

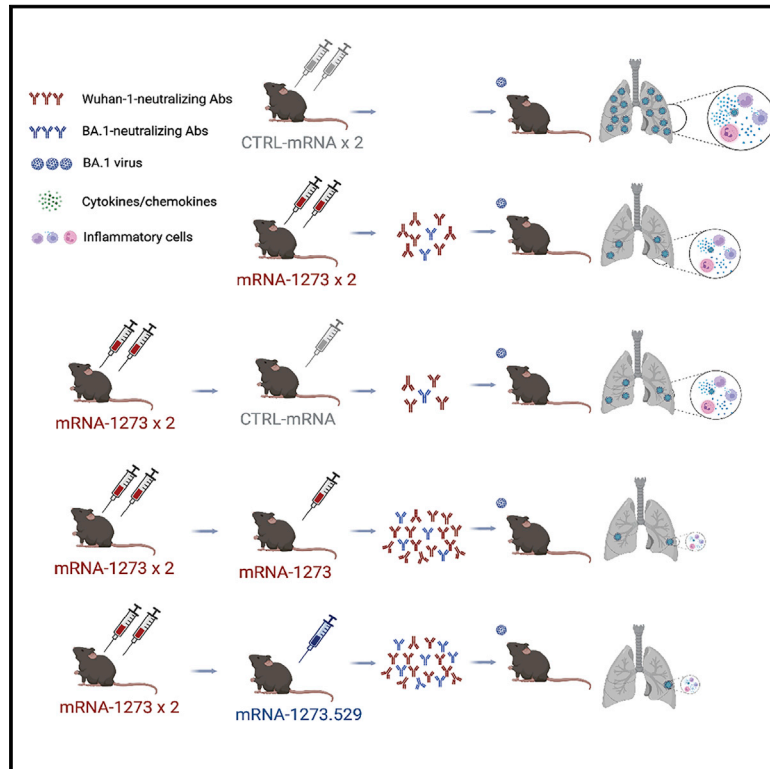


Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Boosting with variant-matched or historical mRNA vaccines protects against Omicron infection in mice

Graphical abstract



Authors

Baoling Ying, Suzanne M. Scheaffer, Bradley Whitener, ..., Darin K. Edwards, Larissa B. Thackray, Michael S. Diamond

Correspondence

lthackray@wustl.edu (L.B.T.),
diamond@wusm.wustl.edu (M.S.D.)

In brief

A comparison of the immunogenicity and efficacy of Omicron BA.1-matched and historical mRNA vaccines as boosters shows that although boosting with either vaccine increased neutralizing titers against Omicron BA.1 and BA.2, slightly greater protection against BA.1 challenge was observed in mice boosted with the BA.1-matched vaccine.

Highlights

- Primary immunization series with mRNA-1273 results in BA.1 breakthrough infection
- Primary immunization with BA.1-matched vaccine neutralizes BA.1 but not Wuhan-1
- Both mRNA-1273 and BA.1-matched boosters protect against BA.1 challenge
- A BA.1-matched booster induces greater neutralizing antibody responses against BA.1



Article

Boosting with variant-matched or historical mRNA vaccines protects against Omicron infection in mice

Baoling Ying,^{1,7} Suzanne M. Scheaffer,^{1,7} Bradley Whitener,^{1,7} Chieh-Yu Liang,^{1,2} Oleksandr Dmytrenko,¹ Samantha Mackin,^{1,2} Kai Wu,³ Diana Lee,³ Laura E. Avena,³ Zhenlu Chong,¹ James Brett Case,¹ LingZhi Ma,³ Thu T.M. Kim,³ Caralyn E. Sein,³ Angela Woods,³ Daniela Montes Berrueta,³ Gwo-Yu Chang,³ Guillaume Stewart-Jones,³ Isabella Renzi,³ Yen-Ting Lai,³ Agata Malinowski,³ Andrea Carfi,³ Sayda M. Elbashir,³ Darin K. Edwards,³ Larissa B. Thackray,^{1,*} and Michael S. Diamond^{1,2,4,5,6,8,*}

¹Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA

²Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO, USA

³Moderna, Inc., Cambridge, MA, USA

⁴Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, USA

⁵Andrew M. and Jane M. Bursky Center for Human Immunology and Immunotherapy Programs, Washington University School of Medicine, St. Louis, MO, USA

⁶Center for Vaccines and Immunity to Microbial Pathogens, Washington University School of Medicine, Saint Louis, MO, USA

⁷These authors contributed equally

⁸Lead contact

*Correspondence: lthackray@wustl.edu (L.B.T.), diamond@wusm.wustl.edu (M.S.D.)
<https://doi.org/10.1016/j.cell.2022.03.037>

SUMMARY

The large number of spike substitutions in Omicron lineage variants (BA.1, BA.1.1., and BA.2) could jeopardize the efficacy of SARS-CoV-2 vaccines. We evaluated in mice the protective efficacy of the Moderna mRNA-1273 vaccine against BA.1 before or after boosting. Whereas two doses of mRNA-1273 vaccine induced high levels of neutralizing antibodies against historical WA1/2020 strains, lower levels against BA.1 were associated with breakthrough infection and inflammation in the lungs. A primary vaccination series with mRNA-1273.529, an Omicron-matched vaccine, potently neutralized BA.1 but inhibited historical or other SARS-CoV-2 variants less effectively. However, boosting with either mRNA-1273 or mRNA-1273.529 vaccines increased neutralizing titers and protection against BA.1 and BA.2 infection. Nonetheless, the neutralizing antibody titers were higher, and lung viral burden and cytokines were slightly lower in mice boosted with mRNA-1273.529 and challenged with BA.1. Thus, boosting with mRNA-1273 or mRNA-1273.529 enhances protection against Omicron infection with limited differences in efficacy measured.

INTRODUCTION

Since the inception of the SARS-CoV-2 pandemic in late 2019, almost 460 million infections and 6.1 million deaths have been recorded (<https://covid19.who.int>). Several vaccines targeting the SARS-CoV-2 spike protein were developed and deployed rapidly with billions of doses administered (<https://covid19.who.int>). These vaccines use the SARS-CoV-2 spike protein from historical strains that circulated during the early phases of the pandemic in 2020 and have reduced the numbers of infections, hospitalizations, and COVID-19-related deaths. Despite the success of COVID-19 vaccines, the continued evolution of more transmissible SARS-CoV-2 variants with amino acid substitutions, deletions, and insertions in the spike protein jeopardizes the efficacy of global vaccination campaigns (Krause et al., 2021).

The SARS-CoV-2 spike protein engages angiotensin-converting enzyme 2 (ACE2) on the surface of human cells to facilitate entry and infection (Letko et al., 2020). The S1 fragment of the spike protein contains the N-terminal (NTD) and receptor binding (RBD) domains, which are targets of neutralizing monoclonal (Barnes et al., 2020; Cao et al., 2020; Pinto et al., 2020; Tortorici et al., 2020; Zost et al., 2020) and polyclonal antibodies (Rathe et al., 2021). In late November of 2021, the Omicron (BA.1) variant emerged, which has the largest number (>30) of amino acid substitutions, deletions, or insertions in the spike protein described to date. These changes in the spike raise concerns for escape from protection by existing vaccines that target early pandemic spike proteins. Indeed, reduced serum neutralization of BA.1 (B.1.1.529) and BA.1.1 (B.1.1.529 with an additional R346K spike substitution) Omicron viruses (Edara et al.,

2022; Pajon et al., 2022) and large numbers of symptomatic breakthrough infections have been reported in vaccinated individuals (Buchan et al., 2022; Christensen et al., 2022; Elliott et al., 2021).

Here, we evaluated the antibody responses and protective activity against Omicron variants of a preclinical version of the current Moderna vaccine, mRNA-1273, or an Omicron-targeted vaccine, mRNA-1273.529, designed with sequences from the historical Wuhan-1 or BA.1 spike genes, respectively, in the context of a primary (two-dose) immunization series or third-dose boosters. We hoped to define serum antibody correlates of protection against Omicron variants, determine the likelihood and significance of breakthrough infections, define the differences in immunogenicity and protection of homologous and heterologous mRNA vaccine boosters, and evaluate the activity of mRNA-1273.529 in the context of a primary immunization series against historical strains and variants of concern that emerged prior to Omicron. In particular, we assessed whether boosting with mRNA-1273 or a BA.1-matched vaccine was differentially effective in eliciting antibodies that neutralize Omicron or prior variants of concern.

After a primary immunization series in mice, mRNA-1273.529 induced antibody responses that efficiently neutralized viruses displaying BA.1 or BA.1.1 spike proteins but poorly inhibited infection of viruses expressing spike proteins of a historical strain or key variants (e.g., Beta (B.1.351) and Delta (B.1.617.2)). Although a primary immunization series with a high-dose mRNA-1273 formulation conferred protection against both historical and BA.1 viruses, a low-dose series, which induced levels of neutralizing antibodies against WA1/2020 strains that correspond to those measured in human serum (Wu et al., 2021), protected against WA1/2020 but did not control viral infection or inflammation in the lungs of BA.1-challenged mice. Although Omicron lineage viruses are less pathogenic in mice, they still replicate to high levels in the lungs and cause inflammation (Halfmann et al., 2022), allowing us to assess vaccine-mediated protection. A third dose of either mRNA-1273 or BA.1-matched mRNA-1273.529 vaccine increased levels of neutralizing antibodies and protection against Omicron variants, although higher levels of neutralizing antibodies and lower levels of BA.1 lung infection and inflammation were observed in mice administered mRNA-1273.529 boosters. Thus, the levels of vaccine-induced immunity that protect against historical or other variant SARS-CoV-2 strains (Ying et al., 2022) fail to prevent breakthrough infection by BA.1 virus, necessitating boosting with either matched or unmatched vaccines.

RESULTS

Vaccination with mRNA-1273 results in diminished neutralizing antibody responses against BA.1 in K18-hACE2 mice

We evaluated the antibody response against BA.1 after immunization with a preclinical version of mRNA-1273 that encodes for the prefusion-stabilized spike protein of SARS-CoV-2 Wuhan-1 strain (Corbett et al., 2020); this vaccine is identical to the clinical material in sequence, lipid composition, and activity but not produced under good manufacturing practice

conditions. We used K18-hACE2 transgenic mice, which are susceptible to severe infection after intranasal inoculation by many SARS-CoV-2 strains (Chen et al., 2021b; Winkler et al., 2020). Groups of 7-week-old female K18-hACE2 mice were immunized twice over 3 weeks by intramuscular route with 5 or 0.1 μg doses of mRNA-1273 or a control mRNA vaccine (Figure 1A). The high 5- μg dose was used as a comparison to prior immunogenicity studies in mice with other variants (Ying et al., 2022), although the levels of immunity are greater than that achieved in humans. The lower 0.1 μg vaccine dose arm was included for evaluating correlates of protection, as we expected breakthrough infections in this group; moreover, the antibody levels induced by lower doses are more similar to that generated by humans. Serum samples were collected 3 weeks after the second dose, and IgG responses against spike proteins (Wuhan-1 and BA.1) were evaluated by ELISA. We confirmed that equivalent amounts of antigenically intact Wuhan-1 and BA.1 spike (proline-stabilized, S2P) and RBD proteins were adsorbed based on detection with a panel of SARS-CoV-2 monoclonal antibodies that cross-react with SARS-CoV (VanBlargan et al., 2021) (Figure 1B). Antibody responses against both the Wuhan-1 and BA.1 spike proteins were robust after two immunizations with mRNA-1273. For the 5- μg dose, mean serum endpoint titers ranged from $\sim 4,000,000$ to 800,000 against the Wuhan-1 and BA.1 spike proteins and 1,000,000 and 40,000 for the Wuhan-1 and BA.1 RBD, respectively (Figures 1C and 1D). For the 0.1- μg dose, approximately 10-fold lower serum IgG responses against the spike and RBD proteins were measured (Figures 1E and 1F). The most noticeable difference in IgG responses after mRNA-1273 vaccination was the reduced titer against the BA.1 RBD compared with Wuhan-1 RBD protein (Figures 1D and 1F).

We characterized functional antibody responses by measuring the inhibitory effects of serum on SARS-CoV-2 infectivity using a focus-reduction neutralization test (FRNT) (Case et al., 2020b) and fully infectious SARS-CoV-2 WA1/2020 D614G and BA.1 strains (Figures 1G, 1H, and S1). We started dilutions at 1/60, which is just above the estimated level of neutralizing antibodies associated with protection in humans (Khoury et al., 2021). For the 5 μg dose, the mRNA-1273 vaccine induced robust serum neutralizing antibody responses against both WA1/2020 D614G and BA.1 (Figures 1G, 1H, and S1). However, the geometric mean titers (GMTs) of neutralization were ~ 8 -fold lower ($p < 0.001$) against BA.1, which agrees with data from studies with human antibodies (Cameroni et al., 2022; Cao et al., 2022; Cele et al., 2021; Dejnirattisai et al., 2022; VanBlargan et al., 2022; Wilhelm et al., 2021). For the 0.1 μg mRNA-1273 vaccine dose, we observed ~ 8 -fold less ($p < 0.01$) serum neutralizing activity against WA1/2020 D614G compared with the higher vaccine dose. Serum from mRNA-1273-vaccinated mice with the 0.1 μg dose also showed larger (~ 21 -fold, $p < 0.001$) reductions in neutralization of BA.1, with all values assigned to the 1/60 limit of detection (Figure 1H).

Two-dose immunization with mRNA-1273 confers limited protection against BA.1 in K18-hACE2 mice

We evaluated the protective activity of the mRNA-1273 vaccine against BA.1 challenge. Although BA.1 and BA.2 strains are less

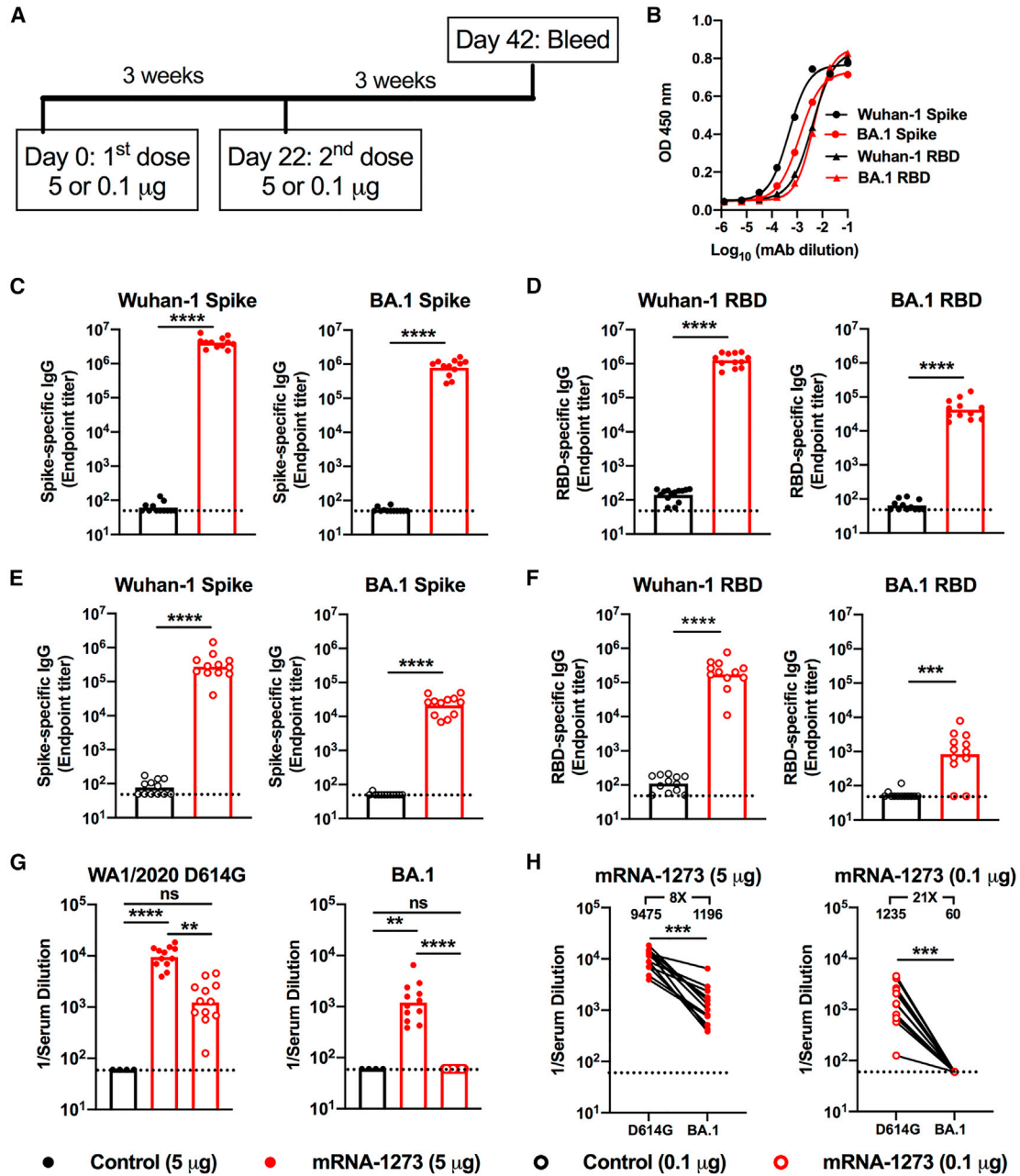


Figure 1. Antibody responses of mRNA vaccines in K18-hACE2 mice

7-week-old female K18-hACE2 mice were immunized with 5 or 0.1 μg of mRNA vaccines.

(A) Scheme of immunizations and blood draw.

(B) Binding of Sarbecovirus cross-reactive mAbs to Wuhan-1 and BA.1 spike and RBD proteins.

(C–F) Serum IgG responses at 3 weeks after the second 5 μg (C and D) or 0.1 μg (E and F) dose of mRNA vaccines (control or mRNA-1273) against indicated spike (C and E) or RBD (D and F) proteins ($n = 12$, two experiments, boxes illustrate geometric mean titers [GMT], dotted lines show limit of detection [LOD]).

