1   **Protection against SARS-CoV-2 Beta Variant in mRNA-1273 Boosted Nonhuman Primates**

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3   **Authors:** Kizzmekia S. Corbett[1,8#], Matthew Gagne[1#], Danielle A. Wagner[1], Sarah O' Connell[1],

4   Sandeep R. Narpala[1], Dillon R. Flebbe[1], Shayne F. Andrew[1], Rachel L. Davis[1], Barbara Flynn[1],

5   Timothy S. Johnston[1], Christopher Stringham[1,9], Lilin Lai[2], Daniel Valentin[3], Alex Van Ry[3],

6   Zackery Flinchbaugh[3], Anne P. Werner[1], Juan I. Moliva[1], Manjari Sriparna[1,10], Sijy O'Dell[1],

7   Stephen D. Schmidt[1], Courtney Tucker[1], Angela Choi[4], Matthew Koch[4], Kevin W. Bock[5],

8   Mahnaz Minai[5], Bianca M. Nagata[5], Gabriela S. Alvarado[1], Amy R. Henry[1], Farida Laboune[1],

9   Chaim A. Schramm[1], Yi Zhang[1], Lingshu Wang[1], Misook Choe[1], Seyhan Boyoglu-Barnum[1],

10   Wei Shi[1], Evan Lamb[1], Saule T. Nurmukhambetova[1], Samantha J. Provost[1], Mitzi M.

11   Donaldson[1], Josue Marquez[1], John-Paul M. Todd[1], Anthony Cook[3], Alan Dodson[3], Andrew

12   Pekosz[6], Eli Boritz[1], Aurélie Ploquin[1], Nicole Doria-Rose[1], Laurent Pessaint[3], Hanne Andersen[3],

13   Kathryn E. Foulds[1], John Misasi[1], Kai Wu[4], Andrea Carfi[4], Martha C. Nason[7], John Mascola[1],

14   Ian N. Moore[5], Darin K. Edwards[4], Mark G. Lewis[3], Mehul S. Suthar[2], Mario Roederer[1], Adrian

15   McDermott[1], Daniel C. Douek[1], Nancy J. Sullivan[1], Barney S. Graham[1*], and Robert A. Seder[1*]

16

17   **Affiliations:**

18   [1]Vaccine Research Center; National Institute of Allergy and Infectious Diseases; National

19   Institutes of Health; Bethesda, Maryland, 20892; United States of America

20   [2]Center for Childhood Infections and Vaccines of Children's Healthcare of Atlanta, Department

21   of Pediatrics, Department of Microbiology and Immunology, Emory Vaccine Center, Emory

22   University, Atlanta, Georgia, 30322, United States of America

23   [3]Bioqual, Inc.; Rockville, Maryland, 20850; United States of America

24   [4]Moderna Inc., Cambridge, MA, 02139; United States of America

25   [5]Infectious Disease Pathogenesis Section; National Institute of Allergy and Infectious Diseases;

26   National Institutes of Health; Bethesda, Maryland, 20892; United States of America

27   [6]Department of Microbiology and Immunology; Johns Hopkins University, Bloomberg School

28   of Public Health, Baltimore, Maryland, 21205, United States of America

29   [7]Biostatistics Research Branch, Division of Clinical Research, National Institute of Allergy and

30   Infectious Diseases, National Institutes of Health; Bethesda, Maryland, 20892; United States of

31   America

32   [8]Current Affiliation: Department of Microbiology and Immunology, Harvard T.H. Chan School

33   of Public Health; Boston, Massachusetts, 02115; United States of America

34   [9]Current Affiliation: Duke University School of Medicine; Medical Sciences Program; Durham,

35   North Carolina, 27705; United States of America

36   [10]Current Affiliation: Medical College of Virginia; Richmond, Virginia, 23223; United States of

37   America

38

39   [#]These authors contributed equally to this manuscript.

40   *Correspondence: rseder@mail.nih.gov and bgraham@nih.gov

41 **ABSTRACT**

42 Neutralizing antibody responses gradually wane after vaccination with mRNA-1273 against

43 several variants of concern (VOC), and additional boost vaccinations may be required to sustain

44 immunity and protection. Here, we evaluated the immune responses in nonhuman primates that

45 received 100 µg of mRNA-1273 vaccine at 0 and 4 weeks and were boosted at week 29 with

46 mRNA-1273 (homologous) or mRNA-1273.β (heterologous), which encompasses the spike

47 sequence of the B.1.351 (beta or β) variant. Reciprocal ID_{50} pseudovirus neutralizing antibody

48 geometric mean titers (GMT) against live SARS-CoV-2 D614G and the β variant, were 4700

49 and 765, respectively, at week 6, the peak of primary response, and 644 and 553, respectively, at

50 a 5-month post-vaccination memory time point. Two weeks following homologous or

51 heterologous boost β-specific reciprocal ID_{50} GMT were 5000 and 3000, respectively. At week

52 38, animals were challenged in the upper and lower airway with the β variant. Two days post-

53 challenge, viral replication was low to undetectable in both BAL and nasal swabs in most of the

54 boosted animals. These data show that boosting with the homologous mRNA-1273 vaccine six

55 months after primary immunization provides up to a 20-fold increase in neutralizing antibody

56 responses across all VOC, which may be required to sustain high-level protection against severe

57 disease, especially for at-risk populations.

58

59 **One-sentence summary:**

60 mRNA-1273 boosted nonhuman primates have increased immune responses and are protected

61 against SARS-CoV-2 beta infection.

62 **INTRODUCTION**

63 SARS-CoV-2 vaccines, such as mRNA-1273, BNT162b2, AD26.COV2.S, AZD1222, and NVX-

64 CoV2373, which deliver the Wuhan-1 spike (S) protein are between 70-94% effective against

65 symptomatic D614G or B.1.1.7 alpha (α) variant infection (*1-5*). Several SARS-CoV-2 variants

66 of concern (VOC) have reduced sensitivity to neutralizing antibodies induced by vaccination or

67 prior infection. The B.1.617.2 delta (δ) variant is notable for its high transmissibility and an

68 intermediate level of reduced neutralization by WA-1 immune sera (*6, 7*). The B.1.351 beta (β)

69 variant - initially isolated in South Africa - and the P.1 gamma (γ) variant - initially isolated in

70 Brazil - show the greatest reduction in neutralizability by vaccinee or convalescent patient sera

71 (*8-13*).

72 Since mRNA-1273 was granted Emergency Use Authorization (EUA) in December 2020 and

73 deployed globally, months have elapsed since the first subjects were vaccinated, and durability

74 of protection remains a concern. Additionally, the question of a need for an additional booster

75 immunization is clinically relevant, particularly as new VOC become more prevalent. mRNA-

76 1273 vaccination in humans induced antibody responses that persisted beyond 6 months (*14*), but

77 serum neutralizing activity was significantly reduced against β and δ variants (*7, 15, 16*). Recent

78 data show that boosting subjects fully vaccinated with mRNA-1273 several months after initial

79 priming with either mRNA-1273, mRNA-1273.β (S sequence based on the β variant), or a 1:1

80 combination of both vaccines (mRNA-1273.211) showed significantly increased neutralizing

81 titers against the prototypic D614G virus and all variants, including β (*17*). While these *in vitro*

82 functional data are promising, there are no available efficacy data to define the level of

83 antibodies that are sufficient to mediate protection against infection since the correlates of

84 protection against VOCs are undetermined. Moreover, in NHP, a higher threshold of neutralizing

85   antibodies was needed for upper compared to lower airway protection (*18*), suggesting a boost

86   could be important to reach this threshold and contribute to reducing virus transmission.

87   Nonhuman primates (NHP) have proven useful for assessing immunogenicity and protection

88   against SARS-CoV-2 to inform vaccine development (*19-24*). Here, NHP immunized with the

89   clinically relevant mRNA-1273 vaccine regimen (100 µg x2) were boosted ~6 months later with

90   mRNA-1273 (homologous) or mRNA-1273.β (heterologous). Antibody, B cell, and T cell

91   responses were assessed temporally to determine how boosting with mRNA-1273 or mRNA-

92   1273.β influences the magnitude of WA-1 or β-specific immune responses and protection

93   against SARS-CoV-2 β challenge. The data show that an additional, delayed boost with mRNA

94   vaccines matched to either the original vaccine strain or to the heterologous challenge strain can

95   have a striking effect on increasing the breadth of neutralization against all VOC tested and

96   protection against the β variant. The data presented here provide a rationale for how boosting can

97   influence immunity and protection in humans.

98

RESULTS

Boosting increases mRNA-1273-induced serum antibody responses

101   The primary focus of this study was to assess the effect of homologous or heterologous boosting

102   with the original WA-1 mRNA-1273 or mRNA-1273.β, respectively, on immunogenicity and

103   protection against infection with the β variant. NHP that received the clinically relevant primary

104   regimen of mRNA-1273 (100 µg at weeks 0 and 4) were boosted with either mRNA-1273

105   (homologous) or mRNA-1273.β (heterologous) (**Fig. S1**). WA-1 and β S- or receptor binding

106   domain (RBD)-specific serum antibody responses were assessed at weeks 6, 24 or 29, 31, and

107   37, which correspond to peak (*19*), memory pre-boost, 2 weeks post-boost, and time of challenge

108   ("pre-challenge") responses, respectively. WA-1 and β S-specific IgG geometric mean titers

109   (GMT) were 53,000 and 39,000 AUC, respectively, at the peak timepoint following a primary

110   regimen of mRNA-1273. These responses dropped 4- and 6-fold, respectively, after ~6 months

111   (week 29). Boosting with mRNA-1273 or mRNA-1273.β induced 4- and ~6-fold increases in

112   WA-1 and β S-specific IgG titers such that at 2 weeks following the memory boost, WA-1 and β

113   S-specific IgG GMT were restored to peak levels **(Fig. 1A-B)**. By week 37, S-specific IgG GMT

114   remained >30,000 and >20,000 AUC, respectively for WA-1 and β **(Fig. 1A-B)**, which, for WA-

115   1, translated to >200,000 international units (IU/mL) **(Table S1)**. RBD-specific responses

116   displayed similar kinetic trends **(Fig. 1C-D)**, and together these data show there is no difference

117   in the ability of mRNA-1273 or mRNA-1273.β to boost primary mRNA-1273-elicited S-binding

118   IgG responses.

119   Next, we assessed the kinetics of neutralizing antibody responses using a lentiviral-based

120   neutralizing antibody assay for D614G, the benchmark strain, as well as several globally

