### Supplementary Appendix

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

Supplement to: Anderson EJ, Jackson LA, Rouphael NG, et al. Safety and Immunogenicity of a Third Dose of SARS-CoV-2 mRNA Vaccine — An Interim Analysis.

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## **Supplemental Methods**

The Advarra institutional review board functioned as a single board and both studies were overseen by independent safety monitoring committees convened for each trial. All participants provided written informed consent before enrollment in the 20-0003 substudy or the 21-0002 trial. Both were conducted under the original Investigational New Drug application submitted to the US FDA. The boost studies were open to participants starting in March 2021.

**Vaccines**

The mRNA-1273 vaccine was co-developed by researchers at the National Institute of Allergy and Infectious Diseases (NIAID, the sponsor of both trials) and at Moderna (Cambridge, MA). The mRNA-1273.351 vaccine was developed by researchers at Moderna. The mRNA-1273 vaccine encodes the S-2P antigen for the Wuhan-Hu-1 SARS-CoV-2 isolate, also known as 614D, that has a genome sequence identical to the first United States isolate (USA WA-1). The mRNA-1273.351 vaccine encodes for the S-2P antigen of the Beta variant. Both antigens were stabilized in the prefusion conformation as previously described.1,2 The lipid nanoparticle capsule was formulated with a fixed ratio of mRNA and lipid. The vaccines were provided as sterile liquid for injection at a concentration of 0.2 mg per mL for mRNA-1273 and 0.5 mg per mL for mRNA-1273.351. Normal saline was used as a diluent to prepare the 0.5 mL doses administered by injection in the deltoid muscle.

**Assessment of SARS-CoV-2 Binding Antibody and Neutralizing Responses**

The 4-plex Mesoscale Discovery (MSD) platform that has completed validation testing and is undergoing regulatory review, as previously described,3,4 against four SARS-CoV-2 proteins: Wa-1 S-2P, Nucleocapsid, B.1.351 S‑2P, and B.1.351 receptor binding domain (RBD).

The DMID 20-0003 substudy participants (monovalent prototype booster group) had pseudovirus neutralization performed using an assay developed at NIH and utilized for the original DMID 20-0003 study.1,3,5-7 Samples from the DMID 21-0002 study (monovalent variant and bivalent booster groups) used the same assay that had been further optimized, qualified and validated and was used to assess Phase 3 COVID-19 vaccine study responses and correlates of protection.8

**T Cell Analyses**

The peptides were synthesized by Biosynthesis and provided as individual lyophilized aliquots and reconstituted at 100mg/ml in DMSO.

As a negative control, cells were incubated with DMSO, the diluent for the peptide pools. As a positive control, cells were stimulated with a polyclonal stimulant, staphylococcal enterotoxin B (SEB). There were no replicates except for the negative control, which had two replicates.

Several criteria were used to determine if data from an assay were acceptable and could be statistically analyzed. The blood draw date must have been within the allowable visit window as determined by the protocol. After sample thawing and overnight incubation, the viability of the PBMCs must have been 66% or greater for testing to have proceeded. If it was not, a new specimen for that participant at that timepoint was thawed for testing. If the PBMC viability of the second thawed aliquot was below this threshold, the ICS assay was not performed, and no data were reported to the statistical center for the participant and timepoint.

The total numbers of CD4+ and CD8+ T cells must also have exceeded certain thresholds. If the number of CD8+ T cells was < 5,000 or CD4+ T cells was < 10,000 for any of the SARS-CoV-2-peptide pools or for one of the negative control replicates for a particular sample, data for that stimulation were filtered. If both negative control replicates failed for number of T cells, the sample was retested. If one negative control replicate failed for number of T cells, the negative control replicate with sufficient cells was used.

Individual-level response calls were determined by statistical testing of the number of cytokine positive CD4 T cells after spike peptide stimulation compared to the frequency of cytokine positive T cells in the negative control stimulation. To assess positivity for a peptide pool within a T-cell subset, a two-by-two contingency table was constructed comparing the SARS-CoV-2-peptide stimulated and negative control data. The four entries in each table were the number of cells positive for IFN-γ and/or IL-2 and the number of cells negative for IFN-γ and IL-2, for both the stimulated and the negative control data. If both negative control replicates were included, then the average number of total cells and the average number of positive cells were used. A one-sided Fisher's exact test was applied to the table, testing whether the number of cytokine-producing cells for the stimulated data was equal to that for the negative control data. Since multiple individual tests (for each peptide pool) were conducted simultaneously, a multiplicity adjustment was made to the individual peptide pool p-values using the Bonferroni-Holm adjustment method. If the adjusted p-value for a peptide pool was ≤0.00001, the response to the peptide pool for the T-cell subset was considered positive. Because the sample sizes (i.e., total cell counts for the T-cell subset) were large, e.g., as high as 100,000 cells, the Fisher’s exact test has high power to reject the null hypothesis for very small differences. Therefore, the adjusted p-value significance threshold was chosen stringently (≤ 0.00001). Plots include data from responders in color and non-responders in gray and the y-axis is truncated at 0.025% and any values below this level are set to this value.

**Analyses**

For both trials, Moderna was involved in discussions of trial design, provided the vaccine candidates, and, as part of the writing group, contributed to drafting this manuscript. The Emmes Company, as a subcontractor to the NIAID, served as the statistical and data coordinating center, developed the statistical analysis plans, and performed the analyses. The manuscript was written entirely by the authors, with the two first authors as the overall lead authors, the last author as the lead and senior NIAID author. The authors had full access to the data reports, which were prepared from the raw data by the statistical and data coordinating center and vouch for the completeness and accuracy of the data and for the fidelity of the trials to their respective protocols.

## **Supplemental Results**

**Vaccine Safety and Reactogenicity**

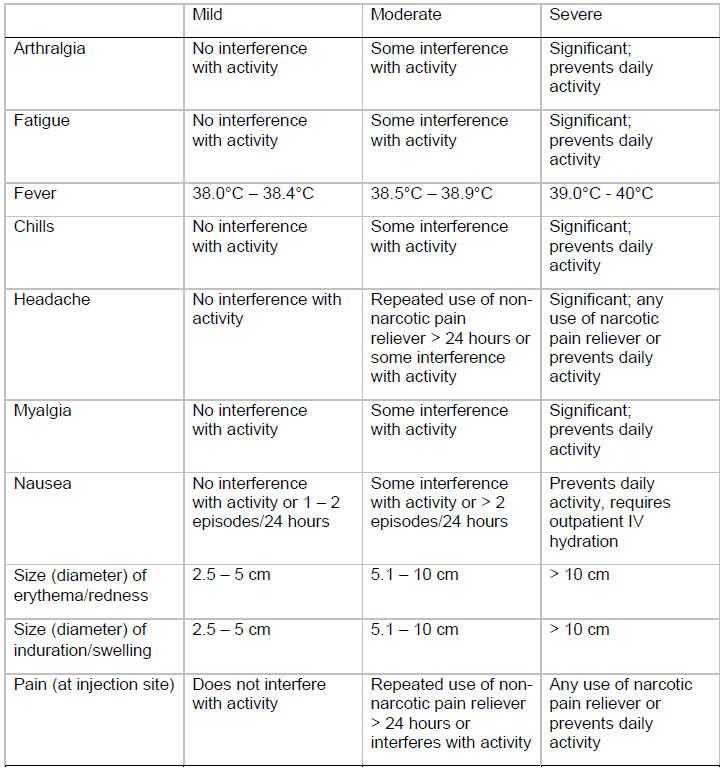
Overall, 45 unsolicited adverse events (all mild to moderate in severity) were reported within 28 days, of which 21 were considered related to the monovalent prototype vaccination. Those events included axillary lymphadenopathy, vomiting, chills, fatigue, gait disturbance, thirst, musculoskeletal chest pain, dizziness, headache, anxiety, oropharyngeal pain, night sweats, sensitive skin, and injection site bruising, paresthesia, pruritus, and pain (**Supplemental Tables S2** and **S5**). All events resolved, typically after 1-2 days, though one individual experienced fatigue for 8 days.

Of the 19 unsolicited adverse events occurring in the monovalent variant vaccine group, ten were judged related to the study vaccination that were mild to moderate in severity except for rash and vomiting (detailed in the manuscript). Related events included axillary lymphadenopathy, dyspepsia, vomiting, chills, swelling, jaw pain, nocturia, cough, epistaxis, and rash.

Of the 12 unsolicited adverse events occurring in the bivalent vaccine group, three were judged related to the study vaccination (vomiting, night sweats, axillary lymphadenopathy), but all were mild or moderate in severity (**Supplemental Tables S4** and **S7**).

A single 58-year-old individual with an underlying history of hypertension, alcohol use, seasonal allergies, anxiety, and brain aneurysm who had was taking budesonide, fluoxetine, montelukast, and loratadine had a serious adverse event of paresthesias of the upper extremity beginning 30 days after vaccination with the bivalent vaccine. At the time, the participant had normal platelets and did not have evidence of thrombosis. This was considered unrelated to vaccination, most likely due to a transient ischemic attack.

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



\*Obtained from a standard toxicity grading scale9

Table S2: Number of All Unsolicited Adverse Events by MedDRA® System Organ Class and Preferred Term, Severity, Relationship, and Vaccination Group – Monovalent Prototype (N=48)

|  | | | Severity | | | Relationship to Study Vaccination | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| System Organ Class (SOC) | Preferred Term (PT) | Total Events (n) | Mild (n) | Moderate (n) | Severe (n) | Not Related (n) | Related (n) |
| Any SOC | **Any PT** | 45 | 28 | 17 | . | 24 | 21 |
| Blood and Lymphatic System Disorders | **Any PT** | 2 | 2 | . | . | 1 | 1 |
| **Leukocytosis** | 1 | 1 | . | . | 1 | . |
| **Lymphadenopathy** | 1 | 1 | . | . | . | 1 |
| Cardiac Disorders | **Bradycardia** | 3 | 3 | . | . | 3 | . |
| Eye Disorders | **Eyelid irritation** | 1 | 1 | . | . | 1 | . |
| Gastrointestinal Disorders | **Any PT** | 2 | 1 | 1 | . | 1 | 1 |
| **Umbilical hernia** | 1 | 1 | . | . | 1 | . |
| **Vomiting** | 1 | . | 1 | . | . | 1 |
| General Disorders and Administration Site Conditions | **Any PT** | 12 | 9 | 3 | . | 1 | 11 |
| **Chills** | 1 | 1 | . | . | . | 1 |
| **Fatigue** | 2 | 1 | 1 | . | . | 2 |
| **Gait disturbance** | 1 | . | 1 | . | . | 1 |
| **Injection site bruising** | 2 | 2 | . | . | 1 | 1 |
| **Injection site paraesthesia** | 1 | 1 | . | . | . | 1 |
| **Injection site pruritus** | 3 | 3 | . | . | . | 3 |
| **Pain** | 1 | 1 | . | . | . | 1 |
| **Thirst** | 1 | . | 1 | . | . | 1 |
| Infections and Infestations | **Any PT** | 2 | . | 2 | . | 2 | . |
| **Lyme disease** | 1 | . | 1 | . | 1 | . |
| **Tooth abscess** | 1 | . | 1 | . | 1 | . |
| Injury, Poisoning and Procedural Complications | **Any PT** | 2 | . | 2 | . | 2 | . |
| **Joint dislocation** | 1 | . | 1 | . | 1 | . |
| **Limb injury** | 1 | . | 1 | . | 1 | . |
| Musculoskeletal and Connective Tissue Disorders | **Any PT** | 6 | . | 6 | . | 5 | 1 |
| **Exostosis** | 1 | . | 1 | . | 1 | . |
| **Musculoskeletal chest pain** | 1 | . | 1 | . | . | 1 |
| **Osteoarthritis** | 1 | . | 1 | . | 1 | . |
| **Scoliosis** | 1 | . | 1 | . | 1 | . |
| **Tendonitis** | 2 | . | 2 | . | 2 | . |
| Nervous System Disorders | **Any PT** | 5 | 4 | 1 | . | 2 | 3 |
| **Dizziness** | 2 | 2 | . | . | . | 2 |
| **Headache** | 2 | 2 | . | . | 1 | 1 |
| **Tremor** | 1 | . | 1 | . | 1 | . |
| Psychiatric Disorders | **Any PT** | 2 | 1 | 1 | . | 1 | 1 |
| **Anxiety** | 1 | 1 | . | . | . | 1 |
| **Generalised anxiety disorder** | 1 | . | 1 | . | 1 | . |
| Renal and Urinary Disorders | **Haematuria** | 1 | 1 | . | . | 1 | . |
| Respiratory, Thoracic and Mediastinal Disorders | **Oropharyngeal pain** | 1 | 1 | . | . | . | 1 |
| Skin and Subcutaneous Tissue Disorders | **Any PT** | 2 | 2 | . | . | . | 2 |
| **Night sweats** | 1 | 1 | . | . | . | 1 |
| **Sensitive skin** | 1 | 1 | . | . | . | 1 |
| Surgical and Medical Procedures | **Tooth extraction** | 1 | . | 1 | . | 1 | . |
| Vascular Disorders | **Any PT** | 3 | 3 | . | . | 3 | . |
| **Hypertension** | 1 | 1 | . | . | 1 | . |
| **Systolic hypertension** | 2 | 2 | . | . | 2 | . |

Table S3: Number of All Unsolicited Adverse Events by MedDRA® System Organ Class and Preferred Term, Severity, Relationship, and Vaccination Group – Monovalent Variant (N=25)

|  | | | Severity | | | Relationship to Study Vaccination | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| System Organ Class (SOC) | Preferred Term (PT) | Total Events (n) | Mild (n) | Moderate (n) | Severe (n) | Not Related (n) | Related (n) |
| Any SOC | **Any PT** | 19 | 12 | 5 | 2 | 9 | 10 |
| Blood and Lymphatic System Disorders | **Lymphadenopathy** | 1 | 1 | . | . | . | 1 |
| Gastrointestinal Disorders | **Any PT** | 2 | . | 1 | 1 | . | 2 |
| **Dyspepsia** | 1 | . | 1 | . | . | 1 |
| **Vomiting** | 1 | . | . | 1 | . | 1 |
| General Disorders and Administration Site Conditions | **Any PT** | 6 | 6 | . | . | 4 | 2 |
| **Chest discomfort** | 1 | 1 | . | . | 1 | . |
| **Chills** | 1 | 1 | . | . | . | 1 |
| **Injection site bruising** | 2 | 2 | . | . | 2 | . |
| **Swelling** | 1 | 1 | . | . | . | 1 |
| **Vessel puncture site pruritus** | 1 | 1 | . | . | 1 | . |
| Immune System Disorders | **Seasonal allergy** | 1 | 1 | . | . | 1 | . |
| Musculoskeletal and Connective Tissue Disorders | **Pain in jaw** | 1 | 1 | . | . | . | 1 |
| Psychiatric Disorders | **Any PT** | 3 | . | 3 | . | 3 | . |
| **Anxiety** | 1 | . | 1 | . | 1 | . |
| **Attention deficit hyperactivity disorder** | 1 | . | 1 | . | 1 | . |
| **Insomnia** | 1 | . | 1 | . | 1 | . |
| Renal and Urinary Disorders | **Nocturia** | 1 | . | 1 | . | . | 1 |
| Reproductive System and Breast Disorders | **Nipple disorder** | 1 | 1 | . | . | 1 | . |
| Respiratory, Thoracic and Mediastinal Disorders | **Any PT** | 2 | 2 | . | . | . | 2 |
| **Cough** | 1 | 1 | . | . | . | 1 |
| **Epistaxis** | 1 | 1 | . | . | . | 1 |
| Skin and Subcutaneous Tissue Disorders | **Rash** | 1 | . | . | 1 | . | 1 |

Table S4: Number of All Unsolicited Adverse Events by MedDRA® System Organ Class and Preferred Term, Severity, Relationship, and Vaccination Group – Bivalent (N=23)

|  | | | Severity | | | Relationship to Study Vaccination | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| System Organ Class (SOC) | Preferred Term (PT) | Total Events (n) | Mild (n) | Moderate (n) | Severe (n) | Not Related (n) | Related (n) |
| Any SOC | **Any PT** | 12 | 4 | 7 | 1 | 9 | 3 |
| Blood and Lymphatic System Disorders | **Lymphadenopathy** | 1 | 1 | . | . | . | 1 |
| Cardiac Disorders | **Bradycardia** | 1 | . | 1 | . | 1 | . |
| Gastrointestinal Disorders | **Vomiting** | 1 | . | 1 | . | . | 1 |
| Injury, Poisoning and Procedural Complications | **Limb injury** | 1 | . | 1 | . | 1 | . |
| Investigations | **Blood pressure increased** | 1 | . | 1 | . | 1 | . |
| Nervous System Disorders | **Paraesthesia** | 1 | . | . | 1 | 1 | . |
| Psychiatric Disorders | **Anxiety** | 1 | . | 1 | . | 1 | . |
| Reproductive System and Breast Disorders | **Heavy menstrual bleeding** | 1 | 1 | . | . | 1 | . |
| Skin and Subcutaneous Tissue Disorders | **Any PT** | 3 | 2 | 1 | . | 2 | 1 |
| **Blister** | 1 | 1 | . | . | 1 | . |
| **Dermatitis** | 1 | 1 | . | . | 1 | . |
| **Night sweats** | 1 | . | 1 | . | . | 1 |
| Vascular Disorders | **Haematoma** | 1 | . | 1 | . | 1 | . |

Table S5: Number and Percentage of Subjects Experiencing Unsolicited Adverse Events MedDRA® System Organ Class and Preferred Term, Severity, Relationship, and Vaccination Group – Monovalent Prototype (N=48)

|  | | | | Severity | | | | | | Relationship to Study Vaccination | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | Any Incidence | | Mild | | Moderate | | Severe | | Not Related | | Related | |
| MedDRA System Organ Class | Preferred Term | n | % | n | % | n | % | n | % | n | % | n | % |
| Any SOC | **Any PT** | 22 | 46 | 17 | 35 | 13 | 27 | . | . | 15 | 31 | 11 | 23 |
| Blood and Lymphatic System Disorders | **Any PT** | 2 | 4 | 2 | 4 | . | . | . | . | 1 | 2 | 1 | 2 |
| **Leukocytosis** | 1 | 2 | 1 | 2 | . | . | . | . | 1 | 2 | . | . |
| **Lymphadenopathy** | 1 | 2 | 1 | 2 | . | . | . | . | . | . | 1 | 2 |
| Cardiac Disorders | **Bradycardia** | 3 | 6 | 3 | 6 | . | . | . | . | 3 | 6 | . | . |
| Eye Disorders | **Eyelid irritation** | 1 | 2 | 1 | 2 | . | . | . | . | 1 | 2 | . | . |
| Gastrointestinal Disorders | **Any PT** | 2 | 4 | 1 | 2 | 1 | 2 | . | . | 1 | 2 | 1 | 2 |
| **Umbilical hernia** | 1 | 2 | 1 | 2 | . | . | . | . | 1 | 2 | . | . |
| **Vomiting** | 1 | 2 | . | . | 1 | 2 | . | . | . | . | 1 | 2 |
| General Disorders and Administration Site Conditions | **Any PT** | 9 | 19 | 8 | 17 | 3 | 6 | . | . | 1 | 2 | 8 | 17 |
| **Chills** | 1 | 2 | 1 | 2 | . | . | . | . | . | . | 1 | 2 |
| **Fatigue** | 2 | 4 | 1 | 2 | 1 | 2 | . | . | . | . | 2 | 4 |
| **Gait disturbance** | 1 | 2 | . | . | 1 | 2 | . | . | . | . | 1 | 2 |
| **Injection site bruising** | 2 | 4 | 2 | 4 | . | . | . | . | 1 | 2 | 1 | 2 |
| **Injection site paraesthesia** | 1 | 2 | 1 | 2 | . | . | . | . | . | . | 1 | 2 |
| **Injection site pruritus** | 3 | 6 | 3 | 6 | . | . | . | . | . | . | 3 | 6 |
| **Pain** | 1 | 2 | 1 | 2 | . | . | . | . | . | . | 1 | 2 |
| **Thirst** | 1 | 2 | . | . | 1 | 2 | . | . | . | . | 1 | 2 |
| Infections and Infestations | **Any PT** | 2 | 4 | . | . | 2 | 4 | . | . | 2 | 4 | . | . |
| **Lyme disease** | 1 | 2 | . | . | 1 | 2 | . | . | 1 | 2 | . | . |
| **Tooth abscess** | 1 | 2 | . | . | 1 | 2 | . | . | 1 | 2 | . | . |
| Injury, Poisoning and Procedural Complications | **Any PT** | 2 | 4 | . | . | 2 | 4 | . | . | 2 | 4 | . | . |
| **Joint dislocation** | 1 | 2 | . | . | 1 | 2 | . | . | 1 | 2 | . | . |
| **Limb injury** | 1 | 2 | . | . | 1 | 2 | . | . | 1 | 2 | . | . |
| Musculoskeletal and Connective Tissue Disorders | **Any PT** | 5 | 10 | . | . | 5 | 10 | . | . | 4 | 8 | 1 | 2 |
| **Exostosis** | 1 | 2 | . | . | 1 | 2 | . | . | 1 | 2 | . | . |
| **Musculoskeletal chest pain** | 1 | 2 | . | . | 1 | 2 | . | . | . | . | 1 | 2 |
| **Osteoarthritis** | 1 | 2 | . | . | 1 | 2 | . | . | 1 | 2 | . | . |
| **Scoliosis** | 1 | 2 | . | . | 1 | 2 | . | . | 1 | 2 | . | . |
| **Tendonitis** | 2 | 4 | . | . | 2 | 4 | . | . | 2 | 4 | . | . |
| Nervous System Disorders | **Any PT** | 4 | 8 | 4 | 8 | 1 | 2 | . | . | 1 | 2 | 3 | 6 |
| **Dizziness** | 2 | 4 | 2 | 4 | . | . | . | . | . | . | 2 | 4 |
| **Headache** | 2 | 4 | 2 | 4 | . | . | . | . | 1 | 2 | 1 | 2 |
| **Tremor** | 1 | 2 | . | . | 1 | 2 | . | . | 1 | 2 | . | . |
| Psychiatric Disorders | **Any PT** | 2 | 4 | 1 | 2 | 1 | 2 | . | . | 1 | 2 | 1 | 2 |
| **Anxiety** | 1 | 2 | 1 | 2 | . | . | . | . | . | . | 1 | 2 |
| **Generalised anxiety disorder** | 1 | 2 | . | . | 1 | 2 | . | . | 1 | 2 | . | . |
| Renal and Urinary Disorders | **Haematuria** | 1 | 2 | 1 | 2 | . | . | . | . | 1 | 2 | . | . |
| Respiratory, Thoracic and Mediastinal Disorders | **Oropharyngeal pain** | 1 | 2 | 1 | 2 | . | . | . | . | . | . | 1 | 2 |
| Skin and Subcutaneous Tissue Disorders | **Any PT** | 2 | 4 | 2 | 4 | . | . | . | . | . | . | 2 | 4 |
| **Night sweats** | 1 | 2 | 1 | 2 | . | . | . | . | . | . | 1 | 2 |
| **Sensitive skin** | 1 | 2 | 1 | 2 | . | . | . | . | . | . | 1 | 2 |
| Surgical and Medical Procedures | **Tooth extraction** | 1 | 2 | . | . | 1 | 2 | . | . | 1 | 2 | . | . |
| Vascular Disorders | **Any PT** | 3 | 6 | 3 | 6 | . | . | . | . | 3 | 6 | . | . |
| **Hypertension** | 1 | 2 | 1 | 2 | . | . | . | . | 1 | 2 | . | . |
| **Systolic hypertension** | 2 | 4 | 2 | 4 | . | . | . | . | 2 | 4 | . | . |
| Note: This table presents number and percentage of subjects. A subject is only counted once per PT and is summarized according to their highest severity and closest relationship. | | | | | | | | | | | | | |