(G) Serum neutralizing antibody responses 3 weeks after second vaccine dose against WA1/2020 D614G (left) and BA.1 (right) in mice immunized with 5 or 0.1 μg of control ($n = 4$) or mRNA-1273 ($n = 12$) vaccines (two experiments, boxes illustrate GMT, dotted lines show the LOD).

(H) Paired analysis of serum neutralizing titers against WA1/2020 D614G and BA.1 from individual mice (data from G) from samples obtained 3 weeks after the second 5 μg (left) or 0.1 μg (right) dose of mRNA-1273 ($n = 12$, two experiments, dotted lines show the LOD). GMT and fold-change values are indicated at the top of the graphs.

(C–F) Mann-Whitney test. (G) One-way ANOVA with Dunn’s post-test. (H) Wilcoxon signed-rank test (** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). See also Figure S1.

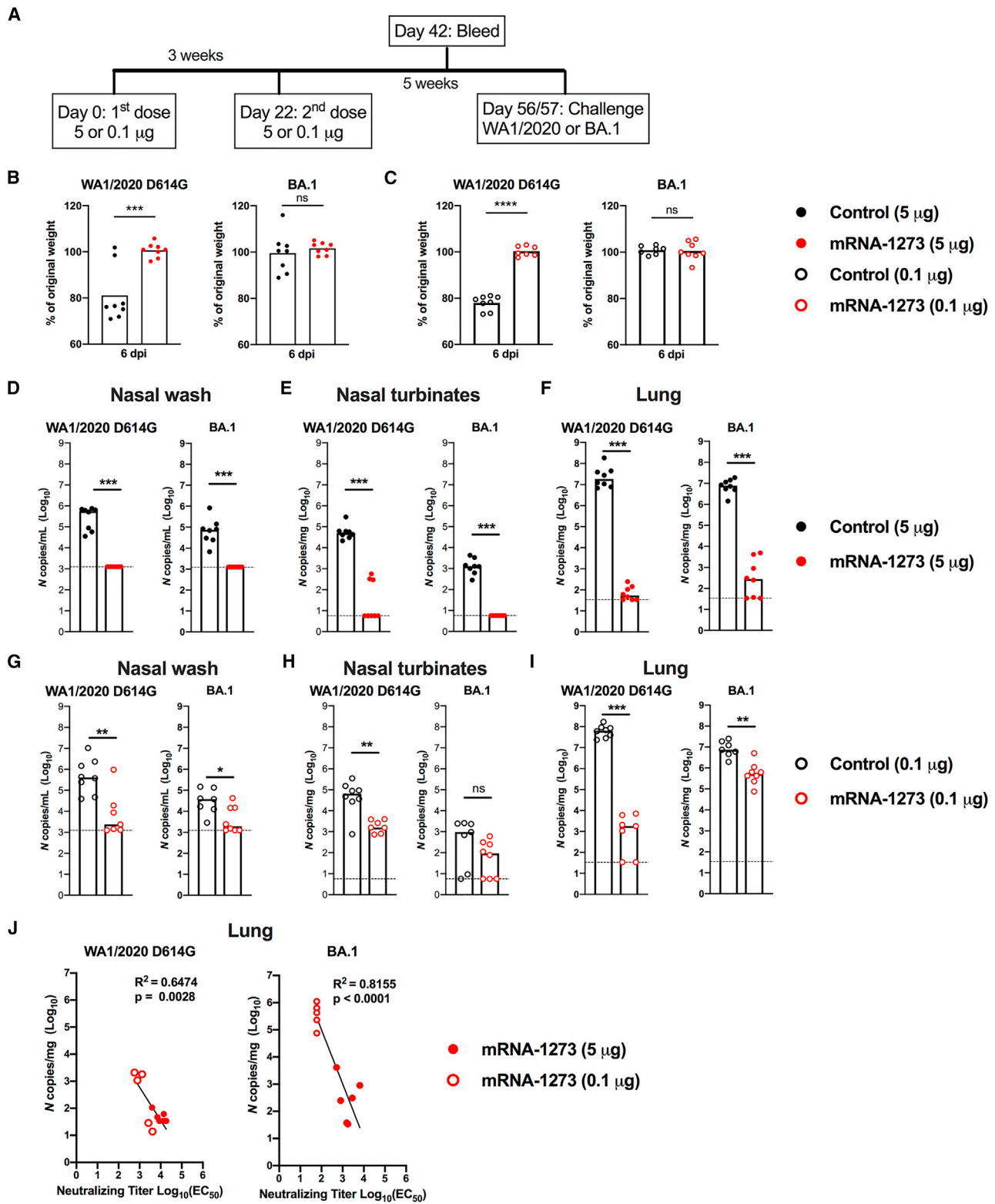


Figure 2. Protection against SARS-CoV-2 infection after mRNA vaccination in K18-hACE2 mice

7-week-old female K18-hACE2 mice were immunized with 5 or 0.1 µg of mRNA vaccines. 5 weeks after a primary vaccination series, mice were challenged with 10^4 focus-forming units (FFU) of WA1/2020 D614G or BA.1.

(legend continued on next page)

pathogenic in rodents (Bentley et al., 2021; Halfmann et al., 2022; Shuai et al., 2022; Yoshihiro et al., 2022), they still replicate to reasonably high levels (approximately 10–100 million copies of *N* gene/mg at 6 days postinfection [dpi]) in the lungs of K18-hACE2 mice (Halfmann et al., 2022). Five weeks after the second vaccine dose, mice were challenged via intranasal route with 10⁴ focus-forming units (FFU) of WA1/2020 D614G or BA.1 (Figure 2A). Compared with the control mRNA vaccine, the 5 and 0.1 μg doses of mRNA-1273 vaccines prevented weight loss at 6 dpi after WA1/2020 D614G infection (Figures 2B and 2C). However, as BA.1-challenged mice failed to lose weight, we could not use this metric to evaluate the protective activity of the mRNA-1273 vaccine.

We next compared the levels of WA1/2020 D614G and BA.1 infection in control mRNA-vaccinated K18-hACE2 mice at 6 dpi (Figures 2D–2I). In the nasal washes of control mRNA-vaccinated K18-hACE2 mice, although some variability was observed, moderate amounts (10⁵–10⁶ copies of *N* per mL) of WA1/2020 D614G RNA were measured; approximately 10-fold lower levels (10⁴–10⁵ copies of *N* per mL) were measured after challenge with BA.1 (Figures 2D and 2G). In the nasal turbinates, a similar pattern was seen with approximately 100-fold lower levels of BA.1 RNA (~10³ versus 10⁵ copies of *N* per mg) (Figures 2E and 2H). In the lungs of control mRNA-vaccinated K18-hACE2 mice, approximately 10-fold less BA.1 RNA was measured compared with WA1/2020 D614G RNA (Figures 2F and 2I).

We assessed the effects of mRNA-1273 vaccination on WA1/2020 D614G and BA.1 infection in respiratory tract samples. The 5 μg dose of mRNA-1273 vaccine protected against WA1/2020 D614G infection with little viral RNA detected at 6 dpi (Figures 2D–2F). In comparison, although BA.1 viral RNA was not detected in the nasal washes or nasal turbinates of animals immunized with 5 μg of mRNA-1273, we observed breakthrough infection, albeit at low levels, in the lungs of most (5 of 8) animals (Figures 2D–2F). Although the 0.1 μg dose of mRNA-1273 vaccine conferred protection against WA1/2020 D614G, several animals had viral RNA in nasal washes (4 of 7 mice), nasal turbinates (7 of 7), and lungs (5 of 7 mice). However, these breakthrough infections generally had reduced (100–100,000-fold) levels compared with the control mRNA vaccine (Figures 2G–2I). After immunization with 0.1 μg of mRNA-1273, BA.1 infection levels were lower in the nasal washes but not in the nasal turbinates (Figures 2G and 2H), in part due to the lower levels of viral RNA in the control mRNA-vaccinated samples. However, in the lungs, 7 of 7 mice vaccinated with the 0.1 μg dose sustained high levels of BA.1 breakthrough infection with ~10⁶ copies of

N per mg, although some limited protection still was observed (Figure 2I).

Serum neutralizing antibody titers showed an inverse correlation with amounts of viral RNA in the lung (Figure 2J) for both viruses, with more infection occurring in BA.1-infected animals with lower neutralization titers. The correlation was most linear for BA.1-challenged animals ($R^2 = 0.8155$, $p < 0.0001$), with a minimum neutralizing titer of approximately 2,000 required to completely prevent infection at 6 dpi. Most of the breakthrough infections occurred with the lower 0.1 μg dose of mRNA vaccines, which models what might be occurring in immunocompromised or elderly individuals, or immunocompetent individuals at times remote from completion of their primary immunization series (Chen et al., 2021a; Choi et al., 2021; Evans et al., 2021).

We tested whether the mRNA-1273 vaccine could suppress cytokine and chemokine responses in the lung at 6 dpi of K18-hACE2 mice after challenge with WA1/2020 D614G or BA.1 (Figures 3A and 3B). WA1/2020 D614G or BA.1 infection of control mRNA-vaccinated K18-hACE2 mice resulted in increased expression of several proinflammatory cytokines and chemokines including G-CSF, GM-CSF, IFN γ , IL-1 β , IL-6, CXCL1, CXCL5, CXCL9, CXCL10, CCL2, CCL4, and TNF- α in lung homogenates (Tables S1 and S2). Proinflammatory cytokine and chemokines in the lung at 6 dpi generally were lower in animals vaccinated with 5 μg dose of mRNA-1273 after challenge with WA1/2020 D614G or BA.1 (Figure 3A). Although K18-hACE2 mice immunized with the lower 0.1 μg dose also showed diminished levels of cytokines and chemokines after WA1/2020 D614G infection, this protection was not observed after BA.1 challenge; levels of proinflammatory cytokines in the lung were similar in mice immunized with the 0.1 μg dose control and mRNA-1273 vaccines after challenge with BA.1 (Figure 3B).

We performed histological analysis of lung tissues from immunized animals challenged with WA1/2020 D614G or BA.1. Lung sections obtained at 6 dpi from mice immunized with either dose of control mRNA vaccine and challenged with WA1/2020 D614G showed severe pneumonia characterized by immune cell infiltration, alveolar space consolidation, vascular congestion, and interstitial edema (Figures 3C and 3D). In comparison, K18-hACE2 mice immunized with the control mRNA vaccines and challenged with BA.1 showed less lung pathology, with focal airspace consolidation and immune cell infiltration, results that are consistent with the lower pathogenicity of Omicron variants in rodents (Abdelnabi et al., 2022; Bentley et al., 2021; Halfmann et al., 2022; Shuai et al., 2022; Yoshihiro et al., 2022).

(A) Scheme of immunizations, blood draws, and virus challenge.

(B and C) Body weight in animals immunized with 5 μg (B) or 0.1 μg (C) of control or mRNA-1273 vaccines between days 0 and 6 after challenge with WA1/2020 D614G or BA.1. Data show mean values ($n = 7$ – 8 , two experiments).

(D–I) Viral burden at 6 dpi in the nasal washes (D and G), nasal turbinates (E and H), and lungs (F and I) as assessed by qRT-PCR of the *N* gene after WA1/2020 D614G or BA.1 challenge of mice immunized with 5 μg (D–F) or 0.1 μg (G–I) of control or mRNA-1273 vaccines ($n = 7$ – 8 , two experiments, boxes illustrate median values, dotted lines show LOD).

(B and C) Unpaired t test. (D–I) Mann-Whitney test (ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

(J) Correlation analyses comparing serum neutralizing antibody concentrations 3 weeks after the second vaccine dose and lung viral titers (6 dpi) after challenge with WA1/2020 D614G (left) or BA.1 (right); Pearson's correlation R^2 and p values are indicated as insets; closed symbols 5 μg vaccine dose; open symbols, 0.1 μg vaccine dose.

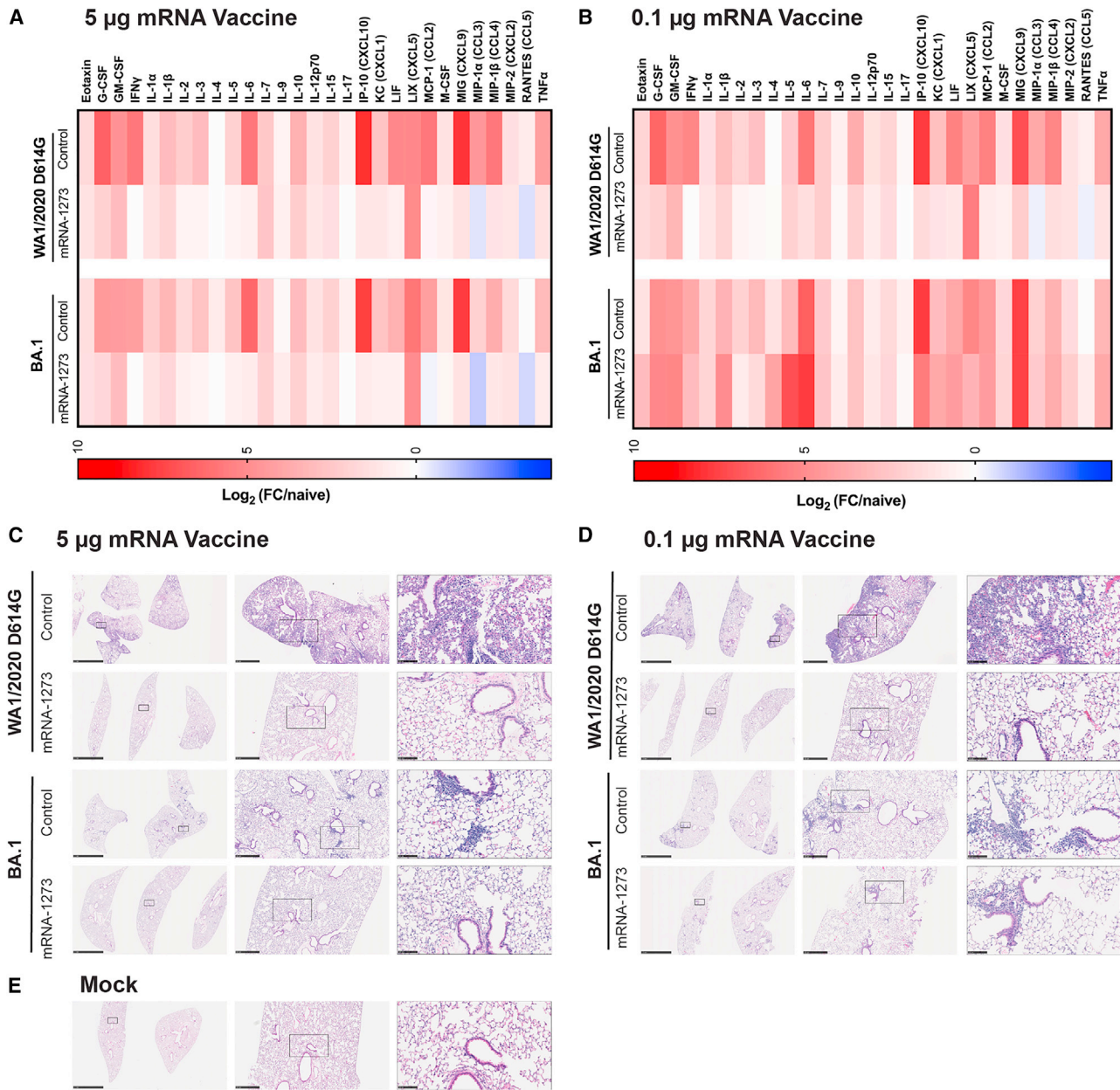


Figure 3. mRNA vaccine protection against disease in K18-hACE2 mice

7-week-old female K18-hACE2 mice were immunized with two 5 or 0.1 µg doses of mRNA vaccines and challenged with WA1/2020 D614G or BA.1, as described in [Figures 1A and 2A](#).

(A and B) Heat-maps of cytokine and chemokine levels in lung homogenates at 6 dpi in animals immunized with 5 µg (A) or 0.1 µg (B) doses of indicated mRNA vaccines. Fold-change was calculated relative to naive uninfected mice, and log₂ values are plotted (2 experiments, n = 7–8 per group except naive, n = 4). The full data set is shown in [Tables S1 and S2](#).

(C–E) Hematoxylin and eosin staining of lung sections harvested from control or mRNA-1273 vaccinated animals (5-µg dose, C; 0.1-µg dose, D) at 6 dpi with WA1/2020 D614G or BA.1. A section from an uninfected animal (E) is shown for comparison. Low (left; scale bars, 1 mm), moderate (middle, scale bars, 200 µm), and high (bottom; scale bars, 50 µm) power images are shown. Representative images of multiple lung sections from n = 3 per group. See also [Tables S1 and S2](#).