121   circulating variants (β, δ, P.1 - γ, B.1.429 - ε , and B.1.526 - ι). Consistent with previous data in

122   NHP and humans (*7, 15*), mRNA-1273 elicited a variant-dependent hierarchy of neutralizing

123   antibody responses; at peak, the ID_{50} GMT for D614G was 4700, followed by 5900, 1500, 1100,

124   834, and 765 for ε, γ, ι, δ, and β, respectively, representing 1-6-fold decreases compared to

125   D614G. At the memory timepoint (week 24), neutralizing antibody titers decreased to 644 ID_{50}

126   GMT for D614G and 808, 278, 252, 738, and 553 for ε, γ, ι, δ, and β, respectively. Of note, there

127   was a greater fold reduction in neutralization titers from week 6 to week 24 for D614G compared

128   to β and δ (*p* <0.0001) **(Fig. 1E-F)**. Similar observations were made using D614G and β VSV-

129   based pseudovirus **(Fig. 1G-H)** or live virus neutralization assays **(Fig. 1I-J)**. Last, WA-1 S-

130   specific antibody avidity, one measure of affinity maturation, was significantly increased over

131   the 6 months following the primary mRNA-1273 immunization series in these NHP (p <0.0001)

132   **(Fig. 1K-L)**. This indicates continued affinity maturation occurs following primary

133   immunization leading to an increase in antibody quality and suggests that, for some variants,

134   affinity maturation of mRNA-1273-elicited neutralizing antibody responses may occur over

135   time, even in the absence of continued antigen exposure.

136   Six months after the primary immunization series, either a homologous (mRNA-1273) or

137   heterologous (mRNA-1273.β) boost induced a 7-21-fold increase in neutralizing antibody titers

138   for all the variants two weeks later, resulting in, for example, 5000 and 3000 β-specific ID_{50}

139   GMT following mRNA-1273 or mRNA-1273.β boosts, respectively **(Fig. 1E-F)**. Of note, these

140   responses to variants were significantly higher than at week 6 for both boost groups (δ-specific

141   neutralizing antibody responses, p = 0.002 and 0.003 for mRNA-1273 and mRNA-1273.β

142   boosts, respectively). This post-boost increase in lentiviral pseudovirus neutralizing titers was

143   confirmed using a VSV-based pseudovirus neutralization assay **(Fig. 1G-H)** and a live virus

144   neutralization assay **(Fig. 1I- J)**. By week 37, live virus neutralizing antibody titers returned to

145   week 6 levels **(Fig. 1I- J)**. Together, these data show that despite waning of mRNA-1273-

146   elicited antibody responses over 6 months, boosting with either homologous or heterologous

147   mRNA was able to restore antibody responses to peak levels and, importantly, with sufficient

148   potency and breadth to neutralize across heterologous virus variants.

149

150   **Boosting increases antibody responses in the airway**

151 Vaccine-induced antibodies localized in the upper and lower airways may play a role in the

152 initial control of SARS-CoV-2 replication (*18, 25, 26*). Therefore, we extended analysis beyond

153 circulating antibody responses to assess WA-1 and β S-specific IgG responses in

154 bronchoalveolar lavage (BAL) and nasal wash samples (NS) at weeks 6 and 36. At week 6, two

155 doses of mRNA-1273 elicited WA-1 and β S-specific GMTs of 13 and 11 AUC, respectively, in

156 the BAL (**Figs. 2A and 2C**). Similar GMT (11 and 10 AUC) of S-specific IgG were also

157 detected in nasal wash samples (**Figs. 2B and 2D**). Interestingly, following a boost with either

158 mRNA-1273 or mRNA-1273.β, the levels of BAL and nasal wash S-specific IgG were also 10-

159 12 AUC GMT **(Figs. 2A-D)**. To detect functional antibodies in these mucosal samples, we

160 utilized an ACE2 binding inhibition assay. In the BAL of NHP boosted with mRNA-1273,

161 ACE2 binding to WA-1 and β S proteins was reduced by a mean of 54% and 37%, respectively

162 (**Fig. 2E**), and 47% and 32% in nasal washes **(Fig. 2F)**. Similarly, in BAL and nasal washes of

163 NHP that were boosted with mRNA-1273.β, ACE2 binding to WA-1 S was reduced by 42% and

164 55% and for β S proteins by 30% and 46%, respectively; ACE2 binding inhibition was reduced

165 for β as compared to WA-1, similar to what was observed for serum antibodies (**Figs. 2E-F**).

166 These data confirm that either homologous or heterologous memory vaccination can induce

167 upper and lower airway functional antibodies that are necessary to mitigate illness and

168 transmission.

169

170 **mRNA-1273.β immunization induces robust serum and mucosal antibody responses**

171 A secondary focus of this study was to evaluate the immunogenicity of mRNA-1273.β as a

172 primary vaccine regimen as this may have relevance for design of future vaccines in naïve

173   individuals; this group additionally served as a homologous vaccine control for the β challenge

174   **(Fig. S1)**. S-specific IgG GMT for WA-1 and β were 5100 and 8900 two weeks after the first

175   immunization of mRNA-1273.β and increased 4-5-fold after a second immunization **(Fig. S2A)**.

176   Two doses of mRNA-1273.β resulted in D614G and β live virus ID_{50} GMT of 198 and 788,

177   respectively **(Fig. S2B)**. Potent S-specific antibody responses against WA-1 or β were also

178   detected in BAL **(Fig. S2C)** and nasal washes **(Fig. S2D)**. Mucosal antibody responses were

179   further exhibited by ACE2 binding inhibition, where mRNA-1273.β induced BAL antibodies

180   that reduced ACE2 binding to WA-1 and β S by a median of 21% and 48%, respectively **(Fig.**

181   **2E)**. In nasal washes, median reduction of ACE2 binding to WA-1 and β S binding was 30% and

182   56%, respectively **(Fig. 2F)**. Together, these data show mRNA-1273.β given as a primary

183   regimen elicits higher β-specific responses as compared to WA-1.

184

185   **Serum antibody repertoire elicited by homologous or heterologous prime boost**

186   To evaluate the impact of homologous (WA-1) or heterologous (β) boosting antigen on serum

187   antibody epitope specificity, the absolute value (RUs) and the relative proportion (percent

188   competition) of serum antibodies against 16 distinct antigenic sites **(Table S2)** on WA-1 SARS-

189   CoV-2 S was measured using surface plasmon resonance (SPR). We evaluated serum antibody

190   specificity at week 35 (6 weeks post-boost), to allow sufficient time for B cell expansion

191   following exposure to naïve antigen in heterologous-boosted animals. The epitope specificity

192   spanned the S2, N-terminal domain (NTD), S1, and RBD subdomains of S protein and the

193   breadth was similar whether the boost was homologous or heterologous **(Fig 3A)**. The specificity

194   of serum antibodies was qualitatively similar across all animals within each group for the NTD

195   subdomain (average standard deviation 32.1-36.9), but not for RBD subdomain (average

196   standard deviation 50.2-56.1) (**Fig 3A**). Between vaccine groups, there was more NTD site A

197   specificity in homologous than in heterologous boosted animals (**Fig. 3A**). Both absolute and

198   relative serum reactivity to NTD site A (mAb 4-8, **Table S2**) were higher in homologous than

199   heterologous boosted animals at all time points post-boost (**Fig. 3B**), indicating mutations found

200   in the β S protein (Δ242-244, R246I) may render the heterologous boost unable to recall a

201   primary mRNA-1273 immunization memory B-cell response to this antigenic site. In contrast,

202   RBD site H (mAb A23-97.1, **Table S2**) showed differences in relative but not absolute serum

203   reactivity, with heterologous inducing higher reactivity than homologous boosting at all post

204   memory boost time points (**Fig 3C**). Because A23-97.1 binds equivalently to both WA-1 and β S

205   (**Fig. S3**), the higher relative (but not absolute) reactivity against this site for β is likely due to the

206   decreased contribution of NTD-A reactivity, as shown by the ratio of mRNA-1273.β to mRNA-

207   1273 relative reactivity (**Fig. 3D**). Overall, mRNA-1273 to β ratios decrease over time,

208   indicating a contraction of the naïve β-directed response and return to a memory, long-lived

209   epitope profile (**Fig. 3D**), and suggesting heterologous boost does not alter absolute epitope

210   reactivity over time. In contrast, following primary immunization with mRNA-1273.β we

211   observed significantly increased absolute serum reactivity to RBD sites B, C, D, and F (**Fig. S4**)

212   as compared to homologous boost, with sites C and D (A19-46.1, A19-61.1, **Table S2**) being

213   associated with strong neutralization potency against β (*27*). These data highlight differences

214   between primary immunization and heterologous boost with mRNA-1273.β and indicate primary

215   immunization plays a key role in shaping the overall serum antibody epitope repertoire.

216

217   **Boosting expands mRNA-1273-induced memory B cell responses**

218   The durability of vaccine-induced antibody responses is an integral component of the pandemic

219   response as efforts continue to mitigate the ongoing spread of SARS-CoV-2. Durable humoral

220   immunity is driven by the ability to generate and sustain memory B cells and long-lived

221   plasmablasts (*28*), and recent data in humans shows the potential for such responses after

222   vaccination with mRNA or primary infection (*29, 30*). To define how B cell specificity matures

223   overtime in mRNA-1273 immunized NHP and to assess how mRNA-1273 priming imprints

224   homologous or heterologous boost, we performed temporal analysis of WA-1 and β S-specific

225   memory B cells **(Fig. S5)**. Following the primary immunization series with mRNA-1273, the

226   frequency of memory B cells expressing antibody receptors dual reactive for both WA-1 and β S

227   at week 6 was 2-3%, with a much lower proportion of single WA-1 or β-specific B cells **(Fig.**

228   **4A-B)**. Six months later, at week 29, there was ~10-fold reduction in the frequency of double-

229   positive WA-1 and β S-specific memory B cells **(Fig. 4A-B)**, but these were restored (~10-fold

230   increase) following a memory boost with mRNA-1273 or mRNA-1273.β **(Fig. 4)**. In contrast,

231   boosting did not cause an increase in the frequency of single-positive WA-1 or β S-specific B

232   cells **(Fig. 4)**. This finding demonstrates a rapid recall response of primary vaccination B cells

233   and coincides with an increase in neutralizing antibody responses **(Fig. 1E-J)**. The mRNA-

234   1273.β immunized NHP had a memory B cell response that also consisted of WA-1 and β S-

235   specific double-positive specificity, but a higher proportion of single positive β S-specific cells

236   **(Fig. 4)**, consistent with β-specific skewing of antibody responses following mRNA-1273.β

237   primary immunization **(Fig. S2)**. These data confirm the observation from serum antibody

238   epitope mapping that both homologous and heterologous boosting can efficiently expand

239   memory B-cell responses that are maintained after primary immunization.

