

# **mRNA-1273 or mRNA-Omicron boost in vaccinated macaques elicits comparable B cell expansion, neutralizing antibodies and protection against Omicron**

## **Authors:**

Matthew Gagne<sup>1,9</sup>, Juan I. Moliva<sup>1,9</sup>, Kathryn E. Foulds<sup>1,9</sup>, Shayne F. Andrew<sup>1,9</sup>, Barbara J. Flynn<sup>1,9</sup>, Anne P. Werner<sup>1,9</sup>, Danielle A. Wagner<sup>1</sup>, I-Ting Teng<sup>1</sup>, Bob C. Lin<sup>1</sup>, Christopher Moore<sup>1</sup>, Nazaire Jean-Baptiste<sup>1</sup>, Robin Carroll<sup>1</sup>, Stephanie L. Foster<sup>2</sup>, Mit Patel<sup>2</sup>, Madison Ellis<sup>2</sup>, Venkata-Viswanadh Edara<sup>2</sup>, Nahara Vargas Maldonado<sup>2</sup>, Mahnaz Minai<sup>3</sup>, Lauren McCormick<sup>1</sup>, Christopher Cole Honeycutt<sup>1</sup>, Bianca M. Nagata<sup>3</sup>, Kevin W. Bock<sup>3</sup>, Caitlyn N. M. Dulan<sup>1</sup>, Jamilet Cordon<sup>1</sup>, John-Paul M. Todd<sup>1</sup>, Elizabeth McCarthy<sup>1</sup>, Laurent Pessaint<sup>4</sup>, Alex Van Ry<sup>4</sup>, Brandon Narvaez<sup>4</sup>, Daniel Valentin<sup>4</sup>, Anthony Cook<sup>4</sup>, Alan Dodson<sup>4</sup>, Katelyn Steingrebe<sup>4</sup>, Dillon R. Flebbe<sup>1</sup>, Saule T. Nurmukhambetova<sup>1</sup>, Sucheta Godbole<sup>1</sup>, Amy R. Henry<sup>1</sup>, Farida Laboune<sup>1</sup>, Jesmine Roberts-Torres<sup>1</sup>, Cynthia G. Lorang<sup>1</sup>, Shivani Amin<sup>1</sup>, Jessica Trost<sup>1</sup>, Mursal Naisan<sup>1</sup>, Manjula Basappa<sup>1</sup>, Jacquelyn Willis<sup>1</sup>, Lingshu Wang<sup>1</sup>, Wei Shi<sup>1</sup>, Nicole A. Doria-Rose<sup>1</sup>, Adam S. Olia<sup>1</sup>, Cuiping Liu<sup>1</sup>, Darcy R. Harris<sup>1</sup>, Andrea Carfi<sup>5</sup>, John R. Mascola<sup>1</sup>, Peter D. Kwong<sup>1</sup>, Darin K. Edwards<sup>5</sup>, Hanne Andersen<sup>4</sup>, Mark G. Lewis<sup>4</sup>, Kizzmekia S. Corbett<sup>6</sup>, Martha C. Nason<sup>7</sup>, Adrian B. McDermott<sup>1</sup>, Mehul S. Suthar<sup>2</sup>, Ian N. Moore<sup>8</sup>, Mario Roederer<sup>1</sup>, Nancy J. Sullivan<sup>1</sup>, Daniel C. Douek<sup>1,10</sup>, and Robert A. Seder<sup>1,10,11</sup>

## **Affiliations:**

<sup>1</sup>Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, 20892, United States of America

<sup>2</sup>Department of Pediatrics, Emory Vaccine Center, Yerkes National Primate Research Center, Emory University School of Medicine, Atlanta, Georgia, 30322, United States of America

<sup>3</sup>Infectious Disease Pathogenesis Section, Comparative Medicine Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland, 20892, United States of America

<sup>4</sup>Bioqual, Inc., Rockville, Maryland, 20850, United States of America

<sup>5</sup>Moderna Inc., Cambridge, MA, 02139, United States of America

<sup>6</sup>Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, 02115, United States of America

<sup>7</sup>Biostatistics Research Branch, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, 20892, United States of America

<sup>8</sup>Division of Pathology, Yerkes National Primate Research Center, Emory University School of Medicine, Atlanta, Georgia, 30329, United States of America

<sup>9</sup>These authors contributed equally to this manuscript.

<sup>10</sup>Correspondence: [ddouek@mail.nih.gov](mailto:ddouek@mail.nih.gov) and [rseder@mail.nih.gov](mailto:rseder@mail.nih.gov)

<sup>11</sup>Lead contact

## 1 **Summary**

2 SARS-CoV-2 Omicron is highly transmissible and has substantial resistance to antibody  
3 neutralization following immunization with ancestral spike-matched vaccines. It is unclear  
4 whether boosting with Omicron-specific vaccines would enhance immunity and protection.  
5 Here, nonhuman primates that received mRNA-1273 at weeks 0 and 4 were boosted at week 41  
6 with mRNA-1273 or mRNA-Omicron. Neutralizing antibody titers against D614G were 4760  
7 and 270 reciprocal ID<sub>50</sub> at week 6 (peak) and week 41 (pre-boost), respectively, and 320 and 110  
8 for Omicron. Two weeks after boost, titers against D614G and Omicron increased to 5360 and  
9 2980, respectively, for mRNA-1273 and 2670 and 1930 for mRNA-Omicron. Following either  
10 boost, 70-80% of spike-specific B cells were cross-reactive against both WA1 and Omicron.  
11 Significant and equivalent control of virus replication in lower airways was observed following  
12 either boost. Therefore, an Omicron boost may not provide greater immunity or protection  
13 compared to a boost with the current mRNA-1273 vaccine.

14

## 15 **Keywords**

16 SARS-CoV-2; Omicron; COVID-19; mRNA vaccine; boost; antibody; B cells; T cells; original  
17 antigenic sin; immune memory

18

## 19 **Introduction**

20 The COVID-19 mRNA vaccines BNT162b2 and mRNA-1273 provide highly effective  
21 protection against symptomatic and severe infection with ancestral SARS-CoV-2 (Baden et al.,

22 2021b; Dagan et al., 2021; Pilishvili et al., 2021; Polack et al., 2020). More recently, protective  
23 efficacy has declined due to both waning vaccine-elicited immunity (Baden et al., 2021a;  
24 Bergwerk et al., 2021; Goldberg et al., 2021) and antigenic shifts in variants of concern (VOC)  
25 including B.1.351 (Beta) and B.1.617.2 (Delta) (Planas et al., 2021; Wang et al., 2021a; Wang et  
26 al., 2021b). Importantly, the introduction of a boost after the initial vaccine regimen enhances  
27 immunity and vaccine efficacy against symptomatic disease, hospitalization and death across a  
28 broad range of age groups (Andrews et al., 2022; Bar-On et al., 2021; Barda et al., 2021; Garcia-  
29 Beltran et al., 2022; Pajon et al., 2022). However, the timing and selection of a boost is a major  
30 scientific and clinical challenge during this evolving pandemic in which emerging VOC have  
31 distinctive patterns of transmission and virulence and against which vaccine-elicited antibody  
32 neutralization is reduced.

33  
34 The most recent VOC, B.1.1.529, henceforth referred to by its WHO designation of Omicron,  
35 was first identified in South Africa in November 2021 and was associated with a dramatic  
36 increase in COVID-19 cases (Cele et al., 2021; Maslo et al., 2021). Omicron is highly  
37 contagious, with a significant transmission advantage compared to Delta, which until recently  
38 was the dominant VOC worldwide (Viana et al., 2022). It remains unclear, however, if this  
39 advantage is due to differences in cell entry, enrichment in respiratory aerosols, or the ability to  
40 evade immunity conferred by vaccination or prior infection. Compared to the ancestral strain,  
41 Omicron contains more than 30 mutations in the spike (S) gene, including S477N, T478K,  
42 E484A, Q493R, G496S, Q498R, N501Y and Y505H in the receptor binding motif (RBM) alone.  
43 Neutralizing antibody titers in sera of individuals recently recovered from previous infection or  
44 shortly after immunization with two doses of an mRNA-based COVID-19 vaccine are

45 dramatically reduced to Omicron as compared to the ancestral strains Wuhan-Hu-1, USA-  
46 WA1/2020 (WA1) and D614G. Numerous studies using both live virus and pseudovirus  
47 neutralization assays report a 60- to 80-fold reduction for convalescent sera and a 20- to 130-fold  
48 reduction for vaccinee sera (Edara et al., 2021a; Hoffmann et al., 2021; Muik et al., 2022;  
49 Schmidt et al., 2021). mRNA-1273 vaccine efficacy against breakthrough cases of Omicron in  
50 the first few months after immunization has been estimated as 30% in California, USA and 37%  
51 in Denmark (Hansen et al., 2021; Tseng et al., 2022) and a complete loss of protection within six  
52 months (Accorsi et al., 2022). Multiple reports have suggested that Omicron has reduced  
53 virulence compared to prior VOC in humans, mice and hamsters (Davies et al., 2022; Halfmann  
54 et al., 2022; Suryawanshi et al., 2022). It is possible that reduced virulence of Omicron may  
55 result from preferential replication in the upper airway compared to the lungs, perhaps due to  
56 altered cellular tropism not reliant on expression of transmembrane serine protease 2  
57 (TMPRSS2) (Meng et al., 2022; Willett et al., 2022). However, the effect of any reduction in  
58 intrinsic viral pathogenicity may be somewhat offset in the context of reduced vaccine efficacy  
59 and enhanced virus transmission in human populations worldwide. Together these data reinforce  
60 the value of boosting to limit the extent of infection from Omicron.

61  
62 Variant-matched boosts have been suggested as a strategy to enhance neutralizing and binding  
63 antibody titers to the corresponding VOC beyond the levels conferred by existing FDA-approved  
64 boosts, which are homologous to the original ancestral WA1-matched primary vaccine regimen.  
65 We previously showed that boosting mRNA-1273 immunized nonhuman primates (NHP) with  
66 mRNA-1273 or a boost matched to the Beta VOC spike (mRNA-1273.351 or mRNA-1273.Beta)  
67 resulted in significant enhancement of neutralizing antibody responses across all VOC tested and

68 an expansion of S-specific memory B cells with ~80-90% expressing WA1 and Beta. Moreover,  
69 both vaccines provided substantial and similar protection against Beta replication (Corbett et al.,  
70 2021a). These NHP data were confirmed in a study in humans which compared an mRNA-1273  
71 boost to mRNA-1273.Beta ~6 months after participants had received the standard two-dose  
72 mRNA-1273 vaccine regimen (Choi et al., 2021). Following either boost, neutralizing titers were  
73 substantially increased against D614G and several variants including Beta and were comparable  
74 between boost groups. Of note, the level of neutralizing antibodies to Beta after either boost were  
75 about 10-fold higher than after the initial vaccination suggesting affinity maturation or epitope  
76 focusing of the B cell response. Together, these data suggest that the variant Beta boost did not  
77 uniquely enhance immunity or protection compared to existing ancestral strain-matched boosts.  
78 However, as Omicron contains more mutations in S compared to Beta and demonstrates even  
79 more substantial escape from vaccine-elicited neutralizing antibodies than does Beta, it is unclear  
80 whether an Omicron-specific boost would provide an additional protective benefit against  
81 Omicron infection beyond that of WA1-matched boosts.

82

83 The nonhuman primate (NHP) model has been useful for demonstrating immune correlates,  
84 mechanisms and durability of vaccine-elicited protection against SARS-CoV-2 and been largely  
85 predictive for what has been observed in humans in terms of protective efficacy (Corbett et al.,  
86 2021b; Gagne et al., 2022; Gilbert et al., 2021). Here, we vaccinated NHP with 100µg mRNA-  
87 1273 at weeks 0 and 4, which is a similar dose and schedule as used in humans. Animals were  
88 then boosted about ~9 months later with 50µg of either a homologous dose of mRNA-1273 or  
89 mRNA-1273.529, which is matched to Omicron S (henceforth referred to as mRNA-Omicron).  
90 For the duration of these 9 months, we collected sera, bronchoalveolar lavage (BAL) and nasal

91 washes to analyze the kinetics of antibody binding and neutralization as well as the frequency of  
92 S-specific B cells for WA1 and Omicron as well as Beta and Delta. Four weeks after boost, NHP  
93 were challenged with Omicron. Viral replication in upper and lower airways and lung  
94 inflammation were measured to compare boost-elicited protection against Omicron.

95

## 96 **Results**

### 97 Kinetics of serum antibody responses following mRNA-1273 immunization and boost

98 Indian-origin rhesus macaques ( $n=8$ ) were immunized with 100 $\mu$ g of mRNA-1273 at weeks 0  
99 and 4 (Fig. S1). Sera were collected at weeks 6 (peak) and 41 (memory) to measure  
100 immunoglobulin G (IgG) binding to WA1 S and a panel of VOC (Fig. 1A). At week 6, we  
101 observed a clear hierarchy of binding titers with WA1>Delta>Beta >Omicron. Geometric mean  
102 titers (GMT) to WA1 and Omicron were  $8 \times 10^{19}$  and  $3 \times 10^{15}$  area under the curve (AUC).  
103 Antibody titers waned markedly by week 41, with GMT of  $2 \times 10^{12}$  and  $2 \times 10^8$  AUC for WA1 and  
104 Omicron, reflecting a 7-log decline for each strain. Similar antibody kinetics and hierarchy of  
105 potency were observed when measuring binding to the receptor binding domain (RBD) of the  
106 same variants, with titers to Omicron of  $7 \times 10^{11}$  AUC at week 6 and  $8 \times 10^7$  AUC at week 41 (Fig.  
107 1B). Nine months after the second dose of mRNA-1273 (week 41), NHP were boosted with  
108 50 $\mu$ g of homologous mRNA-1273 or heterologous virus challenge-matched mRNA-Omicron  
109 ( $n=4$ /group). S-binding titers were restored to the same level as observed at week 6 following  
110 either a homologous or heterologous boost, and titers to Omicron were still lower than all other  
111 variants.

