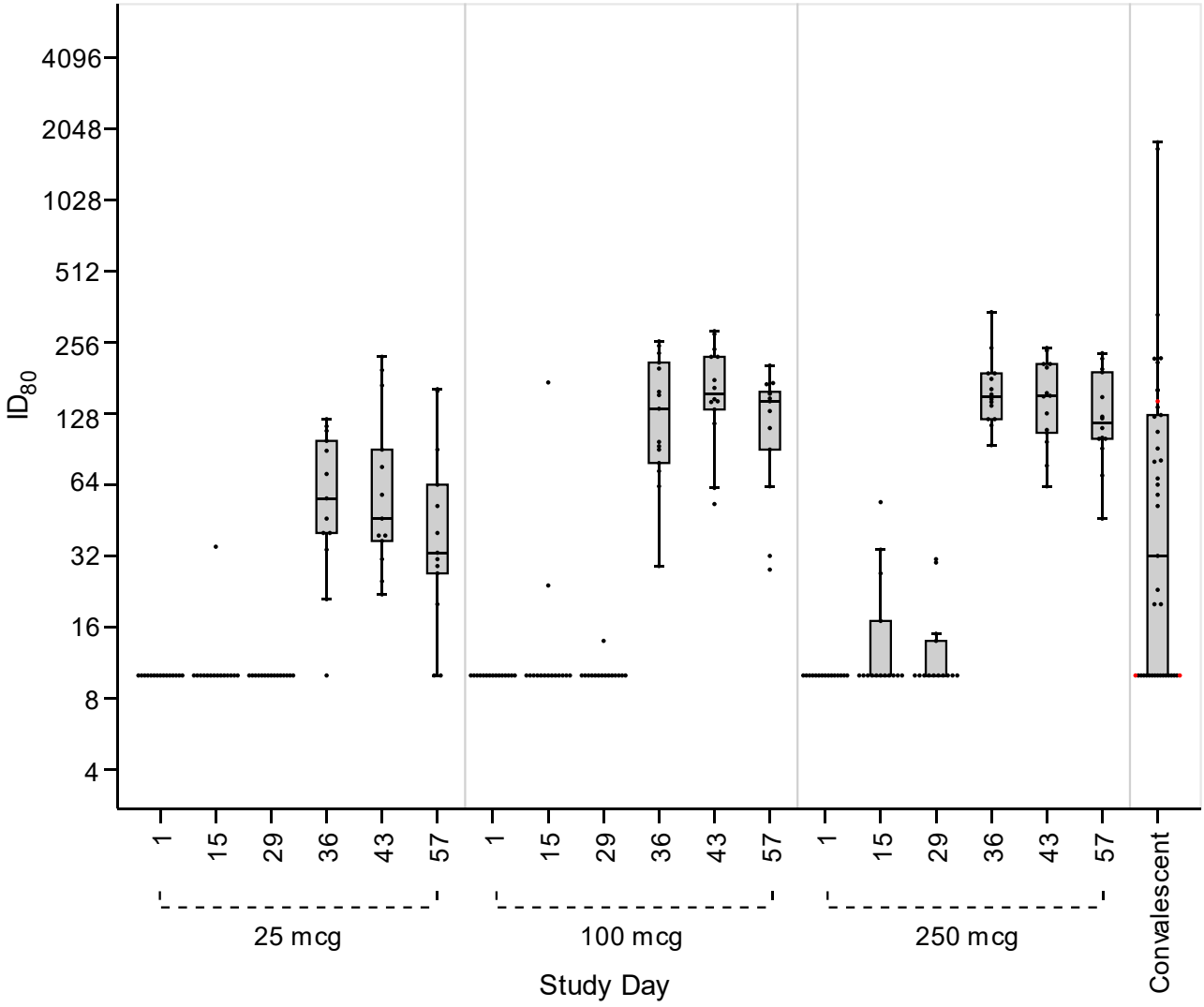


**Figure S2. Pseudovirus neutralization assay responses by time point and vaccination group - ID80.**




Boxes and horizontal bars denote interquartile range (IQR) and median $ID_{80}$, respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median +/- 1.5 x IQR. The convalescent sera panel includes specimens from 41 individuals; red dots indicate the 3 specimens that were cross-tested in PRNT assays. The other 38 specimens were used to calculate summary statistics for the box plot in the convalescent panel.

**Figure S3. Binding to SARS-CoV-2 spike proteins in ELISA expressed as area-under-the-curve (AUC) is highly correlated with binding expressed as endpoint dilution titer.**

**A**, vaccinee sera binding to S-2P expressed as endpoint dilution titer vs AUC. **B**, vaccinee sera binding to RBD expressed as endpoint dilution titer vs AUC.**C**, convalescent sera binding to S-2P expressed as endpoint dilution titer vs AUC. **D**, convalescent sera binding to RBD expressed as endpoint dilution titer vs AUC.

