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1   **Title: Immune Correlates of Protection by mRNA-1273 Immunization against**

2   **SARS-CoV-2 Infection in Nonhuman Primates**

3

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39 **Abstract:** Immune correlates of protection can be used as surrogate endpoints for vaccine

40 efficacy. The nonhuman primate (NHP) model of SARS-CoV-2 infection replicates key features

41 of human infection and may be used to define immune correlates of protection following

42 vaccination. Here, NHP received either no vaccine or doses ranging from 0.3 – 100 µg of

43 mRNA-1273, a mRNA vaccine encoding the prefusion-stabilized SARS-CoV-2 spike (S-2P)

44 protein encapsulated in a lipid nanoparticle. mRNA-1273 vaccination elicited robust circulating

45 and mucosal antibody responses in a dose-dependent manner. Viral replication was significantly

46 reduced in bronchoalveolar lavages and nasal swabs following SARS-CoV-2 challenge in

47 vaccinated animals and was most strongly correlated with levels of anti-S antibody binding and

48 neutralizing activity. Consistent with antibodies being a correlate of protection, passive transfer

49 of vaccine-induced IgG to naïve hamsters was sufficient to mediate protection. Taken together,

50 these data show that mRNA-1273 vaccine-induced humoral immune responses are a mechanistic

51 correlate of protection against SARS-CoV-2 infection in NHP.

52 **One-Sentence Summary:** mRNA-1273 vaccine-induced antibody responses are a mechanistic

53 correlate of protection against SARS-CoV-2 infection in NHP.

54

55   Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of

56   coronavirus disease 2019 (COVID-19), has led to more than 138 million infections and 3 million

57   deaths worldwide as of April 15, 2021 (*1*). Mass vaccination offers the most efficient public

58   health intervention to control the pandemic. Two mRNA-based vaccines, Moderna's mRNA-

59   1273 and Pfizer/BioNTech's BNT 162b2, both of which encode the prefusion-stabilized spike

60   glycoprotein (S-2P) (*2, 3*), showed >94% efficacy against symptomatic COVID-19 in interim

61   Phase 3 analyses (*4, 5*) and are currently being administered globally. Several other vaccines

62   have shown 60-80% efficacy against COVID-19 in Phase 3 trials (*6, 7*), and a number of

63   candidate vaccines are in earlier stages of clinical development (*8*). A critical issue for

64   optimizing the use of COVID-19 vaccines is defining an immune correlate of protection. This

65   surrogate of vaccine efficacy can be used to inform potential dose reduction, advance approval of

66   other vaccine candidates in lieu of Phase 3 efficacy data, extend indications for use to other age

67   groups, and provide insights into the immune mechanisms of protection (*9*).

68   The nonhuman primate (NHP) model has been used to demonstrate immunogenicity and

69   protective efficacy against SARS-CoV-2 with a number of vaccine candidates (*10-13*). The high

70   level of protection achieved with mRNA vaccines in NHP using clinically relevant dose

71   regimens has been consistent with results from human trials. This model exhibits upper and

72   lower airway infection and pathology similar to clinical presentations of mild COVID-19 disease

73   in humans (*14*). While assessment of immune correlates of viral load after primary infection has

74   been completed in NHP (*15*), there are no studies to date that have specifically defined immune

75 correlates of protection in upper and lower airways after vaccination with any product approved

76 for use in humans.

77 We used immunogenicity and protection assessments from our previous NHP mRNA-1273

78 vaccine study (*13*) to hypothesize that serum antibody measurements serve as immune correlates

79 of protection. Here, in a dose de-escalation study, we evaluated how multiple measurements of

80 humoral and cellular immunity correlate with the reduction of viral replication in the upper and

81 lower airway following challenge. Antibody analyses were also performed on bronchoalveolar

82 lavages (BAL) and nasal washes after vaccination to assess site-specific immune correlates. Last,

83 we demonstrated the ability of passively transferred IgG from mRNA-immunized NHP to

84 mediate protection in a highly pathogenic Syrian hamster SARS-CoV-2 challenge model.

85 Together, these studies support spike (S)-specific antibodies as a correlate of protection,

86 highlight the ability of localized mucosal antibodies to control upper and lower airway viral

87 replication, and confirm mRNA-1273-induced IgG to be sufficient for protection against SARS-

88 CoV-2 infection in preclinical models.

89

90 **Results**

91 ***mRNA-1273 vaccination elicits robust antibody responses in a dose-dependent manner***

92 We previously demonstrated dose-dependency of serum antibody responses in NHP following

93 vaccination with 10 or 100 µg of mRNA-1273, with high-level protection against SARS-CoV-2

94 challenge in both dose groups (Fig. S1A) (*13*). These and other immunogenicity outcomes from

95   an additional NHP study in which animals were vaccinated with 30 μg of mRNA-1273 (Fig.

96   S1B) were used to design a study to evaluate immune correlates of protection following mRNA-

97   1273 vaccination in the current study (Fig. S1C). Doses of mRNA-1273 ranging from 0.3 to 30

98   μg were administered in the standard primary regimen at weeks 0 and 4 to generate a range of

99   immune responses and protective outcomes.

100   We first assessed temporal serum S-specific antibody binding or avidity and pseudovirus

101   neutralization responses post-prime and -boost. Consistent with our previous report (*13*), S-

102   specific IgG binding to the conformationally defined prefusion S-2P protein (*2, 3*) was increased

103   over baseline after each immunization, reaching 7,900 and 64,000 median reciprocal endpoint

104   titers by 4 weeks post-prime and -boost, respectively, following immunization with 30 μg of

105   mRNA-1273 (Fig. S2A). There was an 8-10-fold increase in S-specific binding antibodies after

106   the boost in all dose groups, except for the 0.3 μg dose for which boosting elicited 300-fold more

107   S-specific antibodies. S-specific antibody avidity was also increased, by 2-fold, after the boost in

108   all vaccine groups except for the 0.3 μg dose, and there were no differences between the vaccine

109   groups (Fig. S2B). D614G pseudovirus neutralizing antibody responses 4 weeks post-prime were

110   only detectable in the 30 μg dose group (median reciprocal $ID_{50} = 76$), increasing by ~1 $\log_{10}$

111   post-boost (Fig. S2C).