Mice immunized with a high or low dose of mRNA-1273 and challenged with WA1/2020 D614G did not develop lung pathology, with histological findings similar to uninfected mice ([Figures 3C–3E](#)). Mice immunized with the high dose of mRNA-

1273 vaccine were protected against the mild pathological changes associated with BA.1 infection ([Figure 3C](#)). However, animals immunized with the lower dose of mRNA-1273 showed similar lung pathology after BA.1 infection as control

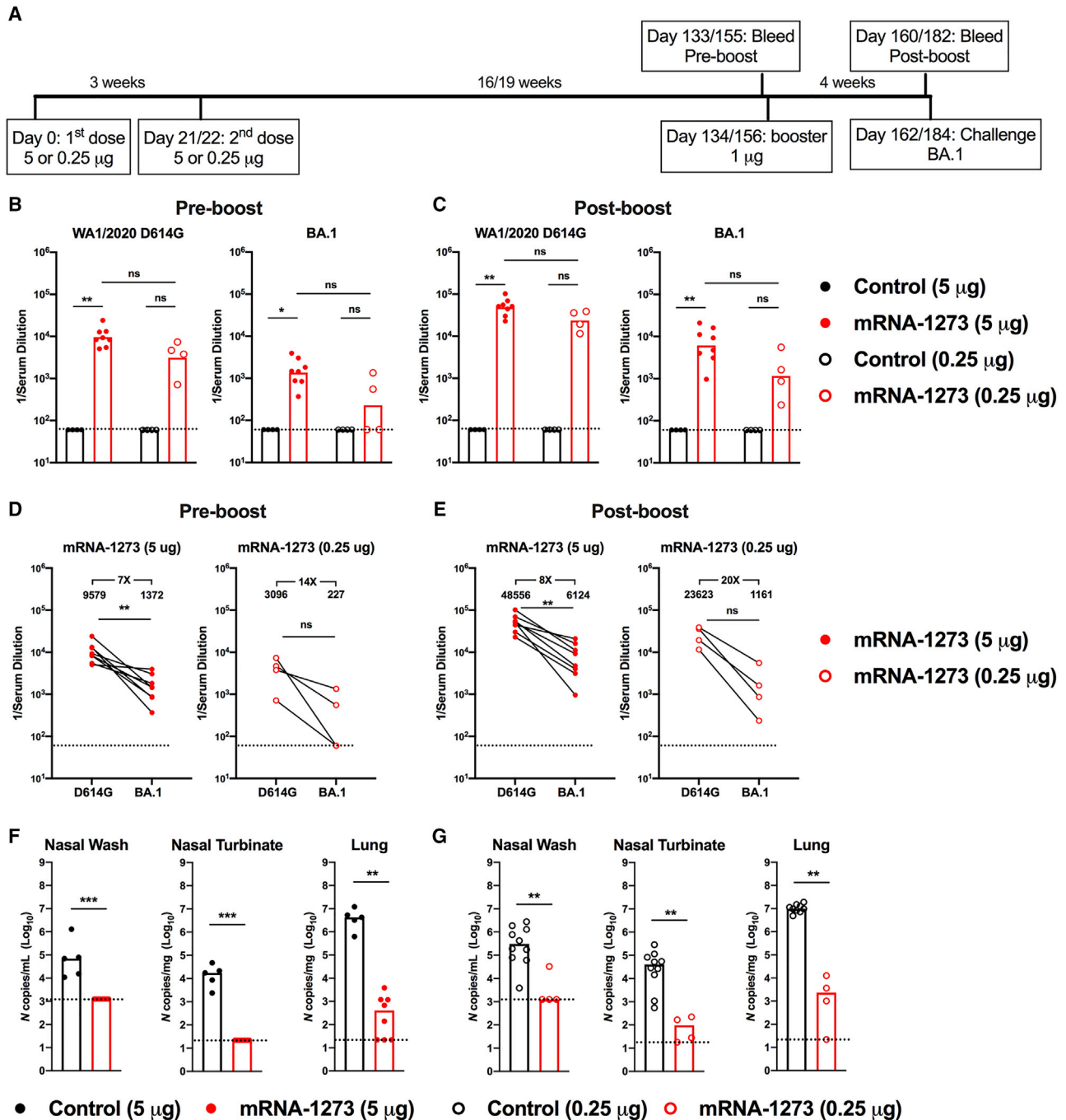


Figure 4. A booster dose of mRNA-1273 enhances neutralizing antibody responses and confers protection in K18-hACE2 mice

7-week-old female K18-hACE2 mice were immunized with 5 or 0.25 μ g of mRNA vaccines and boosted 16–19 weeks later with 1 μ g of mRNA-1273.

(A) Scheme of immunizations, blood draws, and virus challenge.

(B and C) Serum neutralizing antibody responses immediately before (B, pre-boost) and 4 weeks after (C, post-boost) a control or mRNA-1273 booster dose against WA1/2020 D614G (left) and BA.1 (right) in mice immunized with 5 or 0.25 μ g of control (n = 4) or mRNA-1273 (5 μ g, n = 8; 0.25 μ g, n = 4) vaccines (one experiment, boxes illustrate GMT, dotted lines show the LOD).

(D and E) Paired analysis of pre-boost (D) and post-boost (E) serum neutralizing titers against WA1/2020 D614G and BA.1 from individual mice (data from B and C) from samples obtained from animals that received a primary 5 μ g (left) or 0.25 μ g (right) dose series of mRNA-1273 vaccine (n = 4–8, one experiment, dotted lines show the LOD). GMT and fold-change values are indicated at the top of the graphs.

(legend continued on next page)

mRNA-vaccinated animals, with patchy immune cell infiltration, airway space thickening, and mild alveolar congestion (Figure 3D).

An mRNA-1273 booster enhances antibody responses and protection against BA.1 in K18-hACE2 mice

As booster doses are now used in humans to augment immunity and protection against variants, including Omicron (Atmar et al., 2022; Bar-On et al., 2021; Pajon et al., 2022), we evaluated their effects in K18-hACE2 mice. A cohort of 7-week-old female K18-hACE2 mice was immunized with a primary series over a 3-week interval with either a 5 or 0.25 μg dose of mRNA-1273 or a control vaccine (Figure 4A). The 0.25 μg dose used was higher than the 0.1 μg dose used above, in part due to the extended interval between the primary immunization series and boosting. After a 16- to 19-week rest period, blood was collected (pre-boost), animals were boosted with mRNA-1273 or a control vaccine, and a second bleed (post-boost) was performed 1 month later (Figure 4A), and sera were tested for neutralizing activity. As expected, 5- to 10-fold lower pre-boost neutralizing titers were observed from animals immunized with the 0.25 μg than the 5 μg dose of mRNA-1273 (Figures 4B–4E and S2). Although the titers against WA1/2020 D614G were high (GMT: 9,579, 5 μg ; 3,096, 0.25 μg), we observed 7- to 14-fold lower levels ($p < 0.01$, 5 μg) of neutralizing antibodies against BA.1, with half of the sera from mice vaccinated with the 0.25- μg formulation showing no inhibitory activity at the 1/60 limit of detection of the assay (Figures 4B, 4D, and S2). One month after boosting with mRNA-1273, serum neutralizing titers rose against both viruses (Figures 4C, 4E, and S2). All mice boosted with mRNA-1273 had neutralizing titers against BA.1 above the estimated threshold (titer of 50) for protection (GMT: 6,124, 5 μg ; 1,161, 0.25 μg).

Four weeks after boosting (Figure 4A), we evaluated the protective effects of the mRNA-1273 booster on BA.1 infection by measuring viral RNA levels at 6 dpi (Figures 4F and 4G). Compared with animals immunized and boosted with the control mRNA vaccine, K18-hACE2 mice vaccinated with the 5 or 0.25- μg primary series and boosted with mRNA-1273 showed reduced levels ($p < 0.001$) of BA.1 viral RNA in the respiratory tract, with little to no detectable *N* gene copies in the nasal wash or turbinates. However, breakthrough infection, albeit at 2,500- to 6,000-fold lower levels ($p < 0.001$) than the control vaccine, was detected in the lungs of the majority (8 of 12) of mRNA-1273 boosted K18-hACE2 mice (Figures 4F and 4G). Thus, boosting of mice with mRNA-1273 vaccine, despite not being matched to the challenge virus, improves neutralizing antibody responses and reduces infection by BA.1 in the upper and lower respiratory tracts.

A BA.1-matched mRNA vaccine induces robust antibody responses against Omicron variants

As an alternative to a third dose of mRNA-1273, boosting with vaccines targeting an Omicron variant spike might provide

enhanced immunity and protection. To begin to address this question, we generated a lipid-encapsulated mRNA vaccine (mRNA-1273.529) encoding a proline-stabilized SARS-CoV-2 spike from the BA.1 virus. As a first test of its activity, we immunized BALB/c mice twice at 3-week intervals with 1 or 0.1 μg of mRNA-1273 or mRNA-1273.529 vaccines (Figure 5A). Three weeks after the first dose (day 21) and 2 weeks after the second dose (day 36), serum was collected (Figure 5A) and analyzed for binding to Wuhan-1 and BA.1 spike proteins by ELISA (Figures 5B and 5C). At day 21 after the first dose, animals receiving 1 μg of mRNA-1273 showed approximately 11-fold higher levels of binding to homologous Wuhan-1 than heterologous BA.1 spike, whereas equivalent responses to Wuhan-1 and BA.1 spike proteins were detected after vaccination with 1 μg of mRNA-1273.529 (Figure 5B). In comparison, mice receiving the lower 0.1 μg dose of either mRNA-1273 or mRNA-1273.529 had low levels (near the limit of detection) of anti-spike antibody at day 21. Serum collected 2 weeks after the second dose of mRNA-1273 or mRNA-1273.529 also was tested for binding to Wuhan-1 and BA.1 spike proteins. Immunization with either dose of mRNA-1273 resulted in higher (8- to 20-fold) serum IgG binding titers to Wuhan-1 than BA.1 spike (Figure 5C). Reciprocally higher levels (3-fold) of serum IgG binding to BA.1 than Wuhan-1 spike were seen after two 0.1 μg doses of mRNA-1273.529; however, immunization with two 1 μg doses of mRNA-1273.529 resulted in equivalent serum antibody binding to Wuhan-1 and BA.1 spike proteins. Serum collected at day 36 was tested for binding to Wuhan-1, BA.1, B.1.351 (Beta), and B.1.617.2 (Delta) RBD proteins (Figure 5D). Although mRNA-1273 or mRNA-1273.529 vaccines induced equivalent serum IgG binding titers to Wuhan-1, B.1.351, and B.1.617.2 RBD, 4-fold higher levels were generated by the BA.1-targeted mRNA-1273.529 vaccines against BA.1 RBD.

We next tested the inhibitory activity of serum antibodies of BALB/c mice that received two 1 μg doses of mRNA-1273 or mRNA-1273.529 using a vesicular stomatitis virus (VSV)-based pseudovirus neutralization assay and spike proteins of Wuhan-1 D614G, BA.1, BA.1.1., B.1.351, or B.1.617.2 (Figure 5E). Two weeks after the second vaccine dose, animals immunized with mRNA-1273 had slightly higher serum neutralizing titers against Wuhan-1 D614G (GMT: 4,967) than against viruses displaying B.1.351 (GMT: 1,310) or B.1.617.2 (GMT: 1,549). However, and consistent with data in K18-hACE2 mice (Figure 4D) and humans (Dejnirattisai et al., 2022; Liu et al., 2022), BALB/c mice vaccinated with mRNA-1273 had substantially lower (59- to 92-fold reduced) neutralizing titers against BA.1 and BA.1.1, with several samples falling near the presumed 1/50 threshold of protection. In comparison, animals immunized with two 1 μg doses of the BA.1-targeted mRNA-1273.529 vaccine showed distinct profiles. Although high neutralization titers were observed against BA.1 (GMT: 3,375) and BA.1.1

(F and G) 4 weeks after boosting with control or mRNA-1273, mice were challenged with 10^4 FFU of BA.1. Viral burden at 6 dpi in the nasal washes, nasal turbinates, and lungs as assessed by *N* gene levels in animals that had received a primary series immunization with 5 μg (F) or 0.25 μg (G) doses of control or mRNA-1273 vaccines ($n = 7-8$, two experiments, boxes illustrate median values, dotted lines show LOD).

(B and C) One-way ANOVA with Kruskal-Wallis post-test. (D and E) Wilcoxon signed-rank test. (F and G) Mann-Whitney test (ns, not significant; ** $p < 0.01$; *** $p < 0.001$). See also Figure S2.

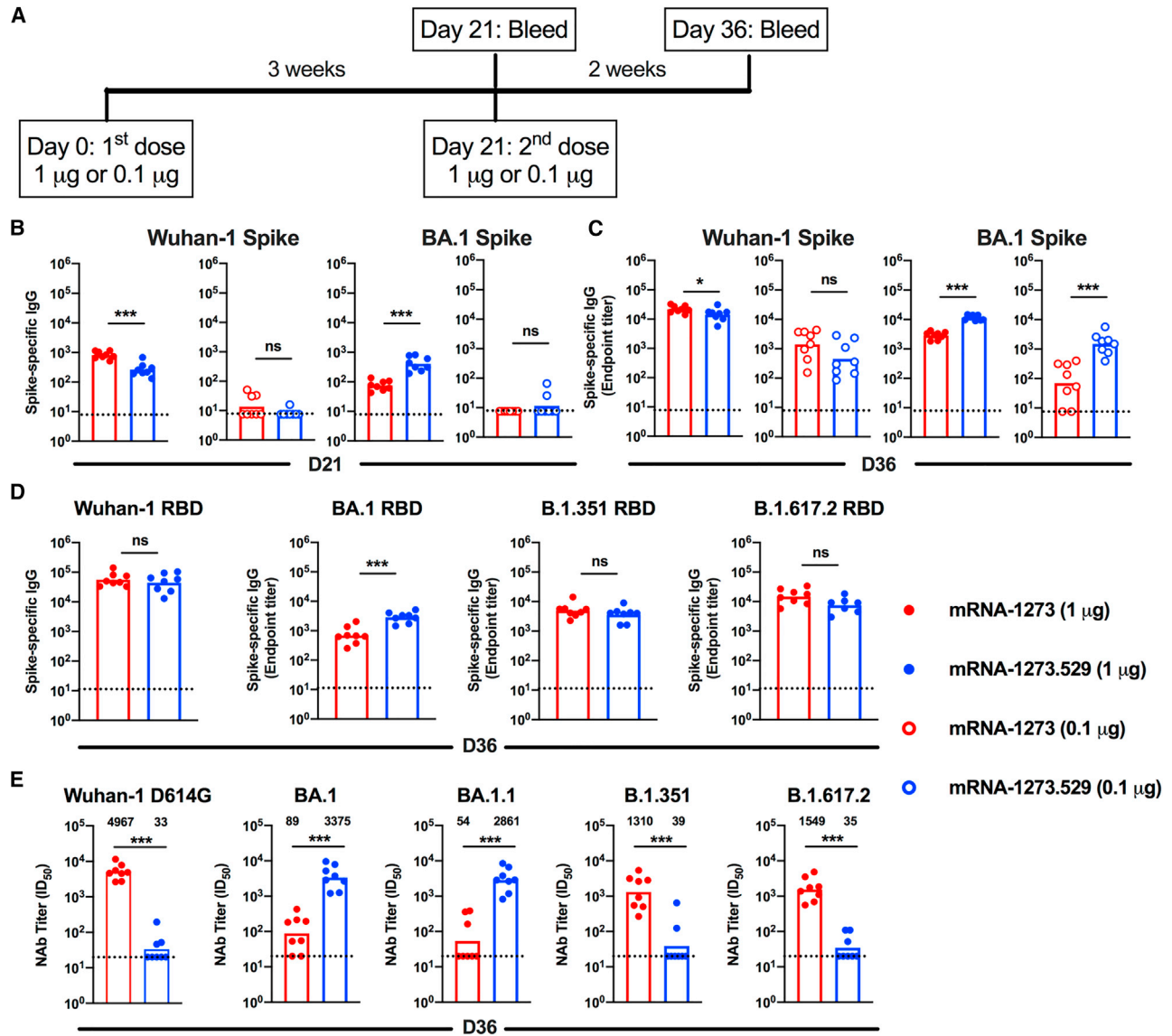


Figure 5. Antibody responses in BALB/c mice after immunization with mRNA-1273 and mRNA-1273.529 vaccines

(A) 6-to-8-week-old female BALB/c mice were immunized twice over a 3-week interval with 1 or 0.1 µg of mRNA-1273 or mRNA-1273.529 vaccine or a PBS control (not shown, all values at the LOD). Immediately before (day 21) or 2 weeks after (day 36) the second vaccine dose, serum was collected.

(B and C) Serum antibody binding to Wuhan-1 or BA.1 spike proteins by ELISA at days 21 (B) and 36 (C) (n = 8, two experiments, boxes illustrate mean values, dotted lines show the LOD).

(D) Serum antibody binding at day 36 to Wuhan-1, BA.1, B.1.351 (Beta), or B.1.617.2 (Delta) RBD proteins by ELISA (n = 8, two experiments, boxes illustrate mean values, dotted lines show the LOD).

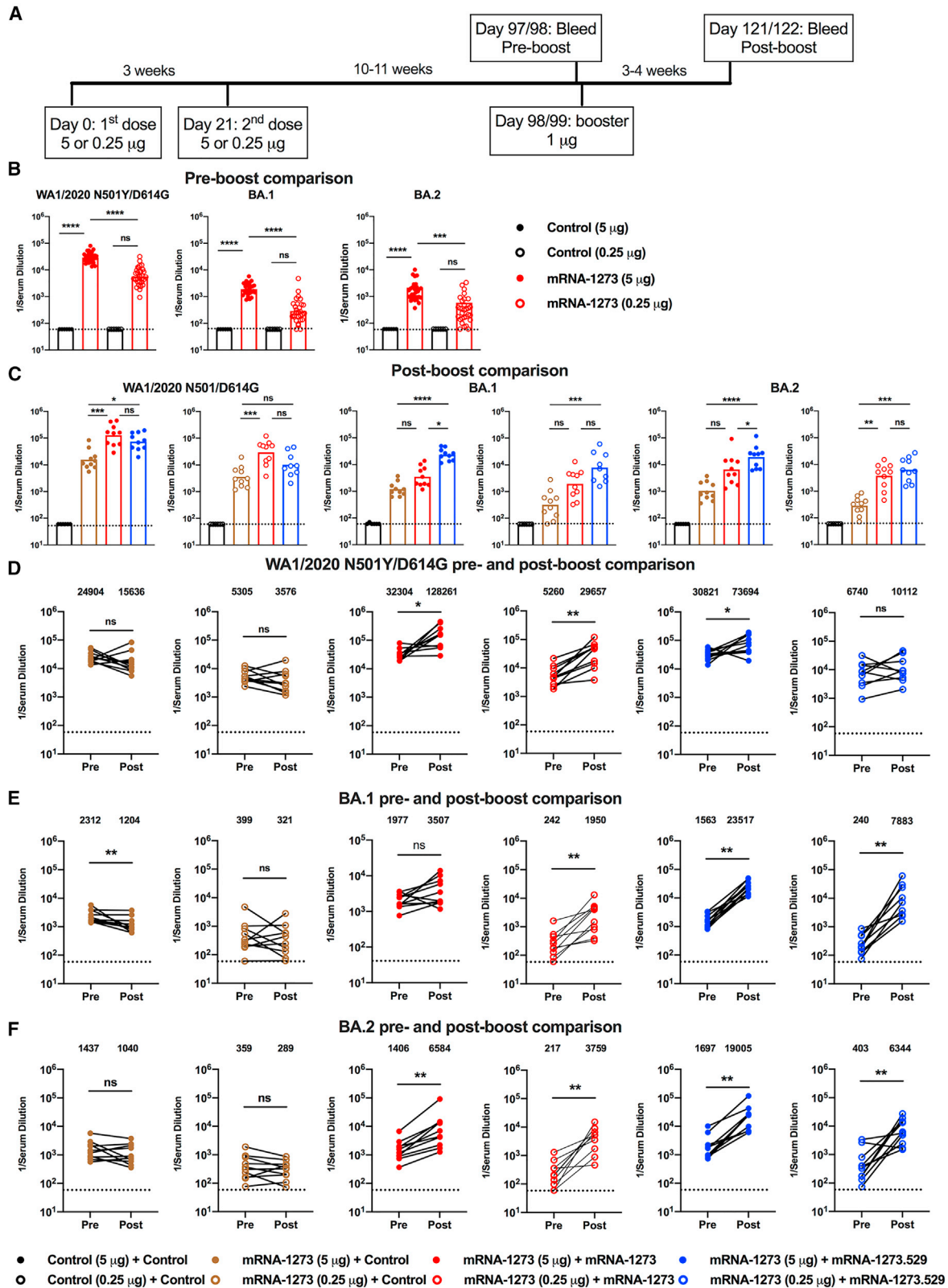
(E) Neutralizing activity of serum at day 36 against VSV pseudoviruses displaying the spike proteins of Wuhan-1 D614G, BA.1, BA.1.1, B.1.351, or B.1.617.2 (n = 8, two experiments, boxes illustrate GMT, dotted lines show the LOD). GMT values are indicated above the columns.

