Variant-specific vaccination induces systems immune responses and potent \textit{in vivo} protection against SARS-CoV-2

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B.1.351 or B.1.617 mRNA-LNP

Mouse immunization

Prime Boost Endpoint

T cell Response
CD8
S peptide
IFNγ, TNFα, IL2...
CD4

Immune Profiling
scRNA-seq scTCR scBCR

In vivo protection
Prime Boost Virus challenge Monitoring Survival
Variant-specific vaccination induces systems immune responses
and potent in vivo protection against SARS-CoV-2

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Abstract
Lipid-nanoparticle (LNP)-mRNA vaccines offer protection against COVID-19. However, multiple variant lineages caused widespread breakthrough infections. Here, we generate LNP-mRNAs specifically encoding wildtype (WT), B.1.351 and B.1.617 SARS-CoV-2 spikes, and systematically study their immune responses. All three LNP-mRNAs induced potent antibody and T cell responses in animal models. However, differences in neutralization activity have been observed between variants. All three vaccines offer potent protection against in vivo challenges of authentic viruses of WA-1, Beta and Delta variant. Single cell transcriptomics of WT- and variant-specific LNP-mRNA vaccinated animals reveal a systematic landscape of immune cell populations and global gene expression. Variant-specific vaccination induces a systemic increase of reactive CD8 T cells and altered gene expression programs in B and T lymphocytes. BCR-seq and TCR-seq unveil repertoire diversity and clonal expansions in vaccinated animals. These data provide assessment of efficacy and direct systems immune profiling of variant-specific LNP-mRNA vaccination in vivo.

Keywords
Lipid nanoparticle, LNP-mRNA, variant-specific COVID-19 vaccine, systems immunology, neutralization, single cell profiling, BCR, TCR, B.1.351, B.1.617, B.1.617.2, Beta variant, Delta variant
Introduction

Severe acute respiratory syndrome coronavirus (SARS-CoV-2), the pathogen of coronavirus disease 2019 (COVID-19), has caused the ongoing global pandemic. Although lipid nanoparticle (LNP) - mRNA based vaccines such as BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna) have demonstrated high efficacy against COVID-19, breakthrough infections have been widely reported in fully vaccinated individuals. Moreover, the virus continues to mutate and multiple dangerous variant lineages have evolved, such as B.1.1.7, B.1.351, and, more recently B.1.617. The B.1.1.7 lineage (Alpha variant, or “UK variant”) has an increased rate of transmission and higher mortality. The B.1.351 lineage (Beta variant, or “South Africa variant”) has an increased rate of transmission, resistance to antibody therapeutics, and reduced vaccine efficacy. The lineage B.1.617 (“Indian variant” lineage, including B.1.617.1 “Kappa variant”, B.1.617.2 “Delta variant” and B.1.617.3) has recently emerged, spread rapidly, and become dominant in multiple regions in the world. The on-going surge of infections in the US is predominantly caused by the Delta variant, originating from the B.1.617 lineage that has >1,000 fold higher viral load in infected individuals. The B.1.617 lineage has an increased rate of transmission, showing reduced serum antibody reactivity in vaccinated individuals, and exhibits resistance to antibody therapeutics. These variants often spread faster than the ancestral “wildtype” (WT) virus (also noted as Wuhan-1 or WA-1, with identical spike sequences), cause more severe disease, are more likely to escape certain host immune response, cause disproportionally higher numbers of breakthrough infections despite the status of full vaccination, and have been designated by WHO and CDC as “variants of concern” (VoCs). Regarding their effects on vaccine efficacy, B.1.351, for example, has been known to reduce the efficacy of the Pfizer-BioNTech vaccine from >90% to near 70%. The Delta variant has also resulted in significant reduction of vaccine efficacy especially for individuals who received only a single dose, and has caused widespread breakthrough infections despite the status of full vaccination.

It has been widely hypothesized that the next-generation of COVID-19 vaccines can be designed to directly target these variants (“variant-specific vaccines”). However, to date, there has been no literature report on any approved or clinical stage variant-specific vaccine. Moreover, the immune responses, specificity, cross-reactivity, and host cell gene expression landscapes upon vaccination have to be rigorously tested for such variant-specific vaccines to be developed. To directly assess the immunogenicity of potential variant-specific SARS-CoV-2 vaccination, we generated LNP-mRNA vaccine candidates that encode the B.1.351 and B.1.617 spikes, along with the WT spike. With these variant-specific LNP-mRNAs, we characterized the immune responses they induce in animals against homologous (cognate) and heterologous spike antigens and SARS-CoV-2 pseudoviruses. To better understand the systematic immune responses induced by variant-specific SARS-CoV-2 spike mRNA-LNP vaccination, we analyzed the combined single-cell transcriptomes and
lymphocyte antigen receptor repertoires of mice immunized with B.1.351 and B.1.617 spike mRNA-LNP vaccine candidates.

Results

Design, generation and physical characterization of variant-specific SARS-CoV-2 spike LNP-mRNAs

We designed and generated nucleotide-modified mRNAs separately encoding full-length SARS-CoV-2 WT, B.1.351 and B.1.617 spikes. The HexaPro mutations were introduced and the furin cleavage site was replaced with a GSAS sequence to stabilize the prefusion state and preserve integrity of spike S1 and S2 subunits (Figure 1A-1B). The protein expression and receptor binding ability of modified spike mRNA were confirmed by in vitro cell transfection and flow cytometry where the spike binding to the human ACE2-Fc fusion protein was detected by PE-anti-Fc antibody (Figure 1C). We encapsulated the spike mRNA with LNP, and evaluated their size and homogeneity by dynamic light scattering (DLS) and transmission electron microscopy (TEM) (Figure 1D). The WT (WA-1), B.1.351 and B.1.617 mRNA LNPs have mean diameters of 80.7 ± 6.9nm, 66.4 ± 5.3 nm and 72.2 ± 5.8 nm with a monodispersed size distribution as determined by DLS and polydispersity indices of 0.08, 0.13 and 0.08, respectively (Figure 1E-F). The immunogenicity of the LNP-mRNA was assessed in C57BL/6Ncr mice by two intramuscular injections (doses) of 1 µg or 10 µg LNP-encapsulated mRNA, separated by 3 weeks (prime and boost, respectively) (Figure 1G). Serum samples were collected two weeks after the prime and boost, and then subjected to ELISA and neutralization assays to evaluate the antibody response. These mice were sacrificed 40 days post vaccination, and the spleen, lymph nodes and blood cells were collected for downstream assays, including single cell transcriptomics sequencing (scRNA-seq), bulk and single cell BCR sequencing (BCR-seq) and TCR sequencing (TCR-seq), as well as flow cytometry. All procedures were standardized across all groups.

Immune responses induced by WT-LNP-mRNA vaccination in mice

WT-LNP-mRNA (WA-1-LNP-mRNA) induced dose-dependent binding antibody responses against spike ECD (Ectodomain of spike protein) and RBD of SARS-CoV-2 WT, B.1.351 and B.1.617 variants after prime and boost (Figure 1H-I). Compared to the post-prime immune response, orders of magnitudes increase in immune response were observed after the boost injection, suggesting that the second dose significantly boosted B cell immunity to SARS-CoV-2 antigens (Figure 1H-I). Using a pseudovirus neutralization assay that has been widely reported to be consistent with authentic virus results, the serum samples from mice receiving WT-LNP-mRNA vaccination also showed potent neutralization activity against all three variants, again with a strong prime-boost effect (Figure 1J). However, the neutralization ability of WT-LNP-mRNA vaccinated sera was found to be several fold lower against either B.1.351 or B.1.617 as compared to the cognate WT
pseudovirus (Figure 1J). These observations are consistent with the series of reports showing dramatic reduction in neutralization of B.1.351 and B.1.617 variants by vaccinated individuals’ sera, convalescent sera, and therapeutic antibodies.\textsuperscript{12,13,23,34}

To evaluate the T cell response to the spike peptides, the splenocytes were isolated from mouse spleens 40 days post vaccination and the antigen-specific CD4\(^+\) and CD8\(^+\) T cell response to S peptide pools were determined by intracellular cytokine staining (Figure S 2). WT-LNP-mRNA, at both low and high doses, induced reactive CD8\(^+\) T cells producing interferon γ (IFN-γ, IFNg), tumor necrosis factor α (TNF-α, TNFα), and interleukin 2 (IL-2) (Figure 1K), at levels consistent with previously reported studies.\textsuperscript{31,35} WT-LNP-mRNA at both doses also induced reactive CD4\(^+\) T cells that produce IFN-γ, but little for TNF-α, IL-2, IL-4 or IL-5 (Figure 1L; Figure S 2B). As technical quality controls, there is no difference in cytokine production between vaccinated groups and the PBS group when cells were treated with vehicle (no peptide) or PMA/ionomycin (Figure S 2C-D). These results suggest that WT-LNP-mRNA vaccines can induce potent spike protein specific CD4 and CD8 T cell responses.

**Binding and neutralizing antibody responses of B.1.617-LNP-mRNA and B.1.351-LNP-mRNA**

Both B.1.617-LNP-mRNA and B.1.351-LNP-mRNA induced dose-dependent binding antibody responses against spike ECD and RBD of SARS-CoV-2 WT, B.1.351 and B.1.617 variants (Figure 2A-2B). The strong boost effect in ELISA was also observed for these two variant-specific LNP-mRNAs (Figure 2A-2B). The dose-dependence effect was observed in both B.1.617-LNP-mRNA and B.1.351-LNP-mRNA groups across three types of ELISA antigens of both RBD and ECD, although the dose effect was less prominent in the post-boost samples, where both doses showed high titers at potential saturation level (Figure 2A-2B; Figure S 1A-B). Relatively speaking, higher antibody responses was often observed with ECD antigen, suggesting an immunogenic domain other than RBD contributed to the additional response to spike ECD (Figure 2A-2B; Figure S 1A-B). Overall, the binding intensity as measured by serum titer between RBD and ECD strongly correlates with each other across all vaccination groups (Figure S 1C).

We then examined pseudovirus-neutralizing antibody response. Both B.1.617-LNP-mRNA and B.1.351-LNP-mRNA elicited potent neutralizing antibodies, of which response mirrored the trend of post-prime and post-boost response reported in ELISA (Figure 2C). The initial level of neutralization was at \(10^2 – 10^3\) level of reciprocal IC50 after priming for most groups (Figure 2C). Consistent with findings in ELISA, an approximately two orders of magnitude increase in neutralization titer by boost was observed across all groups (for both vaccine candidates and for all three pseudovirus types) in the low dose (1 µg) setting, and there was
an approximately one order of magnitude increase in the high dose (10 µg) setting (Figure 2C). The dose

effect of serum neutralization activity for both B.1.617-LNP-mRNA and B.1.351-LNP-mRNA was observed

at priming for most groups, but negligible post boost (i.e. both 1 µg and 10 µg dose groups reach reciprocal

IC50 titer of $10^4$ level after boost) (Figure 2C). Both B.1.617-LNP-mRNA and B.1.351-LNP-mRNA
effectively neutralize pseudoviruses of all three SARS-CoV-2 pseudoviruses post boost at titers of $10^4$ level

(Figure 2C). Interestingly, B.1.351-LNP-mRNA vaccinated animals neutralize pseudoviruses of all three
SARS-CoV-2 at similar levels post boost at both doses (Figure 2C); while B.1.617-LNP-mRNA vaccinated
animals showed significantly higher titer against its cognate B.1.617 pseudovirus (by several folds). Compared
to WT LNP-mRNA, B.1.617 LNP-mRNA displayed significantly higher post-boost binding and neutralizing
antibody titers against its cognate antigen, B.1.617 variant (Figure 2D-2E). The B.1.351, on the other hand,
showed higher post-boost binding, but not neutralization titers against B.1.351 antigen. Overall, across all
vaccination groups, the neutralization activity strongly correlates with binding intensity for ECD binding
(Figure 2E), which also holds true for RBD binding (Figure S 1D). Comparison of WT-LNP-mRNA, B.1.617-
LNP-mRNA and B.1.351-LNP-mRNA for their effects in ELISA and neutralization titers corroborated with
the observations above (Figure S 1E-G).

B.1.617-LNP-mRNA and B.1.351-LNP-mRNA elicited strong systemic T cell response against SARS-
CoV-2 spike

Similarly, to evaluate the T cell response to the spike peptides, the splenocytes were isolated from mouse
spleens 40 days post vaccination and the antigen-specific CD4⁺ and CD8⁺ T cell responses to S peptide pools
were determined by intracellular cytokine staining (Figure S 2E-G). Positive control PMA/ionomycin
treatment group and negative control no peptide group were both validated (Figure S 2F-G). Both B.1.617-
LNP-mRNA and B.1.351-LNP-mRNA, at low and high doses, induced potent reactive CD8⁺ T cell responses
in terms of cellular production of IFN-γ, TNF-α, and IL-2 (Figure 3A-3C). Both LNP-mRNAs at both doses
also induced reactive CD4⁺ T cells that produce IFN-γ, but only minimally for TNF-α, and had no effect on
IL-2, IL-4 or IL-5 (Figure 3D; Figure 2E). Multi-channel flow analysis showed that both B.1.617-LNP-
mRNA and B.1.351-LNP-mRNA, at both low and high doses, induced polyfunctional CD8⁺ T cells
subpopulation that simultaneously produce two cytokines, such as IFN-γ⁺;TNF-α⁺, IFN-γ⁺;IL-2, or TNF-
α⁺;IL-2⁺ CD8⁺ T cells (Figure 3E-G). Both LNP-mRNAs at both doses also induced polyfunctional CD4⁺ T
cells that produce IFN-γ and TNF-α at the same time (Figure 3H).

