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Supplemental information

Multiplexed LNP-mRNA vaccination against pathogenic coronavirus species

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Supplemental information

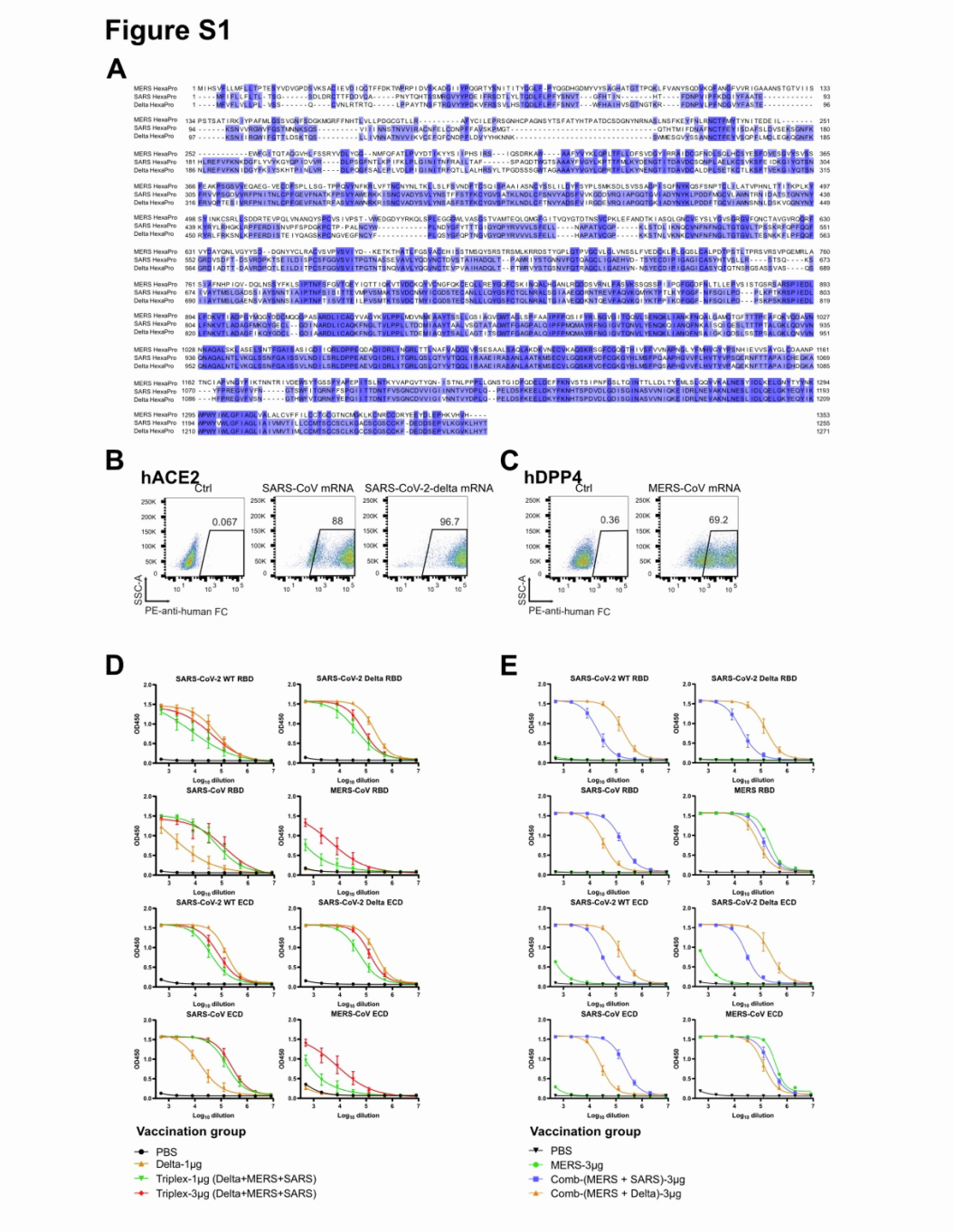

Figure S1 | Sequence alignment, functional validation and ELISA titration curves for engineered mRNA-encoded spike proteins of three pathogenic human coronavirus species.
(A) Sequence alignment of spikes of SARS-CoV-2 Delta variant, SARS-CoV and MERS-CoV used in the LNP-mRNA vaccine. The full-length spike sequences of these three pathogenic human coronavirus species were aligned and their degree of identity at each residue was color coded by a gradient blue color.