(B–E) Mann-Whitney test (ns, not significant; *p < 0.05; **p < 0.01; ***p < 0.001).

(GMT: 2,861), lower titers (87- to 102-fold less, p < 0.01) were detected against Wuhan-1 D614G (GMT: 33), B.1.351 (GMT: 39), and B.1.617.2 (GMT: 35), with most of the samples at the limit of detection of the neutralizing assay. Thus, and as suggested in another preliminary study (Lee et al., 2022), a primary vaccination series with an Omicron-specific mRNA vaccine induces potently neutralizing antibodies against Omicron variants but not against historical SARS-CoV-2 or other variants.

A sequence-matched mRNA vaccine confers enhanced protection against BA.1 infection in mice

As additional BALB/c and K18-hACE2 mice administered a primary mRNA-1273 series were unavailable, we took advantage of an existing cohort of female 129S2 mice that had received two 5 or 0.25 µg doses of mRNA-1273 or control mRNA vaccine over a three-week interval and then were rested for 10–11 weeks (Figure 6A). Blood was collected (pre-boost sample), and groups



(legend on next page)

of animals were boosted with control mRNA, mRNA-1273, or mRNA-1273.529 vaccines. Three to four weeks later, a second post-boost blood sample was collected (Figure 6A), and the neutralizing activities of pre- and post-boost serum antibodies were measured and compared. Mice that received 5 or 0.25 μg doses of mRNA-1273 had high levels of pre-boost neutralizing antibodies against WA1/2020 N501Y/D614G (GMT: 29,161, 5 μg ; 5,749, 0.25 μg) and B.1.351 (GMT: 15,093, 5 μg ; 3,958, 0.25 μg) (Figures 6B, S3, and S4B). We used the WA1/2020 N501Y/D614G strain because the N501Y substitution enables productive infection of conventional strains of laboratory mice (Gu et al., 2020; Liu et al., 2021b; Rathnasingh et al., 2021; Ying et al., 2022). Mice that received 5 or 0.25 μg doses of mRNA-1273 had 9- to 18-fold lower serum pre-boost neutralizing titers against BA.1 (GMT: 1,891, 5 μg ; 317, and 0.25 μg) and BA.2 (GMT: 2,132, 5 μg ; 580, and 0.25 μg) (Figures 6B and S3). Three to four weeks after boosting with mRNA-1273 or mRNA-1273.529, neutralizing titers against WA1/2020 N501Y/D614G and B.1.351, respectively, were approximately 2- to 6-fold and 4- to 22-fold higher (Figures 6C, 6D, S3, and S4B–S4D). Boosting with mRNA-1273.529 resulted in 15- to 32-fold and 11- to 18-fold higher neutralizing titers against BA.1 and BA.2, respectively. In comparison, the mRNA-1273 booster increased neutralizing titers against BA.1 by 2 to 8-fold and BA.2 by 5- to 17-fold (Figures 6C, 6E, 6F, and S3). Thus, although both mRNA-1273 and mRNA-1273.529 boosters augmented serum neutralizing activity against BA.1 and BA.2, a BA.1-matched vaccine induced greater responses.

Three or four days after the post-boost bleed, 129S2 mice were challenged by the intranasal route with 10^5 FFU of WA1/2020 N501Y/D614G or BA.1 (Figure 7A), and viral RNA levels at 3 dpi were measured in the nasal washes, nasal turbinates, and lungs. BA.1 is less pathogenic in 129S2 mice (Halfmann et al., 2022) with \sim 100-fold lower levels of viral RNA in the upper and lower respiratory tracts than after WA1/2020 N501Y/D614G infection (Figure 7B). Nonetheless, substantial viral replication occurs allowing for evaluation of vaccine protection. Mice vaccinated with either 5 or 0.25 μg doses of mRNA-1273 and boosted with either mRNA-1273 or mRNA-1273.529 showed almost complete protection against WA1/2020 N501Y/D614G infection in the nasal washes, nasal turbinates, and lungs, with approximately 100,000- to 1,000,000-fold reductions in viral RNA levels compared with mice immunized with control mRNA vaccine. Mice primed with the 5 μg doses of mRNA-1273 and boosted with either mRNA-1273 or mRNA-1273.529 analogously showed

robust and equivalent protection against BA.1 infection (Figure 7B). In comparison, animals primed with the lower 0.25 μg dose of mRNA-1273 showed some differences after boosting and BA.1 challenge (Figure 7B). Although BA.1 viral RNA levels were reduced in upper respiratory tract tissues after boosting with either mRNA-1273 or mRNA-1273.529, there was a trend ($p = 0.07$) toward lower levels in the nasal turbinates in animals boosted with mRNA-1273.529. Moreover, a 27-fold reduction ($p < 0.01$) in BA.1 infection in the lungs was observed in mice boosted with the BA.1-matched mRNA-1273.529 compared with the mRNA-1273 vaccine.

As an independent metric of protection, we measured cytokine and chemokine levels in lung homogenates of the vaccinated and challenged 129S2 mice at 3 dpi (Figures 7C and 7D, Tables S3 and S4). Mice immunized with 5 or 0.25 μg doses of mRNA-1273 and then given a booster dose of either control, mRNA-1273, or mRNA-1273.529 vaccines generally showed lower levels of proinflammatory cytokines and chemokines after WA1/2020 N501Y/D614G infection than animals that received three doses of control mRNA vaccine. In comparison, after BA.1 challenge, several differences were noted: (1) the inflammatory response after BA.1 infection was lower in magnitude in control mRNA-vaccinated mice than after WA1/2020 N501Y/D614G infection, consistent with its lower pathogenicity in mice (Halfmann et al., 2022); (2) animals immunized with 5 μg doses of mRNA-1273 and then boosted with control, mRNA-1273, or mRNA-1273.529 vaccines showed reduced levels of cytokines and chemokines compared with those receiving three doses of control mRNA, indicating protection against inflammation by homologous or heterologous boosting (Figure 7C); and (3) mice immunized with two 0.25 μg doses of mRNA-1273 and boosted with control vaccine showed little reduction in cytokine and chemokine levels compared with mice receiving three doses of control mRNA vaccine following BA.1 challenge (Figure 7D). Although boosting with mRNA-1273 reduced the levels of most lung inflammatory mediators, the effect was greater in animals boosted with mRNA-1273.529. Thus, and consistent with the virological data, protection against BA.1-induced lung inflammation was modestly improved in animals boosted with the BA.1-matched mRNA-1273.529 vaccine.

DISCUSSION

The emergence of SARS-CoV-2 variants with constellations of amino acid changes in the NTD and RBD of the spike protein

Figure 6. Booster doses of mRNA-1273 or mRNA-1273.529 enhance neutralizing antibody responses in 129S2 mice

7-week-old female 129S2 mice were immunized with 5 or 0.25 μg of mRNA vaccines and then boosted 10–11 weeks later with 1 μg of control mRNA, mRNA-1273, or mRNA-1273.529.

(A) Scheme of immunizations and blood draws.

(B and C) Serum neutralizing antibody responses immediately before (B, pre-boost) and 3–4 weeks after (C, post-boost) a control, mRNA-1273, or mRNA-1273.529 booster dose against WA1/2020 N501Y/D614G (left), BA.1 (middle), and BA.2 (right) in 129S2 mice that received primary series immunizations with 5 or 0.25 μg of control ($n = 6$) or mRNA-1273 ($n = 30$) vaccines (two experiments, boxes illustrate GMTs, dotted lines show the LOD).

(D–F) Paired analysis of pre- and post-boost serum neutralizing titers against WA1/2020 D614G (D), BA.1 (E), and BA.2 (F) viruses from samples obtained from animals (data from B and C) that received the following primary and booster immunizations: mRNA-1273 (5 or 0.25 μg) + control booster, mRNA-1273 (5 or 0.25 μg) + mRNA-1273 booster, and mRNA-1273 (5 or 0.25 μg) + mRNA-1273.529 booster ($n = 10$, two experiments, dotted lines show the LOD). GMT values are indicated at the top of the graphs.

(B and C) One-way ANOVA with Dunn's post-test. (D–F) Wilcoxon signed-rank test (ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). See also Figures S3 and S4.

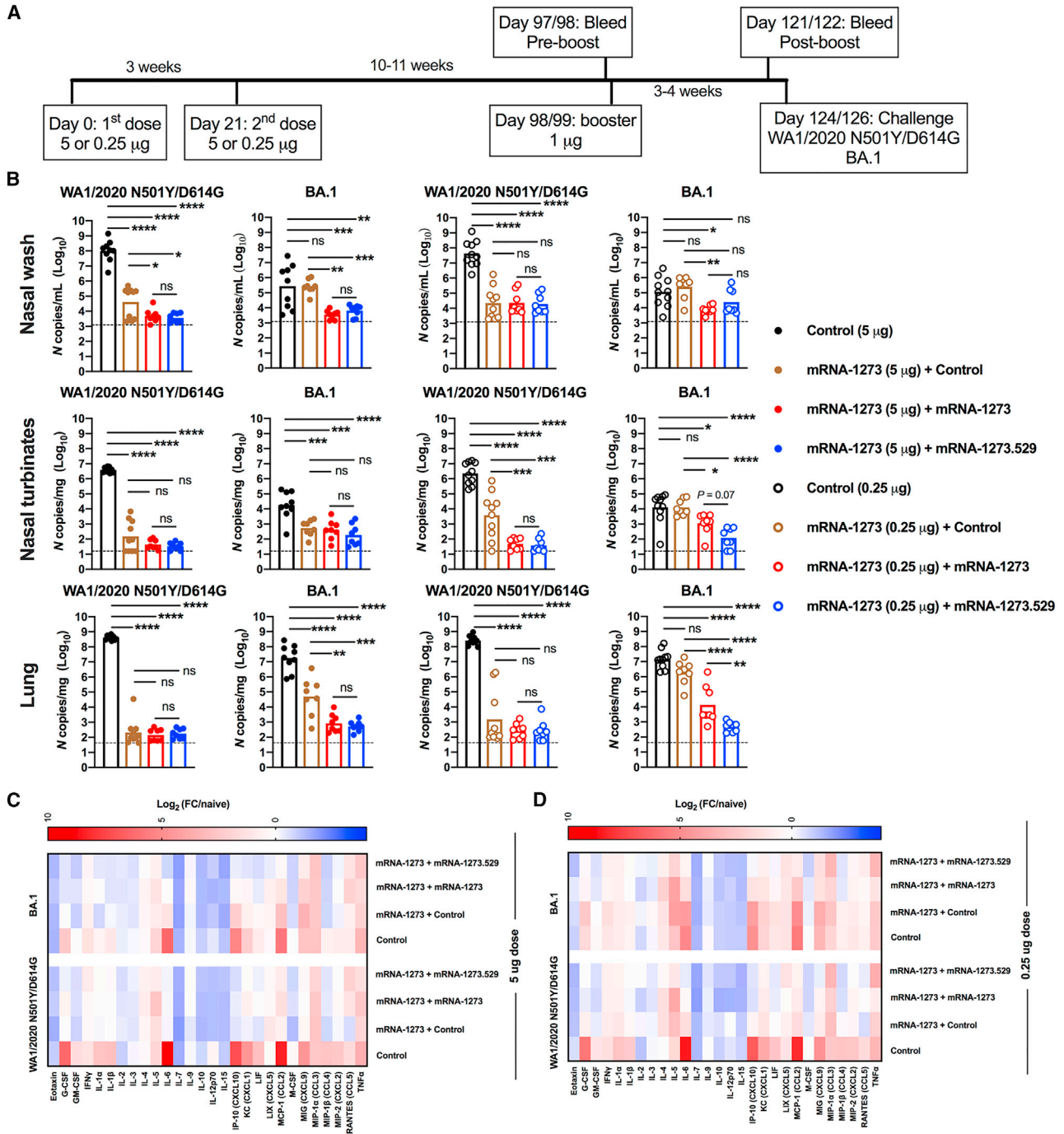


Figure 7. Booster doses of mRNA-1273 or mRNA-1273.529 enhance protection against BA.1 infection in 129S2 mice

7-week-old female 129S2 mice were immunized with 5 or 0.25 μ g of mRNA vaccines, boosted with 1 μ g of control mRNA, mRNA-1273, or mRNA-1273.529 and challenged with WA1/2020 N501Y/D614G or BA.1.

(A) Scheme of immunizations, blood draws, and virus challenge.

(B) Viral RNA levels at 3 dpi in the nasal washes, nasal turbinates, and lungs after WA1/2020 N501Y/D614G or BA.1 challenge of mice immunized with 5 μ g (left) or 0.25 μ g (right) of control or mRNA-1273 vaccines and boosted with control, mRNA-1273, or mRNA-1273.529 vaccines (n = 8–10 per group, two experiments, boxes illustrate mean values, dotted lines show LOD; one-way ANOVA with Tukey's post-test: ns, not significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

(C and D) Heat-maps of cytokine and chemokine levels in lung homogenates at 3 dpi with WA1/2020 N501Y/D614G or BA.1 in animals immunized with 5 μ g (C) or 0.25 μ g (D) doses of control or mRNA-1273 vaccines and then boosted with control, mRNA-1273, or mRNA-1273.529 vaccines. Fold-change was calculated relative to naive mice, and log₂ values are plotted (2 experiments, n = 8 per group except naive, n = 4). See also Tables S3 and S4.

jeopardizes vaccines designed against historical SARS-CoV-2 strains. In this study, we evaluated in mice the protective activity of high- and low-dose formulations of Moderna mRNA-1273 vaccine and matched (mRNA-1273.529) and nonmatched (mRNA-1273) booster doses against Omicron variants. Immunization of mice with a high-dose formulation of mRNA-1273 induced neutralizing antibodies that inhibited infection in cell culture of both the historical WA1/2020 D614G and Omicron BA.1 variant, although we observed reduced efficacy against BA.1, as seen with human sera (Cameroni et al., 2022; Cao et al., 2022; Dejnirattisai et al., 2022; Liu et al., 2022; Xia et al., 2022). Challenge studies in K18-hACE2 and 129S2 mice showed robust protection against both SARS-CoV-2 strains with a high-dose primary series of mRNA-1273 vaccine. In comparison, the neutralizing antibodies induced by the low-dose series of mRNA-1273 vaccine showed less inhibitory activity against BA.1, which correlated with breakthrough infection in the upper and lower respiratory tracts. The low-dose vaccine arms induce levels of neutralizing antibody in mice comparable with those measured in human sera after completion of a primary two-dose vaccination series with mRNA-1273 (Anderson et al., 2020; Widge et al., 2021; Wu et al., 2021). Analysis of cytokines and histology corroborated the low-to-minimal protection against BA.1 in K18-hACE2 mice by the low-dose series of mRNA-1273 vaccine.

We extended these studies by evaluating the effects of matched and nonmatched mRNA vaccine boosters on antibody responses and protection. In a study with a small cohort of K18-hACE2 mice, we observed increased serum neutralizing titers 1 month after boosting with mRNA-1273, although the response to BA.1 was lower than against WA1/2020. These data are consistent with data from humans who received a primary mRNA-1273 or BNT162b2 (Pfizer/BioNTech) series and homologous booster; although neutralizing titers were increased against Omicron viruses after boosting, levels were lower than that against WA1/2020 D614G (Pajon et al., 2022; Xia et al., 2022). Because of this pattern, some have advocated for an Omicron variant-matched booster (Waltz, 2022). In our studies in BALB/c mice, as a primary immunization series, a BA.1-targeted mRNA-1273-529 vaccine induced neutralizing antibodies in serum against BA.1 and BA.1.1. However, neutralizing antibody titers against heterologous WA1/2020 D614G, B.1.351, and B.1.617.2 strains were much lower, as reported by another group (Lee et al., 2022) and also described in unvaccinated humans that experienced a primary BA.1 infection (Rössler et al., 2022). Thus, in unvaccinated, uninfected individuals, multivalent mRNA vaccines (Pajon et al., 2022; Ying et al., 2022) may be required to achieve necessary breadth of humoral responses.