112   For analyses of immune correlates, we used data from six different qualified antibody assays

113   performed at the time of SARS-CoV-2 challenge, 4 weeks post-boost. Anti-S-specific (Fig. 1A)

114   and anti-receptor binding domain (RBD) (Fig. 1B) responses were measured using Meso-Scale

115   Discovery Multiplex ELISA, validated for use in Phase 3 clinical SARS-CoV-2 vaccine trials;

116   here, antibody binding titers are reported in international units (IU) defined by World Health

117   Organization (WHO) standards. Binding antibody titers increased compared to control animals in

118   a dose-dependent manner ranging from a median of 55 to 5,800 IU/mL at 0.3 and 100 µg,

119   respectively, for S-specific IgG, and 66 to 10,400 IU/mL for RBD-specific IgG (Fig. 1A-B).

120   There was also a dose-dependent reduction in median ACE2 binding inhibition comparing 100

121   µg to 1 µg of mRNA-1273 (Fig. 1C), reaching a maximum of 270-fold. *In vitro* neutralizing

122   activity was determined using three orthogonal assays. First, for the lentiviral-based D614G

123   pseudovirus neutralization assay qualified for use in Phase 3 clinical studies, there was a dose-

124   dependent decrease with a median reciprocal ID_{50} titer of 23,000 at the 100 µg dose and 49

125   following immunization with 1 µg of mRNA-1273 (Fig. 1D). VSV-based pseudovirus (Fig. 1E)

126   and live virus (Fig. 1F) neutralization followed the same significant dose-dependency trend.

127   Assessments of antibody binding and neutralization responses were highly correlated with one

128   another, suggesting mRNA-1273 immunization elicits high titer S-binding antibody responses

129   and high-level functional antibody responses (Fig. 2).

130   Given the increasing circulation of SAR-CoV-2 variants of concern, some of which have shown

131   a significant reduction in neutralization sensitivity to vaccine-elicited and convalescent sera (*16-*

132   *20*), we also assessed the ability of mRNA-1273 immune NHP sera to neutralize two of the

133   SAR-CoV-2 variants of concern. Live viral neutralization of the B.1.1.7 variant (*21*), which is

134   highly transmissible and currently circulating around the world (*22*), was not appreciably

135   decreased as compared to D614G (Fig. S3A). For the B.1.351 variant, which contains multiple

136   mutations in RBD and NTD and shows the greatest reduction of neutralization by vaccine sera

137   (*16, 23, 24*), there was a 9-fold reduction compared to D614G in the 100 µg mRNA-1273 dose

138   group. Notably, 9 of 12 animals immunized with 30 or 100 µg of mRNA-1273 had reciprocal

139   ID_{50} titers > 100, while only 1 of 4 animals in the 10 µg dose group had detectable neutralization

140   activity to B.1.351 (Fig. S3B). The reduction in B.1.351 neutralization capacity of mRNA-1273-

141   induced antibodies mirrors what has been previously shown in NHP or humans using only 30 or

142   100 µg (*16, 20*), but these data further suggest that mRNA-1273 dose may have a profound

143   effect on eliciting neutralizing antibodies against the B.1.351 variant.

144   ***mRNA-1273 vaccination elicits upper and lower airway antibodies***

145   To provide additional immune data on correlates of protection at the site of infection, antibody

146   responses in the lower and upper airway were assessed from BAL and nasal wash samples,

147   respectively, at 2 weeks post-boost. There was a dose-dependent increase in BAL and nasal wash

148   S-specific IgG and IgA following two doses of mRNA-1273 (Fig. 1G-J). BAL S-specific IgG

149   titers following 0.3 and 30 µg of mRNA-1273 ranged from a median of 110 to 280,000 area

150   under the curve (AUC) (Fig. 1G), and nasal wash S-specific IgG titers ranged from 86 to

151   142,200 AUC (Fig. 1H). For S-specific IgA, the dose-dependent trend was similar albeit to lower

152   titers where 30 µg of mRNA-1273 elicited 1400 and 21,300 AUC IgA in BAL (Fig. 1I) and nasal

153   washes (Fig. 1J), respectively. Additionally, upper and lower airway antibody responses

154   correlated with each other and the two Phase 3 qualified humoral antibody measurements, S-

155   specific IgG and lentiviral-based pseudovirus neutralization activity; the one exception was that

156   there was no correlation with BAL and nasal wash S-specific IgA (Fig. S4). In all, mRNA-1273

157 vaccination elicits S-specific IgG and IgA antibodies in both the upper and lower airways, which

158 potentially provide immediate protection at the site of infection.

159 ***mRNA-1273 vaccination elicits S-specific CD4 T cell responses***

160 S-specific CD4 and CD8 T cell responses were assessed 2 weeks post-boost. A direct correlation

161 between dose and Th1 responses was observed (p=0.006), where all animals in the 30 μg dose

162 group had Th1 responses (Fig. S5A). In contrast, Th2 responses were low to undetectable in all

163 vaccine dose groups (Fig. S5B). CD8 T cells at these doses of mRNA-1273 were also

164 undetectable. Given the importance of T follicular helper (Tfh) in regulating antibody responses,

165 we extended the analysis to S-specific Tfh cells that express the surface marker CD40L or the

166 canonical cytokine IL-21. Most vaccinated animals had S-specific CD40L+ CD4 Tfh cell

167 responses - the magnitude of which was directly correlated with dose (p<0.001) (Fig. S5C). A

168 direct correlation between dose and magnitude of S-specific IL-21 Tfh cell responses was also

169 observed (p=0.010). (Fig. S5D). Consistent with previous results (*13, 25, 26*), these data show

170 that mRNA-1273 induced Th1- and Tfh-skewed CD4 responses.

171 ***mRNA-1273 vaccination protects against upper and lower airway SARS-CoV-2 replication***

172 To evaluate the protective efficacy of mRNA-1273 vaccination, all animals in experiment VRC-

173 20-857.4 (Fig. S1C) were challenged 4 weeks post-boost with a total dose of $8x10^5$ PFU of a

174 highly pathogenic stock of SARS-CoV-2 (USA-WA1/2020) by combined intranasal and

175 intratracheal routes for upper and lower airway infection, respectively. This challenge dose was

176 chosen to induce viral loads similar to or higher than those detected in nasal secretions of

177   humans following SARS-CoV-2 infection (*27*). The primary efficacy endpoint analysis used

178   subgenomic RNA (sgRNA) qRT-PCR for the nucleocapsid (N) gene (Fig. 3). N sgRNA is the

179   most highly expressed sgRNA species as a result of discontinuous transcription and thus

180   provides greater sensitivity than the envelope (E) gene (Fig. S6) (*28*), which is most commonly

181   used in other NHP SARS-CoV-2 vaccine studies (*13*) to quantify replicating virus.