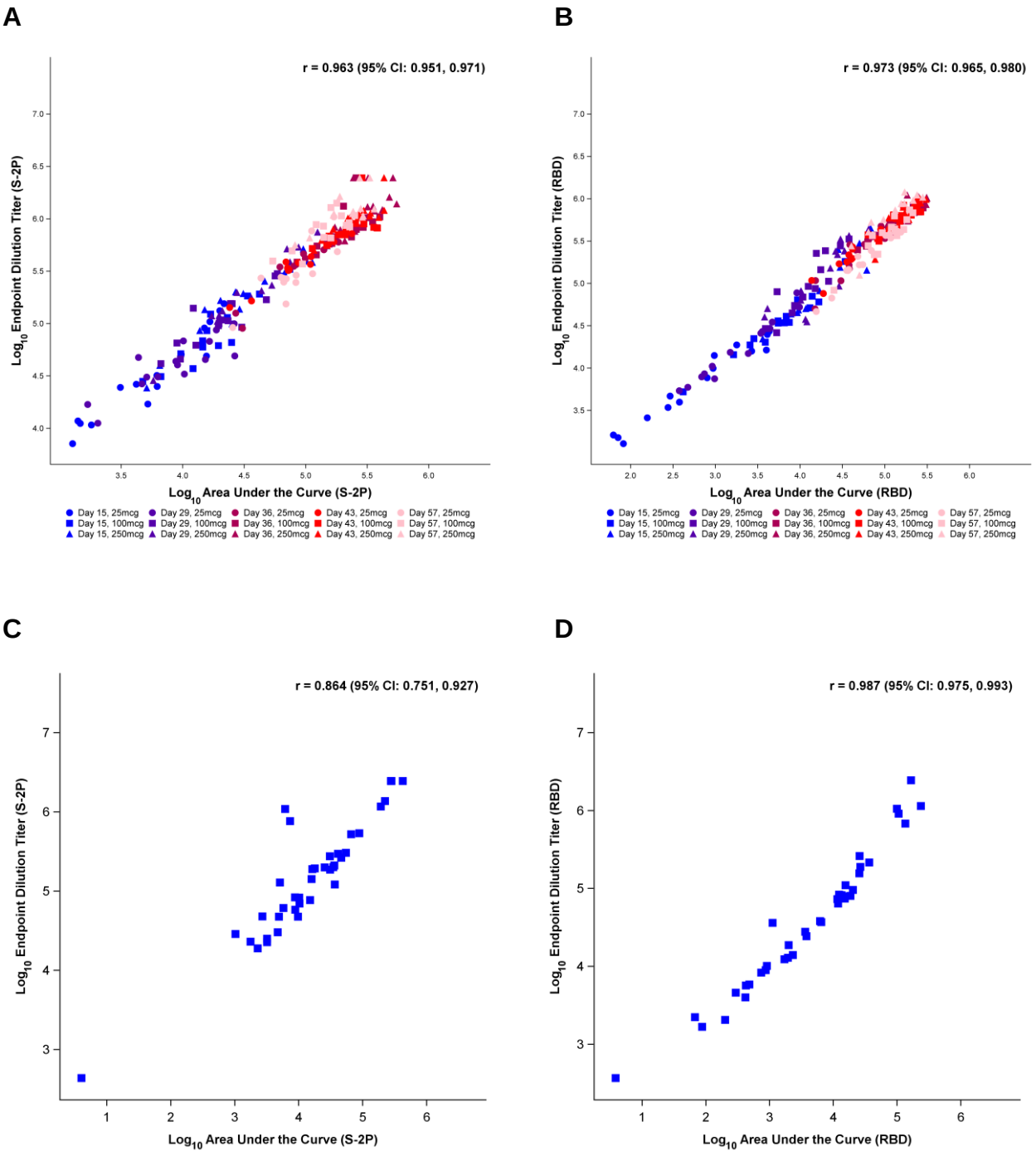


**Figure S4. Binding to S-2P and RBD proteins are highly correlated.**

**A**, vaccinee sera binding to S-2P vs RBD, expressed as area under the curve (AUC). **B**, vaccinee sera binding to S-2P vs RBD, expressed as endpoint dilution titer. **C**, convalescent sera binding to S-2P vs RBD, expressed as AUC. **D**, convalescent sera binding to S-2P vs RBD, expressed as endpoint dilution titer.

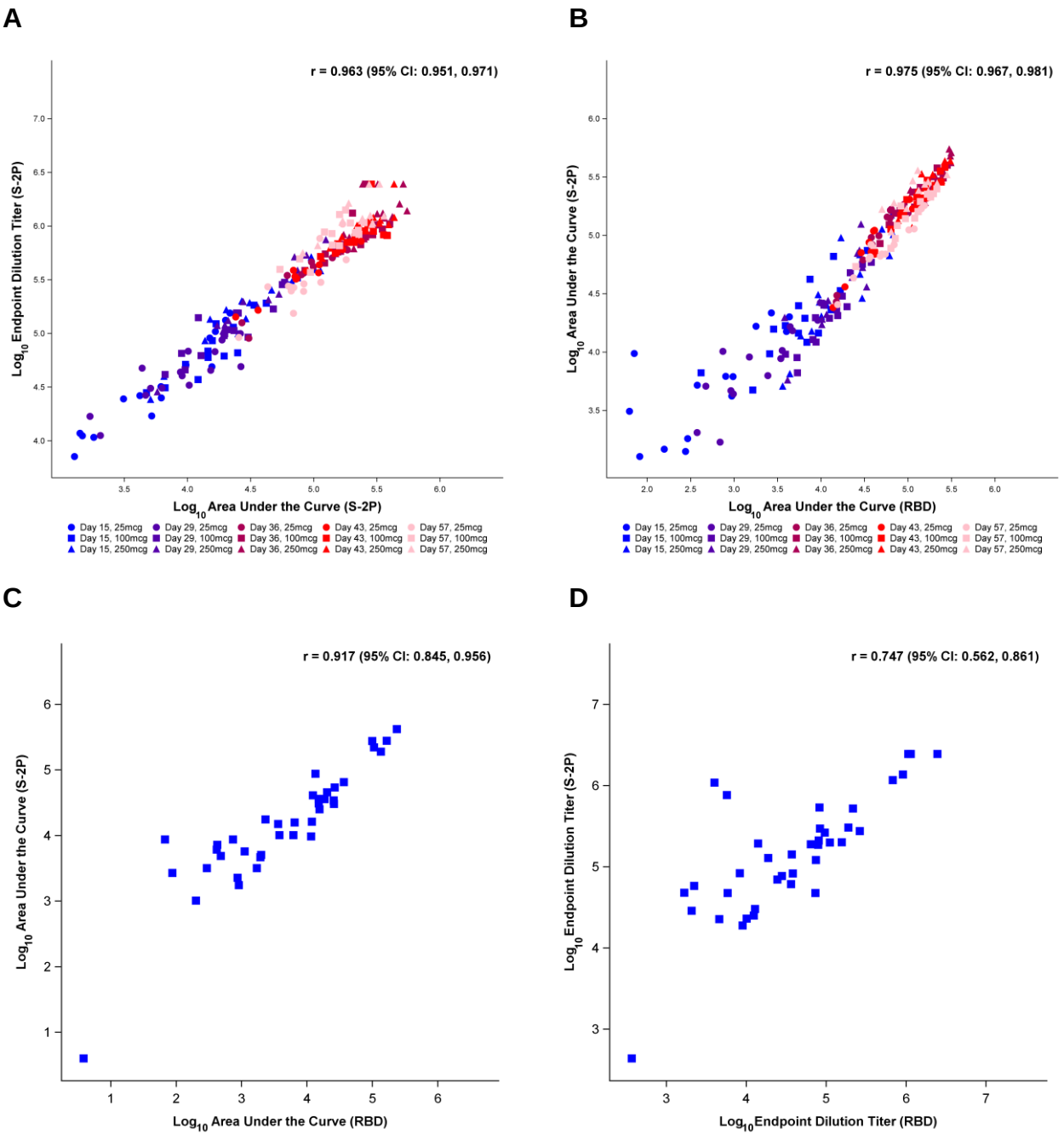


**Figure S5. Pseudovirus neutralization correlates with binding in ELISA.**

**A**, vaccinee sera pseudovirus neutralization (ID50) vs S-2P binding (AUC). **B**, vaccinee sera pseudovirus neutralization ID80 vs S-2P AUC. **C**, convalescent sera ID50 vs S-2P AUC. **D**, convalescent sera ID80 vs S-2P AUC.

