Supplemental Materials

Supplemental Methods

SERA assay

Serum was incubated with a randomized *Escherichia coli* peptide library displaying unique peptides of 12 amino acids (1×10^{10}, 10-fold oversampled) at a 1:25 dilution in a 96-well, deep well plate. Antibody-bound bacterial clones were selected using 50 µL Protein A/G Sera-Mag SpeedBeads (GE Life Sciences, #17152104010350) immunoglobulin (Ig)G or by incubation with biotinylated anti-human IgM antibody (Jackson ImmunoResearch, #709-066-073) at 1:100 dilution, followed by incubation with 50 µL Dynabead MyOne Streptavidin T1 conjugated magnetic beads (IgM) (Thermo-Fisher, #65602). Selected bacterial pools were resuspended in growth media and incubated overnight at 37 °C, 300 rpm to propagate. Plasmid purification, polymerase chain reaction (PCR) amplification, and DNA barcoding with well-specific indices was performed as described previously.[1] Sample concentrations were normalized to 4 nM for each pool and analyzed with Illumina NextSeq500. Healthy control standards were included for every 96-well plate of samples processed to evaluate reproducibility.[2]

S protein RBD ELISA

SARS-CoV-2 spike (S) receptor binding domain (RBD) (alpha variant strain) immobilized on a 96-well plates (Nunc MaxiSorp) at 0.5 µg/mL were incubated overnight at 4 °C. Following incubation, plates were washed with PBS and blocked with 5% non-fat milk in PBS for 2 hours at room temperature. Plates were then incubated with serum (1:250 dilution) for 1 hour at room temperature before being washed and incubated with anti-human IgG-HRP conjugated secondary antibody (Jackson ImmunoResearch) at 1:10,000 in blocking reagent for 1 hour at room temperature. After washing, the reaction was developed with exposure to 3,3',5,5'-tetramethylbenzidine (TMB) reagent (ThermoFisher) for 15 minutes and then stopped with 1M

hydrochloric acid. Absorbance was measured on a Tecan Spectrafluor plus plate reader at 450 nm.[2]

IMUNE-based motif discovery

Booster day 29 samples for each of the different booster vaccine formulations were compared to matching subject samples before primary vaccination (day 1 or baseline) and at >6 months after primary vaccination (booster day 1) to discover S protein-specific motifs that were not previously identified or motifs that were specific to particular booster formulations. Enrichment comparisons were performed for all IMUNE discovered motifs to identify epitopes that are specific to boosted samples.

PIWAS analysis

Protein-based immunome wide association studies (PIWAS) analysis was performed on individual samples, with comparisons drawn longitudinally for each participant and among different booster vaccine formulations using the control cohort for signal normalization. Approximately 1 to 3 million 12-mers per sample were obtained from the serum epitope repertoire analysis (SERA) assay and decomposed into 5-mers and 6-mers. The enrichment score for each k-amino acid (k-mer) was calculated by dividing the number of unique 12-mers containing the k-mer divided by the number of expected k-mers for the sample (based on amino acid proportions) as previously described.[2] Each PIWAS score at a specific amino acid represents an averaged score within a 5 amino acid frame using the tiling data of 5-mers and 6-mers spanning the sequence. The z-score was calculated on comparisons of values from the sample with those from a large cohort control (n=2766). Antigens were ranked using the Mann–Whitney U false-discovery rate (FDR) and tiling data was generated for top ranked antigens in both sample and control data. The 95th quantile bands were calculated for each population and the most prominent S protein epitopes were identified.

PIE analysis

PIE methodology for epitope identification was performed by locating epitopes that had stronger signal in the study samples relative to control data from a large cohort population (n=2766). The distribution of sample values relative to the control was analyzed for each position on the S protein. Outlier threshold was calculated according to $Q_{75}+1.5*(Q_{75}-Q_{25})$, where Q_x is the x^{th} percentile of the control values at that specific sequence location. The outlier sum statistic was approximately the sum of signal above the outlier threshold in the study samples. A null distribution for the outlier sum value was calculated by scrambling case/control labels and recalculating many times. A *P* value for the study samples and control comparison was calculated based on the null distribution, with the significant value set to 0.001 and the outlier sum threshold was set to the 99.5th percentile value of all positions, with an FDR of *P*>0.001. All sequence locations that exceeded both thresholds were included in the final plot. Further details on the outlier sum calculation and FDR are described elsewhere.[3, 4]