(B-C) Surface expression of functional spike proteins in 293T cells after electroporation of corresponding mRNA, as detected by human ACE2 **(B)** or human DPP4 **(C)** Fc fusion protein bound to PE anti-Fc antibody. **(D-E)** ELISA titration curves over serial log10-transformed dilution points of plasma samples from mice treated with spike antigens of SARS2 WT/WA1, SARS2 Delta, SARS and MERS. RBD and ECD ELISA spike antigens were used to evaluate the potency of binding antibodies induced by LNP-mRNA vaccines (top and bottom four panels, respectively). The mice were intramuscularly injected with two doses (x2, 2 weeks apart) of the following: **(D)** PBS, 1μg SARS-CoV-2 Delta variant LNP-mRNA (delta), 1μg or 3μg equal mass mixtures (Delta, SARS and MERS mRNA) delivered by LNP (Triplex-CoV); **(D)** PBS, 3μg MERS LNP-mRNA, 3μg equal mass mixture of MERS mRNA in combination with SARS or Delta mRNA delivered by LNP (Comb).
Related to: Figures 1-3

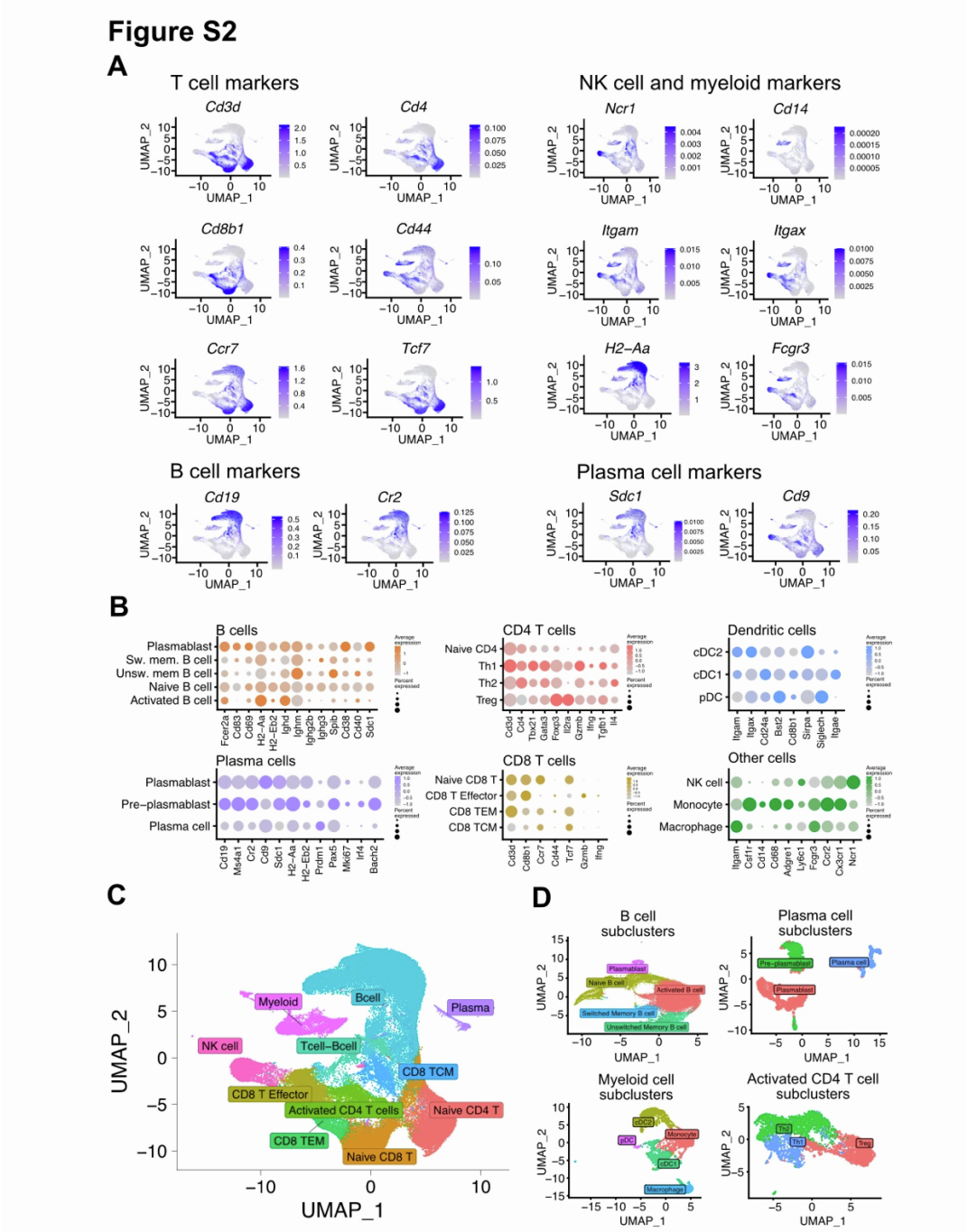

Figure S2 | Single cell transcriptomics visualization, clustering and cell type identification.
(a) UMAP visualization, colored by the scaled expression of representative cell type-specific markers in T cells, NK cells, myeloid cells, B cells, and plasma cells.
(b) Bubble plots showing cell population clusters and their respective feature markers.
(c) UMAP clustering, color-coded by major immune cell populations.
(d) UMAP visualizations of sub-clustering, performed in pooled B cells, plasma cells, myeloid cells, and activated CD4 T cells. Cell subclusters were identified as the indicated immune populations using the markers presented in the main Figures.
Related to: Figure 4