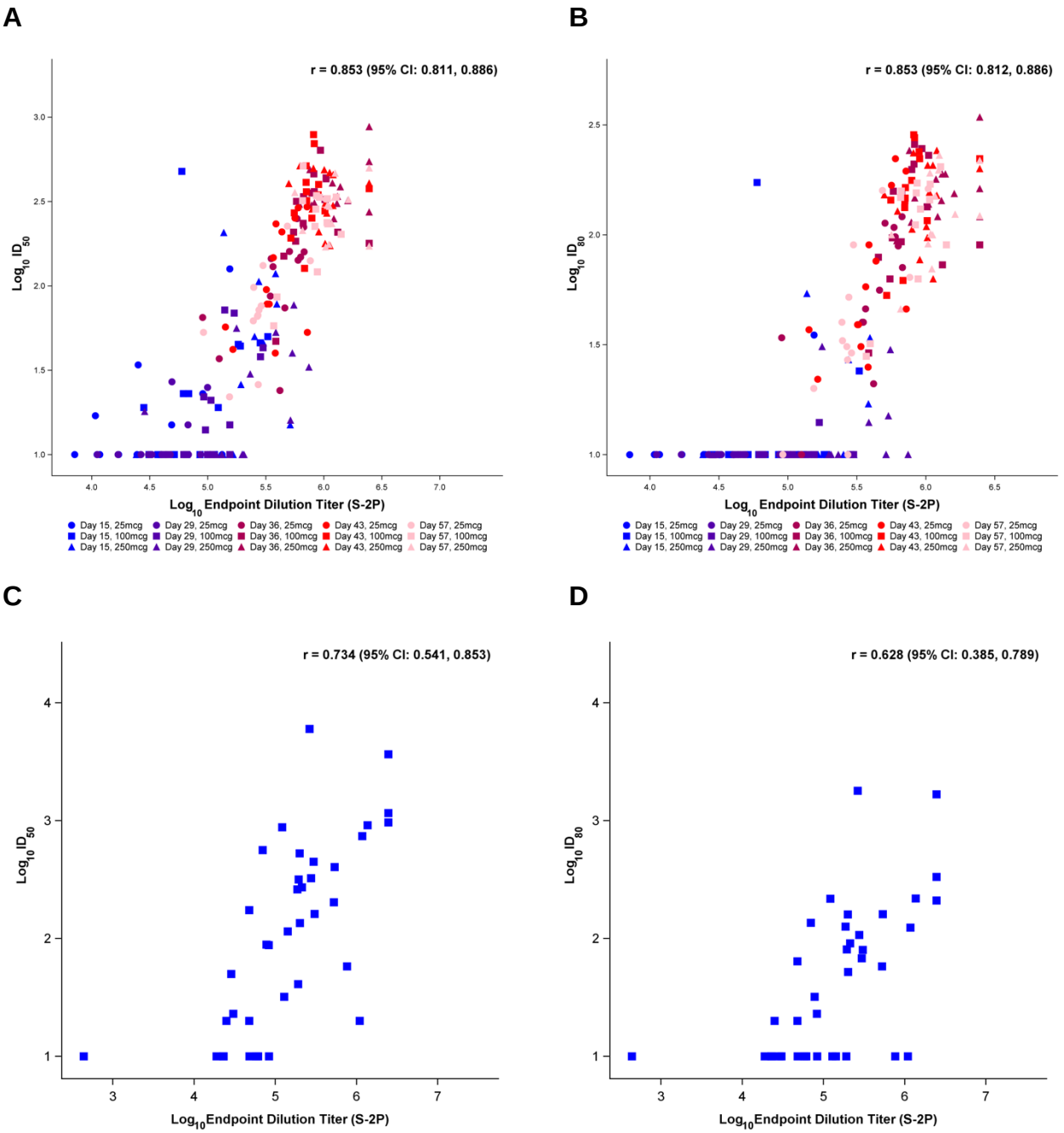
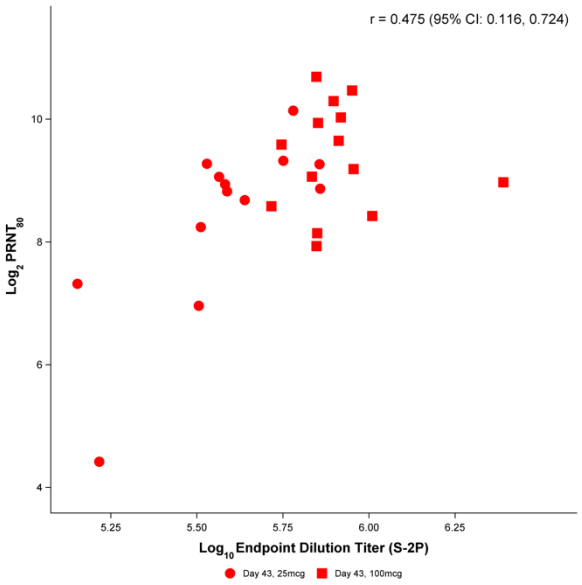


**Figure S6. Live-virus neutralization (PRNT80) correlates with binding in ELISA.**

Data are from sera of vaccinees 43 days after receiving 25 or 100 mcg of mRNA-1273. **A**, live-virus neutralization (PRNT80) vs S-2P binding (AUC) . **B**, PRNT80 vs RBD binding (AUC).

**A**




**B**

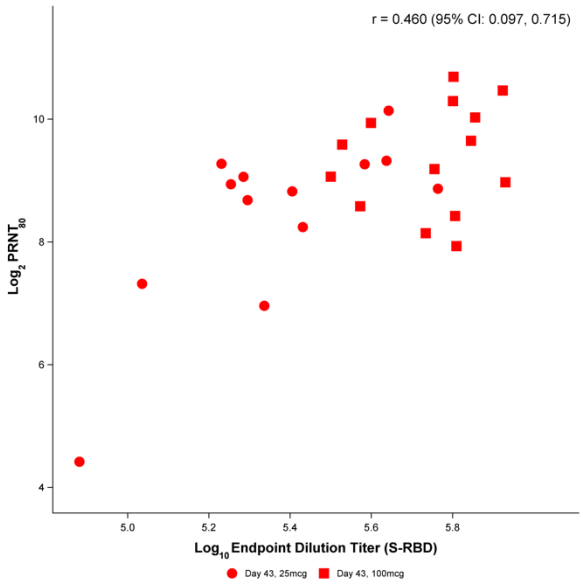


**Figure S7. Live-virus neutralization (PRNT80) correlates with pseudovirus neutralization (ID50 or ID80).**

Values shown are for Day 43 samples from 25 mcg and 100 mcg groups. **A**, Pseudovirus neutralization ID50 vs PRNT80; **B**, Pseudovirus neutralization ID80 vs PRNT80.