When mRNA-1273.529 was administered as a boost after a mRNA-1273 primary series, we observed enhanced neutralizing responses against BA.1 and BA.2, and this was associated with protection against BA.1 infection in the upper and lower respiratory tracts. Boosting with mRNA-1273.529 also enhanced neutralizing antibody responses against the historical WA1/2020 D614G strain, albeit less so, which suggests no detriment in breadth of the mRNA-1273.529 response in the setting of boosting in contrast to the primary immunization series. When compared with boosting with the historical mRNA-1273 vaccine,

which increased neutralizing antibody responses against BA.1 and BA.2 to a lesser degree, the differences in virological protection in the upper and lower airways were modest. When mice were given a high-dose mRNA-1273 primary vaccination series, there was no benefit of the BA.1-matched booster compared with the mRNA-1273 booster, which agrees with recent data from nonhuman primates (Gagne et al., 2021). However, when mice were given a low-dose primary vaccination series, protection against lung infection and inflammation was slightly greater in animals boosted with the BA.1-matched mRNA-1273.529 vaccine. The relative disparity between the larger differences in boosting of neutralizing antibodies by mRNA-1273 and mRNA-1273.529 vaccines and the smaller effects on infection at 1 month post-boost could reflect the protective effects of anamnestic cross-reactive T cells (Gao et al., 2022; Keeton et al., 2022) or Fc effector function and immune cell engagement by cross-reactive non-neutralizing antibodies (Bartsch et al., 2021). Further studies examining the durability of protection afforded by mRNA-1273 versus BA.1-matched mRNA-1273.529 boosters are warranted.

Limitations of study

We note several limitations in our study. (1) Female K18-hACE2 and 129S2 mice were used to allow for group caging, and some of our studies had smaller cohorts, due to animal availability. Follow-up experiments in male mice and with larger cohorts are needed to extend these results. (2) We used a BA.1 Omicron isolate that lacks an R346K mutation; this substitution might further affect vaccine-induced virus neutralization and protection. Although we analyzed antibody responses against BA.2, challenge studies with this strain need to be performed. (3) Our analysis did not account for cross-reactive T cell responses, which could impact protective immunity. (4) BA.1 and BA.2 viruses are less pathogenic in mice (Abdelnabi et al., 2022; Bentley et al., 2021; Halfmann et al., 2022), which could lead to overestimation of protection. Although the attenuated disease caused by Omicron strains in mice is a limitation, we nonetheless observe parallels to human studies (Andrews et al., 2022), including breakthrough infection with two mRNA vaccine doses and enhanced protection with a booster dose. (5) We analyzed mRNA-1273 and mRNA-1273.529 booster responses and protection 1 month after administration. A time course analysis is needed to assess the durability of the enhanced neutralizing antibody responses and protection against Omicron viruses. In humans boosted with the BNT162b2 mRNA vaccine, neutralization of BA.1 by serum antibodies remained robust 4 months later (Xia et al., 2022). (6) Vaccination, boosting, and Omicron virus challenge studies in other animal models and ultimately humans are required for corroboration. (7) We did not evaluate the impact of mRNA-1273 and mRNA-1273.529 boosting on mucosal immune responses, which are more challenging to achieve with intramuscularly delivered vaccines. These responses likely limit virus transmission.

In summary, our studies in mice show protection against BA.1 infection and resultant inflammation when mRNA-1273 or BA.1-matched mRNA-1273.529 boosters are administered. Although the low-dose primary immunization series of mRNA-1273 protected against WA1/2020 challenge, we observed a loss of

serum neutralizing activity against BA.1, and this was associated with breakthrough infection in the lung after BA.1 challenge. Despite diminished cross-variant neutralization responses by BA.1-matched and historical mRNA vaccines when administered as primary series immunizations, third doses improved responses against BA.1 and BA.2, with slightly better protection conferred by the BA.1-matched mRNA-1273.529 booster. Boosting with historical vaccines, variant-matched mRNA vaccines (Choi et al., 2021; Ying et al., 2022) or possibly heterologous platforms targeting historical spike proteins could minimize Omicron or other variant breakthrough infections by increasing the magnitude of neutralizing anti-SARS-CoV-2 antibodies (Atmar et al., 2021; Munro et al., 2021) or expanding the breadth of the antibody repertoire (Falsey et al., 2021; Naranbhai et al., 2022; Wang et al., 2021). Although our data suggest that certain cohorts (those with lower starting antibody responses) might benefit more from an Omicron-matched vaccine, studies evaluating the magnitude and durability of the boosted immune responses are needed, especially in vulnerable populations including the elderly and immunocompromised.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
 - Lead contact
 - Materials availability
 - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
 - Cells
 - Viruses
 - Mice
- METHOD DETAILS
 - Pre-clinical vaccine mRNA and lipid nanoparticle production process
 - Viral antigens
 - ELISA
 - Focus reduction neutralization test
 - Pseudovirus neutralization assay
 - Mouse experiments
 - Measurement of viral burden
 - Cytokine and chemokine protein measurements
 - Lung histology
- QUANTIFICATION AND STATISTICAL ANALYSES

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cell.2022.03.037>.

ACKNOWLEDGMENTS

This study was supported by the NIH (R01 AI157155, NIAID Centers of Excellence for Influenza Research and Response (CEIRR) contracts HHSN272201400008C, 75N93021C00014, and 75N93019C00051). We thank Marciela DeGrace for help in study design, Pei-Yong Shi for the WA1/2020

strains, and Peter Halfmann and Yoshihiro Kawaoka for the BA.1 and BA.2 isolates. We acknowledge the Pulmonary Morphology Core at Washington University School of Medicine for tissue sectioning and slide imaging and thank Michael Whitt for support on VSV-based Pseudovirus production.

AUTHOR CONTRIBUTIONS

B.Y. and S.M.S. performed and analyzed live virus neutralization assays. B.Y., S.M.S., B.W., C.-Y.L., O.D., S.M., Z.C., and J.B.C. performed mouse experiments. B.W. and B.Y. performed and analyzed viral burden analyses. B.Y., L.M., T.T.M.K., C.E.S., and A.W. performed ELISA binding experiments and analysis. G.-Y.C., G.S.-J., I.R., and Y.-T.L. performed variant monitoring and mRNA-1273.529 variant vaccine design and quality control. A.M. enabled rapid preclinical production of mRNA-1273.529 vaccine material. K.W., D.L., D.M.B., and L.E.A. performed pseudovirus neutralization assays. A.C., S.M.E., and D.K.E. provided mRNA vaccines and helped design experiments. L.B.T. and M.S.D. designed studies and supervised the research. M.S.D. and L.B.T. wrote the initial draft, with the other authors providing editorial comments.

DECLARATION OF INTERESTS

M.S.D. is a consultant for Inbios, Vir Biotechnology, Senda Biosciences, and Carnival Corporation, and on the scientific advisory boards of Moderna and Immunome. The Diamond laboratory has received unrelated funding support in sponsored research agreements from J. Virol. Biotechnol., Kaleido, and Emergent BioSolutions and past support from Moderna not related to these studies. K.W., D.L., L.E.A., L.M., T.K., C.S., A.W., A.C., S.M.E., G.-Y.C., G.S.-J., I.R., A.M., Y.-T.L., and D.K.E. are employees of and shareholders in Moderna.

Received: February 8, 2022

Revised: March 16, 2022

Accepted: March 23, 2022

Published: March 28, 2022

REFERENCES

- Abdelnabi, R., Foo, C.S., Zhang, X., Lemmens, V., Maes, P., Slechten, B., Raymenants, J., André, E., Weynand, B., Dallmeier, K., et al. (2022). The omicron (B.1.1.529) SARS-CoV-2 variant of concern does not readily infect Syrian hamsters. *Antiviral Res.* 198, 2021.2012.2024.474086.
- Amanat, F., Thapa, M., Lei, T., Ahmed, S.M.S., Adelsberg, D.C., Carreño, J.M., Strohmeier, S., Schmitz, A.J., Zafar, S., Zhou, J.Q., et al. (2021). SARS-CoV-2 mRNA vaccination induces functionally diverse antibodies to NTD, RBD, and S2. *Cell* 184, 3936–3948.e10.
- Anderson, E.J., Roupael, N.G., Widge, A.T., Jackson, L.A., Roberts, P.C., Makhene, M., Chappell, J.D., Denison, M.R., Stevens, L.J., Pruijssers, A.J., et al. (2020). Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. *N. Engl. J. Med.* 383, 2427–2438.
- Andrews, N., Stowe, J., Kirsebom, F., Toft, S., Rickeard, T., Gallagher, E., Gower, C., Kall, M., Groves, N., O'Connell, A.M., et al. (2022). Covid-19 vaccine effectiveness against the omicron (B.1.1.529) variant. *N. Engl. J. Med.* Published online March 2, 2022. <https://doi.org/10.1056/NEJMoa2119451>.
- Atmar, R.L., Lyke, K.E., Deming, M.E., Jackson, L.A., Branche, A.R., El Sahly, H.M., Rostad, C.A., Martin, J.M., Johnston, C., Rupp, R.E., et al. (2021). Heterologous SARS-CoV-2 booster vaccinations - preliminary report. Preprint at medRxiv. <https://doi.org/10.1101/2021.10.10.21264827>.
- Atmar, R.L., Lyke, K.E., Deming, M.E., Jackson, L.A., Branche, A.R., El Sahly, H.M., Rostad, C.A., Martin, J.M., Johnston, C., Rupp, R.E., et al. (2022). Homologous and heterologous Covid-19 booster vaccinations. *N. Engl. J. Med.* 386, 1046–1057.
- Barnes, C.O., Jette, C.A., Abernathy, M.E., Dam, K.A., Esswein, S.R., Gristick, H.B., Malyutin, A.G., Sharaf, N.G., Huey-Tubman, K.E., Lee, Y.E., et al. (2020).

- SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. *Nature* 588, 682–687.
- Bar-On, Y.M., Goldberg, Y., Mandel, M., Bodenheimer, O., Freedman, L., Kalkstein, N., Mizrahi, B., Alroy-Preis, S., Ash, N., Milo, R., et al. (2021). Protection of BNT162b2 vaccine booster against Covid-19 in Israel. *N. Engl. J. Med.* 385, 1393–1400.
- Bartsch, Y., Tong, X., Kang, J., Avendaño, M.J., Serrano, E.F., García-Salum, T., Pardo-Roa, C., Riquelme, A., Medina, R.A., and Alter, G. (2021). Preserved Omicron Spike specific antibody binding and Fc-recognition across COVID-19 vaccine platforms. Preprint at medRxiv. <https://doi.org/10.1101/2021.12.24.21268378>.
- Bentley, E.G., Kirby, A., Sharma, P., Kipar, A., Mega, D.F., Bramwell, C., Penrice-Randal, R., Prince, T., Brown, J.C., Zhou, J., et al. (2021). SARS-CoV-2 Omicron-B.1.1.529 Variant leads to less severe disease than Pango B and Delta variants strains in a mouse model of severe COVID-19. Preprint at bioRxiv, 2021.2012.2026.474085.
- Buchan, S.A., Chung, H., Brown, K.A., Austin, P.C., Fell, D.B., Gubbay, J.B., Nasreen, S., Schwartz, K.L., Sundaram, M.E., Tadrous, M., et al. (2022). Effectiveness of COVID-19 vaccines against Omicron or Delta infection. Preprint at medRxiv, 2021.2012.2030.21268565.
- Cameroni, E., Bowen, J.E., Rosen, L.E., Saliba, C., Zepeda, S.K., Culap, K., Pinto, D., VanBlargan, L.A., De Marco, A., di Iulio, J., et al. (2022). Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron antigenic shift. *Nature* 602, 664–670.
- Cao, Y., Su, B., Guo, X., Sun, W., Deng, Y., Bao, L., Zhu, Q., Zhang, X., Zheng, Y., Geng, C., et al. (2020). Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent patients' B cells. *Cell* 182, 73–84.e16.
- Cao, Y., Wang, J., Jian, F., Xiao, T., Song, W., Yisimayi, A., Huang, W., Li, Q., Wang, P., An, R., et al. (2022). Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. *Nature* 602, 657–663.
- Case, J.B., Bailey, A.L., Kim, A.S., Chen, R.E., and Diamond, M.S. (2020a). Growth, detection, quantification, and inactivation of SARS-CoV-2. *Virology* 548, 39–48.
- Case, J.B., Rothlauf, P.W., Chen, R.E., Liu, Z., Zhao, H., Kim, A.S., Bloyet, L.M., Zeng, Q., Tahan, S., Droit, L., et al. (2020b). Neutralizing antibody and soluble ACE2 inhibition of a replication-competent VSV-SARS-CoV-2 and a clinical isolate of SARS-CoV-2. *Cell Host Microbe* 28, 475–485.e5.
- Case, J.B., Rothlauf, P.W., Chen, R.E., Kafai, N.M., Fox, J.M., Smith, B.K., Shrihari, S., McCune, B.T., Harvey, I.B., Keeler, S.P., et al. (2020c). Replication-Competent Vesicular Stomatitis Virus Vaccine Vector Protects against SARS-CoV-2-Mediated Pathogenesis in Mice. *Cell Host Microbe* 28, 465–474.e4.
- Cele, S., Jackson, L., Khan, K., Khoury, D.S., Moyo-Gwete, T., Tegally, H., Scheepers, C., Amoako, D., Karim, F., Bernstein, M., et al. (2021). SARS-CoV-2 Omicron has extensive but incomplete escape of Pfizer BNT162b2 elicited neutralization and requires ACE2 for infection. Preprint at medRxiv, 2021.2012.2008.21267417.
- Chen, R.E., Gorman, M.J., Zhu, D.Y., Carreño, J.M., Yuan, D., VanBlargan, L.A., Burdess, S., Lauffenburger, D.A., Kim, W., Turner, J.S., et al. (2021a). Reduced antibody activity against SARS-CoV-2 B.1.617.2 delta virus in serum of mRNA-vaccinated individuals receiving tumor necrosis factor- α inhibitors. *Med (N Y)* 2, 1327–1341.e4.
- Chen, R.E., Winkler, E.S., Case, J.B., Aziati, I.D., Bricker, T.L., Joshi, A., Darling, T.L., Ying, B., Errico, J.M., Shrihari, S., et al. (2021b). In vivo monoclonal antibody efficacy against SARS-CoV-2 variant strains. *Nature* 596, 103–108.
- Chen, R.E., Zhang, X., Case, J.B., Winkler, E.S., Liu, Y., VanBlargan, L.A., Liu, J., Errico, J.M., Xie, X., Suryadevara, N., et al. (2021c). Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal antibodies. *Nat. Med.* 27, 717–726.
- Choi, A., Koch, M., Wu, K., Chu, L., Ma, L., Hill, A., Nunna, N., Huang, W., Oestreicher, J., Colpitts, T., et al. (2021). Safety and immunogenicity of SARS-CoV-2 variant mRNA vaccine boosters in healthy adults: an interim analysis. *Nat. Med.* 27, 2025–2031.
- Christensen, P.A., Olsen, R.J., Long, S.W., Snehal, R., Davis, J.J., Ojeda Saavedra, M., Reppond, K., Shyer, M.N., Cambric, J., Gadd, R., et al. (2022). Signals of significantly increased vaccine breakthrough, decreased hospitalization rates, and less severe disease in patients with COVID-19 caused by the Omicron variant of SARS-CoV-2 in Houston, Texas. Preprint at medRxiv, 2021.2012.2030.21268560.
- Corbett, K.S., Edwards, D.K., Leist, S.R., Abiona, O.M., Boyoglu-Barnum, S., Gillespie, R.A., Himansu, S., Schäfer, A., Ziwawo, C.T., DiPiazza, A.T., et al. (2020). SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. *Nature* 586, 567–571.
- Dejnirattisai, W., Huo, J., Zhou, D., Zahradnik, J., Supasa, P., Liu, C., Duyvesteyn, H.M.E., Ginn, H.M., Mentzer, A.J., Tuekprakhon, A., et al. (2022). SARS-CoV-2 Omicron-B.1.1.529 leads to widespread escape from neutralizing antibody responses. *Cell* 185, 467–484.e15.
- Edara, V.V., Manning, K.E., Ellis, M., Lai, L., Moore, K.M., Foster, S.L., Floyd, K., Davis-Gardner, M.E., Mantus, G., Nyhoff, L.E., et al. (2022). mRNA-1273 and BNT162b2 mRNA vaccines have reduced neutralizing activity against the SARS-CoV-2 omicron variant. *Cell Rep. Med.*, 100529.
- Elliott, P., Bodinier, B., Eales, O., Wang, H., Haw, D., Elliott, J., Whitaker, M., Jonnerby, J., Tang, D., Walters, C.E., et al. (2021). Rapid increase in Omicron infections in England during December 2021: REACT-1 study. Preprint at medRxiv, 2021.2012.2022.21268252.
- Evans, J.P., Zeng, C., Carlin, C., Lozanski, G., Saif, L.J., Oltz, E.M., Gumina, R.J., and Liu, S.L. (2021). Loss of neutralizing antibody response to mRNA vaccination against SARS-CoV-2 variants: differing kinetics and strong boosting by breakthrough infection. Preprint at bioRxiv. <https://doi.org/10.1101/2021.12.06.471455>.
- Falsey, A.R., Frenck, R.W., Walsh, E.E., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Bailey, R., Swanson, K.A., Xu, X., et al. (2021). SARS-CoV-2 neutralization with BNT162b2 vaccine Dose 3. *N. Engl. J. Med.* 385, 1627–1629.
- Gagne, M., Corbett, K.S., Flynn, B.J., Foulds, K.E., Wagner, D.A., Andrew, S.F., Todd, J.M., Honeycutt, C.C., McCormick, L., Nurmukhambetova, S.T., et al. (2021). Protection from SARS-CoV-2 Delta one year after mRNA-1273 vaccination in nonhuman primates is coincident with an anamnestic antibody response in the lower airway. Preprint at bioRxiv, 2022.2002.2003.479037.
- Gao, Y., Cai, C., Grifoni, A., Müller, T.R., Niessl, J., Olofsson, A., Humbert, M., Hansson, L., Österborg, A., Bergman, P., et al. (2022). Ancestral SARS-CoV-2-specific T cells cross-recognize the Omicron variant. *Nat. Med.* 28, 472–476.
- Gu, H., Chen, Q., Yang, G., He, L., Fan, H., Deng, Y.Q., Wang, Y., Teng, Y., Zhao, Z., Cui, Y., et al. (2020). Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine efficacy. *Science* 369, 1603–1607.
- Halfmann, P.J., Iida, S., Iwatsuki-Horimoto, K., Maemura, T., Kiso, M., Scheaffer, S.M., Darling, T.L., Joshi, A., Loeber, S., Singh, G., et al. (2022). SARS-CoV-2 Omicron virus causes attenuated disease in mice and hamsters. *Nature* 603, 687–692.
- Hassett, K.J., Benenato, K.E., Jacquinet, E., Lee, A., Woods, A., Yuzhakov, O., Himansu, S., Deterling, J., Geilich, B.M., Ketova, T., et al. (2019). Optimization of lipid nanoparticles for intramuscular administration of mRNA vaccines. *Mol. Ther. Nucleic Acids* 15, 1–11.
- Imai, M., Iwatsuki-Horimoto, K., Hatta, M., Loeber, S., Halfmann, P.J., Nakajima, N., Watanabe, T., Ujje, M., Takahashi, K., Ito, M., et al. (2020). Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. *Proc. Natl. Acad. Sci. USA* 117, 16587–16595.
- Keeton, R., Tincho, M.B., Ngomti, A., Baguma, R., Benede, N., Suzuki, A., Khan, K., Cele, S., Bernstein, M., Karim, F., et al. (2022). T cell responses to SARS-CoV-2 spike cross-recognize Omicron. *Nature* 603, 488–492.
- Khoury, D.S., Cromer, D., Reynaldi, A., Schlub, T.E., Wheatley, A.K., Juno, J.A., Subbarao, K., Kent, S.J., Triccas, J.A., and Davenport, M.P. (2021). Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat. Med.* 27, 1205–1211.