WT- and variant-specific- LNP-mRNA vaccinations offer strong protection against authentic SARS-
CoV-2 ancestral and VoC viruses in vivo
To further evaluate the protective potency of WT-LNP-mRNA, B.1.617-LNP-mRNA and B.1.351-LNP-mRNA against the challenge of authentic SARS-CoV-2 ancestral virus (WA-1) and VoC viruses (Beta and Delta), we perform in vivo vaccination and infection experiments in a biosafety level 3 (BSL3) setting. We first immunized K18-hACE2 mice with two doses of WT-LNP-mRNA, B.1.617-LNP-mRNA and B.1.351-LNP-mRNA, a prime (day 0) and a boost (day 21) (Figure 4A). One week after boost, we randomly divided these three types of LNP-mRNA vaccinated mice into three subgroups each (Figure 4B) and challenged K18-hACE2 mice with 10^3 plaque forming units (PFUs), a dose that’s 10x of half lethal dose (10x LD50), of SARS-CoV-2 WA-1 virus (Figure 4B).

As a result, virtually all mice in the placebo group developed severe disease due to viral challenge, and consistently decreased body weight, for all three viruses (Figure 4C-D). Despite differences of severity between viruses, in the placebo treated group, the majority of animals succumbed from the disease by day 10 post infection, with 80% death in WA-1 virus group, 100% death in Beta virus group and 60% in Delta virus group, respectively (Figure 4C-D). In sharp contrast, all mice receiving any of the three vaccine (WT-LNP-mRNA, B.1.617-LNP-mRNA or B.1.351-LNP-mRNA) were free of severe disease symptoms, largely maintained their body weight throughout the duration of the study, and all (100%) survived from SARS-CoV-2 infection in the time course of experiment (Figure 4C-D). These data demonstrated that all three LNP-mRNAs, including both WT- and variant-specific- LNP-mRNA vaccinations, can protect the animals from lethal SARS-CoV-2 challenges.

**Single cell immune repertoire mapping of WT- and variant-specific- LNP-mRNA vaccinated animals**

In order to gain insights on the global composition and transcriptional landscape of the immune cells, we performed single cell transcriptomics (scRNA-seq) on the spleen samples of 24 animals from all three vaccination groups (WT/WA-1-LNP-mRNA, B.1.351-LNP-mRNA, and B.1.617-LNP-mRNA, both 1 µg and 10 µg dose groups), plus a control group (PBS treated). Gene expression profiling was performed in a total of 141,729 single cells, as projected on a Uniform Manifold Approximation and Projection (UMAP) (Figure 5A). Cells were clustered by generating a shared nearest neighbors (SNN) graph, and optimizing the modularity using the Louvain algorithm with multilevel refinement algorithm with an empirically chosen resolution, based on the best spatial separation of major immune populations cells via Cd3d, Cd19, Ncr1, Itgam, Itgax, and Sdc1 expression via UMAP visualization (Figure 5B). The clusters were then labeled based on the expression of different immune cell markers (Figures S3-S6, Dataset S1). For better resolution of complex cell types, B cells, T cells and dendritic cells (DCs) (Cd45+Cd19+, Cd45+Cd3d+, and Cd45+Itgax+ clusters, respectively) were separately subset, rescaled, visualized in low dimensional UMAP space, clustered, and populations were
identified using the method above (Figure 5F; Figures S4-S6). Labeled cell types were tested for homogeneity by performing Wilcoxon rank sum testing of scaled data and assessing discreet hierarchical clustering of populations using the top 10 DEGs in each cell type compared to all others (Figure 5C). Using these methods, we identified 32 populations of immune cells, including 7 B cell, 13 T cell, and 4 DC subsets, as well as endothelial cells and a population of Cd19+CD3d+ cells, labeled as B cell-T cell doublets, based on similar observations of Cd19+Cd3+ splenocytes doublets via flow cytometry. Comparisons between different vaccination groups showed vaccine-specific shifts in immune population proportions. Of note, macrophage (Mφ) populations were significantly increased in B.1.351-LNP-mRNA and B.1.617-LNP-mRNA, but decreased in WT/WA-1-LNP-mRNA groups, relative to the PBS control group (Figure 5D). Most notably, all three variant groups had significantly increased levels of activated cytotoxic T cells (Tc1) and significantly decreased follicular (Fo) B cells compared to the PBS control group (Figure 5G). The shift from Fo B cells appears to be accompanied by increases in activated germinal center (GC) B cells for B.1.351-LNP-mRNA and B.1.617-LNP-mRNA groups and B1 cells for the WT/WA-1-LNP-mRNA group, although to non-significant extents (Figure 5G). Together, these immune shifts in macrophages, T cells, and B cells are consistent with an increased immune response.

**Gene expression signatures of B cell and T cell populations of B.1.617-LNP-mRNA and B.1.351-LNP-mRNA vaccinated animals**

Because B and T cells are the cornerstones of adaptive immunity against SARS-CoV-2, we further investigated the B cell sub-populations and T cell sub-populations, respectively. Specifically, we performed differential expression analyses in activated B cells (Activated and GC B cells), activated CD4 T cells, and activated CD8 T cells across vaccination groups. In each comparison, we accounted for the complexities amongst experimental conditions, heterogeneous immune cell populations, and inconsistent transcriptional sampling between cells using scRNA-seq technologies by fitting the data to generalized linear models and assessing how each vaccine variant influences each activated cell type, relative to the PBS treatment. The top upregulated genes in activated B cells represent transcription and translation machineries, which are consistent between WT/WA-1-, B.1.351- and B.1.617- specific LNP-mRNA vaccination groups (Figure 6A). This strong signature was also observed in T cells (Figure 6D), consistent with the phenomenon of active lymphocyte activation upon vaccination. Next, we identified vaccine-specific pathway changes in each cell type using a pathway analysis, in which significant gene set enrichment analysis results are aggregated into “supra-pathway” clusters to highlight unique pathways amongst highly redundant gene ontologies. Our results show upregulation of transcription/translation-related pathways in B cells and CD8 T cells from all three vaccination groups (Figures 6B, C, E, F), while the variant vaccination groups showed significantly enhanced effector
functions in CD8 T cells, including leukocyte differentiation and cellular extravasation in the B.1.351 group and cell killing in the B.1.617 group (Figures 6E-F). We next investigated how the B.1.351-LNP-mRNA and B.1.617-LNP-mRNA variant vaccines compared to WT/WA-1-LNP-mRNA. Results from differential analyses showed broad differences in immune response and cellular metabolism pathways across each cell type, in either comparison (Figures S8 and S9). These data characterize the immune responses generated from each variant vaccine and how these compare to the WT/WA-1 vaccination. In particular, these results provide evidence for a broadly altered immune populations and transcriptomic signatures upon vaccination, including activation of B cell and CD8 T cell subsets, as well as enhanced effector function in CD8 T cells. The differential analyses between variant-specific vaccination and WT vaccination also showed differences in the gene expression in B and T cells.

TCR and BCR diversity mapping of B.1.617-LNP-mRNA and B.1.351-LNP-mRNA vaccinated animals

To reveal the B and T cell clonal diversity and influence by vaccination, we performed VDJ repertoire mapping and clonal analyses of B cell and T cell populations of WA-1-LNP-mRNA, B.1.351-LNP-mRNA and B.1.617-LNP-mRNA vaccinated animals. We performed both single cell BCR sequencing (scBCR-seq) and single cell TCR sequencing (scTCR-seq) on the spleen samples of all groups (6 vaccination groups and a PBS group, n = 24 mice total). We sequenced a total of 154,203 single B cells and 77,699 single T cells. Clonal composition showed the BCR repertoire in the single cell BCR-seq dataset, revealing a trend towards decreased clonal diversity in variant-specific vaccine treated animals, a signal of clonal expansion (Figure 7A-B, e; Figure S10A). The clonal composition of single cell TCR showed a similar decrease in clonal diversity (Figure 7C-D, e; Figure S10B). This phenomenon is consistent with the clonal expansion of stimulated lymphocytes upon vaccination.

To further validate the observations, we also performed bulk BCR-seq and bulk TCR-seq for all these mice on additional tissue samples, including spleen, peripheral blood cells and lymph node (LN). The bulk BCR-seq and TCR-seq data revealed systematic clonality maps of IGH, IGK, IGL, TRA, and TRB repertoires from the spleen, blood, and LN samples of the variant-specific LNP-mRNA vaccinated along with PBS treated animals (Figure 7F; Figures S11A and S12A). Analyses of IGK, IGL, TRA, and TRB repertoires showed trends of decreasing unique clonotype numbers in B.1.351-LNP-mRNA and B.1.617-LNP-mRNA vs PBS peripheral blood samples (Figures S11B and S12B), concomitant with an increased proportion of hyperexpanded clonotypes (> 1% total clones) (Figures S11C and S12C). There is also an increased percent of the repertoire occupied by the top 10 and top 50 most abundant IGK, TRA, and TRB clonotypes in the blood, significantly in TRA chains of 10 µg variant vaccinated and 1 µg B.1.351 vaccinated samples (Figure 7G). Lastly, true
diversity estimates of TRA and TRB chains were significantly decreased in the blood samples of all 1µg B.1.351 and both 10µg variant vaccinated samples, relative to PBS controls (Figure 7H). These combined data unveiled BCR and TCR repertoire clonality, diversity and respective shifts in variant-specific LNP-mRNA vaccinated animals as compared to placebo-treated. In addition, these results are consistent with the observation of decreased clonal diversity from single cell VDJ profiling, which together suggest a clonal expansion of B cells and more notably, T cells.

Discussion

Although efficacious COVID-19 mRNA vaccines have been deployed globally, the rapid spread of SARS-CoV-2 VoCs with higher contagiousness as well as resistance to therapies and vaccines demands evaluation of next-generation COVID-19 vaccines specifically targeting these evolving VoCs. Mounting evidence has suggested that the B.1.351 and B.1.617 lineage variants of SARS-CoV-2 possesses much stronger immune escape capability than the original wildtype virus. The lower neutralizing titers in fully vaccinated patients were found associated with breakthrough infections. It has been speculated that the waning immunity from early vaccination and emergence of more virulent SARS-CoV-2 variants may lead to reduction in vaccine protection and increase of breakthrough infections. It has been reported that mRNA vaccines’ efficacy against B.1.351 and B.1.617.2 dropped significantly. Moreover, for individuals receiving only a single dose of vaccine, the protective efficacy can be dramatically lower. It is worth noting that efficacy value and definition may vary from study to study, which were conducted in different regions and populations. All these factors prompted us to evaluate the next-generation mRNA vaccine candidates encoding the B.1.351 and B.1.617 spike as antigens.

While the findings of differential antibody responses of vaccination against cognate vs. heterologous antigens is in line with the effect of dampening immunity by variants for WT vaccines in human study, however, prior studies were done using WT vaccines against VoCs, not variant-specific vaccines, which are entirely different drug compositions. Currently there is limited published work or immunological data on variant-specific vaccines. Our study directly produced, characterized, and systematically profiled the immunity of variant-specific vaccines. It’s critical to learn their potential protective benefits against wildtype or variants of SARS-CoV-2. In fact, this becomes increasingly important due to the continuous rise of new variants of concern. In reaction to the VoCs, major vaccine producers are actively developing variant-specific vaccines and test their effect in clinical trials (e.g. Pfizer/BioNTech and Moderna), highlighting the clinical relevance.
Our study characterized the titers and cross-reactivity of sera from mice vaccinated with WT- / WA-1-, B.1.351- or B.1.617-LNP-mRNAs to all three WT, B.1.351 and B.1.617 spike antigens, pseudoviruses and authentic viruses. In agreement with findings in patients’ sera, we found that the neutralizing titers of WT vaccine sera were several folds lower against the two variants of concerns than against WT pseudovirus. Interestingly, the B.1.617-LNP-mRNA vaccinated sera also showed particularly strong neutralization activity against its cognate B.1.617 pseudovirus, while the B.1.351-LNP-mRNA showed similar neutralization activity against all three pseudoviruses. It is worth noting that all three forms of vaccine candidates can induce potent B and T cell responses to WT as well as the two VoCs’ spikes. The in vivo challenge experiments showed that all three vaccine candidates, i.e. WT/WA-1-, B.1.351- and B.1.617- LNP-mRNAs offer strong protection against all three authentic viruses (WA-1, Beta and Delta) in mice.

The T cell-biased immune response is important for antiviral immunity and thereby the efficacy and safety of viral vaccines. To evaluate the Th1 and Th2 immune response by the variant vaccines, we performed intracellular staining of Th1 and Th2 cytokines in splenocytes. After stimulation with peptide pools covering the entire S protein, the splenocytes from three mRNA vaccine groups produced more hallmark Th1 cytokine IFN-γ in both CD4+ and CD8+ T cells than those from PBS group. Our flow cytometry data suggested that the two variant vaccine candidates induced strong Th1-biased immune responses, just like the WT vaccine, of which Th1 response had been observed by previous studies in animal models.