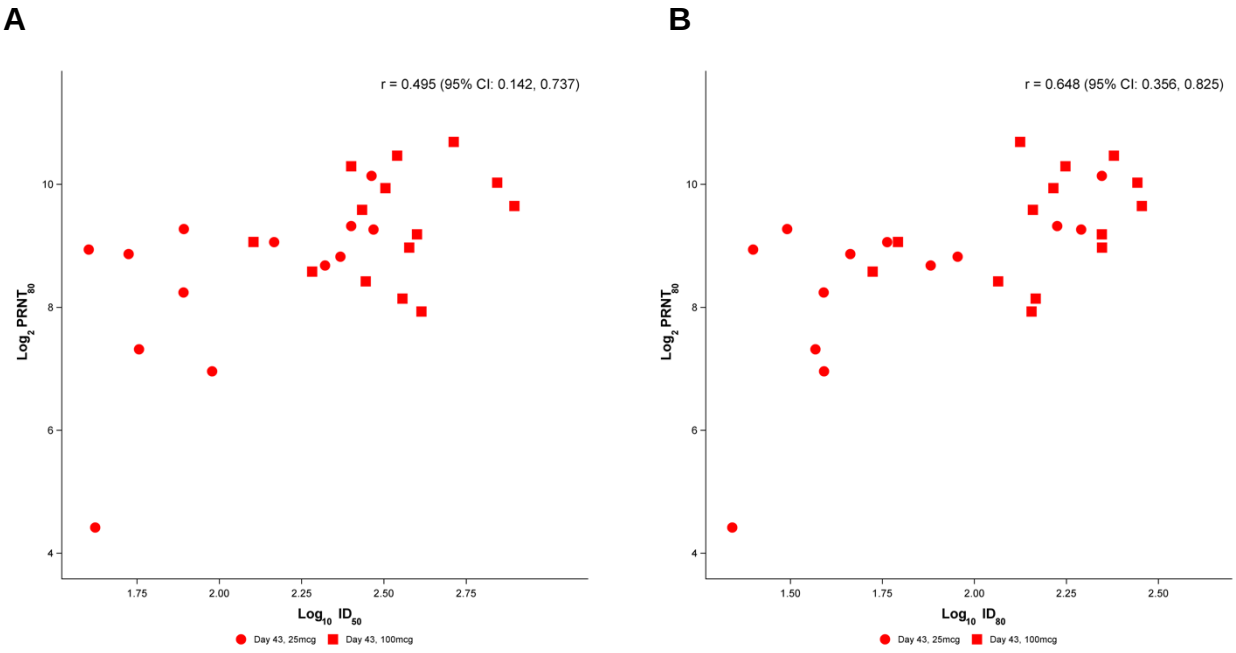


**Fig S8. Time course of immune responses to mRNA-1273**.

Immunizations were administered on days 1 and 29. **A**, binding to S-2P spike protein (endpoint dilution titer) for each subject. **B**, binding to RBD protein (endpoint dilution titer) for each subject. **C**, pseudovirus neutralization (PsVNA (ID50)) for each subject. **D**, pseudovirus neutralization (ID80) for each subject. Gray area indicates values below the lower limit of quantification (LLOQ). Values are geometric mean of replicate assays; single values below the LLOQ are plotted as 10.

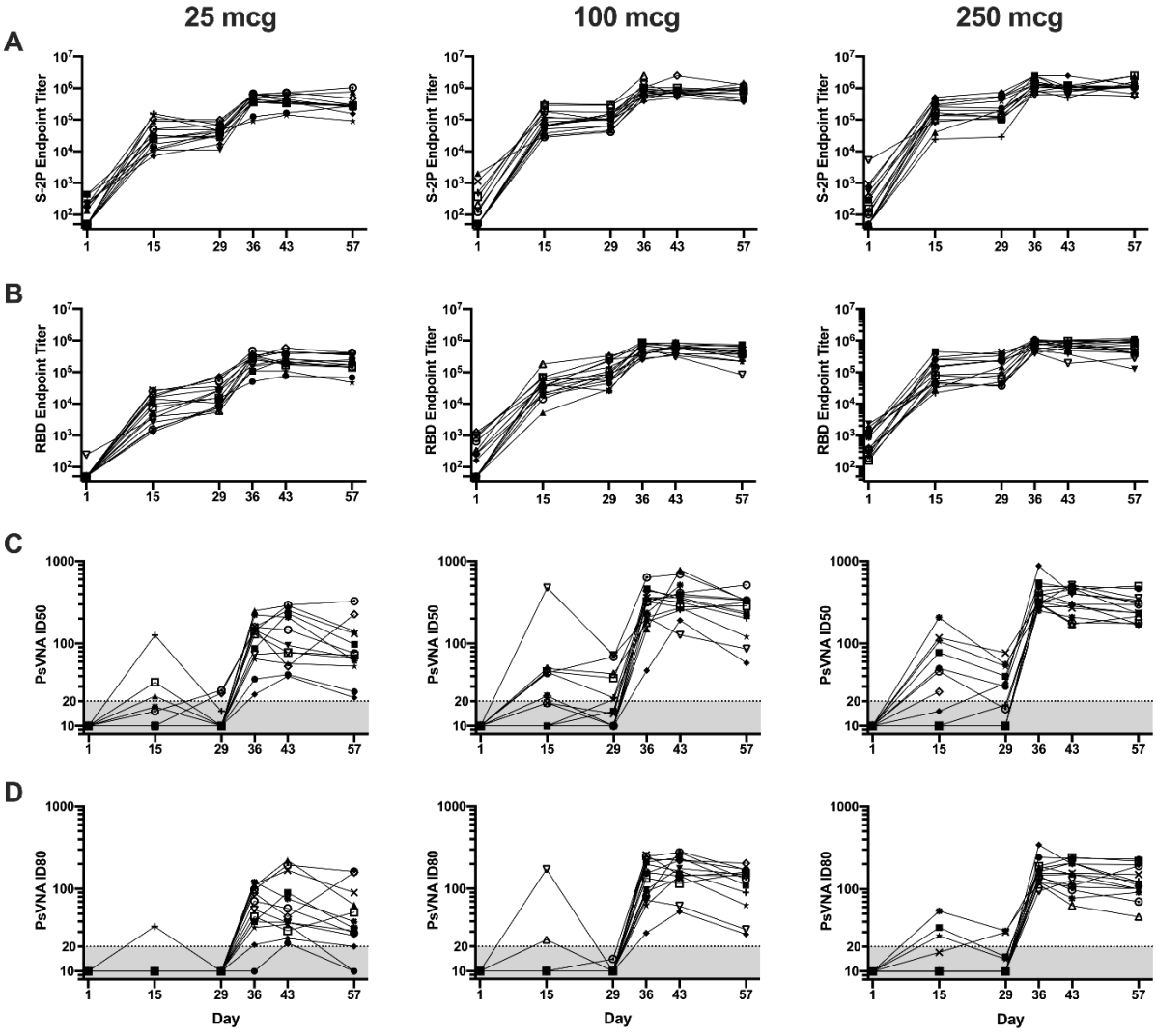


**Fig S9. mRNA-1273-specific CD4 T cell responses (S1 peptide pool).**

Frequencies of CD4 T cells producing the indicated cytokines from 25mcg (**A**) or 100mcg (**B**) dose groups following stimulation with SARS-CoV-2 S1 peptide pool. For Th1 responses (left) red circles indicate any combination of IFN-γ, IL-2 and TNF, blue squares indicate IFN-γ, green triangles indicate IL-2, and orange diamonds indicate TNF. For Th2 cytokine responses (right), red circles indicate any combination of IL-4 and IL-13, blue squares indicate IL-4, and green triangles indicate IL-13.