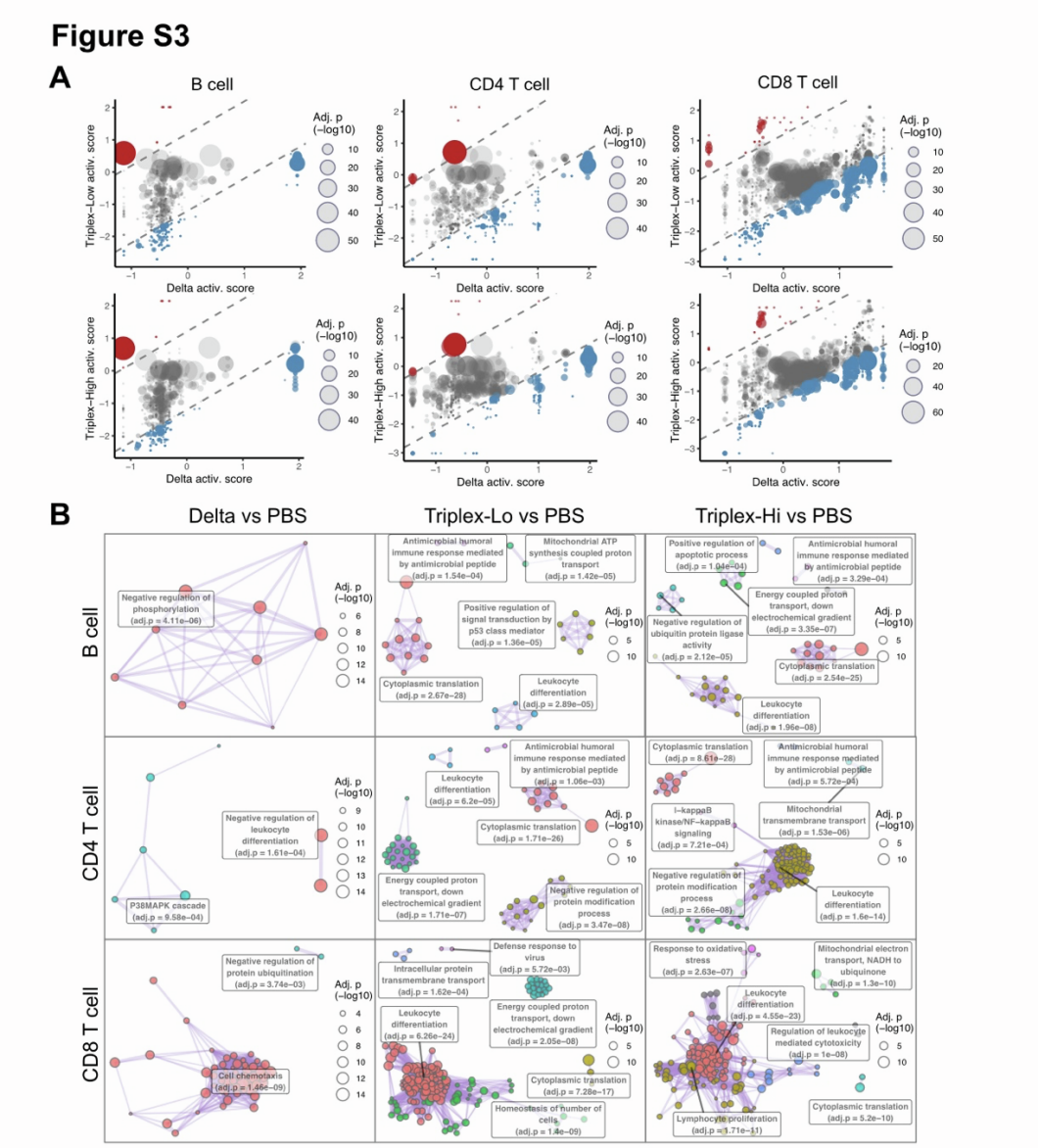

Figure S3 | Additional pathway analysis of differentially expressed genes compared between vaccination groups in different cell types in the single cell RNA-seq data.

(A) Bubble plots of overall biological process pathways of differentially expressed genes compared between vaccination groups in different cell types.

Each dot is a pathway presented with a color and size that represent the respective log fold change and -log10 adjusted p value, while the dot position compares the activation score (mean expression log fold change of pathway genes) in the analysis of mixCoV-vs-PBS (y axis), relative to the Delta-vs-PBS (x-axis).

(B) Network plots of enriched pathways of differentially expressed genes between the vaccination groups and PBS, in different cell types.

Each dot is a pathway with the size and color representing the -log10 adjusted p value and the pathway cluster, respectively. Clusters are labeled with the most significantly enriched member pathway (meta-pathway). Colored representative meta-pathway clusters correspond to the colored text boxes.

