

1 **Protective activity of mRNA vaccines against ancestral and variant SARS-CoV-2**  
2 **strains**

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24 **SUMMARY (150 words)**

25         Although mRNA vaccines prevent COVID-19, variants jeopardize their efficacy as  
26         immunity wanes. Here, we assessed the immunogenicity and protective activity of historical  
27         (mRNA-1273, designed for Wuhan-1 spike) or modified (mRNA-1273.351, designed for B.1.351  
28         spike) preclinical Moderna mRNA vaccines in 129S2 and K18-hACE2 mice. Immunization with  
29         high or low dose formulations of mRNA vaccines induced neutralizing antibodies in serum  
30         against ancestral SARS-CoV-2 and several variants, although levels were lower particularly  
31         against the B.1.617.2 (Delta) virus. Protection against weight loss and lung pathology was  
32         observed with all high-dose vaccines against all viruses. Nonetheless, low-dose formulations of  
33         the vaccines, which produced lower magnitude antibody and T cell responses, and serve as a  
34         possible model for waning immunity, showed breakthrough lung infection and pneumonia with  
35         B.1.617.2. Thus, as levels of immunity induced by mRNA vaccines decline, breakthrough  
36         infection and disease likely will occur with some SARS-CoV-2 variants, suggesting a need for  
37         additional booster regimens.

38 **INTRODUCTION**

39 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the  
40 Coronavirus Disease 2019 (COVID-19) syndrome. More than 211 million infections and 4.4  
41 million deaths have been recorded worldwide (<https://covid19.who.int>) since the start of the  
42 pandemic. The extensive morbidity and mortality associated with the COVID-19 pandemic made  
43 the development of SARS-CoV-2 vaccines a global health priority. In a short period of less than  
44 one year, several highly effective vaccines targeting the SARS-CoV-2 spike protein  
45 encompassing multiple platforms (lipid nanoparticle encapsulated mRNA, inactivated virion, or  
46 viral-vectored vaccine platforms (Graham, 2020)) gained Emergency Use Authorization or Food  
47 and Drug Administration approval and were deployed with hundreds of millions of doses given  
48 worldwide (<https://covid19.who.int>). The currently used vaccines all were designed against the  
49 spike protein of strains that were circulating early in the pandemic. In localities with high rates of  
50 vaccination, markedly reduced numbers of infections, hospitalizations, and deaths were initially  
51 observed.

52 Despite the success of COVID-19 vaccines and their potential for curtailing the  
53 pandemic, the continued evolution of more transmissible SARS-CoV-2 variants of concern  
54 (VOC) including B.1.1.7 (Alpha), B.1.351 (Beta), B.1.1.28 (Gamma), and B.1.617.2 (Delta) with  
55 substitutions in the spike protein jeopardizes the efficacy of vaccination campaigns (Krause et  
56 al., 2021). Experiments in cell culture suggest that neutralization by vaccine-induced sera is  
57 diminished against variants expressing mutations in the spike gene at positions L452, E484,  
58 and elsewhere (Chen et al., 2021b; McCallum et al., 2021a; Tada et al., 2021; Wang et al.,  
59 2021a; Wang et al., 2021b; Wibmer et al., 2021). Moreover, viral-vectored (ChAdOx1 nCoV-19  
60 and Ad26.Cov2) and protein nanoparticle (NVX-CoV2373)-based vaccines showed reduced  
61 activity (10 to 60%) against symptomatic infection caused by the B.1.351 variant in clinical trials  
62 in humans (Madhi et al., 2021; Sadoff et al., 2021; Shinde et al., 2021), whereas mRNA-based

63 vaccines (e.g., BNT162b2) retained substantial (~75%) efficacy against the B.1.351 variant in  
64 humans with almost complete protection against severe disease (Abu-Raddad et al., 2021).