- Krause, P.R., Fleming, T.R., Longini, I.M., Peto, R., Briand, S., Heymann, D.L., Beral, V., Snape, M.D., Rees, H., Roper, A.M., et al. (2021). SARS-CoV-2 variants and vaccines. *N. Engl. J. Med.* **385**, 179–186.
- Lee, I.-J., Sun, C.-P., Wu, P.-Y., Lan, Y.-H., Wang, I.-H., Liu, W.-C., Tseng, S.-C., Tsung, S.-I., Chou, Y.-C., Kumar, M., et al. (2022). Omicron-specific mRNA vaccine induced potent neutralizing antibody against Omicron but not other SARS-CoV-2 variants. Preprint at bioRxiv, 2022.2001.2031.478406.
- Letko, M., Marzi, A., and Munster, V. (2020). Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat. Microbiol.* **5**, 562–569.
- Liu, L., Iketani, S., Guo, Y., Chan, J.F., Wang, M., Liu, L., Luo, Y., Chu, H., Huang, Y., Nair, M.S., et al. (2022). Striking antibody evasion manifested by the omicron variant of SARS-CoV-2. *Nature* **602**, 676–681.
- Liu, Y., Hu, G., Wang, Y., Ren, W., Zhao, X., Ji, F., Zhu, Y., Feng, F., Gong, M., Ju, X., et al. (2021b). Functional and genetic analysis of viral receptor ACE2 orthologs reveals a broad potential host range of SARS-CoV-2. *Proc. Natl. Acad. Sci. USA* **118**, e2025373118.
- Munro, A.P.S., Janani, L., Cornelius, V., Aley, P.K., Babbage, G., Baxter, D., Bula, M., Cathie, K., Chatterjee, K., Dodd, K., et al. (2021). Safety and immunogenicity of seven COVID-19 vaccines as a third dose (booster) following two doses of ChAdOx1 nCoV-19 or BNT162b2 in the UK (COV-BOOST): a blinded, multicentre, randomised, controlled, phase 2 trial. *Lancet* **398**, 2258–2276.
- Naranbhai, V., St Denis, K.J., Lam, E.C., Ofoman, O., Garcia-Beltran, W.F., Mairena, C.B., Bhan, A.K., Gainor, J.F., Balazs, A.B., Iafate, A.J., et al. (2022). Neutralization breadth of SARS-CoV-2 viral variants following primary series and booster SARS-CoV-2 vaccines in patients with cancer. *Cancer Cell* **40**, 103–108.e2.
- Nelson, J., Sorensen, E.W., Mintri, S., Rabideau, A.E., Zheng, W., Besin, G., Khatwani, N., Su, S.V., Miracco, E.J., Issa, W.J., et al. (2020). Impact of mRNA chemistry and manufacturing process on innate immune activation. *Sci. Adv.* **6**, eaaz6893.
- Pajon, R., Doria-Rose, N.A., Shen, X., Schmidt, S.D., O'Dell, S., McDanal, C., Feng, W., Tong, J., Eaton, A., Magliano, M., et al. (2022). SARS-CoV-2 omicron variant neutralization after mRNA-1273 booster vaccination. *N. Engl. J. Med.* **386**, 1088–1091.
- Pinto, D., Park, Y.J., Beltramello, M., Walls, A.C., Tortorici, M.A., Bianchi, S., Jaconi, S., Culap, K., Zatta, F., De Marco, A., et al. (2020). Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* **583**, 290–295.
- Plante, J.A., Liu, Y., Liu, J., Xia, H., Johnson, B.A., Lokugamage, K.G., Zhang, X., Muruato, A.E., Zou, J., Fontes-Garfias, C.R., et al. (2021). Spike mutation D614G alters SARS-CoV-2 fitness. *Nature* **592**, 116–121.
- Rathe, J.A., Hemann, E.A., Eggenberger, J., Li, Z., Knoll, M.L., Stokes, C., Hsiang, T.Y., Netland, J., Takehara, K.K., Pepper, M., et al. (2021). SARS-CoV-2 serologic assays in control and unknown populations demonstrate the necessity of virus neutralization testing. *J. Infect. Dis.* **223**, 1120–1131.
- Rathnasinghe, R., Jangra, S., Cupic, A., Martínez-Romero, C., Mulder, L.C.F., Kehrer, T., Yildiz, S., Choi, A., Mena, I., De Vrieze, J., et al. (2021). The N501Y mutation in SARS-CoV-2 spike leads to morbidity in obese and aged mice and is neutralized by convalescent and post-vaccination human sera. Preprint at medRxiv. <https://doi.org/10.1101/2021.01.19.21249592>.
- Rössler, A., Knabl, L., Laer, D.v., and Kimpel, J. (2022). Neutralization profile of Omicron variant convalescent individuals. Preprint at medRxiv, 2022.2002.2001.22270263.
- Shuai, H., Chan, J.F., Hu, B., Chai, Y., Yuen, T.T., Yin, F., Huang, X., Yoon, C., Hu, J.C., Liu, H., et al. (2022). Attenuated replication and pathogenicity of SARS-CoV-2 B.1.1.529 Omicron. *Nature* **603**, 693–699.
- Stadlbauer, D., Amanat, F., Chromikova, V., Jiang, K., Strohmaier, S., Arunkumar, G.A., Tan, J., Bhavsar, D., Capuano, C., Kirkpatrick, E., et al. (2020). SARS-CoV-2 seroconversion in humans: A detailed protocol for a serological assay, antigen production, and test setup. *Curr. Protoc. Microbiol.* **57**, e100.
- Tortorici, M.A., Beltramello, M., Lempp, F.A., Pinto, D., Dang, H.V., Rosen, L.E., McCallum, M., Bowen, J., Minola, A., Jaconi, S., et al. (2020). Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. *Science* **370**, 950–957.
- VanBlargan, L.A., Adams, L.J., Liu, Z., Chen, R.E., Gilchuk, P., Raju, S., Smith, B.K., Zhao, H., Case, J.B., Winkler, E.S., et al. (2021). A potentially neutralizing SARS-CoV-2 antibody inhibits variants of concern by utilizing unique binding residues in a highly conserved epitope. *Immunity* **54**, 2399–2416.e6.
- VanBlargan, L.A., Errico, J.M., Halfmann, P.J., Zost, S.J., Crowe, J.E., Jr., Purcell, L.A., Kawaoka, Y., Corti, D., Fremont, D.H., and Diamond, M.S. (2022). An infectious SARS-CoV-2 B.1.1.529 Omicron virus escapes neutralization by therapeutic monoclonal antibodies. *Nat. Med.* **28**, 490–495.
- Waltz, E. (2022). Does the world need an Omicron vaccine? What researchers say. *Nature* **602**, 192–193.
- Wang, Z., Muecksch, F., Schaefer-Babajew, D., Finkin, S., Viant, C., Gaebler, C., Hoffmann, H.H., Barnes, C.O., Cipolla, M., Ramos, V., et al. (2021). Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. *Nature* **595**, 426–431.
- Whitt, M.A. (2010). Generation of VSV pseudotypes using recombinant ΔG-VSV for studies on virus entry, identification of entry inhibitors, and immune responses to vaccines. *J. Virol. Methods* **169**, 365–374.
- Widge, A.T., Roupaphel, N.G., Jackson, L.A., Anderson, E.J., Roberts, P.C., Makhene, M., Chappell, J.D., Denison, M.R., Stevens, L.J., Pruijssers, A.J., et al. (2021). Durability of responses after SARS-CoV-2 mRNA-1273 vaccination. *N. Engl. J. Med.* **384**, 80–82.
- Wilhelm, A., Widera, M., Grikscheit, K., Toptan, T., Schenk, B., Pallas, C., Metzler, M., Kohmer, N., Hoehl, S., Helfritz, F.A., et al. (2021). Reduced neutralization of SARS-CoV-2 omicron variant by vaccine sera and monoclonal antibodies. Preprint at medRxiv, 2021.2012.2007.21267432.
- Winkler, E.S., Bailey, A.L., Kafai, N.M., Nair, S., McCune, B.T., Yu, J., Fox, J.M., Chen, R.E., Earnest, J.T., Keeler, S.P., et al. (2020). SARS-CoV-2 infection of human ACE2-transgenic mice causes severe lung inflammation and impaired function. *Nat. Immunol.* **21**, 1327–1335.
- Wu, K., Werner, A.P., Koch, M., Choi, A., Narayanan, E., Stewart-Jones, G.B.E., Colpitts, T., Bennett, H., Boyoglu-Barnum, S., Shi, W., et al. (2021). Serum neutralizing activity elicited by mRNA-1273 vaccine. *N. Engl. J. Med.* **384**, 1468–1470.
- Xia, H., Zou, J., Kurhade, C., Cai, H., Yang, Q., Cutler, M., Cooper, D., Muik, A., Jansen, K.U., Xie, X., et al. (2022). Neutralization and durability of 2 or 3 doses of the BNT162b2 vaccine against Omicron SARS-CoV-2. *Cell Host Microbe*. <https://doi.org/10.1016/j.chom.2022.02.015>.
- Ying, B., Whitener, B., VanBlargan, L.A., Hassan, A.O., Shrihari, S., Liang, C.Y., Karl, C.E., Mackin, S., Chen, R.E., Kafai, N.M., et al. (2022). Protective activity of mRNA vaccines against ancestral and variant SARS-CoV-2 strains. *Sci. Transl. Med.* **14**, eabm3302.
- Yoshihiro, K., Ryuta, U., Maki, K., Shun, I., Masaki, I., Emi, T., Makoto, K., Peter, H., Samantha, L., Tadashi, M., et al. (2022). Characterization and antiviral susceptibility of SARS-CoV-2 Omicron/BA.2. Nature portfolio. <https://doi.org/10.21203/rs.3.rs-1375091/v1>.
- Zang, R., Gomez Castro, M.F., McCune, B.T., Zeng, Q., Rothlauf, P.W., Sonnek, N.M., Liu, Z., Brulois, K.F., Wang, X., Greenberg, H.B., et al. (2020). TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. *Sci. Immunol.* **5**, eabc3582.
- Zost, S.J., Gilchuk, P., Chen, R.E., Case, J.B., Reidy, J.X., Trivette, A., Nargi, R.S., Sutton, R.E., Suryadevara, N., Chen, E.C., et al. (2020). Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein. *Nat. Med.* **26**, 1422–1427.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
SARS2-02	VanBlargan et al., 2021	N/A
SARS2-08	VanBlargan et al., 2021	N/A
SARS2-09	VanBlargan et al., 2021	N/A
SARS2-10	VanBlargan et al., 2021	N/A
SARS2-11	VanBlargan et al., 2021	N/A
SARS2-13	This paper	N/A
SARS2-14	VanBlargan et al., 2021	N/A
SARS2-16	VanBlargan et al., 2021	N/A
SARS2-17	This paper	N/A
SARS2-20	This paper	N/A
SARS2-26	This paper	N/A
SARS2-27	VanBlargan et al., 2021	N/A
SARS2-28	This paper	N/A
SARS2-31	VanBlargan et al., 2021	N/A
SARS2-38	VanBlargan et al., 2021	N/A
SARS2-41	VanBlargan et al., 2021	N/A
SARS2-42	VanBlargan et al., 2021	N/A
SARS2-49	VanBlargan et al., 2021	N/A
SARS2-57	VanBlargan et al., 2021	N/A
SARS2-62	VanBlargan et al., 2021	N/A
SARS2-64	This paper	N/A
SARS2-65	VanBlargan et al., 2021	N/A
SARS2-67	VanBlargan et al., 2021	N/A
SARS2-71	VanBlargan et al., 2021	N/A
Goat anti-mouse IgG, human adsorbed-HRP	Southern Biotech	Cat # 1030-05; RRID: AB_2619742
Anti-mouse IgG-HRP	Sigma	A8924; RRID:AB_258426
Bacterial and virus strains		
VSV-SARS-CoV-2 Pseudoviruses	Case et al., 2020b and Moderna	N/A
SARS-CoV-2 D614G	Chen et al., 2021c	N/A
SARS-CoV-2 N501Y/D614G	Chen et al., 2021c	N/A
SARS-CoV-2 B.1.351	Chen et al., 2021c	N/A
SARS-CoV-2 BA.1	Kawaoka Laboratory (University of Wisconsin)	N/A
SARS-CoV-2 BA.2	Kawaoka Laboratory (University of Wisconsin)	N/A
Chemicals, peptides, and recombinant proteins		
SARS-CoV-2 Wuhan spike protein	Moderna	N/A
SARS-CoV-2 BA.1 spike	Moderna	N/A
mRNA -1273, mRNA1273.529	Moderna	N/A
Experimental models: Cell lines		
Vero+TMPRSS2	Chen et al., 2021	N/A
Vero+TMPRSS2+ACE2	Chen et al., 2021	N/A

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Organisms/strains		
K18-hACE2 transgenic mice	Jackson laboratory	strain # 34860
129S2/SvPasCrl	Charles River	Strain 287
BALB/c	Charles River	Strain 028
Oligonucleotides		
SARS-CoV-2 qPCR primer 5'- ATGCTGCAATCGTGCTACAA-3'	Case et al., 2020c	N/A
SARS-CoV-2 qPCR primer 5'- GACTGCCGCCTCTGCTC-3'	Case et al., 2020c	N/A
SARS-CoV-2 qPCR probe 5'-/56-FAM/ TCAAGGAAC/Zen/ AACATTGCCAA/3IABkFQ-3'	Case et al., 2020c	N/A
Software and algorithms		
GraphPad Prism	GraphPad	v 8.4.3
Adobe Illustrator	Adobe	V 24.2.3
NDP.View2	Hamamatsu	N/A
Biorender	Biorender.com	N/A

RESOURCE AVAILABILITY**Lead contact**

Further information and requests for resources and reagents should be directed to the lead contact, Michael S. Diamond (mdiamond@wustl.edu).

Materials availability

All requests for resources and reagents should be directed to the Lead Contact author. This includes viruses, vaccines, and primer-probe sets. All reagents will be made available on request after completion of a Materials Transfer Agreement (MTA). The mRNA vaccines (control, mRNA-1273, and mRNA-1273.529) can be obtained under an MTA with Moderna (contact: Darin Edwards, darin.edwards@modernatx.com).

Data and code availability

All data supporting the findings of this study are available within the paper and are available from the corresponding author upon request. This paper does not include original code. 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**Cells**

African green monkey Vero-TMPRSS2 ([Zang et al., 2020](#)) and Vero-hACE2-TMPRSS2 ([Chen et al., 2021c](#)) cells were cultured at 37°C in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 10 mM HEPES pH 7.3, 1 mM sodium pyruvate, 1 × non-essential amino acids, and 100 U/mL of penicillin–streptomycin. Vero-TMPRSS2 cells were supplemented with 5 µg/mL of blasticidin. Vero-hACE2-TMPRSS2 cells were supplemented with 10 µg/mL of puromycin. All cells routinely tested negative for mycoplasma using a PCR-based assay.

Viruses

The WA1/2020 recombinant strain with D614G substitution was described previously ([Plante et al., 2021](#)). The BA.1 (B.1.1.529) isolate (hCoV-19/USA/WI-WSLH-221686/2021) was obtained from an individual in Wisconsin as a mid-turbinate nasal swab and passaged once on Vero-TMPRSS2 cells ([Imai et al., 2020](#)). The BA.2 isolate (CoV-19/Japan/UT-NCD1288-2N/2022) was obtained from a subject in Japan as a generous gift of Y. Kawaoka (University of Wisconsin). The B.1.351 isolate has been described previously ([Chen et al., 2021b](#)). All viruses were subjected to next-generation sequencing ([Chen et al., 2021c](#)) to confirm the introduction and stability of substitutions. All virus experiments were performed in an approved biosafety level 3 (BSL-3) facility.