Related to: Figure 4

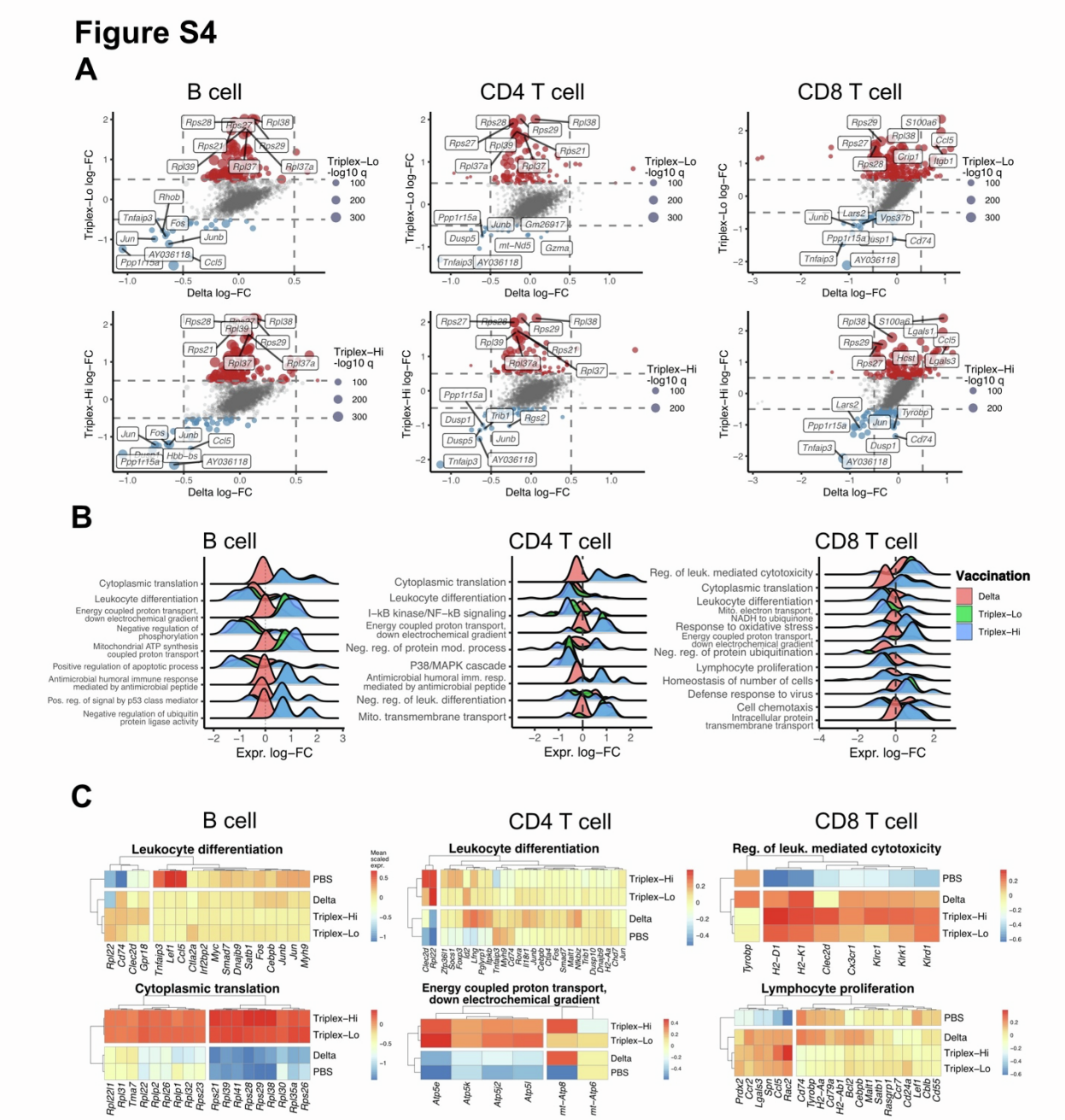

Figure S4 | Differential expression, pathway signature and gene set cluster analyses of single cell transcriptomics for animals vaccinated by multiplexed LNP-mRNAs.

(A) Square plots compare differential expression (DE) of mixCoV-vs-PBS (y axis) to Delta-vs-PBS (x axis) analyses (n = 3, one independent experiment). Each gene is presented by a dot, positioned by the log2(x+1) fold change in either DE analysis and sized by the -log10 FDR-adjusted p value. Genes that are upregulated or down regulated in mixCoV-vs-PBS are shown as red or blue dots, respectively. Analyses were done for B cell, CD4 T cell and CD8 T cell populations.

(B) Ridge density plots showing the expression log fold change meta-pathway genes between different vaccination groups in different cell types. Each plot presents the top five meta-pathways in either mixCoV-vs-PBS analysis, and only differentially expressed genes of either analysis were selected for each meta-pathway ridgeplot.

(C) Heatmaps of differentially expressed genes between different vaccination groups of representative pathways in different cell types.

Notes:

Each indicated cell type in the analysis represents the pooled activated immune cell subsets from the overall UMAP: B cell = activated B cells, switched memory B cells, and unswitched memory B cells; CD4 T cells = Th1, Th2 and Treg; CD8 T cells = CD8 effector T cells, CD8 TEM, and CD8 TCM.
Related to: Figure 4