112

113 Neutralizing antibody titers were then assessed using a live virus assay (Fig. 1C and Table S1).  
114 At week 6, neutralizing titers were highest to D614G followed by Delta, then Beta and Omicron.  
115 Titers to all variants markedly declined by week 41, including a drop in reciprocal 50%  
116 inhibitory dilution ( $ID_{50}$ ) titers for D614G from 5560 at week 6 to 330 at week 41 and for  
117 Omicron from 110 at week 6 to 33 at week 41. However, following either boost, neutralizing  
118 titers to D614G and Delta were increased similar to week 6 and titers to Beta and Omicron were  
119 greater than they had been at week 6 (Beta:  $P=0.05$  and  $0.035$ ; Omicron:  $P=0.041$  and  $0.01$  for  
120 mRNA-1273 and mRNA-Omicron, respectively). We substantiated these findings using a  
121 lentiviral pseudovirus neutralization assay similar to the one used to assess immune responses in  
122 human clinical trials (Fig. 1D). Following either boost, pseudovirus neutralizing titers were  
123 greater to Beta and Omicron than they had been at the week 6 time-point, including an increase  
124 in Omicron titers from 320 GMT to 2980 GMT in the mRNA-1273 boost group and 1930 GMT  
125 in the mRNA-Omicron boost group (Beta:  $P=0.022$  and  $<0.0001$ ; Omicron:  $P=0.049$  and  $0.002$   
126 for mRNA-1273 and mRNA-Omicron, respectively).

127

128 The increase in neutralizing titers to all VOC tested after the third dose could suggest continued  
129 antibody maturation (Gaebler et al., 2021). To extend this analysis, we measured antibody  
130 avidity over time following immunization (Fig. 1E). Serum antibody avidity to WA1 S-2P  
131 increased from a geometric mean avidity index of 0.43 to 0.61 from weeks 6 to 41 ( $P=0.0016$ ), a  
132 comparable increase to our previous findings (Corbett et al., 2021a; Gagne et al., 2022).  
133 Similarly, avidity to Omicron S-2P rose from 0.44 to 0.67 ( $P<0.0001$ ). Following the boost, no  
134 further change was observed ( $P>0.05$  for both boost groups).

135



136 Collectively, these data show that boosting with the homologous mRNA-1273 or mRNA-  
137 Omicron leads to comparable and significant increases in neutralizing antibody responses against  
138 all VOC including Omicron.

139

140 mRNA-1273 and mRNA-Omicron boosting increase mucosal antibody responses to Omicron

141 Upper and lower airway antibody response are critical for mediating protection against SARS-  
142 CoV-2 and were assessed following immunization. Nasal washes (NW) and bronchoalveolar  
143 lavage fluid (BAL) were collected at weeks 8 (four weeks after the initial mRNA-1273  
144 immunizations), 39 (pre-boost) and 43 (two weeks after the boost). At all timepoints, BAL and  
145 NW IgG S-binding titers followed the hierarchy of WA1>Delta>Beta>Omicron (Fig. 2A-B), the  
146 same trend detected in our serological assays. In BAL, immediately prior to the boost, GMT  
147 were  $6.8 \times 10^6$ ,  $4.0 \times 10^6$ ,  $1.3 \times 10^6$  and  $2.5 \times 10^4$  AUC for WA1, Delta, Beta and Omicron,  
148 respectively. These titers correlated with a 2-fold reduction for Delta compared to WA1, a 5-fold  
149 reduction for Beta, and a 275-fold reduction for Omicron. Following either boost, titers were  
150 increased by 3-4 logs for all variants. In NW, titers decreased from  $\sim 10^{11}$  for WA1, Delta and  
151 Beta at week 8 to  $1.3 \times 10^6$ ,  $3.7 \times 10^5$  and  $1.9 \times 10^5$  for WA1, Delta and Beta, respectively. GMT to  
152 Omicron similarly declined from  $8.8 \times 10^8$  to  $8.7 \times 10^3$  AUC and were lower than WA1 and all  
153 other variants. Consistent with the findings in the BAL, either boost increased nasal antibody  
154 titers  $\sim 6$ -7 logs, with GMT of  $\sim 10^{12}$  for WA1, Delta and Beta and  $\sim 10^{10}$  for Omicron.

155

156 In a number of prior NHP studies, we have not been able to detect antibody neutralizing titers  
157 using pseudo- or live-virus assays from NW or BAL. However, based on its high sensitivity, we  
158 have used the angiotensin converting enzyme 2 receptor (ACE2) inhibition assay to measure

159 antibody function as a surrogate for neutralization capacity (Corbett et al., 2021a; Gagne et al.,  
160 2022). While the antigen for determination of binding titers was wildtype S, our ACE2 inhibition  
161 assay used stabilized S-2P (Table S2). In the BAL, 25-50% median binding inhibition was  
162 observed for all variants at week 8, except for Omicron S-2P in which binding inhibition was  
163 low to undetectable (Fig. 2C). ACE2 binding inhibition declined to a median of <15% for all  
164 variants by week 39 and then increased after either the homologous or mRNA-Omicron boost to  
165 levels above those at week 8. Of note, although ACE2 inhibition of Omicron S-2P increased  
166 following the boost, it remained lower than all other variants. In the upper airway, ACE2  
167 inhibition was low to undetectable at week 39 following the initial vaccine regimen for all  
168 variants. However, after either boost, there was an increase across all variants including Omicron  
169 to values higher than the initial peak at week 8 (Fig. 2D). Thus, boosting with either vaccine was  
170 important for enhancing mucosal antibody binding and neutralization responses.

171

#### 172 Similar expansion of cross-reactive S-2P-specific memory B cells following boosting

173 The observation of rapid and significant increases in binding and neutralizing antibody titers to  
174 Omicron in both blood and mucosal sites after homologous or heterologous mRNA boost  
175 suggests an anamnestic response involving the mobilization of cross-reactive memory B cells.  
176 Thus, we measured B cell binding to pairs of fluorochrome-labeled S-2P probes with different  
177 VOC including Omicron at weeks 6, 41 and 43 (2 weeks post-boost) (Fig. S2). Of the total S-2P  
178 specific memory B cell responses at week 6, 63% were dual-specific and capable of binding both  
179 WA1 and Omicron probes, with 33% binding WA1 alone and only 4% which bound Omicron  
180 alone (Fig. 3A, 4A). By week 41, the total S-specific memory B cell compartment had declined

181 ~90% as a fraction of all class-switched memory B cells (Fig. S3A), although the dual-specific  
182 population remained the largest fraction within the S-binding pool (Fig. 4A).

183

184 Two weeks after boosting, there was an expansion of the total S-specific memory B cell  
185 compartment similar to that observed at week 6. Following an mRNA-1273 boost, 24% of all S-  
186 2P-specific memory B cells were specific for WA1 alone and 71% were dual-specific for WA1  
187 and Omicron. After the mRNA-Omicron boost, 81% were dual-specific for WA1 and Omicron.,  
188 with 12% specific for WA1 only (Fig. 4A). Of note, we did not observe a population of  
189 Omicron-only memory B cells before or after the boost that was clearly distinct from background  
190 staining (Fig. 3A). These data suggest a marked expansion of cross-reactive dual-specific WA1-  
191 and Omicron-positive B cells for either boost, with mRNA-1273 also expanding WA1-only B  
192 cell responses. The increase in cross-reactive B cells for WA1 and Omicron is consistent with the  
193 comparable and high-level of neutralizing titers against D614G and Omicron by either boost  
194 (Fig. 1C-D). To extend these data, serologic mapping of antigenic sites on Omicron and WA1  
195 RBD was performed. This analysis revealed that boosting with either mRNA-1273 or mRNA-  
196 Omicron elicited serum antibody reactivity with similar RBD specificities (Fig S4).

197

198 To further explore the effect of boosting on anamnestic B cell responses, we phenotyped the  
199 activation status of S-binding memory B cells (Fig. 4E). WA1 S-2P- and/or Omicron S-2P-  
200 binding memory B cells predominantly had an activated memory phenotype immediately after  
201 both the second and third doses.

202

203 It has recently been shown that individuals in South Africa with or without prior vaccination had  
204 increased neutralizing antibody titers to Delta and Omicron following Omicron infection (Khan  
205 et al., 2022). Thus, we determined whether there was cross-reactivity of B cells for Delta and  
206 Omicron following vaccination. Indeed, 68% of all Delta S-2P and/or Omicron S-2P memory B  
207 cells were also dual-specific at week 6 and the remainder of S-binding memory B cells largely  
208 bound Delta alone (Fig. 3B, 4B). Following a third dose, the frequency of dual-specific cells  
209 increased to 76% for mRNA-1273 and 85% for mRNA-Omicron, consistent with our findings on  
210 cross-reactive B cells using WA1 and Omicron S-2P probes.

211

212 To complete the analysis and demonstrate cross-reactivity of B cells across other variants, we  
213 assessed the frequencies of memory B cells specific for WA-1 and Delta or Beta. We have  
214 previously reported that dual-specific WA1 S-2P and Delta S-2P memory B cells accounted for  
215 greater than 85% of all memory B cells which bound either spike after two immunizations with  
216 mRNA-1273 (Gagne et al., 2022). Here we confirmed and extended these findings and show that  
217 after either boost, ~95% of all WA1- and/or Delta-binding memory B cells are dual-specific (Fig.  
218 3C, 4C). Similar findings were obtained with WA1 and Beta S-2P probes, in which the dual-  
219 specific population was 85% at week 6 and 90% following either boost (Fig. 3D, 4D). Of note  
220 following the mRNA-Omicron boost, very few B cells were detected that only bound WA1  
221 epitopes when co-staining for Delta or Beta. Overall, the data show that cross-reactive cells were  
222 expanded following a boost with either mRNA-1273 or mRNA-Omicron while only mRNA-  
223 1273 was capable of boosting memory B cells specific for WA1 alone (Fig. S3).

224

225 S-2P-specific T cell responses in blood and BAL following vaccination

226 We have previously shown that mRNA-1273 immunization elicits T<sub>H1</sub>, T<sub>FH</sub> and a low frequency  
227 of CD8 responses to S peptides in NHP (Corbett et al., 2020; Corbett et al., 2021a; Corbett et al.,  
228 2021c; Gagne et al., 2022; Jackson et al., 2020). Consistent with the prior studies we show that  
229 mRNA-1273 elicits T<sub>H1</sub>, T<sub>FH</sub> and low-level CD8 T cell responses at the peak of the response  
230 (week 6) that decline over time (Fig. S5 and S6). Boosting with either mRNA-1273 or mRNA-  
231 Omicron increased T<sub>FH</sub> responses which could be important for expanding the S-specific  
232 memory B cell population following the boost (Johnston et al., 2009; Nurieva et al., 2009;  
233 Tangye et al., 2002). We also detected T<sub>H1</sub> and CD8 T cells in BAL at week 8 that decreased to  
234 undetectable levels at week 39. Such responses were increased with either mRNA-1273 or  
235 mRNA-Omicron (Fig. S5).

236

237 Boosting with mRNA-1273 or mRNA-Omicron provides equivalent protection in the lungs  
238 against Omicron challenge

239 To determine the extent of protection provided by a homologous mRNA-1273 or challenge  
240 virus-matched mRNA-Omicron boost following the two-dose mRNA-1273 immunization series,  
241 we obtained a new viral stock of Omicron, which was sequenced and confirmed to contain the  
242 canonical mutations present in the dominant Omicron sub-lineage BA.1 (Fig. S7).

243

244 Four weeks after administration of either boost, we challenged these NHP and 8 control NHPs  
245 with  $1 \times 10^6$  plaque forming units (PFU) via both intratracheal (IT) and intranasal (IN) routes. The  
246 control NHP had previously been administered 50 $\mu$ g of control mRNA formulated in lipid  
247 nanoparticles at the time of boost and had never been vaccinated.

248

249 BAL, nasal swabs (NS) and oral swabs (OS) were collected following challenge. Copy numbers  
250 of SARS-CoV-2 subgenomic RNA (sgRNA) were measured to determine the extent of viral  
251 replication. As sgRNA encoding for the N gene (sgRNA\_N) are the most abundant transcripts  
252 produced due to the viral discontinuous transcription process (Kim et al., 2020), the sgRNA\_N  
253 qRT-PCR assay was chosen for its enhanced sensitivity. On day 2 post-infection in the BAL,  
254 unvaccinated NHP had geometric mean copy numbers of  $1 \times 10^6$  sgRNA\_N per mL whereas the  
255 vaccinated NHP had  $3 \times 10^2$  and  $2 \times 10^2$  for the mRNA-1273 and mRNA-Omicron cohorts,  
256 respectively (Fig. 5A). By day 4, all vaccinated NHP had undetectable levels of sgRNA\_N while  
257 copy numbers in the unvaccinated group had only declined to  $3 \times 10^5$  per mL (mRNA-1273 vs  
258 control on days 2 and 4:  $P < 0.0001$ ; mRNA-Omicron vs control on days 2 and 4:  $P < 0.0001$ ).

259

260 In the nose, sgRNA\_N copy numbers at days 1 to 4 were low for most animals and were not  
261 different between the control and vaccinated cohorts, so protection following vaccination could  
262 not be determined (Fig. 5B). At day 4, 5/8 controls had detectable virus in the nose as compared  
263 to 3/8 vaccinated NHP, with no clear difference between the boost cohorts. However, by day 8,  
264 4/8 controls still had detectable sgRNA\_N including 2 animals with increased copy numbers  
265 while none of the vaccinated NHP had detectable sgRNA.

266

267 In assessing sgRNA\_N in the throat, it is noteworthy that 2 days after challenge, only 1/8  
268 vaccinated NHP (in either boost group) had detectable virus in the throat compared to 6/8 control  
269 NHP (Fig. 5C).

270

271 We also measured the amount of culturable virus using a tissue culture infectious dose assay  
272 (TCID<sub>50</sub>). No virus was detected in the BAL of any vaccinated NHP, while 8/8 and 7/8 control  
273 NHP had detectable virus 2 and 4 days after challenge, respectively (Fig. 5D). In the NS, 1/8  
274 boosted animals had culturable virus at any timepoint. In the unvaccinated control animals, 2/8  
275 and 3/8 NHP had culturable virus in the nose 2 and 4 days after challenge, respectively (Fig. 5E).

276

### 277 Viral antigen and pathology in the lungs after challenge

278 To assess lung pathology in NHP, 2 of the animals in each group were euthanized on day 8  
279 following Omicron challenge, and the amount of viral antigen (SARS-CoV-2 N) and  
280 inflammation in the lungs were assessed (Fig. 6). N antigen was detected in variable amounts in  
281 the lungs of both control animals. When present, viral antigen was often associated with the  
282 alveolar capillaries and, occasionally, nearby immune cells. There was no evidence of viral  
283 antigen in the lungs of the vaccinated NHP.

284

285 Animals from both boost groups displayed histopathologic alterations that were classified as  
286 minimal to mild or moderate. Inflammation was largely characterized by mild and patchy  
287 expansion of alveolar capillaries, generalized alveolar capillary hypercellularity, mild and  
288 regional type II pneumocyte hyperplasia and, less frequently, scattered collections of immune  
289 cells within some alveolar spaces. In contrast, unvaccinated animals were characterized as  
290 having a moderate to severe pathology. Lung sections from controls included features  
291 characterized by moderate and often diffuse alveolar capillary expansion, diffuse  
292 hypercellularity, moderate type II pneumocyte hyperplasia and multiple areas of perivascular

293 cellular infiltration. Together, these data indicate that protection against Omicron was robust in  
294 the lungs regardless of boost selection.