65 Immunization of humans with two 100 µg doses of the lipid nanoparticle-encapsulated  
66 mRNA-1273 vaccine encoding a proline-stabilized full-length SARS-CoV-2 spike glycoprotein  
67 corresponding to the historical Wuhan-Hu-1 virus conferred 94% efficacy against symptomatic  
68 COVID-19 in clinical trials performed in the United States (Baden et al., 2021). More recent data  
69 in non-human primates shows that vaccination with two doses of mRNA-1273 results in an  
70 effective immune response that controls upper and lower respiratory tract infection after  
71 challenge with the SARS-CoV-2 B.1.351 viral variant (Corbett et al., 2021). As an alternative  
72 approach, several manufacturers have designed modified vaccines that target specific VOC  
73 including B.1.351 for possible immunization or boosting. Indeed, a mRNA-1273.351 vaccine  
74 recently was generated, which encodes a proline stabilized full-length SARS-CoV-2 spike  
75 glycoprotein from the B.1.351 virus. Here, we evaluated the immunogenicity and protective  
76 activity of lipid-encapsulated mRNA-1273 and mRNA-1273.351 Moderna vaccines in the  
77 context of challenge of wild-type 129S2 and human ACE2 (hACE2) transgenic (K18-hACE2)  
78 mice with historical and emerging SARS-CoV-2 strains including several key VOC.

79 **RESULTS**

80 **Immunogenicity of mRNA vaccines in 129S2 mice.** We first tested preclinical  
81 versions of the Moderna mRNA-1273 and mRNA-1273.351 vaccines encoding sequenced-  
82 optimized prefusion-stabilized spike proteins of Wuhan-1 and B.1.351, respectively, in  
83 immunocompetent 129S2 mice. These animals are permissive to infection by some SARS-CoV-  
84 2 variants (e.g., B.1.1.7, B.1.1.28, and B.1.351) or mouse-adapted strains (Chen et al., 2021a;  
85 Gu et al., 2020; Rathnasinghe et al., 2021) that encode an N501Y mutation, which enables  
86 engagement of endogenous murine ACE2 (Liu et al., 2021b). Infection of 129S2 mice with  
87 SARS-CoV-2 results in mild to moderate lung infection and clinical disease with subsequent  
88 recovery (Chen et al., 2021a; Rathnasinghe et al., 2021). To assess the immunogenicity of the  
89 vaccines, groups of 7 to 9-week-old female 129S2 mice were immunized and boosted three  
90 weeks later by an intramuscular route with 5  $\mu$ g (high) or 0.25  $\mu$ g (low) doses of mRNA-1273,  
91 mRNA-1273.351, mRNA-1273.211 (1:1 mixture [total 5 or 0.25  $\mu$ g] of mRNA-1273 and mRNA-  
92 1273.351), or a control non-coding mRNA (**Fig 1A**); we included the mRNA-1273.211 mixture  
93 since it is being tested in humans (NCT04927065 (Wu et al., 2021)). Serum samples were  
94 collected three weeks after boosting, and IgG responses against recombinant spike proteins of  
95 ancestral (Wuhan-1) or variant (B.1.1.7, B.1.351, or B.1.617.2) viruses (Amanat et al., 2021)  
96 were evaluated by ELISA (**Fig 1B**). As expected, the control mRNA did not generate spike-  
97 specific IgG (values below the limit of detection), whereas antibody responses against the spike  
98 proteins from all other mRNA vaccines were robust. For the 5  $\mu$ g dose, mean endpoint titers of  
99 serum ranged from 619,650 to 1,503,560 against the different spike proteins with little variation  
100 between the mRNA vaccines. For the 0.25  $\mu$ g dose, approximately 5-fold lower serum IgG  
101 responses were observed with mean endpoint titers ranging from 126,900 to 382,725, again  
102 with little difference between the mRNA vaccines. The responses trended slightly higher against  
103 the ancestral spike protein and lower against the B.1.617.2 spike protein although most of these

104 differences did not attain statistical significance. Overall, both doses and all spike-based vaccine  
105 formulations generated strong anti-spike protein IgG responses in 129S2 mice.