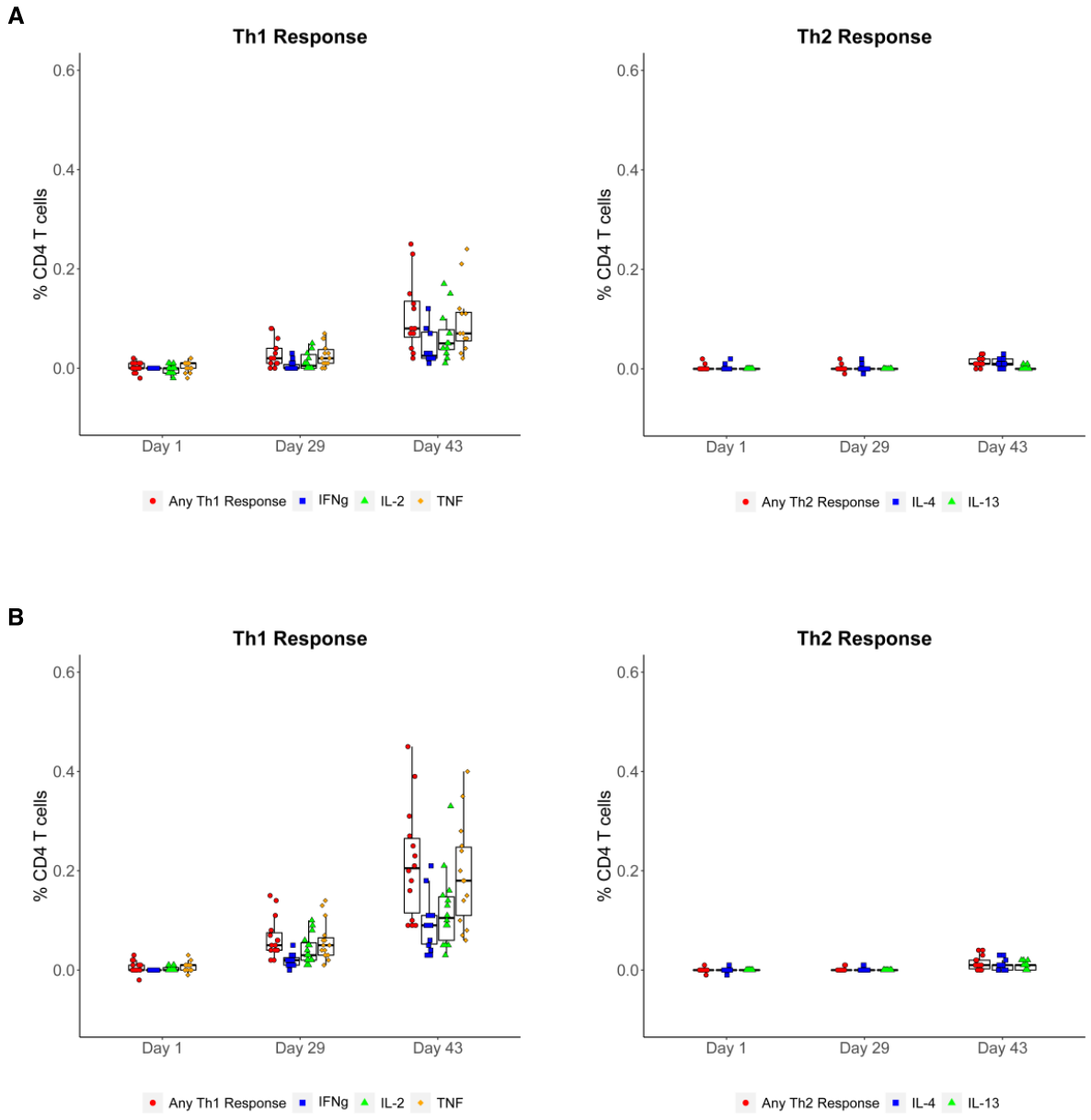


**Fig S10. mRNA-1273-specific CD4 T cell responses (S2 peptide pool).**

Frequencies of CD4 T cells producing the indicated cytokines from 25mcg (**A**) or 100mcg (**B**) dose groups following stimulation with SARS-CoV-2 S2 peptide pool. For Th1 responses (left) red circles indicate any combination of IFN-γ, IL-2 and TNF, blue squares indicate IFN-γ, green triangles indicate IL-2, and orange diamonds indicate TNF. For Th2 cytokine responses (right), red circles indicate any combination of IL-4 and IL-13, blue squares indicate IL-4, and green triangles indicate IL-13.