182   We observed a vaccine dose effect for protection against viral replication in the upper and lower

183   airway. On days 2 and 4 post challenge, there were ~2 and 5 $\log_{10}$ reductions in sgRNA_N in

184   BAL compared to control animals at doses of 1 µg and 30 µg, respectively (Fig. 3A). Moreover,

185   by day 4 post-challenge, the majority of animals vaccinated with 1 µg or higher had low to

186   undetectable sgRNA_E in BAL (Fig. S6A). By contrast, the reduction in sgRNA in nasal swabs

187   was primarily limited to animals receiving 30 µg of mRNA-1273 as compared to control animals

188   (Fig. 3B, Fig. S5B). These data highlight differences in immune responses required for reduction

189   in viral replication for upper and lower airway protection. Post-challenge, there was a strong

190   correlation between sgRNA in the upper and lower airways; however, the virus was more rapidly

191   cleared from the BAL compared to the nasal swab samples. Thus, there was a time-dependent

192   loss of concordance in the correlations with upper and lower airways samples (Fig. 3C-E),

193   suggesting distinct mechanisms for viral clearance in the two compartments.

194   ***mRNA-1273-vaccinated NHP have limited virus and inflammation in lungs***

195   Animals in each of the dose groups were assessed for detection of virus in the lung and

196   histopathology 7- or 8-days post SARS-CoV-2 challenge. In the control animals, SARS-CoV-2

197   infection caused moderate to severe inflammation that often involved the small airways and the

198   adjacent alveolar interstitium consistent with previous reports (*29-31*). Alveolar air spaces

199   occasionally contained inflammatory cell infiltrates, alveolar capillary septa were moderately

200   thickened, and moderate and diffuse type II pneumocyte hyperplasia was observed. Multiple

201   pneumocytes in the lung sections from the control group were positive for SARS-CoV-2 viral

202   antigen by immunohistochemistry (IHC) (Fig. S7, Table S1). Viral antigen was detected in both

203   control animals but only sporadically across vaccinated animals in various dose groups (Table

204   S1). These observations show that NHP develop mild inflammation in the lung over 1 week

205   following SARS-CoV-2 infection and that vaccination limits or completely prevents

206   inflammation or detection of viral antigen in the lung tissue.

207   ***Post-challenge anamnestic antibody responses are increased in low dose vaccine groups***

208   Following SARS-CoV-2 challenge, we assessed antibody responses in blood, BAL, and nasal

209   washes for up to 28 days to determine if there were anamnestic or primary responses to S or N

210   proteins, respectively (Fig. S8). This analysis provides a functional immune assessment of

211   whether the virus detected in the upper and lower airways by PCR following challenge is

212   sufficient to boost vaccine-induced S-specific antibody responses or elicit primary N responses.

213   In sera, there was no post-challenge increase in S-specific (Fig. S8A), RBD-specific (Fig. S8B),

214   or neutralizing antibodies (Fig. S8C) in the 3, 10, or 30 µg dose groups. In contrast, at doses

215   below 1 µg, there were increased primary S-specific (Fig. S8A), RBD-specific (Fig. S8B), and

216   neutralizing antibody responses (Fig. S8C) at day 28 post-challenge compared to pre-challenge.

217   Similar primary S-specific antibody response trends were also apparent with BAL and nasal

218   wash IgG and IgA responses (Fig. S9). Of note, in comparing pre-challenge N-specific IgG

219   responses to those post-challenge, we only observed seroconversion in the control animals and

220   animals immunized with <3 µg of mRNA-1273 (Fig. S8D).

221   The reduction of viral replication as determined by sgRNA coupled with limited pathology in the

222   lung and no detectable anamnestic S responses or induction of primary responses to N provide

223   three distinct measures suggesting that vaccine-elicited immune responses, particularly at high

224   doses, were protective. To understand this further, and to establish immune correlates of

225   protective immunity, we explored relationships between immune parameters and viral load.

226   ***Antibody responses correlate with protection against SARS-CoV-2 replication***

227   Prior to conducting study VRC-20-857.4 (Fig. S1C), we pre-specified that our analysis of a

228   potential correlate would focus primarily on the relationship between S-specific binding

229   antibodies and sgRNA levels in NS. Correlations with sgRNA levels in BAL served as an

230   important secondary analysis. The pre-defined primary hypothesis of the study was that S-

231   specific IgG at 4 weeks post-boost (pre-challenge) would inversely correlate with viral

232   replication in the NS at day 2 post-challenge and that vaccine dose may not be predictive of viral

233   replication after adjustment for S-specific IgG. The hypotheses were analogous for the

234   relationship between S-specific IgG at 4 weeks post-boost and day 2 BAL sgRNA.

235   S-specific IgG at week 8 correlated strongly with sgRNA in both the NS (p=0.001) (Fig. 4G,

236   Table S2) and BAL (p<0.001) (Fig. 4A, Table S2) at day 2. As shown in Table S2, a 1 $\log_{10}$

237   change in S-specific IgG corresponds to a 1 $\log_{10}$ change in sgRNA at day 2 in the NS, and a 0.9

238    $\log_{10}$ change in the sgRNA in the BAL at day 2. Once the S-specific IgG was included in a linear

239   model predicting sgRNA, including dose in the model did not substantially increase the adjusted

240   R^2, nor was the coefficient significant (p=0.115 for NS and p=0.214 for BAL). This suggests that

241   the effect of dose on day 2 sgRNA in NS and BAL is fully captured by the adjustment for S-

242   specific IgG and that, in this model, S-specific IgG meets our pre-specified criteria to be

243   considered as a correlate of sgRNA levels in NS and BAL.

244   As RBD-specific IgG, ACE2 binding inhibition, pseudovirus neutralization, and live virus

245   neutralization correlated with S-specific IgG (Fig. 2), analyses of these as potential correlates of

246   sgRNA were also planned. All six antibody measurements were highly correlated with each

247   other (Fig. 2), with vaccine dose (Fig. 1), and with sgRNA in BAL (Fig. 4AF) and NS (Fig. 4G-

248   L). As shown in Table S2A, for all six antibody measurements, dose was not significantly

249   predictive of sgRNA in the BAL after adjusting for antibody levels; for NS, dose remained

250   significantly predictive after adjusting for VSV-based pseudovirus neutralization and marginally

251   significant after adjusting for live virus neutralization. This suggests that in addition to S-specific

252   IgG, RBD-specific IgG, ACE2 binding inhibition, and lentiviral-based pseudovirus

253   neutralization meet our criteria for potential correlates of protection. Furthermore, lower and

254   upper airway S-specific antibodies in the BAL and NS negatively correlated with BAL (Fig.