Mice

Animal studies were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. For studies (K18-hACE2 and 129S2 mice) at Washington University School of Medicine, the

protocols were approved by the Institutional Animal Care and Use Committee at the Washington University School of Medicine (assurance number A3381-01). Virus inoculations were performed under anesthesia that was induced and maintained with ketamine hydrochloride and xylazine, and all efforts were made to minimize animal suffering. For studies with BALB/c mice, animal experiments were carried out in compliance with approval from the Animal Care and Use Committee of Moderna, Inc. Sample size for animal experiments was determined on the basis of criteria set by the institutional Animal Care and Use Committee. Experiments were neither randomized nor blinded.

Heterozygous K18-hACE2 C57BL/6J mice (strain: 2B6.Cg-Tg(K18-ACE2)2PrImn/J, Cat # 34860) were obtained from The Jackson Laboratory. 129S2 mice (strain: 129S2/SvPasCrl, Cat # 287) and BALB/c mice (strain: BALB/cAnNCrl, Cat # 028) were obtained from Charles River Laboratories. Animals were housed in groups and fed standard chow diets.

METHOD DETAILS

Pre-clinical vaccine mRNA and lipid nanoparticle production process

A sequence-optimized mRNA encoding prefusion-stabilized Wuhan-Hu-1 (mRNA-1273) SARS-CoV-2 S-2P or BA.1 (mRNA-1273.529) protein was synthesized *in vitro* using an optimized T7 RNA polymerase-mediated transcription reaction with complete replacement of uridine by N1m-pseudouridine (Nelson et al., 2020). The pre-clinical mRNA-1273.529 vaccine encoded the following substitutions: A67V, Δ 69-70, T95I, G142D, Δ 143-145, Δ 211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493K, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F. A non-translating control mRNA was synthesized and formulated into lipid nanoparticles as previously described (Corbett et al., 2020). The reaction included a DNA template containing the immunogen open-reading frame flanked by 5' untranslated region (UTR) and 3' UTR sequences, and was terminated by an encoded polyA tail. After RNA transcription, the cap-1 structure was added using the vaccinia virus capping enzyme and 2'-O-methyltransferase (New England Biolabs). The mRNA was purified by oligo-dT affinity purification, buffer exchanged by tangential flow filtration into sodium acetate, pH 5.0, sterile filtered, and kept frozen at -20°C until further use.

The mRNA was encapsulated in a lipid nanoparticle through a modified ethanol-drop nanoprecipitation process described previously (Hassett et al., 2019). Ionizable, structural, helper, and polyethylene glycol lipids were briefly mixed with mRNA in an acetate buffer, pH 5.0, at a ratio of 2.5:1 (lipid:mRNA). The mixture was neutralized with Tris-HCl, pH 7.5, sucrose was added as a cryoprotectant, and the final solution was sterile-filtered. Vials were filled with formulated lipid nanoparticle and stored frozen at -20°C until further use. The pre-clinical vaccine product underwent analytical characterization, which included the determination of particle size and polydispersity, encapsulation, mRNA purity, double-stranded RNA content, osmolality, pH, endotoxin, and bioburden, and the material was deemed acceptable for *in vivo* study. The mRNA-1273 and mRNA-1273.529 preclinical drug products used in this study are identical in sequence and prepared with similar methods to the clinical mRNA-1273 and mRNA-1273.529 Drug Products.

Viral antigens

Recombinant soluble S and RBD proteins from Wuhan-1, BA.1, B.1.351 and B.1.617.2 SARS-CoV-2 strains were expressed as described (Amanat et al., 2021; Stadlbauer et al., 2020). Recombinant proteins were produced in Expi293F cells (ThermoFisher) by transfection of DNA using the ExpiFectamine 293 Transfection Kit (ThermoFisher). Supernatants were harvested 3 days post-transfection, and recombinant proteins were purified using Ni-NTA agarose (ThermoFisher), then buffer exchanged into PBS and concentrated using Amicon Ultracel centrifugal filters (EMD Millipore). SARS-CoV-2 B.1.617.2 RBD protein was purchased from Sino Biological (Cat. # 40592-V08H90).

ELISA

Assays were performed using one of two methods: (a) 96-well microtiter plates (Thermo Fisher) were coated with 100 μL of recombinant Wuhan-1, BA.1, B.1.351, or B.1.617.2 spike or RBD proteins. Plates were incubated at 4°C overnight and then blocked for 1.5 h at 4°C using SuperBlock (Thermo). Sera were serially diluted in 5% goat serum in PBST, added to plates, incubated for 2 h at 37°C , and then washed 3 times with PBST. Goat anti-mouse IgG-HRP (Southern Biotech Cat. #1030-05) was diluted in 5% goat serum in PBST before adding to the wells and incubating for 1 h at 37°C . Plates were washed 3 times with PBST before the addition of 3,3',5,5'-tetramethylbenzidine (TMB) substrate (Thermo). Reactions were stopped by the addition of TMB stop solution (Sera Care Cat. #5150-0021). Optical density (OD) measurements were taken at 450 nm, and titers were determined using a 4-parameter logistic curve fit in Prism v.8 (GraphPad 112 Software, Inc.) and defined as the reciprocal dilution at approximately optical density 450 of 1 (normalized to a mouse standard on each plate). (b) Purified recombinant Wuhan-1 or BA.1 spike or RBD proteins were coated onto 96-well Maxisorp clear plates at 2 $\mu\text{g}/\text{mL}$ (spike) or 4 $\mu\text{g}/\text{mL}$ (RBD) in 50 mM Na_2CO_3 pH 9.6 (50 μL) overnight at 4°C . Coating buffers were aspirated, and wells were blocked with 200 μL of 1X PBS + 0.05% Tween-20 + 2% BSA + 0.02% Na₃N (Blocking buffer, PBSTBA) overnight at 4°C . Sera or culture supernatant of an equal volume mixture of a panel of murine anti-SARS-CoV-2 mAbs that cross-react with SARS-CoV spike protein (SARS2-08, -09, -10, -13, -14, -17, -20, -26, -27, -28, -31, -41, -42, -44, -49, -62, -64, -65, and -67 (VanBlargan et al., 2021)) were serially diluted in blocking buffer and added to the plates. Plates were incubated for 1 h at room temperature and then washed 3 times with PBST, followed by addition of 50 μL of 1:2000 anti-mouse IgG-HRP (Southern Biotech Cat. #1030-05) in PBST. Following a 1 h incubation at room temperature, plates were washed 3 times

with PBST and 50 μ L of 1-Step Ultra TMB-ELISA was added (ThermoFisher Cat. #34028). Following a 5 to 10-min incubation, reactions were stopped with 50 μ L of 2 M sulfuric acid. The absorbance of each well at 450 nm was determined using a microplate reader (BioTek) within 5 min of addition of sulfuric acid. The endpoint serum dilution was calculated with curve fit analysis of optical density (OD) values for serially diluted sera with a cut-off value set to mean plus six times the standard deviation of the background signal.

Focus reduction neutralization test

Serial dilutions of sera were incubated with 10^2 focus-forming units (FFU) of WA1/2020 D614G, BA.1, BA.2, or B.1.351 for 1 h at 37°C. Antibody-virus complexes were added to Vero-TMPRSS2 cell monolayers in 96-well plates and incubated at 37°C for 1 h. Subsequently, cells were overlaid with 1% (w/v) methylcellulose in MEM. Plates were harvested 30 h (WA1/2020 D614G and B.1.1.351) or 72 h (BA.1 and BA.2) later by removing overlays and fixed with 4% PFA in PBS for 20 min at room temperature. Plates were washed and sequentially incubated with an oligoclonal pool (SARS2-02, -08, -09, -10, -11, -13, -14, -17, -20, -26, -27, -28, -31, -38, -41, -42, -44, -49, -57, -62, -64, -65, -67, and -71 (VanBlargan et al., 2021)) of anti-S murine antibodies (including cross-reactive mAbs to SARS-CoV) and HRP-conjugated goat anti-mouse IgG (Sigma Cat # A8924, RRID: AB_258426) in PBS supplemented with 0.1% saponin and 0.1% bovine serum albumin. SARS-CoV-2-infected cell foci were visualized using TrueBlue peroxidase substrate (KPL) and quantitated on an ImmunoSpot microanalyzer (Cellular Technologies).

Pseudovirus neutralization assay

Codon-optimized full-length spike genes (Wuhan-1 with D614G, B.1.351, B.1.617.2, and BA.1) were cloned into a pCAGGS vector. Spike genes contained the following mutations: B.1.351: L18F-D80A-D215G-L242-244del-R246I-K417N-E484K-N501Y-D614G-A701V; B.1.617.2: T19R-T95I-G142D-E156G-F157del-R158del-L452R-T478K-D614G-P681R-D950N; and BA.1: A67V, Δ 69-70, T95I, G142D/ Δ VYY143-145, Δ N211/L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F. To generate VSV Δ G-based SARS-CoV-2 pseudovirus, BHK-21/WI-2 cells were transfected with the spike expression plasmid and infected by VSV Δ G-firefly-luciferase as previously described (Whitt, 2010). A549-hACE2-TMPRSS2 cells were used as target cells for the neutralization assay and maintained in DMEM supplemented with 10% fetal bovine serum and 1 μ g/mL puromycin. To perform neutralization assay, mouse serum samples were heat-inactivated for 45 min at 56°C, and serial dilutions were made in DMEM supplemented with 10% FBS. The diluted serum samples or culture medium (serving as virus only control) were mixed with VSV Δ G-based SARS-CoV-2 pseudovirus and incubated at 37°C for 45 min. The inoculum virus or virus-serum mix was subsequently used to infect A549-hACE2-TMPRSS2 cells for 18 h at 37°C. At 18 h post infection, an equal volume of One-Glo reagent (Promega; E6120) was added to culture medium for readout using BMG PHERastar-FS plate reader. The percentage of neutralization was calculated based on relative light units of the virus control, and subsequently analyzed using four parameter logistic curve (Prism 8.0).

Mouse experiments

K18hACE2 transgenic mice

Seven-week-old female K18-hACE2 C57BL/6 mice were immunized three weeks apart with 5, 0.25, or 0.1 μ g of mRNA vaccines (control or mRNA-1273) in 50 μ L of PBS via intramuscular injection in the hind leg. Animals were bled three to four weeks after the second vaccine dose for immunogenicity analysis. In some experiments, four weeks after completing the primary series immunization, mice were challenged with 10^4 FFU of WA1/2020 D614G or BA.1 of SARS-CoV-2 strains by the intranasal route. In other experiments, 17 to 19 weeks after completing the primary series immunization, animals were bled for antibody analysis, and then boosted with 1 μ g of control or mRNA-1273 vaccine. Four weeks later, K18-hACE2 mice were challenged with 10^4 FFU of BA.1 by the intranasal route. For some studies, weights were measured at day 0 and 6, and in all experiments, animals were euthanized at 6 dpi. Tissues were harvested for virological, immunological, and pathological analyses.

BALB/c mice

6 to 8-week-old female BALB/c mice were immunized three weeks apart with 1 or 0.1 μ g of mRNA vaccines (mRNA-1273 or mRNA-1273.529) or PBS (in 50 μ L) via intramuscular injection in the quadriceps muscle of the hind leg under isoflurane anesthesia. Blood was sampled three weeks after the first immunization and two weeks after the second immunization, and anti-spike and neutralizing antibody levels were measured by ELISA and a VSV-based pseudovirus neutralization assay.

129S2 mice

Seven-week-old female 129S2 mice were immunized three weeks apart with 5 or 0.25 μ g of mRNA vaccines (control, mRNA-1273) in 50 μ L of PBS via intramuscular injection in the hind leg. Ten to eleven weeks later, animals were bled (pre-boost), and subsequently boosted with 1 μ g of control, mRNA-1273, or mRNA-1273.529 vaccine. Three to four weeks later, animals were bled again (post-boost), and then challenged with 10^5 FFU of BA.1 by the intranasal route. Animals were euthanized at 3 dpi, and tissues were harvested for virological and cytokine/chemokine analyses.

Measurement of viral burden

Tissues were weighed and homogenized with zirconia beads in a MagNA Lyser instrument (Roche Life Science) in 1 ml of DMEM medium supplemented with 2% heat-inactivated FBS. Tissue homogenates were clarified by centrifugation at 10,000 rpm for 5 min and stored at -80°C . RNA was extracted using the MagMax mirVana Total RNA isolation kit (Thermo Fisher Scientific) on the Kingfisher Flex extraction robot (Thermo Fisher Scientific). RNA was reverse transcribed and amplified using the TaqMan RNA-to-CT 1-Step Kit (Thermo Fisher Scientific). Reverse transcription was carried out at 48°C for 15 min followed by 2 min at 95°C . Amplification was accomplished over 50 cycles as follows: 95°C for 15 s and 60°C for 1 min. Copies of SARS-CoV-2 N gene RNA in samples were determined using a published assay (Case et al., 2020a).

Cytokine and chemokine protein measurements

Lung homogenates were incubated with Triton-X-100 (1% final concentration) for 1 h at room temperature to inactivate SARS-CoV-2. Homogenates were analyzed for cytokines and chemokines by Eve Technologies Corporation (Calgary, AB, Canada) using their Mouse Cytokine Array/Chemokine Array 31-Plex (MD31) platform.

Lung histology

Lungs of euthanized mice were inflated with ~ 2 mL of 10% neutral buffered formalin using a 3-mL syringe and catheter inserted into the trachea and kept in fixative for 7 days. Tissues were embedded in paraffin, and sections were stained with hematoxylin and eosin. Images were captured using the Nanozoomer (Hamamatsu) at the Alafi Neuroimaging Core at Washington University.

QUANTIFICATION AND STATISTICAL ANALYSES

Statistical significance was assigned when p values were < 0.05 using GraphPad Prism version 9.3. Tests, number of animals, median values, and statistical comparison groups are indicated in the Figure legends. Changes in infectious virus titer, viral RNA levels, or serum antibody responses were compared to unvaccinated or mRNA-control immunized animals and were analyzed by one-way ANOVA with a multiple comparisons correction, Mann-Whitney test, or Wilcoxon signed-rank test depending on the type of results, number of comparisons, and distribution of the data.

Supplemental figures

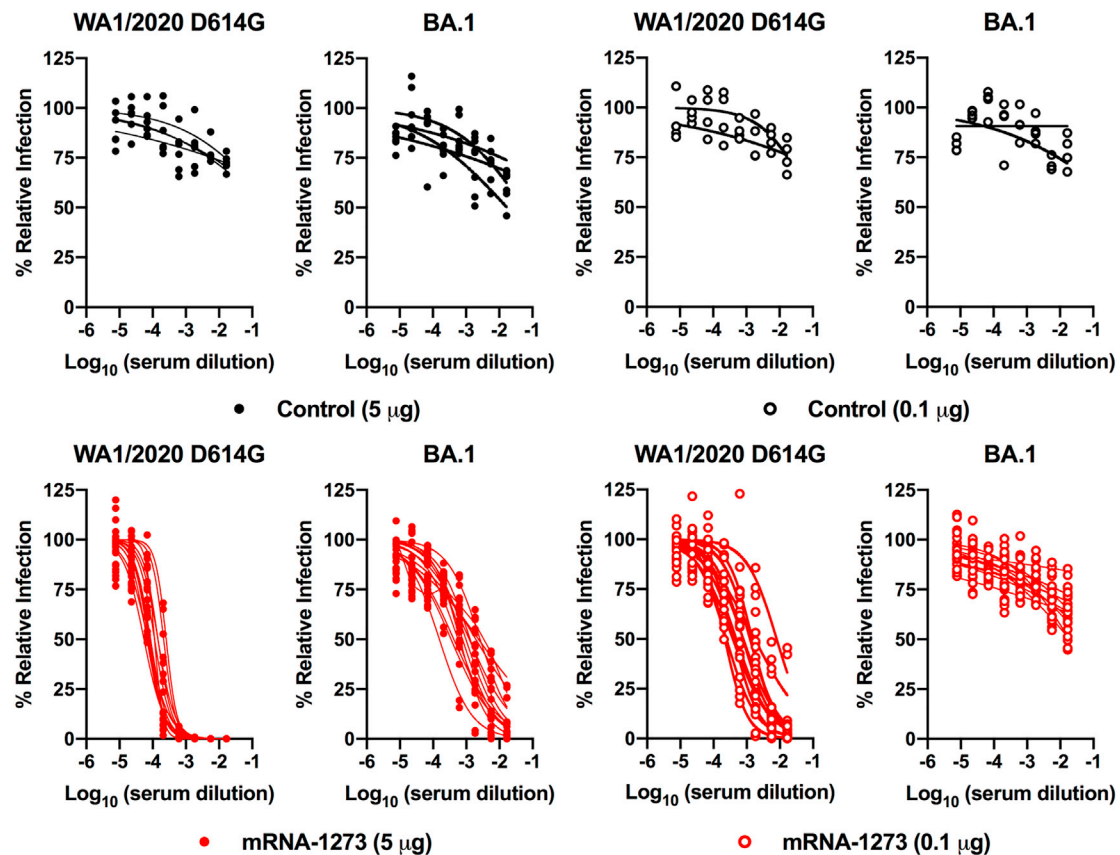
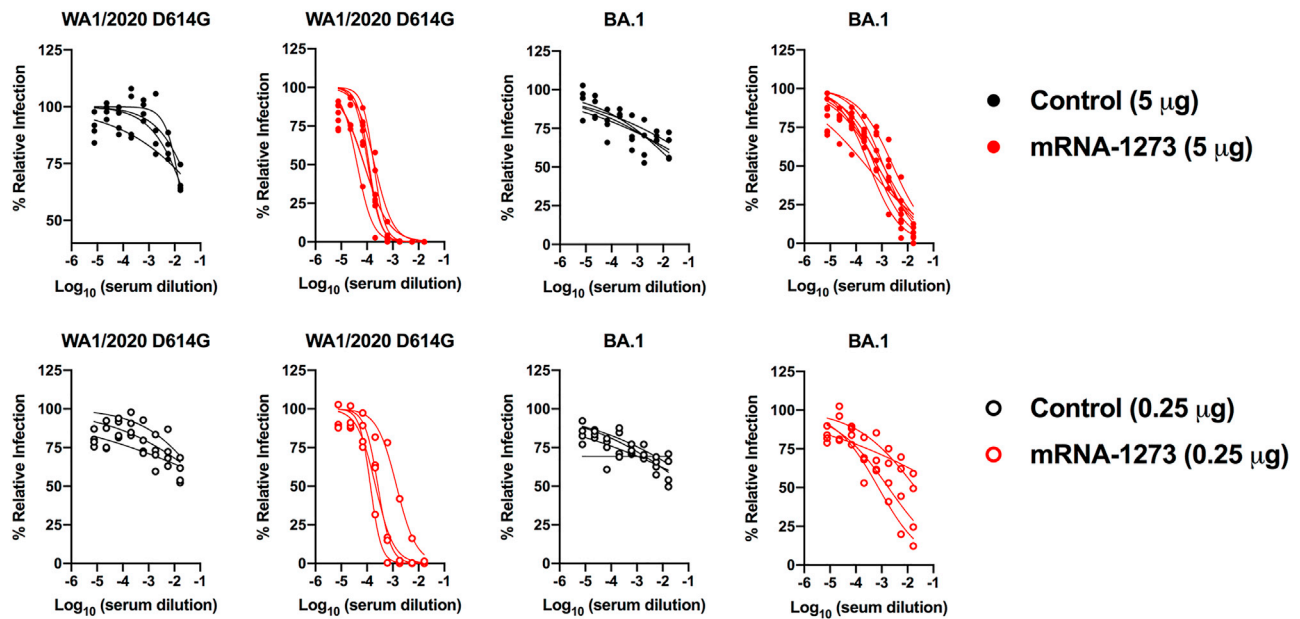


Figure S1. Serum neutralization of WA1/2020 D614G and BA.1 viruses, related to Figure 1

Seven-week-old female K18-hACE2 mice were immunized with 5 or 0.1 µg of mRNA vaccines. Serum neutralizing antibody responses against WA1/2020 D614G and BA.1 were assessed 3 weeks after the second vaccine dose (control mRNA, top; mRNA-1273, bottom) from mice immunized with 5 or 0.1 µg of control (n = 4) or mRNA-1273 (n = 12) vaccines. Neutralization curves corresponding to individual mice are shown for the indicated vaccines. Each point represents the mean of two technical replicates.