Figure S5

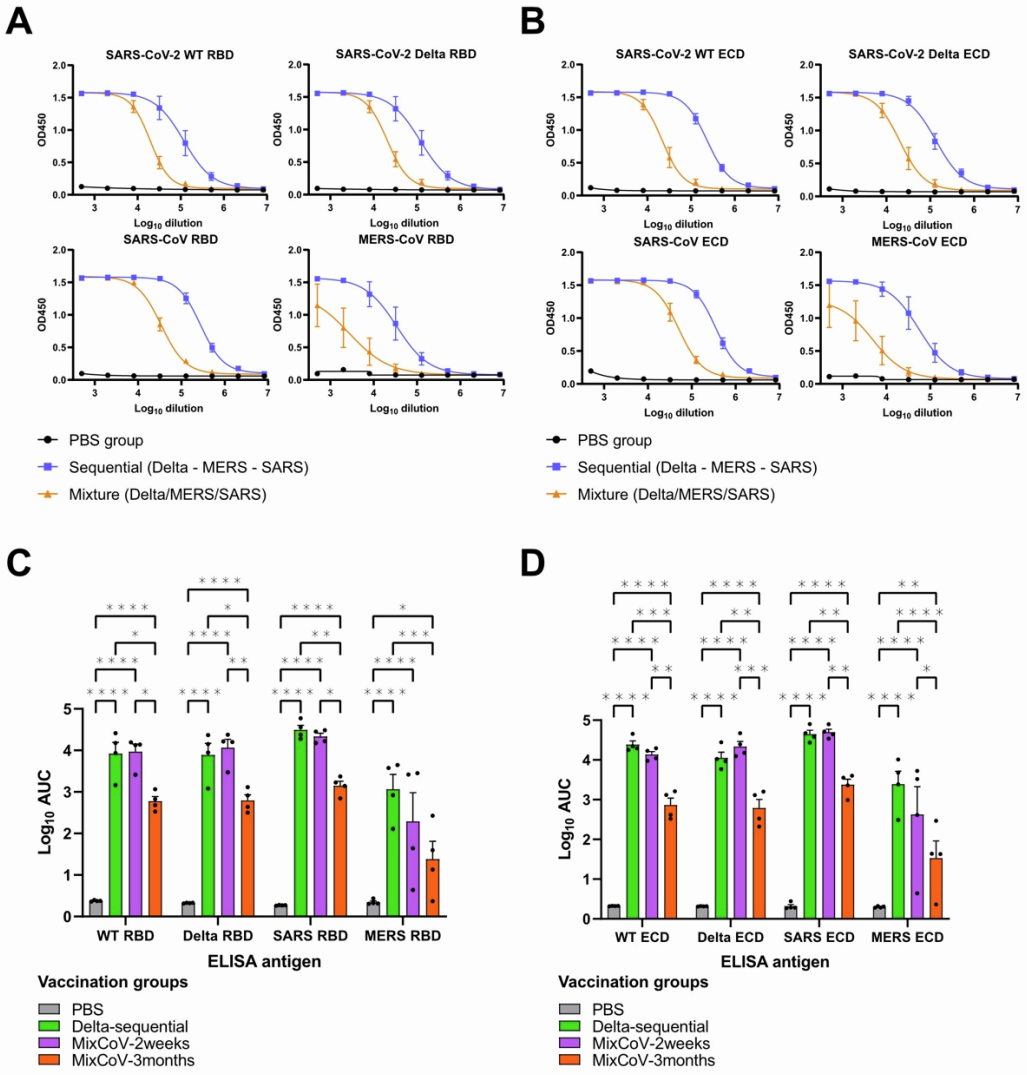

Figure S5 | Analyses of antibody responses induced by sequential and Triplex LNP-mRNA vaccinations.
(A-B) ELISA OD450 titration curves over serial log10-transformed dilution points of plasma from mice treated with PBS, sequential or mixture LNP-mRNA vaccinations. ELISA antibody titers are against RBDs or ECDs of SARS2 WT/WA1, SARS2 Delta, SARS and MERS.
The Sequential vaccination mice were intramuscularly injected with two doses (x2, 3 weeks between prime and boost) of 1 μg SARS-CoV-2 Delta, MERS, SARS LNP-mRNA, three weeks apart, in this sequence (Sequential Delta-MERS-SARS). The Mixture vaccination mice were intramuscularly injected with two doses (3 weeks between prime and boost) 3μg equal mass mixture (1μg each) of Delta, SARS and MERS LNP-mRNA (Mixture Delta/MERS/SARS).
(C-D) Comparative analyses of antibody responses induced by Triplex LNP-mRNA vaccination against SARS-CoV-2 Delta, SARS-CoV and MERS-CoV *in vivo*. ELISA antibody titers are against **(C)** RBDs or **(D)** ECDs of SARS2 WT/WA1, SARS2 Delta, SARS and MERS.
Related to: Figure 5