Table S6: Number and Percentage of Subjects Experiencing Unsolicited Adverse Events MedDRA® System Organ Class and Preferred Term, Severity, Relationship, and Vaccination Group – Monovalent Variant (N=25)

|  | | | | Severity | | | | | | Relationship to Study Vaccination | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | Any Incidence | | Mild | | Moderate | | Severe | | Not Related | | Related | |
| MedDRA System Organ Class | Preferred Term | n | % | n | % | n | % | n | % | n | % | n | % |
| Any SOC | **Any PT** | 14 | 56 | 10 | 40 | 4 | 16 | 2 | 8 | 6 | 24 | 10 | 40 |
| Blood and Lymphatic System Disorders | **Lymphadenopathy** | 1 | 4 | 1 | 4 | . | . | . | . | . | . | 1 | 4 |
| Gastrointestinal Disorders | **Any PT** | 2 | 8 | . | . | 1 | 4 | 1 | 4 | . | . | 2 | 8 |
| **Dyspepsia** | 1 | 4 | . | . | 1 | 4 | . | . | . | . | 1 | 4 |
| **Vomiting** | 1 | 4 | . | . | . | . | 1 | 4 | . | . | 1 | 4 |
| General Disorders and Administration Site Conditions | **Any PT** | 6 | 24 | 6 | 24 | . | . | . | . | 4 | 16 | 2 | 8 |
| **Chest discomfort** | 1 | 4 | 1 | 4 | . | . | . | . | 1 | 4 | . | . |
| **Chills** | 1 | 4 | 1 | 4 | . | . | . | . | . | . | 1 | 4 |
| **Injection site bruising** | 2 | 8 | 2 | 8 | . | . | . | . | 2 | 8 | . | . |
| **Swelling** | 1 | 4 | 1 | 4 | . | . | . | . | . | . | 1 | 4 |
| **Vessel puncture site pruritus** | 1 | 4 | 1 | 4 | . | . | . | . | 1 | 4 | . | . |
| Immune System Disorders | **Seasonal allergy** | 1 | 4 | 1 | 4 | . | . | . | . | 1 | 4 | . | . |
| Musculoskeletal and Connective Tissue Disorders | **Pain in jaw** | 1 | 4 | 1 | 4 | . | . | . | . | . | . | 1 | 4 |
| Psychiatric Disorders | **Any PT** | 2 | 8 | . | . | 2 | 8 | . | . | 2 | 8 | . | . |
| **Anxiety** | 1 | 4 | . | . | 1 | 4 | . | . | 1 | 4 | . | . |
| **Attention deficit hyperactivity disorder** | 1 | 4 | . | . | 1 | 4 | . | . | 1 | 4 | . | . |
| **Insomnia** | 1 | 4 | . | . | 1 | 4 | . | . | 1 | 4 | . | . |
| Renal and Urinary Disorders | **Nocturia** | 1 | 4 | . | . | 1 | 4 | . | . | . | . | 1 | 4 |
| Reproductive System and Breast Disorders | **Nipple disorder** | 1 | 4 | 1 | 4 | . | . | . | . | 1 | 4 | . | . |
| Respiratory, Thoracic and Mediastinal Disorders | **Any PT** | 2 | 8 | 2 | 8 | . | . | . | . | . | . | 2 | 8 |
| **Cough** | 1 | 4 | 1 | 4 | . | . | . | . | . | . | 1 | 4 |
| **Epistaxis** | 1 | 4 | 1 | 4 | . | . | . | . | . | . | 1 | 4 |
| Skin and Subcutaneous Tissue Disorders | **Rash** | 1 | 4 | . | . | . | . | 1 | 4 | . | . | 1 | 4 |
| Note: This table presents number and percentage of subjects. A subject is only counted once per PT and is summarized according to their highest severity and closest relationship. | | | | | | | | | | | | | |

Table S7: Number and Percentage of Subjects Experiencing Unsolicited Adverse Events MedDRA® System Organ Class and Preferred Term, Severity, Relationship, and Vaccination Group – Bivalent (N=23)

|  | | | | Severity | | | | | | Relationship to Study Vaccination | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | Any Incidence | | Mild | | Moderate | | Severe | | Not Related | | Related | |
| MedDRA System Organ Class | Preferred Term | n | % | n | % | n | % | n | % | n | % | n | % |
| Any SOC | **Any PT** | 8 | 35 | 4 | 17 | 6 | 26 | 1 | 4 | 7 | 30 | 3 | 13 |
| Blood and Lymphatic System Disorders | **Lymphadenopathy** | 1 | 4 | 1 | 4 | . | . | . | . | . | . | 1 | 4 |
| Cardiac Disorders | **Bradycardia** | 1 | 4 | . | . | 1 | 4 | . | . | 1 | 4 | . | . |
| Gastrointestinal Disorders | **Vomiting** | 1 | 4 | . | . | 1 | 4 | . | . | . | . | 1 | 4 |
| Injury, Poisoning and Procedural Complications | **Limb injury** | 1 | 4 | . | . | 1 | 4 | . | . | 1 | 4 | . | . |
| Investigations | **Blood pressure increased** | 1 | 4 | . | . | 1 | 4 | . | . | 1 | 4 | . | . |
| Nervous System Disorders | **Paraesthesia** | 1 | 4 | . | . | . | . | 1 | 4 | 1 | 4 | . | . |
| Psychiatric Disorders | **Anxiety** | 1 | 4 | . | . | 1 | 4 | . | . | 1 | 4 | . | . |
| Reproductive System and Breast Disorders | **Heavy menstrual bleeding** | 1 | 4 | 1 | 4 | . | . | . | . | 1 | 4 | . | . |
| Skin and Subcutaneous Tissue Disorders | **Any PT** | 3 | 13 | 2 | 9 | 1 | 4 | . | . | 2 | 9 | 1 | 4 |
| **Blister** | 1 | 4 | 1 | 4 | . | . | . | . | 1 | 4 | . | . |
| **Dermatitis** | 1 | 4 | 1 | 4 | . | . | . | . | 1 | 4 | . | . |
| **Night sweats** | 1 | 4 | . | . | 1 | 4 | . | . | . | . | 1 | 4 |
| Vascular Disorders | **Haematoma** | 1 | 4 | . | . | 1 | 4 | . | . | 1 | 4 | . | . |
| Note: This table presents number and percentage of subjects. A subject is only counted once per PT and is summarized according to their highest severity and closest relationship. | | | | | | | | | | | | | |

## **Table S8: Serum IgG Binding Assay Area Under the Curve Geometric Mean Titers by Variant and Prime Series Dose at Baseline before a Third Dose of mRNA Vaccination with 95% Confidence Intervals**

| Variants | Statistic | 25 μg  (N=29) | 50 μg  (N=23) | 100 μg  (N=31) | 250 μg  (N=13) |
| --- | --- | --- | --- | --- | --- |
| 614D (Wa-1) | n | 28 | 21 | 27 | 12 |
| GM | 4948 | 9268 | 12861 | 14409 |
| 95% CI | 3742, 6543 | 7327, 11724 | 10004, 16534 | 10578, 19627 |
| Beta (B.1.351) | n | 28 | 21 | 27 | 12 |
| GM | 1953 | 3723 | 5100 | 5777 |
| 95% CI | 1513, 2522 | 3001, 4618 | 3952, 6581 | 4407, 7574 |
| Delta (B.1.617.2) | n | 28 | 21 | 27 | 12 |
| GM | 2354 | 4724 | 6686 | 7553 |
| 95% CI | 1798, 3083 | 3852, 5792 | 5249, 8516 | 5603, 10183 |
| Alpha (B.1.1.7) | n | 28 | 21 | 27 | 12 |
| GM | 3509 | 6309 | 8972 | 9539 |
| 95% CI | 2578, 4776 | 4954, 8034 | 6898, 11670 | 7074, 12862 |
| Gamma (P.1) | n | 28 | 21 | 27 | 12 |
| GM | 1577 | 3419 | 4531 | 5340 |
| 95% CI | 1169, 2128 | 2614, 4472 | 3457, 5938 | 3886, 7337 |
| Note: N=Number of Subjects n=Number of subjects with results available at time point Confidence intervals of the geometric means were calculated with the Student’s t distribution on log-transformed data | | | | | |

**Table S9.** **Serum IgG Binding Assay Area Under the Curve (AUC) Geometric Means for Variants by Time Point with 95% Confidence Intervals**

1. IgG Binding Assay Area Under the Curve GM by Time Point - 614D

| Time Point | Statistic | Monovalent prototype | Monovalent variant | Bivalent | P201 |
| --- | --- | --- | --- | --- | --- |
| Booster Dose, Day 1 | n | 41 | 25 | 22 | 30 |
| GM | 5865 | 13412 | 12225 | 14066 |
| 95% CI | 4711, 7303 | 10064, 17872 | 10049, 14873 | 11813, 16747 |
| Day 15 | n | 41 | 25 | 22 | 30 |
| GM | 62272 | 61373 | 62025 | 61650 |
| 95% CI | 59973, 64659 | 58622, 64254 | 59468, 64691 | 59609, 63762 |
| Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | |

1. **Serum IgG Binding Assay Area Under the Curve GM by Time Point – Beta (B.1.351)**

| Time Point | Statistic | Monovalent prototype | Monovalent variant | Bivalent | P201 |
| --- | --- | --- | --- | --- | --- |
| Booster Dose, Day 1 | n | 41 | 25 | 22 | 30 |
| GM | 2362 | 5438 | 4649 | 5608 |
| 95% CI | 1926, 2898 | 4117, 7183 | 3761, 5748 | 4713, 6675 |
| Day 15 | n | 41 | 25 | 22 | 30 |
| GM | 47733 | 49768 | 48126 | 43097 |
| 95% CI | 44932, 50710 | 46282, 53517 | 44728, 51781 | 40878, 45437 |
| Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | |

1. Serum IgG Binding Assay Area Under the Curve GM by Time Point - Alpha (B.1.1.7)

| Time Point | Statistic | Monovalent prototype | Monovalent variant | Bivalent | P201 |
| --- | --- | --- | --- | --- | --- |
| Booster Dose, Day 1 | n | 41 | 25 | 22 | 30 |
| GM | 4151 | 9406 | 7999 | 9780 |
| 95% CI | 3281, 5252 | 6990, 12657 | 6495, 9851 | 8115, 11787 |
| Day 15 | n | 41 | 25 | 22 | 30 |
| GM | 59512 | 58549 | 58930 | 56993 |
| 95% CI | 56677, 62490 | 55031, 62292 | 55613, 62446 | 54362, 59751 |
| Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | |

1. **Serum IgG Binding Assay Area Under the Curve GM by Time Point – Delta (B.1.617.2)**

| Time Point | Statistic | Monovalent prototype | Monovalent variant | Bivalent | P201 |
| --- | --- | --- | --- | --- | --- |
| Booster Dose, Day 1 | n | 41 | 25 | 22 | 30 |
| GM | 2857 | 6978 | 6310 | 6982 |
| 95% CI | 2311, 3533 | 5347, 9106 | 5125, 7767 | 5836, 8353 |
| Day 15 | N | 41 | 25 | 22 | 30 |
| GM | 51698 | 50352 | 50377 | 46313 |
| 95% CI | 48378, 55247 | 46399, 54641 | 46889, 54124 | 43760, 49016 |
| Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | |

1. Serum IgG Binding Assay Area Under the Curve GM by Time Point – Gamma (P.1)

| Time Point | Statistic | Monovalent prototype | Monovalent variant | Bivalent | P201 |
| --- | --- | --- | --- | --- | --- |
| Booster Dose, Day 1 | n | 41 | 25 | 22 | 30 |
| GM | 1982 | 4840 | 4281 | 4968 |
| 95% CI | 1549, 2536 | 3594, 6519 | 3373, 5434 | 4005, 6163 |
| Day 15 | n | 41 | 25 | 22 | 30 |
| GM | 47496 | 49096 | 48283 | 43370 |
| 95% CI | 44002, 51267 | 45048, 53508 | 44330, 52588 | 40735, 46175 |
| Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | |

Table S10. Serum IgG Binding Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group

1. Serum IgG Binding Antibody Units/mL Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - S2P-614D

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent Prototype**  **(N=48)** | **Monovalent Variant**  **(N=25)** | **Bivalent**  **(N=23)** | **P201 50 μg mRNA-1273** |
| Day 1  (Pre-Vaccination 1) | n | 41 | 25 | 22 | 30 |
| GM | 118 | 365 | 319 | 409 |
| 95% CI | 92, 151 | 265, 502 | 249, 407 | 329, 509 |
| Day 15  (14 Days Post Vaccination 1) | n | 41 | 25 | 22 | 30 |
| GM | 8396 | 9591 | 9046 | 7477 |
| 95% CI | 6912, 10199 | 7485, 12289 | 7299, 11212 | 6416, 8713 |
| Day 29 Post Vaccination 1  (28 Days Post Vaccination 1) | n | 41 | 25 | 22 | 30 |
| GM | 8197 | 11487 | 10588 | 6399 |
| 95% CI | 6534, 10284 | 8970, 14711 | 8375, 13386 | 5486, 7465 |
| Note: N=Number of Subjects  n=Number of subjects with results available at time point  Confidence intervals of the geometric means were calculated with the Student’s t distribution on log-transformed data  P201=Subjects from Moderna Phase 2 study that received a 2 dose regimen of 100 ug mRNA-1273 followed by a booster of 50 ug mRNA-1273 | | | | | |

1. Serum IgG Binding Assay Arbitrary Units/mL Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - S2P-614D (Wa-1)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent Prototype**  **(N=48)** | **Monovalent Variant**  **(N=25)** | **Bivalent**  **(N=23)** | **P201 50 μg mRNA-1273** |
| Day 1  (Pre-Vaccination 1) | n | 41 | 25 | 22 | 30 |
| GM | 13085 | 40556 | 35394 | 45466 |
| 95% CI | 10173, 16831 | 29458, 55833 | 27704, 45219 | 36581, 56509 |
| Day 15  (14 Days Post Vaccination 1) | n | 41 | 25 | 22 | 30 |
| GM | 932918 | 1065643 | 1005139 | 830765 |
| 95% CI | 767982, 1133277 | 831652, 1365469 | 811001, 1245749 | 712901, 968115 |
| Day 29 Post Vaccination 1  (28 Days Post Vaccination 1) | n | 41 | 25 | 22 | 30 |
| GM | 910785 | 1276348 | 1176460 | 711048 |
| 95% CI | 725953, 1142677 | 996653, 1634535 | 930536, 1487377 | 609587, 829396 |
| Note: N=Number of Subjects  n=Number of subjects with results available at time point  Confidence intervals of the geometric means were calculated with the Student’s t distribution on log-transformed data  P201=Subjects from Moderna Phase 2 study that received a 2 dose regimen of 100 ug mRNA-1273 followed by a booster of 50 ug mRNA-1273 | | | | | |

1. IgG Binding Assay Arbitrary Units/mL Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - S2P-Beta (B.1.351)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent Prototype**  **(N=48)** | **Monovalent Variant**  **(N=25)** | **Bivalent**  **(N=23)** | **P201 50 μg mRNA-1273** |
| Day 1  (Pre-Vaccination 1) | n | 41 | 25 | 22 | 30 |
| GM | 5381 | 16714 | 14462 | 17287 |
| 95% CI | 4282, 6763 | 12487, 22373 | 11244, 18603 | 13839, 21594 |
| Day 15  (14 Days Post Vaccination 1) | n | 41 | 25 | 22 | 30 |
| GM | 437626 | 598972 | 475346 | 341216 |
| 95% CI | 360968, 530564 | 463183, 774570 | 383415, 589319 | 292176, 398488 |
| Day 29 Post Vaccination 1  (28 Days Post Vaccination 1) | n | 41 | 25 | 22 | 30 |
| GM | 426872 | 633385 | 502900 | 287748 |
| 95% CI | 344255, 529318 | 495342, 809897 | 401635, 629698 | 245603, 337126 |
| Note: N=Number of Subjects  n=Number of subjects with results available at time point  Confidence intervals of the geometric means were calculated with the Student’s t distribution on log-transformed data  P201=Subjects from Moderna Phase 2 study that received a 2 dose regimen of 100 ug mRNA-1273 followed by a booster of 50 ug mRNA-1273 | | | | | |

1. Serum IgG Binding Assay Arbitrary Units/mL Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - RBD-Beta (B.1.351)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent Prototype**  **(N=48)** | **Monovalent Variant**  **(N=25)** | **Bivalent**  **(N=23)** | **P201 50 μg mRNA-1273** |
| Day 1  (Pre-Vaccination 1) | n | 41 | 25 | 22 | 30 |
| GM | 1566 | 4860 | 3956 | 4678 |
| 95% CI | 1195, 2052 | 3567, 6622 | 3131, 4997 | 3544, 6176 |
| Day 15  (14 Days Post Vaccination 1) | n | 41 | 25 | 22 | 30 |
| GM | 131242 | 213451 | 164860 | 111025 |
| 95% CI | 104905, 164192 | 165067, 276019 | 132745, 204745 | 89227, 138148 |
| Day 29 Post Vaccination 1  (28 Days Post Vaccination 1) | n | 41 | 25 | 22 | 30 |
| GM | 123658 | 209466 | 161428 | 94756 |
| 95% CI | 96261, 158853 | 161815, 271148 | 126982, 205218 | 76166, 117883 |
| Note: N=Number of Subjects  n=Number of subjects with results available at time point  Confidence intervals of the geometric means were calculated with the Student’s t distribution on log-transformed data  P201=Subjects from Moderna Phase 2 study that received a 2 dose regimen of 100 ug mRNA-1273 followed by a booster of 50 ug mRNA-1273 | | | | | |

**Table S11.** **Serum Pseudovirus Neutralization ID50 Assay Geometric Mean (GM) Titers at Baseline before a Third Dose of mRNA Vaccination by Variant with 95% Confidence Intervals**

| Variants | Statistic | 25 μg  (N=29) | 50 μg  (N=23) | 100 μg  (N=31) | 250 μg  (N=13) |
| --- | --- | --- | --- | --- | --- |
| D614G | n | 28 | 21 | 27 | 12 |
| GM | 32 | 70 | 129 | 117 |
| 95% CI | 21, 47 | 44, 110 | 102, 163 | 83, 167 |
| Seropositive | 17/28 (60.7%) | 19/21 (90.5%) | 27/27 (100%) | 12/12 (100%) |
| 95% CI | 40.6%, 78.5% | 69.6%, 98.8% | 87.2%, 100% | 73.5%, 100% |
| Beta (B.1.351) | n | 28 | 21 | 27 | 12 |
| GM | 12 | 17 | 22 | 28 |
| 95% CI | 10, 14 | 12, 22 | 16, 31 | 19, 43 |
| Seropositive | 3/28 (10.7%) | 7/21 (33.3%) | 18/27 (66.7%) | 9/12 (75%) |
| 95% CI | 2.3%, 28.2% | 14.6%, 57% | 46%, 83.5% | 42.8%, 94.5% |
| Delta (B.1.617.2) | n | 15 | 10 | 22 |  |
| GM | 10 | 14 | 83 |  |
| 95% CI | 10, 11 | 10, 21 | 53, 128 |  |
| Seropositive | 0/15 (0%) | 3/10 (30%) | 22/22 (100%) |  |
| 95% CI | 0%, 21.8% | 6.7%, 65.2% | 84.6%, 100% |  |
| Alpha (B.1.1.7) | n | 15 | 10 | 22 |  |
| GM | 34 | 38 | 89 |  |
| 95% CI | 18, 64 | 17, 86 | 64, 126 |  |
| Seropositive | 9/15 (60%) | 6/10 (60%) | 21/22 (95.5%) |  |
| 95% CI | 32.3%, 83.7% | 26.2%, 87.8% | 77.2%, 99.9% |  |
| Gamma (P.1) | n | 15 | 10 | 22 |  |
| GM | 17 | 22 | 44 |  |
| 95% CI | 11, 26 | 11, 44 | 29, 67 |  |
| Seropositive | 4/15 (26.7%) | 4/10 (40%) | 19/22 (86.4%) |  |
| 95% CI | 7.8%, 55.1% | 12.2%, 73.8% | 65.1%, 97.1% |  |
| Note: N=Number of Subjects n=Number of subjects with results available at time point Confidence intervals of the geometric means were calculated with the Student’s t distribution on log-transformed data | | | | | |

## **Table S12.** **Serum responses following the booster vaccination assessed by a pseudovirus neutralizing assay – ID50 by Timepoint and Group:A) D614G; B) Beta (B.1.351); C) Alpha (B.1.1.7); D) Delta (B.1.617.2); E) Gamma (P.1).**

1. Pseudovirus Neutralization Assay ID₅₀ - D614G Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group

| Time Point | Statistic | Monovalent prototype | Monovalent variant | Bivalent | P201 | Peak Neutralization (100 μg) | Peak Neutralization (25/50 μg) |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Booster Dose, Day 1 | n | 41 | 25 | 22 | 30 | 20 | 41 |
| GM | 39 | 127 | 110 | 190 | 1399 | 645 |
| 95% CI | 28, 54 | 96, 168 | 84, 143 | 147, 244 | 1111, 1762 | 504, 826 |
| Day 15, +/- 2 | n | 41 | 25 | 22 | 30 |  |  |
| GM | 2842 | 2111 | 2435 | 2321 |  |  |
| 95% CI | 2270, 3560 | 1615, 2760 | 1912, 3102 | 1890, 2851 |  |  |
| Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003 Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | | | |

1. Pseudovirus Neutralization Assay ID₅₀ - Beta (B.1.351) Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group

| Time Point | Statistic | Monovalent prototype | Monovalent variant | Bivalent | P201 | Peak Neutralization (100 μg) | Peak Neutralization (25/50 μg) |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Booster Dose, Day 1 | n | 41 | 25 | 22 | 30 | 20 | 41 |
| GM | 13 | 23 | 23 | 28 | 165 | 48 |
| 95% CI | 11, 15 | 16, 33 | 16, 32 | 20, 39 | 105, 259 | 35, 64 |
| Day 15, +/- 2 | n | 41 | 25 | 22 | 30 |  |  |
| GM | 865 | 1100 | 866 | 539 |  |  |
| 95% CI | 672, 1113 | 867, 1395 | 659, 1139 | 428, 680 |  |  |
| Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003 Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | | | |

1. Pseudovirus Neutralization Assay ID₅₀ - Alpha (B.1.1.7) Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group

| Time Point | Statistic | Monovalent prototype | Monovalent variant | Bivalent | P201 | Peak Neutralization  (100 μg) |
| --- | --- | --- | --- | --- | --- | --- |
| Booster Dose, Day 1 | n | 25 | 12 | 10 | 16 | 17 |
| GM | 36 | 110 | 70 | 77 | 925 |
| 95% CI | 23, 56 | 69, 176 | 40, 121 | 50, 118 | 662, 1292 |
| Day 15, +/- 2 | n | 25 | 12 | 10 | 16 |  |
| GM | 3425 | 3278 | 2882 | 1539 |  |
| 95% CI | 2543, 4613 | 2549, 4215 | 2005, 4143 | 1082, 2188 |  |
| Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003 Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | | |

1. Pseudovirus Neutralization Assay ID₅₀ - Delta (B.1.617.2) Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group

| Time Point | Statistic | Monovalent prototype | Monovalent variant | Bivalent | P201 | Peak Neutralization (100 μg) | Peak Neutralization (25/50 μg) |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Booster Dose, Day 1 | n | 25 | 12 | 10 | 16 | 17 | 25 |
| GM | 12 | 119 | 53 | 69 | 592 | 260 |
| 95% CI | 10, 14 | 58, 242 | 35, 82 | 41, 115 | 413, 849 | 197, 342 |
| Day 15, +/- 2 | n | 25 | 12 | 10 | 16 |  |  |
| GM | 517 | 2045 | 1509 | 951 |  |  |
| 95% CI | 361, 740 | 1379, 3034 | 885, 2571 | 618, 1463 |  |  |
| Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003 Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | | | |

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1. Pseudovirus Neutralization Assay ID₅₀ - Gamma (P.1) Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group

| Time Point | Statistic | Monovalent prototype | Monovalent variant | Bivalent | P201 | Peak Neutralization  (100 μg) |
| --- | --- | --- | --- | --- | --- | --- |
| Booster Dose, Day 1 | n | 25 | 12 | 10 | 16 | 16 |
| GM | 19 | 45 | 43 | 33 | 485 |
| 95% CI | 13, 27 | 23, 90 | 24, 75 | 21, 53 | 321, 733 |
| Day 15, +/- 2 | n | 25 | 12 | 10 | 16 |  |
| GM | 2197 | 4609 | 3055 | 1157 |  |
| 95% CI | 1595, 3028 | 3054, 6956 | 1809, 5161 | 783, 1710 |  |
| Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003 Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | | |

**Table S13.** **Serum Pseudovirus Neutralization ID80 Assay Geometric Mean (GM) Titers at Baseline before a Third Dose of mRNA Vaccination by Variant with 95% Confidence Intervals**

| Variants | Statistic | 25 μg  (N=29) | 50 μg  (N=23) | 100 μg  (N=31) | 250 μg  (N=13) |
| --- | --- | --- | --- | --- | --- |
| D614G | n | 28 | 21 | 27 | 12 |
| GM | 18 | 28 | 47 | 47 |
| 95% CI | 13, 24 | 20, 41 | 38, 60 | 32, 68 |
| Seropositive | 10/28 (35.7%) | 12/21 (57.1%) | 24/27 (88.9%) | 10/12 (83.3%) |
| 95% CI | 18.6%, 55.9% | 34%, 78.2% | 70.8%, 97.6% | 51.6%, 97.9% |
| Beta (B.1.351) | n | 28 | 21 | 27 | 12 |
| GM | 11 | 11 | 10 | 11 |
| 95% CI | 10, 11 | 8, 13 | 8, 13 | 8, 15 |
| Seropositive | 1/28 (3.6%) | 2/21 (9.5%) | 3/27 (11.1%) | 1/12 (8.3%) |
| 95% CI | 0.1%, 18.3% | 1.2%, 30.4% | 2.4%, 29.2% | 0.2%, 38.5% |
| Delta (B.1.617.2) | n | 15 | 10 | 22 |  |
| GM | 10 | 10 | 21 |  |
| 95% CI | NE | 10, 11 | 15, 28 |  |
| Seropositive | 0/15 (0%) | 0/10 (0%) | 11/22 (50%) |  |
| 95% CI | 0%, 21.8% | 0%, 30.8% | 28.2%, 71.8% |  |
| Alpha (B.1.1.7) | n | 15 | 10 | 22 |  |
| GM | 20 | 18 | 27 |  |
| 95% CI | 14, 29 | 10, 31 | 19, 39 |  |
| Seropositive | 8/15 (53.3%) | 4/10 (40%) | 15/22 (68.2%) |  |
| 95% CI | 26.6%, 78.7% | 12.2%, 73.8% | 45.1%, 86.1% |  |
| Gamma (P.1) | n | 15 | 10 | 22 |  |
| GM | 12 | 14 | 18 |  |
| 95% CI | 10, 15 | 9, 20 | 13, 25 |  |
| Seropositive | 2/15 (13.3%) | 2/10 (20%) | 8/22 (36.4%) |  |
| 95% CI | 1.7%, 40.5% | 2.5%, 55.6% | 17.2%, 59.3% |  |
| Note: N=Number of Subjects n=Number of subjects with results available at time point Confidence intervals of the geometric means were calculated with the Student’s t distribution on log-transformed data | | | | | |

## **Table S14.** **Neutralizing responses following the booster vaccination assessed by a pseudovirus neutralizing assay – ID80 by Timepoint and Group.**

1. **Pseudovirus Neutralization Assay ID₈₀ - D614G Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent prototype** | **Monovalent variant** | **Bivalent** | **P201** |
| Booster  Dose,  Day 1 | n | 41 | 25 | 22 | 30 |
| GM | 20 | 47 | 43 | 63 |
| 95% CI | 15, 25 | 36, 62 | 33, 55 | 50, 79 |
| Day 15,  +/- 2 | n | 41 | 25 | 22 | 30 |
| GM | 1315 | 758 | 886 | 768 |
| 95% CI | 1049, 1648 | 588, 979 | 688, 1142 | 625, 944 |
| Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003  Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | |

1. **Pseudovirus Neutralization Assay ID₈₀ - Beta (B.1.351) Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent prototype** | **Monovalent variant** | **Bivalent** | **P201** |
| Booster  Dose,  Day 1 | n | 41 | 25 | 22 | 30 |
| GM | 11 | 10 | 10 | 12 |
| 95% CI | 10, 12 | 8, 13 | 7, 12 | 9, 16 |
| Day 15,  +/- 2 | n | 41 | 25 | 22 | 30 |
| GM | 422 | 392 | 311 | 221 |
| 95% CI | 321, 553 | 323, 477 | 233, 415 | 173, 283 |
| Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003  Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | |

1. **Pseudovirus Neutralization Assay ID₈₀ - Alpha (B.1.1.7) Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent prototype** | **Monovalent variant** | **Bivalent** | **P201** |
| Booster  Dose,  Day 1 | n | 25 | 12 | 10 | 16 |
| GM | 19 | 31 | 24 | 28 |
| 95% CI | 14, 25 | 19, 51 | 13, 42 | 19, 42 |
| Day 15,  +/- 2 | n | 25 | 12 | 10 | 16 |
| GM | 1485 | 1224 | 1106 | 575 |
| 95% CI | 1076, 2051 | 885, 1693 | 733, 1669 | 402, 822 |
| Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003  Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | |

1. **Pseudovirus Neutralization Assay ID₈₀ - Delta (B.1.617.2) Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent prototype** | **Monovalent variant** | **Bivalent** | **P201** |
| Booster  Dose,  Day 1 | n | 25 | 12 | 10 | 16 |
| GM | 10 | 25 | 16 | 18 |
| 95% CI | 10, 10 | 15, 43 | 11, 24 | 12, 28 |
| Day 15,  +/- 2 | n | 25 | 12 | 10 | 16 |
| GM | 309 | 681 | 485 | 339 |
| 95% CI | 212, 450 | 493, 940 | 299, 788 | 246, 465 |
| Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003  Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | |

1. **Pseudovirus Neutralization Assay ID₈₀ - Gamma (P.1) Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent prototype** | **Monovalent variant** | **Bivalent** | **P201** |
| Booster  Dose,  Day 1 | n | 25 | 12 | 10 | 16 |
| GM | 13 | 18 | 17 | 16 |
| 95% CI | 11, 15 | 11, 31 | 10, 27 | 11, 22 |
| Day 15,  +/- 2 | n | 25 | 12 | 10 | 16 |
| GM | 817 | 1322 | 972 | 399 |
| 95% CI | 598, 1118 | 887, 1971 | 597, 1585 | 294, 541 |
| Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003  Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | |

**Table S15.** **Serum FRNT ID50 Assay Geometric Mean (GM) Titers at Baseline before a Third Dose of mRNA Vaccination by Variant with 95% Confidence Intervals**

| Variants | Statistic | 25 μg  (N=29) | 50 μg  (N=23) | 100 μg  (N=31) |
| --- | --- | --- | --- | --- |
| D614G | n | 15 | 10 | 22 |
| GM | 34 | 63 | 95 |
| 95% CI | 22, 54 | 31, 127 | 65, 139 |
| Seropositive | 11/15 (73.3%) | 9/10 (90%) | 20/22 (90.9%) |
| 95% CI | 44.9%, 92.2% | 55.5%, 99.7% | 70.8%, 98.9% |
| Beta (B.1.351) | n | 15 | 10 | 22 |
| GM | 14 | 12 | 27 |
| 95% CI | 9, 20 | 9, 17 | 18, 39 |
| Seropositive | 3/15 (20%) | 1/10 (10%) | 14/22 (63.6%) |
| 95% CI | 4.3%, 48.1% | 0.3%, 44.5% | 40.7%, 82.8% |
| Delta (B.1.617.2) | n | 15 | 10 | 22 |
| GM | 15 | 57 | 43 |
| 95% CI | 10, 22 | 25, 130 | 31, 61 |
| Seropositive | 4/15 (26.7%) | 7/10 (70%) | 18/22 (81.8%) |
| 95% CI | 7.8%, 55.1% | 34.8%, 93.3% | 59.7%, 94.8% |
| Alpha (B.1.1.7) | n | 15 | 10 | 22 |
| GM | 31 | 58 | 102 |
| 95% CI | 16, 59 | 26, 129 | 71, 146 |
| Seropositive | 8/15 (53.3%) | 8/10 (80%) | 22/22 (100%) |
| 95% CI | 26.6%, 78.7% | 44.4%, 97.5% | 84.6%, 100% |
| Gamma (P.1) | n | 15 | 10 | 22 |
| GM | 11 | 13 | 33 |
| 95% CI | 9, 13 | 9, 20 | 20, 55 |
| Seropositive | 2/15 (13.3%) | 2/10 (20%) | 14/22 (63.6%) |
| 95% CI | 1.7%, 40.5% | 2.5%, 55.6% | 40.7%, 82.8% |
| Note: N=Number of Subjects n=Number of subjects with results available at time point Confidence intervals of the geometric means were calculated with the Student’s t distribution on log-transformed data | | | | |

**Table S16.** **Serum FRNT Assay Titers Distribution by Time Point – ID50 – Versus Variants with 95% Confidence Intervals**

1. **FRNT Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₅₀ - D614G**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent prototype** | **Monovalent variant** | **Bivalent** | **P201** | **Peak Neutralization** |
| Day 1 | n | 25 | 12 | 10 | 16 | 18 |
| GM | 44 | 97 | 93 | 79 | 750 |
| 95% CI | 30, 64 | 58, 161 | 46, 187 | 53, 117 | 549, 1024 |
| Day 15,  +/- 2 | n | 25 | 12 | 11 | 16 |  |
| GM | 3375 | 2829 | 2392 | 2084 |  |
| 95% CI | 2374, 4796 | 2139, 3740 | 1664, 3438 | 1564, 2777 |  |
| NE=Not Estimable  Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003  Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | | |

1. **FRNT Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₅₀ - Beta (B.1.351)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent prototype** | **Monovalent variant** | **Bivalent** | **P201** | **Peak Neutralization** |
| Day 1 | n | 25 | 12 | 10 | 16 | 17 |
| GM | 13 | 28 | 25 | 29 | 163 |
| 95% CI | 10, 17 | 17, 48 | 13, 48 | 20, 42 | 93, 286 |
| Day 15,  +/- 2 | n | 25 | 12 | 10 | 16 |  |
| GM | 1063 | 2020 | 969 | 533 |  |
| 95% CI | 717, 1575 | 1222, 3339 | 609, 1540 | 360, 788 |  |
| NE=Not Estimable  Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003  Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | | |

1. **FRNT Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₅₀ - Alpha (B.1.1.7)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent prototype** | **Monovalent variant** | **Bivalent** | **P201** | **Peak Neutralization** |
| Day 1 | n | 25 | 12 | 10 | 16 | 18 |
| GM | 40 | 135 | 73 | 87 | 624 |
| 95% CI | 25, 64 | 81, 224 | 43, 123 | 44, 172 | 452, 861 |
| Day 15,  +/- 2 | n | 25 | 12 | 11 | 16 |  |
| GM | 2527 | 3196 | 2230 | 1600 |  |
| 95% CI | 1780, 3587 | 2351, 4344 | 1236, 4023 | 1078, 2375 |  |
| NE=Not Estimable  Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003  Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | | |

1. **FRNT Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₅₀ - Delta (B.1.617.2)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent prototype** | **Monovalent variant** | **Bivalent** | **P201** | **Peak Neutralization** |
| Day 1 | n | 25 | 12 | 10 | 16 |  |
| GM | 26 | 45 | 41 | 31 |  |
| 95% CI | 16, 41 | 26, 78 | 25, 68 | 19, 52 |  |
| Day 15,  +/- 2 | n | 25 | 12 | 11 | 16 |  |
| GM | 1756 | 1712 | 1139 | 807 |  |
| 95% CI | 1262, 2443 | 1251, 2343 | 671, 1932 | 507, 1284 |  |
| NE=Not Estimable  Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003  Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | | |

1. **FRNT Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₅₀ - Gamma (P.1)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent prototype** | **Monovalent variant** | **Bivalent** | **P201** | **Peak Neutralization** |
| Day 1 | n | 25 | 12 | 10 | 16 |  |
| GM | 12 | 38 | 28 | 25 |  |
| 95% CI | 10, 14 | 19, 79 | 12, 65 | 14, 45 |  |
| Day 15,  +/- 2 | n | 25 | 12 | 11 | 16 |  |
| GM | 1348 | 1796 | 1113 | 742 |  |
| 95% CI | 903, 2011 | 1221, 2643 | 494, 2510 | 468, 1178 |  |
| NE=Not Estimable  Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003  Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | | |

**Table S17.** **Serum FRNT ID80 Assay Geometric Mean (GM) Titers at Baseline before a Third Dose of mRNA Vaccination by Variant with 95% Confidence Intervals**

| Variants | Statistic | 25 μg  (N=29) | 50 μg  (N=23) | 100 μg  (N=31) |
| --- | --- | --- | --- | --- |
| D614G | n | 15 | 10 | 22 |
| GM | 15 | 21 | 32 |
| 95% CI | 11, 21 | 11, 39 | 23, 43 |
| Seropositive | 4/15 (26.7%) | 5/10 (50%) | 16/22 (72.7%) |
| 95% CI | 7.8%, 55.1% | 18.7%, 81.3% | 49.8%, 89.3% |
| Beta (B.1.351) | n | 15 | 10 | 22 |
| GM | 10 | 10 | 11 |
| 95% CI | NE | 9, 12 | 10, 13 |
| Seropositive | 0/15 (0%) | 0/10 (0%) | 1/22 (4.5%) |
| 95% CI | 0%, 21.8% | 0%, 30.8% | 0.1%, 22.8% |
| Delta (B.1.617.2) | n | 15 | 10 | 22 |
| GM | 10 | 11 | 15 |
| 95% CI | NE | 9, 15 | 11, 19 |
| Seropositive | 0/15 (0%) | 1/10 (10%) | 6/22 (27.3%) |
| 95% CI | 0%, 21.8% | 0.3%, 44.5% | 10.7%, 50.2% |
| Alpha (B.1.1.7) | n | 15 | 10 | 22 |
| GM | 11 | 17 | 25 |
| 95% CI | 10, 13 | 10, 27 | 16, 37 |
| Seropositive | 1/15 (6.7%) | 4/10 (40%) | 12/22 (54.5%) |
| 95% CI | 0.2%, 31.9% | 12.2%, 73.8% | 32.2%, 75.6% |
| Gamma (P.1) | n | 15 | 10 | 22 |
| GM | 10 | 11 | 14 |
| 95% CI | NE | 9, 13 | 11, 18 |
| Seropositive | 0/15 (0%) | 1/10 (10%) | 6/22 (27.3%) |
| 95% CI | 0%, 21.8% | 0.3%, 44.5% | 10.7%, 50.2% |
| Note: N=Number of Subjects n=Number of subjects with results available at time point Confidence intervals of the geometric means were calculated with the Student’s t distribution on log-transformed data | | | | |

**Table S18. Serum FRNT Assay Titers Distribution by Time Point – ID80 – Versus Variants with 95% Confidence Intervals**

1. **FRNT Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₈₀ - D614G**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent prototype** | **Monovalent variant** | **Bivalent** | **P201** | **Peak Neutralization** |
| Day 1 | n | 25 | 12 | 10 | 16 |  |
| GM | 17 | 33 | 30 | 30 |  |
| 95% CI | 13, 23 | 21, 54 | 19, 49 | 19, 46 |  |
| Day 15,  +/- 2 | n | 25 | 12 | 11 | 16 |  |
| GM | 757 | 1067 | 1075 | 653 |  |
| 95% CI | 512, 1121 | 798, 1427 | 734, 1574 | 461, 924 |  |
| NE=Not Estimable  Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003  Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | | |

1. **FRNT Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₈₀ - Beta (B.1.351)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent prototype** | **Monovalent variant** | **Bivalent** | **P201** | **Peak Neutralization** |
| Day 1 | n | 25 | 12 | 10 | 16 |  |
| GM | 10 | 11 | 12 | 11 |  |
| 95% CI | 10, 11 | 10, 11 | 8, 16 | 10, 13 |  |
| Day 15,  +/- 2 | n | 25 | 12 | 10 | 16 |  |
| GM | 220 | 570 | 234 | 154 |  |
| 95% CI | 156, 311 | 332, 980 | 121, 449 | 102, 232 |  |
| NE=Not Estimable  Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003  Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | | |

1. **FRNT Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₈₀ - Alpha (B.1.1.7)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent prototype** | **Monovalent variant** | **Bivalent** | **P201** | **Peak Neutralization** |
| Day 1 | n | 25 | 12 | 10 | 16 |  |
| GM | 13 | 27 | 22 | 25 |  |
| 95% CI | 11, 16 | 15, 51 | 11, 41 | 16, 41 |  |
| Day 15,  +/- 2 | n | 25 | 12 | 11 | 16 |  |
| GM | 732 | 1230 | 942 | 543 |  |
| 95% CI | 533, 1006 | 945, 1601 | 464, 1914 | 348, 847 |  |
| NE=Not Estimable  Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003  Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | | |

1. **FRNT Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₈₀ - Delta (B.1.617.2)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent prototype** | **Monovalent variant** | **Bivalent** | **P201** | **Peak Neutralization** |
| Day 1 | n | 25 | 12 | 10 | 16 |  |
| GM | 11 | 16 | 13 | 11 |  |
| 95% CI | 9, 12 | 11, 24 | 9, 20 | 9, 14 |  |
| Day 15,  +/- 2 | n | 25 | 12 | 11 | 16 |  |
| GM | 479 | 521 | 459 | 147 |  |
| 95% CI | 335, 684 | 362, 751 | 236, 894 | 73, 295 |  |
| NE=Not Estimable  Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003  Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | | |

1. **FRNT Assay Geometric Mean (GM) Results with 95% Confidence Intervals by Time Point and Vaccination Group - ID₈₀ - Gamma (P.1)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Time Point** | **Statistic** | **Monovalent prototype** | **Monovalent variant** | **Bivalent** | **P201** | **Peak Neutralization** |
| Day 1 | n | 25 | 12 | 10 | 16 |  |
| GM | 10 | 14 | 14 | 12 |  |
| 95% CI | 10, 11 | 10, 20 | 9, 21 | 10, 16 |  |
| Day 15,  +/- 2 | n | 25 | 12 | 11 | 16 |  |
| GM | 334 | 634 | 345 | 183 |  |
| 95% CI | 227, 490 | 403, 998 | 132, 903 | 106, 317 |  |
| NE=Not Estimable  Peak Neutralization = Neutralization 2 weeks post second dose of 100 ug mRNA-1273 in DMID Protocol 20-0003  Confidence intervals of the geometric means were calculated with the student’s t distribution on log-transformed data | | | | | | |

**Table S19. Mean Percentages of CD4 T Cells Expressing Cytokines with 95% CI**

See separate table.

**Table S20. Mean Percentages of CD8 T Cells Expressing Cytokines with 95% CI**

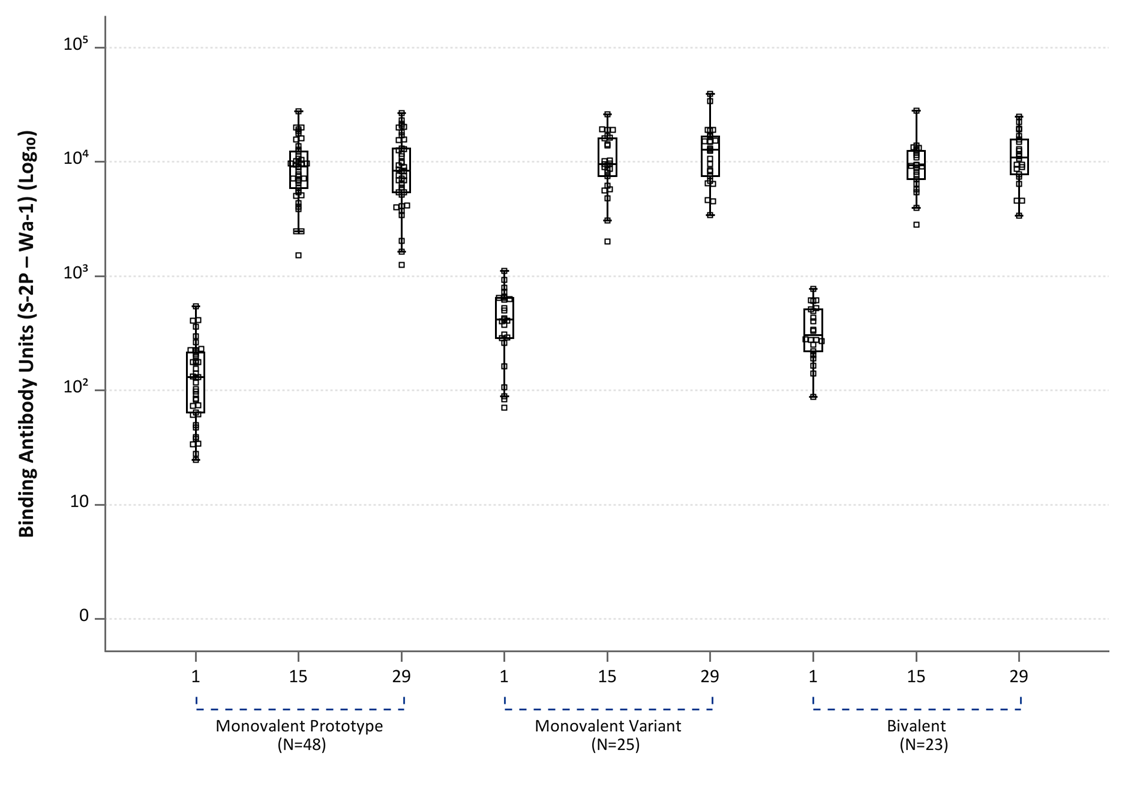
See separate table.

## **Figure S1. Serum IgG Binding Assays Distribution by Time Point and Treatment Group**

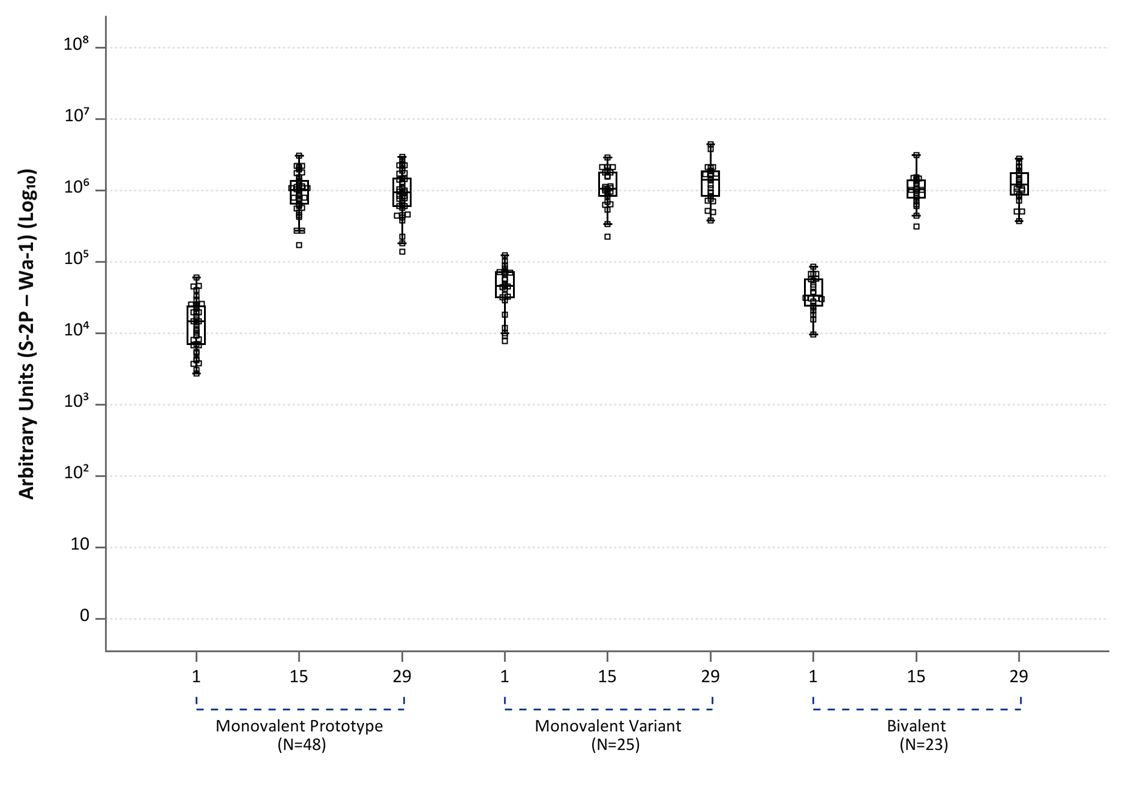
IgG S-2P antibodies were low in many participants at baseline, particularly in those who had received 25 or 50 mcg doses of mRNA-1273 for their primary 2 dose series (monovalent prototype group) and against the Beta and Delta variants. All participants had robust increases in S-2P IgG antibody titers by 2 weeks after receipt of vaccine across all the variants.