295

## 296 **Discussion**

297 Omicron has become the dominant global variant of SARS-CoV-2 due to its transmission  
298 advantage relative to Delta and its ability to evade prior immunity (Grabowski et al., 2022; Viana  
299 et al., 2022). Vaccine efficacy against infection with Omicron has declined and boosting with a  
300 third dose of an mRNA COVID-19 vaccine matched to the prototype strain has been shown to  
301 restore immunity and protection (Accorsi et al., 2022; Garcia-Beltran et al., 2022; Hansen et al.,  
302 2021; Pajon et al., 2022; Tseng et al., 2022). Here, we immunized NHP with 2 doses of mRNA-  
303 1273 (100 $\mu$ g) and boosted them ~9 months later with 50 $\mu$ g of either mRNA-1273 or mRNA-  
304 Omicron prior to challenge with Omicron virus. The principal findings were: (1) 9 months after  
305 the two-dose regimen, neutralizing and binding antibody titers to Omicron had declined  
306 substantially in blood and mucosal airways; (2) after the boost, neutralizing antibody titers to  
307 ancestral strains were restored and those to Omicron were increased compared to the peak  
308 response after the initial prime and boost; (3) both boosts expanded cross-reactive memory B  
309 cells but only the homologous boost was capable of expanding B cells specific for epitopes  
310 unique to the ancestral strain; and (4) both boosts provided complete protection in the lungs and  
311 limited protection in the upper airway after Omicron challenge.

312

313 Following either mRNA-1273 or mRNA-Omicron boost, there was essentially complete  
314 protection in the lower airway with no culturable virus by day 2 and no detectable sgRNA\_N by  
315 day 4. These data are comparable to our previous findings of equivalent upper and lower airway



316 protection following Beta challenge in NHP boosted with either mRNA-1273 or mRNA-  
317 1273. Beta 1-2 months after immunization with 2 doses of mRNA-1273 (Corbett et al., 2021a). In  
318 contrast to the lower airway, there were no clear and consistent differences in sgRNA\_N copy  
319 number at days 2 or 4 in the upper airway of vaccinated or control NHP. Of note, more of the  
320 control animals had detectable sgRNA at day 4 and increased sgRNA at day 8 as compared to  
321 the boosted animals. We would also note that the amount of Omicron replication as assessed by  
322 sgRNA or culturable virus in the control animals is demonstrably different than in our prior  
323 studies in which NHP were challenged with WA1, Delta or Beta (Corbett et al., 2020; Corbett et  
324 al., 2021c; Gagne et al., 2022). These findings compliment a growing body of evidence for  
325 reduced overall severity of Omicron infection in animal models of COVID-19 compared to other  
326 variants (Bentley et al., 2021; Halfmann et al., 2022; Suryawanshi et al., 2022). Overall, the  
327 findings here of high-level protection in the lungs recapitulate observations in mRNA-1273-  
328 vaccinated humans of reduced disease severity following infection with Omicron (Abdullah et  
329 al., 2021; Sigal, 2022; Wolter et al., 2022).

330

331 Neutralizing antibodies to Omicron in the blood or ACE2 binding inhibitory antibodies in the  
332 airway mucosa were low after the first 2 doses of mRNA-1273 at weeks 6-8 and low to  
333 undetectable ~9 months later. Importantly, either mRNA-1273 or mRNA-Omicron boosts were  
334 able to significantly increase neutralizing antibody titers against Omicron and Beta beyond their  
335 initial peak consistent with a rapid recall B cell response. This also implies that neutralizing  
336 antibody titers at extended times after vaccination may not be a reliable surrogate either for  
337 vaccine efficacy in the lower airway or for predicting responses following a boost or infection as  
338 they may not reflect the recall capacity of the underlying memory B cell population.

339  
340 The observation that boosting with either mRNA-1273 or mRNA-Omicron resulted in the  
341 expansion of a similarly high frequency of cross-reactive B cells likely stems from the principle  
342 of original antigenic sin, otherwise termed antigenic imprinting, whereby prior immune memory  
343 is recalled by a related antigenic encounter (Davenport and Hennessy, 1957; Davenport et al.,  
344 1953). Recall of prior immunity may be either deleterious or beneficial as exemplified by the  
345 impact of the circulating influenza A subtypes at the time of an individual's first exposure after  
346 birth on patterns of disease susceptibility to subsequent pandemic influenza A outbreaks (Gostic  
347 et al., 2016; Worobey et al., 2014). The current worldwide distribution and evolution of SARS-  
348 CoV-2, however, is quite different from that of influenza A. Whereas multiple subtypes of  
349 influenza A circulate with different levels of co-dominance, SARS-CoV-2 distribution has  
350 generally become rapidly dominated by a single variant — currently Omicron — before  
351 replacement by another that, for various reasons, is more transmissible. The question therefore is  
352 whether there is added value from boosting with a heterologous vaccine matched to the dominant  
353 circulating variant, or whether cross-reactive B cell recall immunity elicited by boosting with the  
354 original vaccine is sufficient to reduce infection and disease severity. As we have now shown in  
355 two different NHP studies, boosting animals with either mRNA-1273.Beta (Corbett et al., 2021a)  
356 or mRNA-Omicron provided no advantage over mRNA-1273 in recalling high titer neutralizing  
357 antibodies across all variants tested and protecting from virus replication after challenge. These  
358 considerations apply to the large numbers of individuals with prior immunity from vaccination or  
359 infection with current and previous variants who may not necessarily benefit from a change in  
360 vaccine design.  
361

362 Looking to the future, however, if Omicron, or a closely antigenically related variant, remains  
363 the dominant circulating variant for some years to come, then it is possible that a change in the  
364 initial vaccine regimen would be warranted, particularly in immunologically naïve populations  
365 such as children as they reach the age of eligibility for approved COVID-19 vaccines.  
366 Importantly, it would need to be established that a switch in COVID-19 vaccine design to match  
367 the current dominant variant would not jeopardize responses against variants which may be  
368 antigenically distant from Omicron but close to the prototype. Indeed a recent study has shown  
369 that immunization of mice with an Omicron mRNA vaccine elicited strong neutralization against  
370 Omicron but not to other variants, consistent with data from primary Omicron infection (Lee et  
371 al., 2022; Roessler et al., 2022; Suryawanshi et al., 2022). Thus, a combination or bivalent  
372 vaccine to generate B cells specific for the current variant as well as cross-reactive to other  
373 variants might ensure greater breadth of neutralization.

374  
375 In summary, our findings highlight two important factors that will impact management of this  
376 pandemic. The first is the design of the vaccine and whether it should be changed based on the  
377 currently circulating variant. At present, boosting with mRNA-1273 provides robust increases in  
378 neutralizing antibodies and appears to be sufficient to prevent severe disease after exposure from  
379 all known variants (Baden et al., 2021a; Bruxvoort et al., 2021a; Bruxvoort et al., 2021b;  
380 Chemaitelly et al., 2021; Corbett et al., 2020; Corbett et al., 2021c; Gagne et al., 2022; Pilishvili  
381 et al., 2021; Tang et al., 2021). Variant-matched vaccines may be preferable in the future if new  
382 variants were to emerge that were even further antigenically distant such that cross-reactive  
383 epitopes are rendered ineffective or if there were differences in the durability of neutralizing  
384 antibody titers elicited by different boosts. Second, as neutralizing antibody titers wane with time

385 after vaccination, their ability to serve as a surrogate for vaccine efficacy or to predict clinical  
386 outcomes against severe disease after infection with VOC may become diminished. Thus the  
387 determination of when to administer a boost may depend on the recall capacity of the underlying  
388 memory B cell population. These considerations will become clear as human clinical data are  
389 made available.

390

### 391 **Limitations of the study**

392 There are several limitations to this study. First, NHP models may not fully recapitulate clinical  
393 data in humans regarding the extent of viral replication necessary for the enhanced transmission  
394 of Omicron compared to prior variants. Even if a significant component of Omicron's growth  
395 advantage is due to immune escape, the role of viral replication kinetics cannot be ruled out.  
396 Here, viral titers were low in the lungs and low to undetectable in the upper airway. Second,  
397 neutralizing antibody titers in NHP are 5- to 10-fold greater than in humans that received the  
398 same dose and regimen of mRNA-1273 with a boost (Edara et al., 2021a; Pajon et al., 2022).  
399 Third, a second dose of mRNA-Omicron may elicit a population of B cells specific only for  
400 Omicron. Finally, since we sought to compare two different mRNA boosts, we did not have an  
401 unboosted group to determine whether the boost enhanced protection. As all the boosted NHP  
402 were completely protected in the lungs, we were unable to determine an immune threshold for  
403 protection.

404

### 405 **Materials and methods**

#### 406 **Resource availability**

#### 407 **Lead contact**

408 Further information and requests for resources should be directed to and will be fulfilled by the  
409 lead contact, Robert A. Seder (rseder@mail.nih.gov).

410

#### 411 **Materials availability**

412 This study did not generate new unique reagents.

413

#### 414 **Data and code availability**

- 415 • All data reported in this paper will be shared by the lead contact upon request.
- 416 • This paper does not report original code.
- 417 • Any additional information required to reanalyze the data reported in this paper is  
418 available from the lead contact upon request.

419

#### 420 **Experimental model and subject details**

##### 421 **Preclinical mRNA and lipid nanoparticle production**

422 A sequence-optimized mRNA encoding prefusion-stabilized SARS-CoV-2 S protein containing  
423 2 proline stabilization mutations (S-2P) (Pallesen et al., 2017; Wrapp et al., 2020) for WA1 and  
424 Omicron were synthesized in vitro and formulated (Hassett et al., 2019). Control mRNA  
425 “UNFIX-01 (Untranslated Factor 9)” was synthesized and similarly formulated into lipid  
426 nanoparticles as previously described (Corbett et al., 2021a).

427

##### 428 **Rhesus macaque model and immunizations**

429 All experiments conducted according to NIH regulations and standards on the humane care and  
430 use of laboratory animals as well as the Animal Care and Use Committees of the NIH Vaccine

431 Research Center and BIOQUAL, Inc. (Rockville, Maryland). All studies were conducted at  
432 BIOQUAL, Inc. Four- to eight-year-old rhesus macaques of Indian origin were stratified into  
433 groups based on sex, age and weight. Eight macaques were immunized with mRNA-1273 at  
434 weeks 0 and 4 with a dose of 100 $\mu$ g delivered intramuscularly in 1mL of formulated lipid  
435 nanoparticles diluted in phosphate-buffered saline (PBS) into the right quadriceps as previously  
436 described (Corbett et al., 2020; Corbett et al., 2021c; Gagne et al., 2022). At week 41 (~9 months  
437 after the second immunization), the eight macaques were split into two groups of 4 and boosted  
438 with 50 $\mu$ g mRNA-1273 or 50 $\mu$ g of mRNA-Omicron. Animals in the control group were  
439 immunized with 50 $\mu$ g control mRNA at the time of the boost.

440

## 441 **Method details**

### 442 **Cells and viruses**

443 VeroE6-TMPRSS2 cells were generated at the Vaccine Research Center, NIH, Bethesda, MD.  
444 Isolation and sequencing of EHC-083E (D614G SARS-CoV-2), Delta, Beta and Omicron for  
445 live virus neutralization assays were previously described (Edara et al., 2021b; Edara et al.,  
446 2021c; Vanderheiden et al., 2020). Viruses were propagated in Vero-TMPRSS2 cells to generate  
447 viral stocks. Viral titers were determined by focus-forming assay on VeroE6-TMPRSS2 cells.  
448 Viral stocks were stored at -80°C until use.

449

### 450 **Sequencing of Omicron virus stock**

451 We used NEBNext Ultra II RNA Prep reagents and multiplex oligos (New England Biolabs) to  
452 prepare Illumina-ready libraries, which were sequenced on a MiSeq (Illumina) as described  
453 previously (Corbett et al., 2021c; Gagne et al., 2022). Demultiplexed sequence reads were

454 analyzed in the CLC Genomics Workbench v.21.0.3 by (1) trimming for quality, length, and  
455 adaptor sequence, (2) mapping to the Wuhan-Hu-1 SARS-CoV-2 reference (GenBank no.  
456 NC\_045512), (3) improving the mapping by local realignment in areas containing insertions and  
457 deletions (indels), and (4) generating both a sample consensus sequence and a list of variants.  
458 Default settings were used for all tools.

459

#### 460 **Omicron challenge**

461 Macaques were challenged at week 45 (4 weeks after the second boost) with a total dose of  $1 \times$   
462  $10^6$  PFU of SARS-CoV-2 Omicron. The viral inoculum was administered as  $7.5 \times 10^5$  PFUs in  
463 3mL intratracheally and  $2.5 \times 10^5$  PFUs in 1mL intranasally in a volume of 0.5mL distributed  
464 evenly into each nostril.

465

#### 466 **Serum and mucosal antibody titers**

467 Quantification of antibodies in the blood and mucosa were performed as previously described  
468 (Corbett et al., 2020). Briefly, total IgG antigen-specific antibodies to variant SARS-CoV-2 S-  
469 and RBD-derived antigens were determined in a multiplex serology assay by MSD V-Plex  
470 SARS-CoV-2 Panel 23 for S and MSD V-Plex SARS-CoV-2 Panel 22 for RBD) according to  
471 manufacturer's instructions, except 25 $\mu$ l of sample and detection antibody were used per well.  
472 Heat inactivated plasma was initially diluted 1:100 and then serially diluted 1:10 for blood S-  
473 and 1:4 for RBD-binding. BAL fluid and nasal washes were concentrated 10-fold with Amicon  
474 Ultra centrifugal filter devices (Millipore Sigma). Concentrated samples were diluted 1:5 prior to  
475 5-fold serial dilutions.

476

477 **S-2P antigens**

478 While S antigens were used for binding ELISAs, S-2P antigens were used for ACE2 inhibition  
479 assays and B cell probe binding. S-2P constructs were made as follows. Biotinylated S probes  
480 were expressed transiently for WA1, Delta, Beta and Omicron strains and purified and  
481 biotinylated in a single in-process step (Teng et al., 2021; Zhou et al., 2020). S-2P for WA1 and  
482 Omicron were made as previously described (Olia et al., 2021).