240

Boosting restimulates mRNA-1273-induced Th1 and Tfh T cell responses

mRNA-1273 has been shown to elicit CD4 responses comprised of Th1 and T follicular helper (Tfh) cells, and a lower frequency of CD8 T cells in both humans and NHP (*18, 19, 26, 31*). However, the definition of longitudinal T-cell development and the potential for boosting contracted memory T cells have not yet been reported in mRNA-1273 immunized NHP. Here, consistent with prior studies, S-specific Th1 and Tfh, but not Th2, responses were induced at week 6, 2 weeks following mRNA-1273 vaccination **(Fig. 5)**. In these NHP, Th1 and Tfh responses were contracted by week 29, but were both boosted by either mRNA-1273 or mRNA-1273.β **(Figs. 5A, and 5C-D)**. Week 36 responses revealed mRNA-1273.β immunization also induced S-specific Th1 and Tfh, but not Th2, responses **(Fig. 5)**. These data suggest that homologous and heterologous mRNA boosts are equally capable of restimulating Th1 and Tfh T cell responses.

NHP are highly protected against SARS-CoV-2 β following a boost

NHP were challenged at week 38, ~8 weeks post-boost in animals receiving three immunizations, and ~5 weeks post primary mRNA-1273.β immunization (two immunizations), with a total dose of $2x10^5$ PFU of SARS-CoV-2 β by intratracheal (IT) and intranasal (IN) routes **(Fig. S1)**. Two days post-challenge, control NHP that received mock mRNA had a median of ~6 $\log_{10}$ SARS-CoV-2 envelope (E) sgRNA (sgRNA _E) copies/mL in BAL. In contrast, all 8 NHP that received primary mRNA-1273.β immunization had undetectable BAL sgRNA_E. In NHP that received a boost after primary immunization with mRNA-1273, there was a hierarchy of protection against viral replication in the lung, where 4/8 NHP that received the homologous

263 mRNA-1273 boost had undetectable BAL sgRNA _E, and 7/8 NHP boosted with heterologous

264 mRNA-1273.β had undetectable BAL sgRNA_E. By day 4, all boosted animals with the

265 exception of one mRNA-1273 boosted NHP had undetectable BAL sgRNA_E **(Fig. 6A)**. In NS,

266 at days 2 and 4 post-challenge, both groups of boosted NHP showed significantly less (~3-4 $\log_{10}$

267 reduction) sgRNA E compared to controls ($p = 0.0014$ and 0.0004 for mRNA-1273 and mRNA-

268 1273.β, respectively). By day 7, the majority (13/16) of boosted NHP had undetectable

269 sgRNA_E compared to control NHP, for which NS sgRNA_E persisted at a median of 4 $\log_{10}$

270 NS sgRNA_E **(Fig. 6B).**

271 While sgRNA is a sensitive measurement of viral replication that can be used diagnostically, we

272 also determined the ability of live virus to be propagated from post-challenge BAL and NS

273 samples, an alternative measure of viral load that is relevant for indicating lung disease or

274 transmission from the upper airway. On day 2 post-challenge, all boosted animals had low to

275 undetectable (<4 $\log_{10}$ TCID$_{50}$/mL) of culturable SARS-CoV-2 in BAL **(Fig. 6C)**. In NS, SARS-

276 CoV-2 was unculturable for 3/8 and 5/8 NHP that were boosted with mRNA-1273 or mRNA-

277 1273.β, respectively, which was similar to the mRNA-1273.β primary immunization group **(Fig.**

278 **6D)**. sgRNA and viral titers were highly correlated **(Fig. 6E-F)**; in fact, there was no culturable

279 SARS-CoV-2 from BAL or NS that had sgRNA_E levels <1.0 x10^4 RNA copies/mL **(Fig. 6E)**

280 and <4.7 x 10^3 RNA copies/swab **(Fig. 6F)**, respectively. Nucleocapsid (N)-specific sgRNA

281 measurements followed the same trend, albeit to with higher detection sensitivity **(Fig. S7)**.

282 We also evaluated lung samples for pathology and detection of viral antigen (VAg) 7-9 days post

283 SARS-CoV-2 β challenge. Inflammation was minimal to mild and was similar across lung

284 samples from vaccinated NHP, with rare cases showing a moderate to severe response **(Fig. S8**

285 **and Table S3)**. The inflammatory changes in the lung were characterized by a mixture of

286 macrophages and polymorphonuclear cells present within some alveolar spaces and mild to

287 moderate expansion of alveolar capillaries with mild Type II pneumocyte hyperplasia at day 7

288 post-challenge to changes more consistent with lymphocytes, histiocytes and fewer

289 polymorphonuclear cells associated with more prominent and expanded alveolar capillaries,

290 occasional areas of perivascular and peribronchiolar inflammation, and Type II pneumocyte

291 hyperplasia at days 8-9 post-challenge. SARS-CoV-2 antigen (Ag) was detected in 6/6 control

292 NHP evaluated at days 7, 8, or 9 after infectious challenge, with 3/6 showing Ag present in

293 multiple lobes. In vaccinated NHP, Ag was detected in only 1/24 animals, and in a single lobe

294 (mRNA-1273x3, **Table S3)**. These results, together with sgRNA data, confirm that boosting

295 mRNA-1273 immunized NHP limits β viral replication in the lower and upper airway.

296

297 **DISCUSSION**

298 The SARS-CoV-2 mRNA-1273 vaccine shows between 90-100% protection against WA-1 (*2*),

299 α, or β variants (*32*) when administered as two doses four weeks apart and assessed within a 2

300 month window. Kinetic analyses of antibody responses following vaccination with mRNA-1273

301 (*7*) or BNT162b2 (*30*) show reduction of neutralizing activity from the peak humoral response

302 after the second immunization through day 209 (*7, 17*). Consistently, the β variant is the most

303 neutralization resistant of all VOC to date. However, it has not yet been established how this

304 resistance affects the durability of protective efficacy in humans. Recent clinical reports from

305 Israel show that cohorts immunized with the BioNTech/Pfizer vaccine over 6 months ago may

306 present with symptomatic disease when infected with VOC (*33*). These data suggest that it may

307 be important to boost antibody responses, especially against VOC, to sustain protection against

308 severe disease, especially in at-risk cohorts and to reduce the potential for transmission. A related

309   issue is whether the additional boost should be matched to a specific newly arising variant

310   (heterologous) or if homologous boosting with the original vaccine based on ancestral strains

311   will be sufficient to generate broadly neutralizing antibody responses against VOC. Here, we

312   show that boosting NHP ~ 6 months after their primary vaccination series with either mRNA-

313   1273 or mRNA-1273.β significantly increased serum neutralizing activity against all variants and

314   mediated high-level protection in the upper and lower airways against challenge with the β

315   variant.

316   Longitudinal neutralizing antibody responses against the benchmark D614G strain compared to β

317   and several additional VOC confirmed data from prior vaccine studies in humans or NHP (*7, 30,*

318   *34*), that there was a 3-10 fold reduction in neutralizing activity against several VOC, with β and

319   γ variants showing the greatest reduction at the peak time point after the primary immunization

320   series. Here, all neutralizing responses were significantly reduced by week 24. Of note, there was

321   a more rapid decay of binding antibodies to VOCs compared to WA-1, but a relatively slower

322   decay in serum neutralizing activity against VOC. These data suggest that, although binding

323   antibody is an established correlate for short term protection, deeper analysis is needed to define

324   correlates of protection with contracted memory responses. These data also raise a possibility

325   that while there is a quantitative decrease in the magnitude of antibody responses over time

326   following vaccination, the loss of functional activity may be partially mitigated by affinity

327   maturation and improved quality of the response. It was recently reported that there may be

328   discordance in antibody evolution between convalescent individuals and those who receive an

329   mRNA vaccine. Specifically, less affinity maturation and a limited increase in breadth to the

330   RBD subdomain was observed in isolated monoclonal antibodies between 2- and 5-months post

331   mRNA vaccination (*30*). Our assessment of polyclonal serum antibody affinities (avidity) using

332 the intact WA-1 S protein showed that vaccination with mRNA-1273 led to an increase in

333 avidity over time, confirming a qualitative improvement in the antibody response in NHP (*29,*

334 *35, 36*).

335 Following a homologous mRNA-1273, or heterologous mRNA-1273.β boost at 6 months after the

336 primary immunization series, there was a ~7-21-fold increase in serum antibody neutralizing

337 activity for D614G, β, and the other VOCs tested, and the increase was not significantly different

338 between homologous or heterologous boosting. These data are consistent with recent studies in

339 humans showing that boosting with mRNA-1273 or mRNA-1273.β approximately 6 months after

340 the primary immunization with mRNA-1273 elicited significantly higher neutralizing titers against

341 the D614G, β, and the other VOC tested compared to pre-boost titers (*37*). Importantly, there was

342 significant boosting of neutralizing responses against the β variant even in individuals that had

343 undetectable responses prior to the boost. Last, neutralizing responses after the boost in our NHP

344 study and in humans were significantly higher than the peak titers observed after the primary

345 vaccination series (*17*).

346 The increase in antibody responses post-boost suggests that there is significant B-cell memory

347 induced by primary mRNA vaccination that can be rapidly recalled following the boost (*7, 29*).

348 Here, we show that after primary immunization with mRNA-1273, the peak response yields a total

349 frequency of WA-1 and β-specific memory B cells of ~ 3%, and the majority (~ 80%) of these

350 responses recognized both S proteins, with a small proportion specific for WA-1 only. There was

351 a very low frequency of B cells specific for β only. After 6 months, all responses contracted to

352 exhibit a 10-fold decrease in the frequency of WA-1 and β-specific memory B cells, but these were

353 rapidly restored to a frequency of 3% after boosting. The relative frequency of B cells specific for

354   WA-1, β, or both did not change post-boost suggesting that priming with mRNA-1273 imprinted

355   the B-cell repertoire.