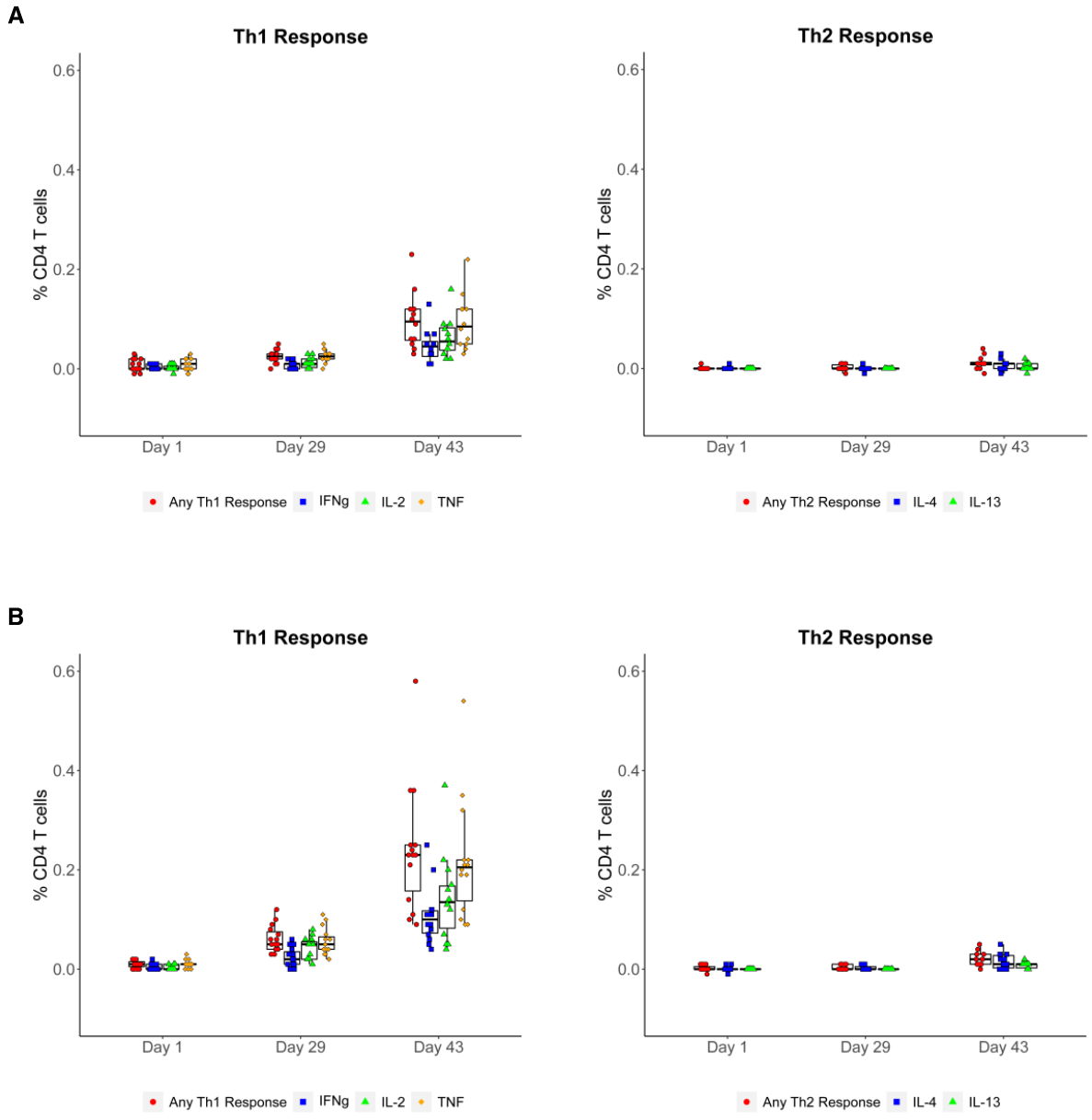


**Fig S11. mRNA-1273-specific CD8 T cell responses.**

Frequencies of CD8 T cells producing the indicated cytokines from 25mcg (**A**) or 100mcg (**B**) dose groups following stimulation with SARS-CoV-2 S1 peptide pool (left) or SARS-CoV-2 S2 peptide pool (right). Red circles indicate any combination of IFN-γ, IL-2 and TNF, blue squares indicate IFN-γ, green triangles indicate IL-2, and orange diamonds indicate TNF.

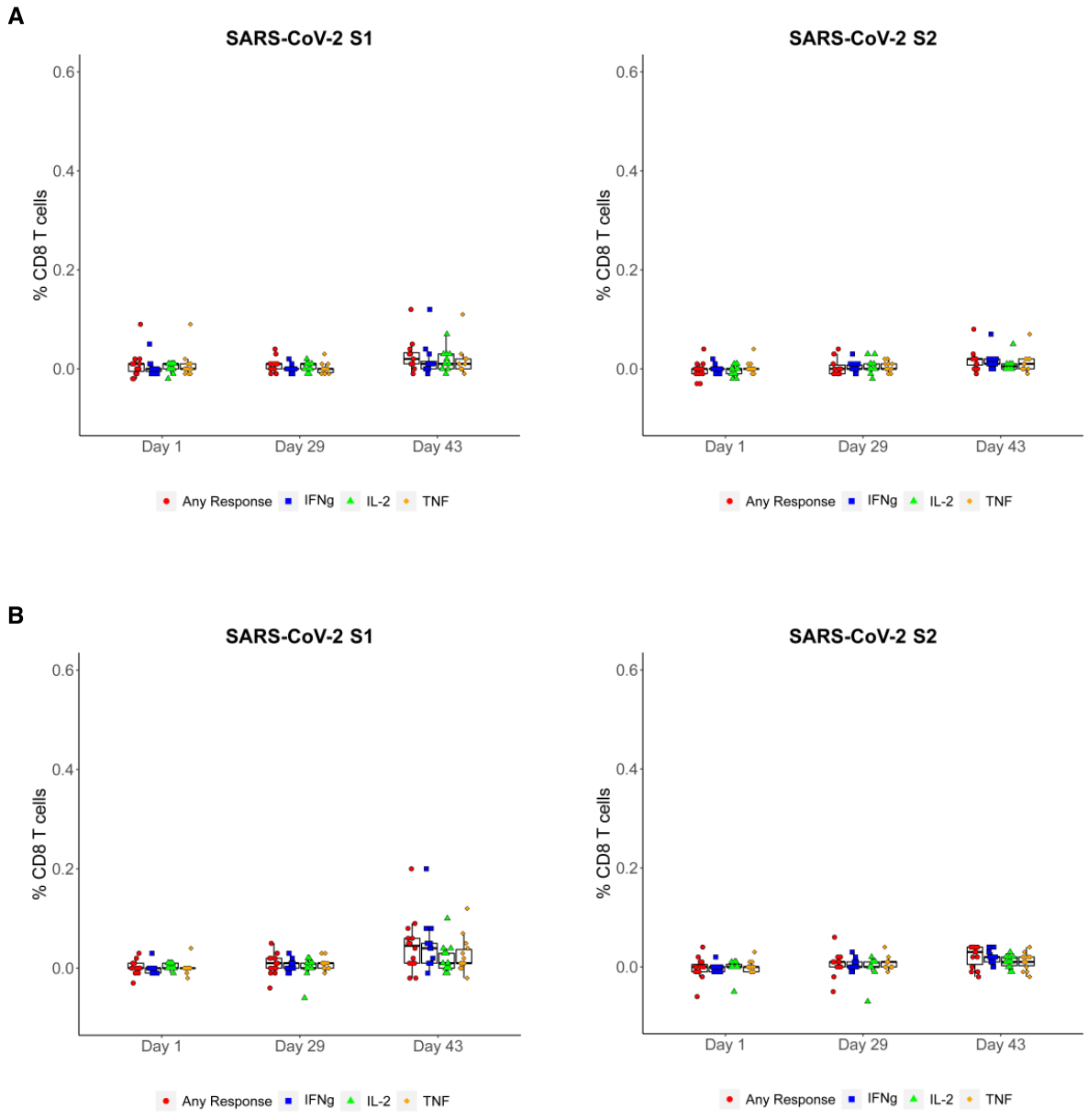


**Table S1. Toxicity grading scales for solicited systemic and local adverse events*.**

| | Mild | Moderate | Severe |
|---|---|---|---|
| Arthralgia | No interference with activity | Some interference with activity | Significant; prevents daily activity |
| Fatigue | No interference with activity | Some interference with activity | Significant; prevents daily activity |
| Fever | 38.0°C – 38.4°C | 38.5°C – 38.9°C | 39.0°C - 40°C |
| Chills | No interference with activity | Some interference with activity | Significant; prevents daily activity |
| Headache | No interference with activity | Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity | Significant; any use of narcotic pain reliever or prevents daily activity |
| Myalgia | No interference with activity | Some interference with activity | Significant; prevents daily activity |
| Nausea | No interference with activity or 1 – 2 episodes/24 hours | Some interference with activity or > 2 episodes/24 hours | Prevents daily activity, requires outpatient IV hydration |
| Size (diameter) of erythema/redness | 2.5 – 5 cm | 5.1 – 10 cm | > 10 cm |
| Size (diameter) of induration/swelling | 2.5 – 5 cm | 5.1 – 10 cm | > 10 cm |
| Pain (at injection site) | Does not interfere with activity | Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity | Any use of narcotic pain reliever or prevents daily activity |