255   S10A-B) and NS sgRNA levels, respectively (Fig. S10C-D).

256   To assess the robustness of these findings, these analyses were repeated using logistic regression

257   to model the probability that the sgRNA was below a threshold, defined as 10,000 sgRNA copies

258   for BAL and 100,000 sgRNA copies for NS. These thresholds were chosen to be below all of the

259   sgRNA values in the control animals and within the range of the values for the mRNA-1273-

260   vaccinated animals. The results of these analyses were similar to the primary analyses done on

261   the (log) linear models. In these data, no animal with S-specific IgG >336 IU/mL had BAL

262   sgRNA >10,000 copies/mL (Fig. 4A), and no animal with S-specific IgG >645 IU/mL had NS

263   sgRNA >100,000 copies/swab (Fig. 4G). Last, no animals with a S-binding binding titer of >488

264   IU/mL had higher N-specific primary antibody responses post-challenge above the background

265   value at the time of challenge; consistent with that, there was a strong negative correlation

266   between pre-challenge S-specific antibodies and post-challenge N-specific antibodies (Fig. 4M).

267   Additionally, there was limited to no lung pathology or viral antigen detection in animals with

268   <10,000 sgRNA copies/mL in BAL, providing additional evidence that mRNA-1273-vaccinated

269   animals were protected from lower airway disease.

270   We also examined the correlations between T cell responses and sgRNA and found that CD40L+

271   Tfh cells and any Th1 response were each univariately associated with reduced sgRNA in both

272   BAL and NS. After adjustment for S-specific IgG, none of these remained significantly

273   associated with sgRNA levels in the BAL, suggesting that these T cell measures do not predict

274   sgRNA independently of the binding antibody measured in BAL. However, IL-21+ Tfh,

275   CD40L+ Tfh, and any Th1 response remained significantly predictive of sgRNA levels in NS

276   (Table S2B) further confirming that clearance of virus from BAL and NS have distinct

277   immunological requirements (Fig. 3C-E).

278   ***Passively transferred mRNA-1273-induced IgG mediates protection against SARS-CoV-2***

279　　High titer antibody responses in blood and upper and lower airways associated with the rapid

280　　control of viral load and lower airway pathology in the lung suggested that antibody was the

281　　primary immunological mechanism of protection. To directly address whether vaccine-induced

282　　antibody was sufficient to mediate protection, mRNA-immune NHP IgG was purified from

283　　pooled sera 2 weeks post-boost of 100 μg of mRNA-1273 (*13*) (Fig. 5A) and passively

284　　transferred to hamsters (Fig. 5B). Two or 10 mg of total mRNA-1273-immune NHP IgG or 10

285　　mg of pre-immune NHP IgG (control) was administered to 8 Syrian hamsters/group, and

286　　immediately before challenge, humoral S-specific IgG (Fig. 5C) and pseudovirus neutralization

287　　titers (Fig. 5D) were assessed to confirm the relative antibody responses prior to the passive

288　　transfer. Of note, while there were different antibody responses based on the two different

289　　amounts of total IgG transferred, this study is only used for showing antibody is sufficient for

290　　protection and not for making any determinations of correlates. At 24 hr post-immunization,

291　　hamsters were inoculated with 3 x 10^4 PFU SARS-CoV-2 (USA-WA1/2020) intranasally.

292　　Control hamsters and hamsters that received 2 mg mRNA-1273-immune IgG showed a ~10%

293　　weight loss by day 6, a defined endpoint for pathogenicity in this model (*32*). By contrast,

294　　hamsters that received 10 mg mRNA-1273-immune NHP IgG showed little to no weight loss

295　　post-challenge (Fig. 5E). These data show that mRNA-1273 immune IgG is sufficient to mediate

296　　protection from disease *in vivo* against SARS-CoV-2 infection.

297

298

Discussion

Defining immune correlates of protection is a critical aspect of vaccine development for extending the use of approved vaccines and facilitating the development of new candidate vaccines, as well as defining potential mechanisms of protection. For SARS-CoV-2, a primary goal of current vaccines is to prevent symptomatic COVID-19. For moderate to severe disease this is likely a consequence of reducing viral load in lower airways and for mild disease reducing viral load in both lower and upper airways. An additional benefit of upper airway protection is that limiting nasal carriage of virus will also reduce transmission risk. Here, we establish that the level of S-specific antibody elicited by mRNA-1273 vaccination correlates with control of upper and lower airway viral replication following SARS-CoV-2 challenge in NHP. Furthermore, we find that CD4 T cell responses elicited by the vaccine did not provide any additional power to predict protection but were associated with reduction of viral load in NS. Finally, we show that vaccine-elicited antibodies are sufficient for protection against disease in hamsters. S-binding antibodies met our prespecified statistical analysis criteria for a correlate of protection as they strongly inversely correlated with lower and upper airway viral loads with no additional predictive power provided by vaccine dose.

A key parameter to assess correlates of protection in NHP is the amount of virus used for challenge. In this study, a challenge dose of 8×10^5 PFU of a newly characterized, pathogenic SARS-CoV-2 USA-WA1/2020 strain was used to achieve a level of viral replication comparable to or exceeding that observed from nasal swabs of humans with symptomatic infection, measured by sgRNA (*33*) or genomic vRNA (*34-36*). The sgRNA levels for N or E of 10^6-10^7 in control

320   animals at day 2 post-challenge are among the highest reported for NHP challenge studies and

321   likely models viral load in humans at the upper end of inoculum size. We used the same qualified

322   antibody binding and pseudovirus neutralization assays for assessing immune responses as in

323   human Phase 3 vaccine trials. Additionally, the use of WHO standards to report binding titers in

324   IU enables comparison of immune responses and outcomes with other NHP vaccine studies and

325   benchmarking to human vaccine clinical trials. We show that a 10-fold increase in S-binding

326   titers was associated with approximately 10-fold reductions in viral replication in BAL and NS

327   post-challenge. No animal with S-specific IgG >336 IU/mL had BAL sgRNA >10,000

328   copies/mL, and no animal with S-specific IgG >645 IU/mL had NS sgRNA >100,000

329   copies/swab, which were chosen as thresholds for protection. These reductions in viral

330   replication, assessed by sgRNA compared to controls, were associated with limited inflammation

331   and viral antigen detection in the lung tissue and may be sufficient to prevent moderate or severe

332   lower airway infection. Notably, even animals in the 1 and 3 μg dose groups, for which the

333   elicited S-specific antibody levels were 81 and 272 IU/mL and reciprocal pseudovirus

334   neutralization titers of 49 and 53, exhibited ~2-4 $\log_{10}$ less viral replication in BAL compared to

335   the control animals at day 2 post-challenge. Last, S-binding titers of >488 IU/mL were associated

336   with no increase in N-specific antibody responses post-challenge, a metric used in human studies

337   for assessing prevention of asymptomatic infection post-vaccination (*34*).