483

484 **S-2P-ACE2 binding inhibition**

485 ACE2 binding inhibition was performed using a modified Meso Scale Discovery (MSD)  
486 platform assay. Briefly, after blocking MSD Streptavidin MULTI-ARRAY 384 well plates with  
487 Blocker A (MSD), the plates were coated with 1 µg/ml of biotinylated SARS-CoV-2 variant S-  
488 2P (WA1, Beta, Delta or Omicron) and incubated for 1 hour at room temperature (RT). The  
489 plates were washed 5 times with wash buffer (1x PBS containing 0.05% Tween-20). Diluted  
490 samples were added to the coated plates and incubated for 1 hour at RT. MSD SULFO-TAG  
491 Human ACE2 protein was diluted 1:200 and added to the plates. After 1 hour incubation at RT,  
492 the plates were washed 5 times with wash buffer and read on MSD Sector S 600 instrument after  
493 the addition of Gold Read Buffer B (MSD). Results are reported as percent inhibition. BAL fluid  
494 and nasal washes were first concentrated 10-fold with Amicon Ultra centrifugal filter devices  
495 (Millipore Sigma) and then diluted 1:5 in Diluent 100 (MSD).

496

497 **Focus reduction neutralization assay**

498 FRNT assays were performed as previously described (Edara et al., 2021b; Edara et al., 2021c;  
499 Vanderheiden et al., 2020). Briefly, samples were diluted at 3-fold in 8 serial dilutions using



500 DMEM (VWR, #45000-304) in duplicates with an initial dilution of 1:10 in a total volume of  
501 60µl. Serially diluted samples were incubated with an equal volume of WA1, Delta, Beta or  
502 Omicron (100-200 foci per well based on the target cell) at 37°C for 45 minutes in a round-  
503 bottomed 96-well culture plate. The antibody-virus mixture was then added to VeroE6-  
504 TMPRSS2 cells and incubated at 37°C for 1 hour. Post-incubation, the antibody-virus mixture  
505 was removed and 100µl of pre-warmed 0.85% methylcellulose overlay was added to each well.  
506 Plates were incubated at 37°C for 18 hours and the methylcellulose overlay was removed and  
507 washed six times with PBS. Cells were fixed with 2% paraformaldehyde in PBS for 30 minutes.  
508 Following fixation, plates were washed twice with PBS and permeabilization buffer (0.1% BSA,  
509 0.1% Saponin in PBS) was added to permeabilized cells for at least 20 minutes. Cells were  
510 incubated with an anti-SARS-CoV S primary antibody directly conjugated to Alexafluor-647  
511 (CR3022-AF647) overnight at 4°C. Foci were visualized and imaged on an ELISPOT reader  
512 (CTL). Antibody neutralization was quantified by counting the number of foci for each sample  
513 using the Viridot program (Katzelnick et al., 2018). The neutralization titers were calculated as  
514 follows:  $1 - (\text{ratio of the mean number of foci in the presence of sera and foci at the highest}$   
515  $\text{dilution of respective sera sample})$ . Each specimen was tested in duplicate. The FRNT-50 titers  
516 were interpolated using a 4-parameter nonlinear regression in GraphPad Prism 9.2.0. Samples  
517 that do not neutralize at the limit of detection (LOD) at 50% are plotted at 20 and was used for  
518 geometric mean and fold-change calculations. The assay LOD was 20.

519

## 520 **Lentiviral pseudovirus neutralization**

521 Neutralizing antibodies in serum or plasma were measured in a validated pseudovirus-based  
522 assay as a function of reductions in luciferase reporter gene expression after a single round of

523 infection with SARS-CoV-2 spike-pseudotyped viruses in 293T/ACE2 cells (293T cell line  
524 stably overexpressing the human ACE2 cell surface receptor protein, obtained from Drs. Mike  
525 Farzan and Huihui Mu at Scripps) as previously described (Gilbert et al., 2021; Shen et al.,  
526 2021). SARS-CoV-2 S-pseudotyped virus was prepared by transfection in 293T/17 cells (human  
527 embryonic kidney cells in origin; obtained from American Type Culture Collection, #CRL-  
528 11268) using a lentivirus backbone vector, a spike-expression plasmid encoding S protein from  
529 Wuhan-Hu-1 strain (GenBank no. NC\_045512) with a p.Asp614Gly mutation, a TMPRSS2  
530 expression plasmid and a firefly Luc reporter plasmid. For pseudovirus encoding the S from  
531 Delta, Beta and Omicron, the plasmid was altered via site-directed mutagenesis to match the S  
532 sequence to the corresponding variant sequence as previously described (Corbett et al., 2021a). A  
533 pre-titrated dose of pseudovirus was incubated with eight serial 5-fold dilutions of serum  
534 samples (1:20 start dilution) in duplicate in 384-well flat-bottom tissue culture plates (Thermo  
535 Fisher, #12-565-344) for 1 hour at 37°C prior to adding 293T/ACE2 cells. One set of 14 wells  
536 received cells and virus (virus control) and another set of 14 wells received cells only  
537 (background control), corresponding to technical replicates. Luminescence was measured after  
538 66-72 hours of incubation using Britelite-Plus luciferase reagent (Perkin Elmer, #6066769).  
539 Neutralization titers are the inhibitory dilution of serum samples at which relative luminescence  
540 units (RLUs) were reduced by 50% (ID<sub>50</sub>) compared to virus control wells after subtraction of  
541 background RLUs. Serum samples were heat-inactivated for 30-45 minutes at 56°C prior to  
542 assay.

543

544 **Serum antibody avidity**

545 Avidity was measured as described previously (Francica et al., 2021) in an adapted ELISA assay.  
546 Briefly, ELISA against S-2P was performed in the absence or presence of sodium thiocyanate  
547 (NaSCN) and developed with HRP-conjugated goat anti-monkey IgG (H+L) secondary antibody  
548 (Invitrogen) and SureBlue 3,3',5,5'-tetramethylbenzidine (TMB) microwell peroxidase substrate  
549 (1-Component; SeraCare) and quenched with 1 N H<sub>2</sub>SO<sub>4</sub>. The avidity index (AI) was calculated  
550 as the ratio of IgG binding to S-2P in the absence or presence of NaSCN.

551

### 552 **Epitope mapping**

553 Serum epitope mapping competition assays were performed, as previously described (Corbett et  
554 al., 2021a), using the Biacore 8K+ surface plasmon resonance system (Cytiva). Briefly, through  
555 primary amine coupling using a His capture kit (Cytiva), anti-histidine antibody was  
556 immobilized on Series S Sensor Chip CM5 (Cytiva) allowing for the capture of his-tagged  
557 SARS-CoV-2 S-2P on active sensor surface.

558

559 Human IgG monoclonal antibodies (mAbs) used for these analyses include: RBD-specific mAbs  
560 B1-182, A19-46.1, A20-29.1, A19-61.1, S309, A23-97.1 and A23-80.1. Negative control  
561 antibody or competitor mAb was injected over both active and reference surfaces. Following  
562 this, NHP sera (diluted 1:50) was flowed over both active and reference sensor surfaces. Active  
563 and reference sensor surfaces were regenerated between each analysis cycle.

564

565 For analysis, sensorgrams were aligned to Y (Response Units) = 0, using Biacore 8K Insights  
566 Evaluation Software (Cytiva) beginning at the serum association phase. Relative “analyte  
567 binding late” report points (RU) were collected and used to calculate fractional competition (%)

568 C) using the following formula:  $\% C = [1 - (100 * ((RU \text{ in presence of competitor mAb}) / (RU$   
569  $\text{ in presence of negative control mAb}))]$ . Results are reported as fractional competition. Assays  
570 were performed in duplicate, with average data point represented on corresponding graphs.

571

## 572 **B cell probe binding**

573 Kinetics of S-specific memory B cells responses were determined as previously described  
574 (Gagne et al., 2022). Briefly, cryopreserved PBMC were thawed and stained with the following  
575 antibodies (monoclonal unless indicated): IgD FITC (goat polyclonal, Southern Biotech), IgM  
576 PerCP-Cy5.5 (clone G20-127, BD Biosciences), IgA Dylight 405 (goat polyclonal, Jackson  
577 Immunoresearch Inc), CD20 BV570 (clone 2H7, Biolegend), CD27 BV650 (clone O323,  
578 Biolegend), CD14 BV785 (clone M5E2, Biolegend), CD16 BUV496 (clone 3G8, BD  
579 Biosciences), CD4 BUV737 (clone SK3, BD Biosciences), CD19 APC (clone J3-119,  
580 Beckman), IgG Alexa 700 (clone G18-145, BD Biosciences), CD3 APC-Cy7 (clone SP34-2, BD  
581 Biosciences), CD38 PE (clone OKT10, Caprico Biotechnologies), CD21 PE-Cy5 (clone B-ly4,  
582 BD Biosciences) and CXCR5 PE-Cy7 (clone MU5UBEE, Thermo Fisher Scientific). Stained  
583 cells were then incubated with streptavidin-BV605 (BD Biosciences) labeled WA1, Beta, Delta  
584 or Omicron S-2P and streptavidin-BUV661 (BD Biosciences) labeled WA1 or Delta S-2P for 30  
585 minutes at 4°C (protected from light). Cells were washed and fixed in 0.5% formaldehyde  
586 (Tousimis Research Corp) prior to data acquisition. Aqua live/dead fixable dead cell stain kit  
587 (Thermo Fisher Scientific) was used to exclude dead cells. All antibodies were previously  
588 titrated to determine the optimal concentration. Samples were acquired on an BD FACSymphony  
589 cytometer and analyzed using FlowJo version 10.7.2 (BD, Ashland, OR).

590

## 591 **Intracellular cytokine staining**

592 Intracellular cytokine staining was performed as previously described (Donaldson et al., 2019;  
593 Gagne et al., 2022). Briefly, cryopreserved PBMC and BAL cells were thawed and rested  
594 overnight in a 37°C/5% CO<sub>2</sub> incubator. The following morning, cells were stimulated with  
595 SARS-CoV-2 S protein (S1 and S2, matched to vaccine insert) peptide pools (JPT Peptides) at a  
596 final concentration of 2 µg/ml in the presence of 3mM monensin for 6 hours. The S1 and S2  
597 peptide pools were comprised of 158 and 157 individual peptides, respectively, as 15 mers  
598 overlapping by 11 amino acids in 100% DMSO. Negative controls received an equal  
599 concentration of DMSO instead of peptides (final concentration of 0.5%). The following  
600 monoclonal antibodies were used: CD3 APC-Cy7 (clone SP34-2, BD Biosciences), CD4 PE-  
601 Cy5.5 (clone S3.5, Invitrogen), CD8 BV570 (clone RPA-T8, BioLegend), CD45RA PE-Cy5  
602 (clone 5H9, BD Biosciences), CCR7 BV650 (clone G043H7, BioLegend), CXCR5 PE (clone  
603 MU5UBEE, Thermo Fisher), CXCR3 BV711 (clone 1C6/CXCR3, BD Biosciences), PD-1  
604 BUV737 (clone EH12.1, BD Biosciences), ICOS Pe-Cy7 (clone C398.4A, BioLegend), CD69  
605 ECD (clone TP1.55.3, Beckman Coulter), IFN-γ Ax700 (clone B27, BioLegend), IL-2 BV750  
606 (clone MQ1-17H12, BD Biosciences), IL-4 BB700 (clone MP4-25D2, BD Biosciences), TNF-  
607 FITC (clone Mab11, BD Biosciences), IL-13 BV421 (clone JES10-5A2, BD Biosciences), IL-17  
608 BV605 (clone BL168, BioLegend), IL-21 Ax647 (clone 3A3-N2.1, BD Biosciences) and CD154  
609 BV785 (clone 24-31, BioLegend). Aqua live/dead fixable dead cell stain kit (Thermo Fisher  
610 Scientific) was used to exclude dead cells. All antibodies were previously titrated to determine  
611 the optimal concentration. Samples were acquired on a BD FACSymphony flow cytometer and  
612 analyzed using FlowJo version 10.8.0 (BD, Ashland, OR).

613

614 **Subgenomic RNA quantification**

615 sgRNA was isolated and quantified by researchers blinded to vaccine history as previously  
616 described (Corbett et al., 2021c), except for the use of a new probe noted below. Briefly, total  
617 RNA was extracted from BAL fluid and nasal swabs using RNAzol BD column kit (Molecular  
618 Research Center). PCR reactions were conducted with TaqMan Fast Virus 1-Step Master Mix  
619 (Applied Biosystems), forward primer in the 5' leader region and gene-specific probes and  
620 reverse primers as follows:

621

622 sgLeadSARSCoV2\_F: 5'-CGATCTCTTGTAGATCTGTTCTC-3'

623

624 *N gene*

625 N2\_P: 5'-FAM- CGATCAAAACAACGTCGGCCCC-BHQ1-3'

626 wtN\_R: 5'-GGTGAACCAAGACGCAGTAT-3'

627

628 Amplifications were performed with a QuantStudio 6 Pro Real-Time PCR System (Applied  
629 Biosystems). The assay lower LOD was 50 copies per reaction.

630

631 **Median Tissue Culture Infectious Dose (TCID<sub>50</sub>) assay**

632 TCID<sub>50</sub> was quantified as previously described (Corbett et al., 2021c). Briefly, Vero-TMPRSS2  
633 cells were plated and incubated overnight. The following day, BAL samples were serially  
634 diluted, and the plates were incubated at 37 °C/5.0% CO<sub>2</sub> for four days. Positive (virus stock of  
635 known infectious titer in the assay) and negative (medium only) control wells were included in

636 each assay setup. The cell monolayers were visually inspected for cytopathic effect. TCID<sub>50</sub>  
637 values were calculated using the Reed–Muench formula.

638

### 639 **Histopathology and immunohistochemistry**

640 Routine histopathology and detection of SARS-CoV-2 virus antigen via immunohistochemistry  
641 (IHC) were performed as previously described (Corbett et al., 2020; Gagne et al., 2022). Briefly,  
642 seven to nine days following Omicron challenge animals were euthanized and lung tissue was  
643 processed and stained with hematoxylin and eosin for pathological analysis or with a rabbit  
644 polyclonal anti-SARS-CoV-2 anti-nucleocapsid antibody (GeneTex, GTX135357) at a dilution  
645 of 1:2000 for IHC. Tissue sections were analyzed by a blinded board-certified veterinary  
646 pathologist using an Olympus BX43 light microscope. Photomicrographs were taken on an  
647 Olympus DP27 camera.

648

### 649 **Quantification and statistical analysis**

650 Comparisons between groups, or between timepoints within a group, are based on unpaired and  
651 paired t-tests, respectively. All analysis for serum epitope mapping was performed using  
652 unpaired, two-tailed t-test. Binding, neutralizing and viral assays are log-transformed as  
653 appropriate and reported with geometric means and corresponding confidence intervals where  
654 indicated. There are no adjustments for multiple comparisons, so all p-values should be  
655 interpreted as suggestive rather than conclusive. All analyses are conducted using R version 4.0.2  
656 and GraphPad Prism version 8.2.0 unless otherwise specified.