356   The boosting of B cells specific for WA-1 and β highlights the overlapping epitopes in S between

357   the two strains and likely accounts for neutralization breadth against VOCs. A notable finding was

358   a limited (<5%) frequency of B cells specific for β only following mRNA-1273 vaccination or

359   after the homologous or heterologous boost. However, naïve animals primed with only mRNA-

360   1273.β showed between 25-40% of the total B-cell response specific for β only. Likewise, serum

361   antibody epitope profiling demonstrated a repertoire that was qualitatively similar following either

362   homologous or heterologous memory boost after mRNA-1273 primary vaccination, while a

363   primary vaccination series with mRNA-1273.β yielded a unique repertoire (both qualitative and

364   quantitative) with marked increases in reactivity to epitopes associated with broad and potent

365   neutralization of VOCs. These data have implications for how future mRNA vaccines can be

366   designed to imprint B-cell repertoires in naïve individuals for increased potency and breadth of

367   neutralizing activity (*38*).

368   While the data presented here focus on the role of boosting vaccine responses with mRNA, as a

369   proof-of-principle we also show that priming with mRNA-1273.β in naïve NHP induced potent

370   neutralizing antibody responses against β and provided high-level protection in the upper and

371   lower airways following challenge. Parallel studies in pre-clinical rodent models have shown that

372   priming with a variant mRNA or a bi-valent vaccine containing the WA-1 and the β variant

373   induces higher neutralizing responses to D614G and a number of variants tested relative to either

374   mRNA-1273 or mRNA-1273.β alone (*38*).

375   In conclusion, the data reported here show that a booster dose can significantly improve the

376   breadth and potency of neutralizing antibody responses sufficient to mediate protection against

377 upper and lower airway infection by a heterologous challenge virus shown to be relatively

378 resistant to *in vitro* neutralization. The potential clinical utility of a boost would be to sustain

379 high-level protection against severe disease and possibly limit the duration and extent of mild

380 infection in the setting of waning immunity especially in the elderly, and other groups with pre-

381 existing health conditions or poor response to vaccination. We have previously shown that there

382 is a higher threshold for antibody-mediated protection in the upper airway (*18*), which would be

383 more important for limiting mild infection and transmission than for protecting the lower airways

384 to limit severe disease. We speculate that new variants with high viral load and transmissibility,

385 such as δ, may require a late boost to increase antibody responses in the upper airway enough to

386 limit transmission. Furthermore, if such variants have more rapid progression to lower airway

387 disease than prior strains, boosting may be more important for mitigating severe disease as serum

388 antibody wanes, because disease progression may outpace otherwise protective anamnestic

389 responses. Last, a late boost will potentially provide more durable and broader immunity and

390 would reduce morbidity and mortality until greater population immunity is achieved.

391

392 **MATERIALS AND METHODS**

393 **Pre-clinical mRNA and lipid nanoparticle production**

394 A sequence-optimized mRNA encoding prefusion-stabilized SARS-CoV-2 S-2P protein (*39, 40*)

395 for WA-1 and β was synthesized *in vitro* and formulated as previously reported (*17, 41*). Control

396 mRNA "UNFIX-01 (Untranslated Factor 9)" was synthesized based on the sequence in **Table S4**

397 and similarly formulated into lipid nanoparticles.

398 **Rhesus macaque model**

399   Animal experiments were carried out in compliance with all pertinent US National Institutes of

400   Health regulations and approval from the Animal Care and Use Committees of the Vaccine

401   Research Center and Bioqual, Inc. (Rockville, MD). Studies were conducted at Bioqual, Inc. The

402   experimental details of VRC-20-857.3b **(Fig. S1)** are similar to prior studies (*18, 19, 26, 34*).

403   Briefly, 3–15-year-old rhesus macaques of Indian origin were stratified into groups based sex,

404   age, and weight. Animals were immunized with mRNA-1273 at week 0 and week 4 with a dose

405   of 100 µg intramuscularly (IM) in 1 ml formulated in PBS into the right hindleg. Placebo-control

406   animals were administered control mRNA. At week 29 (~ 25 weeks after the second

407   immunization), a group of animals were boosted with 50 µg of mRNA-1273 or 50 µg of mRNA-

408   1273.β. An additional group of animals were immunized at week 29 and 33 with 50 µg of

409   mRNA-1273.β. At week 38 (9 weeks after the homogolous or heterologous mRNA boost or 5

410   weeks after the mRNA-1273.β prime and boost) all animals were challenged with a total dose of

411    $2x10^5$ PFU SARS-CoV-2 β (JHU P2) as previously described (*34*). The viral inoculum was

412   administered as $1.5x10^5$ PFU in 3 mL intratracheally (IT) and $0.5x10^5$ PFU in 1 mL intranasally

413   (IN) in a volume of 0.5 mL into each nostril. Pre- and post-challenge sample collection is

414   detailed in **Fig. S1**.

415   **Quantification of SARS-CoV-2 subgenomic RNA (sgRNA)**

416   BAL and nasal swab (NS) subgenomic SARS-CoV-2 E mRNA was quantified via reverse

417   transcription-polymerase chain reaction (RT-PCR) as previously described (*26*). Subgenomic

418   SARS-CoV-2 N mRNA was quantified similarly, as described in (*34*). The lower limit of

419   quantification was 50 copies.

420   **TCID50 quantification of SARS-CoV-2 from BAL**

421   Viral load (TCID50) from BAL samples was calculated using previously described methods

422   (*34*).

Histopathology and immunohistochemistry (IHC)

424   Post-challenge, on days 7-9, animals were euthanized, and lung tissue was processed and stained

425   with hematoxylin and eosin (H&E) for routine histopathology and analyzed for detection of

426   SARS-CoV-2 virus antigen as previously described (*34*). All samples were blinded and

427   evaluated by a board-certified veterinary pathologist.

Multiplex meso-scale ELISA for serum antibody responses

429   For 10-plex ELISA, 96-well plates precoated with SARS-CoV-2 S-2P (*39*) and RBD proteins

430   from multiple variants, SARS-CoV-2 N protein, and Bovine Serum Albumin (BSA) and supplied

431   by the manufacturer [Meso Scale Display (MSD)]. Determination of serum antibody binding was

432   performed as previously described (*7*) , and reagent details are in **Table S5**. All calculations are

433   performed within Excel and the GraphPad Prism software, v7.0. Readouts are provided as Area

434   Under Curve (AUC). For 4-plex ELISA, 96-well plates were pre-coated with SARS-CoV-2 S-

435   2P, RBB, and N and a BSA control in each well. Determination of serum antibody binding was

436   performed as previously described (*7, 18, 26*). Calculated ECLIA parameters to measure binding

437   antibody activities included interpolated concentrations or assigned arbitrary units (AU/ml) read

438   from the standard curve. International units (IU) were established for each antigen based on

439   parallelism between MSD reference standard and the WHO international standard. S-specific

440   IgG lower limit of detection = 0.3076 IU/mL. RBD-specific IgG lower limit of detection =

441   1.5936 IU/mL.

Meso-scale ELISA for mucosal antibody responses

443   Total S-specific IgG in BAL and nasal washes was determined by meso-scale ELISA (MSD) as

444   previously described (*46*).

445   **Serum antibody avidity assay**

446   Avidity was assessed using a sodium thiocyanate (NaSCN)-based avidity ELISA against SARS-

447   CoV-2 S-2P as previously described (*18, 26*). The avidity index (AI) was calculated using the

448   ratio of IgG binding to S-2P in the absence or presence of NaSCN and reported as the average of

449   two independent experiments, each containing duplicate samples.

450   **Serum antibody epitope definition**

451   Serum epitope mapping competition assays were performed using a Biacore 8K+ (Cytiva)

452   surface plasmon resonance (SPR) spectrometer. According to manufacturer's protocol, anti-

453   histidine IgG1 antibody was immobilized on Series S Sensor Chip CM5 (Cytiva) through

454   primary amine coupling using a His capture kit (Cytiva). His-tagged SARS-CoV-2 WA.1 S

455   protein containing 2 proline stabilization mutations (S-2P) was captured on active sensor surface.

456   Competitor monoclonal antibody (mAb) or a negative control antibody at set concentrations

457   were injected over both active and reference surfaces to saturation. Competitor human IgG mAbs

458   used include: S2-specific mAb S652-112, NTD-specific mAbs 4-8, S652-118, and N3C, S1-

459   specific mAb A20-36.1 and RBD-specific mAbs B1-182, CB6, A20-29.1, A19-46.1, LY-

460   COV555, A19-61.1, S309, A23-97.1, A19-30.1, A23-80.1 and CR3022. Non-human primate

461   sera were flowed over both active and reference sensor surfaces for 40 minutes. 1X PBS-P+

462   (Cytiva) was used as the running buffer and diluent for all samples. Active and reference sensor

463   surfaces were regenerated between each analysis cycle using 10mM glycine, pH 1.5 (Cytiva).

464   Prior to analysis, sensorgrams were aligned to Y (Response Units) = 0, beginning at the serum

465   association phase using Biacore 8K Insights Evaluation Software (Cytiva). Reference-subtracted

466   relative "analyte binding late" report points (RU) were collected and used to calculate absolute

467   and relative competition. Relative percent competition (% C) was calculated using the following

468   formula: % C = [1 – (100 * ((RU in presence of competitor mAb) / (RU in presence of negative

469   control mAb))]. Absolute competition (ΔRUs) was calculated with the following formula: ΔRUs

470   = [(RU in presence of negative control mAb) – (RU in presence of competitor mAb)]. Results

471   are reported as absolute serum epitope reactivity and percent competition and statistical analysis

472   was performed using unpaired, two-tailed t-test (Graphpad Prism v.8). All assays were

473   performed in duplicate, with average data point for each animal represented on corresponding

474   graphs.

475   **Surface plasmon resonance binding assay**

476   Series S CM5 Sensor Chips (Cytiva) were activated by immobilizing anti-histidine IgG1

477   antibody on surface using a His capture kit (Cytiva), according to manufacturer's protocol. His-

478   tagged SARS-CoV-2 WA.1 or β S protein containing 2 proline stabilization mutations (S-2P)

479   was captured on active sensor surface at a set concentration for 10 minutes. Monoclonal

480   antibodies (mAbs) were injected over both active and reference surfaces and allowed to bind to

481   saturation. Graphs represent reference-subtracted relative "analyte binding late" report points

482   (RUs), determined by aligning sensorgrams to Y (Response Units) = 0, beginning at the mAb

483   association stage using Biacore 8K Insights Evaluation Software (Cytiva). All assays were

484   performed using a Biacore 8K+ (Cytiva) surface plasmon resonance (SPR) spectrometer.