338   The antibody titers required in this high-dose challenge NHP model for a reduction in viral

339   replication may be a conservative estimate for what is required to prevent clinical disease in

340   humans. The strong correlation and proportional changes between S-specific binding titers with

341   serum neutralizing activity support using easy-to-measure binding titers as the primary metric for

342   defining a correlate of protection in humans at least for mRNA-based vaccines delivering similar

343   antigens and eliciting similar patterns of immunogenicity.

344   Mucosal antibody responses are thought to be an important mechanism of protection against a

345   variety of upper respiratory viral infections (*37-41*). Both BAL and nasal wash S-specific IgG

346   and IgA were predictive for reducing sgRNA in these compartments. Serum antibody levels were

347   a strong predictor of IgG and IgA responses in BAL and nasal washes as well as for protection

348   measured by viral replication in these sites. Given mRNA-1273 is administered via

349   intramuscular delivery, these data suggest that localized upper and lower airway antibodies are

350   transudated from serum and suggest serum antibody levels to be a surrogate for BAL and NS

351   antibody levels following mRNA-1273 vaccination.

352   Additionally, here we considered whether the infection boosted vaccine-induced antibodies.

353   There were no anamnestic S-specific responses or increase in N-specific responses in blood or

354   BAL within 28 days post infection in the >3 µg dose groups compared to pre-challenge,

355   consistent with these doses inducing higher antibody responses. By contrast, there were

356   increased anamnestic S-binding antibody responses in the <1 µg dose groups. These data suggest

357   that boosting of vaccine-induced antibodies can occur following upper airway infection in

358   animals that have minimal viral replication in the lower airway. Therefore, the necessity and

359   timing of subsequent vaccine boosting will depend on whether the goal is to prevent severe

360   disease and lower airway infection while allowing community exposure to provide mucosal

361   immunity from upper airway infection or to achieve sterilizing immunity through vaccination to

362   more rapidly reduce transmission.

363   Based on the rapid control of viral replication in the lower airway by day 2 post-challenge and

364   the presence of robust airway IgG responses, we hypothesized that antibodies are not only a

365   correlate but also the primary mechanism of protection. The critical role of antibodies for

366   mediating protection is also consistent with multiple human cohort studies assessing antibody

367   responses in people after prior exposure showing that subsequent infection is reduced (*42-45*).

368   The level of serum neutralizing activity has also been suggested as a predictor of efficacy from

369   COVID-19 (*45*). The demonstration here that purified mRNA-1273 immune NHP IgG is

370   associated with dose-dependent protection from weight loss in the highly pathogenic hamster

371   model provides direct evidence that vaccine-elicited antibody is sufficient to mediate protection

372   from disease.

373   To conclude, this study establishes a critical role of functional antibodies as a correlate of

374   protection against SARS-CoV-2 in a NHP and that for mRNA expressing this particular spike

375   antigen, binding antibody is a surrogate marker of protection. Furthermore, this study establishes

376   that a lower antibody level is needed for reduction of viral replication in the lower airway than in

377   the upper airway. Ongoing NHP studies will assess durability of mRNA-1273-elicited protection

378   and efficacy of mRNA-1273 vaccination against global SARS-CoV-2 variants. These findings

379   anticipate the correlates analysis comparing virus replication in NS with serum antibody being

380   performed on samples from vaccinated subjects in Phase 3 clinical trials who experienced

381   breakthrough infection.

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509

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541   S.B-B., K.L., W.S., E.S.Y., Y.Z., and L.W. provided critical published reagents/analytic tools.

542   K.S.C., M.C.N., N.J.S., M.R., B.S.G, and R.A.S. wrote the manuscript. K.S.C., M.C.N. M.G.,

543   and G.A. prepared figures and tables. All authors contributed to discussions about and editing of

544   the manuscript.

545   **Competing Interests:** K.S.C. and B.S.G. are inventors on U.S. Patent No. 10,960,070 B2 and

546   International Patent Application No. WO/2018/081318 entitled "Prefusion Coronavirus Spike

547   Proteins and Their Use." K.S.C., O.M.A., and B.S.G. are inventors on US Patent Application No.

548   62/972,886 entitled "2019-nCoV Vaccine".

549   **Data and materials availability:** All data are available in the main text or the supplementary

550   materials.
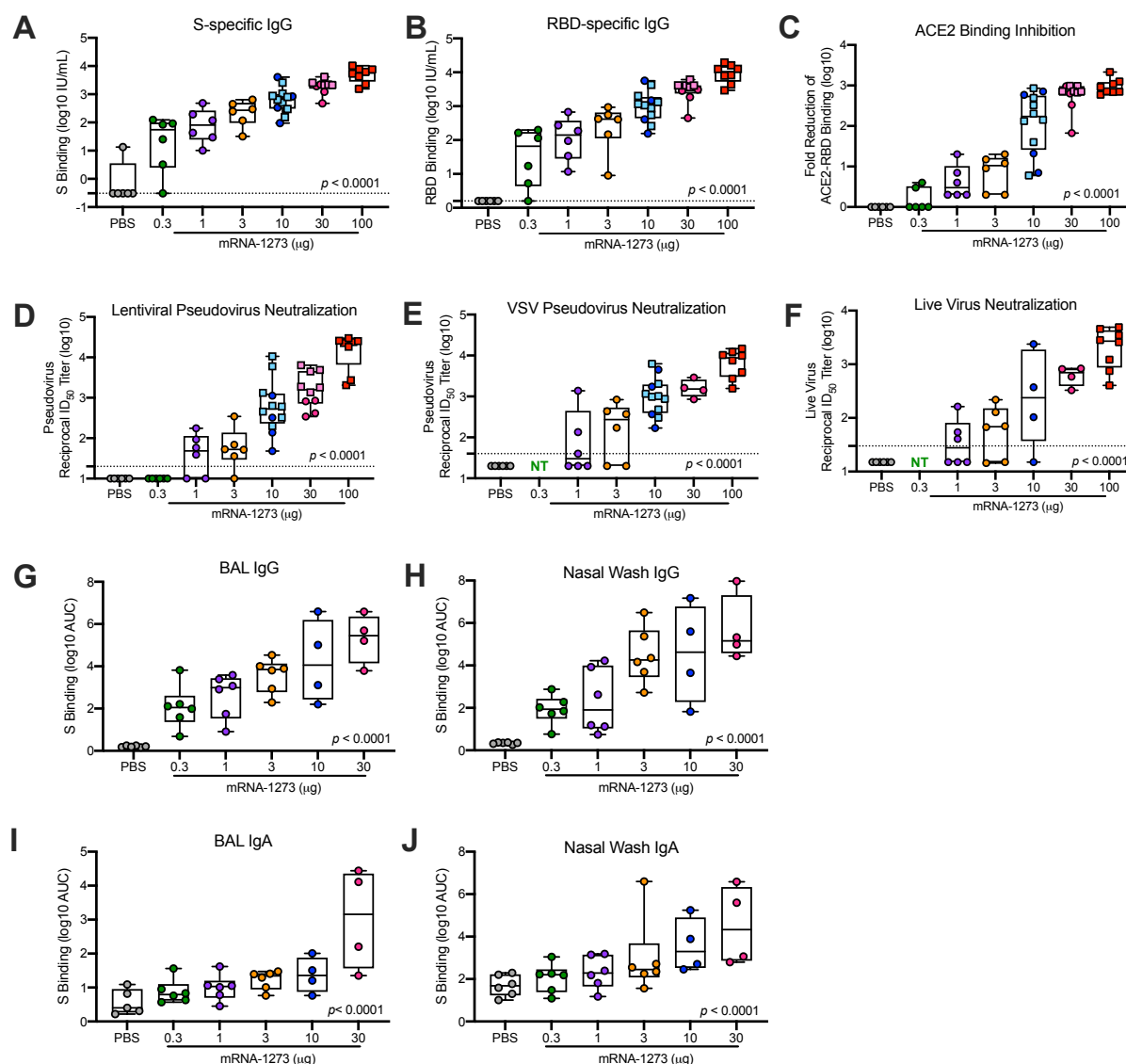
Fig. 1. Antibody responses following mRNA-1273 immunization. Rhesus macaques were immunized according to Fig. S1 with PBS (gray) or mRNA-1273 (0.3 µg – green, 1 µg – purple, 3 µg – orange, 10 µg – blue, 30 µg – pink, or 100 µg – red). Sera collected 4 weeks post-boost, immediately before challenge, were assessed for SARS-CoV-2 S-specific (A) and RBD-specific (B) IgG by MULTI-ARRAY ELISA, inhibition of ACE2 binding to RBD (C), SARS-CoV-2 lentiviral-based pseudovirus neutralization (D), SARS-CoV-2 VSV-based pseudovirus