Paired PIE analysis

Paired PIE was used to determine changes in outlier signals for individual participants across 2 time points. The distribution of differences in PIWAS signal was first calculated for each subject on a non-S protein control portion of the SARS-CoV-2 proteome and the outlier thresholds were defined individually for each subject to determine an increase or decrease to outlier signal:

$$T_I^i = I_{75}^i + 1.5 \cdot (I_{75}^i - I_{25}^i)$$

$$T_D^i = D_{75}^i + 1.5 \cdot (D_{75}^i - D_{25}^i)$$

Increased and absolute value decreases across time points were calculated for each participant and compared to the thresholds to determine if signals were classed as outlier increase or decrease.

Sample QC

All samples were subjected to multiple analysis metrics for quality control (QC) after sequencing was performed to ensure adequate antibody capture, lack of cross-contamination, and plate-to-plate consistency. All plates used in this study had consistent QC metrics and all samples screened passed internal and well-specific QC metrics. Antibody fingerprinting was used to compare antibody signals across all same-subject samples for determining possible sample mismatch.

Supplemental Tables

Table S1. Participant samples

Booster Vaccine	Clinical Trial Participants	Trial Participants, n (Samples)
mRNA-1273 (Wuhan-Hu-1)	Enrolled in the open-label interventional phase (Part B) of a phase 2 study (NCT04405076),[5] where participants received a booster dose of mRNA-1273 6 to 8 months after completing the mRNA-1273 primary series	40 (160)
mRNA-1273.351 (beta variant)	Enrolled in the proof-of-concept rollover study (Part C) of a phase 2 study (NCT04405076), where participants who had received 2 doses of mRNA-1273 in the phase 3 clinical trial (NCT04470427) at least 6 months earlier were rolled over into Part C to receive a booster dose of mRNA-1273.351	20 (80)
mRNA-1273.211 (Wuhan-Hu-1 and beta strains)	Enrolled in Part A of a phase 2 study (NCT04927065), where participants who had received 2 doses of mRNA-1273 in the phase 3 clinical trial (NCT04470427) at least 6 months earlier were administered a booster dose of mRNA-1273.211	37 (148)
mRNA-1273.617.2 (delta variant)	Enrolled in Part C of a phase 2 study (NCT04927065), where participants who had received 2 doses of mRNA-1273 in the phase 3 clinical trial (NCT04470427) at least 6 months earlier were administered a booster dose of mRNA-1273.617.2	39 (156)
mRNA-1273.213 (beta and delta strains)	Enrolled in Part D of a phase 2 study (NCT04927065), where participants who had received 2 doses of mRNA-1273 in the phase 3 clinical trial (NCT04470427) at least 6 months earlier were administered a booster dose of mRNA-1273.213	38 (152)

Supplemental Figures

Figure S1. Antibody signals toward SARS-CoV-2 S-specific motifs at each time point after vaccination by booster vaccine formulation**.**
Motif z-scores for individual S protein motifs are shown from before vaccination (day 1) to after booster vaccination (booster day 29) time points. At time points prior to booster vaccination, all samples were plotted as a single point since samples were either baseline (before vaccination) or primary mRNA-1273 vaccination only (error bars represent range of motif median z-scores from participants in each booster vaccine group). After booster vaccination, the plot branches into each of the booster vaccine formulation types to compare motif z-scores in each group. Newly discovered motifs are included for comparison to motif variants already present in the panel. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