Boxes and horizontal bars denote interquartile range (IQR) and median AU, respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median +/- 1.5 x IQR. Titers for participants that had received 100 mcg for their initial two doses in the Phase I study are highlighted with red triangles. All other participants that had received other initial doses (25, 50, 250 mcg) are denoted by black circles.

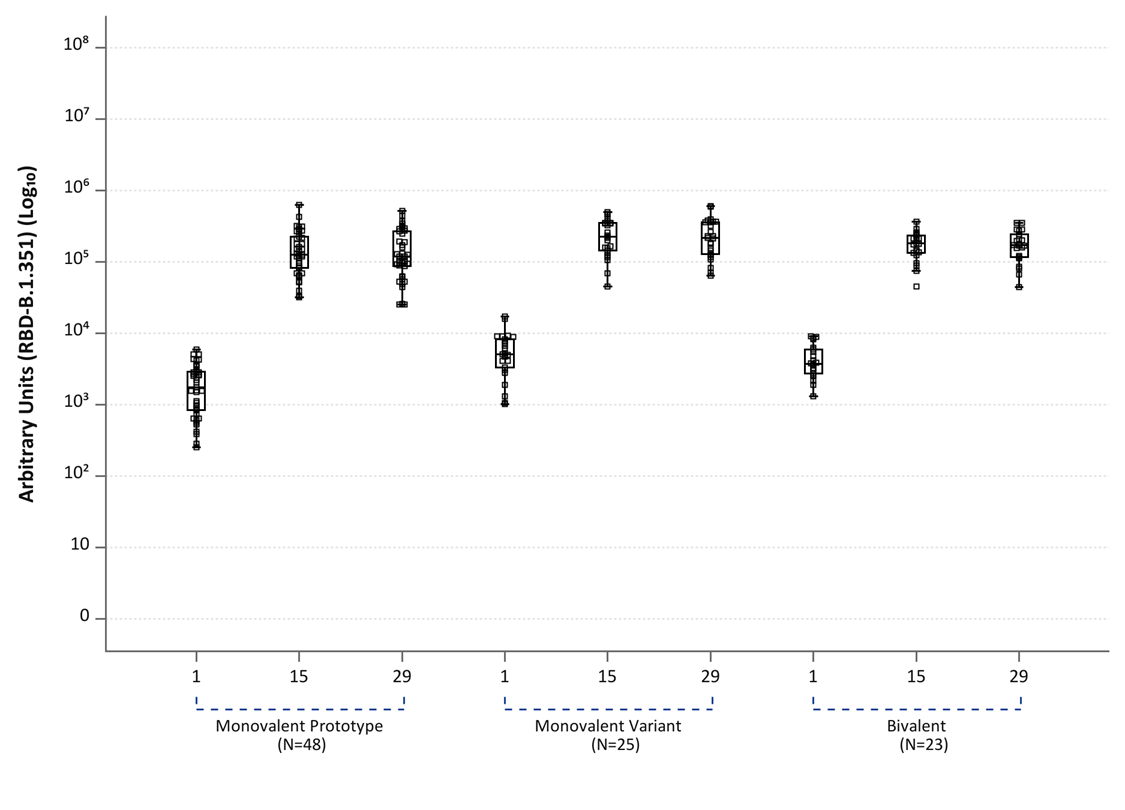
1. **Serum IgG S-2P Binding Assay Binding Antibody Units/mL Distribution by Time Point and Treatment Group – 614D**



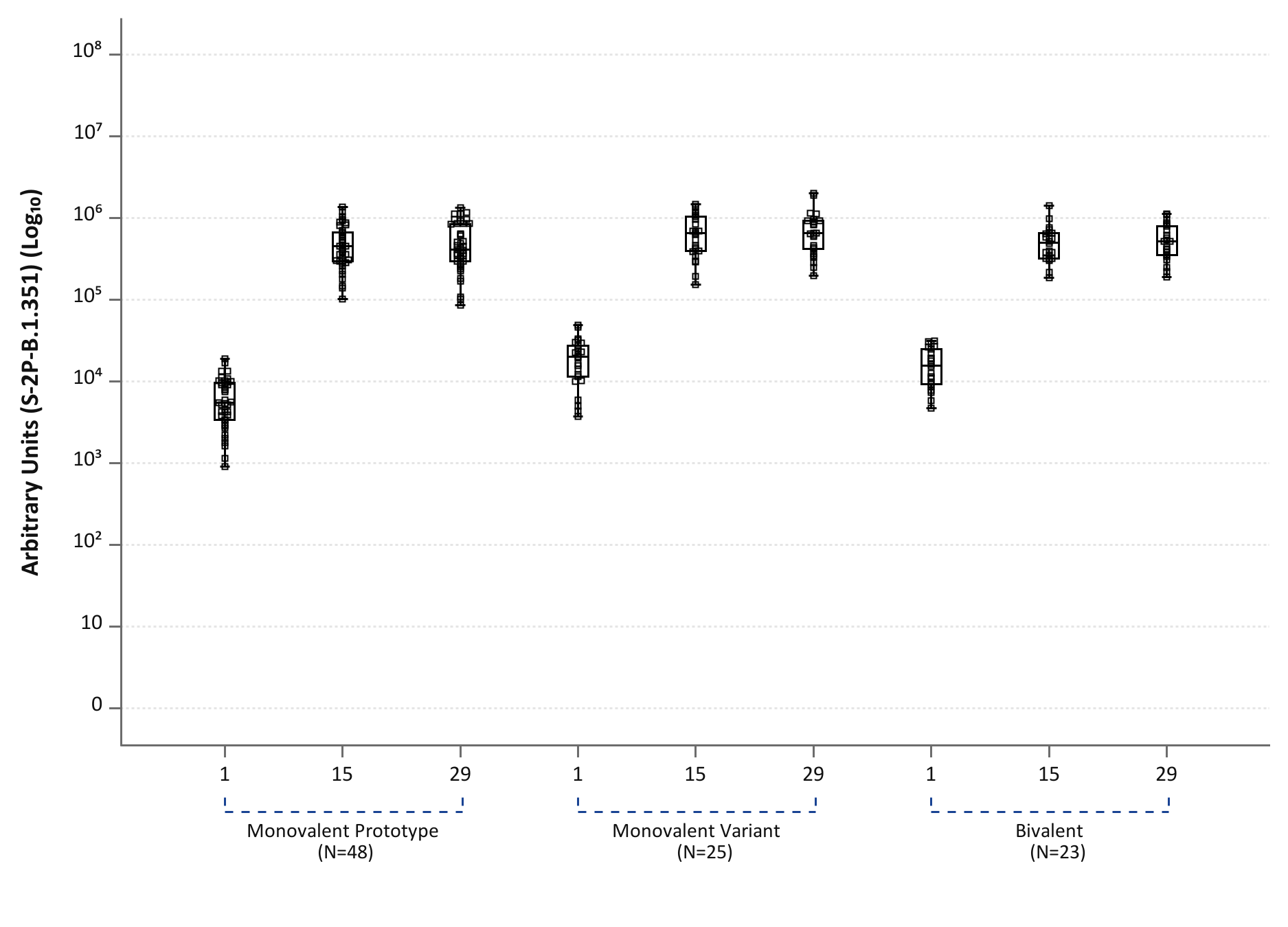
1. **Serum IgG S-2P Binding Assay Arbitrary Units/mL Distribution by Time Point and Treatment Group – 614D**



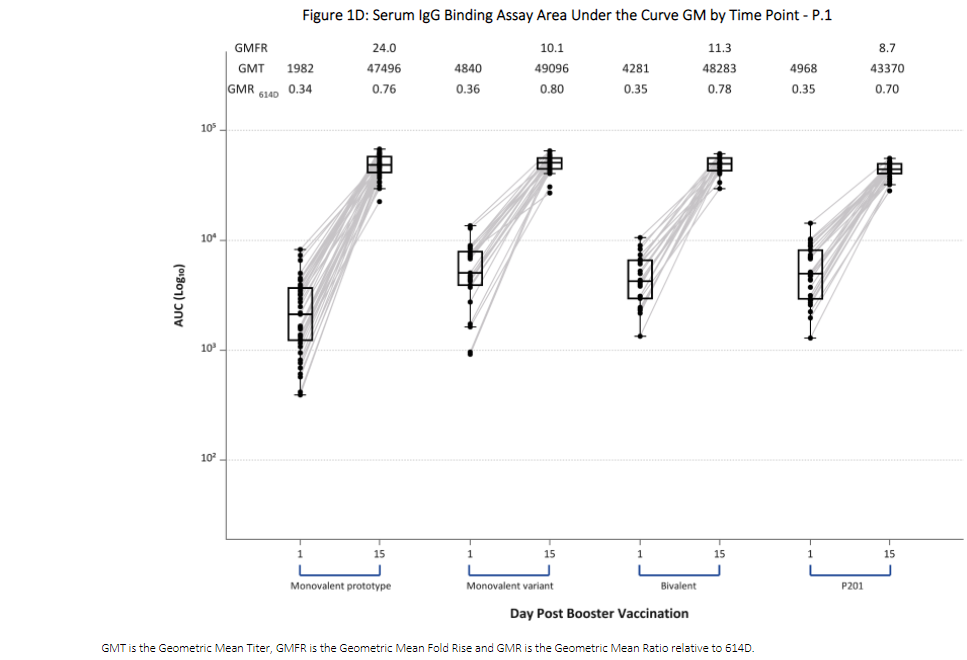
1. **Serum IgG RBD Binding Assay Arbitrary Units/mL Distribution by Time Point and Treatment Group – Beta (B.1.351)**



1. **Serum IgG S-2P Binding Assay Arbitrary Units/mL Distribution by Time Point and Treatment Group - Beta (B.1.351)**



1. **Serum IgG Binding Area Under the Curve (AUC) Titers Distributed by Timepoint for Gamma (P.1)**

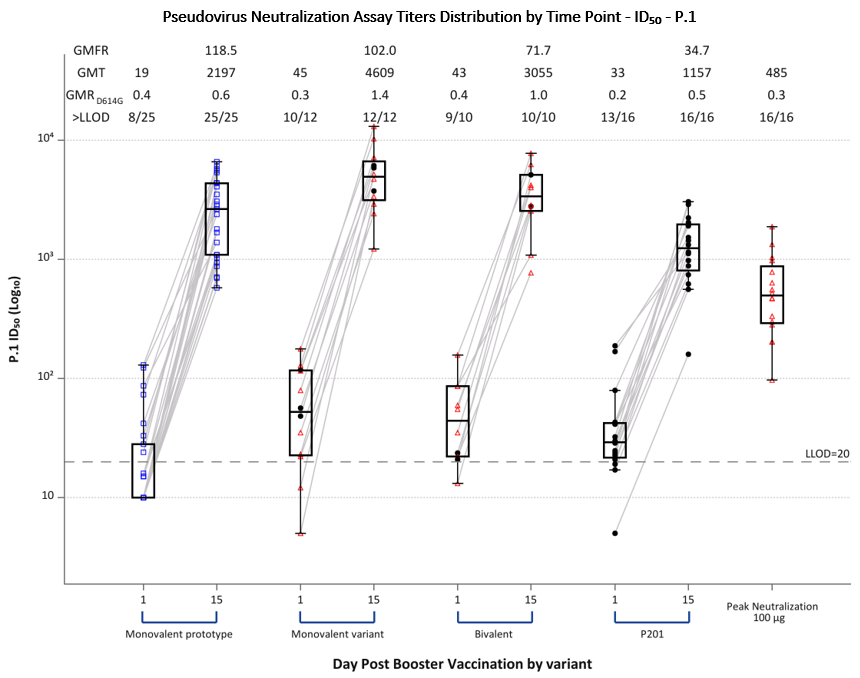


GMT (geometric mean titer), GMFR (geometric mean fold rise), GMR614D (the ratio of GMTs for the variant of concern divided by the GMT of 614D strain). Boxes and horizontal bars denote interquartile range (IQR) and median respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median +/- 1.5 x IQR.

## **Figure S2. Serum Pseudovirus Neutralization Assay ID50 Titers Distributed by Timepoint for the Gamma Variant (P.1)**

Serum Pseudovirus Neutralization Assay ID50 Titerswere low to undetectable in many participants at baseline, particularly in those who had received 25 or 50 mcg doses of mRNA-1273 for their primary 2 dose series (blue squares in monovalent prototype group) for the Gamma Variant (P.1). All participants had robust increases in Pseudovirus Neutralization Assay ID50 antibody titers by 2 weeks after receipt of vaccine. Red triangles denote those that had received an initial 2 dose series of 100 mcg of mRNA-1273. For comparison, pseudovirus neutralization assayID50 titers were similar or greater than those of 30 adults that had received an initial 100 mcg of mRNA-1273 in the Phase 2 study followed by a 50 mcg third dose (P201). Peak pseudovirus neutralization titers observed (at 2 or 4 weeks) after the second dose in 100 mcg recipients in the Phase 1 mRNA-1273 participants at provided as a comparator (Peak Neutralization 100 mcg).

Boxes and horizontal bars denote interquartile range (IQR) and median AU, respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median +/- 1.5 x IQR. LLOD is the lower limit of detection of the assay.

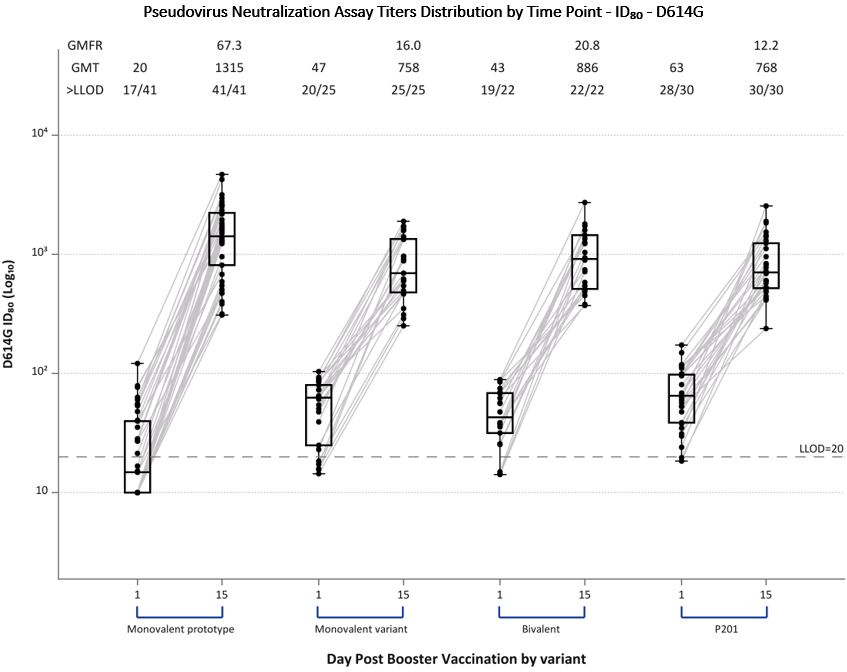
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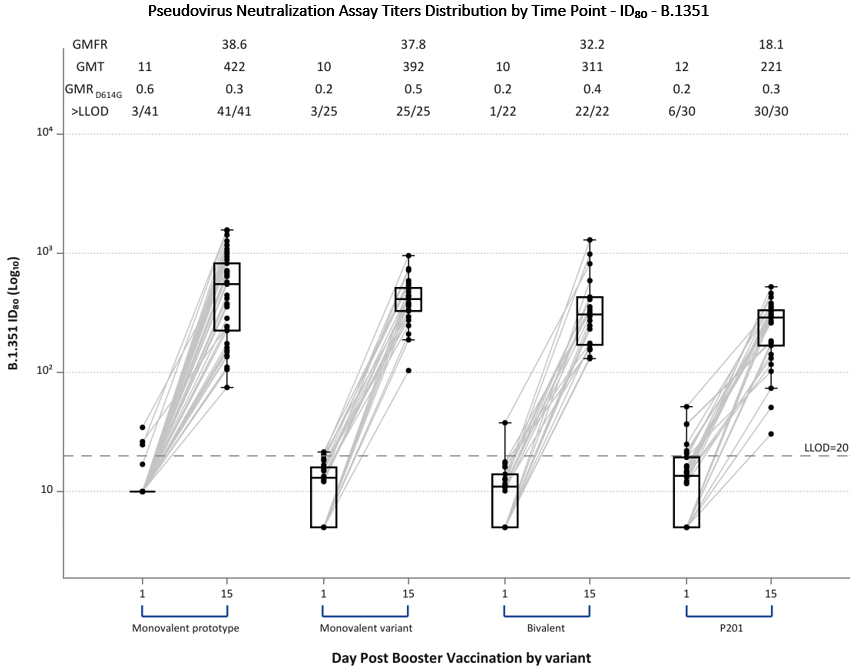
## **Figure S3. Serum Pseudovirus Neutralization Assay ID80 Titers Distributed by Timepoint by Variant. a: D614G, b: Beta (B.1.351), c: Alpha (B.1.1.7); d: Delta (B.1.617.2), and e: Gamma (P.1).**

Serum Pseudovirus Neutralization Assay ID80 Titers before and at 14 days after receiving a third dose of mRNA by study group: Monovalent prototype (100 mcg of mRNA-1273), Monovalent variant (50 mcg of mRNA-1273.351), and Bivalent (25 mcg of mRNA-1273, 25 mcg of mRNA-1273.351). Serum Pseudovirus Neutralization Assay ID80 Titers were low to undetectable in many participants at baseline, particularly in those who had received 25 or 50 mcg doses of mRNA-1273 for their primary 2 dose series (blue squares in monovalent prototype group), particularly for Beta, Delta, and Gamma variants. All participants had robust increases in Pseudovirus Neutralization Assay ID80 antibody titers by 2 weeks after receipt of vaccine across all the variants. For comparison, titers were similar or greater than those of 30 adults that had received an initial 100 mcg of mRNA-1273 in the Phase 2 study followed by a 50 mcg third dose (P201). Peak pseudovirus neutralization titers observed (at 2 or 4 weeks) after the second dose in 100 mcg recipients in the Phase 1 mRNA-1273 participants at provided as a comparator (Peak Neutralization 100 mcg).

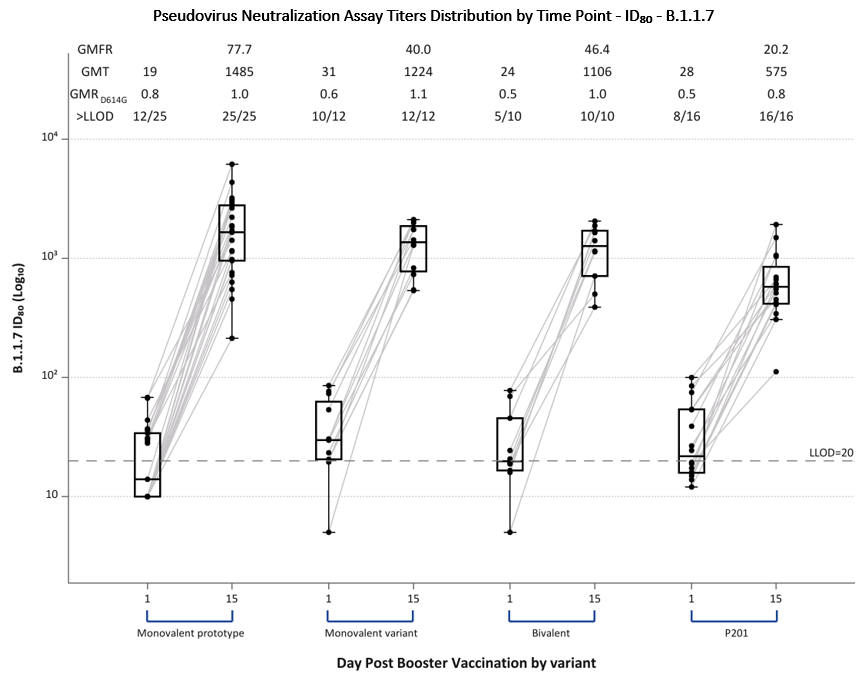
Boxes and horizontal bars denote interquartile range (IQR) and median AU, respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median +/- 1.5 x IQR. LLOD is the lower limit of detection of the assay.

1. **Serum Pseudovirus Neutralization Assay ID80 Titers Distributed by Timepoint by Variant – D614G**

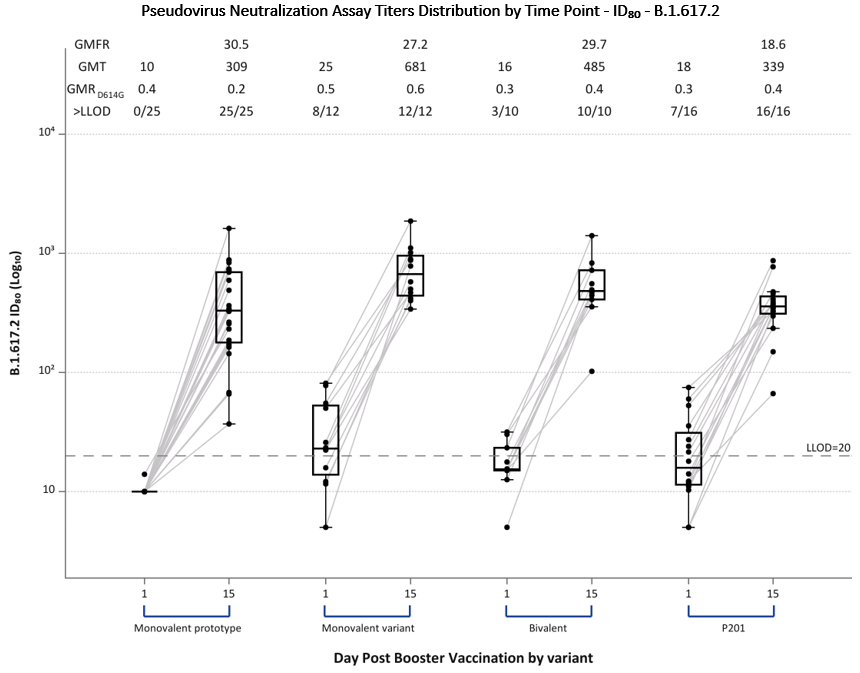


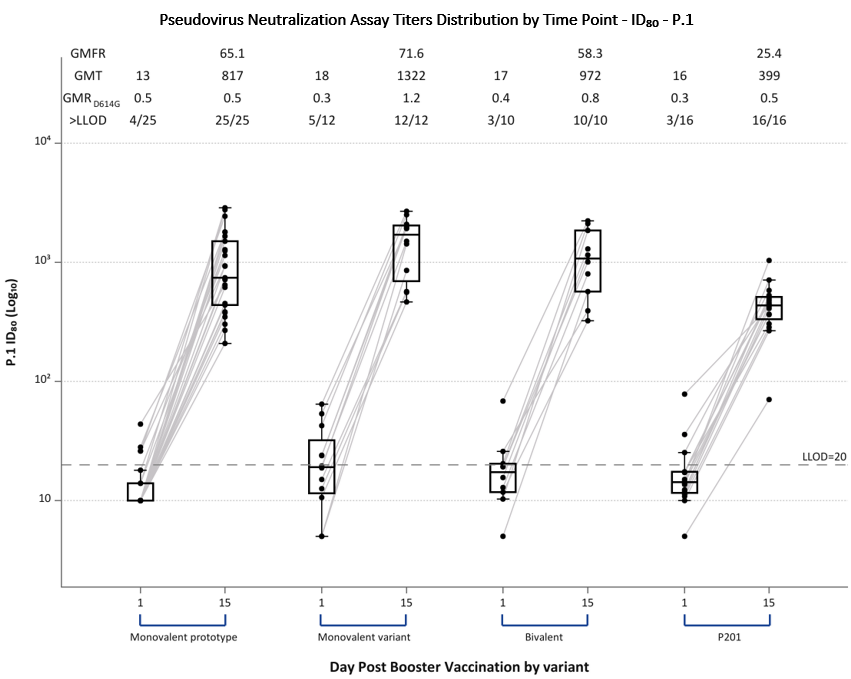
1. **Serum Pseudovirus Neutralization Assay ID80 Titers Distributed by Timepoint by Variant – Beta Variant (B.1.351)**