485   **ACE2-binding inhibition assay**

486 ACE2-binding inhibition was completed, as previously described (*47*), using 1:40 diluted BAL

487 and nasal wash samples.

Lentiviral pseudovirus neutralization assay

489 Pseudotyped lentiviral reporter viruses were produced by the co-transfection of plasmids

490 encoding SARS-CoV-2 proteins from multiple variants, a luciferase reporter, lentivirus

491 backbone, and human transmembrane protease serine 2 (TMPRSS2) genes as previously

492 described (*17, 37*); reagent details are in **Table S5**. Sera were tested, in duplicate, for

493 neutralizing activity against the pseudoviruses and percent neutralization was calculated in Prism

494 v9.0.2 (GraphPad). The lower limit of quantification was 1:40 ID_{50}.

VSV pseudovirus neutralization assay

496 SARS-CoV-2 pseudotyped recombinant VSV-ΔG-firefly luciferase viruses were made by co-

497 transfection of plasmid expressing full-length S, and subsequent infection with VSVΔG-firefly-

498 luciferase and neutralization assays were completed on sera samples as previously described (*15,*

499 *42*); reagent details are in **Table S5**. The lower limit of quantification was 1:40 ID_{50}.

Focus reduction neutralization test (FRNT)

501 FRNT assays were performed on sera samples, in duplicate, as previously described (*34*); and

502 reagent details are in **Table S5**. For samples that do not neutralize 50% of virus at the limit of

503 detection, 5 was plotted and used for geometric mean calculations.

B cell Probe Binding Assay

505 Cryopreserved PBMC were thawed and washed in wash buffer (4% Heat-inactivated newborn

506 calf serum/0.02% NaN₃/ phenol-free RPMI). Cells were incubated with streptavidin-BV605 (BD

507 Biosciences) labeled β S-2P and streptavidin-BUV661 (BD Biosciences) labeled WA-1 S-2P for

508   30 minutes at 4°C, washed twice in 1X PBS, and incubated with Aqua live/dead fixable dead cell

509   stain (Thermo Fisher Scientific) for 20 minutes at room temperature. After two washes in wash

510   buffer, cells were incubated with primary antibodies for 20 minutes at room temperature. The

511   following antibodies were used (monoclonal unless indicated): IgD FITC (goat polyclonal,

512   Southern Biotech), IgM PerCP-Cy5.5 (clone G20-127, BD Biosciences), IgA Dylight 405 (goat

513   polyclonal, Jackson Immunoresearch Inc), CD20 BV570 (clone 2H7, Biolegend), CD27 BV650

514   (clone O323, Biolegend), CD14 BV785 (clone M5E2, Biolegend), CD8 BUV395 (clone RPA-

515   T8, BD Biosciences), CD16 BUV496 (clone 3G8, BD Biosciences), CD4 BUV737 (clone SK3,

516   BD Biosciences), CD19 APC (clone J3-119, Beckman Coulter), IgG Alexa 700 (clone G18-145,

517   BD Biosciences), CD3 APC-Cy7 (clone SP34.2, BD Biosciences), CD38 PE (clone OKT10,

518   Caprico Biotechnologies), CD21 PE-Cy5 (clone B-ly4, BD Biosciences), and CXCR5 PE-Cy7

519   (clone MU5UBEE, Thermo Fisher Scientific). Cells were washed twice in wash buffer and

520   residual red blood cells were lysed using BD FACS Lysing Solution (BD Biosciences) for 10

521   minutes at room temperature. Following two additional washes, cells were fixed in 0.5%

522   formaldehyde (Tousimis Research Corp.) All antibodies were previously titrated to determine the

523   optimal concentration. Samples were acquired on an BD FACSymphony flow cytometer and

524   analyzed using FlowJo version 10.7.2 (BD, Ashland, OR).

525   **Intracellular cytokine staining**

526   Cryopreserved PBMC were thawed and rested overnight in a 37C/5% CO2 incubator. The next

527   morning, cells were stimulated with SARS-CoV-2 Spike protein peptide pools (S1 and S2) that

528   were matched to the vaccine insert (comprised of 158 and 157 individual peptides, respectively,

529   as 15mers overlapping by 11 aa in 100% DMSO, JPT Peptides) at a final concentration of 2

530   µg/ml in the presence of 3 mM monensin for 6 hours. Negative controls received an equal

531   concentration of DMSO instead of peptides (final concentration of 0.5%). Intracellular cytokine

532   staining was performed as described (*43*). The following monoclonal antibodies were used: CD3

533   APC-Cy7 (clone SP34.2, BD Biosciences), CD4 PE-Cy5.5 (clone S3.5, Invitrogen), CD8 BV570

534   (clone RPA-T8, Biolegend), CD45RA PE-Cy5 (clone 5H9, BD Biosciences), CCR7 BV650

535   (clone G043H7, Biolegend), CXCR5 PE (clone MU5UBEE, Thermo Fisher), CXCR3 BV711

536   (clone 1C6/CXCR3, BD Biosciences), PD-1 BUV737 (clone EH12.1, BD Biosciences), ICOS

537   Pe-Cy7 (clone C398.4A, Biolegend), CD69 ECD (cloneTP1.55.3, Beckman Coulter), IFN-g

538   Ax700 (clone B27, Biolegend), IL-2 BV750 (clone MQ1-17H12, BD Biosciences), IL-4 BB700

539   (clone MP4-25D2, BD Biosciences), TNF-FITC (clone Mab11, BD Biosciences), IL-13 BV421

540   (clone JES10-5A2, BD Biosciences), IL-17 BV605 (clone BL168, Biolegend), IL-21 Ax647

541   (clone 3A3-N2.1, BD Biosciences), and CD154 BV785 (clone 24-31, BioLegend). Aqua

542   live/dead fixable dead cell stain kit (Thermo Fisher Scientific) was used to exclude dead cells.

543   All antibodies were previously titrated to determine the optimal concentration. Samples were

544   acquired on an BD FACSymphony flow cytometer and analyzed using FlowJo version 9.9.6

545   (Treestar, Inc., Ashland, OR). Gating schema is represented in **Fig. S6**.

546   **Statistical Analysis**

547   Graphs show data from individual animals with dotted lines indicating assay limits of detection.

548   Groups were compared for viral load and immune responses using Welch's t-tests; data were

549   analyzed on the log10 scale for viral loads and appropriate immune assays. Changes over time

550   were summarized using the change on the $\log_{10}$ scale, and statistical significance was determined

551   using paired t-tests. When more than two variants are compared, as in Figure 1 A-F, a Holm's

552   adjustment for multiple comparisons was used on the set of p-values to determine significance;

553   there were no adjustments for multiple comparisons across different analyses in this descriptive

554   study. Comparisons drawn between vaccination groups for serum antibody epitope analysis were

555   performed using unpaired *t* tests, within each time point evaluated. Analyses were performed in

556   R version 4.0.2 and Prism (GraphPad) versions 8.2 and 9.0.2.

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681   K.S.C., D.W., M.S., K.E.F., S.F.A., and G.A. prepared figures and tables. All authors contributed

682   to discussions about and editing of the manuscript. **Competing Interests:** K.S.C. and B.S.G. are

683   inventors on U.S. Patent No. 10,960,070 B2 and International Patent Application No.

684   WO/2018/081318 entitled "Prefusion Coronavirus Spike Proteins and Their Use." K.S.C. and

685   B.S.G. are inventors on US Patent Application No. 62/972,886 entitled "2019-nCoV Vaccine".

686   J.M., L.W., C.A.S., J.R.M, D.D, N.J.S., A.R.H., W.S., Y.Z. and M.R. are inventors on US patent

687   application No. 63/147,419 entitled "Antibodies Targeting the Spike Protein of Coronaviruses".