Single cell sequencing is a powerful technology for immune and gene expression profiling, which has been utilized for mapping immune responses to COVID-19 infection. In order to gain insights on the transcriptional landscape of the immune cells, and clonal repertoire changes specifically in B and T cells, we performed single cell transcriptomics, as well as BCR and TCR repertoire sequencing. The single cell transcriptomics data revealed a systematic landscape of immune cell populations in B.1.351-LNP-mRNA and B.1.617-LNP-mRNA vaccinated animals. We mapped out the repertoires and associated global gene expression status of the immune populations including B cells, T cells, and innate immune cells. From the overall splenocyte population, we observed a distinct and significant increase in the CD8 T cell, activated B cell, and macrophage cell populations in vaccinated animals. Interestingly, differential expression between vaccinated and placebo-treated animals showed a strong signature of increased expression of transcriptional and translational machinery in both B and T cells. While the actual mechanism awaits future studies, these phenomena are potentially reflective of the active proliferation and immune responses in these lymphocytes.
BCR and TCR sequencing are efficient tools for mapping of clonal repertoire diversity, which has been rapidly utilized for sequencing COVID-19 patients\textsuperscript{46,47}. BCR-seq and TCR-seq unveiled the diversity and clonality and respective shifts in variant-specific LNP-mRNA inoculated animals as compared to placebo-treated. The decrease in VDJ clonal diversity, along with clonal expansion of a small number of clones, are observed in vaccinated animals as compared to placebo group. Vaccinated animals from both B.1.351-LNP-mRNA and B.1.617-LNP-mRNA groups have clonal TCR expansion, especially pronounced in peripheral blood samples. The induction of diverse and expanding clones is a signature of vaccine induced protective immunity\textsuperscript{38}. The goal of this experiment is to profile the global repertoire of BCR and TCR, rather than just the antigen-specific cells. Alternatively, antigen-specific sorting will zoom into the picture of those clones that are reactive to the spike antigen, but may miss other antigenic or bystander clones. The population of B or plasma cells contain antigen-specific clones in the vaccinated animals, as suggested by positive ELISA, neutralization and protection data. While outside the scope of this study, it is of future interest to further dissect the clonal expanded populations of B cells or plasma cell for antigen-specific responses, for example, whether they are monoclonal, oligoclonal, polyclonal and whether there are increased mutations for GC selection. In addition, the T cell clonal evolution is complex as it involves responses to both structural proteins (S, M, N, E) and non-structural proteins (NSPs). We performed the unsorted single cell and bulk BCR/TCR-seq analysis using the entire populations from the samples to charter a comprehensive landscape of the BCR/TCR repertoires in the WT- and variant-specific vaccinated animals.

In summary, our study provided direct assessment of in vivo immune responses to vaccination using LNP-mRNAs encoding specific SARS-CoV-2 variant spikes in pre-clinical animal models. The single cell and bulk VDJ repertoire mapping also provided unbiased datasets and robust systems immunology of SARS-CoV-2 vaccination by LNP-mRNA specifically encoding B.1.351 and B.1.617 spikes. Last but not least, all three vaccine candidates, including WT-LNP-mRNA, B.1.351-LNP-mRNA, and B.1.617-LNP-mRNA showed full protective potency for mice against the challenge of authentic SARS-CoV-2 viruses, not only the ancestral WA-01, but also two variants of concern, Beta and Delta. These original data may offer valuable insights for the development of the next-generation COVID-19 vaccines against the SARS-CoV-2 pathogen and especially its emerging variants of concern\textsuperscript{21}.

**Limitation of the Study**

We note a few limitations of our study. 1) The characterized VoC-specific vaccine candidates target the WT and the two variants dominant in 2020-2021, while new VoCs continue to emerge and evolve. Investigation of vaccine candidates targeting emerging variants are warranted. 2) Although commonly used, the animal
model in this study is mouse, which has certain species specific immune response different from human. Non-human primate and clinical studies are necessary to further advance the development of variant-specific vaccines. 3) In our experimental setting, mice were challenged shortly after boost. However, in real world setting, individuals might face viral exposure at various time points before, during, or after vaccination. The efficacy and safety of the variant-specific vaccine candidates need to be rigorously tested in future translational and clinical studies.
Acknowledgments

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Declaration of Interest:

A patent application has been filed by Yale University related to the data described here (inventors: SC, LP, ZF, JP, XZ, MD). Yale University has committed to rapidly executable nonexclusive royalty-free licenses to intellectual property rights for the purpose of making and distributing products to prevent, diagnose, and treat COVID-19 infection during the pandemic and for a short period thereafter. SC is a scientific Founder of EvolveImmune Tx and Cellinfinity Bio, unrelated to this study. The remaining authors declare no competing interests.

Author Contributions:

LP*: Developed vaccination systems, performed majority of experiments, manuscript prep
PAR*: Developed NGS pipelines, performed majority of NGS analyses, manuscript prep
AÖ*: Performed BL3 in vivo challenge experiment
ZF*: Prepared LNPs, performed ELISAs, participated in animal experiments, manuscript prep
JJP*: Developed NGS pipelines, performed initial NGS analyses, manuscript prep
XZ*: Developed vaccination immune assays, performed flow experiments
QL, MBD, QX: assisted experiments or assay setup
RF, PC: assisted experimental logistics
CL: provided TEM resources
CBW: Design and supervised BL3 work, manuscript prep, funding
SC: Conceptualization, overall design and supervision, manuscript prep, funding
STAR Methods

KEY RESOURCES TABLE

Key resources table is provided as a separate supplementary file.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Sidi Chen (sidi.chen@yale.edu).

Materials Availability Statement

All unique/stable reagents generated in this study are available from the lead contact. Certain materials such as vaccine candidates will be shared with a completed Materials Transfer Agreement.

Data and Code Availability

All data generated or analyzed during this study are included in this article, supplementary information, and source data files. Specifically, source data and statistics for non-high-throughput experiments are provided in a supplementary table excel file. Processed data and statistics for NGS experiments are provided in Data S1. The raw NGS data have been deposited at SRA and are publicly available. Additional Supplemental flow cytometry raw data are available from Mendeley Data at http://dx.doi.org/10.17632/2m6hvhhmr4.1. The original codes of data analysis are available from the lead contact upon reasonable request. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Institutional approval

This study has received institutional regulatory approval. All recombinant DNA (rDNA) and biosafety work were performed under the guidelines of Yale Environment, Health and Safety (EHS) Committee with approved protocols (Chen-15-45, 18-45, 20-18, 20-26). All animal work was performed under the guidelines of Yale University Institutional Animal Care and Use Committee (IACUC) with approved protocols (Chen-2018-20068; Chen-2020-20358; Wilen 2021-20198).
Animals

*M. musculus* (mice), 6-8 weeks old females of C57BL/6Ncr were purchased from Charles River. *M. musculus* (mice), 6-8 weeks old females of K18-hACE2 mice (B6.Cg-Tg(K18-ACE2)2Prlmn/J) were purchased from Jackson Laboratory and used for immunogenicity study. Animals were housed in individually ventilated cages in a dedicated vivarium with clean food, water, and bedding. Animals are housed with a maximum of 5 mice per cage, at regular ambient room temperature (65-75°F, or 18-23°C), 40-60% humidity, and a 14 h:10 h light cycle. All experiments utilize randomized littermate controls.

Cell Lines

HEK293T (ATCC) and 293T-hACE2 (gifted from Dr Bieniasz’ lab) cell lines were cultured in complete growth medium, Dulbecco’s modified Eagle’s medium (DMEM; Thermo fisher) supplemented with 10% Fetal bovine serum (FBS, Hyclone), 1% penicillin-streptomycin (Gibco) (D10 media for short). Cells were typically passaged every 1-2 days at a split ratio of 1:2 or 1:4 when the confluency reached at 80%.

**In vivo efficacy testing against challenges of authentic SARS-CoV-2 and variant viruses**

The protective efficacy of SARS-CoV-2 WT and variant mRNA-LNP against replication-competent SARS-CoV-2 virus and variant virus were evaluated in vivo. These experiments were performed in an animal BSL3 (ABSL3) facility. Replication competent SARS-CoV-2 (USA-WA1/2020) virus and the Beta variant (B.1.351) were produced in VeroE6-ACE2-TMPRSS2 cells. The Delta variant (B.1.617.2) was produced in WT Vero E6 cells. Titers for all three viruses were determined by plaque assay using WT Vero E6.

8-week old littermate controlled female K18-hACE2 mice (B6.Cg-Tg(K18-ACE2)2Prlmn/J) were purchased from the Jackson Laboratory. Mice were randomly distributed into groups, received 10 μg of WT-LNP mRNA, B.1.351-LNP-mRNA or B.1.617-LNP-mRNA via the intramuscular route on day 0 (Prime) and day 21 (Boost). One week after boost, the LNP-mRNA vaccinated, and control mice were subdivided into three groups randomly, then sedated with isoflurane. SARS-CoV-2 isolated USA-WA1/2020, Beta variant, or Delta variant was inoculated intranasally at a dose of 10^3 PFU/mouse (determined using WT Vero E6) in 50 ul of DPBS. Survival, body conditions, and weights of mice were monitored daily for 10 consecutive days.

**METHOD DETAILS**
Plasmid construction

The DNA sequences of B.1.351 and B.1.617 SARS-CoV-2 spikes for the mRNA transcription and pseudovirus assay were synthesized as gBlocks (IDT) and cloned by Gibson Assembly (NEB) into pcDNA3.1 plasmids. To improve expression and retain prefusion conformation, six prolines (HexaPro variant, 6P) were introduced to the SARS-CoV-2 spike sequence in the mRNA transcription plasmids. The plasmids for the pseudotyped virus assay including pHIVNLGagPol and pCCNanoLuc2AEGFP are gifts from Dr. Bieniasz’ lab. The C-terminal 19 amino acids were deleted in the SARS-CoV-2 spike sequence for the pseudovirus assay.

Cell culture

HEK293T (ATCC) and 293T-hACE2 (gifted from Dr Bieniasz’ lab) cell lines were cultured in complete growth medium, Dulbecco’s modified Eagle’s medium (DMEM; Thermo fisher) supplemented with 10% Fetal bovine serum (FBS, Hyclone), 1% penicillin-streptomycin (Gibco) (D10 media for short). Cells were typically passaged every 1-2 days at a split ratio of 1:2 or 1:4 when the confluency reached at 80%.

mRNA production by in vitro transcription and vaccine formulation

A sequence-optimized mRNA encoding B.1.351 variant (6P) or B.1.617 variant (6P) protein was synthesized in vitro using an HiscribeTM T7 ARCA mRNA Kit (with tailing) (NEB), with 50% replacement of uridine by N1-methyl-pseudouridine. A linearized DNA template containing the B.1.351 variant (6P) or B.1.617 variant (6P) open reading frame flanked by 5’ untranslated region (UTR) and 3’ UTR sequences and was terminated by an encoded polyA tail was used as template. The above DNA templates were obtained from circulated plasmids pVP22b (B.1351 variant (6P)) and pVP29b (B.1.617 variant (6P)). pVP22b and pVP29b plasmids were linearized with BbsI restriction enzyme digestion and cleaned up with gel purification.

The mRNA was synthesized and purified by following the manufacturer’s instructions and kept frozen at -80 °C until further use. The mRNA was encapsulated in a lipid nanoparticle (Genvoy-ILM™, Precision Nanosystem) using the NanoAssembler® Ignite™ machine (Precision Nanosystems). All procedures are following the guidance of manufacturers. In brief, Genvoy-ILM™, containing 50% PNI ionizable lipid, 10% DSPC, 37.5% cholesterol, 2.5% PNI stabilizer, were mixed with mRNA in acetate buffer, pH 5.0, at a ratio of 6:1 (Genvoy-ILM™: mRNA). The mixture was neutralized with Tris-Cl pH 7.5, sucrose was added as a cryoprotectant. The final solution was sterile filtered and stored frozen at -80 °C until further use. The particle size of mRNA-LNP was determined by DLS machine (DynaPro NanoStar, Wyatt, WDPN-06). The encapsulation and mRNA concentration were measured by using Quant-iT™ RiboGreen™ RNA Assay Kit (Thermofisher).
Negative-stain TEM

5 μl of the sample was deposited on a glow-discharged formvar/carbon-coated copper grid (Electron Microscopy Sciences, catalog number FCF400-Cu-50), incubated for 1 min and blotted away. The grid was washed briefly with 2% (w/v) uranyl formate (Electron Microscopy Sciences, catalog number 22450) and stained for 1 min with the same uranyl formate buffer. Images were acquired using a JEOL JEM-1400 Plus microscope with an acceleration voltage of 80 kV and a bottom-mount 4k × 3k charge-coupled device camera (Advanced Microscopy Technologies, AMT).

In vitro mRNA expression

HEK293T cells were electroporated with mRNA encoding B.1.351 variant (6P) or B.1.617 variant (6P) proteins using Neon™ Transfection System 10 μL Kit following the standard protocol provided by manufacturer. After 12 h, the cells were collected and resuspended in MACS buffer (D-PBS with 2 mM EDTA and 0.5% BSA). To detect surface-protein expression, the cells were stained with 10 μg/mL ACE2–Fc chimera (Genescript, Z03484) in MACS buffer for 30 min on ice. Thereafter, cells were washed twice in MACS buffer and incubated with PE–anti-human FC antibody (Biolegend, M1310G05) in MACS buffer for 30 min on ice. Live/Dead aqua fixable stain (Invitrogen) were used to assess viability. Data acquisition was performed on BD FACSARia II Cell Sorter (BD). Analysis was performed using FlowJo software.

Mice immunization and sample collection

A standard two-dose schedule given 21 days apart was adopted. 1 μg or 10 μg LNP-mRNA were diluted in 1X PBS and inoculated into mice intramuscularly for prime and boost. Control mice received PBS. Two weeks post-prime (day14) and two weeks post-boost (day 35), sera were collected from experimental mice and utilized for following ELISA and neutralization assay of pseudovirus. Forty days (day 40) after prime, mice were euthanized for endpoint data collection. Splenocytes were collected for T cell stimulation and cytokine analysis, and single cell profiling. Lymphocytes were separately collected from mouse blood, spleen and draining lymph nodes and applied for Bulk BCR and TCR profiling.

Cell isolation from animals

For every mouse treated with either LNP-mRNA or PBS. Blood, spleens and draining lymph nodes were separately collected. Spleen and lymph node were homogenized gently and filtered with a 100 μm cell strainer (BD Falcon, Heidelberg, Germany). The cell suspension was centrifuged for 5 min with 400 g at 4 °C. Erythrocytes were lysed briefly using ACK lysis buffer (Lonza) with 1mL per spleen for 1~2 mins before
adding 10 mL PBS containing 2% FBS to restore iso-osmolarity. The single-cell suspensions were filtered through a 40 μm cell strainer (BD Falcon, Heidelberg, Germany).