**C) Serum Pseudovirus Neutralization Assay ID80 Titers Distributed by Timepoint – Alpha Variant (B.1.1.7)**

****

**D) Serum Pseudovirus Neutralization Assay ID80 Titers Distributed by Timepoint – Delta Variant (B.1.617.2)**

****

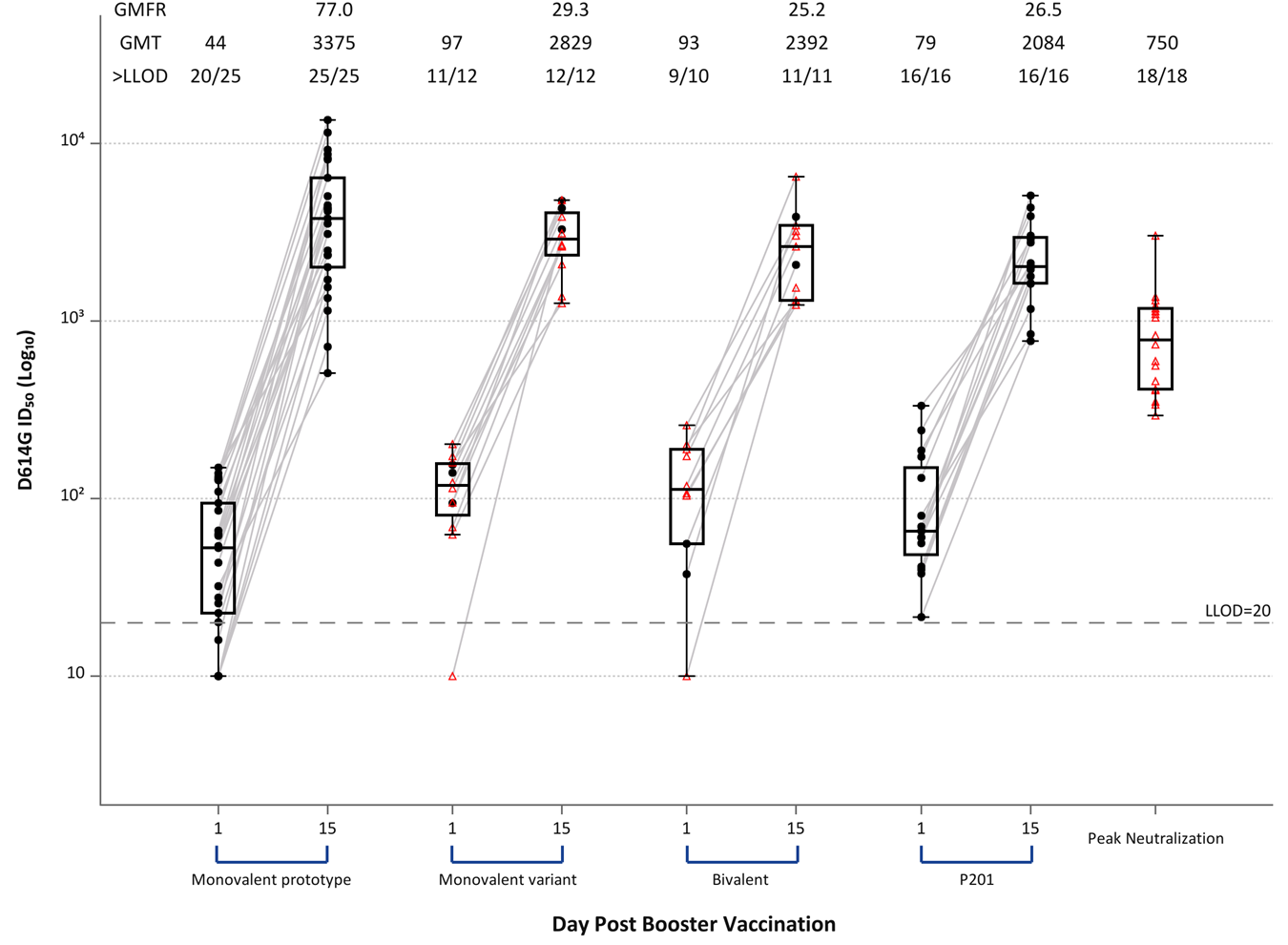
**E) Serum Pseudovirus Neutralization Assay ID80 Titers Distributed by Timepoint – Gamma Variant (P.1) **

## **Figure S4. Serum FRNT50 Titers Distributed by Timepoint and Group. Panel A: D614G, b: Beta (B.1.351), c: Alpha (B.1.1.7); d: Delta (B.1.617.2), and e: Gamma (P.1).**

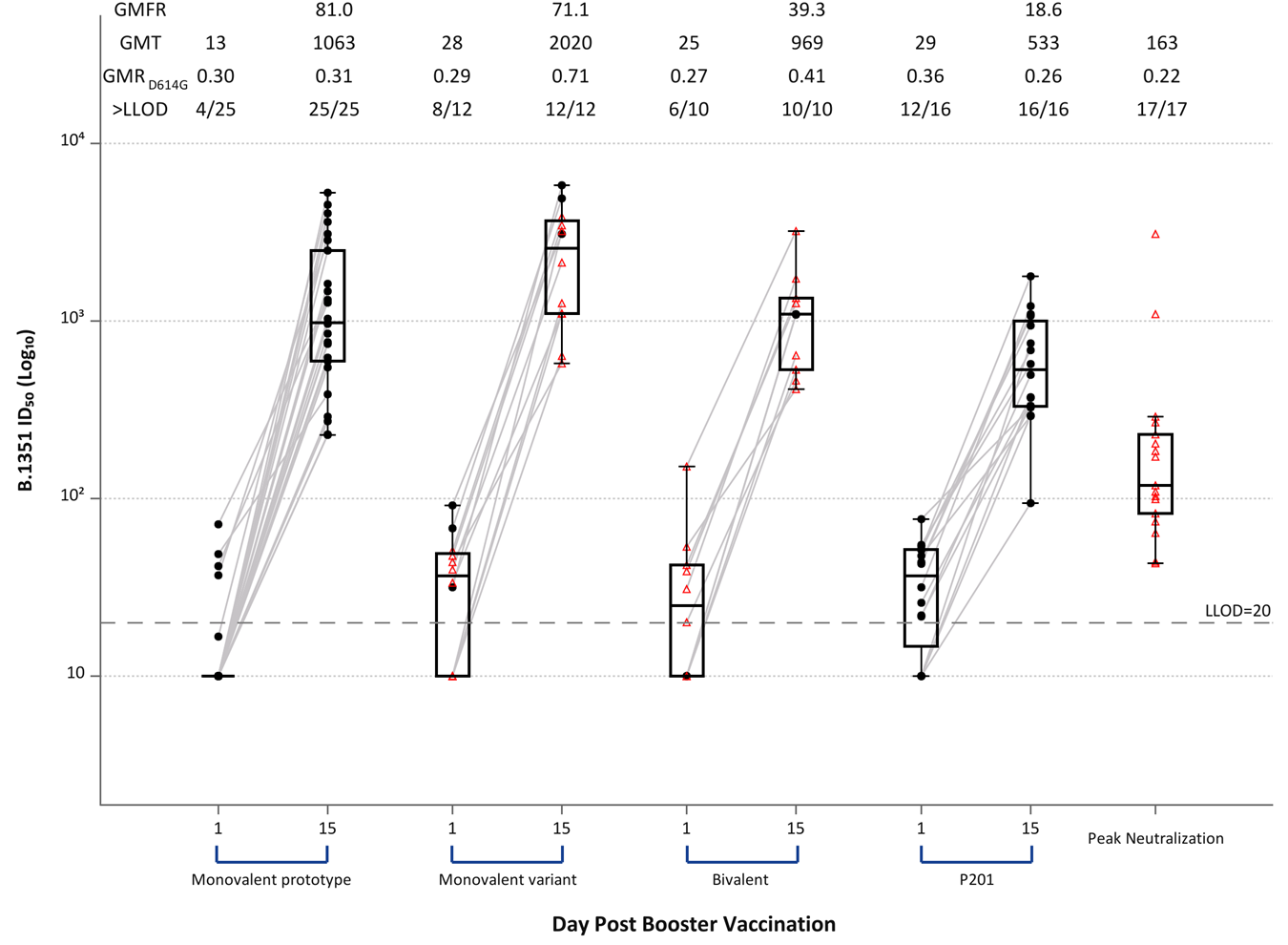
Serum FRNT**50** antibody titers area under the curve before and at 14 days after receiving a third dose of mRNA by study group: Monovalent prototype (100 mcg of mRNA-1273), Monovalent variant (50 mcg of mRNA-1273.351), and Bivalent (25 mcg of mRNA-1273, 25 mcg of mRNA-1273.351). FRNT50 antibodies were low to undetectable in many participants at baseline, particularly in those who had received 25 or 50 mcg doses of mRNA-1273 for their primary 2 dose series (monovalent prototype group) and against the Beta, Delta, and Gamma variants. All participants had robust increases in FRNT50antibody titers by 2 weeks after receipt of vaccine across all the variants. For comparison, FRNT50 titers were similar to those of 30 adults that had received an initial 100 mcg of mRNA-1273 in the Phase 2 study followed by a 50 mcg third dose were included (P201). FRNT50 titers were generally greater than the peak responses observed at 2 or 4 weeks after a second 100 mcg dose of mRNA-1273 in those that had enrolled in the original Phase I study that were used for comparison. FRNT ID50 titers after a third dose are generally greater than those observed (at 2 or 4 weeks) after the second dose in 100 mcg recipients in the Phase 1 mRNA-1273 participants (Peak Neutralization 100 mcg).

GMT (geometric mean titer), GMFR (geometric mean fold rise), GMR614D is the ratio of GMTs for the variant of concern divided by the GMT of 614D strain, >LLOD is the number of subjects with titers greater than the lower limit of detection at that timepoint. Boxes and horizontal bars denote interquartile range (IQR) and median respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median +/- 1.5 x IQR. Titers for participants that had received 100 mcg for their initial two doses in the Phase I study are highlighted with red triangles. All other participants that had received other initial doses (25, 50, 250 mcg) are denoted by black circles.

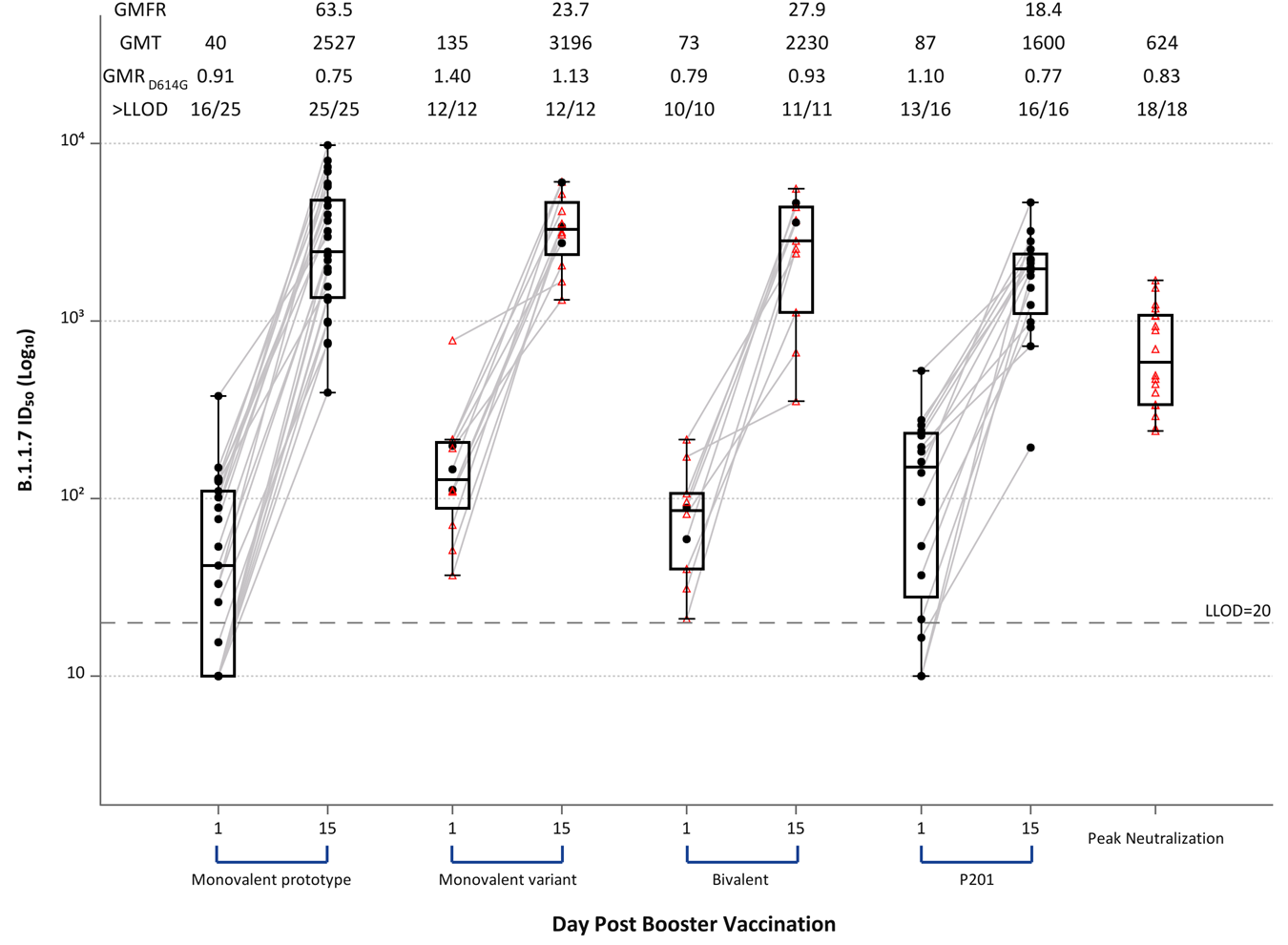
1. **FRNT Assay Titers Distribution by Time Point - ID₅₀ - D614G**



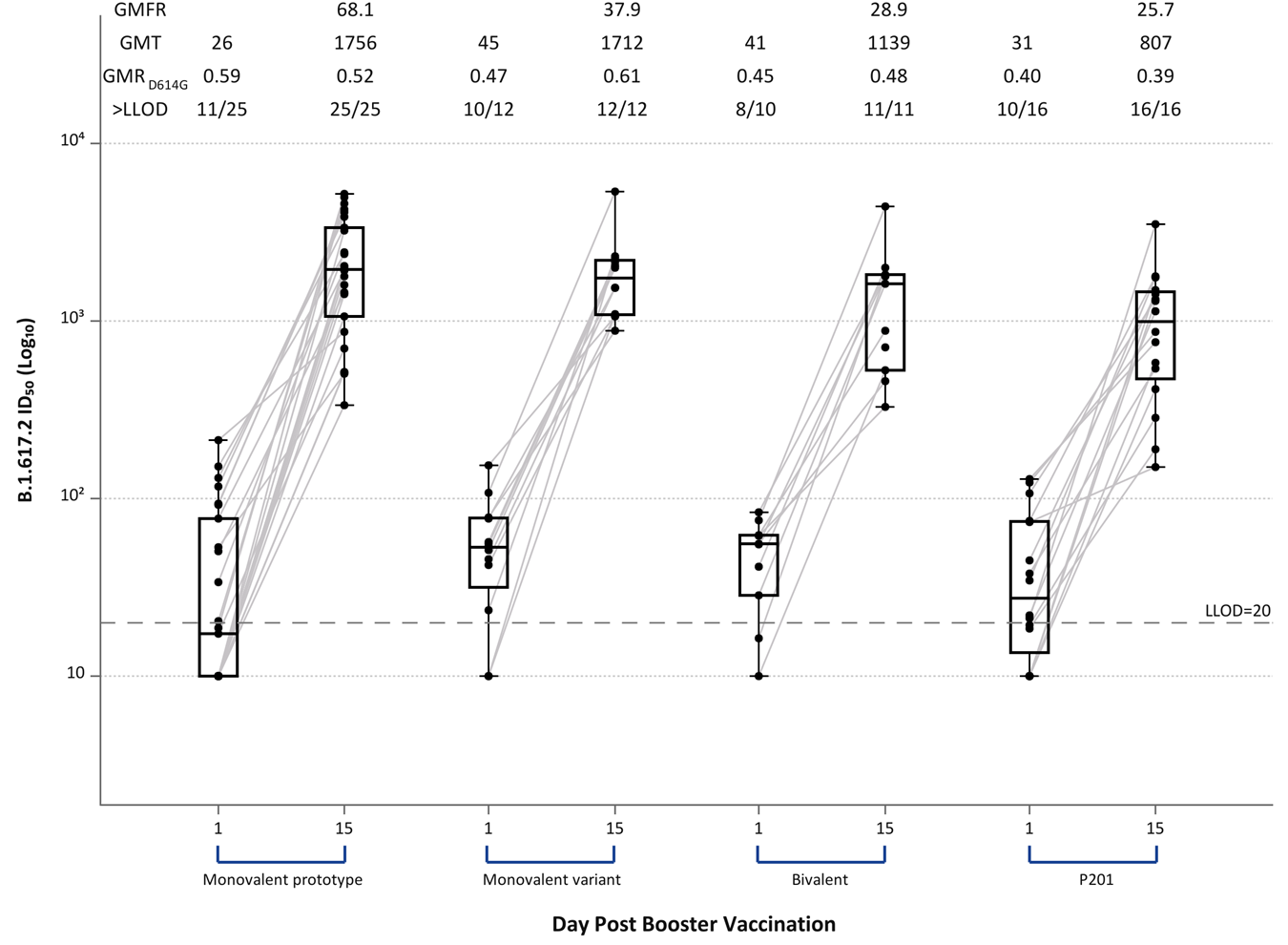
1. **FRNT Assay Titers Distribution by Time Point - ID₅₀ - Beta (B.1.351)**



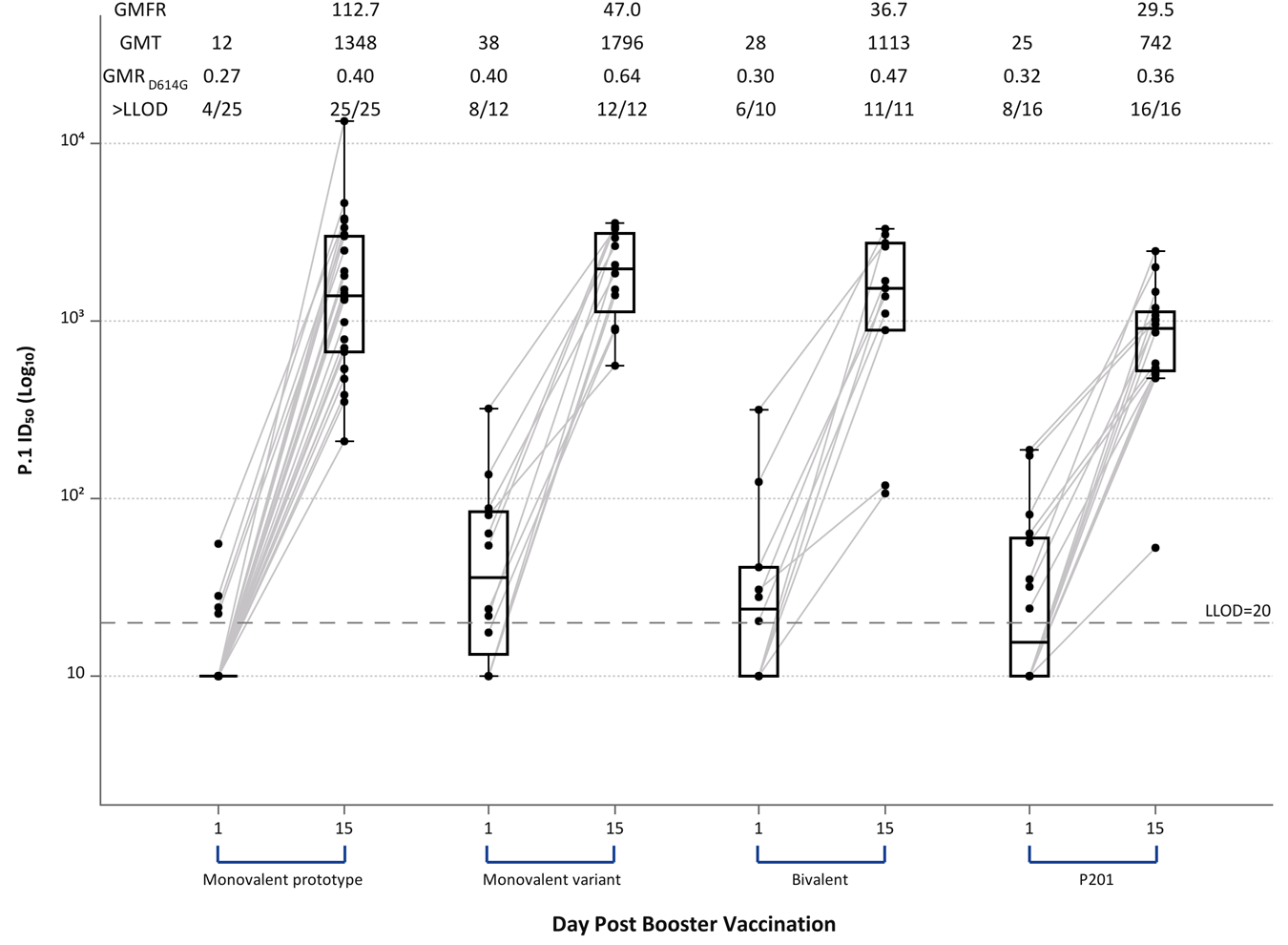
1. **FRNT Assay Titers Distribution by Time Point - ID₅₀ - Alpha (B.1.1.7)**



1. **FRNT Assay Titers Distribution by Time Point - ID₅₀ - Delta (B.1.617.2)**



1. **FRNT Assay Titers Distribution by Time Point - ID₅₀ - Gamma (P.1)**

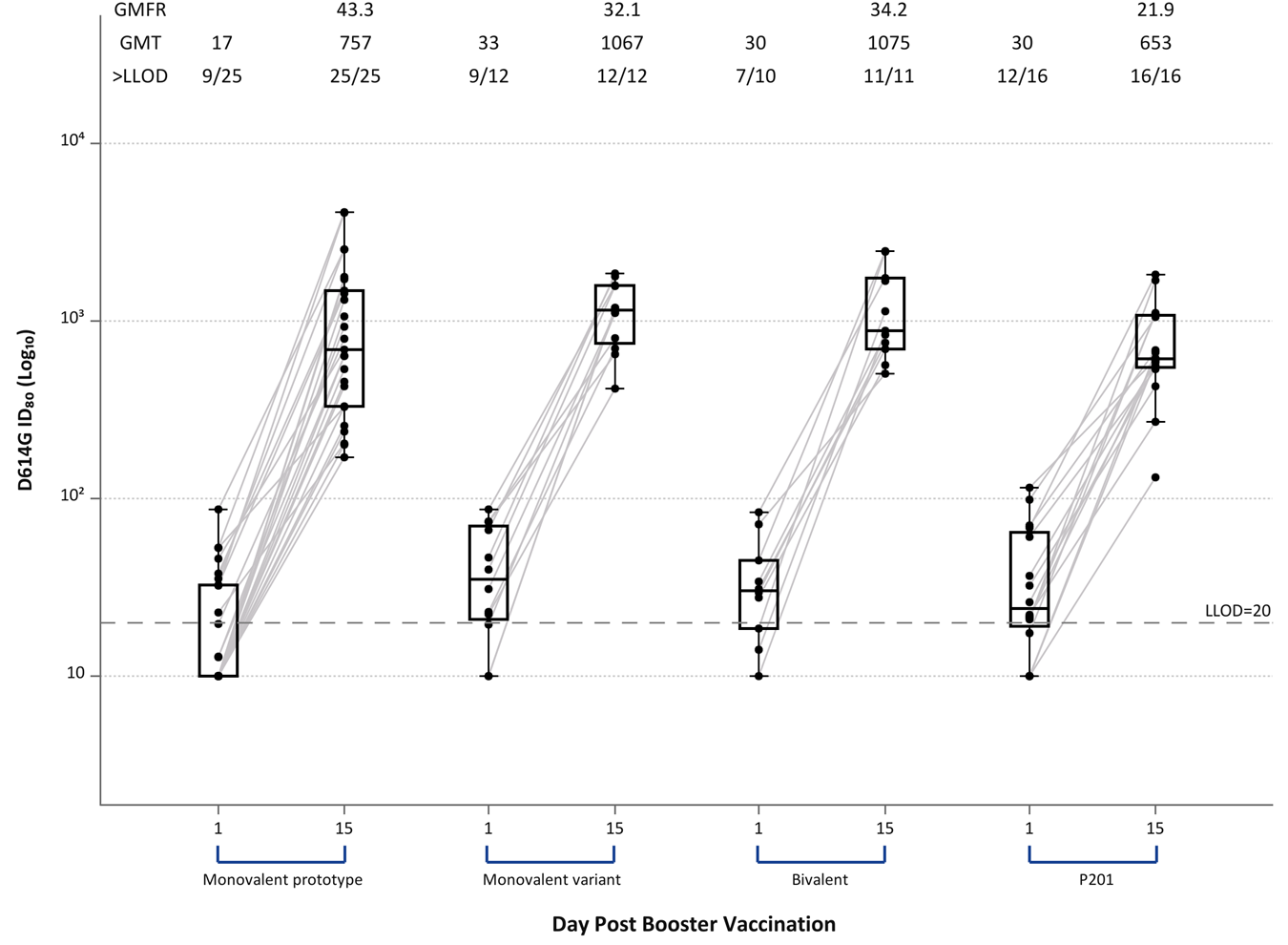


## **Figure S5. Serum FRNT80 Titers Distributed by Timepoint and Group. Panel A: D614G, b: Beta (B.1.351), c: Alpha (B.1.1.7); d: Delta (B.1.617.2), and e: Gamma (P.1).**