657

658 *P* values are stated in the text, and the sample *n* is listed in corresponding figure legends. For all  
659 data presented, *n*=4 for individual boost cohorts and *n*=7-8 for controls and vaccinated NHP at  
660 pre-boost timepoints.

661

## 662 **Acknowledgments**

663 We would like to thank G. Alvarado for experimental organization and administrative support.

664 The VRC Production Program (VPP) provided the WA1 protein for the avidity assay. VPP

665 contributors include C. Anderson, V. Bhagat, J. Burd, J. Cai, K. Carlton, W. Chuenchor, N.

666 Clbelli, G. Dobrescu, M. Figur, J. Gall, H. Geng, D. Gowetski, K. Gulla, L. Hogan, V. Ivleva, S.

667 Khayat, P. Lei, Y. Li, I. Loukinov, M. Mai, S. Nugent, M. Pratt, E. Reilly, E. Rosales-Zavala, E.

668 Scheideman, A. Shaddeau, A. Thomas, S. Upadhyay, K. Vickery, A. Vinitsky, C. Wang, C.

669 Webber and Y. Yang.

670

671 This project has been funded in part by both the Intramural Program of the National Institute of

672 Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human

673 Services and under HHSN272201400004C (NIAID Centers of Excellence for Influenza

674 Research and Surveillance, CEIRS) and NIH P51 OD011132 awarded to Emory University. This

675 work was also supported in part by the Emory Executive Vice President for Health Affairs

676 Synergy Fund award, COVID-Catalyst-I3 Funds from the Woodruff Health Sciences Center and

677 Emory School of Medicine, the Pediatric Research Alliance Center for Childhood Infections and

678 Vaccines and Children's Healthcare of Atlanta, and Woodruff Health Sciences Center 2020

679 COVID-19 CURE Award.

680



681 **Author contributions**

682 M.R., N.J.S., D.C.D. and R.A.S. designed experiments. M.G., J.I.M, K.E.F., S.F.A., B.J.F.,  
683 A.P.W, D.A.W., B.C.L., C.M., N.J-B., R.C., S.L.F., M.P., M.E., V-V.E., N.V.M., M.M., L.M.,  
684 C.C.H., B.M.N., K.W.B., C.N.M.D., J.C., J-P.M.T., E.M., L.P., A.V.R., B.N., D.V., A.C., A.D.,  
685 K.S., D.R.F., S.T.N., S.G., A.R.H., F.L., J.R-T., C.G.L, S.A., D.K.E., H.A., M.G.L., K.S.C.,  
686 M.C.N., A.B.M., M.S.S., I.N.M., M.R., N.J.S., D.C.D. and R.A.S. performed, analyzed, and/or  
687 supervised experiments. M.G., J.I.M., K.E.F., S.F.A., D.A.W., I.N.M. and D.C.D. designed  
688 figures. I-T.T., J.T., M.N., M.B., J.W., L.W., W.S., N.A.D-R., A.S.O., C.L., D.R.H., A.C.,  
689 J.R.M. and P.D.K. provided critical reagents. M.G., J.I.M., D.C.D. and R.A.S. wrote manuscript.  
690 All authors edited the manuscript and provided feedback on research.

691

692 **Declaration of interests**

693 K.S.C. is an inventor on U.S. Patent No. 10,960,070 B2 and International Patent Application No.  
694 WO/2018/081318 entitled “Prefusion Coronavirus Spike Proteins and Their Use”. K.S.C. is an  
695 inventor on U.S. Patent Application No. 62/972,886 entitled “2019-nCoV Vaccine”. L.W., W.S.,  
696 J.R.M., M.R., N.J.S. and D.C.D are inventors on U.S. Patent Application No. 63/147,419 entitled  
697 “Antibodies Targeting the Spike Protein of Coronaviruses”. L.P., A.V.R., B.N., D.V., A.C.,  
698 A.D., K.S., H.A. and M.G.L. are employees of Bioqual. K.S.C, L.W. and W.S. are inventors on  
699 multiple U.S. Patent Applications entitled “Anti-Coronavirus Antibodies and Methods of Use”.  
700 A.C. and D.K.E. are employees of Moderna. M.S.S. serves on the scientific board of advisors for  
701 Moderna and Ocugen. The other authors declare no competing interests.

702

## 703 **References**

- 704 Abdullah, F., Myers, J., Basu, D., Tintinger, G., Ueckermann, V., Mathebula, M., Ramlall, R.,  
705 Spoor, S., de Villiers, T., Van der Walt, Z., *et al.* (2021). Decreased severity of disease during the  
706 first global omicron variant covid-19 outbreak in a large hospital in tshwane, south africa. *Int J*  
707 *Infect Dis* *116*, 38-42.
- 708 Accorsi, E.K., Britton, A., Fleming-Dutra, K.E., Smith, Z.R., Shang, N., Derado, G., Miller, J.,  
709 Schrag, S.J., and Verani, J.R. (2022). Association Between 3 Doses of mRNA COVID-19 Vaccine  
710 and Symptomatic Infection Caused by the SARS-CoV-2 Omicron and Delta Variants. *JAMA*.  
711 Andrews, N., Stowe, J., Kirsebom, F., Toffa, S., Sachdeva, R., Gower, C., Ramsay, M., and Bernal,  
712 J.L. (2022). Effectiveness of COVID-19 booster vaccines against covid-19 related symptoms,  
713 hospitalisation and death in England. *Nat Med*.
- 714 Baden, L.R., El Sahly, H.M., Essink, B., Follmann, D., Neuzil, K.M., August, A., Clouting, H., Fortier,  
715 G., Deng, W., Han, S., *et al.* (2021a). Phase 3 Trial of mRNA-1273 during the Delta-Variant Surge.  
716 *N Engl J Med*.
- 717 Baden, L.R., El Sahly, H.M., Essink, B., Kotloff, K., Frey, S., Novak, R., Diemert, D., Spector, S.A.,  
718 Roupheal, N., Creech, C.B., *et al.* (2021b). Efficacy and Safety of the mRNA-1273 SARS-CoV-2  
719 Vaccine. *N Engl J Med* *384*, 403-416.
- 720 Bar-On, Y.M., Goldberg, Y., Mandel, M., Bodenheimer, O., Freedman, L., Alroy-Preis, S., Ash, N.,  
721 Huppert, A., and Milo, R. (2021). Protection against Covid-19 by BNT162b2 Booster across Age  
722 Groups. *N Engl J Med* *385*, 2421-2430.
- 723 Barda, N., Dagan, N., Cohen, C., Hernan, M.A., Lipsitch, M., Kohane, I.S., Reis, B.Y., and Balicer,  
724 R.D. (2021). Effectiveness of a third dose of the BNT162b2 mRNA COVID-19 vaccine for  
725 preventing severe outcomes in Israel: an observational study. *Lancet* *398*, 2093-2100.
- 726 Bentley, E.G., Kirby, A., Sharma, P., Kipar, A., Mega, D.F., Bramwell, C., Penrice-Randal, R.,  
727 Prince, T., Brown, J.C., Zhou, J., *et al.* (2021). SARS-CoV-2 Omicron-B.1.1.529 Variant leads to  
728 less severe disease than Pango B and Delta variants strains in a mouse model of severe COVID-  
729 19. *bioRxiv*, 2021.2012.2026.474085.
- 730 Bergwerk, M., Gonen, T., Lustig, Y., Amit, S., Lipsitch, M., Cohen, C., Mandelboim, M., Gal Levin,  
731 E., Rubin, C., Indenbaum, V., *et al.* (2021). Covid-19 Breakthrough Infections in Vaccinated  
732 Health Care Workers. *N Engl J Med*.
- 733 Bruxvoort, K.J., Sy, L.S., Qian, L., Ackerson, B.K., Luo, Y., Lee, G.S., Tian, Y., Florea, A., Aragonés,  
734 M., Tubert, J.E., *et al.* (2021a). Effectiveness of mRNA-1273 against delta, mu, and other  
735 emerging variants of SARS-CoV-2: test negative case-control study. *BMJ* *375*, e068848.
- 736 Bruxvoort, K.J., Sy, L.S., Qian, L., Ackerson, B.K., Luo, Y., Lee, G.S., Tian, Y., Florea, A., Takhar,  
737 H.S., Tubert, J.E., *et al.* (2021b). Real-world effectiveness of the mRNA-1273 vaccine against  
738 COVID-19: Interim results from a prospective observational cohort study. *Lancet Reg Health*  
739 *Am*, 100134.
- 740 Cele, S., Jackson, L., Khoury, D.S., Khan, K., Moyo-Gwete, T., Tegally, H., San, J.E., Cromer, D.,  
741 Scheepers, C., Amoako, D.G., *et al.* (2021). Omicron extensively but incompletely escapes Pfizer  
742 BNT162b2 neutralization. *Nature*.
- 743 Chemaitelly, H., Yassine, H.M., Benslimane, F.M., Al Khatib, H.A., Tang, P., Hasan, M.R., Malek,  
744 J.A., Coyle, P., Ayoub, H.H., Al Kanaani, Z., *et al.* (2021). mRNA-1273 COVID-19 vaccine

745 effectiveness against the B.1.1.7 and B.1.351 variants and severe COVID-19 disease in Qatar.  
746 Nat Med 27, 1614-1621.

747 Choi, A., Koch, M., Wu, K., Chu, L., Ma, L., Hill, A., Nunna, N., Huang, W., Oestreicher, J., Colpitts,  
748 T., *et al.* (2021). Safety and immunogenicity of SARS-CoV-2 variant mRNA vaccine boosters in  
749 healthy adults: an interim analysis. Nat Med 27, 2025-2031.

750 Corbett, K.S., Flynn, B., Foulds, K.E., Francica, J.R., Boyoglu-Barnum, S., Werner, A.P., Flach, B.,  
751 O'Connell, S., Bock, K.W., Minai, M., *et al.* (2020). Evaluation of the mRNA-1273 Vaccine against  
752 SARS-CoV-2 in Nonhuman Primates. N Engl J Med 383, 1544-1555.

753 Corbett, K.S., Gagne, M., Wagner, D.A., S, O.C., Narpala, S.R., Flebbe, D.R., Andrew, S.F., Davis,  
754 R.L., Flynn, B., Johnston, T.S., *et al.* (2021a). Protection against SARS-CoV-2 beta variant in  
755 mRNA-1273 vaccine-boosted nonhuman primates. Science, eabl8912.

756 Corbett, K.S., Nason, M.C., Flach, B., Gagne, M., O'Connell, S., Johnston, T.S., Shah, S.N., Edara,  
757 V.V., Floyd, K., Lai, L., *et al.* (2021b). Immune correlates of protection by mRNA-1273 vaccine  
758 against SARS-CoV-2 in nonhuman primates. Science, eabj0299.

759 Corbett, K.S., Werner, A.P., Connell, S.O., Gagne, M., Lai, L., Moliva, J.I., Flynn, B., Choi, A., Koch,  
760 M., Foulds, K.E., *et al.* (2021c). mRNA-1273 protects against SARS-CoV-2 beta infection in  
761 nonhuman primates. Nat Immunol.

762 Dagan, N., Barda, N., Kepten, E., Miron, O., Perchik, S., Katz, M.A., Hernan, M.A., Lipsitch, M.,  
763 Reis, B., and Balicer, R.D. (2021). BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass  
764 Vaccination Setting. N Engl J Med 384, 1412-1423.

765 Davenport, F.M., and Hennessy, A.V. (1957). Predetermination by infection and by vaccination  
766 of antibody response to influenza virus vaccines. J Exp Med 106, 835-850.

767 Davenport, F.M., Hennessy, A.V., and Francis, T., Jr. (1953). Epidemiologic and immunologic  
768 significance of age distribution of antibody to antigenic variants of influenza virus. J Exp Med  
769 98, 641-656.

770 Davies, M.-A., Kassanjee, R., Rousseau, P., Morden, E., Johnson, L., Solomon, W., Hsiao, N.-Y.,  
771 Hussey, H., Meintjes, G., Paleker, M., *et al.* (2022). Outcomes of laboratory-confirmed SARS-  
772 CoV-2 infection in the Omicron-driven fourth wave compared with previous waves in the  
773 Western Cape Province, South Africa. medRxiv, 2022.2001.2012.22269148.

774 Donaldson, M.M., Kao, S.F., and Foulds, K.E. (2019). OMIP-052: An 18-Color Panel for  
775 Measuring Th1, Th2, Th17, and Tfh Responses in Rhesus Macaques. Cytometry A 95, 261-263.

776 Edara, V.-V., Manning, K.E., Ellis, M., Lai, L., Moore, K.M., Foster, S.L., Floyd, K., Davis-Gardner,  
777 M.E., Mantus, G., Nyhoff, L.E., *et al.* (2021a). mRNA-1273 and BNT162b2 mRNA vaccines have  
778 reduced neutralizing activity against the SARS-CoV-2 Omicron variant. bioRxiv,  
779 2021.2012.2020.473557.

780 Edara, V.V., Norwood, C., Floyd, K., Lai, L., Davis-Gardner, M.E., Hudson, W.H., Mantus, G.,  
781 Nyhoff, L.E., Adelman, M.W., Fineman, R., *et al.* (2021b). Infection- and vaccine-induced  
782 antibody binding and neutralization of the B.1.351 SARS-CoV-2 variant. Cell Host Microbe 29,  
783 516-521 e513.

784 Edara, V.V., Pinsky, B.A., Suthar, M.S., Lai, L., Davis-Gardner, M.E., Floyd, K., Flowers, M.W.,  
785 Wrammert, J., Hussaini, L., Ciric, C.R., *et al.* (2021c). Infection and Vaccine-Induced Neutralizing-  
786 Antibody Responses to the SARS-CoV-2 B.1.617 Variants. N Engl J Med.

787 Francica, J.R., Flynn, B.J., Foulds, K.E., Noe, A.T., Werner, A.P., Moore, I.N., Gagne, M., Johnston,  
788 T.S., Tucker, C., Davis, R.L., *et al.* (2021). Protective antibodies elicited by SARS-CoV-2 spike

789 protein vaccination are boosted in the lung after challenge in nonhuman primates. *Sci Transl*  
790 *Med*.

791 Gaebler, C., Wang, Z., Lorenzi, J.C.C., Muecksch, F., Finkin, S., Tokuyama, M., Cho, A., Jankovic,  
792 M., Schaefer-Babajew, D., Oliveira, T.Y., *et al.* (2021). Evolution of antibody immunity to SARS-  
793 CoV-2. *Nature* *591*, 639-644.