**Flow Cytometry**

Spleens from three mice in LNP mRNA vaccine groups and four mice in PBS group were collected five days post boost. Mononuclear single-cell suspensions from whole mouse spleens were generated using the above method. 0.5 million splenocytes were resuspended with 200μl into RPMI1640 supplemented with 10% FBS, 1% penicillin–streptomycin antibiotic, Glutamax and 2mM 2-mercaptoethanol, anti-mouse CD28 antibody (Biolegend, Clone 37.51) and seed into 96- well plate for overnight. The splenocytes were incubated for 6 hr at 37°C in vitro with BrefeldinA (Biolegend) under three conditions: no peptide, PMA/Ionomycin, and PepTivator® SARS-CoV-2 Prot_S Complete peptide pool (Miltenyi Biotec, 15 mers with 11 amino acid overlap) covering the entire SARS-CoV-2 S protein. Peptide pools were used at a final concentration of 200 ng/ml. Following stimulation, cells were washed with PBS before surface staining with LIVE/DEAD Fixable Dead Cell Stain (Invitrogen, 1:1000) and a surface stain cocktail containing the following antibodies: CD3 PE/Cy7 (Biolegend, Clone 17A2, 1:200), CD8a BV421 (Biolegend, Clone QA17A07, 1:200), CD4 FITC (Biolegend, Clone GK1.5, 1:200) in MACS buffer (D-PBS with 2 mM EDTA and 0.5% BSA) on ice for 20 min, cells were washed with MACS buffer then fixed and permeabilized using the BD Cytofix/Cytoperm fixation/permeabilization solution kit according to the manufacturer’s instructions. Cells were washed in perm/wash solution for 5 min, and stained by intracellular staining for 30 min at 4 °C using a cocktail of the following antibodies: IFN-γ PE (Biolegend, Clone W18272D, 1:500), TNF Percp-Cy5.5 (Biolegend, Clone MP6-XT22, 1:500), IL2 BV510 (Biolegend, Clone JES6-5H4, 1:500), IL4 BV605 (Biolegend, Clone 11B11, 1:500), IL5 APC (Biolegend, Clone TRFK5, 1:500) in MACS buffer. Finally, cells were washed in MACS for twice and resuspended in MACS buffer before running on BD FACS Aria II Cell Sorter (BD). Analysis was performed using FlowJo software according to the gating strategy outlined in a Supplemental Figure. Polyfunctional T cells were analyzed by examination of cellular populations expressing multiple markers.

**ELISA**

The 384-well ELISA plates were coated with 3 μg/ml of antigens overnight at 4 degree. The antigen panel used in the ELISA assay includes SARS-CoV-2 spike S1+S2 ECD and RBD of 2019-nCoV (SINO, ECD 40589-V08B1 and RBD 40592-V08B), Indian variant B.1.617 (SINO, ECD 40589-V08B12 and RBD 40592-V08H88), South African variant (SINO, ECD 40589-V08B07 and RBD 40592-V08H85) and spike RBD of wild-type, South African variant and Indian variant. Plates were washed with PBS plus 0.5% Tween 20 (PBST)
three times using the 50TS microplate washer (Fisher Scientific, NC0611021) and blocked with 0.5% BSA in PBST at room temperature for one hour. Plasma was serially diluted twofold or fourfold starting at a 1:2000 dilution. Samples were added to the coated plates and incubated at room temperature for one hour, followed by washes with PBST five times. Anti-mouse secondary antibody was diluted to 1:2500 in blocking buffer and incubated at room temperature for one hour. Plates were washed five times and developed with tetramethylbenzidine substrate (Biolegend, 421101). The reaction was stopped with 1 M phosphoric acid, and OD at 450 nm was determined by multimode microplate reader (PerkinElmer EnVision 2105). The binding response (OD450) were plotted against the dilution factor in log10 scale to display the dilution-dependent response. The area under curve of the dilution-dependent response (Log10 AUC) was calculated to evaluate the potency of the serum antibody binding to spike antigens.

SARS-CoV-2 pseudovirus reporter and neutralization assays

HIV-1 based SARS-CoV-2 WT, B.1.351 variant, and B.1.617 variant pseudotyped virions were generated using respective spike sequences and applied in neutralization assays. Plasmid expressing a C-terminally truncated SARS-CoV-2 S protein (pSARS-CoV-2Δ19) was from Dr Bieniasz’ lab. Plasmids expressing a C-terminally truncated SARS-CoV-2 B.1.351 variant S protein (B.1.351 variant-Δ19) and SARS-CoV-2 B.1.617 variant S protein (B.1.617 variant-Δ19) were generated as above. The three plasmids-based HIV-1 pseudotyped virus system were utilized to generate (HIV-1/NanoLuc2AEGFP)-SARS-CoV-2 particles, (HIV-1/NanoLuc2AEGFP)-B.1.351 variant particles, and B.1.617 variant particles. The reporter vector, pCCNanoLuc2AEGFP, and HIV-1 structural/regulatory proteins (pHIVNLGagPol) expression plasmid were gifts from Dr Bieniasz’s lab. Briefly, 293T cells were seeded in 150 mm plates, and transfected with 21 µg pHIVNLGagPol, 21 µg pCCNanoLuc2AEGFP, and 7.5 µg of a SARS-CoV-2 SAΔ19 or B.1.351 variant-Δ19 or SARS-CoV-2 SA SAΔ19 plasmid, utilizing 198 µl PEI. At 48 h after transfection, the 20-ml supernatant was harvested and filtered through a 0.45-µm filter, and concentrated before aliquoted and frozen in -80°C.

The pseudovirus neutralization assays were performed on 293T-hACE2 cell. One day before, 293T-hACE2 cells were plated in a 96 well plate, 0.01 x10⁶ cells per well. The following day, serial dilution serum plasma, collected from PBS or LNP-mRNA vaccine immunized mice and started from 1:100 (5-fold serial dilution using complete growth medium), 55 µL aliquots were mixed with the same volume of SARS-CoV-2 WT, B.1.351 variant, and B.1.617 variant pseudovirus. The mixture was incubated for 1 hr at 37 °C incubator, supplied with 5% CO₂. Then 100 µL of the mixtures were added into 96-well plates with 293T-hACE2 cells. Plates were incubated at 37°C supplied with 5% CO₂. 48 hr later, 293T-hACE2 cells were collected and the GFP+ cells were analyzed with Attune NxT Acoustic Focusing Cytometer (Thermo Fisher). The 50%
inhibitory concentration (IC50) was calculated with a four-parameter logistic regression using GraphPad Prism (GraphPad Software Inc.).

**Bulk BCR and TCR sequencing**

Lymphocytes from blood, draining lymph node, spleen of each mRNA-LNP vaccinated and control mice were collected as described above for mouse immunization and sample collection. mRNA of lymphocytes from three tissues were extracted using a commercial RNeasy® Plus Mini Kit (Qiagen). Following bulk BCR and TCR are prepared using SMARTer Mouse BCR IgG H/K/L Profiling Kit and SMARTer Mouse TCR a/b profiling kit separately (Takara). Based on the extracted mRNA amount of each sample, the input RNA amounts for bulk BCR libraries were as follows: lymphocytes from blood (100 ng), lymphocytes from lymph node (1000 ng), and lymphocytes from spleen (1000 ng). The input RNA amounts for bulk TCR libraries were as follows: lymphocytes from blood (100 ng), lymphocytes from lymph node (500 ng), and lymphocytes from spleen (500 ng). All procedures followed the standard protocol of the manufacture. The pooled library was sequenced using MiSeq (Illumina) with 2*300 read length.

**Single cell profiling**

Splenocytes were collected from mRNA-LNP vaccinated and control mice were collected as described above for mouse immunization and sample collection, and normalized to 1000 cells/µL. Standard volumes of cell suspension were loaded to achieve targeted cell recovery to 10000 cells. The samples were subjected to 14 cycles of cDNA amplification. Following this, gene expression (GEX), TCR-enriched and BCR-enriched libraries were prepared according to the manufacturer’s protocol (10x Genomics). All libraries were sequenced using a NovaSeq 6000 (Illumina) with 2*150 read length.

**Single cell transcriptomics data analysis for immune repertoire profiling**

Single cell gene expression data were pre-processed with a standard Cell Ranger v6.0.1 (10x Genomics) pipeline, aligning reads to the mm10 mouse reference transcriptome. Data set integration and cell population analyses were then performed using the Seurat v4.0.5 package for the R statistical programming language. Specifically, each data set was filtered (cells with 200-2500 RNA features and < 5% mitochondrial RNA), log-normalized, scaled then integrated using the method by Stuart et al., using PBS vaccination samples as the reference group (reciprocal-PCA method, 2000 anchors, k = 20). The integrated data was rescaled, centered, then visualized in low-dimensional space by uniform manifold approximation and projection (UMAP), using the first 15 dimensions from a principal components analysis (PCA), chosen based on the elbow plot method.
Cells were clustered by generating a shared nearest neighbors (SNN) graph (k = 20, first 15 PCA dimensions) and optimizing modularity using the Louvain algorithm with multilevel refinement algorithm and an empirically chosen resolution, based on the best spatial separation of major immune populations cells via \( Cd3d, Cd19, Ncr1, Itgam, Itgax, \) and \( Sdc1 \) expression on UMAP visualization. The clusters were then labeled using the expression patterns of immune cell markers (Dataset S1), based on (a) the proportion of cells within each cluster that express the markers (>10% of cells with scaled expression > 1) and (b) the cluster-averaged scaled expression > 0 (Figures 5B, S3-6). For better resolution of complex cell types, B cells, T cells and dendritic cells (DCs) (\( Cd45+Cd19+, Cd45+Cd3d+, \) and \( Cd45+Itgax+ \) clusters, respectively) were separately subset, rescaled, visualized in low dimensional UMAP space, clustered, and populations were identified using the method above. Labeled cell types were tested for homogeneity by performing Wilcoxon rank sum testing of scaled data and assessing discreet hierarchical clustering of populations using the top 10 DEGs in each cell type compared to all others.

Differential expression analyses compared the effect of different variant vaccines on activated B cells (Activated and germinal center B cells), activated CD4 T cells (Th1, Th2, Treg, Th17, Tfh, and exhausted CD4 T cells), and activated CD8 T cells (Tc1, Tcm, and exhausted CD8 T cells) using a modified edgeR analysis pipeline. Briefly, low-expression genes (< 5% detection across cells) were excluded, TMM-wsp size factors were calculated, data were fit to a gamma-Poisson generalized linear model (~ scaled cell detection rate + WA-1 + B.1.351 + B.1.617 + vaccine concentration + cell type) (Dataset S1), and the fitted data were assessed by quasi-likelihood F tests \(^{53,54} \). Model fitting and DE was performed using the glmGamPoi package for R \(^{55} \), and the following vaccination statuses were used as the coefficient equal to zero under the null hypothesis: (1) WA-1, (2) B.1.351, (3) B.1.617, (4) B.1.351 - WA-1, or (5) B.1.617 - WA-1.

Downstream analyses were performed using differentially expressed genes (DEGs) with an FDR-adjusted p value < 0.01 and a log fold-change (log-FC) > 0.5 or < -0.5 for upregulated and downregulated genes, respectively. First, DEGs were sorted by significance and analyzed by the gProfiler2 R package with biological process gene ontology (GO) terms for mus musculus, against known genes as the analysis domain \(^{56,57} \). Analysis results were filtered to include those with an adjusted p value (gProfiler gSCS method) < 0.01, GO terms <= 600 genes, and terms that included > 4 DEGs. If there were more than 3 filtered terms, results were clustered into “supra-pathways” by constructing an undirected network graph with (1) edges weighted by filtered pathway similarity coefficients (coefficient = Jaccard + Overlap of genes between GO terms; coefficients > 0.375), (2) a layout calculated via Fruchterman-Reingold algorithm, and (3) terms clustered by the Leiden algorithm (modularity function, 1000 iterations, resolution = 0.8), all of which using the iGraph,
network, and sna R packages. The clustered pathways were labeled by the most significant pathway from each cluster.

**VDJ sequencing data analysis**

Bulk VDJ sequencing data had adapters trimmed by Trimmomatic v0.39 in single-end mode, clipping Illumina TruSeq adapters with default settings and filtering reads with an average quality score < 30. Clonotypes were called using MiXCR v2.1.5 with the recommended settings for 5’ RACE (RNA alignment to V gene transcripts with P region). Single-cell sequencing data was processed using the Cellranger v5.0.1 (10x Genomics) pipeline and aligned to the mm10 VDJ reference. The MiXCR clonotype output or Cell Ranger AIRR-formated output (bulk and single cell VDJ analyses, respectively) were used as inputs to Immunarch v0.6.6 R package for calculating summary statistics, diversity metrics, and repertoire overlaps.

**Standard Statistical analysis**

The statistical methods are described in figure legends and/or supplementary Excel tables. The statistical significance was labeled as follows: n.s., not significant; * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001. Prism (GraphPad Software) and RStudio were used for these analyses. Additional information can be found in the Nature Research Reporting Summary.