*Obtained from a standard toxicity grading scale.[7]

**Table S2. Percentage of subjects experiencing solicited adverse events by symptom, maximum severity, vaccination number, and dose group.**

| Symptom | Vaccination | Dose group | N | Maximum Severity % Mild | % Moderate | % Severe |
|---|---|---|---|---|---|---|
| Any Systemic Symptom | 1 | 25 mcg | 15 | 20.0 | 13.3 | - |
| | | 100 mcg | 15 | 53.3 | 13.3 | - |
| | | 250 mcg | 15 | 26.7 | 26.7 | - |
| | 2 | 25 mcg | 13 | 30.8 | 23.1 | - |
| | | 100 mcg | 15 | 20.0 | 80.0 | - |
| | | 250 mcg | 14 | 14.3 | 64.3 | 21.4 |
| Arthralgia | 1 | 25 mcg | 15 | - | - | - |
| | | 100 mcg | 15 | 6.7 | 6.7 | - |
| | | 250 mcg | 15 | 6.7 | - | - |
| | 2 | 25 mcg | 13 | | 15.4 | - |
| | | 100 mcg | 15 | 6.7 | 6.7 | - |
| | | 250 mcg | 14 | 35.7 | 21.4 | - |
| Fatigue | 1 | 25 mcg | 15 | 13.3 | 13.3 | - |
| | | 100 mcg | 15 | 20.0 | 6.7 | - |
| | | 250 mcg | 15 | 20.0 | 13.3 | - |
| | 2 | 25 mcg | 13 | 30.8 | 7.7 | - |
| | | 100 mcg | 15 | 40.0 | 40.0 | - |
| | | 250 mcg | 14 | 14.3 | 42.9 | 14.3 |
| Fever | 1 | 25 mcg | 15 | - | - | - |
| | | 100 mcg | 15 | - | - | - |
| | | 250 mcg | 15 | - | - | - |
| | 2 | 25 mcg | 13 | - | - | - |
| | | 100 mcg | 15 | 33.3 | 6.7 | |
| | | 250 mcg | 14 | 35.7 | 14.3 | 7.1 |
| Chills | 1 | 25 mcg | 15 | - | - | - |
| | | 100 mcg | 15 | 6.7 | - | - |
| | | 250 mcg | 15 | 13.3 | - | - |
| | 2 | 25 mcg | 13 | 7.7 | - | - |
| | | 100 mcg | 15 | 53.3 | 26.7 | - |
| | | 250 mcg | 14 | 28.6 | 35.7 | 21.4 |
| Headache | 1 | 25 mcg | 15 | 20.0 | - | - |
| | | 100 mcg | 15 | 26.7 | - | - |
| | | 250 mcg | 15 | 26.7 | 20.0 | - |
| | 2 | 25 mcg | 13 | 15.4 | 7.7 | - |
| | | 100 mcg | 15 | 33.3 | 26.7 | - |
| | | 250 mcg | 14 | 64.3 | 28.6 | 7.1 |
| Myalgia | 1 | 25 mcg | 15 | 6.7 | - | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 100 mcg | 15 | 6.7 | - | - |
| | | 250 mcg | 15 | 26.7 | - | - |
| | 2 | 25 mcg | 13 | 15.4 | 7.7 | - |
| | | 100 mcg | 15 | 13.3 | 40.0 | - |
| | | 250 mcg | 14 | 35.7 | 50.0 | 7.1 |
| Nausea | 1 | 25 mcg | 15 | - | 6.7 | - |
| | | 100 mcg | 15 | - | - | - |
| | | 250 mcg | 15 | 6.7 | - | - |
| | 2 | 25 mcg | 13 | - | - | - |
| | | 100 mcg | 15 | 40.0 | 6.7 | |
| | | 250 mcg | 14 | 7.1 | 14.3 | 7.1 |
| Any Local Symptom | 1 | 25 mcg | 15 | 66.7 | - | - |
| | | 100 mcg | 15 | 73.3 | 13.3 | 6.7 |
| | | 250 mcg | 15 | 60.0 | 33.3 | 6.7 |
| | 2 | 25 mcg | 13 | 69.2 | 7.7 | - |
| | | 100 mcg | 15 | 66.7 | 26.7 | 6.7 |
| | | 250 mcg | 14 | 50.0 | 42.9 | 7.1 |
| Size of Erythema/Redness | 1 | 25 mcg | 15 | - | - | - |
| | | 100 mcg | 15 | - | 6.7 | 6.7 |
| | | 250 mcg | 15 | - | - | 6.7 |
| | 2 | 25 mcg | 13 | - | - | - |
| | | 100 mcg | 15 | 6.7 | - | 6.7 |
| | | 250 mcg | 14 | - | 14.3 | 7.1 |
| Size of Induration/Swelling | 1 | 25 mcg | 15 | - | - | - |
| | | 100 mcg | 15 | 13.3 | - | - |
| | | 250 mcg | 15 | 6.7 | 6.7 | 6.7 |
| | 2 | 25 mcg | 13 | - | - | - |
| | | 100 mcg | 15 | - | 6.7 | - |
| | | 250 mcg | 14 | 7.1 | 14.3 | - |
| Pain | 1 | 25 mcg | 15 | 66.7 | - | - |
| | | 100 mcg | 15 | 80.0 | 13.3 | - |
| | | 250 mcg | 15 | 60.0 | 40.0 | - |
| | 2 | 25 mcg | 13 | 69.2 | 7.7 | - |
| | | 100 mcg | 15 | 73.3 | 26.7 | - |
| | | 250 mcg | 14 | 71.4 | 28.6 | - |