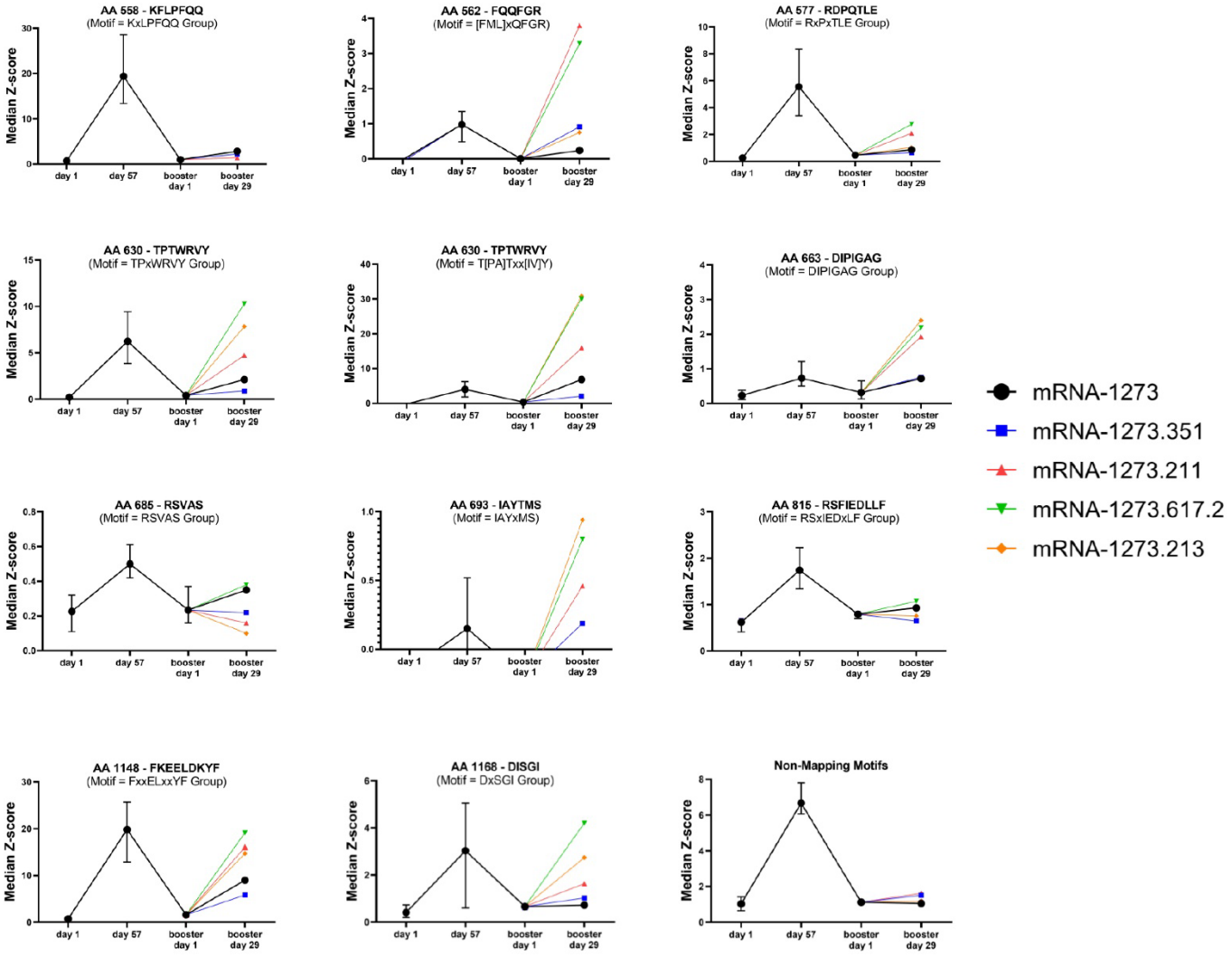


Figure S2. Trends in SARS-CoV-2 epitope signals after vaccination.
(**a**) Plots demonstrating the 95th percentile PIWAS value for epitope regions 577-583 (booster vaccination restores previously strong epitope signal); 693-698 (booster vaccination enhances a previously weak or absent signal); or 607-612 (strong signal after primary vaccination is not enhanced by booster vaccination) across the study time points. (**b**) Tiling plots of individual samples of the corresponding epitopes (a) at day 57 and booster day 29, where the PIWAS value of a sample is shown along the amino acid position of the SARS-CoV-2 S protein. PIWAS, protein-based immunome wide association studies.