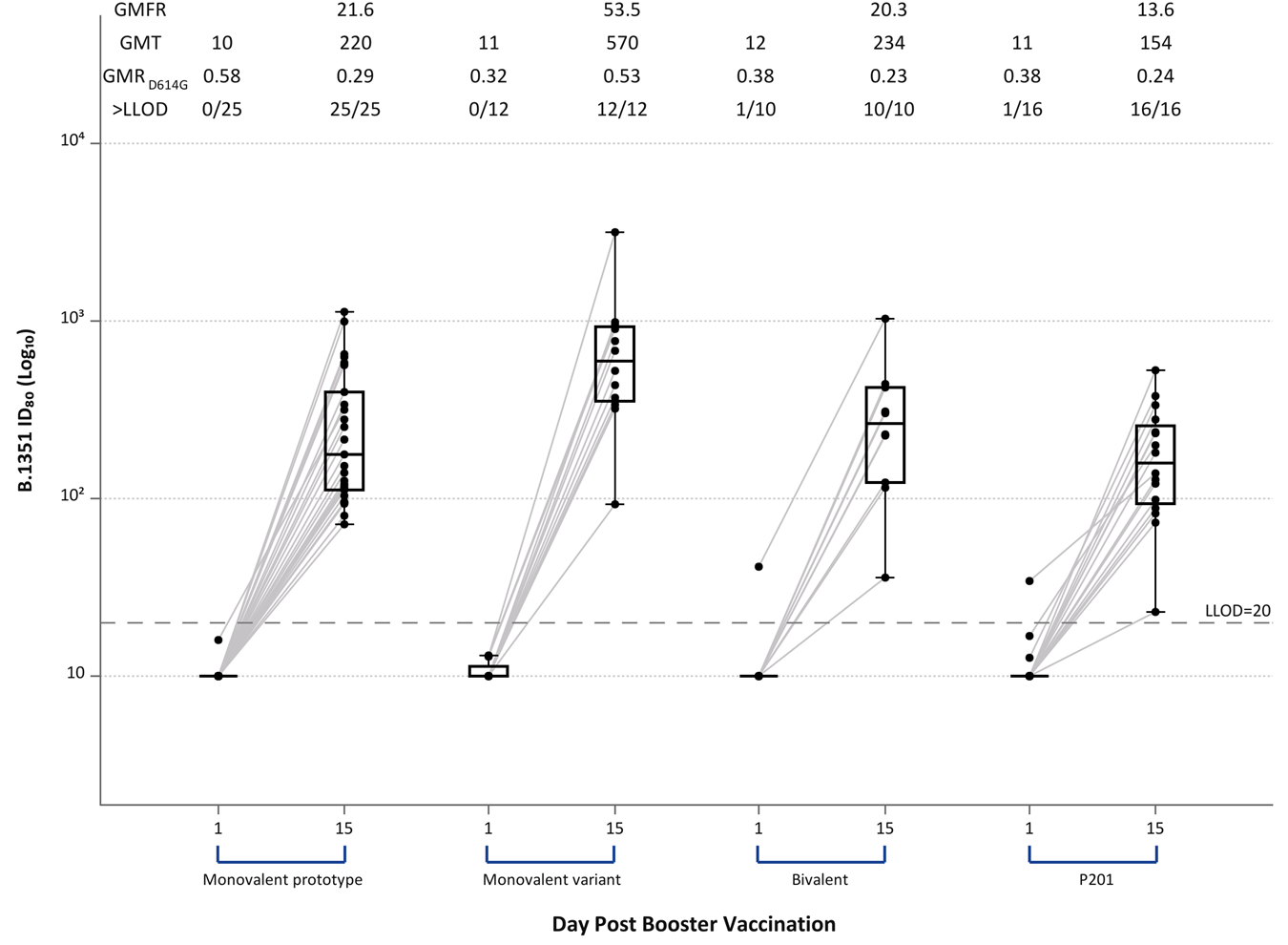
Serum FRNT**80** antibody titers area under the curve before and at 14 days after receiving a third dose of mRNA by study group: Monovalent prototype (100 mcg of mRNA-1273), Monovalent variant (50 mcg of mRNA-1273.351), and Bivalent (25 mcg of mRNA-1273, 25 mcg of mRNA-1273.351). FRNT80 antibodies were low to undetectable in many participants at baseline, particularly in those who had received 25 or 50 mcg doses of mRNA-1273 for their primary 2 dose series (monovalent prototype group) and against the Beta, Delta, and Gamma variants. All participants had robust increases in FRNT80antibody titers by 2 weeks after receipt of vaccine across all the variants. For comparison, FRNT80 titers were similar to those of 30 adults that had received an initial 100 mcg of mRNA-1273 in the Phase 2 study followed by a 50 mcg third dose were included (P201). FRNT80 titers were generally greater than the peak responses observed at 2 or 4 weeks after a second 100 mcg dose of mRNA-1273 in those that had enrolled in the original Phase I study that were used for comparison. FRNT ID80 titers after a third dose are generally greater than those observed (at 2 or 4 weeks) after the second dose in 100 mcg recipients in the Phase 1 mRNA-1273 participants (Peak Neutralization 100 mcg).

GMT (geometric mean titer), GMFR (geometric mean fold rise), GMR614D is the ratio of GMTs for the variant of concern divided by the GMT of 614D strain, >LLOD is the number of subjects with titers greater than the lower limit of detection at that timepoint. Boxes and horizontal bars denote interquartile range (IQR) and median respectively. Whisker endpoints are equal to the maximum and minimum values below or above the median +/- 1.5 x IQR. Titers for participants that had received 100 mcg for their initial two doses in the Phase I study are highlighted with red triangles. All other participants that had received other initial doses (25, 50, 250 mcg) are denoted by black circles. LLOD is the lower limit of detection of the assay.

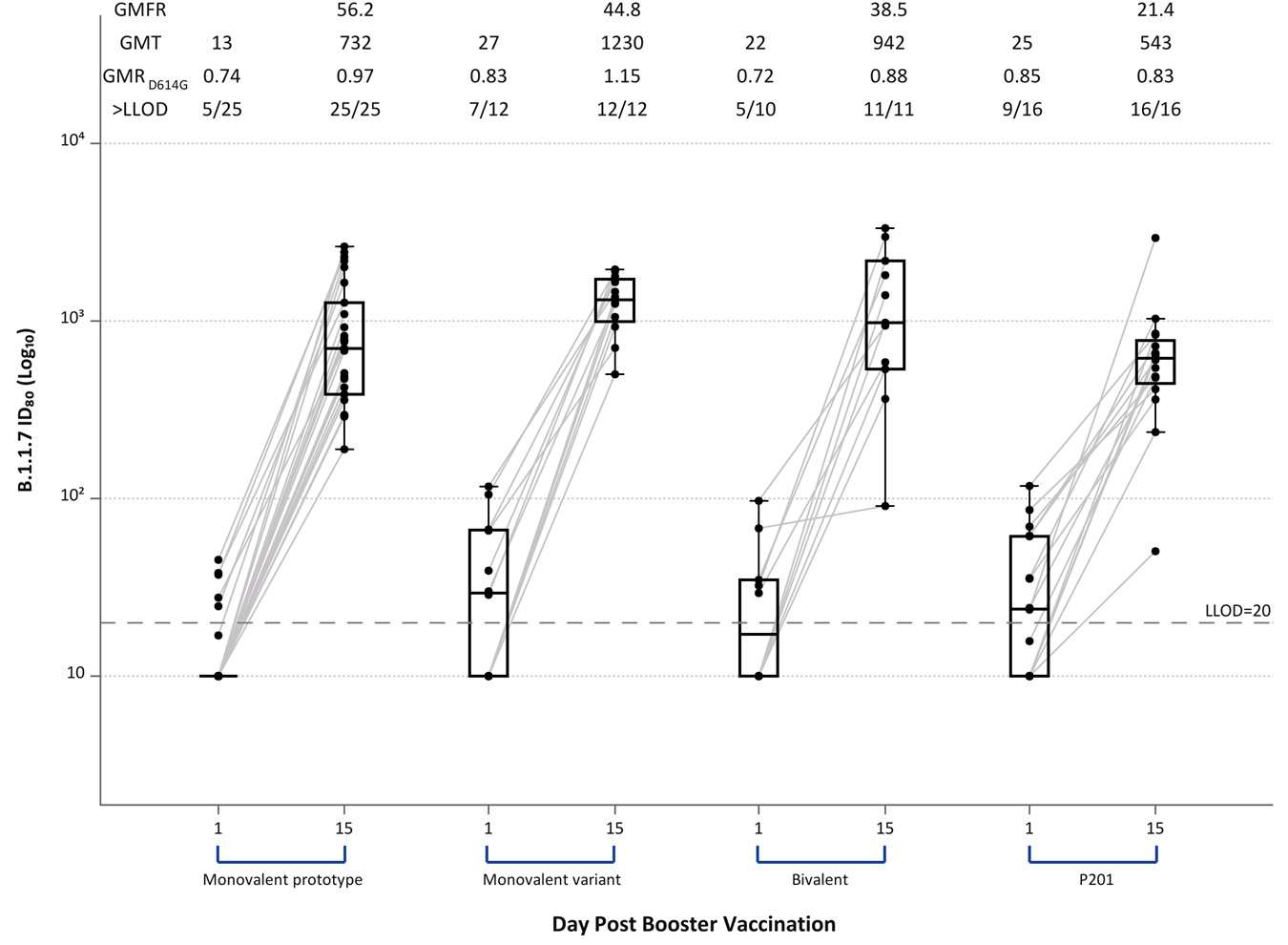
1. **FRNT Assay Titers Distribution by Time Point - ID₈₀ - D614G**



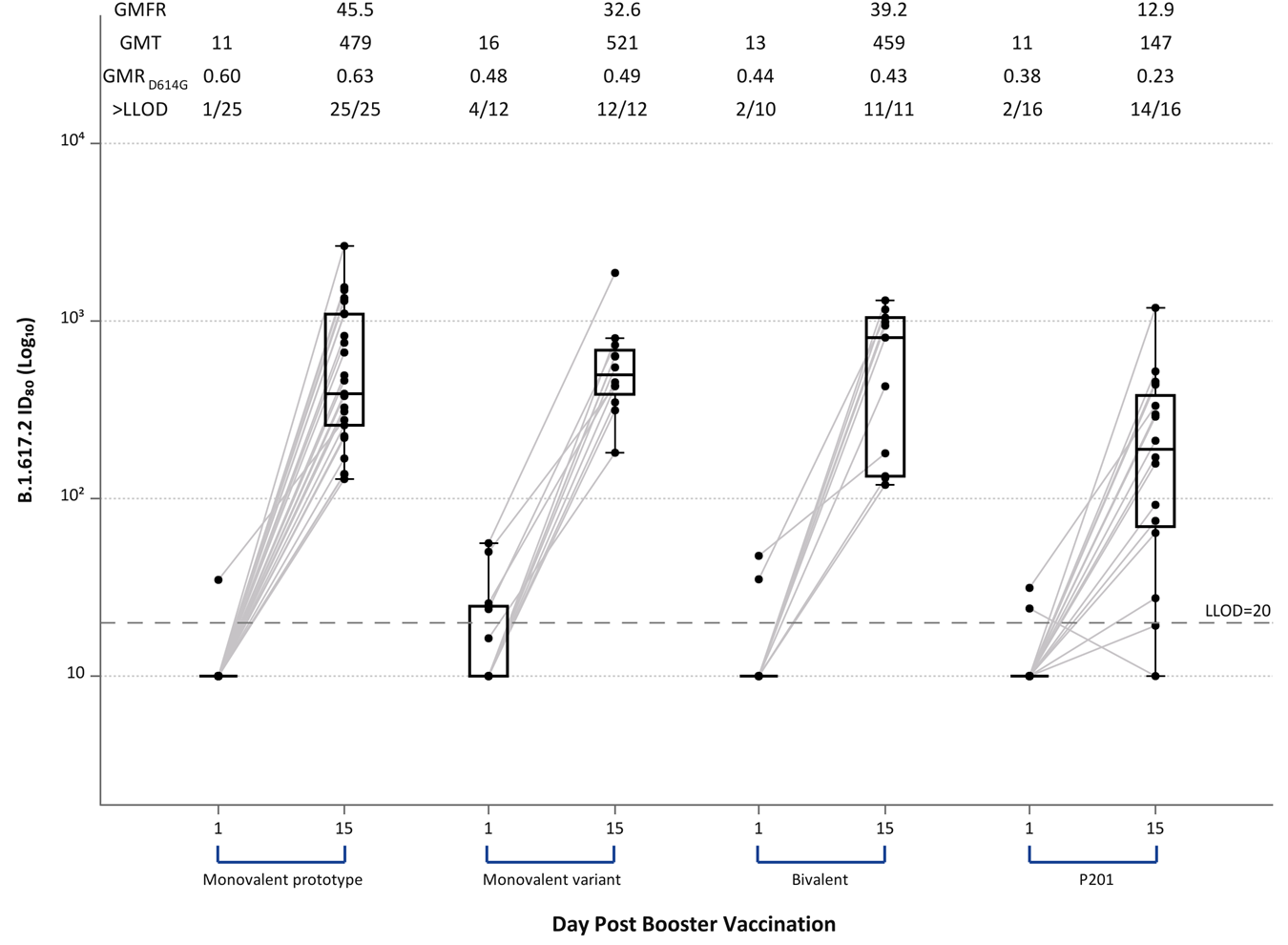
1. **FRNT Assay Titers Distribution by Time Point - ID₈₀ - Beta (B.1.351)**



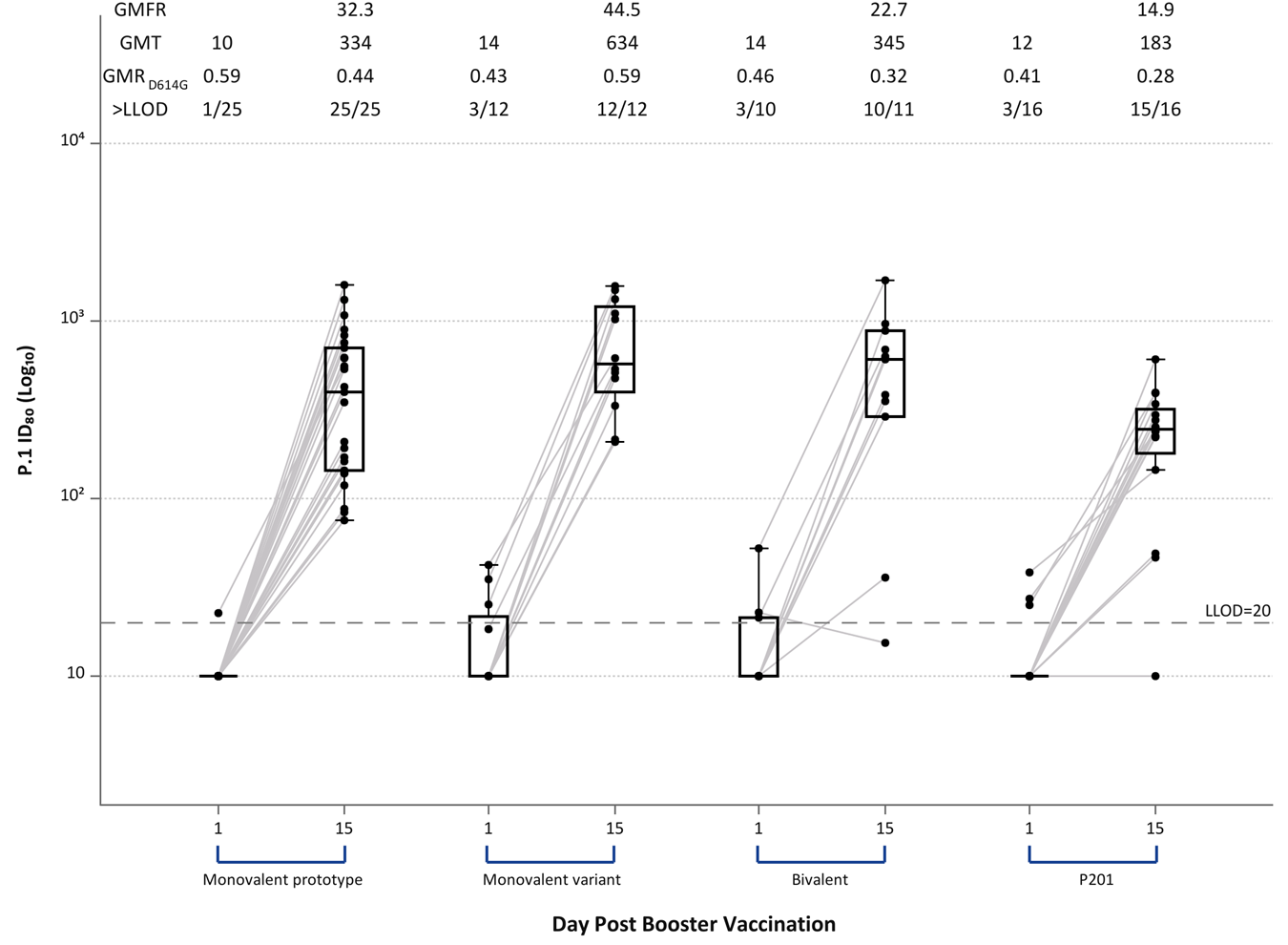
1. **FRNT Assay Titers Distribution by Time Point - ID₈₀ - Alpha (B.1.1.7)**



1. **Assay Titers Distribution by Time Point - ID₈₀ - Delta (B.1.617.2)**



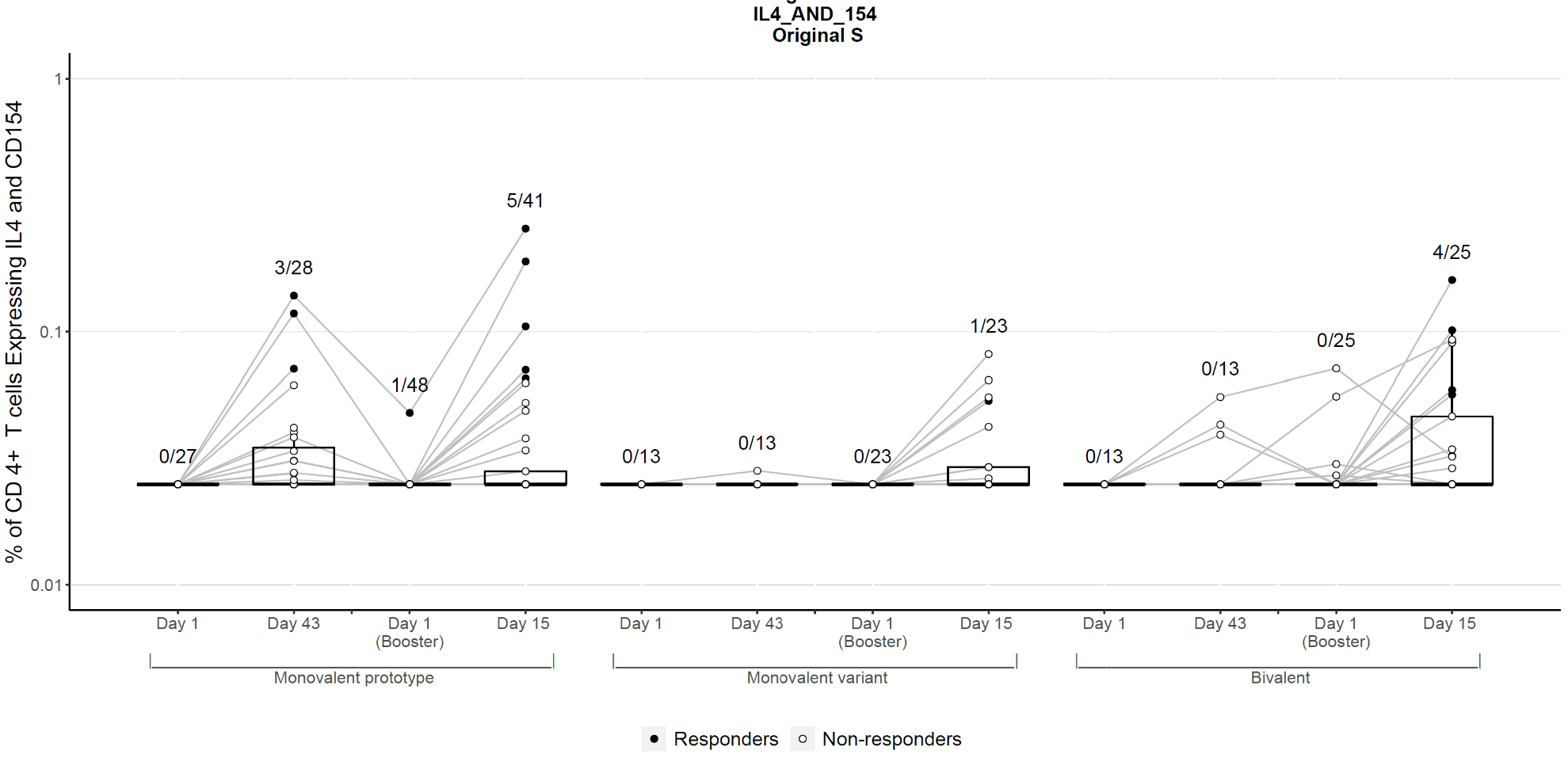
1. **FRNT Assay Titers Distribution by Time Point - ID₈₀ - P.1**



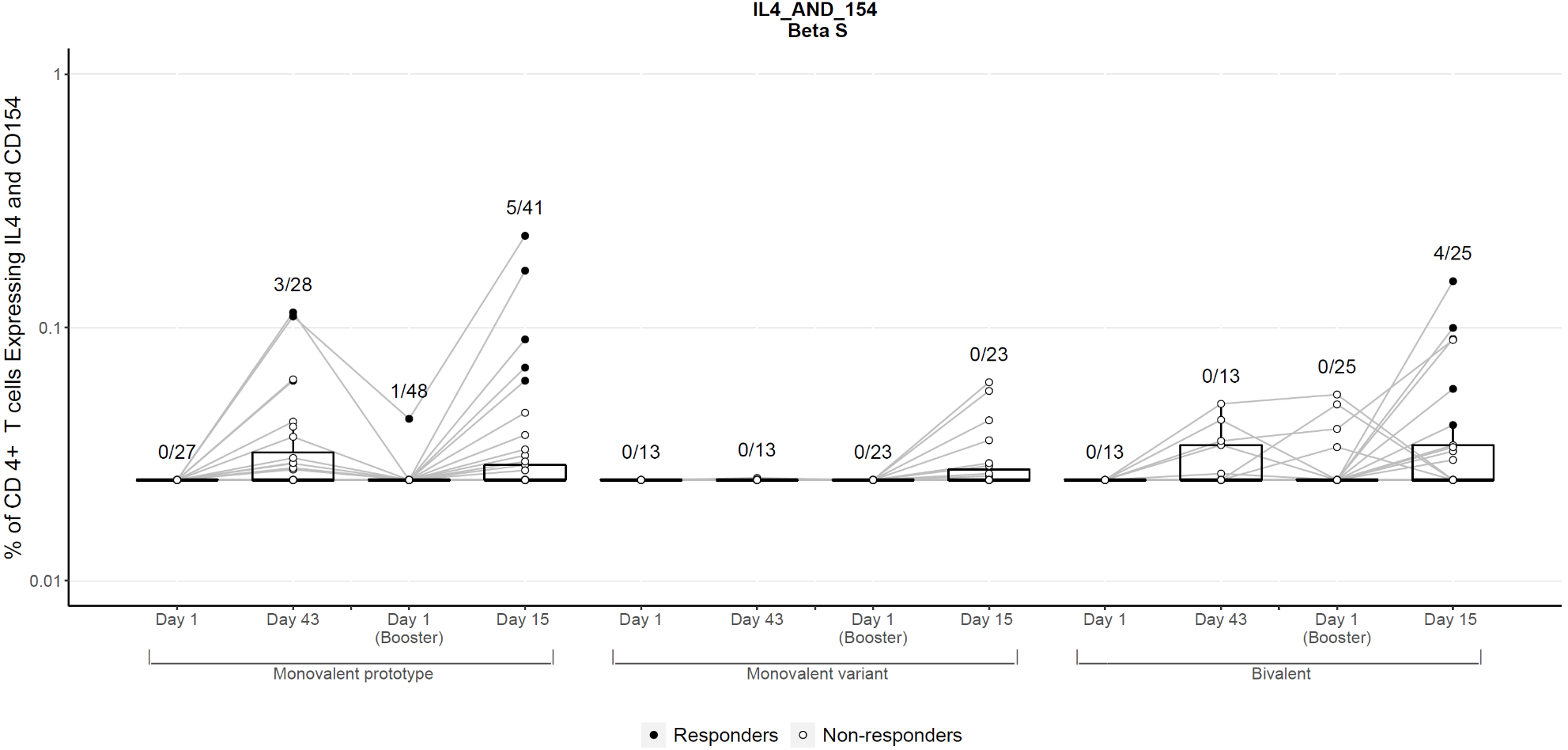
## **Figure S6. CD4 T Cell TH2 Responses by Group and Timepoint.**

Background adjusted % of CD4 T cells expressing IL-4 in response to peptides from the A) 614D spike and B) the beta variant spike, respectively. Data was thresholded at 0.025%. Data points below 0.025% are displayed at 0.025%. Closed circles indicate positive spike-specific IL-4 responses and open circles indicate negative spike-specific IL-4 responses by Fisher’s exact test. The numbers on the graph indicate the number of positive responders over the total number tested for each group and timepoint.