neutralization (E), and SARS-CoV-2 EHC-83E focus reduction neutralization (F). BAL (G, I) and nasal washes (H, J) collected 2 weeks post-boost were assessed for SARS-CoV-2 S-specific IgG (A-B) and IgA (C-D) by MULTI-ARRAY ELISA. Squares represent NHP in previous experiments (S1A, VRC-20-857.1 and S1B, VRC-20-857.2); circles represent individual NHP in experiment S1C, VRC-20-857.4. Boxes and horizontal bars denote the IQR and medians, respectively; whisker end points are equal to the maximum and minimum values. Dotted lines indicate assay limits of detection, where applicable. NT = not tested. All measures were significantly correlated with dose ($p < 0.0001$), as determined by a test of Spearman's correlation.
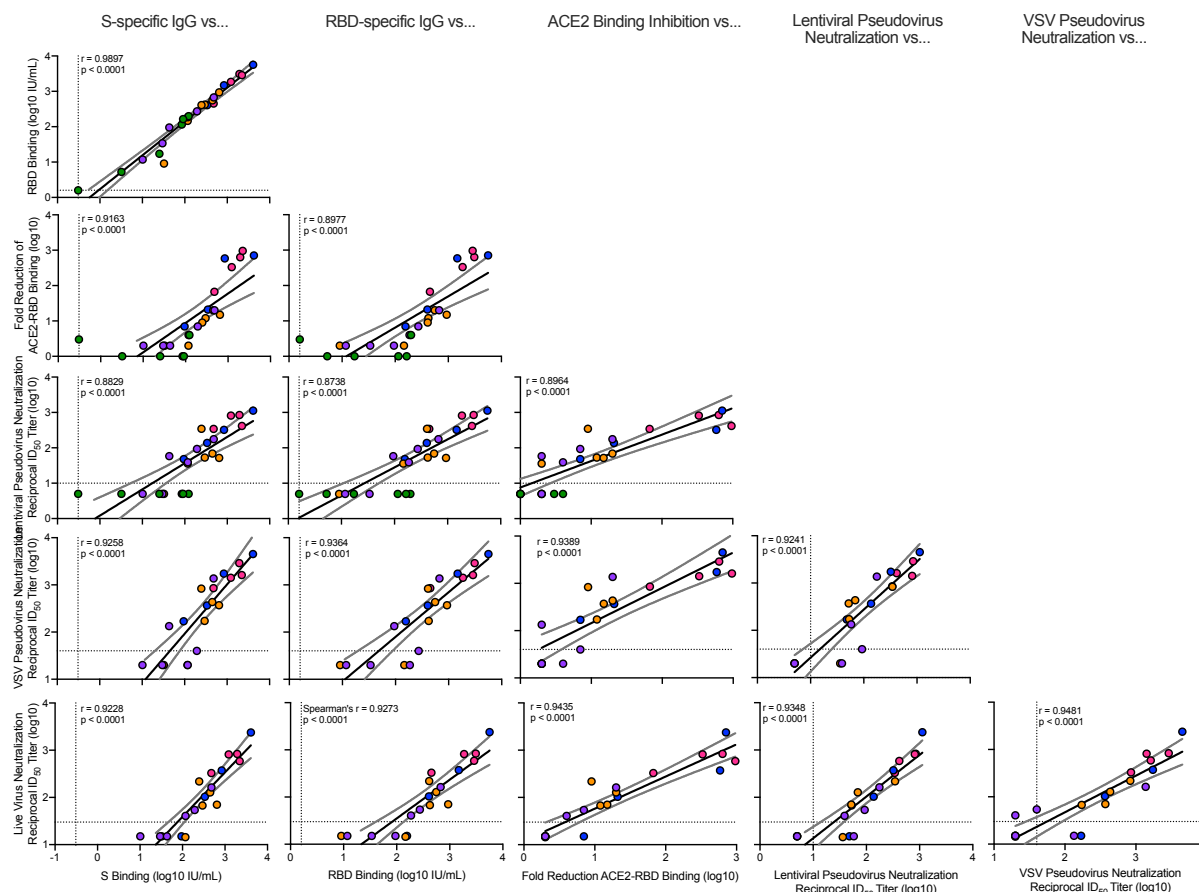
Fig. 2. **Correlations of humoral antibody analyses.** Rhesus macaques were immunized according to Fig. S1C. Plots show correlations between SARS-CoV-2 S-specific IgG, RBD-specific IgG, ACE2 binding inhibition, lentiviral-based pseudovirus neutralization, VSV-based pseudovirus neutralization, and EHC-83E focus reduction neutralization at 4 weeks post-boost. Circles represent individual NHP, where colors indicate mRNA-1273 dose as defined in Fig. S1C. Dotted lines indicate assay limits of detection. Black and gray lines indicate linear regression and 95% confidence interval, respectively. 'r' represents Spearman's correlation coefficients, and 'p' the corresponding p-values.
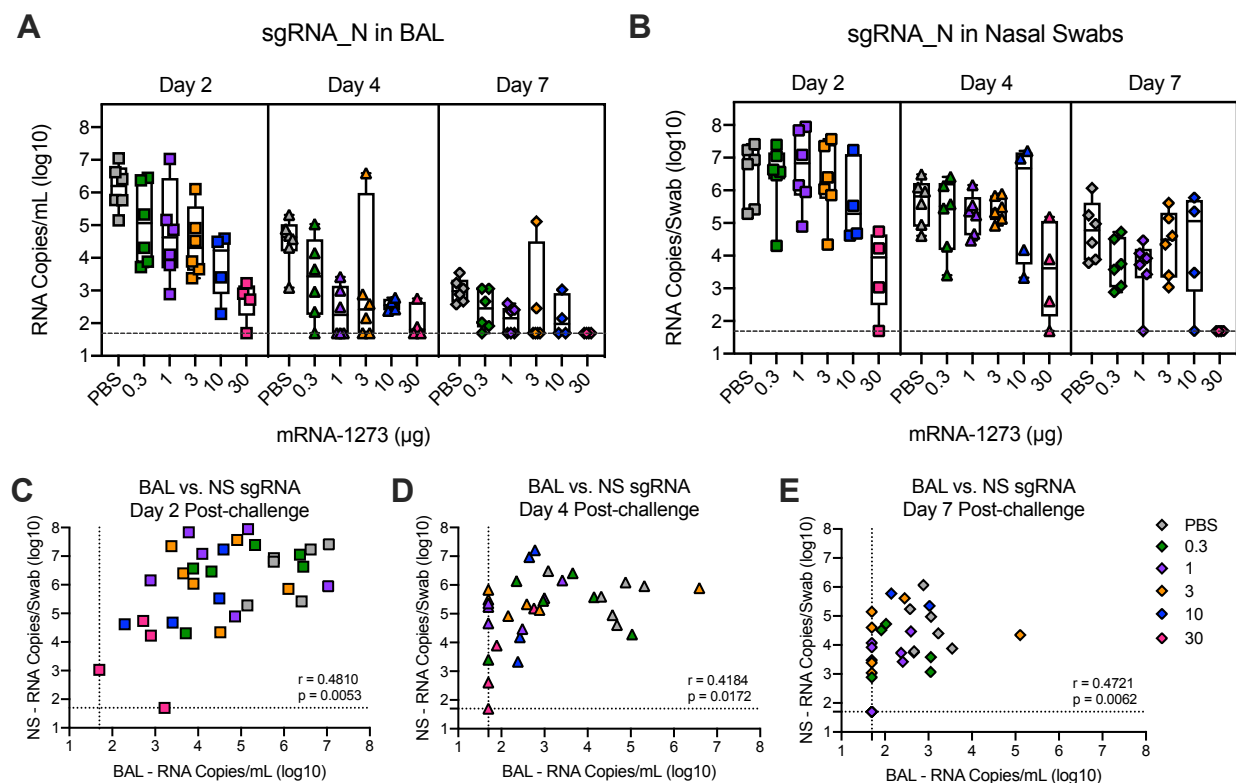