106 We characterized serum antibody responses functionally by assaying inhibition of  
107 SARS-CoV-2 infectivity using a focus-reduction neutralization test (FRNT) (Case et al., 2020b).  
108 We tested a panel of sera from each group of vaccinated mice against several fully-infectious  
109 SARS-CoV-2 strains including an ancestral Washington strain with a single D614G substitution  
110 (WA1/2020 D614G) or one with both D614G and N501Y substitutions (WA1/2020  
111 D614G/N501Y), a B.1.1.7 isolate encoding an E484K mutation (B.1.1.7/E484K), a B.1.351  
112 isolate, and a B.1.617.2 isolate (**Fig 1C-L**). Due to the limited amount of sera recovered from  
113 live animals, we started dilutions at 1/180. As expected, serum from all control mRNA-  
114 immunized mice did not inhibit infection of the SARS-CoV-2 strains (**Fig 1C-L**). For the 5  $\mu$ g  
115 dose, all three spike gene vaccines (mRNA-1273, mRNA-1273.351, and mRNA-1273.211)  
116 induced robust serum neutralizing antibody responses (**Fig 1C-G**). In general, these titers were  
117 similar with the exception of ~4-fold lower geometric mean titers (GMTs) against WA1/2020  
118 D614G and ~2-fold higher GMTs against B.1.351 induced by mRNA-1273.351 compared to the  
119 mRNA-1273 and mRNA-1273.211 vaccines. Lower neutralizing responses (~4- to 5-fold) were  
120 seen against the B.1.617.2 strain by all three mRNA vaccines (**Fig 1G**). For the 0.25  $\mu$ g vaccine  
121 dose, we observed ~10-fold lower levels of serum neutralizing activity against each of the  
122 viruses (**Fig 1H-L**) and noted the following trends: (a) the mRNA-1273.351 vaccine induced  
123 lower levels of neutralizing antibody against WA1/2020 D614G and WA1/2020 D614G/N501Y  
124 than the mRNA-1273 vaccine (**Fig 1H-I**); (b) the mRNA-1273.211 mixture generally induced  
125 neutralizing antibodies that were equivalent to one of the two vaccine components; (c) serum  
126 from mRNA-1273-vaccinated mice showed smaller reductions in neutralization against B.1.351  
127 than anticipated based on prior studies in humans and C57BL6 mice (Chen et al., 2021b; Wang  
128 et al., 2021a) (**Fig 1J**); (d) serum from mRNA-1273.351-vaccinated mice trended toward higher

129 neutralization against B.1.351; and (e) serum neutralizing antibody levels from all vaccinated  
130 mice were lower against B.1.617.2 than other strains, although responses from animals  
131 administered mRNA-1273 were somewhat higher (**Fig 1L**). Overall, these differences were  
132 visualized best in a comparative analysis of the inhibitory activity of each serum sample for the 5  
133  $\mu$ g (**Fig S1A-C**) and 0.25  $\mu$ g (**Fig S1D-F**) doses.

134         Using the neutralization data from mRNA vaccinated 129S2 mice, we created antigenic  
135 maps to visualize the relationships between the WA1/2020 D614G, WA1/2020 D614G/N501Y,  
136 B.1.1.7/E484K, B.1.351, and B.1.617.2 SARS-CoV-2 strains (**Fig 1M-N**). Neutralization titers  
137 obtained after 5 or 0.25  $\mu$ g dosing with mRNA-1273 and mRNA-1273.351 vaccines were used  
138 to position the serum relative to each virus using antigenic cartography (a modification of  
139 multidimensional scaling for binding assay data), such that higher neutralization titers are  
140 represented by shorter distances between serum and the virus. Each gridline, or antigenic unit,  
141 of the map corresponds to a 2-fold difference in neutralization titer of a given virus. Three  
142 antigen clusters were observed: (a) WA1/2020 D614G and WA1/2020 D614G/N501Y grouped  
143 together; (b) viruses containing E484K mutations (B.1.1.7/E484K and B.1.351) had a similar  
144 antigenic position; and (c) B.1.617.2 was the most distant antigenically, which is consistent with  
145 the lower levels of serum neutralization induced by all of the mRNA vaccines against this VOC.

146         **Protection by mRNA vaccines in 129S2 mice.** We tested the protective activity of the  
147 different mRNA vaccines in 129S2 mice. Three weeks after boosting, mice were challenged via  
148 an intranasal route with WA1/2020 N501Y/D614G, B.1.1.7/E484K, or B.1.351. The WA1/2020  
149 D614G and B.1.617.2 viruses were not used for challenge in this model since they lack the  
150 mouse-adapting N501Y substitution and cannot infect conventional laboratory mice (Gu et al.,  
151 2020). Compared to the control mRNA vaccine, the 5  $\mu$ g or 0.25  $\mu$ g doses of mRNA-1273,  
152 mRNA-1273.351, or mRNA-1273.211 vaccines all prevented weight loss between 2 and 4 dpi,

153 although protection was not statistically significant for some groups immunized with the mRNA-  
154 1273 vaccine and challenged with B.1.351 or B.1.1.7/E484K viruses (**Fig 2A-B**).