**Table S3. Number of unsolicited, non-serious, adverse events classified by MedDRA® System Organ Class, severity, and investigator-assigned relationship to study vaccination.**

| Medra System Organ Class | Severity | Not related to vaccination | Related to vaccination |
|---|---|---|---|
| Any System Organ Class | Mild | 48 | 21 |
| | Moderate | 7 | 12 |
| | Severe | - | 2 |
| Cardiac Disorders | Mild | 3 | - |
| | Moderate | - | - |
| | Severe | - | - |
| Ear and Labyrinth Disorders | Mild | - | - |
| | Moderate | - | - |
| | Severe | - | - |
| Eye Disorders | Mild | - | 2 |
| | Moderate | - | - |
| | Severe | - | - |
| Gastrointestinal Disorders | Mild | 7 | 2 |
| | Moderate | 1 | 3 |
| | Severe | - | - |
| General Disorders and Administration Site Conditions | Mild | 10 | 5 |
| | Moderate | - | 3 |
| | Severe | - | - |
| Infections and Infestations | Mild | - | - |
| | Moderate | - | 1 |
| | Severe | - | - |
| Injury, Poisoning, and Procedural Complications | Mild | 8 | - |
| | Moderate | 1 | - |
| | Severe | - | - |
| Investigations | Mild | 1 | - |
| | Moderate | - | - |
| | Severe | - | - |
| Metabolism and Nutrition Disorders | Mild | - | 3 |
| | Moderate | 1 | 2 |
| | Severe | - | - |
| Musculoskeletal and Connective Tissue Disorders | Mild | 2 | 2 |
| | Moderate | 1 | 1 |
| | Severe | - | - |
| Nervous System Disorders | Mild | 3 | - |
| | Moderate | 3 | - |
| | Severe | - | 2* |

| Medra System Organ Class | Severity | Not related to vaccination | Related to vaccination |
|---|---|---|---|
| Psychiatric Disorders | Mild | 1 | 1 |
| | Moderate | - | - |
| | Severe | - | - |
| Reproductive System and Breast Disorders | Mild | 3 | 1 |
| | Moderate | - | - |
| | Severe | - | - |
| Respiratory, Thoracic and Mediastinal Disorders | Mild | 5 | 2 |
| | Moderate | - | - |
| | Severe | - | - |
| Skin and Subcutaneous Tissue Disorders | Mild | 2 | 2 |
| | Moderate | - | 2 |
| | Severe | - | - |
| Vascular Disorders | Mild | 3 | 1 |
| | Moderate | - | - |
| | Severe | - | - |

*These two events judged related to vaccination were syncope and lightheadedness described in the case report in this supplement.

**Table S4. ELISA S-2P area under the curve IgG Williams mean, with 95% confidence intervals, by time point and dose group, and in a panel of convalescent sera.**

| Time point | N | 25 mcg | N | 100 mcg | N | 250 mcg | Convalescent Sera (N=38) |
|---|---|---|---|---|---|---|---|
| Day 1 | 15 | 0.5<br>(0.1 – 1.2) | 15 | 1.3<br>(0 - 4.0) | 15 | 1.4<br>(0- 4.7) | 14,196<br>(7,283 – 27,670) |
| Day 15 | 15 | 5,674<br>(3,224 – 9,983) | 15 | 19,068<br>(12,424 – 29,264) | 15 | 30,641<br>(18,028 – 52,078) | |
| Day 29 | 15 | 8,304<br>(5,221 – 13,209) | 15 | 20,525<br>(14,234 – 29,595) | 14 | 38,448<br>(22,899 – 64,555) | |
| Day 36 | 13 | 94,998<br>(64,997 – 138,817) | 15 | 213,076<br>(165,185 – 274,852) | 14 | 315,723<br>(255,688– 389,853) | |
| Day 43 | 13 | 91,081<br>(61,317 – 135,293) | 14 | 221,956<br>(182,108 – 270,524) | 14 | 254,374<br>(200,737 – 322,342) | |
| Day 57 | 13 | 77,904<br>(56,717 – 107,006) | 14 | 147,332<br>(113,898 – 190,579) | 13 | 215,140<br>(164,749 – 280,943) | |

All participants seroconverted at Day 15.

**Table S5. ELISA RBD area under the curve IgG Williams mean, with 95% confidence intervals, by time point and dose group, and in a panel of convalescent sera.**

| Time point | N | 25 mcg | N | 100 mcg | N | 250 mcg | Convalescent Sera (N=38) |
|---|---|---|---|---|---|---|---|
| Day 1 | 15 | 0<br>(0 – 0.1) | 15 | 1.5<br>(0.3 – 3.8) | 15 | 14.1<br>(5.8 - 32.8) | 4,216<br>(1,905 – 9,330) |
| Day 15 | 15 | 596<br>(258 – 1,376) | 15 | 5,642<br>(3,130 – 10,170) | 15 | 15,337<br>(9,094 – 25,866) | |
| Day 29 | 15 | 2,110<br>(1,130 – 3,939) | 15 | 12,130<br>(8,447 – 17,418) | 14 | 17,556<br>(10,869 – 28,358) | |
| Day 36 | 13 | 43,973<br>(30,848 – 62,681) | 15 | 126,250<br>(91,696 – 173,824) | 14 | 200,639<br>(160,276 – 251,167) | |
| Day 43 | 13 | 45,791<br>(30,246 – 69,327) | 14 | 141,713<br>(110,096 – 182,410) | 14 | 170,112<br>(133,715 – 216,415) | |
| Day 57 | 13 | 55,071<br>(36,135 – 83,930) | 14 | 106,248<br>(76,429 – 147,701) | 14 | 118,611<br>(84,180 – 111,486) | |

All participants seroconverted at Day 15.

**Table S6. Pseudovirus neutralization assay ID80 geometric mean results with 95% confidence intervals by time point and dose group, and in a panel of convalescent sera.**

| Time point | N | 25 mcg | N | 100 mcg | N | 250 mcg | Convalescent Sera (N=38) |
|---|---|---|---|---|---|---|---|
| Day 1 | 15 | 10 | 15 | 10 | 15 | 10 | 43.1 (26.4 – 70.5) |
| Day 15 | 15 | 10.9 (9.1 – 13.0) | 15 | 12.8 (8.4 – 19.5) | 15 | 13.4 (9.9 – 18.3) | |
| Day 29 | 15 | 10 | 15 | 10.2 (9.7 – 10.7) | 14 | 12.4 (9.8 – 15.6) | |
| Day 36 | 13 | 53.1 (34.0 – 82.9) | 15 | 120.7 (85.9 – 169.7) | 14 | 158.0 (130.6 – 191.1) | |
| Day 43 | 13 | 59.9 (37.4 – 95.8) | 14 | 153.8 (115.0 – 205.6) | 14 | 140.9 (110.5 – 179.7) | |
| Day 57 | 13 | 39.1 (22.8 – 66.9) | 14 | 110.3 (76.7 – 158.6) | 14 | 120.8 (92.9 – 157.0) | |

Samples that do not neutralize at the 80% level are expressed as <20 and plotted at half that dilution, *i.e.,* 10.

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