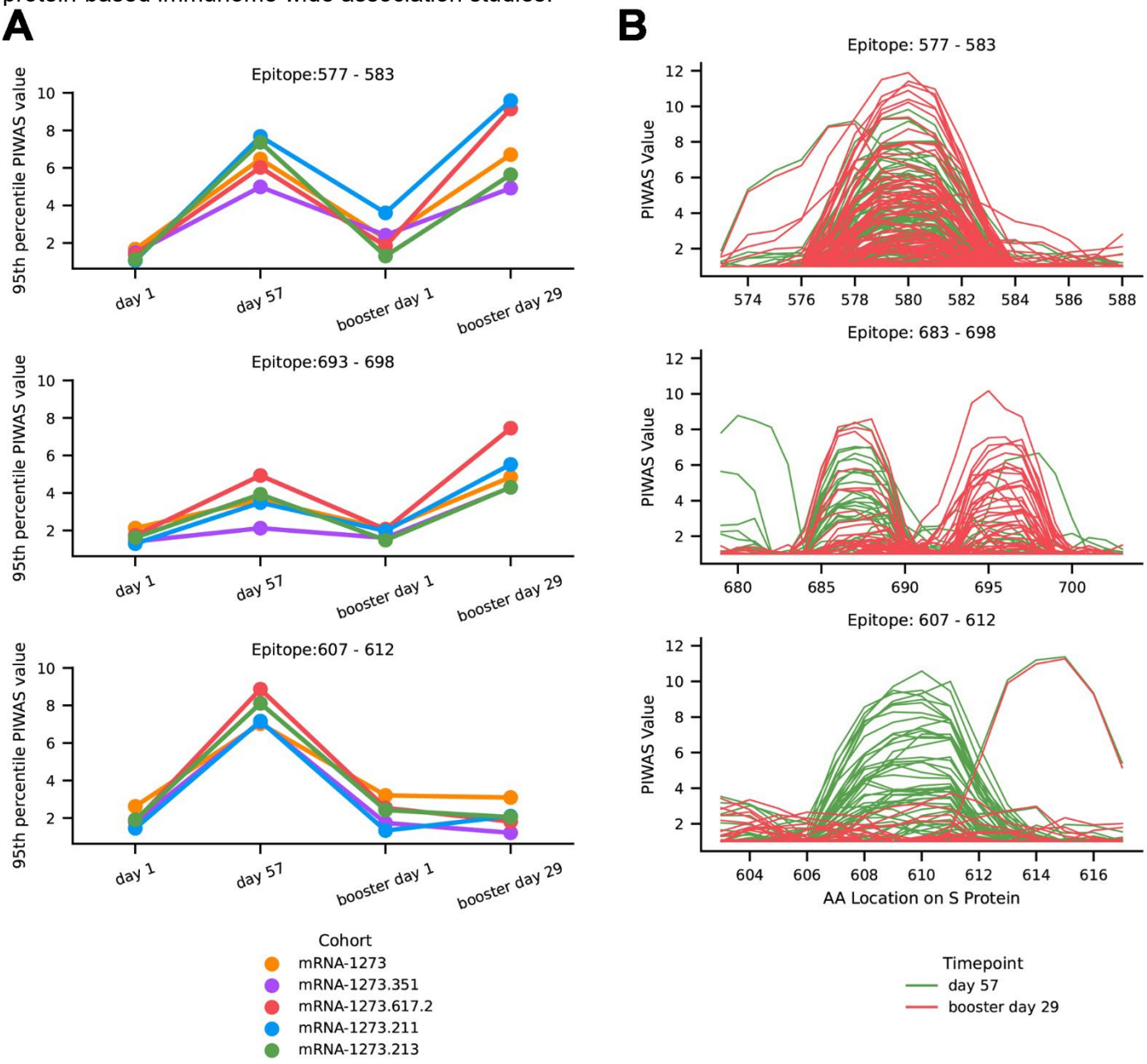


Figure S3. High resolution longitudinal signal from an individual participant.
(**a**) PIWAS signal showing longitudinal changes in epitope signal at region 558-569 observed for an individual participant in the mRNA-1273.211 booster group. (**b**) Enrichment logo plots for the same individual participant at region 558-569 from day 1 (before vaccination) to after booster vaccination (booster day 29). Note that baseline is shown but no signal was observed, while booster day 29 was rendered with a larger y-axis for resolution. PIWAS, protein-based immunome wide association studies.