794 Gagne, M., Corbett, K.S., Flynn, B.J., Foulds, K.E., Wagner, D.A., Andrew, S.F., Todd, J.M.,  
795 Honeycutt, C.C., McCormick, L., Nurmukhambetova, S.T., *et al.* (2022). Protection from SARS-  
796 CoV-2 Delta one year after mRNA-1273 vaccination in rhesus macaques coincides with  
797 anamnestic antibody response in the lung. *Cell* *185*, 113-130 e115.

798 Garcia-Beltran, W.F., St Denis, K.J., Hoelzemer, A., Lam, E.C., Nitido, A.D., Sheehan, M.L.,  
799 Berrios, C., Ofoman, O., Chang, C.C., Hauser, B.M., *et al.* (2022). mRNA-based COVID-19 vaccine  
800 boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant. *Cell*.

801 Gilbert, P.B., Montefiori, D.C., McDermott, A.B., Fong, Y., Benkeser, D., Deng, W., Zhou, H.,  
802 Houchens, C.R., Martins, K., Jayashankar, L., *et al.* (2021). Immune correlates analysis of the  
803 mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science*, eab3435.

804 Goldberg, Y., Mandel, M., Bar-On, Y.M., Bodenheimer, O., Freedman, L., Haas, E.J., Milo, R.,  
805 Alroy-Preis, S., Ash, N., and Huppert, A. (2021). Waning Immunity after the BNT162b2 Vaccine  
806 in Israel. *N Engl J Med*.

807 Gostic, K.M., Ambrose, M., Worobey, M., and Lloyd-Smith, J.O. (2016). Potent protection  
808 against H5N1 and H7N9 influenza via childhood hemagglutinin imprinting. *Science* *354*, 722-  
809 726.

810 Grabowski, F., Kočańczyk, M., and Lipniacki, T. (2022). The Spread of SARS-CoV-2 Variant  
811 Omicron with a Doubling Time of 2.0-3.3 Days Can Be Explained by Immune Evasion. *Viruses* *14*,  
812 294.

813 Halfmann, P.J., Iida, S., Iwatsuki-Horimoto, K., Maemura, T., Kiso, M., Scheaffer, S.M., Darling,  
814 T.L., Joshi, A., Loeber, S., Singh, G., *et al.* (2022). SARS-CoV-2 Omicron virus causes attenuated  
815 disease in mice and hamsters. *Nature*.

816 Hansen, C.H., Schelde, A.B., Moustsen-Helm, I.R., Emborg, H.-D., Krause, T.G., Mølbak, K.,  
817 Valentiner-Branth, P., and Institut, o.b.o.t.I.D.P.G.a.S.S. (2021). Vaccine effectiveness against  
818 SARS-CoV-2 infection with the Omicron or Delta variants following a two-dose or booster  
819 BNT162b2 or mRNA-1273 vaccination series: A Danish cohort study. *medRxiv*,  
820 2021.2012.2020.21267966.

821 Hassett, K.J., Benenato, K.E., Jacquinet, E., Lee, A., Woods, A., Yuzhakov, O., Himansu, S.,  
822 Deterling, J., Geilich, B.M., Ketova, T., *et al.* (2019). Optimization of Lipid Nanoparticles for  
823 Intramuscular Administration of mRNA Vaccines. *Mol Ther Nucleic Acids* *15*, 1-11.

824 Hoffmann, M., Kruger, N., Schulz, S., Cossmann, A., Rocha, C., Kempf, A., Nehlmeier, I.,  
825 Graichen, L., Moldenhauer, A.S., Winkler, M.S., *et al.* (2021). The Omicron variant is highly  
826 resistant against antibody-mediated neutralization: Implications for control of the COVID-19  
827 pandemic. *Cell*.

828 Jackson, L.A., Anderson, E.J., Roupheal, N.G., Roberts, P.C., Makhene, M., Coler, R.N.,  
829 McCullough, M.P., Chappell, J.D., Denison, M.R., Stevens, L.J., *et al.* (2020). An mRNA Vaccine  
830 against SARS-CoV-2 - Preliminary Report. *N Engl J Med* *383*, 1920-1931.

831 Johnston, R.J., Poholek, A.C., DiToro, D., Yusuf, I., Eto, D., Barnett, B., Dent, A.L., Craft, J., and  
832 Crotty, S. (2009). Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular  
833 helper cell differentiation. *Science* 325, 1006-1010.

834 Katzelnick, L.C., Coello Escoto, A., McElvany, B.D., Chavez, C., Salje, H., Luo, W., Rodriguez-  
835 Barraquer, I., Jarman, R., Durbin, A.P., Diehl, S.A., *et al.* (2018). Viridot: An automated virus  
836 plaque (immunofocus) counter for the measurement of serological neutralizing responses with  
837 application to dengue virus. *PLoS Negl Trop Dis* 12, e0006862.

838 Khan, K., Karim, F., Cele, S., San, J.E., Lustig, G., Tegally, H., Rosenberg, Y., Bernstein, M., Ganga,  
839 Y., Jule, Z., *et al.* (2022). Omicron infection of vaccinated individuals enhances neutralizing  
840 immunity against the Delta variant. *medRxiv*, 2021.2012.2027.21268439.

841 Kim, D., Lee, J.Y., Yang, J.S., Kim, J.W., Kim, V.N., and Chang, H. (2020). The Architecture of  
842 SARS-CoV-2 Transcriptome. *Cell* 181, 914-921 e910.

843 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,  
844 Y.-C., Kumari, M., *et al.* (2022). Omicron-specific mRNA vaccine induced potent neutralizing  
845 antibody against Omicron but not other SARS-CoV-2 variants. *bioRxiv*, 2022.2001.2031.478406.

846 Maslo, C., Friedland, R., Toubkin, M., Laubscher, A., Akaloo, T., and Kama, B. (2021).  
847 Characteristics and Outcomes of Hospitalized Patients in South Africa During the COVID-19  
848 Omicron Wave Compared With Previous Waves. *JAMA*.

849 Meng, B., Ferreira, I., Abdullahi, A., Goonawardane, N., Saito, A., Kimura, I., Yamasoba, D.,  
850 Kemp, S.A., Papa, G., Fatihi, S., *et al.* (2022). SARS-CoV-2 Omicron spike mediated immune  
851 escape, infectivity and cell-cell fusion. *bioRxiv*, 2021.2012.2017.473248.

852 Muik, A., Lui, B.G., Wallisch, A.K., Bacher, M., Muhl, J., Reinholz, J., Ozhelvaci, O., Beckmann, N.,  
853 Guimil Garcia, R.C., Poran, A., *et al.* (2022). Neutralization of SARS-CoV-2 Omicron by BNT162b2  
854 mRNA vaccine-elicited human sera. *Science*, eabn7591.

855 Nurieva, R.I., Chung, Y., Martinez, G.J., Yang, X.O., Tanaka, S., Matskevitch, T.D., Wang, Y.H., and  
856 Dong, C. (2009). Bcl6 mediates the development of T follicular helper cells. *Science* 325, 1001-  
857 1005.

858 Olia, A.S., Tsybovsky, Y., Chen, S.J., Liu, C., Nazzari, A.F., Ou, L., Wang, L., Kong, W.P., Leung, K.,  
859 Liu, T., *et al.* (2021). SARS-CoV-2 S2P spike ages through distinct states with altered  
860 immunogenicity. *J Biol Chem* 297, 101127.

861 Pajon, R., Doria-Rose, N.A., Shen, X., Schmidt, S.D., O'Dell, S., McDanal, C., Feng, W., Tong, J.,  
862 Eaton, A., Maglinao, M., *et al.* (2022). SARS-CoV-2 Omicron Variant Neutralization after mRNA-  
863 1273 Booster Vaccination. *N Engl J Med*.

864 Pallesen, J., Wang, N., Corbett, K.S., Wrapp, D., Kirchdoerfer, R.N., Turner, H.L., Cottrell, C.A.,  
865 Becker, M.M., Wang, L., Shi, W., *et al.* (2017). Immunogenicity and structures of a rationally  
866 designed prefusion MERS-CoV spike antigen. *Proc Natl Acad Sci U S A* 114, E7348-E7357.

867 Pilishvili, T., Gierke, R., Fleming-Dutra, K.E., Farrar, J.L., Mohr, N.M., Talan, D.A., Krishnadasan,  
868 A., Harland, K.K., Smithline, H.A., Hou, P.C., *et al.* (2021). Effectiveness of mRNA Covid-19  
869 Vaccine among U.S. Health Care Personnel. *N Engl J Med*.

870 Planas, D., Veyer, D., Baidaliuk, A., Staropoli, I., Guivel-Benhassine, F., Rajah, M.M., Planchais,  
871 C., Porrot, F., Robillard, N., Puech, J., *et al.* (2021). Reduced sensitivity of SARS-CoV-2 variant  
872 Delta to antibody neutralization. *Nature* 596, 276-280.

873 Polack, F.P., Thomas, S.J., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Perez, J.L., Perez  
874 Marc, G., Moreira, E.D., Zerbini, C., *et al.* (2020). Safety and Efficacy of the BNT162b2 mRNA  
875 Covid-19 Vaccine. *N Engl J Med* **383**, 2603-2615.

876 Roessler, A., Knabl, L., von Laer, D., and Kimpel, J. (2022). Neutralization profile of Omicron  
877 variant convalescent individuals. medRxiv, 2022.2002.2001.22270263.

878 Schmidt, F., Muecksch, F., Weisblum, Y., Da Silva, J., Bednarski, E., Cho, A., Wang, Z., Gaebler, C.,  
879 Caskey, M., Nussenzweig, M.C., *et al.* (2021). Plasma Neutralization of the SARS-CoV-2 Omicron  
880 Variant. *New England Journal of Medicine*.

881 Shen, X., Tang, H., McDanal, C., Wagh, K., Fischer, W., Theiler, J., Yoon, H., Li, D., Haynes, B.F.,  
882 Sanders, K.O., *et al.* (2021). SARS-CoV-2 variant B.1.1.7 is susceptible to neutralizing antibodies  
883 elicited by ancestral spike vaccines. *Cell Host Microbe* **29**, 529-539 e523.

884 Sigal, A. (2022). Milder disease with Omicron: is it the virus or the pre-existing immunity? *Nat*  
885 *Rev Immunol*.

886 Suryawanshi, R.K., Chen, I.P., Ma, T., Syed, A.M., Simoneau, C.R., Ciling, A., Khalid, M.M.,  
887 Sreekumar, B., Chen, P.-Y., George, A.F., *et al.* (2022). Limited cross-variant immunity after  
888 infection with the SARS-CoV-2 Omicron variant without vaccination. medRxiv,  
889 2022.2001.2013.22269243.

890 Tang, P., Hasan, M.R., Chemaitelly, H., Yassine, H.M., Benslimane, F.M., Al Khatib, H.A.,  
891 AlMukdad, S., Coyle, P., Ayoub, H.H., Al Kanaani, Z., *et al.* (2021). BNT162b2 and mRNA-1273  
892 COVID-19 vaccine effectiveness against the SARS-CoV-2 Delta variant in Qatar. *Nat Med*.

893 Tangye, S.G., Ferguson, A., Avery, D.T., Ma, C.S., and Hodgkin, P.D. (2002). Isotype switching by  
894 human B cells is division-associated and regulated by cytokines. *J Immunol* **169**, 4298-4306.

895 Teng, I.-T., Nazari, A.F., Choe, M., Liu, T., de Souza, M.O., Petrova, Y., Tsybovsky, Y., Wang, S.,  
896 Zhang, B., Artamonov, M., *et al.* (2021). Molecular probes of spike ectodomain and its  
897 subdomains for SARS-CoV-2 variants, Alpha through Omicron. bioRxiv, 2021.2012.2029.474491.

898 Tseng, H.F., Ackerson, B.K., Luo, Y., Sy, L.S., Talarico, C.A., Tian, Y., Bruxvoort, K.J., Tubert, J.E.,  
899 Florea, A., Ku, J.H., *et al.* (2022). Effectiveness of mRNA-1273 against SARS-CoV-2 omicron and  
900 delta variants. medRxiv, 2022.2001.2007.22268919.

901 Vanderheiden, A., Edara, V.V., Floyd, K., Kauffman, R.C., Mantus, G., Anderson, E., Roupael, N.,  
902 Edupuganti, S., Shi, P.Y., Menachery, V.D., *et al.* (2020). Development of a Rapid Focus  
903 Reduction Neutralization Test Assay for Measuring SARS-CoV-2 Neutralizing Antibodies. *Curr*  
904 *Protoc Immunol* **131**, e116.

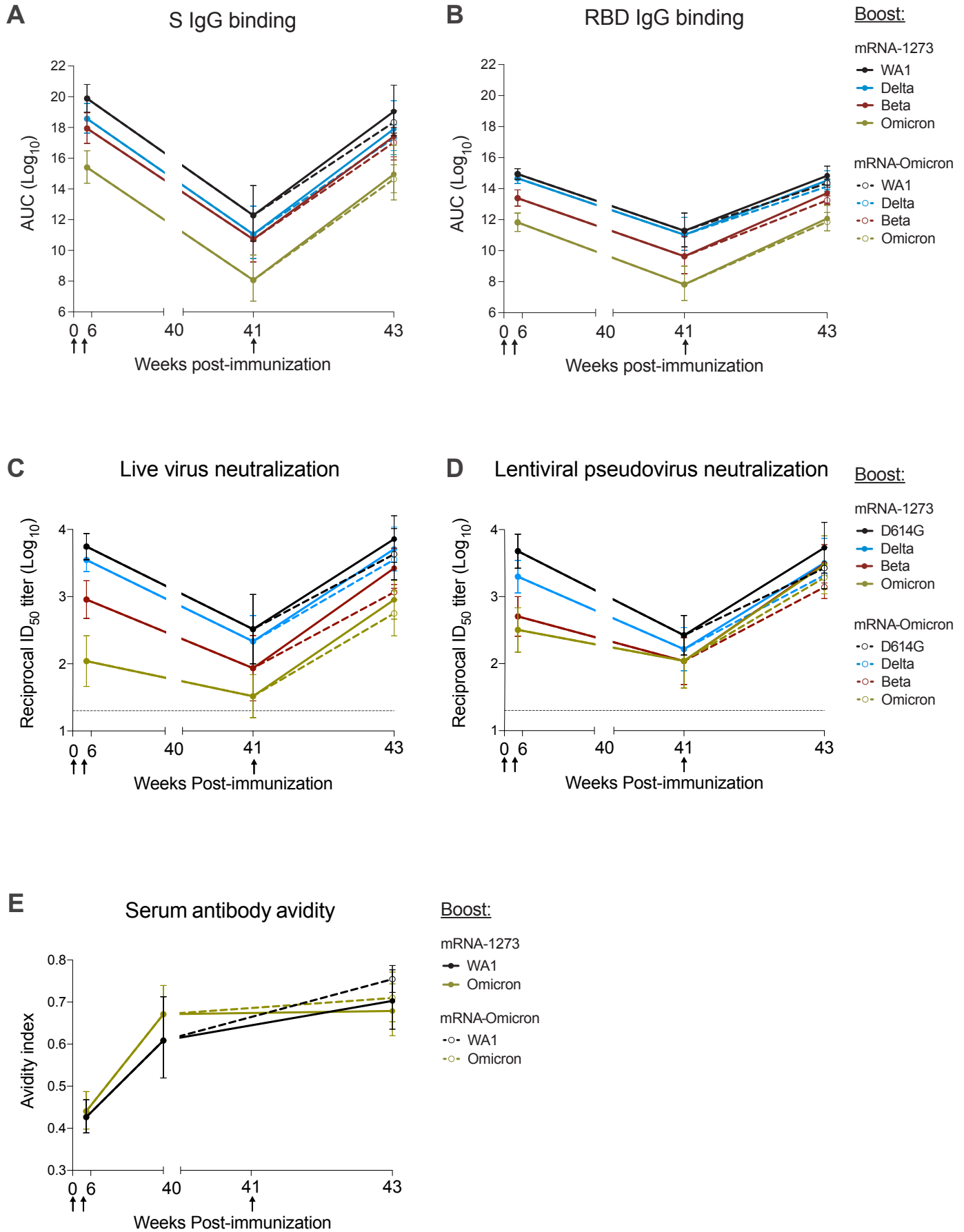
905 Viana, R., Moyo, S., Amoako, D.G., Tegally, H., Scheepers, C., Althaus, C.L., Anyaneji, U.J., Bester,  
906 P.A., Boni, M.F., Chand, M., *et al.* (2022). Rapid epidemic expansion of the SARS-CoV-2 Omicron  
907 variant in southern Africa. *Nature*.