155 At 4 days post-infection (dpi), mice were euthanized, and nasal washes, lungs, and  
156 spleen were collected for viral burden analysis. In the nasal washes or lungs from control  
157 mRNA-vaccinated 129S2 mice, high levels ( $\sim 10^7$  copies of *N* per mL or mg) of viral RNA were  
158 measured after challenge with WA1/2020 N501Y/D614G, B.1.1.7/E484K, or B.1.351 (**Fig 2C-**  
159 **D**). Lower levels of SARS-CoV-2 RNA ( $\sim 10^2$  to  $10^4$  copies of *N* per mg) were measured in the  
160 spleen (**Fig S2A**). In general, the mRNA-1273, mRNA-1273.351, and the mRNA-1273.211  
161 vaccines conferred robust protection against infection in nasal washes, lungs, and spleens by  
162 the challenge SARS-CoV-2 strains, although some breakthrough was noted. After the 5  $\mu$ g dose  
163 immunization with mRNA-1273, moderate B.1.1.7/E484K infection was detected in nasal  
164 washes in 5 of 8 mice, although viral RNA was absent from the lungs. Some (3 of 8) mice  
165 immunized with the mRNA-1273.211 mixture also showed breakthrough in the lungs, albeit at  
166 greater than 100-fold lower levels than the control vaccine. In comparison, the 5  $\mu$ g dose of  
167 mRNA-1273.351 was protective in the nasal wash and lungs against all viruses, with little, if  
168 any, viral RNA measured.

169 As expected, the 0.25  $\mu$ g dose of the mRNA vaccines showed less protective efficacy  
170 against SARS-CoV-2 challenge. With few exceptions (2 mice, mRNA-1273.351), the protection  
171 conferred by the 0.25  $\mu$ g dose against WA1/2020 N501Y/D614G and B.1.1.7/E484K challenge  
172 was strong in the nasal washes at 4 dpi (**Fig 2C**). In comparison, after B.1.351 challenge, 8 of 8  
173 mice immunized with mRNA-1273 showed viral RNA in nasal washes, with 3 of 8 showing  
174 levels that approached those seen in control-vaccinated mice. Greater protection was generated  
175 against B.1.351 by mRNA-1273.351 or the mRNA-1273.211 mixture vaccine, although  
176 breakthrough infections were detected. In the lungs, strong protection against infection with  
177 WA1/2020 N501Y/D614G was generated by all three spike mRNA vaccines (**Fig 2D**). However,

178 some infection was seen after B.1.1.7/E484K or B.1.351 challenge. For example, 6 of 8 mice  
179 immunized with mRNA-1273 had moderate to high levels of B.1.351 viral RNA in their lungs at 4  
180 dpi.

181 We assessed for correlations between vaccine-induced neutralizing antibody titers and  
182 protection against SARS-CoV-2 infection in the lung after virus challenge. Serum levels of  
183 neutralizing antibody were associated inversely with SARS-CoV-2 RNA levels in the lung (**Fig**  
184 **2E**) with a minimum neutralizing titer of approximately 5,000 required to prevent infection in the  
185 lung at 4 dpi. Most of the breakthrough infections occurred with the B.1.351 challenge at the  
186 0.25 µg dose of vaccines. For reasons that remains unclear, the threshold for complete  
187 protection in the lung after challenge with WA1/2020 N501Y/D614G was lower (2 to 7-fold) than  
188 against the other viruses. Moreover, when we compared body weight change at 4 dpi with  
189 neutralizing titers, only animals challenged with B.1.351 showed a linear correlation (**Fig S2B**),  
190 possibly because of the greater number of breakthrough infections in this group.

191 We also assessed the effect of the mRNA vaccines on lung disease at 4 dpi in 129S2  
192 mice. For these studies, we analyzed lung sections from the group of mice that received the  
193 lower 0.25 µg vaccine dose and the B.1.351 challenge virus, as this combination resulted in the  
194 greatest number of breakthrough infections. As expected, mice immunized with the control  
195 mRNA vaccine and challenged with B.1.351 developed mild pneumonia characterized by  
196 immune cell accumulation in perivascular and alveolar locations, vascular congestion, and  
197 interstitial edema. In contrast, animals immunized with mRNA-1273, mRNA-1273.351, or  
198 mRNA-1273.211 vaccines did not show these pathological changes (**Fig 3**). Thus, immunization  
199 with even the low dose of the mRNA vaccines was sufficient to mitigate SARS-CoV-2-induced  
200 lung injury in immunocompetent 129S2 mice challenged with some VOC.