Pre-boost neutralization activity



Post-boost neutralization activity

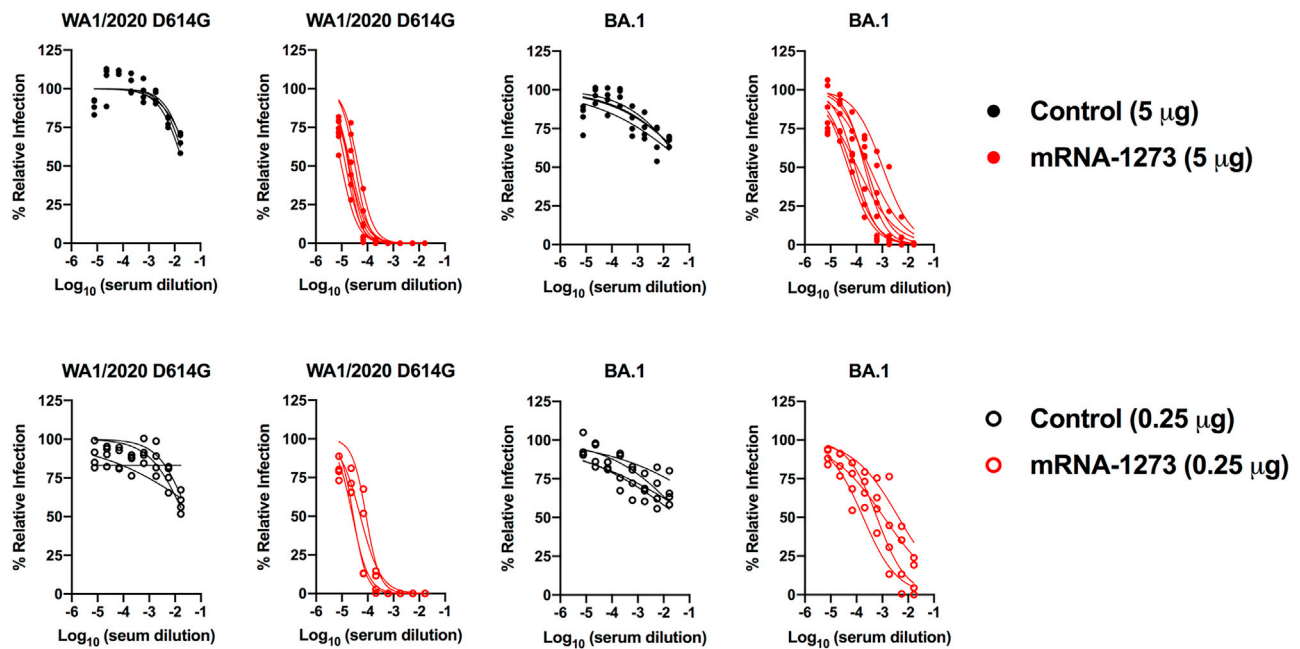
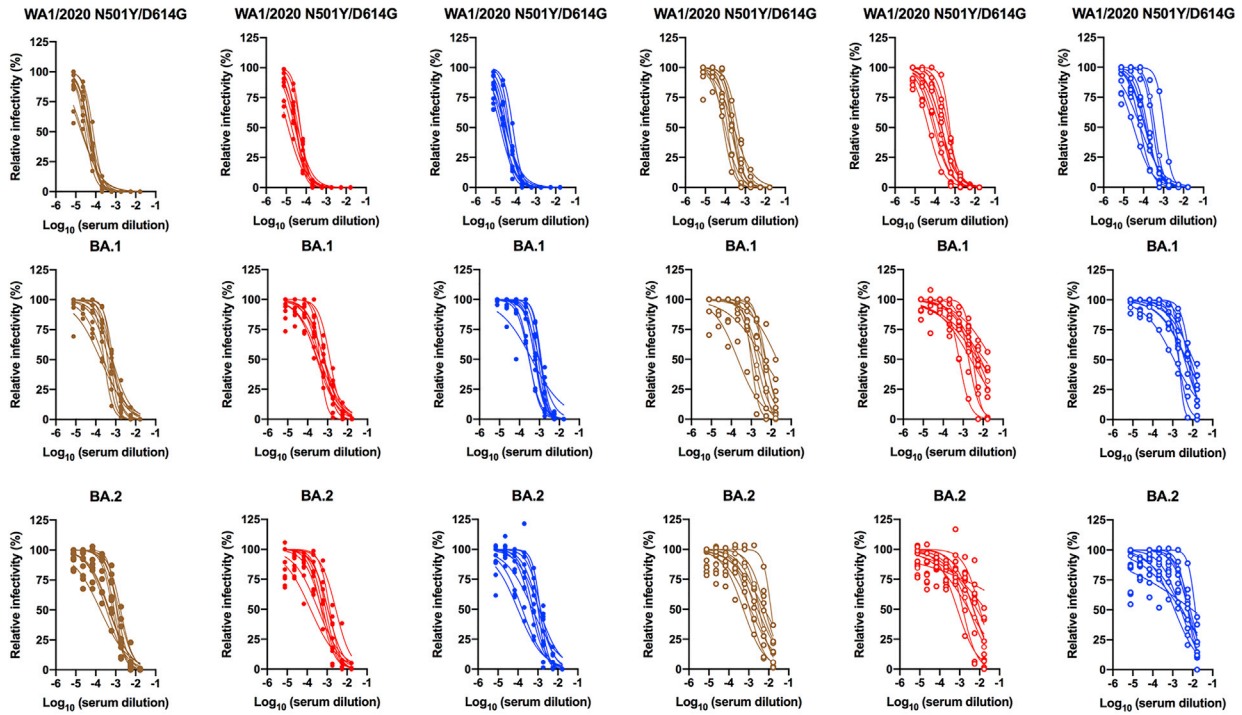


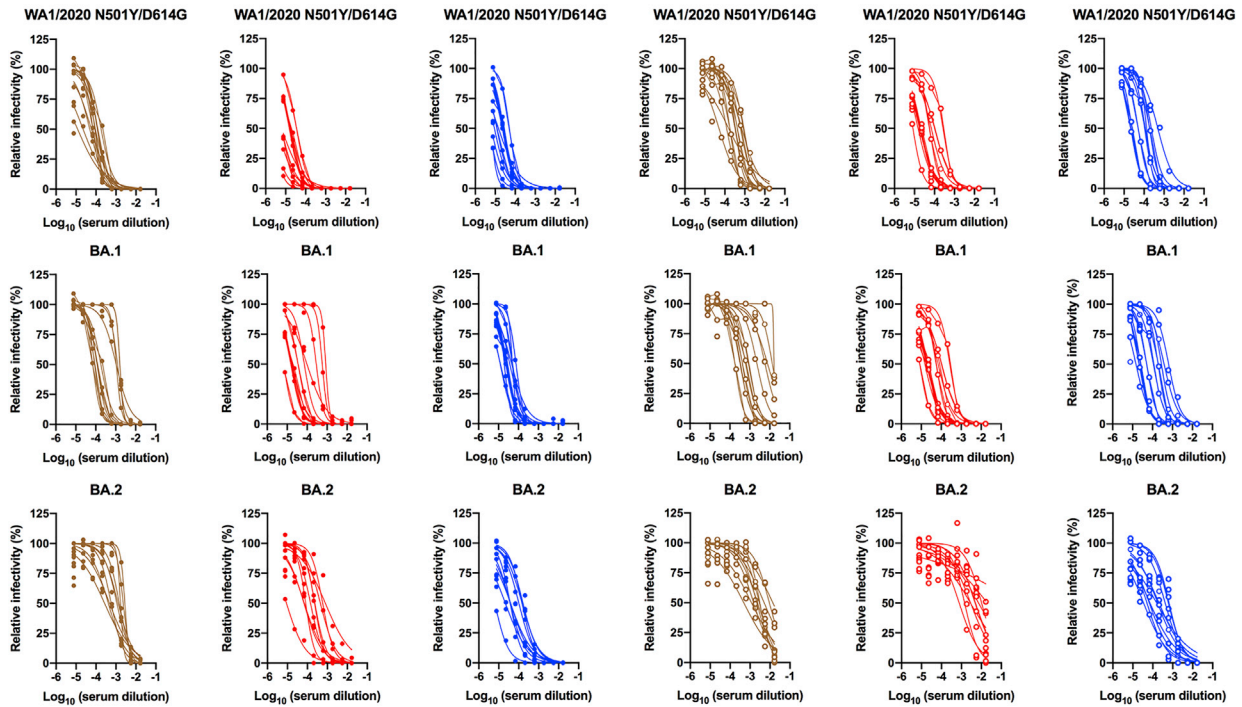
Figure S2. Serum neutralization of WA1/2020 D614G and BA.1 viruses, related to Figure 4

Seven-week-old female K18-hACE2 mice were immunized with 5 or 0.25 µg of mRNA vaccines and then boosted approximately 17–19 weeks later with 1 µg of mRNA-1273. Serum neutralizing antibody responses against WA1/2020 D614G and BA.1 immediately before (top, pre-boost) and 4 weeks after (bottom, post-boost) a control or mRNA-1273 booster dose from mice immunized with 5 or 0.25 µg of control ($n = 4$) or mRNA-1273 (5 µg, $n = 8$; 0.25 µg, $n = 4$) vaccines. Neutralization curves corresponding to individual mice are shown for the indicated immunizations. Each point represents the mean of two technical replicates.

Pre-boost neutralization activity



Post-boost neutralization activity



- mRNA-1273 (5 µg) + Control
- mRNA-1273 (5 µg) + mRNA-1273
- mRNA-1273 (5 µg) + mRNA-1273.529
- mRNA-1273 (0.25 µg) + Control
- mRNA-1273 (0.25 µg) + mRNA-1273
- mRNA-1273 (0.25 µg) + mRNA-1273.529

(legend on next page)

Figure S3. Pre- and post-boost serum neutralization of WA1/2020 N501Y/D614G, BA.1, and BA.2 viruses, related to Figure 6

Seven-week-old female 129S2 mice were immunized with 5 or 0.25 μg of mRNA vaccines and then boosted 10–11 weeks later with 1 μg of control mRNA, mRNA-1273, or mRNA-1273.529. Neutralizing antibody responses against WA1/2020 N501Y/D614G, BA.1, and BA.2 from serum immediately before (top, pre-boost) or 1 month after boosting (bottom, post-boost) with indicated vaccines from 129S2 mice that had received primary series immunizations with 5 or 0.25 μg of control or mRNA-1273 vaccines. Neutralization curves corresponding to individual mice are shown for the indicated immunizations. Sera are from two independent experiments, and each point represents the mean of two technical replicates.

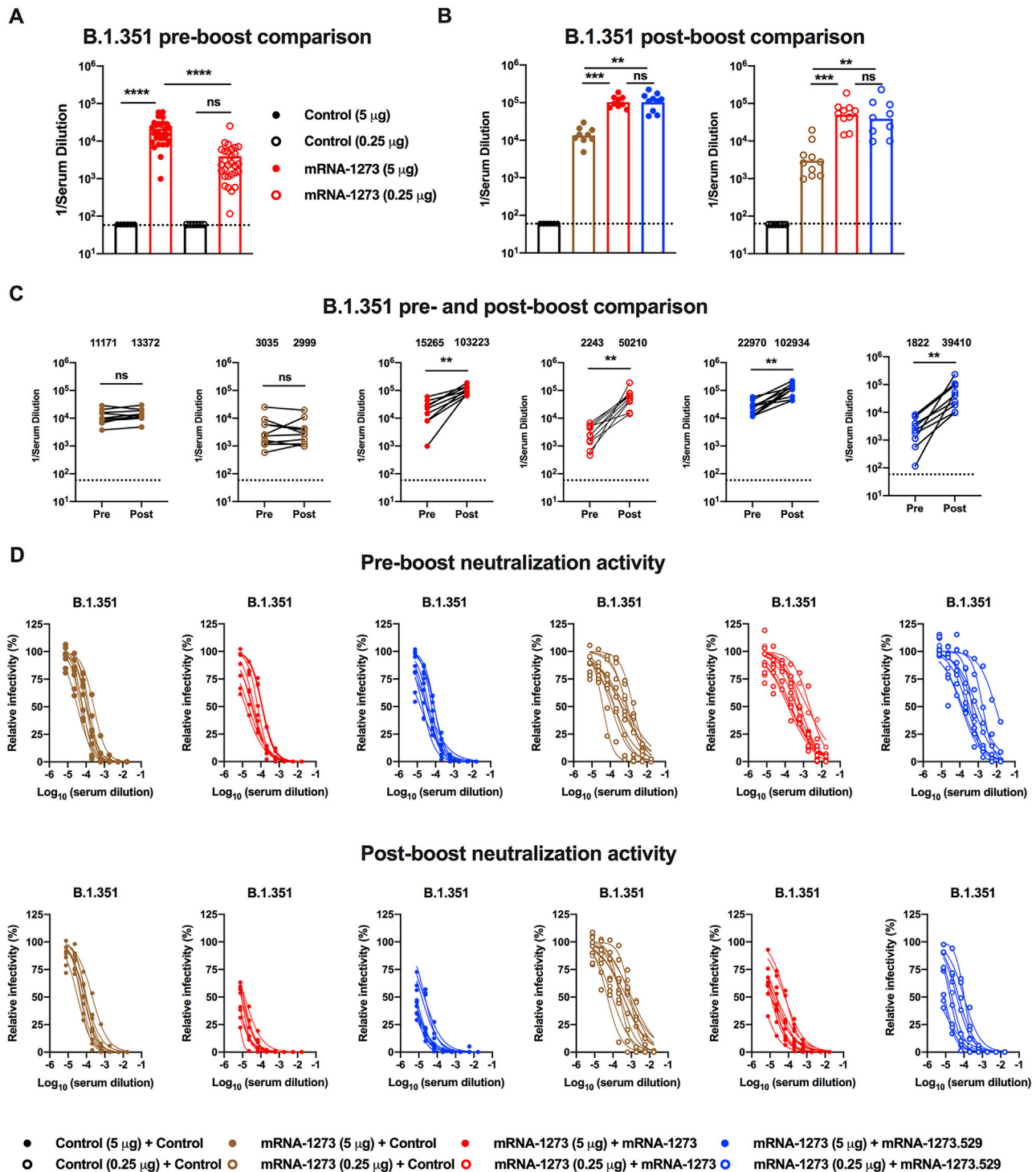


Figure S4. Pre- and post-boost serum neutralization of B.1.351 virus, related to Figure 6

7-week-old female 129S2 mice were immunized with 5 or 0.25 μ g of mRNA vaccines and boosted 10–11 weeks later with 1 μ g of control mRNA, mRNA-1273, or mRNA-1273.529.

(A and B) Serum neutralizing antibody responses immediately before (A, pre-boost) and 3–4 weeks after (B, post-boost) administering a control, mRNA-1273, or mRNA-1273.529 booster dose as judged by FRNT with B.1.351 (two experiments, boxes illustrate GMT, dotted lines show the LOD).

(C) Paired analysis of pre- and post-boost serum neutralizing titers against B.1.351 from samples obtained from animals (data from A to B) that received the following primary and booster immunizations: mRNA-1273 (5 or 0.25 μ g) + control booster, mRNA-1273 (5 or 0.25 μ g) + mRNA-1273 booster,

(legend continued on next page)

mRNA-1273 (5 or 0.25 μ g) + mRNA-1273.529 booster (n = 10, two experiments, dotted lines show the LOD). GMT values are indicated at the top of the graphs.

(D) Neutralizing antibody responses against B.1.351 from serum before (top, pre-boost) or 1 month after boosting (bottom, post-boost) with indicated vaccines from 129S2 mice that had received primary series immunizations with 5 or 0.25 μ g of control or mRNA-1273 vaccines. Neutralization curves corresponding to individual mice are shown. Sera are from two independent experiments, and each point represents the mean of two technical replicates.

(B and C) One-way ANOVA with Dunn's post-test. (D–F) Wilcoxon signed-rank test (ns, not significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).