908 Wang, L., Zhou, T., Zhang, Y., Yang, E.S., Schramm, C.A., Shi, W., Pegu, A., Oloniniyi, O.K., Henry,  
909 A.R., Darko, S., *et al.* (2021a). Ultrapotent antibodies against diverse and highly transmissible  
910 SARS-CoV-2 variants. *Science*.

911 Wang, R., Zhang, Q., Ge, J., Ren, W., Zhang, R., Lan, J., Ju, B., Su, B., Yu, F., Chen, P., *et al.*  
912 (2021b). Analysis of SARS-CoV-2 variant mutations reveals neutralization escape mechanisms  
913 and the ability to use ACE2 receptors from additional species. *Immunity* **54**, 1611-1621 e1615.

914 Willett, B.J., Grove, J., MacLean, O.A., Wilkie, C., Logan, N., Lorenzo, G.D., Furnon, W., Scott, S.,  
915 Manali, M., Szemiel, A., *et al.* (2022). The hyper-transmissible SARS-CoV-2 Omicron variant

916 exhibits significant antigenic change, vaccine escape and a switch in cell entry mechanism.  
917 medRxiv, 2022.2001.2003.21268111.  
918 Wolter, N., Jassat, W., Walaza, S., Welch, R., Moultrie, H., Groome, M., Amoako, D.G., Everatt,  
919 J., Bhiman, J.N., Scheepers, C., *et al.* (2022). Early assessment of the clinical severity of the  
920 SARS-CoV-2 omicron variant in South Africa: a data linkage study. *Lancet*.  
921 Worobey, M., Han, G.Z., and Rambaut, A. (2014). Genesis and pathogenesis of the 1918  
922 pandemic H1N1 influenza A virus. *Proc Natl Acad Sci U S A* *111*, 8107-8112.  
923 Wrapp, D., Wang, N., Corbett, K.S., Goldsmith, J.A., Hsieh, C.L., Abiona, O., Graham, B.S., and  
924 McLellan, J.S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.  
925 *Science* *367*, 1260-1263.  
926 Zhou, T., Teng, I.T., Olia, A.S., Cerutti, G., Gorman, J., Nazzari, A., Shi, W., Tsybovsky, Y., Wang,  
927 L., Wang, S., *et al.* (2020). Structure-Based Design with Tag-Based Purification and In-Process  
928 Biotinylation Enable Streamlined Development of SARS-CoV-2 Spike Molecular Probes. *Cell Rep*  
929 *33*, 108322.





## **Figure 1 Kinetics of serum antibody responses following mRNA-1273 immunization and boost**

(A-E) Sera were collected at weeks 6, 40 or 41, and 43 post-immunization.

(A-B) IgG binding titers to (A) variant S and (B) variant RBD expressed in AUC.

(C-D) Neutralizing titers to (C) live virus and (D) lentiviral pseudovirus expressed as the reciprocal ID<sub>50</sub>.

(E) Avidity index for WA1 S-2P- and Omicron S-2P-binding serum antibodies.

Circles represent geometric means (A-D) or arithmetic means (E). Error bars represent 95% confidence intervals (CI). Assay LOD indicated by dotted lines. Break in X-axis indicates a change in scale without a break in the range depicted. Responses to variants are color-coded as WA1 or D614G (black), Delta (blue), Beta (red) and Omicron (green). Arrows represent timepoints of immunizations. Following the boost at week 41, mRNA-1273-boosted NHP indicated by solid lines and mRNA-Omicron-boosted NHP indicated by dashed lines. 4 NHP per boost group.

See also Figure S1 for experimental schema and Table S1 for detailed neutralizing titers.

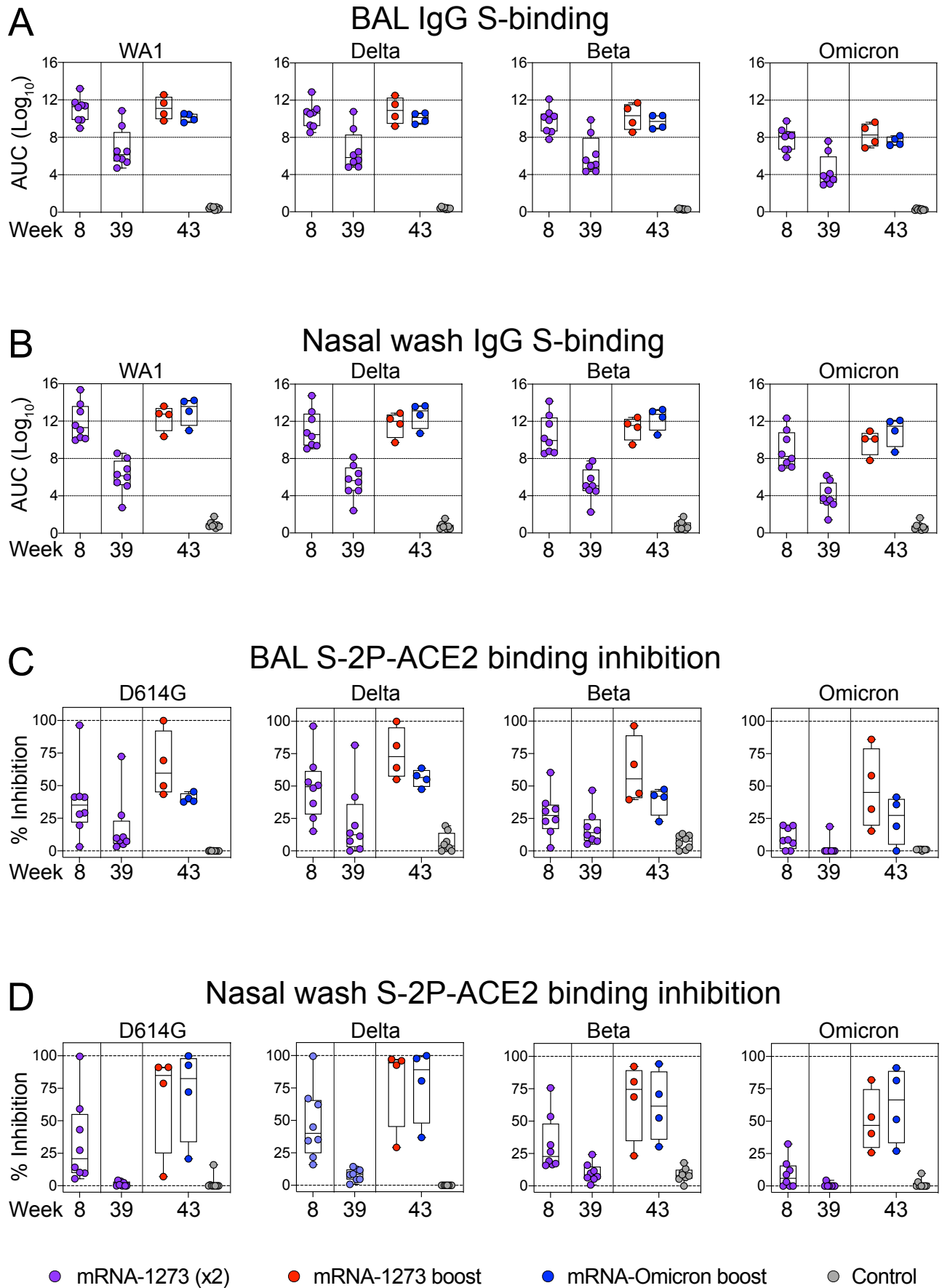


Figure 2

**Figure 2 Kinetics of mucosal antibody responses following mRNA-1273 immunization and boost**

(A-D) BAL (A, C) and NW (B, D) were collected at weeks 8, 39 and 43 post-immunization.

(A-B) IgG binding titers to WA1, Delta, Beta and Omicron expressed in AUC.

(C-D) D614G, Delta, Beta and Omicron S-2P-ACE2 binding inhibition in the presence of mucosal fluids. All samples diluted 1:5.

Circles indicate individual NHP. Boxes represent interquartile range with the median denoted by a horizontal line. Dotted lines are for visualization purposes and denote  $4\text{-log}_{10}$  increases in binding titers (A-B) or 0 and 100% inhibition (C-D). 8 controls and 8 vaccinated NHP, split into 2 cohorts post-boost.

See also Table S2 for list of mutations in variant-specific S-2P-ACE2 inhibition assays.

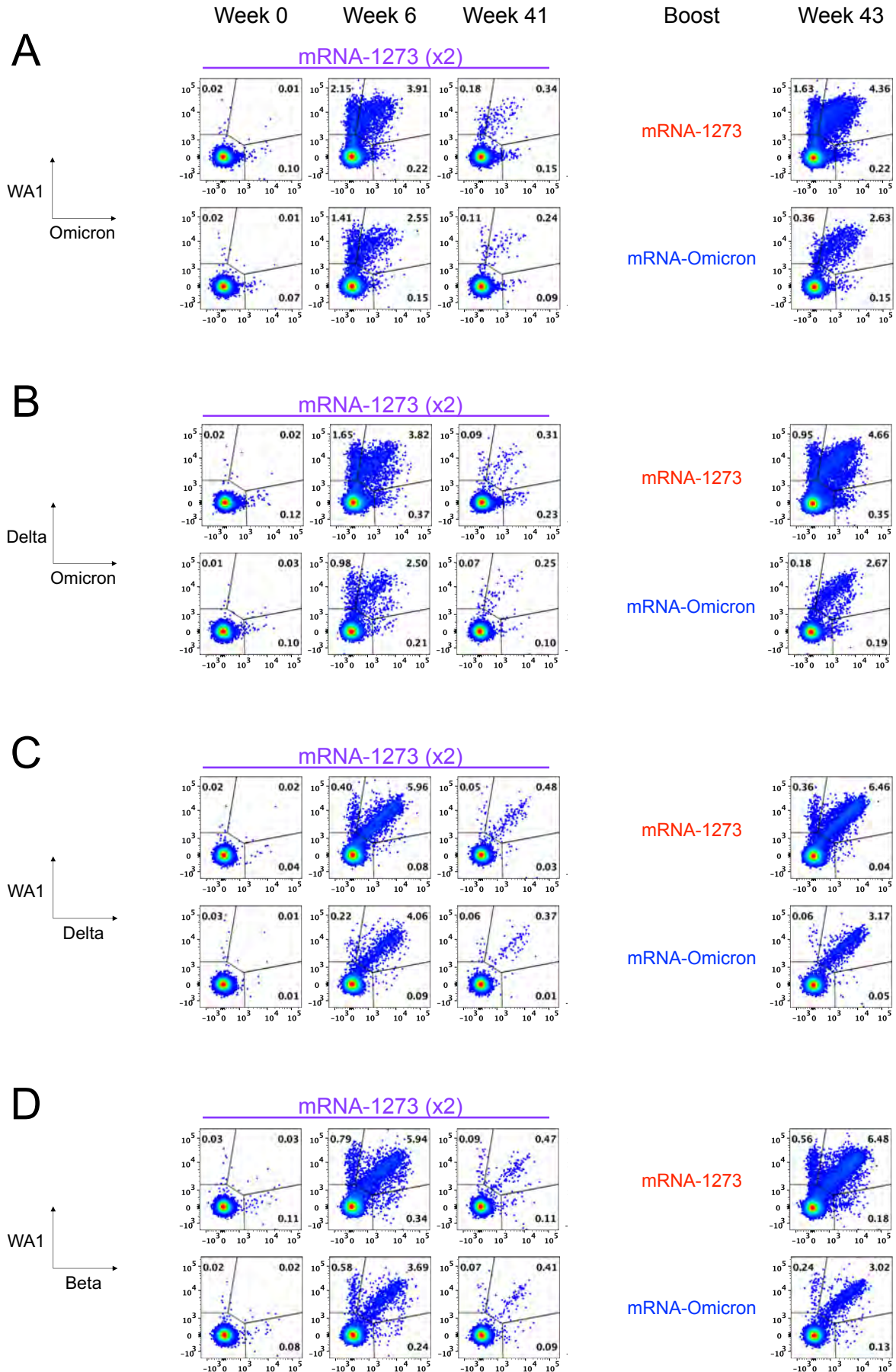


Figure 3

### **Figure 3 Memory B cell specificities following immunization and boosting**

(A-D) Representative flow cytometry plots showing single variant-specific (top left and bottom right quadrant) and dual-variant specific (top right quadrant) memory B cells at weeks 0, 6, 41 and 43 post-immunization. Event frequencies per gate are expressed as a percentage of all class-switched memory B cells. Cross-reactivity shown for (A) WA1 and Omicron S-2P, (B) Delta and Omicron S-2P, (C) WA1 and Delta S-2P and (D) WA1 and Beta S-2P. 4-7 NHP per group.

See also Figure S2 for B cell gating strategy.