**Replication, randomization, blinding and reagent validations**

Replicate experiments have been performed for all key data shown in this study. Biological or technical replicate samples were randomized where appropriate. In animal experiments, mice were randomized by littermates. Experiments were not blinded. NGS data processing were blinded using metadata. Subsequent analyses were not blinded. Commercial antibodies were validated by the vendors, and re-validated in house as appropriate. Custom antibodies were validated by specific antibody - antigen interaction assays, such as ELISA. Isotype controls were used for antibody validations. Cell lines were authenticated by original vendors, and re-validated in lab as appropriate. All cell lines tested negative for mycoplasma.
Supplemental Data items

Data S1. A zip file that contains the following excels:
1. Compiled single cell gene expression data (Figure 5-6)
2. Compiled single cell gene differential expression data (Figure 5-6)
3. Compiled single cell BCR and TCR sequencing data (Figure 7)
4. Compiled bulk BCR sequencing data (Figure 7)
5. Compiled bulk TCR sequencing data (Figure 7)

Related to Figures 5, 6, 7

Source data and statistics.
An excel file that contains source data and statistics of non-NGS experiments
“VarVac original data and statistics non-NGS 20220420.xlsx”
Related to Figures 1, 2, 3, 4


Figure Legends

**Figure 1 | Overview of the primary experimental design, and the B and T cell responses induced by WT-LNP-mRNA vaccination against SARS-CoV-2 WT, B.1.351 and B.1.617 spikes in mice.**

A, Schematic of the designs of three variant-specific LNP-mRNA vaccine candidates. Functional elements were shown in the spike mRNA and translated protein of SARS-CoV-2 WT, B.1.351 and B.1.617 spikes, including protein domains, HexaPro and variant-specific mutations.

B, 3D structure highlighting certain variant-specific mutations in B.1.351 and B.1.617 spikes. Distribution of mutations of B.1.351 and B.1.617 were shown in the structure of SARS-CoV-2 (PDB: 6VSB). Mutations of B.1.351 and B.1.617 were shown as spheres, except for those in the unstructured loop regions. Certain mutations were not visible in the structure as they fall into floppy regions of spike.

C, Graphical representation of B.1.351-LNP-mRNA complex and B.1.617-LNP-mRNA complex formation. The spike mRNAs of B.1.351 and B.1.617 were encapsulated by LNP via NanoAssemblr Ignite. The size and encapsulation rate of the mRNA-LNP complex were measured by dynamic light scatter (DLS) and Ribogreen assay, respectively.

D, After electroporated into 293FT cells, *In vitro* expression of B.1.351-spike or B.1.617-spike mRNA were detected by flowcytometry using the human ACE2-Fc fusion protein and PE-anti-Fc antibody.

E-F, DLS (e) and TEM (f) of size and monodispersity characterization of LNP-mRNAs.

G, Schematic of overall design of primary experiments. Six- to 8-week-old C57BL/6Ncr mice (B.1.351-LNP-mRNA (top) and B.1.617-LNP-mRNA, n = 6 mice per group; WT-LNP-mRNA, n = 4 mice; PBS, n = 9) received 1 or 10 μg of WT-LNP mRNA, B.1.351-LNP-mRNA or B.1.617-LNP-mRNA via the intramuscular route on day 0 (Prime) and day 21 (Boost). Blood was collected twice, two weeks post prime and boost. The binding and pseudovirus-neutralizing antibody responses induced by LNP-mRNA were evaluated by ELISA and neutralization assay. Mice were euthanized at day 40. The spleen, lymph node and blood samples were collected to analyze immune responses in by flow cytometry, bulk BCR and TCR profiling and single cell profiling.

H-I, Serum ELISA titers of WT-LNP mRNA vaccinated animals (n = 4). Serum antibody titer as area under curve (AUC) of log_{10}-transformed curve (10^{log_{10} AUC}) to spike RBDs (h) and ECDs (i) of SARS-CoV-2 WT, B.1.351 and B.1.617. Two-way ANOVA with Tukey's multiple comparisons test was used to assess statistical significance.

J, Serum neutralization titers of WT-LNP mRNA vaccinated animals (n = 4). Cross neutralization of SARS-CoV-2 WT, B.1.351 or B.1.617 pseudovirus infection of ACE2-overexpressed 293T cells. Two-way ANOVA with Tukey's multiple comparisons test was used to assess statistical significance.
K-L, T cell response of WT-LNP mRNA vaccinated animals (n = 4). CD8⁺ (k) and CD4⁺ (l) T cell responses were measured by intracellular cytokine staining 6 hours after addition of BFA. The unpaired parametric t test was used to evaluate the statistical significance.

Notes:
In this figure:
Each dot represents data from one mouse.
Data are shown as mean ± s.e.m. plus individual data points in dot plots.
Statistical significance labels: n.s., not significant; * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001
Source data and additional statistics for experiments are provided in a supplemental excel file.

See also: Figure(s) S1, S2

Figure 2 | B.1.351-LNP-mRNA and B.1.617-LNP-mRNA elicit robust binding and pseudovirus-neutralizing antibody response against all three variants in mice

A, Serum ELISA titers of animals vaccinated by B.1.351-LNP-mRNA (top) and B.1.617-LNP-mRNA (bottom), against RBD from three different spikes (WT, B.1.351, and B.1.617) of SARS-CoV-2 (n = 6).
B, Serum ELISA titers of animals vaccinated by B.1.351-LNP-mRNA (top) and B.1.617-LNP-mRNA (bottom), against ECD from three different spikes (WT, B.1.351, and B.1.617) of SARS-CoV-2 (n = 6).
C, Serum neutralization titers of animals vaccinated by B.1.351-LNP-mRNA (top) and B.1.617-LNP-mRNA (bottom), against three pseudoviruses (WT, B.1.351, and B.1.617) of SARS-CoV-2 (n = 6).
D-E, Direct comparison of serum ELISA (D) and neutralization (E) titers of animals boosted by WT, B.1.351-LNP-mRNA and B.1.617-LNP-mRNA, against WT, B.1.351, and B.1.617 spikes or pseudoviruses of SARS-CoV-2.
F, Heatmap of neutralization titers of animals vaccinated by all three LNP-mRNAs, against three pseudoviruses (WT, B.1.351, and B.1.617) of SARS-CoV-2.
G, Correlation X-Y scatterplots of ELISA ECD log10AUC vs neutralization log10IC50 for all vaccine groups

Notes:
In this figure:
Each dot represents data from one mouse.
Data are shown as mean ± s.e.m. plus individual data points in dot plots.
Statistical significance labels: n.s., not significant; * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001
Source data and additional statistics for experiments are provided in a supplemental excel file.

See also: Figure(s) S1
Figure 3 | B.1.351-LNP-mRNA and B.1.617-LNP-mRNA induced S protein-specific T cell response

A-C Percentage of CD8⁺ T cells expressing IFN-γ (a), TNFα (b), and IL-2 (c), in response to stimulation of S peptide pools (n=3). Left panels, representative flow plots; right panels, dot-bar plots for statistics of the left panels.

D, Percentage of CD4⁺ T cells expressing IFN-γ in response to stimulation of S peptide pools (n = 3). Left panels, representative flow plots; right panels, dot-bar plots for statistics of the left panels.

E-H, B.1.351-LNP-mRNA and B.1.617-LNP-mRNA induced S protein-specific polyfunctional CD8 and CD4 T cells

E-G, Percentage of CD8⁺ T cells expressing both IFN-γ and TNFα (E), both IFN-γ and IL-2(F), TNFα and IL-2 (G), in response to stimulation of S peptide pools (n = 3). Left panels, representative flow plots; right panels, dot-bar plots for statistics of the left panels.

H, Percentage of CD4⁺ T cells expressing both IFN-γ and TNFα in response to stimulation of S peptide pools (n = 3). Left panels, representative flow plots; right panels, dot-bar plots for statistics of the left panels.

Notes:

In this figure:

Each dot represents data from one mouse.

Data are shown as mean ± s.e.m. plus individual data points in dot plots.

Statistical significance labels: n.s., not significant; * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001

Source data and additional statistics for experiments are provided in a supplemental excel file.

See also: Figure(s) S2

Figure 4 | B.1.351-LNP-mRNA and B.1.617-LNP-mRNA showed in vivo protect efficacy against the challenge of replication competent authentic SARS-CoV-2 and variant viruses

A, Schematic of authentic virus challenge experiments on mRNA-LNP vaccinated mice. hACE2-K18 mice were separated randomly and received 10 μg of WT-LNP mRNA, B.1.351-LNP-mRNA or B.1.617-LNP-mRNA via the intramuscular route on day 0 (Prime) and day 21 (Boost). One week after boost (day28), the mRNA-LNP vaccinated, and control mice were distributed into three groups and challenged with WA-1, beta, and delta authentic live virus. Survival, body conditions, and weights of mice were monitored daily for 10 consecutive days.

B, A numeric summary of the number of hACE2-K18 mice that vaccinated with WT-LNP mRNA, B.1.351-LNP-mRNA or B.1.617-LNP-mRNA and challenged with three different authentic virus WA01, Beta (B.1.351) and Delta (B.1.617.2).
C. Body weight curves of WT-LNP mRNA, B.1.351-LNP-mRNA, B.1.617-LNP-mRNA vaccinated, and control hACE2 transgenic mice under lethal challenges with different authentic virus WA-01 (Left), Beta (Middle), and Delta (Right).

D. Survival curves of WT-LNP mRNA, B.1.351-LNP-mRNA or B.1.617-LNP-mRNA vaccinated, and control hACE2 transgenic mice under lethal challenges with different authentic virus WA-01 (Left), Beta (Middle), and Delta (Right).

Notes:
Each dot represents data from one mouse.
Data are shown as mean ± s.e.m. plus individual data points in dot plots.
Statistical significance labels: n.s., not significant; * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001
Source data and additional statistics for experiments are provided in a supplemental excel file.

Figure 5 | Single cell transcriptomics of variant-specific LNP-mRNA vaccinated animals
A. UMAP visualizations of all 141,729 cells pooled across samples and conditions. Cells are color-labeled by vaccine, concentration, and unsupervised clustering in each panel, top to bottom. Clusters are labeled by cell types that were assigned, based on the expression of cell type-specific markers.

B. UMAP heatmaps of the expression of major cell type-specific markers across all cells.

C. Heatmap of differentially expressed genes (DEGs) across indicated cell types. Differential expression analyses were performed using Wilcoxon rank sum test for each cell type vs all other cells, and the heatmap includes the 10 DEGs from each analysis (absolute log2-FC > 4, q < 0.01).

D. Boxplots of overall cell type proportions compared across vaccine groups (n = 6 for each). Comparisons were performed using a 2-way ANOVA test, accounting for vaccine and cell type as covariates, with Dunnet’s post-hoc analysis for multiple comparisons against PBS as the control. Data were analyzed together, but displayed separately for clarity.

E. Stacked bar chart of cell proportions between different vaccination groups (n = 6 for each).

F. UMAP visualization of T cell and B cell sub-populations across all samples and conditions. Sub-clusters are labeled by cell types, assigned by the expression of cell type-specific markers.

G. Boxplots of B and T subset proportions compared across vaccine groups (n = 6 for each). Comparisons were performed using a 2-way ANOVA test, accounting for vaccine and cell type as covariates, with Dunnet’s post-hoc analysis for multiple comparisons against PBS as the control. Data were analyzed together, but displayed separately for clarity.

Notes:
In this figure, panels D and G:

Each dot represents data from one mouse.

The high dose (n = 3 each) and low dose (n = 3 each) groups for each vaccine were merged (n = 6 total) in single cell data analysis, same thereafter.

Statistical significance labels: n.s., not significant; * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001

See also: Figures S3-6

**Figure 6 | Single cell analysis of activated B cell and CD8 T cell populations with gene expression signatures of variant-specific LNP-mRNA vaccinated animals**

A, Volcano plots of differential expression (DE) analyses for each vaccination group vs PBS in B cells. Analyses were performed using quasi-likelihood F tests of scRNA-seq data fitted with gamma-Poisson generalized linear models.

B, Network plots of clustered terms from pathway analyses of upregulated genes in the indicated in B cell DE analysis. Pathway enrichment analyses were performed by gProfiler2, and significantly enriched pathways were clustered with Leiden algorithm. Pathway clusters (supra-pathways) are labeled by their most significant member term along with its enrichment q value. The top five supra-pathways are shown for each plot.

C, Expression heatmaps of DE genes from selected upregulated supra-pathways in B cell DE analysis. Single-cell expression values were scaled then averaged across vaccination groups.

D, Volcano plots of differential expression (DE) analyses for each vaccination group vs PBS in CD8 T cells. Analyses were performed using quasi-likelihood F tests of scRNA-seq data fitted with gamma-Poisson generalized linear models.

E, Network plots of clustered terms from pathway analyses of upregulated genes in the indicated in CD8 T cell DE analysis. Pathway enrichment analyses were performed by gProfiler2, and significantly enriched pathways were clustered with Leiden algorithm. Pathway clusters (supra-pathways) are labeled by their most significant member term along with its enrichment q value. The top five supra-pathways are shown for each plot.

F, Expression heatmaps of DE genes from selected upregulated supra-pathways in CD8 T cell DE analysis. Single-cell expression values were scaled then averaged across vaccination groups.

See also: Figures S7-9

**Figure 7 | VDJ repertoire and clonal analyses of B cell and T cell populations from variant-specific LNP-mRNA vaccinated animals**
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A, Clonal composition bar plot depicting proportion of the BCR repertoire occupied by the clones of a given size for all samples in the single cell BCR-seq dataset.

B, Bar plot of Chao1 indices for each condition for repertoires in the single cell BCR-seq dataset (n = 6 for each group).

C, Clonal composition bar plot depicting proportion of the TCR repertoire occupied by the clones of a given size for all samples in the single cell TCR-seq dataset.

D, Bar plot of unique clonotypes for each for repertoires in the single cell TCR-seq.

E, Circos plots of V-J clonotype distribution for single cell BCR-seq dataset (left panel) and single cell TCR-seq dataset (right panel). The 20 most abundant V-J combinations are shown for pooled vaccination group.

F, Clonal composition bar plot depicting proportion of the BCR repertoire occupied by the clones of a given size for all samples in the bulk BCR-seq dataset (left panel) and bulk TCR-seq dataset (right panel).