Figure S6

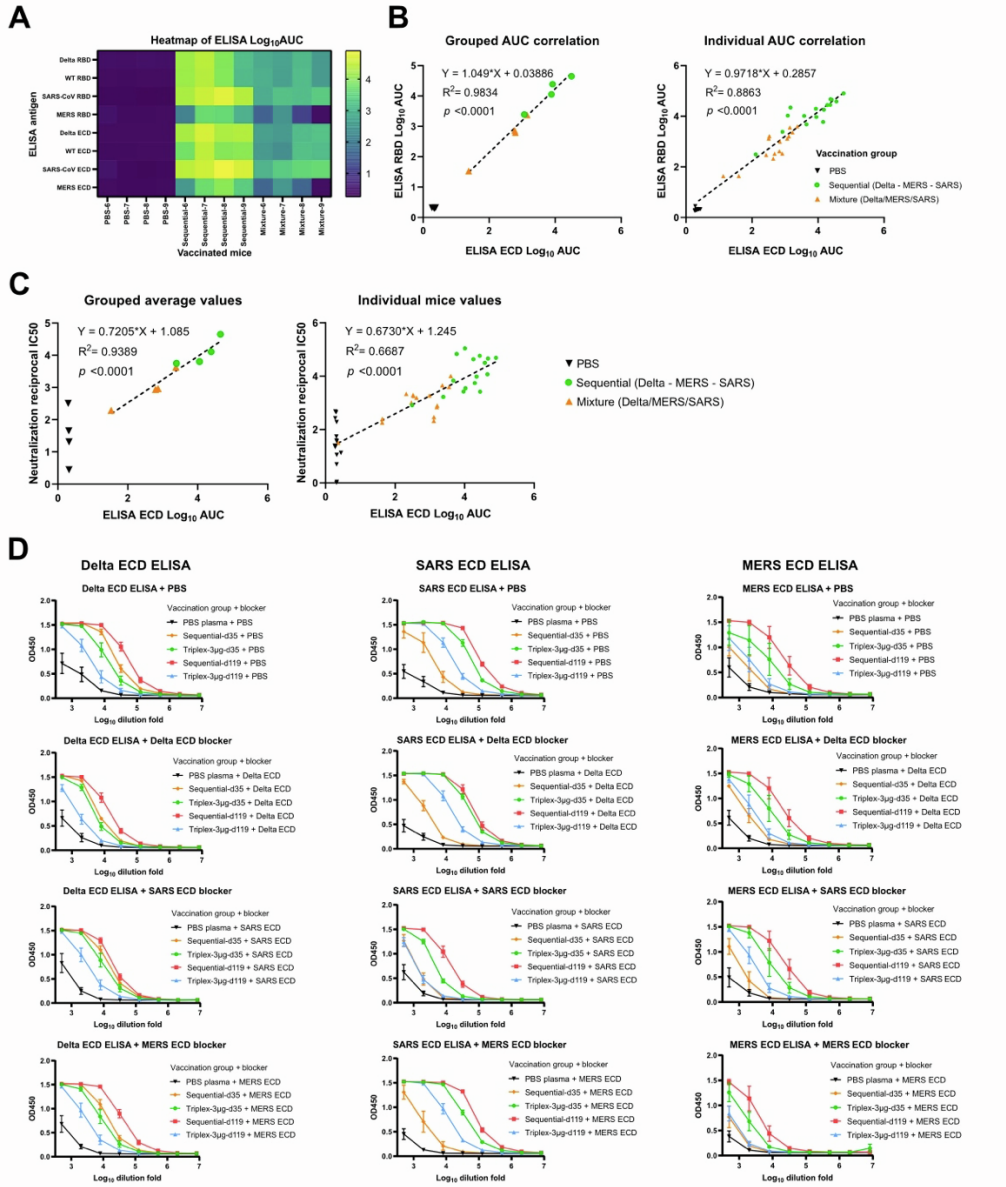

Figure S6 | Correlation analysis of neutralization datasets; Blocking ELISA titration curves.
(A) Heatmap of antibody titers of individual mice (one column represents one mouse) against eight spike antigens in ELISA (one row represents one antigen).
(B) Correlation of antibody titers against RBD (y value) and ECD (x value) of same coronavirus spike, by individual mouse, or by averaged group.
(C) Correlation of neutralization IC50 vs. antibody titers against ECD of same coronavirus spike, by individual mouse, or by averaged group.
(D) Blocking ELISA titration curve in response to the Delta, SARS or MERS ECD antigen in the presence of various competing agents or blockers: PBS, Delta ECD, SARS ECD and MERS ECD.
Related to: Figure 5