1. **614D Spike-Specific TH2 CD4 T Cells**



1. **Beta Spike-Specific TH2 CD4 T Cells**



## **Figure S7. Memory CD4 TH1 Responses by Group and Timepoint for 614D spike.**

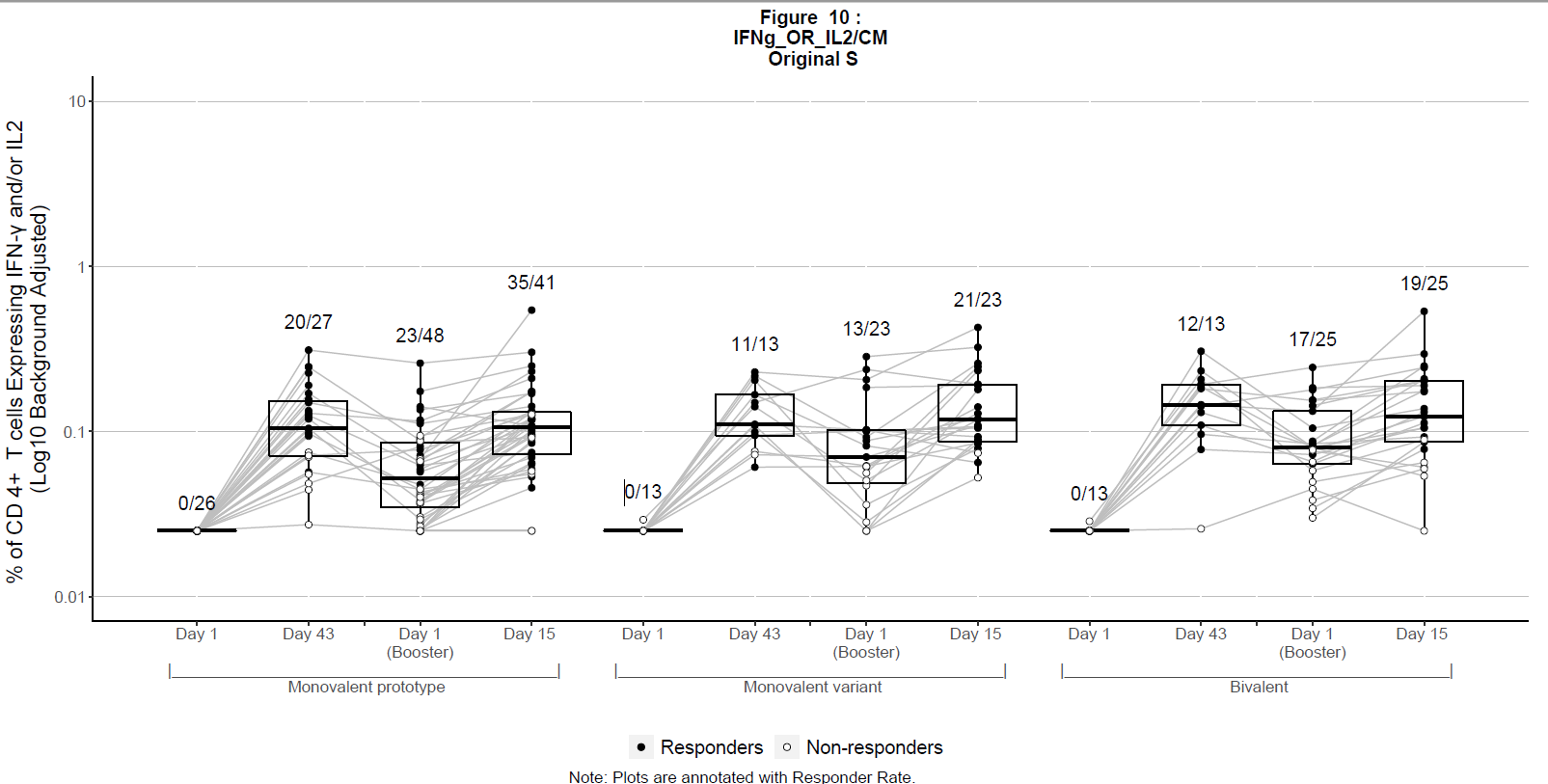
Background adjusted % of CD4 T cells that are **A)** effector memory (EM), **B)** central memory (CM), **C)** TEMRA and **D)** overlay of EM, CM, and TEMRA, and express IFN**-** and/or IL-2 in response to peptides from the 614D spike, respectively, out of total CD4 T cells. Data was thresholded at 0.025%. Data points below 0.025% are displayed at 0.025%. Closed circles indicate positive spike-specific responses and open circles indicate negative spike-specific responses by Fisher’s exact test. The numbers on the graph indicate the number of positive responders over the total number tested for each group and timepoint.

* 1. **Spike-Specific Effector Memory CD4 T Cells**

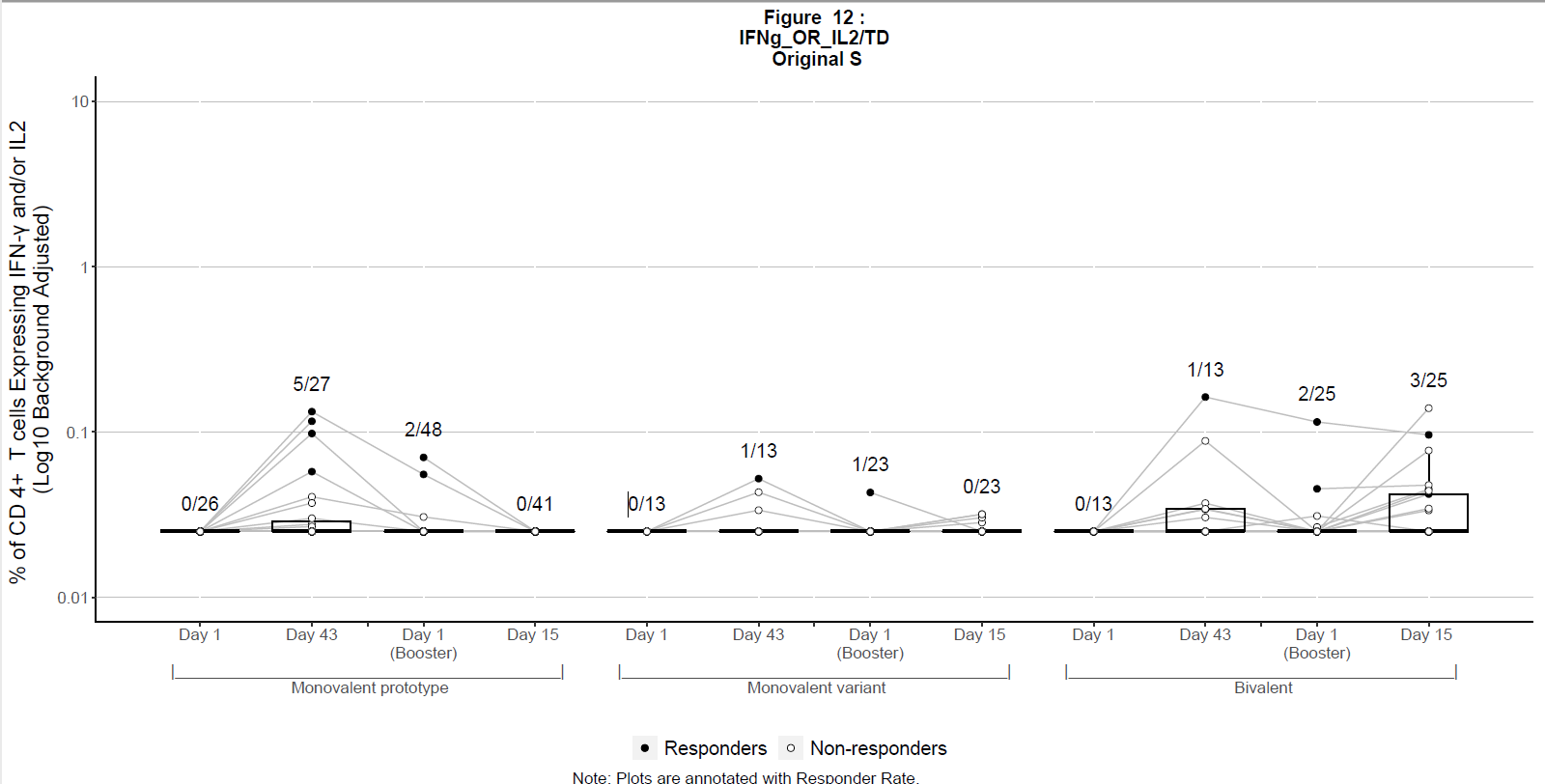
Diagram, engineering drawing

Description automatically generated**:**

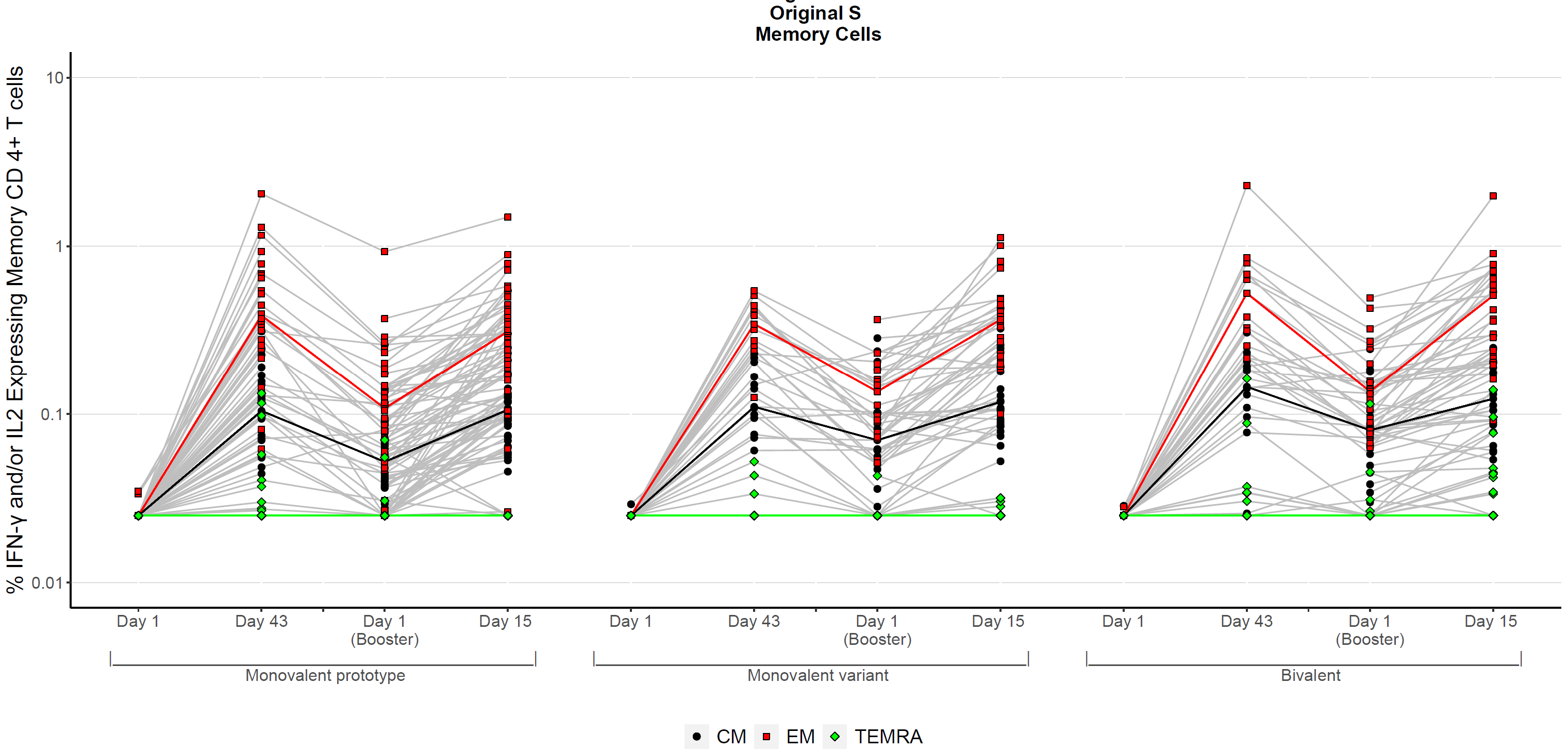
* 1. **Spike-Specific Central Memory CD4 T Cells**



* 1. **Spike-Specific TEMRA Memory CD4 T Cells**

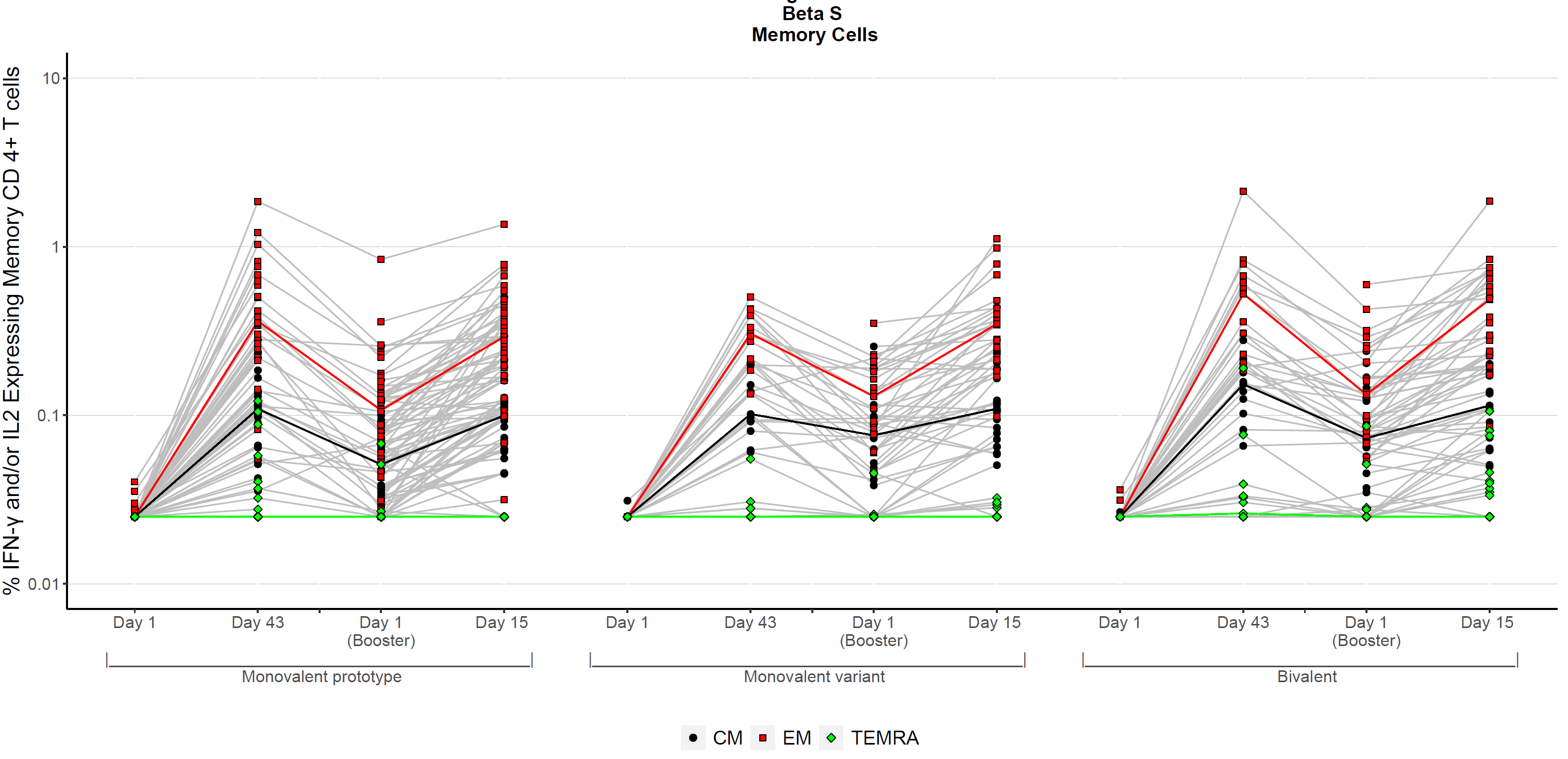


* 1. **Overlay of Spike-specific CD4 effector memory (EM), central memory (CM), and TEMRA for 614D**

****

## **Figure S8. Memory CD4 TH1 Responses by Group and Timepoint for Beta spike.**

Background adjusted % of CD4 T cells that are the overlay of EM, CM, and TEMRA, and express IFN**-** and/or IL-2 in response to peptides from the Beta spike, respectively, out of total CD4 T cells. Data was thresholded at 0.025%. Data points below 0.025% are displayed at 0.025%. Closed circles indicate positive spike-specific responses and open circles indicate negative spike-specific responses by Fisher’s exact test. The numbers on the graph indicate the number of positive responders over the total number tested for each group and timepoint.



## **Figure S9. Memory CD8 T Cell Responses to 614D by Group and Timepoint.**

Background adjusted % of CD8 T cells that are **A)** effector memory (EM), **B)** central memory (CM), **C)** TEMRA, and **D)** Overlay of Spike-specific CD8 effector memory (EM), central memory (CM), and TEMRA and express IFN- and/or IL-2 in response to peptides from the 614D spike out of total CD8 T cells. Data was thresholded at 0.025%. Data points below 0.025% are displayed at 0.025%. Closed circles indicate positive spike-specific responses and open circles indicate negative spike-specific responses by Fisher’s exact test. The numbers on the graph indicate the number of positive responders over the total number tested for each group and timepoint.