G, Bar plots depicting relative abundances of IGH, IGK, IGL, TRA, TRB, and TRD clonotypes within specific frequency ranges in the bulk BCR/TCR-seq data from different tissues of different vaccination groups. Relative abundances are presented for individual and grouped samples in e and f, respectively.

H, Bar plots of the effective clone numbers (true diversity estimates) for selected BCR and TCR chain repertoires in the bulk TCR-seq dataset across vaccination and tissue groups.

Notes:
For the single-cell BCR/TCR-seq datasets, n = 6 samples for the PBS, and n=3 for WA-1 1µg, WA-1 10 µg, B.1.351 1 µg, B.1.351, B.1.617 1µg, and B.1.617 10 µg groups. For the bulk BCR/TCR-seq datasets, n = 4 PBS samples, and n=3 for B.1.351 1µg, B.1.351, B.1.617 1µg, and B.1.617 10 µg groups.
Statistics for F and G performed using two-way ANOVA tests with Dunnet’s multiple comparison test.
Statistical significance labels: n.s., not significant; * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001

See also: Figure S10-12
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**Figure 1**

**A**

Reciprocal IC50

**B**

Log AUC

**C**

%Intensity

**D**

Radius (nm)

**E**

Neutralization assay

**F**

ELISA antigen

**G**

Vaccination group

**H**

S peptide CD8 T cells

**I**

CD8+ T cells (%)

**J**

Neutralization

**K**

S peptide CD4 T cells
Figure 2

A

ELISA RBD

B

B.1.351 vaccine

ELISA ECD

C

Neutralization

LNP-mRNA Vaccination group

B.1.351-1 µg-Prime

B.1.351-10 µg-Prime

B.1.351-1 µg-boost

B.1.351-10 µg-Boost

D

ELISA WT

ELISA B.1.351 ECD

ELISA B.1.617 ECD

E

Neutralization log10 IC50

WT pseudovirus

B.1.351 pseudovirus

B.1.617 pseudovirus

F

Neutralization mean log10 IC50

y=0.7403*x + 2.067

Model p-value: 4.4e-14

Adj. R-squared: 0.8114

Pearson corr: 0.90376
Figure 3

A. CD8 T cells

CD8 T cells were analyzed by flow cytometry for IFN-γ and TNFα expression after stimulation with B.1.351-LNPmRNA and B.1.617-LNPmRNA vaccines. The data were normalized to the PBS control and presented as the percentage of CD8+ T cells expressing IFN-γ, TNFα, or both.

B. CD8 T cells

CD8 T cells were analyzed for TNFα expression after stimulation with B.1.351-LNPmRNA and B.1.617-LNPmRNA vaccines. The data were normalized to the PBS control and presented as the percentage of CD8+ T cells expressing TNFα.

C. CD8 T cells

CD8 T cells were analyzed for IL-2 expression after stimulation with B.1.351-LNPmRNA and B.1.617-LNPmRNA vaccines. The data were normalized to the PBS control and presented as the percentage of CD8+ T cells expressing IL-2.

D. CD4 T cells

CD4 T cells were analyzed for IFN-γ expression after stimulation with B.1.351-LNPmRNA and B.1.617-LNPmRNA vaccines. The data were normalized to the PBS control and presented as the percentage of CD4+ T cells expressing IFN-γ.

E. CD8 T cells

CD8 T cells were analyzed for IFN-γ and TNFα expression after stimulation with B.1.351-LNPmRNA and B.1.617-LNPmRNA vaccines. The data were normalized to the PBS control and presented as the percentage of CD8+ T cells expressing both cytokines.

F. CD8 T cells

CD8 T cells were analyzed for IFN-γ and IL-2 expression after stimulation with B.1.351-LNPmRNA and B.1.617-LNPmRNA vaccines. The data were normalized to the PBS control and presented as the percentage of CD8+ T cells expressing both cytokines.

G. CD8 T cells

CD8 T cells were analyzed for TNFα and IL-2 expression after stimulation with B.1.351-LNPmRNA and B.1.617-LNPmRNA vaccines. The data were normalized to the PBS control and presented as the percentage of CD8+ T cells expressing both cytokines.

H. CD4 T cells

CD4 T cells were analyzed for IFN-γ and TNFα expression after stimulation with B.1.351-LNPmRNA and B.1.617-LNPmRNA vaccines. The data were normalized to the PBS control and presented as the percentage of CD4+ T cells expressing both cytokines.
## Figure 4

### A

- **mRNA-LNP injection**
- **virus challenge**

**Prime**

**Boost**

**Survival Weight measurement**

Days D0 → D21 → D28 → D38

### B

|                  | WA-1 virus | Beta virus | Delta virus |
|------------------|------------|------------|-------------|
| PBS              | 5 mice     | 6 mice     | 5 mice      |
| WT-mRNA-LNP      | 5 mice     | 5 mice     | 6 mice      |
| B.1,351-mRNA-LNP | 6 mice     | 6 mice     | 5 mice      |
| B.1,617-mRNA-LNP | 5 mice     | 5 mice     | 5 mice      |

### C

#### WA-1

- LNP-mRNA Vaccinate group

- PBS
- WT
- B.1,351
- B.1,617

#### Beta

- LNP-mRNA Vaccinate group

- PBS
- WT
- B.1,351
- B.1,617

#### Delta

- LNP-mRNA Vaccinate group

- PBS
- WT
- B.1,351
- B.1,617

### D

#### WA-1

- LNP-mRNA Vaccinate group

- PBS
- WT
- B.1,351
- B.1,617

#### Beta

- LNP-mRNA Vaccinate group

- PBS
- WT
- B.1,351
- B.1,617

#### Delta

- LNP-mRNA Vaccinate group

- PBS
- WT
- B.1,351
- B.1,617
Figure 6

A. Activated B cells

WA-1 vs PBS

B.1.351 vs PBS

B.1.617 vs PBS

B. Upreg. pathways

Adj. p value (−log10)

C. Covalent chromatin modification

D. Activated CD8 T cells

WA-1 vs PBS

B.1.351 vs PBS

B.1.617 vs PBS

E. Upreg. pathways

F. Oxidative phosphorylation

Cell killing
Highlights

- WT LNP-mRNA shows reduced neutralization to the B.1.351 and B.1.617 variants
- B.1.617 LNP-mRNA elicits B.1.617-neutralizing activity stronger than WT LNP-mRNA
- WT, B.1.351 and B.1.617 LNP-mRNA protect against WA-1, Beta and Delta VoC challenges
- Single cell and BCR/TCR-seq reveal systems immune profiles of vaccinated animals

eTOC
Peng et. al. characterize the B cell and T cell responses elicited by WT, B.1.351 and B.1.617 mRNA vaccine candidates. All three LNP-mRNA protect against WA-1, Beta and Delta variants-of-concern challenges. Single cell, BCR and TCR sequencing reveals systems immune profiles of variant-specific LNP-mRNA vaccinated animals.
### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibody**        |        |            |
| Antibody            |        |            |
| Anti-mouse secondary antibody | Fisher Scientific | Cat#31439 |
| PE-anti-human FC antibody | Biolegend | CatM1310G05 |
| Anti-mouse CD28 antibody Clone 37.51 | Biolegend | Cat#102116 |
| CD3 PE/Cy7 Clone 17A2 | Biolegend | Cat#100320 |
| CD8a BV421 Clone QA17A07 | Biolegend | Cat#155010 |
| CD4 FITC Clone GK1.5 | Biolegend | Cat#100406 |
| IFN-γ PE Clone W18272D | Biolegend | Cat#163503 |
| TNF Percp-Cy5.5 Clone MP6-XT22 | Biolegend | Cat#506322 |
| IL2 BV510 Clone JES6-5H4 | Biolegend | Cat#503833 |
| IL4 BV605 Clone 11B11 | Biolegend | Cat#504126 |
| IL5 APC Clone TRFK5 | Biolegend | Cat#504306 |
| **Bacterial and Virus Strains** |        |            |
| SARS-CoV-2 WT pseudovirus | This study | This study |
| B.1.351 variant pseudovirus | This study | This study |
| B.1.617 variant pseudovirus | This study | This study |
| **Biological Samples** |        |            |
| **Chemicals, Peptides, and Recombinant Proteins** |        |            |
| DPBS                | Kline   | Cat#14190144 |
| RPMI 1640 Medium    | Gibco   | Cat#11875-093 |
| Fetal Bovine Serum | Sigma Aldrich | Cat#F4135-500ML |
| DMEM                | Kline   | Cat#11995065 |
| Penicillin-Streptomycin (10,000 U/mL) | Gibco | Cat#15140122 |
| Glutamax            | Med School | Cat#35050061 |
| 2-mercaptoethanol   | Sigma   | M6250       |
| Brefeldin A         | Biolegend | Cat#420601 |
| TWEEN-20            | Sigma-Aldrich | Cat# P1379 |
| 50TS microplate washer | Fisher Scientific | Cat#BT50TS16 |
| Neon™ Transfection System 10 μL Kit | ThermoFisher | Cat# MPK1025 |
| BD Cytofix/Cytoperm fixation/permeabilization solution kit | Fisher Scientific | Cat#BDB554714 |
| ACK Lysing Buffer   | Lonza   | Cat#BP10-548E |
| ACE2–Fc chimera     | Genescript | Cat#Z03484 |
| Gibson Assembly Master Mix - 50 rxn | NEB | Cat#E2611L |
| Hiscr ipt™ T7 ARCA mRNA Kit (with tailing) | NEB | Cat#E2060S |
| Phusion Flash High-Fidelity PCR Master Mix | ThermoFisher | Cat#F548L |
| E-Gel™ Low Range Quantitative DNA Ladder | ThermoFisher | Cat#12373031 |
| QIAquick Gel Extraction Kit | Qiagen | Cat#28706 |
| QIAamp Fast DNA Tissue Kit | Qiagen | Cat#51404 |
| EndoFree® Plasmid Maxi Kit | Qiagen | Cat#12362 |
| Quant-i™ RiboGreen™ RNA Assay Kit | ThermoFisher | Cat#R11490 |
| Tetramethylbenzidine substrate | Biolegend | Cat#421101 |
| Item                                                                 | Supplier          | Catalog Number |
|----------------------------------------------------------------------|-------------------|----------------|
| SMARTer Mouse BCR IgG H/K/L Profiling Kit                           | Takara            | Cat#634424     |
| SMARTer Mouse TCR a/b profiling kit                                 | Takara            | Cat#634404     |
| RNeasy® Plus Mini Kit                                               | Qiagen            | Cat#74134      |
| Glow-discharged formvar/carbon-coated copper grid                   | Electron Microscopy Sciences | FCF400-Cu-50   |
| 2% (w/v) uranyl formate                                             | Electron Microscopy Sciences | Cat#22450     |
| Library Construction Kit, 16 rxns                                  | 10X Genomics      | Cat#1000190    |
| Live/Dead Aqua fixable stain                                       | Thermofisher      | Cat#L34976     |
| GenVoy-ILM T Cell Kit for mRNA with Spark Cartridges                | Precision Nanosystems | Cat#1000683   |
| GenVoy-ILM                                                         | Precision Nanosystems | Cat#NWW0042   |
| BSA                                                                | Fisher Scientific | BP1600-100     |
| 100 μm cell strainer                                               | Corning           | Cat#352360     |
| 40 μm cell strainer                                                | Corning           | Cat#352340     |
| BbSI                                                               | Kline             | Cat#R3539L     |
| Bovine Serum Albumin                                                | Sigma Aldrich     | Cat#A9418-100G |
| EDTA                                                               | Kline             | Cat#AB00502-01000 |
| Macron™ 2796-05 Phosphoric Acid, 85%                                | Avantor           | Cat#MK-2796-05 |
| Polyethylenimine HCl MAX, Linear, Mw 40,000 (PEI MAX 40000)        | POLYSCIENCES INC  | Cat#24765-1    |
| Tris-Ci pH 7.5                                                     | Boston Bioproducts | Cat#IBB-594    |
| N1-Methylpseudouridine-5’-Triphosphate - (N-1081)                  | TriLink (NC)      | Cat#N-1081-1   |
| Sucrose                                                            | Thomas            | Cat#C987K85 (EA/1) |
| Tetramethylbenzidine                                               | Biolegend         | Cat#421101     |
| PepTivator SARS-CoV-2 Prot_S Complete, research grade               | Miltenyi Biotec   | Cat#130-127-951 |
| SARS-CoV-2 (2019-nCoV) Spike S1+S2 ECD-His Recombinant Protein     | SINO              | Cat#40589-V08B1 |
| SARS-CoV-2 (2019-nCoV) Spike RBD                                   | Quote UQ7100      | Cat#40592-V08B |
| SARS-CoV-2 Spike RBD (L452R,T478K)                                  | SINO              | Cat#40592-V08H90 |
| SARS-CoV-2 Spike S1+S2 (E154K, L452R, E484Q, D614G, P681R, E1072K, K1073R) Protein (ECD, His Tag) | SINO              | Cat#40589-V08B12 |
| SARS-CoV-2 (2019-nCoV) Spike RBD (L452R, E484Q) Protein (His Tag) | SINO              | Cat#40592-V08H88 |
| SARS-CoV-2 (2019-nCoV) Spike S1+S2 (L18F, D80A, D215G, LAL242-244 deletion, R246I, K417N, E484K, N501Y, D614G, A701V) Protein (ECD, His Tag) | SINO              | Cat#40589-V08B07 |
| SARS-CoV-2 (2019-nCoV) Spike RBD(N501Y)-His Recombinant Protein    | SINO              | Cat#40592-V08H82 |
| SARS-CoV-2 (2019-nCoV) Spike RBD(K417N, E484K, N501Y)-His Recombinant Protein | SINO              | Cat#40592-V08H85 |
| Chromium Next GEM Single Cell 5’ Kit v2, 16 rxns                  | 10X Genomics      | Cat#PN-1000263 |
| Chromium Next GEM Chip K Single Cell Kit, 16 rxns                  | 10X Genomics      | Cat#PN-1000287 |
| Dual Index Kit TT Set A, 96 rxns PN-1000215                          | 10X Genomics      | Cat#PN-1000215 |
| Mouse BCR Amplification Kit, 16 rxns PN-1000255                     | 10X Genomics      | Cat#PN-1000255 |
| SPRselect - 60 mL                                                   | Beckman Coulter   | Cat#B23318     |
| Chromium Single Cell Mouse TCR Amplification Kit, 16 rxns          | 10X Genomics      | Cat#PN-1000254 |
### Critical commercial assays