Fig. 3. Efficacy of mRNA-1273 against upper and lower respiratory viral replication. Rhesus macaques were immunized and challenged as described in Fig. S1C. BAL (A) and nasal swabs (NS) (B) were collected on days 2 (squares), 4 (triangles), and 7 (diamonds) post-challenge, and viral replication was assessed by detection of SARS-CoV-2 N-specific sgRNA. (A-B) Boxes and horizontal bars denote the IQR and medians, respectively; whisker end points are equal to the maximum and minimum values. (C-E) Correlations shown between BAL and NS sgRNA at days 2 (C), 4 (D), and 7 (E) post-challenge are Spearman's correlation coefficients (r) and corresponding p-values. Symbols represent individual NHP and may overlap, ie. n=6 animals plotted at assay limit (dotted line) for both BAL and NS in (E).
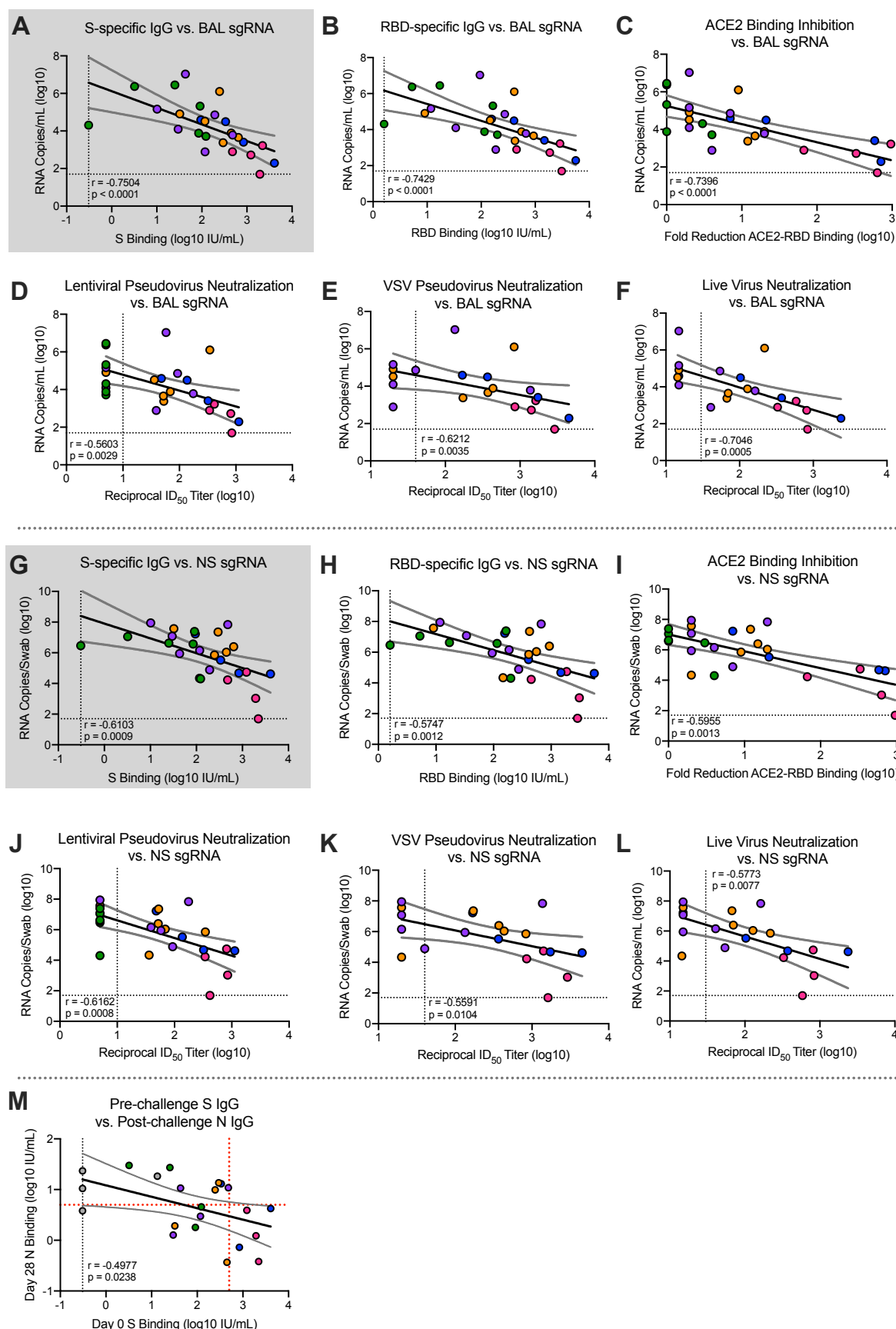
Fig. 4. **Antibody correlates of protection.** Rhesus macaques were immunized and challenged as described in Fig. S1C. Plots show correlations between SARS-CoV-2 N-specific sgRNA in BAL (A-F) and NS (G-L) at day 2 post-challenge and pre-challenge (week 4 post-boost) SARS-CoV-2 S-specific IgG (A, G), RBD-specific IgG (B, H), ACE2 binding inhibition (C, I), SARS-CoV-2 lentiviral-based pseudovirus neutralization (D, J), SARS-CoV-2 VSV-based pseudovirus neutralization (E, K) and SARS-CoV-2 EHC-83E focus reduction neutralization (F, L). Gray shading for S-specific IgG represents the use of this assessment as primary predictor of protection outcome as stated in primary hypothesis. (M) Plot shows correlation between pre-challenge (week 4 post-boost) SARS-CoV-2 S-specific IgG with day 28 post-challenge SARS-CoV-2 N-specific IgG. Circles