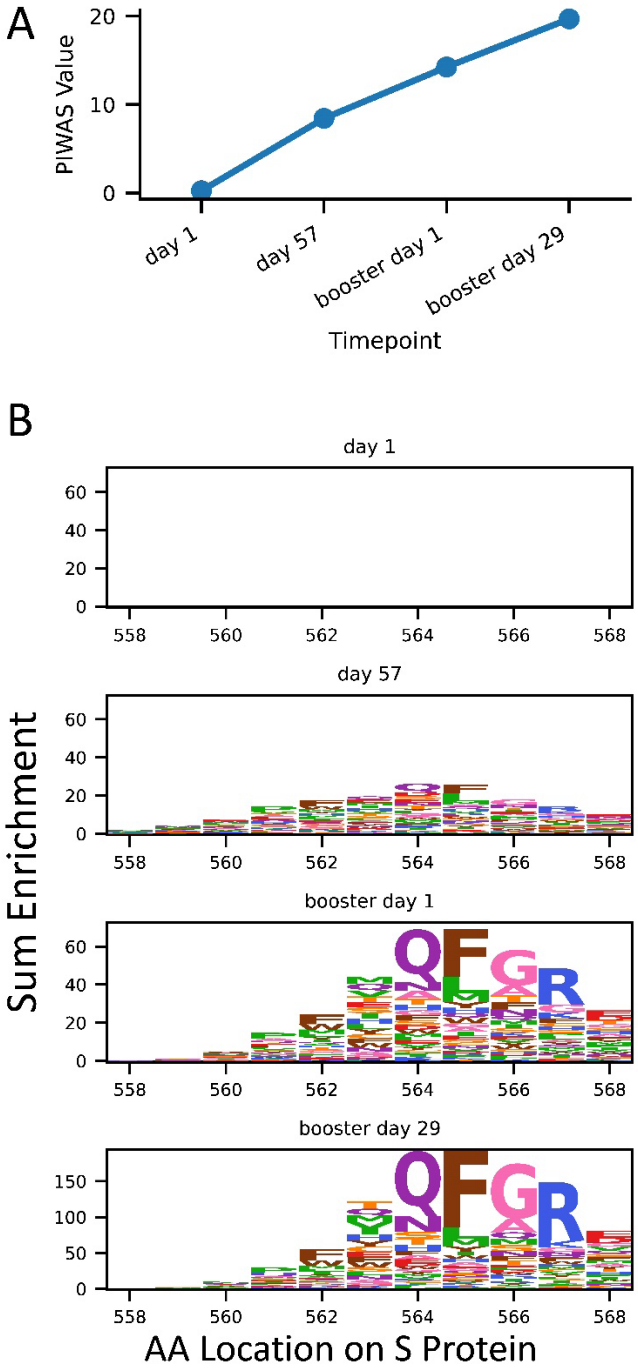
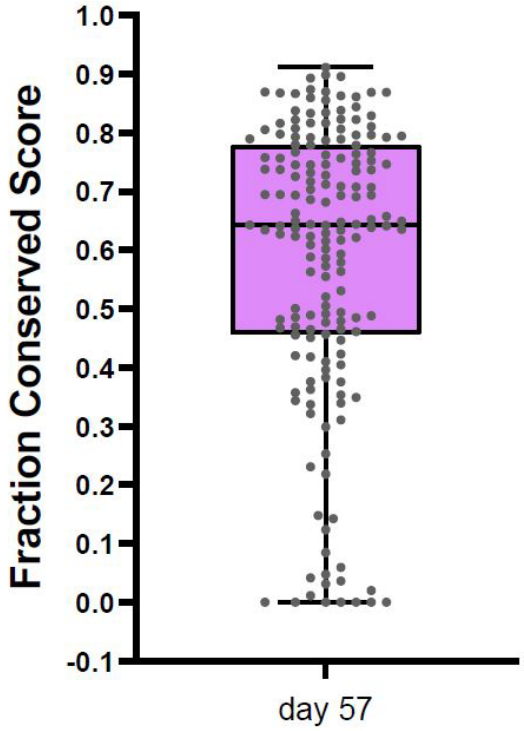


Figure S4. Fraction of antibody signal toward conserved epitopes after primary vaccination (day 57).
Conserved scores were calculated for each participant by integrating paired PIE signal at day 57 (with baseline signal removed) in shared amino acid regions identified with PIE. Individual epitope scores were calculated by integrating signal in regions of probable individual epitopes that were defined in all amino acid positions where paired PIE outlier signal increased from baseline to day 57. The ratio of conserved score to conserved plus individual scores at day 57 is provided for each participant. PIE, protein-wide identification of epitopes.



References

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2. Haynes, W.A. *et al.* High-resolution epitope mapping and characterization of SARS-CoV-2 antibodies in large cohorts of subjects with COVID-19. *Commun Biol* **4**, 1317 (2021).

3. Tibshirani, R. & Hastie, T. Outlier sums for differential gene expression analysis. *Biostatistics* **8**, 2-8 (2006).

4. Benjamini, Y. & Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B (Methodological)* **57**, 289-300 (1995).

5. Chu, L. *et al.* Immune response to SARS-CoV-2 after a booster of mRNA-1273: an open-label phase 2 trial. *Nat Med* **28**, 1042-1049 (2022).