- Deposited data
  - Single cell RNA-seq data of Vaccinated animals: GEO/SRA
  - Single cell VDJ-seq data of Vaccinated animals: GEO/SRA
  - Bulk VDJ-seq data of Vaccinated animals: GEO/SRA
  - Flow cytometry data of Vaccinated animals: Mendeley Data, DOI: 10.17632/2m6hvhhmr4.2

### Experimental models: Cell lines
- HEK293FT: ThermoFisher, Catalog Number: R70007
- HKE293T-hACE2: Schmidt et al., 2020, Gift from Dr Bieniasz' lab
- Vero-E6: ATCC, Catalog Number: CRL-1586™

### Experimental Models: Organisms/Strains
- C57BL/6Ncr: Charles River, strain #556
- B6.Cg-Tg(K18-ACE2)2Prlmn/J: Jackson Laboratory, strain #034860

### Oligonucleotides
- gBlocks: IDT, Custom, sequence specific, various
- primers: IDT, Custom, sequence specific, various

### Recombinant DNA
- pcDNA3.1: Addgene, Cat# V790-20
- pHIVNLGagPol: Schmidt et al., 2020, Gift from Dr Bieniasz' lab
- pCCNanoLuc2AEGFP: Schmidt et al., 2020, Gift from Dr Bieniasz' lab
- pSARS-CoV-2 SΔ19: Schmidt et al., 2022, Gift from Dr Bieniasz' lab
- pVP22b (B.1.351 variant (6P)): This study
- pVP29b (B.1.617 variant (6P)): This study
- pVP31b (WT spike (6P)): This study
- pCCNanoLuc2AEGFP plasmid: Schmidt et al., Gift from Dr Bieniasz' lab
- Polyethylenimine: POLYSCIENCES INC, Cat#24765-1
- (HIV-1/NanoLuc2AEGFP)-SARS-CoV-2 plasmid: This study
- (HIV-1/NanoLuc2AEGFP)-B.1.351 variant plasmid: This study
- (HIV-1/NanoLuc2AEGFP)-B.1.617 variant plasmid: This study

### Software and Algorithms
- FlowJo software 9.9.6: FlowJo, LLC, https://www.flowjo.com
- GraphPad Prism 8.0: GraphPad Software Inc, https://www.graphpad.com/scientific-software/prism/
- Pymol: Schrödinger, http://www.pymol.org/
- Cell Ranger v3.1.0: 10X Genomics, https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/installation
| Package                  | Description                                                                 | Website                                                                 |
|-------------------------|------------------------------------------------------------------------------|-------------------------------------------------------------------------|
| Loupe V(D)J Browser     | 10X Genomics                                                                  | https://support.10xgenomics.com/single-cell-vdj/software/visualization/latest/installation |
| Trimmomomatic           | Bolger et al., Bioinformatics, 2014                                          | https://github.com/timflutre/trimmomatic                                |
| mixcr                   | Bolotin et al., Nat Methods, 2015                                            | https://github.com/milaboratory/mixcr                                   |
| R                       | R project                                                                     | https://www.r-project.org                                               |
| Seurat R package        | Satija et al., Nat Biotechnol 2015                                            | https://satijalab.org/seurat/index.html                                  |
| plyr R package          | Wickham. (2011). Journal of Statistical Software                             | http://www.jstatsoft.org/v40/i01/                                       |
| dplyr R package         | Wickham et al., (2021). dplyr: A Grammar of Data Manipulation. R package version 1.0.7 | https://CRAN.R-project.org/package=dplyr                                |
| patchwork R package     | Pedersen (2020). patchwork: The Composer of Plots. R package version 1.1.1   | https://CRAN.R-project.org/package=patchwork                             |
| ggrepel R package       | Slowikowski (2021). ggrepel: Automatically Position Non-Overlapping Text Labels with ‘ggplot2’. R package version 0.9.1 | https://CRAN.R-project.org/package=ggrepel                              |
| dplyr R package         | Wickham. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York | https://ggplot2.tidyverse.org                                             |
| limma R package         | Ritchie et al., (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research 43(7), e47 | https://git.bioconductor.org/packages/limma                               |
| edgeR R package         | Robinson et al., Bioinformatics 2010; McCarthy et al., Nucleic Acid Research 2012 | https://git.bioconductor.org/packages/edgeR                               |
| stringr R package       | Hadley Wickham (2019). stringr: Simple, Consistent Wrappers for Common String Operations. R package version 1.4.0 | https://CRAN.R-project.org/package=stringr                               |
| ggrides R package       | Claus O. Wilke (2021). ggrrides: Ridgeline Plots in ‘ggplot2’. R package version 0.5.3 | https://CRAN.R-project.org/package=ggrides                               |
| igraph R package        | Csardi, G., & Nepusz, T. (2006). The Igraph Software Package for Complex Network Research. InterJournal | https://igraph.org                                                        |
| Package                | Description                                                                 | Reference                                                                 | URL                                                                 |
|------------------------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------|----------------------------------------------------------------------|
| network R package      | Butts C. (2008). network: a Package for Managing Relational Data in R. Journal of Statistical Software, 24 (2) | https://CRAN.R-project.org/package=network                                 |
| sna R package          | Carter T. Butts (2020). sna: Tools for Social Network Analysis. R package version 2.6 | https://CRAN.R-project.org/package=sna                                     |
| Immunarch R package    | ImmunoMind Team, Zenodo, 2019                                               | https://github.com/immunomind/immunarch                                   |
| Circlize R package     | Gu et al., Bioinformatics, 2014                                             | https://cran.r-project.org/package=circlize                                |
| Pheatmap R package     | Kolde, 2019                                                                 | https://cran.r-project.org/package=pheatmap                                |
| Future R package       | Bengtsson, 2021                                                             | https://cran.r-project.org/package=future                                  |
| SeuratWrappers R package | Satija et al., 2020                                                      | https://github.com/satijalab/seurat-wrappers                              |
| glmGamPoi R package    | Ahlmann-Eltze and Huber, Bioinformatics, 2021                               | https://github.com/constae(glmGamPoi)                                      |
| Other                  |                                                                             |                                                                           |
| SARS-CoV-2 WT-LNP-mRNA vaccine candidate | This study                                                                 | This study                                                                |
| B.1.351-LNP-mRNA vaccine candidate | This study                                                                 | This study                                                                |
| B.1.617-LNP-mRNA vaccine candidate | This study                                                                 | This study                                                                |
Supplemental Information for

Variant-specific vaccination induces systems immune responses and potent in vivo protection against SARS-CoV-2
Figure S1 | Workflow of production and physical characterization of the spike mRNA and lipid nanoparticles used for the LNP-mRNA vaccine candidates; and additional data on antibody responses of all three groups of LNP-mRNA vaccinated animals

A, Heatmap of ELISA titers of animals vaccinated by all three LNP-mRNAs, against the RBDs from three different spikes of SARS-CoV-2.

B, Heatmap of ELISA titers of animals vaccinated by all three LNP-mRNAs, against the ECDs from three different spikes of SARS-CoV-2.

C, Correlation X-Y scatterplots of ELISA and neutralization titers between ELISA RBD log10AUC vs ELISA ECD log10AUC for all vaccine groups.

D, Correlation X-Y scatterplots of ELISA and neutralization titers between ELISA RBD log10AUC vs neutralization log10IC50 for all vaccine groups.

E-G, ELISA and neutralization titers of animals vaccinated by WT-LNP-mRNA, B.1.351-LNP-mRNA and B.1.617-LNP-mRNA (same data as in Figures 1-2, plot for visualizing comparisons between antigens.

E, Serum ELISA titers of vaccinated animals against RBD from three different spikes (WT, B.1.351, and B.1.617) of SARS-CoV-2 (n = 6).

F, Serum ELISA titers of vaccinated animals against ECD from three different spikes (WT, B.1.351, and B.1.617) of SARS-CoV-2 (n = 6).

G, Serum neutralization titers of vaccinated animals against three pseudoviruses (WT, B.1.351, and B.1.617) of SARS-CoV-2 (n = 6).

Related to: Figure(s) 1, 2
Supplemental Figure 2

A

B

C

D

E

F

G
Figure S 2 | Additional flow cytometry analysis of WT-LNP-mRNA, B.1.351-LNP-mRNA and B.1.617-LNP-mRNA induced S protein-specific T cell response

A, Flow cytometry panel and gating strategy to quantify SARS-CoV-2 S-specific T cells in B.1.351-mRNA-LNP and B.1.617-mRNA-LNP vaccinated group and PBS group.

B, Percentage of CD4 T cells expressing TNFα, IL-2, IL4, and IL5 in response to S peptide pools of WT-mRNA-LNP vaccines treated mice and PBS treated control mice.

C-D, Percentage of CD8 T cells (left) and CD4 cells (right) expressing IFN-γ, TNFα, IL-2, IL-4, and IL-5 of splenocytes from WT-LNP-mRNA vaccinated mice and PBS treated control mice without peptide stimulation (C), or in response to PMA/ionomycin stimulation (D).

E, Percentage of CD4 T cells expressing TNFα, IL-2, IL4, and IL5 in response to S peptide pools of B.1.351-mRNA-LNP and B.1.617-mRNA-LNP vaccinated group and PBS group.

F-G, Percentage of CD8 T cells (left) and CD4 cells (right) expressing IFN-γ, TNFα, IL-2, IL-4, and IL-5 of splenocytes from B.1.351-mRNA-LNP and B.1.617-mRNA-LNP vaccinated mice and PBS treated control mice without peptide stimulation (F), or in response to PMA/ionomycin stimulation (G).

Notes:

In this figure:

Each dot represents data from one mouse.

Statistical significance labels: n.s., not significant; * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001

Related to: Figure(s) 1, 3
Figure S3 | Additional analysis of single cell transcriptomics and immune repertoire profiling of variant-specific LNP-mRNA vaccinated animals

A, UMAP visualization of all 141,729 cells pooled across samples and conditions. Cells colored and labelled by cluster.

B, Heatmap of common immune marker gene expression across indicated clusters. Expression values are scaled across cells and averaged by cluster. NA values were given for < 1% detectable expression amongst a cluster population.

C, UMAP heatmaps and violin plots of the expression of cell type-specific markers used for assignment of cell identity.

Normalized, unscaled expression values used for each plot.

Related to: Figure 5
Supplemental Figure 4

A  B cell population clustering

B  B cell marker expression

C  Developmental markers

Cq83  Cd1d1  Cx2  Fceir2a  Cd24a

Cq83  Cd1d1  Cx2  Fceir2a  Cd24a

Cq83  Cd1d1  Cx2  Fceir2a  Cd24a

GC B cell markers

Ighd  Igha  Iggb2b  Bcl6  Cx27
Figure S 4 | Additional analysis of B cell subpopulations in scRNA-seq profiling of variant-specific LNP-mRNA vaccinated animals

A, UMAP visualization of CD19+ B cell populations pooled across samples and conditions. Cells colored and labelled by cluster.

B, Heatmap of B cell subset-specific gene expression across indicated clusters. Expression values were scaled across cells and averaged by cluster. NA values were given for < 1% detectable expression amongst a cluster population.

C, UMAP heatmaps and violin plots of the expression of cell type-specific markers used to assign cell identity. Normalized, unscaled expression values used for each plot.

Related to: Figure 5
Supplemental Figure 5

A. T cell population clustering

B. T cell marker expression

C. Common T cell markers

Th cell transcription factors

Cytokines and effector molecules
Figure S 5 | Additional analysis of T cell subpopulations in scRNA-seq profiling of variant-specific LNP-mRNA vaccinated animals

A, UMAP visualization of CD3d+ T cell populations pooled across samples and conditions. Cells colored and labelled by cluster.

B, Heatmap of T cell subset-specific gene expression across indicated clusters. Expression values are scaled across cells and averaged by cluster. NA values were given for < 1% detectable expression amongst a cluster population.

C, UMAP heatmaps and violin plots of the expression of cell type-specific markers used to assign cell identity. Normalized, unscaled expression values were used for each plot.

Related to: Figure 5
**Figure S 6 | Additional analysis of dendritic cell subpopulations in scRNA-seq profiling of variant-specific LNP-mRNA vaccinated animals**

**A**, UMAP visualization of Itgax (Cd11c) + dendritic cell (DC) populations pooled across samples and conditions. Cells colored and labelled by cluster.

**B**, Heatmap of DC cell subset-specific gene expression across indicated clusters. Expression values are scaled across cells and averaged by cluster. NA values were given for < 1% detectable expression amongst a cluster population.

**C**, UMAP heatmaps and violin plots of the expression of cell type-specific markers used to assign cell identity. Normalized, unscaled expression values used for each plot.