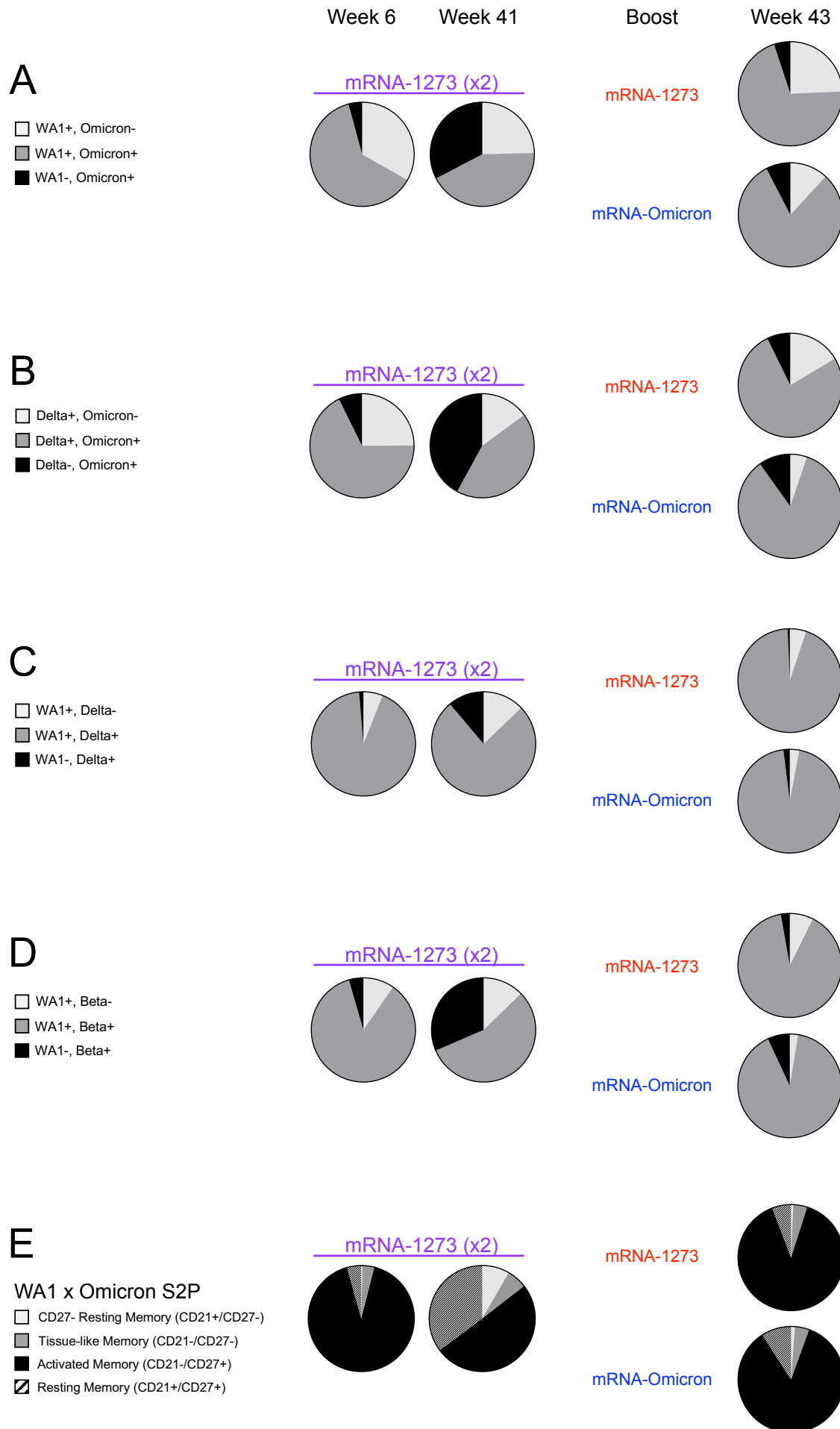


Figure 4

**Figure 4 Similar expansion of cross-reactive S-2P-specific memory B cells following boosting**

(A-D) Pie charts indicating the proportion of total S-binding memory B cells that are cross-reactive (dark gray) or specific for the indicated variants (black or light gray) for all NHP (geomean) at weeks 6, 41 and 43 post-immunization. Where applicable, memory B cells specific only for WA1 or Delta are represented by the light gray segment. Cross-reactivity shown for (A) WA1 and Omicron S-2P, (B) Delta and Omicron S-2P, (C) WA1 and Delta S-2P and (D) WA1 and Beta S-2P.

(E) Pie charts indicating the proportion of total S-2P-binding memory B cells (geomean) that have a phenotype consistent with resting memory (pattern), activated memory (black), tissue-like memory (dark gray) or CD27-negative resting memory (light gray) B cells at weeks 6, 41 and 43 post-immunization. Analysis shown here for memory B cells that bind to WA1 and/or Omicron S-2P. 4-7 NHP per group.

See also Figure S3 for frequencies of cross-reactive S-2P memory B cells, Figure S4 for serum epitope reactivity and Figures S4 and S5 for T cell responses after boosting.

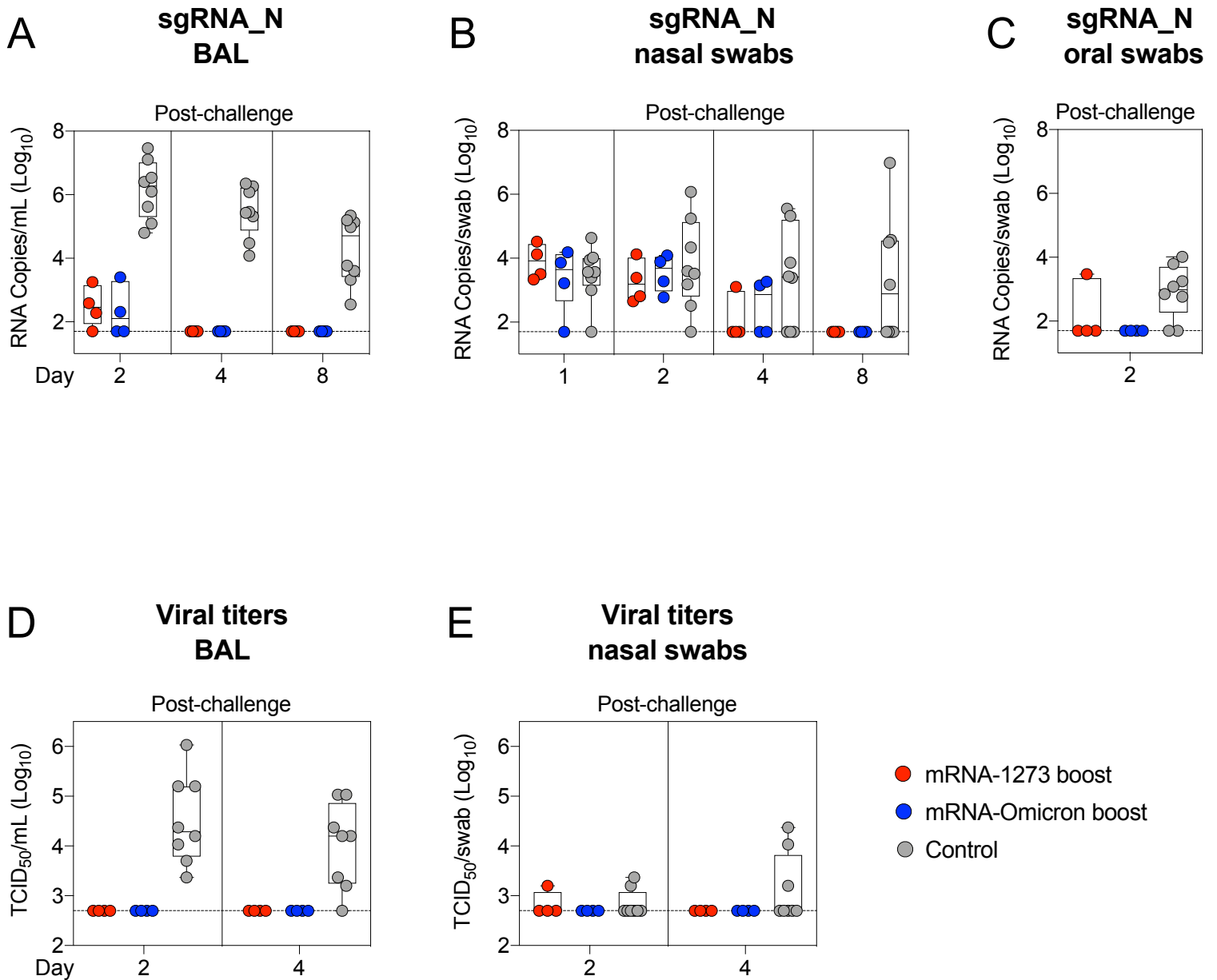


Figure 5



## **Figure 5 Boosting provides equivalent protection in the lungs against Omicron challenge**

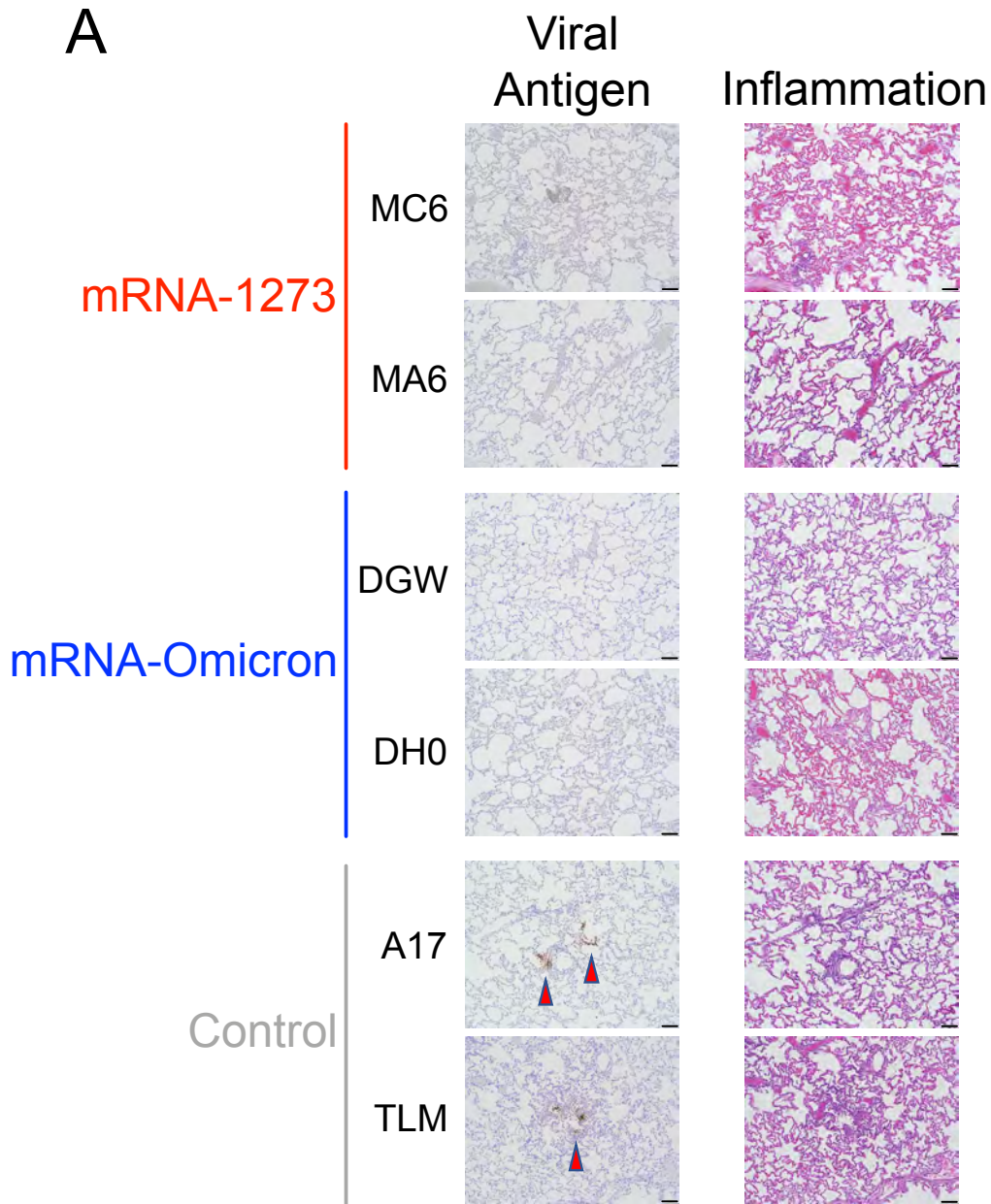
(A-E) BAL (A, D), NS (B, E) and OS (C) were collected at the indicated times following challenge with  $1 \times 10^6$  PFU Omicron.

(A-C) Omicron sgRNA\_N copy numbers per mL of BAL or per swab.

(D-E) Viral titers per mL of BAL or per swab.

Circles indicate individual NHP. Boxes represent interquartile range with the median denoted by a horizontal line. Assay LOD indicated by dotted lines. 8 controls and 4 vaccinated NHP per boost cohort.

See also Figure S7 for Omicron challenge stock sequence.



**B**

		Lc	Rmid	Rc		Lc	Rmid	Rc
mRNA-1273	MC6	-	-	-		+	+	+
	MA6	-	-	-		+/-	+/-	+/-
mRNA-Omicron	DGW	-	-	-		+/-	+/-	+/-
	DH0	-	-	-		+	+/-	+
Control	A17	+/-	+	+		+	++	++
	TLM	+	+	+/-		++	++	++

Figure 6

## Figure 6 Viral antigen and pathology in the lungs after challenge

(A-B) 2 NHP per group were euthanized on day 8 post-challenge and tissue sections taken from lungs.

(A) *Left*. Representative images indicating detection of SARS-CoV-2 N antigen by immunohistochemistry with a polyclonal anti-N antibody. Antigen-positive foci are marked by a red arrow. *Right*. Hematoxylin and eosin stain (H&E) illustrating the extent of inflammation and cellular infiltrates. Images at 10x magnification with black bars for scale (100 $\mu$ m).

(B) SARS-CoV-2 antigen and inflammation scores in the left cranial lobe (Lc), right middle lobe (Rmid) and right caudal lobe (Rc) of the lungs. Antigen scoring legend: - no antigen detected; +/- rare to occasional foci; + occasional to multiple foci; ++ multiple to numerous foci; +++ numerous foci. Inflammation scoring legend: - minimal to absent inflammation; +/- minimal to mild inflammation; + mild to moderate inflammation; ++ moderate to severe inflammation; +++ severe inflammation. Horizontal rows correspond to individual NHP depicted above (A).