represent individual NHP, where colors indicate mRNA-1273 dose. Dotted lines indicate assay limits of detection. Black and gray lines indicate linear regression and 95% confidence interval, respectively. In (M), red dotted horizontal line represents 6, the maximum of all pre-challenge values across all groups, and the red dotted vertical line represents a reciprocal S-specific IgG titer of 500, above which none of the animals had day 28 N Binding titers above 6. 'r' represents Spearman's correlation coefficient, and 'p' the corresponding p-value.
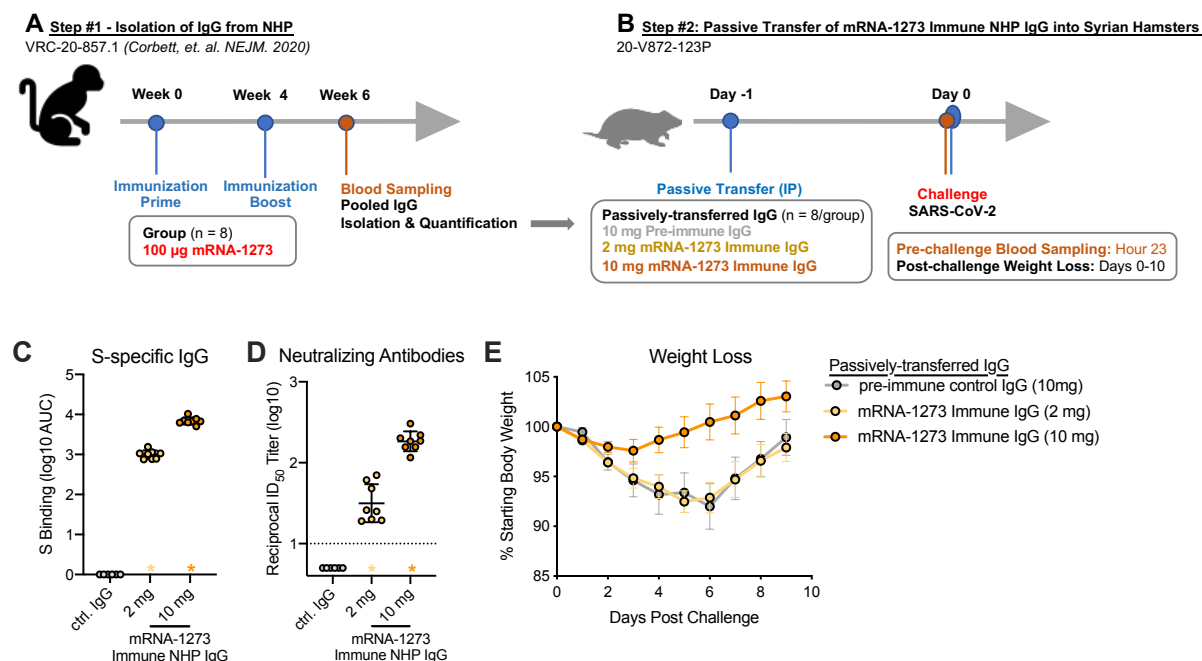
Fig. 5. Passive transfer of mRNA-1273 immune NHP IgG into Syrian hamsters. (A) Sera were pooled from all NHP that received 100 µg of mRNA-1273 in a primary vaccination series. (B) mRNA-1273 immune NHP IgG (2 mg, yellow or 10 mg, orange) or pre-immune NHP IgG (10 mg, gray) was passively transferred to Syrian hamsters (n = 8/group) 24 hours prior to SARS-CoV-2 challenge. Twenty-three hours post-immunization, hamsters were bled to quantify circulating S-specific IgG (C) and SARS-CoV-2 pseudovirus neutralizing antibodies (D). Following challenge, hamsters were monitored for weight loss (E). (C-D) Circles represent individual NHP. Bars and error bars represent GMT and geometric SD, respectively. Asterisks at the axis represent animals that did not receive adequate IgG via passive transfer and were thus excluded from weight loss analyses. (D) The dotted line indicates the neutralization assay limit of detection. (E) Circle and error bars represent mean and SEM, respectively.