688   D.V., A.V.R., Z.F., A.C., L.P., H.A., and M.G.L. are employees of Bioqual. K.S.C, B.S.G, L.W.,

689   W.S., and Y.Z. are inventors on multiple US Patent Applications entitled "Anti-Coronavirus

690   Antibodies and Methods of Use". A.C., M.K., A.C., and D.K.E. are employees of Moderna.

691   M.S.S. serves on the scientific board of advisors for Moderna. **Data and materials availability:**

692   All data are available in the main text or the supplementary materials.
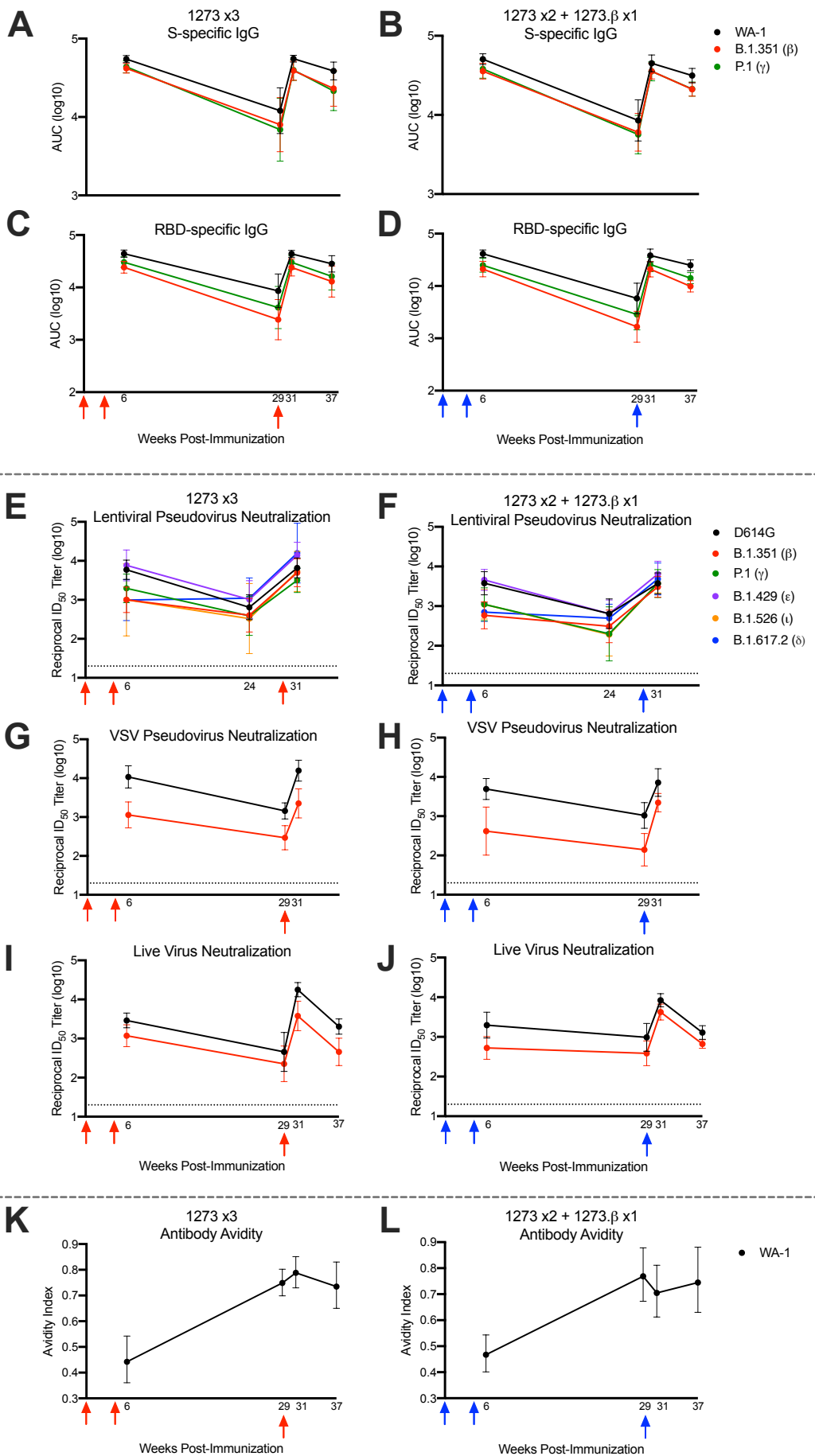
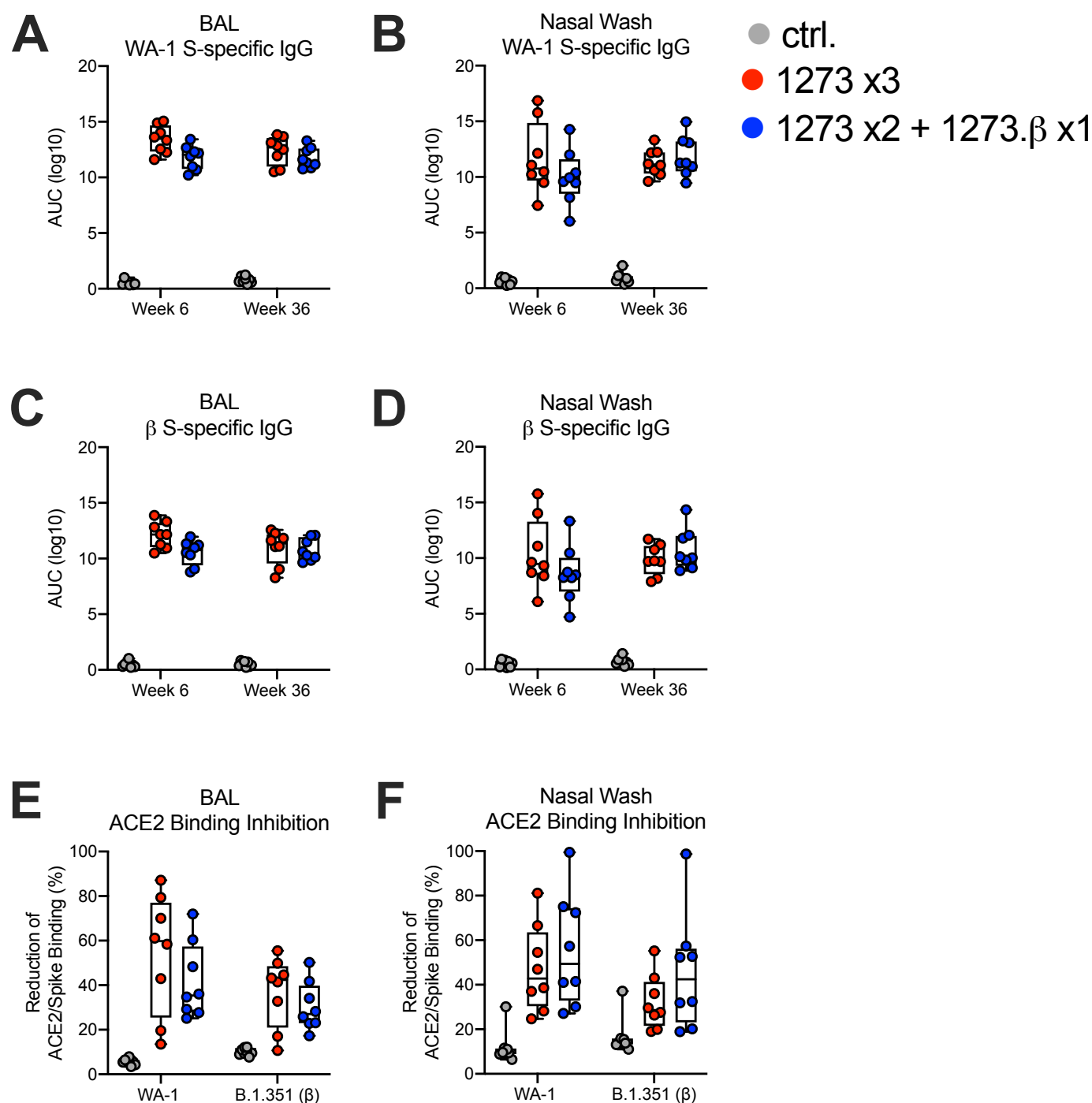**<u>Figure 1</u>. Temporal serum antibody responses to SARS-CoV-2 variants.** Rhesus macaques (n = 8/group) were immunized according to Figure S1. Sera were collected at weeks 6, 24 or 29, 31, and 37 and assessed, where applicable, for SARS-CoV-2 WA-1 (black), β (red), and P.1 – gamma, γ (green), B.1.429 – epsilon, ε (purple), B.1.526 – iota, ι (orange), and B.1.617.2 – delta, δ (blue) S-specific (A-B) and RBD-specific (C-D) IgG by MULTI-ARRAY ELISA, lentiviral-based pseudovirus neutralization (E-F), VSV-based pseudovirus neutralization (G-H), live virus neutralization (I-J), and WA-1 S-specific antibody avidity (K-L). Lines represent geometric mean titer (GMT) and geometric error. Dotted lines indicate neutralization assay limits of detection. Arrows point to immunization timepoints.

Figure 2. Mucosal antibody responses to SARS-CoV-2 variants. Rhesus macaques (n = 8/group) were immunized according to Figure S1. BAL (A, C, E) and nasal washes (B, D, F) collected at weeks 6 and 36 were assessed for S-specific IgG to SARS-CoV-2 WA-1 (A-B) and β (C-D) by MULTI-ARRAY ELISA. Week 36 BAL and nasal washes were assessed for inhibition of ACE2 binding to WA-1 and β S (E-F). Boxes and horizontal

bars denote interquartile ranges (IQR) and medians, respectively; whisker end points are equal to the maximum