**Related to: Figure 5**
## Supplemental Figure 7

### A

- **WA-1 vs PBS**
  - CD6 T cells
  - Act. B cells
  - B.1.351 vs PBS
  - CD8 T cells
  - Act. B cells

### B

- **CD4 T cells**
  - WA-1 vs PBS
  - B.1.351 vs PBS
  - B.1.617 vs PBS

### D

- **Uptreg. pathways**
  - Ribosome dysfunction
  - Mitogen-activated protein kinase signaling pathway
  - Cell division

### E

- **Oxidative stress**
  - Glutathione peroxidase activity
  - Cell division
  - Response to reactive oxygen species

### F

- **Downreg. pathways**
  - Negative regulation of phosphatase activity
  - Positive regulation of gene expression
  - Regulation of leukocyte cell-cell adhesion
  - Entry into host

- **Supplemental Information**
  - Peng et al.
Figure S 7 | Additional analysis of differential gene expression in the B and T cell subpopulations of variant-specific LNP-mRNA vaccinated animals

A, D, F, Network plots of clustered terms from pathway analyses of upregulated genes in the indicated cell type and DE comparative analysis. Pathway enrichment analyses were performed by gProfiler2, and significantly enriched pathways were clustered with Leiden algorithm. Pathway clusters (supra-pathways) are labeled by their most significant member term along with its enrichment q value. The top five supra-pathways are shown for each plot.

B, E, G, Expression heatmaps of DE genes from a selected upregulated supra-pathway. Single-cell expression values were scaled then averaged across vaccination groups.

Related to: Figure 6
Supplemental Figure 8

A. B.1.351 vs WA-1
Act. B cells vs CD4 T cells vs CD8 T cells

B. Upreg. pathways

- Positive regulation of apoptotic process (Adj. p = 1.8e-04)
- Regulation of cell division (Adj. p = 3.3e-04)
- Cellular response to calcium ion (Adj. p = 1.0e-10)

C. Cellular response to calcium ion

D. Downreg. pathways

- Generation of precursor metabolites and energy (Adj. p = 8.9e-08)
- Generation of precursor metabolites and energy (Adj. p = 1.4e-06)

E. Generation of precursor metabolites and energy vs Negative regulation of immune response
Figure S 8 | Differential expression analysis in the B and T cell subpopulations between B.1.351-LNP-mRNA and WA-1-LNP-mRNA vaccinated animals

A, Volcano plots of differential expression (DE) analyses in B cells or CD8 T cells from B.1.351-LNP-mRNA vs WA-1-LNP-mRNA vaccinated animals. Analyses were performed using quasi-likelihood F tests of scRNA-seq data fitted with gamma-Poisson generalized linear models.

B, D, Network plots of clustered terms from pathway analyses of (B) upregulated and (D) downregulated genes in the indicated DE comparative analysis. Pathway enrichment analyses were performed by gProfiler2, and significantly enriched pathways were clustered with Leiden algorithm. Pathway clusters (supra-pathways) are labeled by their most significant member term along with its enrichment q value. The top five supra-pathways are shown for each plot.

C, E, Expression heatmaps of DE genes from a selected (C) upregulated and (E) downregulated supra-pathway. Single-cell expression values were scaled then averaged across vaccination groups.

Related to: Figure 6
Supplemental Figure 9

A. B.1.617 vs WA-1
1. Act. B cells
2. CD4 T cells
3. CD8 T cells

B. Ureg. pathways
- Positive regulation of apoptotic process (adj. p = 3.64e-06)
- Cellular response to calcium ion (adj. p = 3.03e-10)
- Negative regulation of protein phosphorylation (adj. p = 4.94e-04)

C. C. Cellular response to calcium
- Response to metal ion (adj. p = 4.59e-06)
- Response to reactive oxygen species (adj. p = 1.5e-06)

D. D. Downreg. pathways
- Antigen processing and presentation of peptide or glycopeptide epitopes via MHC class II (adj. p = 4.86e-15)
- Aerobic respiration (adj. p = 1.81e-05)
- Negative regulation of leukocyte activation (adj. p = 1.1e-06)

E. E. Cell growth
- Negative regulation of leukocyte activation (adj. p = 1.1e-06)
- Aerobic respiration (adj. p = 1.81e-05)
Figure S 9 | Differential expression analysis in the B and T cell subpopulations between B.1.617-LNP-mRNA and WA-1-LNP-mRNA vaccinated animals

A, Volcano plots of differential expression (DE) analyses in B cells or CD8 T cells from B.1.617-LNP-mRNA vs WA-1-LNP-mRNA vaccinated animals. Analyses were performed using quasi-likelihood F tests of scRNA-seq data fitted with gamma-Poisson generalized linear models.

B, D, Network plots of clustered terms from pathway analyses of (B) upregulated and (D) downregulated genes in the indicated DE comparative analysis. Pathway enrichment analyses were performed by gProfiler2, and significantly enriched pathways were clustered with Leiden algorithm. Pathway clusters (supra-pathways) are labeled by their most significant member term along with its enrichment q value. The top five supra-pathways are shown for each plot.

C, E, Expression heatmaps of DE genes from a selected (C) upregulated and (E) downregulated supra-pathway. Single-cell expression values were scaled then averaged across vaccination groups.

Related to: Figure 6
Supplemental Figure 10

A  Single cell BCR
Reertoire overlap

B  Single cell TCR
Reertoire overlap
Figure S 10 | Additional analysis of single cell VDJ BCR-seq and TCR-seq data between variant-specific LNP-mRNA vaccinated animals

A, Heatmaps of BCR repertoire overlap between single cell BCR-seq datasets, represented by Jaccard indices.

B, Heatmaps of TCR repertoire overlap between single cell TCR-seq datasets, represented by Jaccard indices.

Related to: Figure 7
Supplemental Figure 11

A

IgH
PBS
B.1.351
B.1.617

IgK
PBS
B.1.351
B.1.617

IgL
PBS
B.1.351
B.1.617

B

Unique clonotypes: IgH

Unique clonotypes: IgK

Unique clonotypes: IgL

C

IgH clones with specific frequencies

IgK clones with specific frequencies

IgL clones with specific frequencies
**Figure S 11 | Additional analysis of bulk VDJ BCR-seq data between variant-specific LNP-mRNA vaccinated animals**

**A**, Circos plots of V-J clonotype distribution for IGH, IGK, and IGL BCR-seq datasets. The 20 most abundant V-J combinations are shown for pooled vaccination group.

**B**, Bar plots of the unique clonotype numbers among blood, spleen, and lymph node samples from different variant-specific LNP-mRNA vaccinated animals.

**C**, Bar plots of the relative abundance of IGH, IGK, and IGL clonotypes from different tissues within specific clonotype frequency ranges.

**Notes:** PBS samples: n = 4; B.1.351 and B.1.617 samples: n = 6.

**Related to:** Figure 7
Supplemental Figure 12

A

TRB

B.1.351

B.1.617

TRB

Unique clonotypes: TRA

Unique clonotypes: TRB

C

TRA clones with specific frequencies

TRB clones with specific frequencies

Small (0 < X < 0.01)

Medium (0.01 < X < 0.05)

Large (0.05 < X < 0.1)

Hyperabundant (0.1 < X < 1)
Figure S 12 | Additional analysis of bulk VDJ TCR-seq data between variant-specific LNP-mRNA vaccinated animals

A, Circos plots of V-J clonotype distribution for TRA, TRB, and TRD TCR-seq datasets. The 20 most abundant V-J combinations are shown for pooled vaccination group.

B, Bar plots of the unique clonotype numbers among blood, spleen, and lymph node samples from different variant-specific LNP-mRNA vaccinated animals.

C, Bar plots of the relative abundance of TRA, TRB, and TRD clonotypes from different tissues within specific clonotype frequency ranges.

Notes: PBS samples: n = 4; B.1.351 and B.1.617 samples: n = 6.

Related to: Figure 7
### Other Supplemental Files

#### Supplemental Source Data and Statistics (non-NGS)

A supplemental excel file contains all original data and statistics for non-high-throughput experiments.

#### Data S1. Supplemental Dataset (NGS)

A zip file containing five excel files for all NGS processed data, including 5 separate datasets.

**Dataset 1 | Single cell GEX of PBS, WA-1-LNP-mRNA, B.1.351-LNP-mRNA, and B.1.617-LNP-mRNA treated animals**

| Tabs in this dataset: |
|-----------------------|
| S01. Metadata of merged single cell GEX dataset. Dataset contains basic statistics, clustering information, cell type assignment, and UMAP embeddings for each cell. |
| S02. Differentially expressed genes (DEGs) for each cell type compared to all others, using Wilcoxon ranked sum test. |
| S03. List of 118 immune gene markers for identification of specific cell types. |
| S04. Assigned cell types and specific markers used for identification. |
| S05. Cell type proportions for each sample. |
| S06. Statistics for comparison of cell type proportions between vaccination groups, using a two-way ANOVA test. |

**Dataset 2 | T cell and B cell specific analyses of single cell GEX of PBS, WA-1-LNP-mRNA, B.1.351-LNP-mRNA, and B.1.617-LNP-mRNA treated animals**

| Tabs in this dataset: |
|-----------------------|
| S07. Design matrices for DE analyses. |
| S08. DE results of WA-1 vaccination effect in different activated immune cell types, using a Quasi-likelihood F (QLF) test for WA-1 vaccination as the coefficient equal to zero in a generalized linear model (GLM). |
| S09. DE results of B.1.351 vaccination effect in different activated immune cell types, using a QLF test for B.1.351 as the GLM coefficient equal to zero. |
| S10. DE results of B.1.617 vaccination effect in different activated immune cell types, using a QLF test for B.1.617 as the GLM coefficient equal to zero. |
| S11. DE results of B.1.351 vs WA-1 vaccination in different activated immune cell types, using a QLF test for B.1.351 – WA-1 as the GLM contrast equal to zero. |
| S12. DE results of B.1.617 vs WA-1 vaccination in different activated immune cell types, using a QLF test for B.1.617 – WA-1 as the GLM contrast equal to zero. |
| S13. Pathway network analysis results for WA-1 DE analysis in B cells. |
| S14. Pathway network analysis results for WA-1 DE analysis in CD8 T cells. |
| S15. Pathway network analysis results for WA-1 DE analysis in CD4 T cells. |
| S16. Pathway network analysis results for B.1.351 DE analysis in B cells. |
| S17. Pathway network analysis results for B.1.351 DE analysis in CD8 T cells. |
| S18. Pathway network analysis results for B.1.351 DE analysis in CD4 T cells. |
| S19. Pathway network analysis results for B.1.617 DE analysis in B cells. |
| S20. Pathway network analysis results for B.1.617 DE analysis in CD8 T cells. |
| S21. Pathway network analysis results for B.1.617 DE analysis in CD4 T cells. |
| S22. Pathway network analysis results for B.1.351 – WA-1 DE analysis in B cells. |
| S23. Pathway network analysis results for B.1.351 – WA-1 DE analysis in CD8 T cells. |
| S24. Pathway network analysis results for B.1.351 – WA-1 DE analysis in CD4 T cells. |
| S25. Pathway network analysis results for B.1.617 – WA-1 DE analysis in B cells. |
| S26. Pathway network analysis results for B.1.617 – WA-1 DE analysis in CD8 T cells. |
| S27. Pathway network analysis results for B.1.617 – WA-1 DE analysis in CD4 T cells. |

**Dataset 3 | Single cell BCR-seq and TCR-seq of PBS, WA-1-LNP-mRNA, B.1.351-LNP-mRNA, and B.1.617-LNP-mRNA treated animals**

| Tabs in this dataset: |
|-----------------------|
| S28. BCR clonotype counts. |
| S29. BCR clonotype statistics, including relative clone abundance and top clone proportions across different ranges. |
| S30. Diversity statistics for BCR clonotypes, including data for Chao1, true diversity and Gini-Simpson methods. |
| S31. BCR clonotype counts. |
| S32. BCR clonotype statistics, including relative clone abundance and top clone proportions across different ranges. |
| S33. Diversity statistics for BCR clonotypes, including data for Chao1, true diversity and Gini-Simpson methods. |

**Dataset 4 | Bulk BCR-seq PBS, WA-1-LNP-mRNA, B.1.351-LNP-mRNA, and B.1.617-LNP-mRNA treated animals**
Tabs in this dataset:
1. S34. IGH clonotype counts.
2. S35. IGH clonotype statistics, including relative clone abundance and top clone proportions across different ranges.
3. S36. Diversity statistics for IGH clonotypes, including data for Chao1, true diversity and Gini-Simpson methods.
4. S37. IGK clonotype counts.
5. S38. IGK clonotype statistics, including relative clone abundance and top clone proportions across different ranges.
6. S39. Diversity statistics for IGK clonotypes, including data for Chao1, true diversity and Gini-Simpson methods. Details of the two-way ANOVA test for true diversity are included.
7. S40. IGL clonotype counts.
8. S41. IGL clonotype statistics, including relative clone abundance and top clone proportions across different ranges.
9. S42. Diversity statistics for IGL clonotypes, including data for Chao1, true diversity and Gini-Simpson methods.

Dataset 5 | Bulk TCR-seq PBS, WA-1-LNP-mRNA, B.1.351-LNP-mRNA, and B.1.617-LNP-mRNA treated animals

Tabs in this dataset:
1. S43. TRA clonotype counts.
2. S44. TRA clonotype statistics, including relative clone abundance and top clone proportions across different ranges.
3. S45. Diversity statistics for TRA clonotypes, including data for Chao1, true diversity and Gini-Simpson methods. Details of the two-way ANOVA test for true diversity are included.
4. S46. TRB clonotype counts.
5. S47. TRB clonotype statistics, including relative clone abundance and top clone proportions across different ranges.
6. S48. Diversity statistics for TRB clonotypes, including data for Chao1, true diversity and Gini-Simpson methods. Details of the two-way ANOVA test for true diversity are included.
7. S49. TRD clonotype counts.
8. S50. TRD clonotype statistics, including relative clone abundance and top clone proportions across different ranges.
9. S51. Diversity statistics for TRD clonotypes, including data for Chao1, true diversity and Gini-Simpson methods. Details of the two-way ANOVA test for true diversity are included.