1. **Spike-Specific Effector Memory CD8 T Cells**

**Diagram, engineering drawing

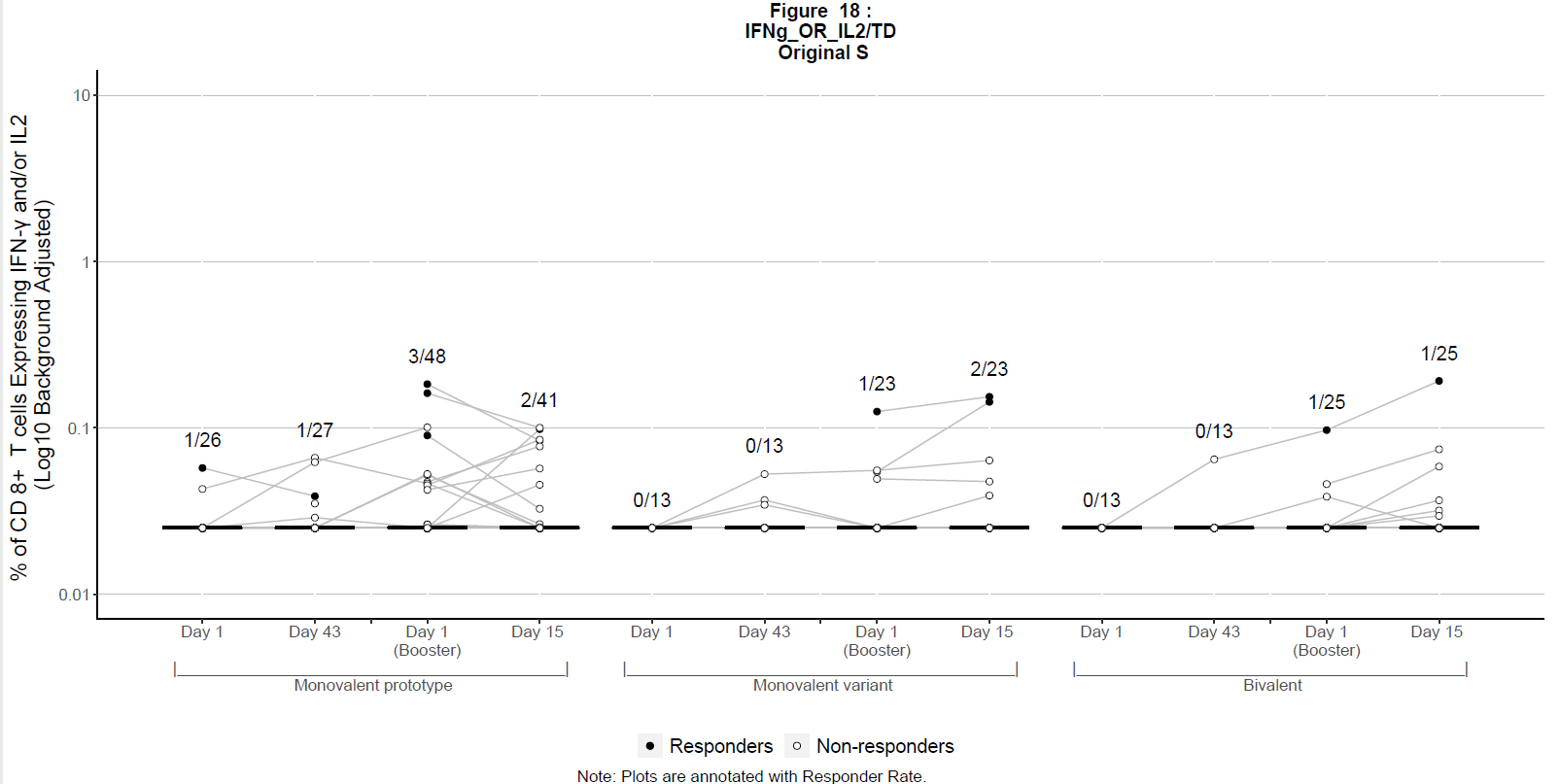
Description automatically generated**

1. **Spike-Specific Central Memory CD8 T Cells**

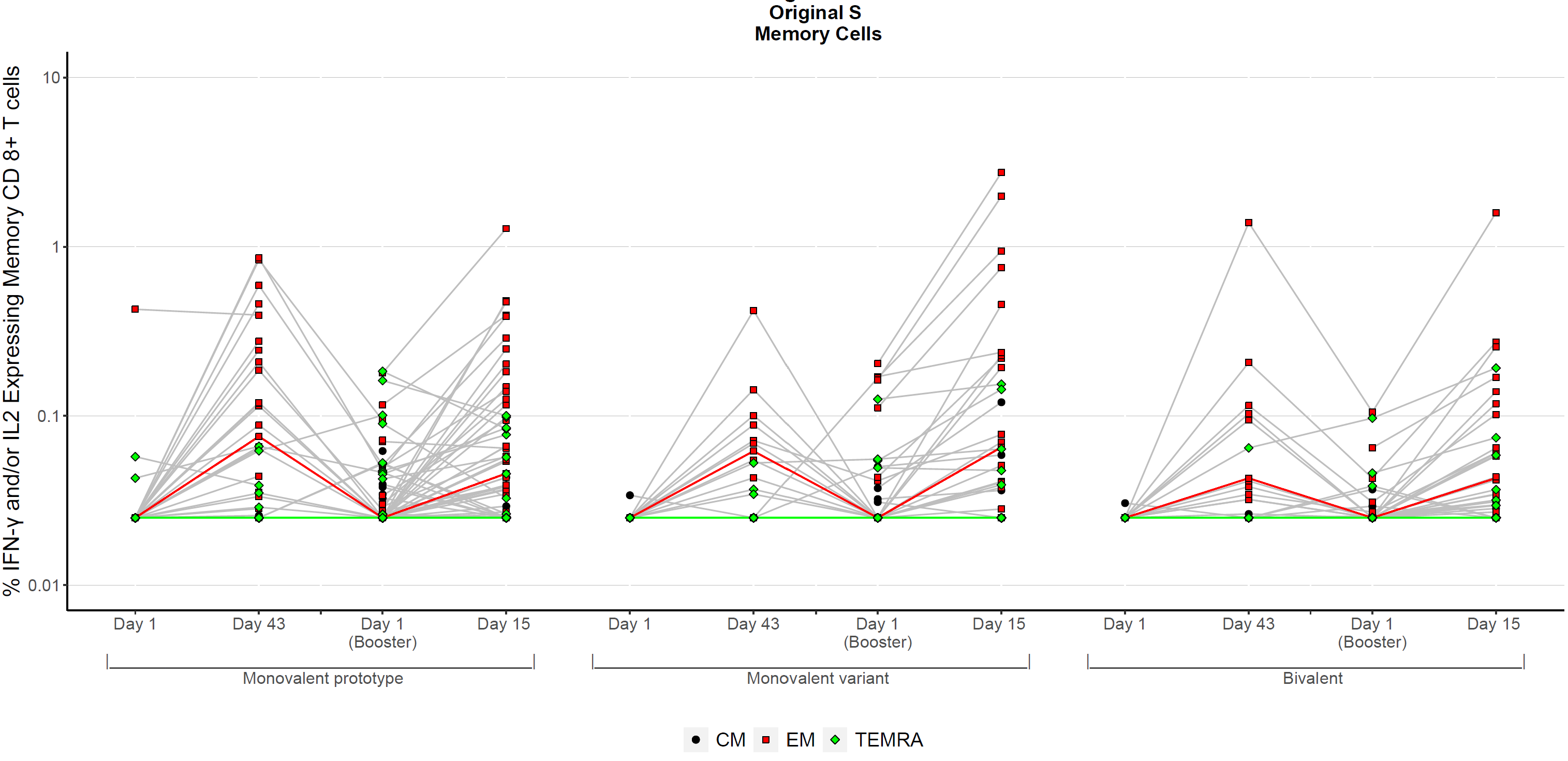
**A picture containing graphical user interface

Description automatically generated**

1. **Spike-Specific TEMRA Memory CD8 T Cells**

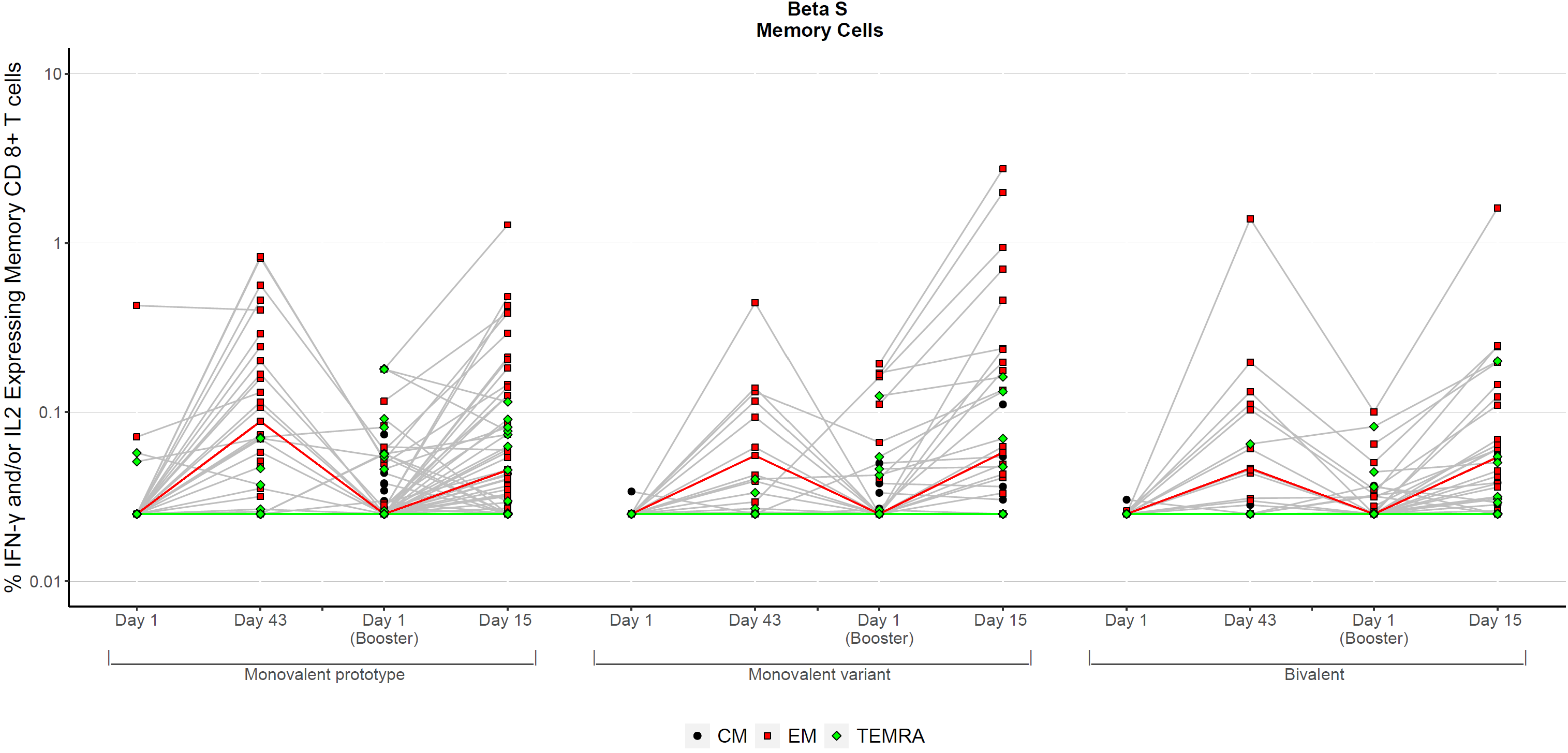
****

1. **Overlay of Spike-specific CD8 effector memory (EM), central memory (CM), and TEMRA for 614D**

****

## **Figure S10. Memory CD8 T Cell Responses by Group and Timepoint for Beta.**

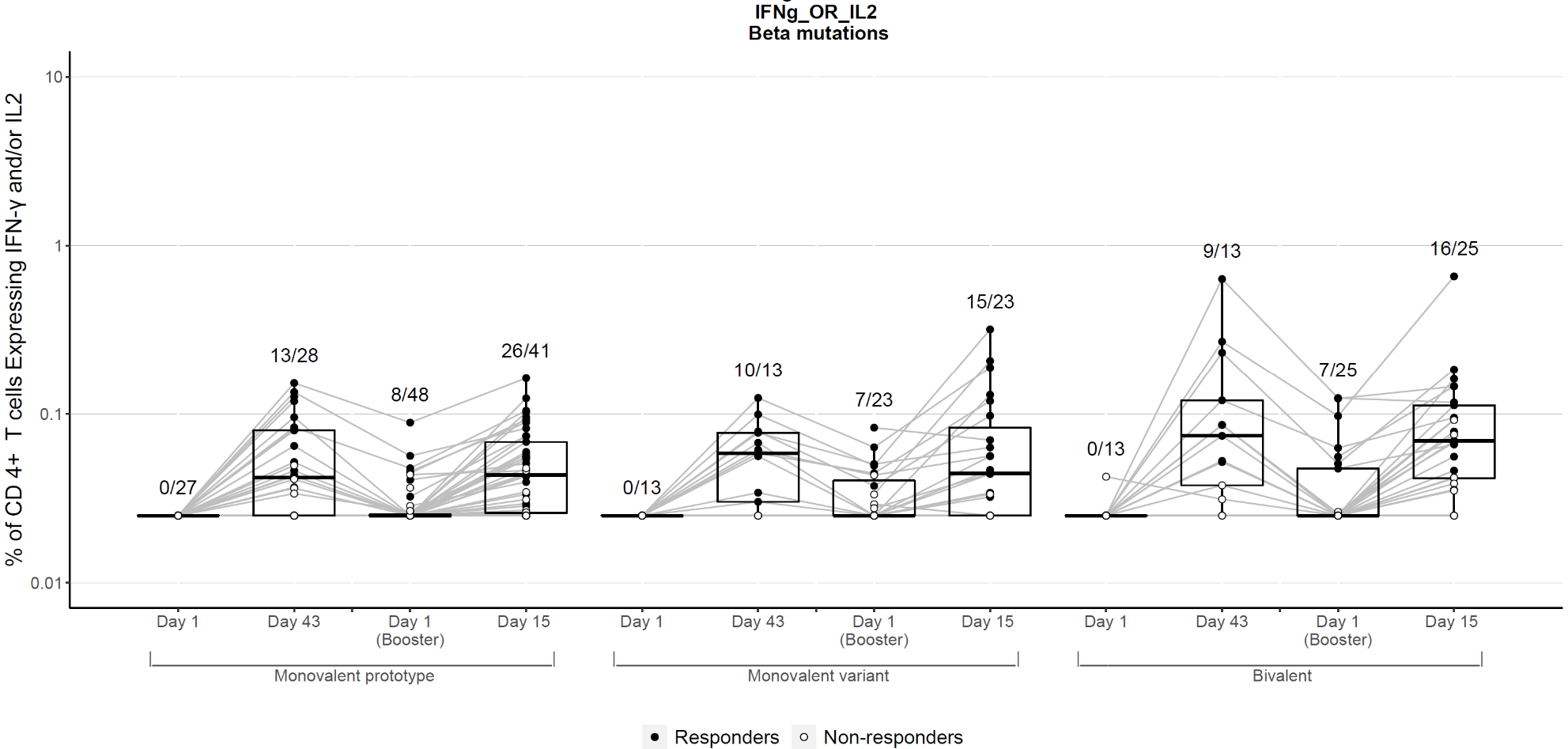
Background adjusted % of CD8 T cells that are the Overlay of Spike-specific CD8 effector memory (EM), central memory (CM), and TEMRA and express IFN- and/or IL-2 in response to peptides from Beta, respectively, out of total CD8 T cells. Data was thresholded at 0.025%. Data points below 0.025% are displayed at 0.025%. Closed circles indicate positive spike-specific responses and open circles indicate negative spike-specific responses by Fisher’s exact test. The numbers on the graph indicate the number of positive responders over the total number tested for each group and timepoint.

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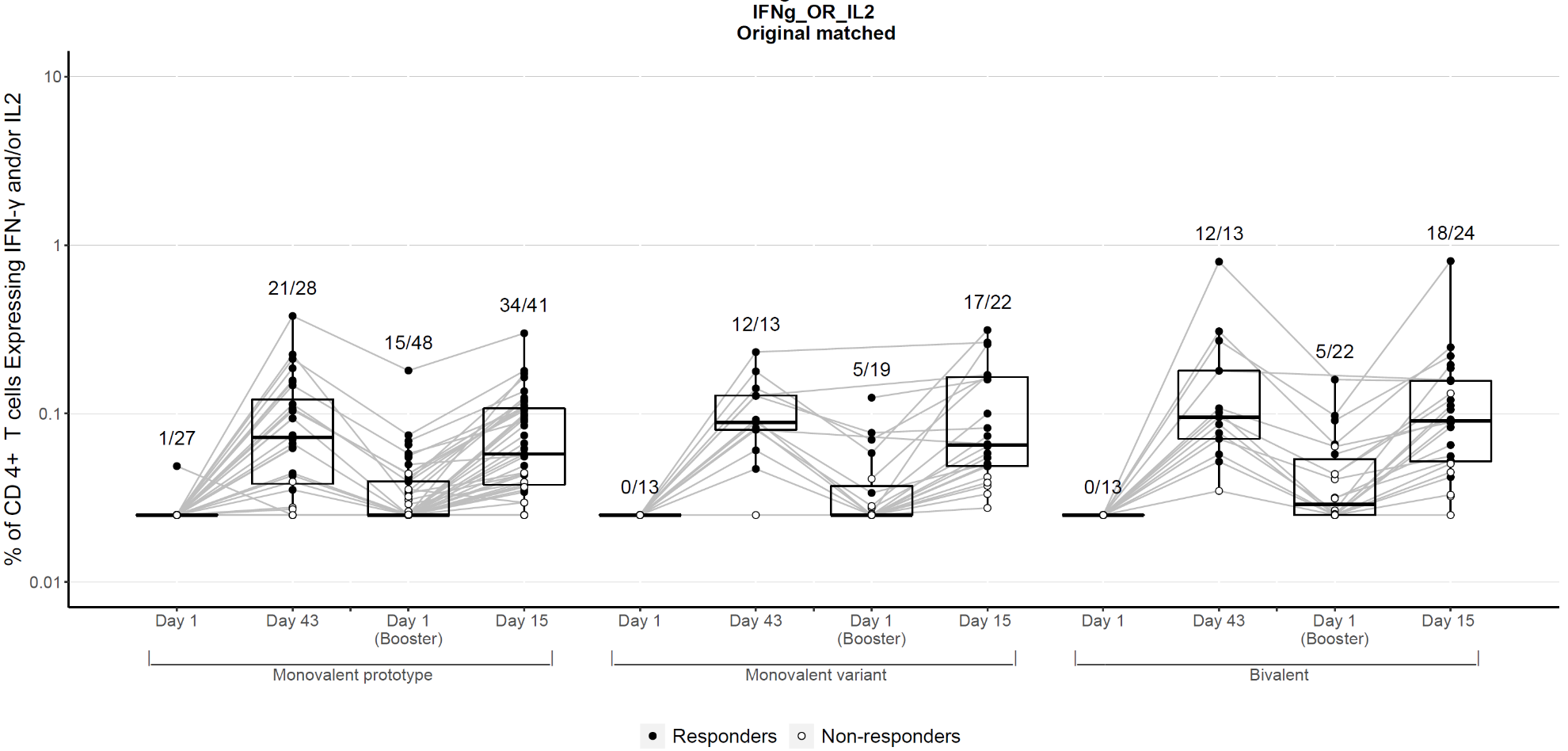
## **Figure S11. CD4 T Cell TH1 Responses by Group and Timepoint to the Variant Regions.**

Background adjusted % of CD4 T cells expressing IFN- and/or IL-2 in response to the subset of peptides which contain mutations in the Beta spike A) and a matching set of peptides covering the same regions in the B) 614D spike, respectively. Data was thresholded at 0.025%. Data points below 0.025% are displayed at 0.025%. Closed circles indicate positive responses and open circles indicate negative responses by Fisher’s exact test. The numbers on the graph indicate the number of positive responders over the total number tested for each group and timepoint.

1. **Beta variant regions**



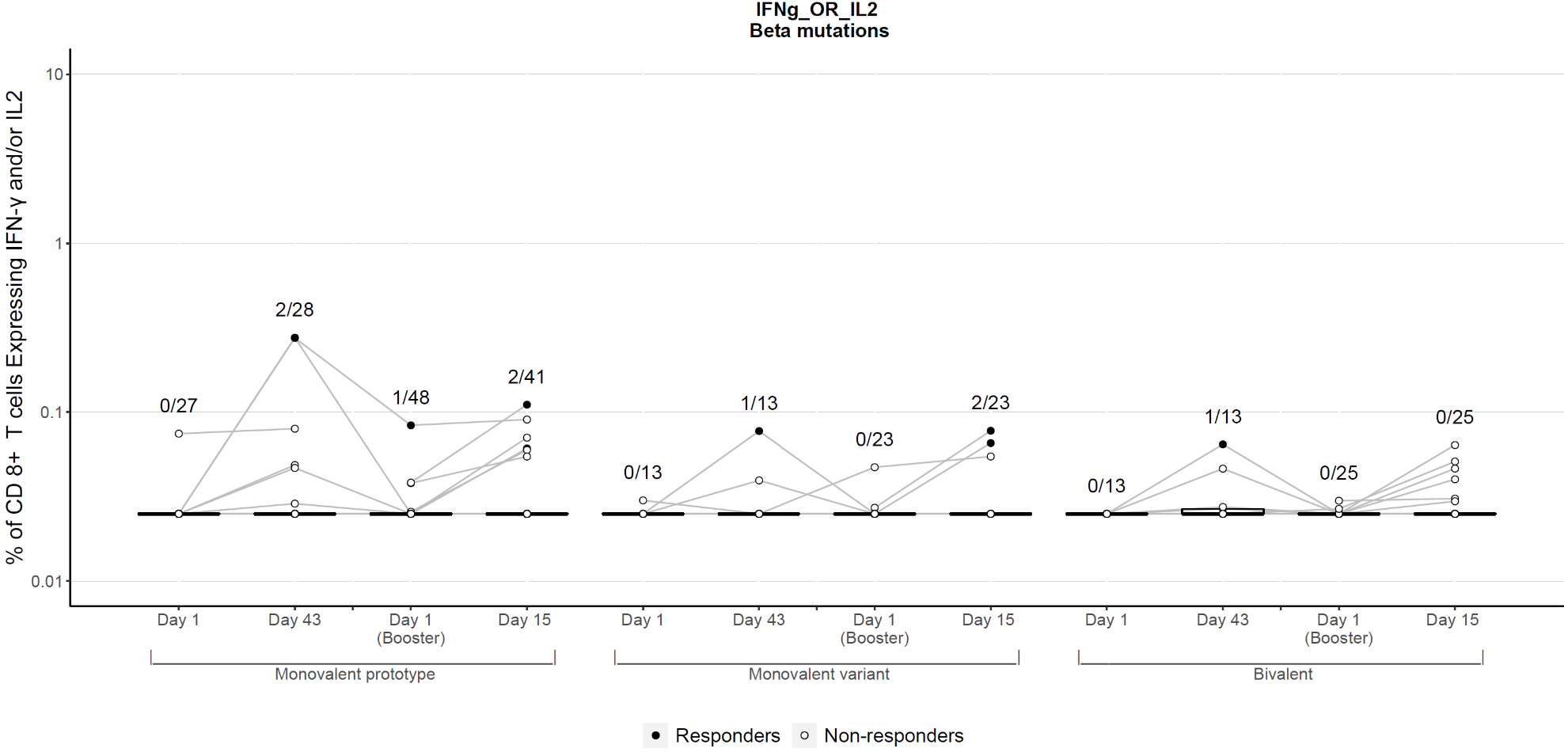
1. **614D matched peptides corresponding to the variant regions**



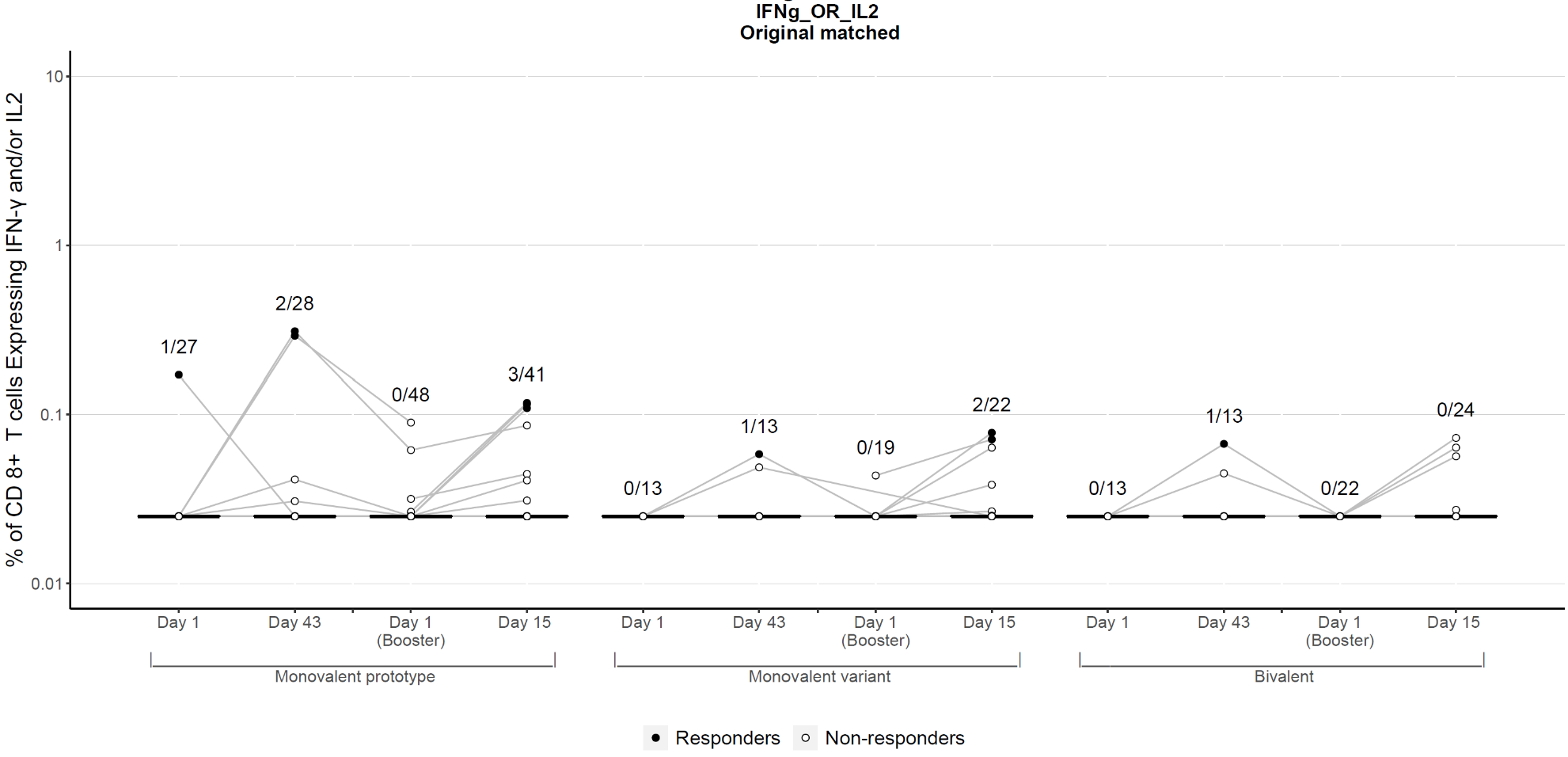
## **Figure S12. CD8 T Cell TH1 Responses by Group and Timepoint to the Variant Regions.**

Background adjusted % of CD8 T cells expressing IFN- and/or IL-2 in response to the subset of peptides which contain mutations in the Beta spike **A)** and a matching set of peptides covering the same regions in the **B)** 614D spike, respectively. Data was thresholded at 0.025%. Data points below 0.025% are displayed at 0.025%. Closed circles indicate positive responses and open circles indicate negative responses by Fisher’s exact test. The numbers on the graph indicate the number of positive responders over the total number tested for each group and timepoint.

1. **Beta variant regions**



1. **614D matched peptides corresponding to the variant regions**



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