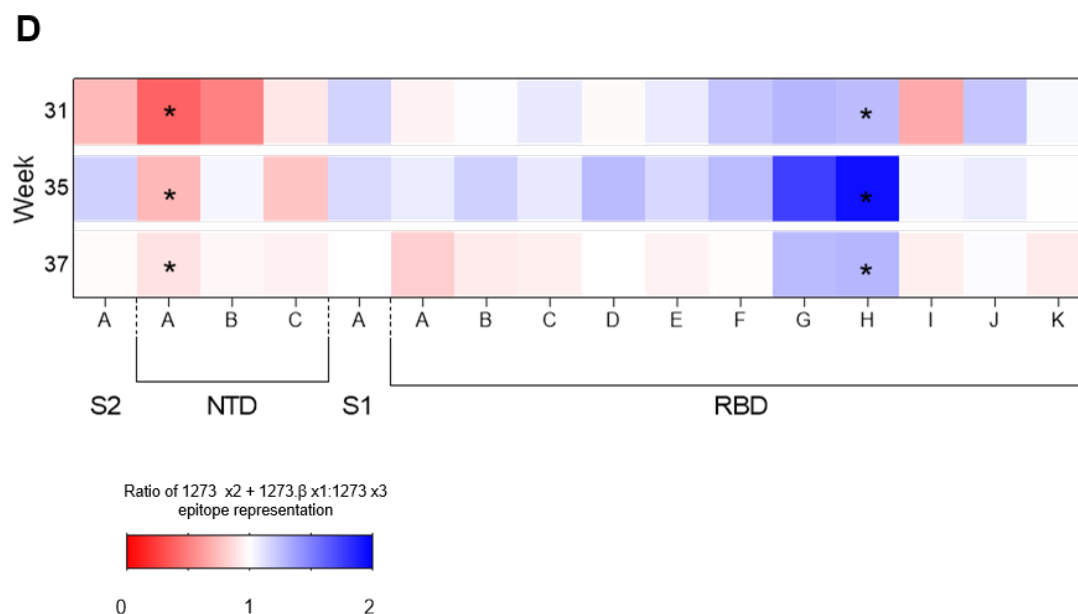and minimum values. Circles represent individual NHP.
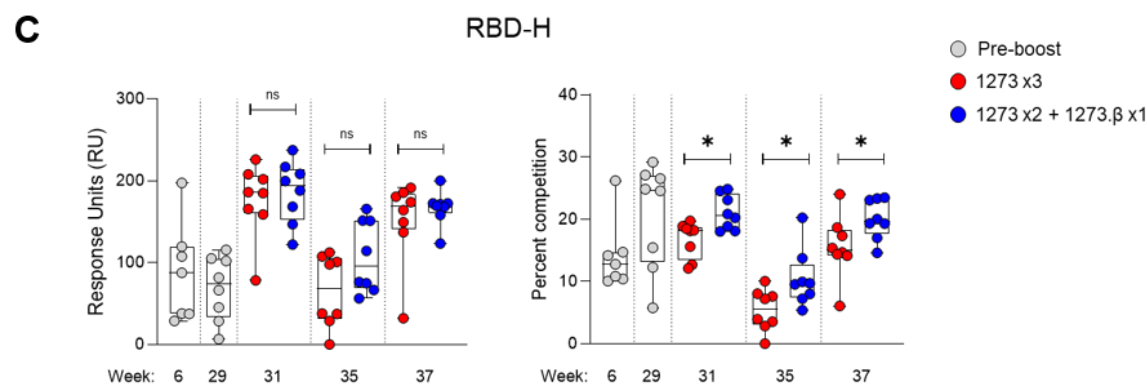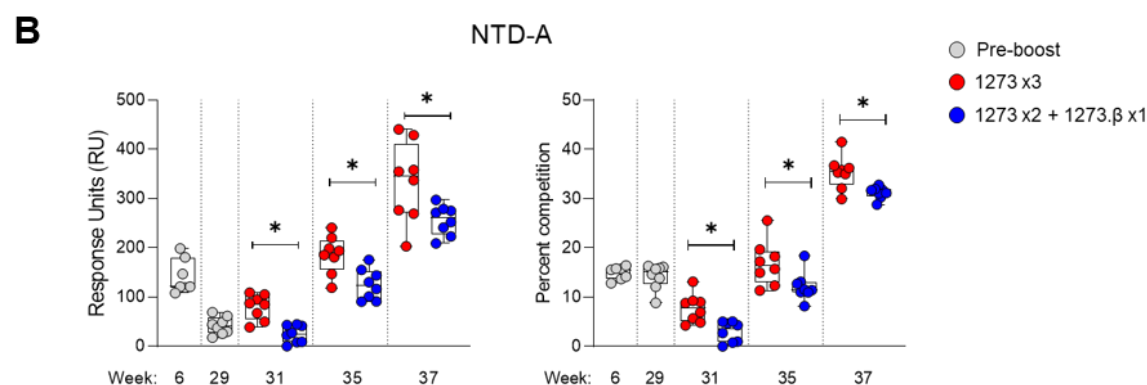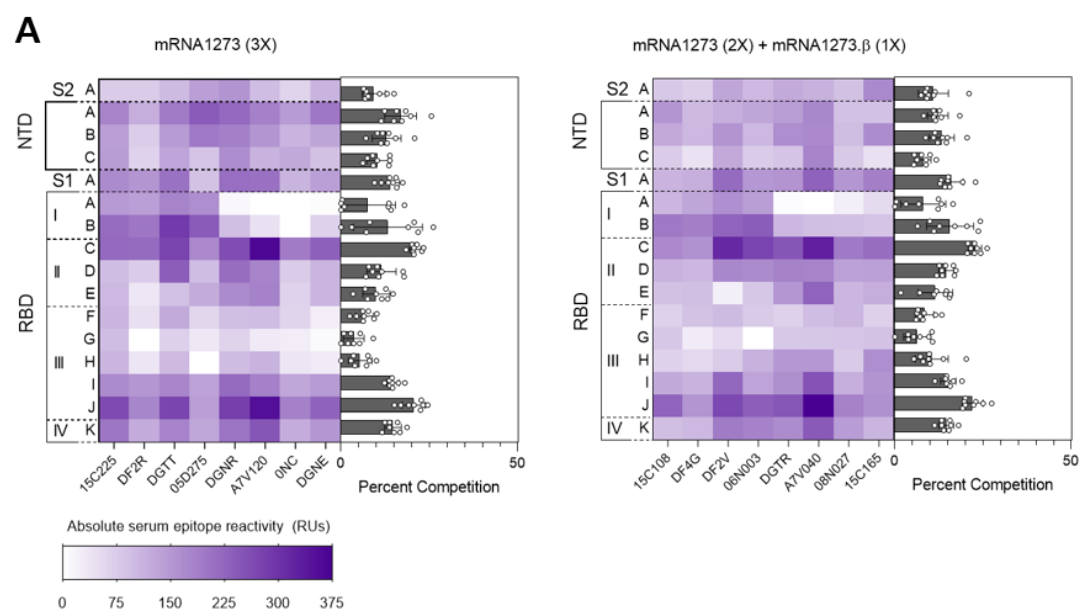
Figure 3. **Serum Epitope Mapping Analysis**. (A) Serum epitope fingerprints at Week 35 (6 weeks post-boost). Absolute serum epitope reactivity (RUs) is indicated from light to dark purple. Columns represent individual NHP, rows represent antigenic site. Bar graphs on the right depict relative serum reactivity (percent competition) for each antigenic site with each animal represented by an open circle (n=8/group). (B, C) Absolute (RUs) and relative (percent competition) serum reactivity for epitopes which show significant ($p \le 0.05$) differences between immunization groups at all time points. NTD-A site is represented by mAb 4-8, RBD site H is represented by mAb A23-97.1 *see Table S2*. (D) Ratio of mRNA-1273 x2 + mRNA-1273.β x1 to mRNA-1273 x3 epitope representation (percent competition) for each antigenic site at week 31, 35 and 37. Significant ($p \le 0.05$) differences determined by unpaired t-test between immunization groups at a single time point.
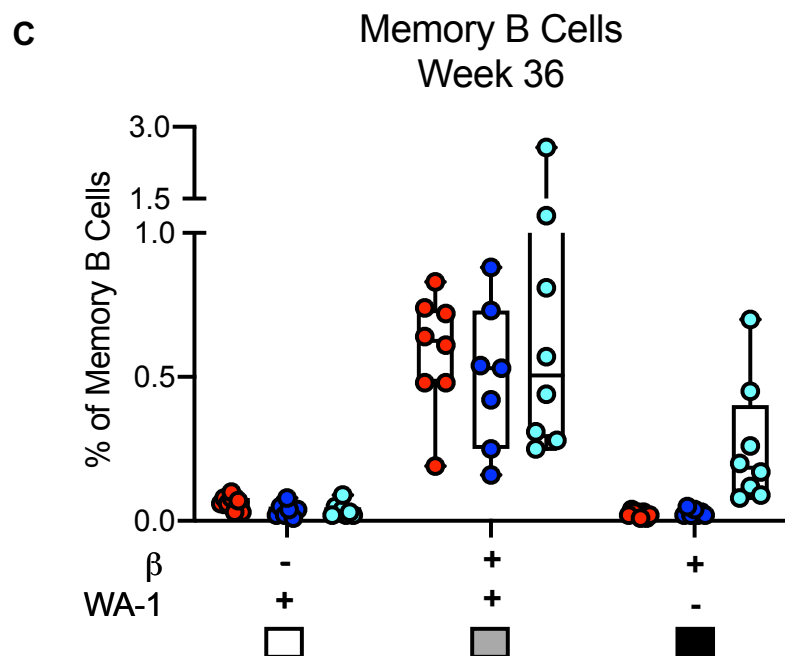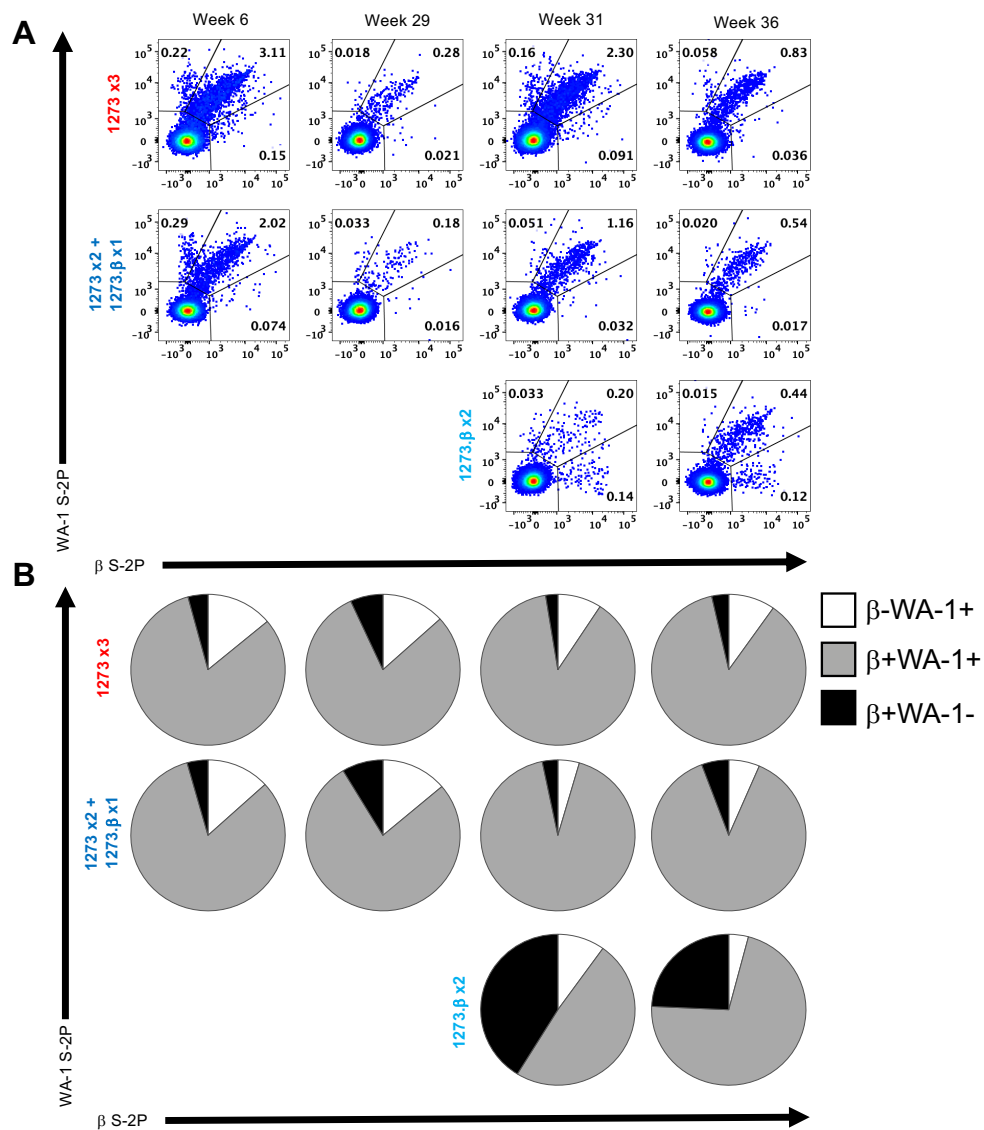
A