201 **Immunogenicity of mRNA vaccines in K18-hACE2 transgenic mice.** We next  
202 evaluated the mRNA-1273 and mRNA-1273.351 vaccines in K18-hACE2 transgenic mice,

203 which sustain higher levels of infection and disease after intranasal inoculation by many SARS-  
204 CoV-2 strains (Winkler et al., 2020) including isolates containing or lacking mouse-adapting  
205 mutations (e.g., N501Y) (Chen et al., 2021a). Due to a limited availability of K18-hACE2 mice  
206 and the need to test two control viruses (WA1/2021 D614G and WA1/2021 D614G/N501Y), we  
207 tested mRNA-1273 and mRNA-1273.351 but not the mRNA-1273.211 mixture vaccine. Groups  
208 of 7-week-old female K18-hACE2 mice were immunized and boosted three weeks later by  
209 intramuscular route with 5 or 0.25 µg doses of mRNA-1273, mRNA-1273.351, or control mRNA  
210 vaccine (**Fig 4A**). Serum samples were collected three weeks after boosting, and IgG  
211 responses against recombinant spike proteins (Wuhan-1, B.1.1.7, B.1.351, or B.1.617.2) were  
212 evaluated by ELISA (**Fig 4B**). Antibody responses against the different spike proteins were  
213 robust although slightly lower (~2 to 3-fold) than that seen in 129S2 mice (**Fig 1B**). Serum mean  
214 endpoint IgG titers ranged from 218,700 to 1,601,425 against the different spike proteins with  
215 little variation observed with the 5 µg doses of different mRNA vaccines. For the 0.25 µg dose,  
216 lower (~6 to 10-fold) IgG titers were measured (24,300 to 101,250) with little difference between  
217 the mRNA-1273 and mRNA-1273.351 vaccines. Although the IgG levels against the B.1.617.2  
218 spike protein were reduced slightly compared to the other SARS-CoV-2 spike proteins, in  
219 general, robust antibody responses were detected in K18-hACE2 mice.

220 We performed FRNTs to assess the neutralizing activity of pre-challenge serum against  
221 WA1/2020 D614G, WA1/2020 D614G/N501Y, B.1.1.7/E484K, B.1.351, and B.1.617.2 SARS-  
222 CoV-2 strains. Because of the limited amount of sera recovered from K18-hACE2 mice, we  
223 initially started dilutions at 1/180. As expected, serum from all control mRNA-immunized mice  
224 did not inhibit infection of the SARS-CoV-2 strains (**Fig 4C-L**). In general, neutralizing antibody  
225 titers induced by 5 or 0.25 µg mRNA vaccine dosing trended lower (~ 3 to 6-fold) in immunized  
226 K18-hACE2 than from 129S2 mice. For the 5 µg dose, while both mRNA-1273 and mRNA-  
227 1273.351 vaccines induced robust serum neutralizing antibody responses, we observed the

228 following (**Fig 4C-G and Fig S3**): (a) the mRNA-1273.351 vaccine induced lower levels of  
229 neutralizing antibody against WA1/2020 D614G and WA1/2020 D614G/N501Y than the mRNA-  
230 1273 vaccine (**Fig 4C and D**); (b) a reciprocal pattern was observed against viruses containing  
231 E484K mutations. The mRNA-1273.351 vaccine induced higher levels of neutralizing antibody  
232 against B.1.1.7/E484K and B.1.351 than the mRNA-1273 vaccine (**Fig 4E and F**); and (c) no  
233 differences in neutralizing activity were observed with the mRNA-1273 and mRNA-1273.351  
234 vaccines against the B.1.617.2 strain. Although responses were elevated, they were lower than  
235 against other strains (**Fig 4G**). Similar patterns were observed for the 0.25  $\mu$ g dose (**Fig 4H-L**),  
236 although ~10-fold lower levels of neutralizing activity were induced by each vaccine against  
237 each of the viruses. Because of this, we started our dilution series at 1/60 for sera derived from  
238 animals immunized with the 0.25  $\mu$ g dose of mRNA vaccines. In general, the pattern of  
239 neutralization paralleled results with the higher dose, with the mRNA-1273 vaccine performing  
240 better against historical WA1/2020 viruses and the mRNA-1273.351 vaccine showing greater  
241 inhibitory titers against B.1.351 (**Fig 4H, I and K**). However, serum from mice vaccinated with  
242 mRNA-1273 or mRNA-1273.351 vaccines neutralized B.1.617.2 less efficiently (**Fig 4L**), with  
243 several data points at the limit of detection (1/60: mRNA-1273, 4 of 24; mRNA-1273.351, 9 of  
244 24) and responses induced by mRNA-1273 trending higher. A comparative analysis of the  
245 inhibitory activity of each serum sample for the 5  $\mu$ g (**Fig S3A-B**) and 0.25  $\mu$ g (**Fig SC-D**) doses  
246 visually showed these differences, as serum induced by the mRNA-1273 vaccine consistently  
247 showed less neutralizing activity against B.1.1.7/E484K, B.1.351, and B.1.617.2, whereas  
248 serum from mRNA-1273.351-vaccinated mice had greater inhibitory activity against B.1.351 and  
249 B.1.1.7/E484K.