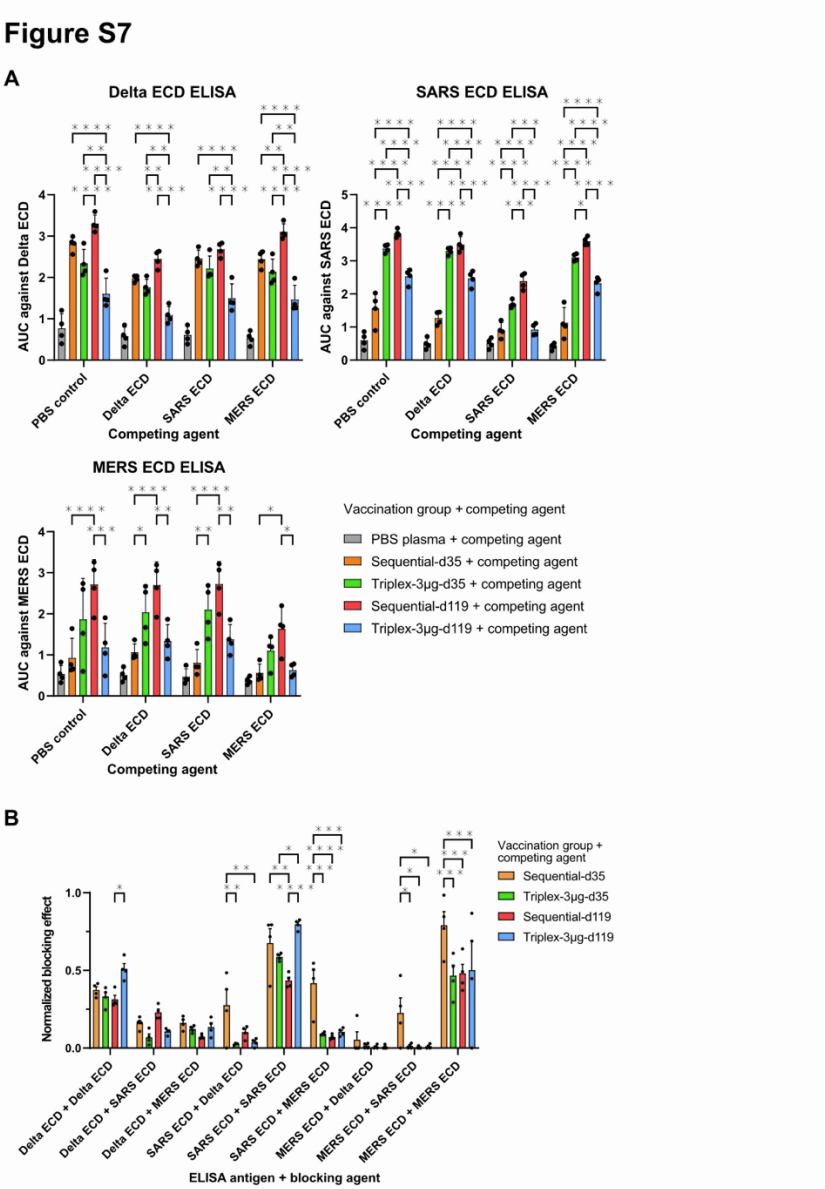

Figure S7 | Blocking ELISA antibody titers of plasma from different vaccination groups.
(A) Blocking ELISA antibody titers against Delta, SARS, and MERS ECDs in the presence of competing reagents including PBS (negative control), Delta, SARS or MERS ECDs. Statistical significance was analyzed between different vaccination groups in the presence of the same blocker. PBS plasma group was excluded in the statistical analysis in order to simplify graph.
(B) Normalized blocking effect induced by different blockers in each vaccination group in response to ELISA antigens of Delta, SARS and MERS ECDs. The blocking effect was quantified by normalizing the blocker-induced AUC reduction with vaccine-specific AUC increase. The vaccine-specific AUC increase (100%) is calculated from AUC difference in PBS plasma group (0% or baseline) and vaccination group under the same antigen and blocker condition. The blocker-induced AUC reduction is the AUC difference between PBS and blocker treatment under the same vaccination and antigen condition.
Related to: Figure 5

Supplemental Source Data and Statistics

Supplemental excel file(s) contains all original data and statistics for non-NGS experiments.

Supplemental Datasets

Dataset 1 | Single cell GEX of multiplexed LNP-mRNA vaccinated animals

Tabs in this dataset:

- Metadata of merged single cell GEX dataset.
- Clustering of scGEX dataset.
- Two-dimensional UMAP embeddings.
- Wilcoxon statistics for cluster-specific differentially expressed genes
- Markers for clustering immune cell subsets.
- Statistics for cell type proportions across treatment groups.
- Results of the differential expression (DE) analyses of treatment vs PBS groups in different cell types.
- Results of the differential expression (DE) analyses of mixCoV vs Delta treatment groups in different cell types.
- Results of the gProfiler pathway analysis in different comparisons.