Week 6 | Week 29 | Week 31 | Week 36

1273 x3

1273 x2 + 1273.β x1

1273.β x2

WA-1 S-2P

β S-2P

B

1273 x3

1273 x2 + 1273.β x1

1273.β x2

WA-1 S-2P

β S-2P

□ β-WA-1+
▩ β+WA-1+
■ β+WA-1-

C

Memory B Cells
Week 36

% of Memory B Cells

β   -   +   +
WA-1 +   +   -

□   ▩   ■

Figure 4. Frequency of SARS-CoV-2 spike-specific B cells in PBMC. (A) Representative flow cytometry plots from one NHP per vaccine group depicting the frequency of memory B cells that bind the WA-1 S, WA-1 S and β S, or β S at various time points after immunization. (B) Pie charts representing the fraction of S-specific B cells that are single positive for the WA-1 (white, β-WA-1+), double positive for WA-1 and β (gray, β+WA-1+), and single positive for β (black, b+WA-1-) for all NHP in each group. (C) Frequency of β-WA-1+, β+WA-1+, and β+WA-1- memory B cells at week 36 in each of the vaccine groups. Boxes and horizontal bars denote the IQR and medians, respectively; whisker end points are equal to the maximum and minimum values. Circles represent individual NHP.
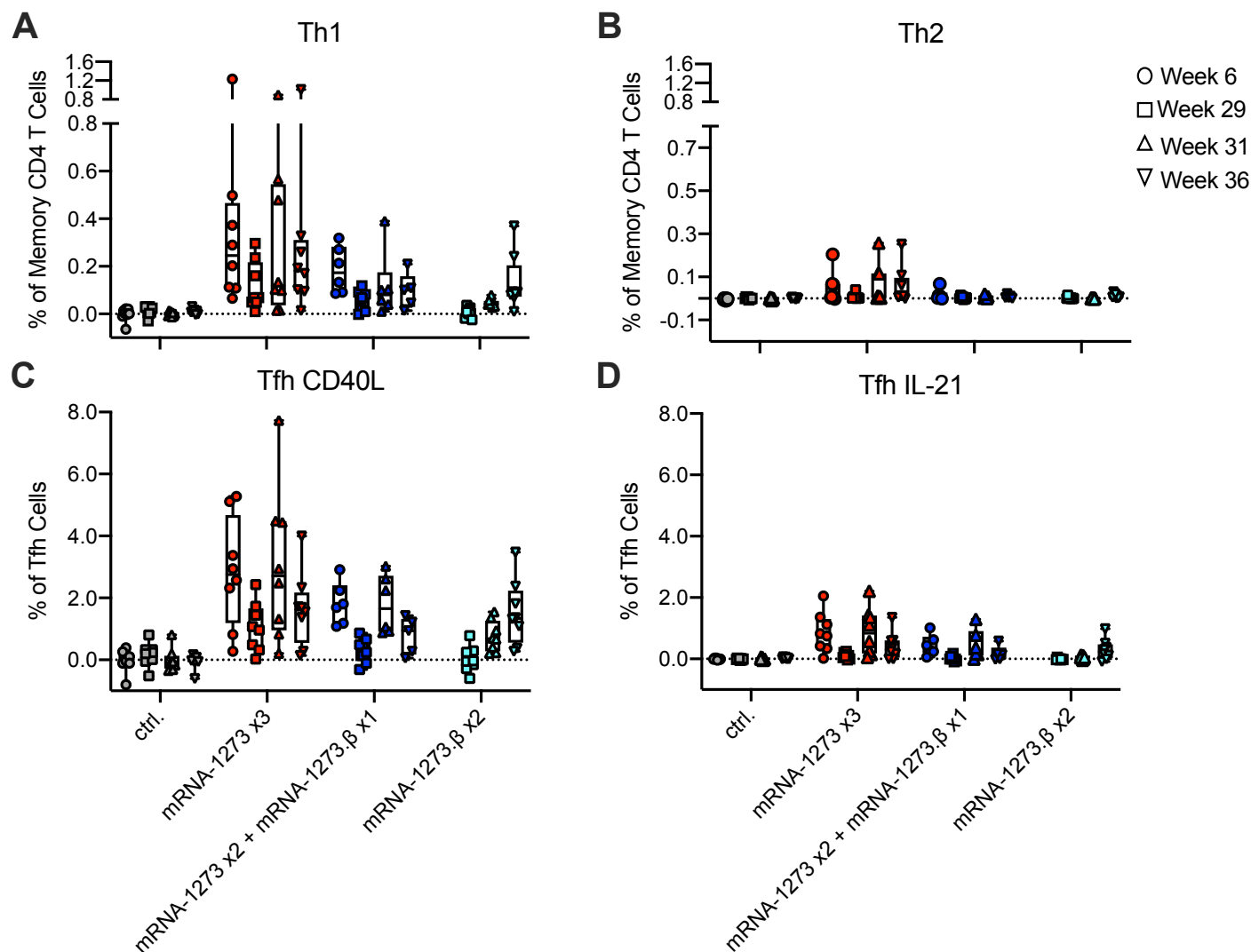
Figure 5. T cell responses following mRNA-1273 immunization. Rhesus macaques were immunized according to Figure S1. Intracellular staining (ICS) was performed on PBMCs at weeks 6, 29, 31, and 36 to assess T cell responses to SARS-CoV-2 S protein peptide pools, S1 and S2. Responses to S1 and S2 individual peptide pools were summed. (A) Th1 responses (IFNg, IL-2, or TNF), (B) Th2 responses (IL-4 or IL-13), (C) Tfh CD40L upregulation (peripheral follicular helper T cells (Tfh) were gated on central memory CXCR5$^+$PD-1$^+$ICOS$^+$ CD4 T cells), (D) Tfh IL-21. Boxes and horizontal bars denote IQR and medians, respectively; whisker end points are equal to the maximum and minimum values. Circles represent individual NHP. Dotted lines are set to 0%.
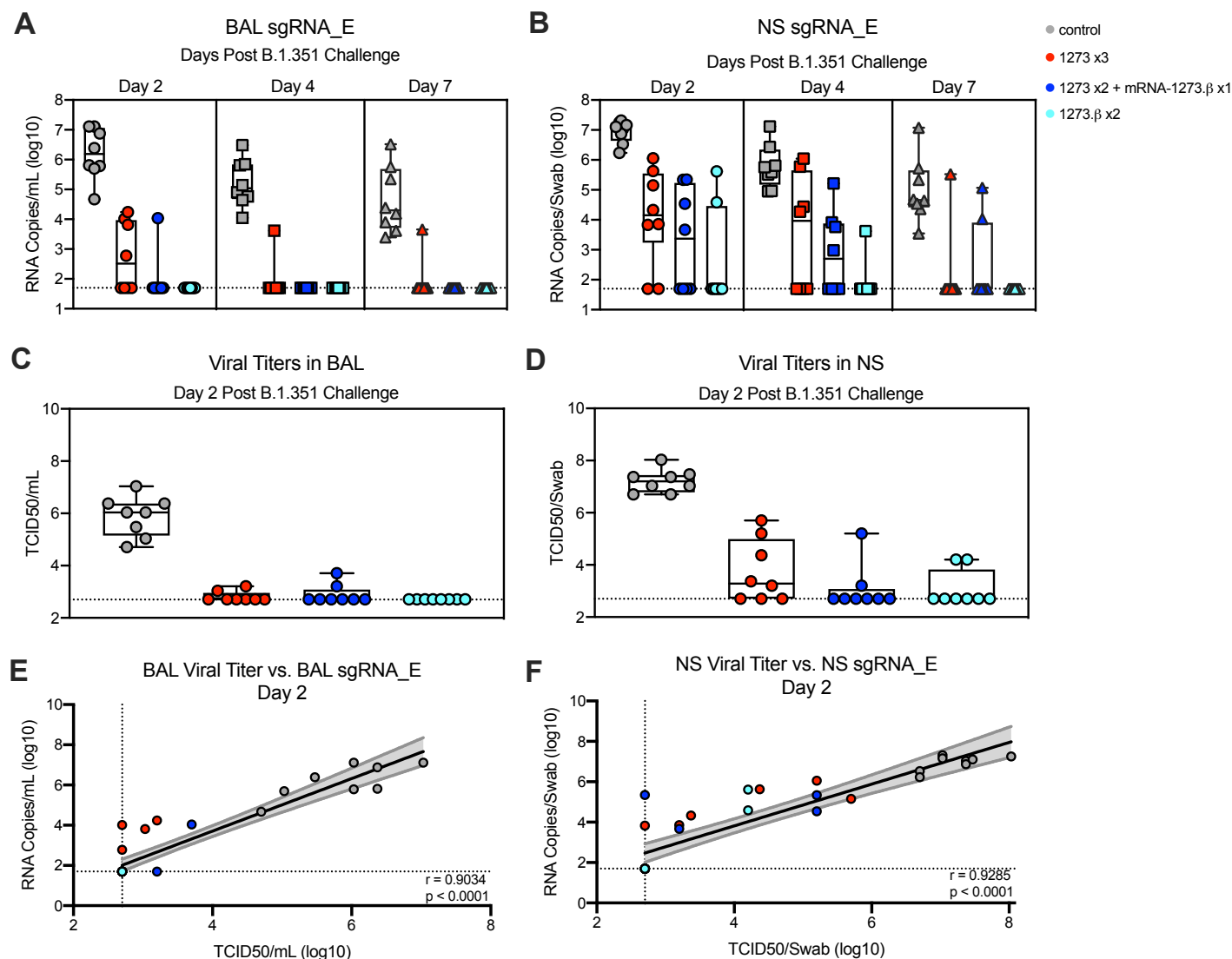
Figure 6. **Efficacy of mRNA-1273 against upper and lower respiratory B.1.351 viral replication.** Rhesus macaques were immunized and challenged as described in Figure S1. BAL (A, C) and nasal swabs (NS) (B, D) were collected on days 2 (circles), 4 (squares), and 7 (triangles) post-challenge, where applicable, and viral replication was assessed by detection of SARS-CoV-2_E sgRNA. Viral titers were assessed by TCID50 assay for BAL (C) and NS (D) collected on day 2 post-challenge. Boxes and horizontal bars denote the IQR and medians, respectively;

whisker end points are equal to the maximum and minimum values. (E-F) Plots show correlations between viral titers and sgRNA_E in BAL (E) and NS (F) 2 days post-challenge. Black and gray lines indicate linear regression and 95% confidence interval, respectively. 'r' and 'p' represent Spearman's correlation coefficients and corresponding p-values, respectively. Symbols represent individual NHP and may overlap for equal values. Dotted lines indicate assay limits of detection.