250 We used the neutralization data from mRNA-vaccinated K18-hACE2 mice to generate  
251 maps defining the antigenic relationships between WA1/2020 D614G, WA1/2020  
252 D614G/N501Y, B.1.1.7/E484K, B.1.351, and B.1.617.2 SARS-CoV-2 strains (**Fig 4M-N**). Serum

253 obtained after 5 or 0.25 µg dosing with mRNA-1273 or mRNA-1273.351 vaccines was analyzed  
254 against the indicated viruses, and each antigenic unit corresponded to a 2-fold difference in  
255 neutralization titer of a given virus. The results were remarkably similar to that seen with 129S2  
256 vaccinated mice (**Fig 1M-N**): (a) WA1/2020 D614G and WA1/2020 D614G/N501Y grouped  
257 together; (b) B.1.1.7/E484K and B.1.351 viruses, which contain E484K mutations, grouped near  
258 each other; and (c) B.1.617.2 localized to a separate antigenic group.

259 We also examined T cell responses in mRNA-vaccinated K18-hACE2 mice two weeks  
260 after boosting (**Fig 4A and M-Q**) using H-2<sup>b</sup> restricted immunodominant peptides in the spike  
261 protein for CD8<sup>+</sup> and CD4<sup>+</sup> T cells. After peptide stimulation *ex vivo* and staining for intracellular  
262 IFN-γ production, we detected a robust CD8<sup>+</sup> T cell (~2 to 4 percent positive) response in the  
263 spleens of animals immunized with 5 µg of the mRNA-1273 or mRNA-1273.351 vaccines (**Fig**  
264 **4O and P**). The response was approximately 10-fold lower in animals immunized with the lower  
265 0.25 µg dose. While we also detected a spike protein-specific CD4<sup>+</sup> T cell response after  
266 immunization (~0.5 to 1.5 percent positive) with the 5 µg dose of mRNA-1273 or mRNA-  
267 1273.351 vaccines, it was lower in magnitude (**Fig 4Q and R**). Moreover, the low 0.25 µg dose  
268 mRNA-1273 or mRNA-1273.351 vaccines induced CD4<sup>+</sup> T cell responses that were barely  
269 greater than the control mRNA vaccine.

270 **Protection by mRNA vaccines in K18-hACE2 transgenic mice.** We next evaluated  
271 the protective activity of the mRNA vaccines in K18-hACE2 mice. Three to four weeks after  
272 boosting, mice were challenged via intranasal route with WA1/2020 D614G, WA1/2020  
273 N501Y/D614G, B.1.1.7/E484K, B.1.351, or B.1.617.2 strains. Compared to the control mRNA  
274 vaccine, the 5 µg and 0.25 µg doses of mRNA-1273 and mRNA-1273.351 vaccines all  
275 prevented the weight loss occurring between 3 and 6 dpi (**Fig 5A-B**).

276 At 6 dpi, mice were euthanized, and nasal washes, lungs, and brains were collected for  
277 viral burden analysis (**Fig 5C-D, and S4A**). In the nasal washes of control mRNA-vaccinated

278 K18-hACE2 mice, although some variability was observed, moderate levels ( $\sim 10^5$  copies of  $N$   
279 per mL) of viral RNA were measured after challenge with WA1/2020 D614G, WA1/2020  
280 N501Y/D614G, B.1.1.7/E484K, B.1.351, or B.1.617.2 strains (**Fig 5C**). In comparison, in the  
281 lungs of control mRNA-vaccinated K18-hACE2 mice, higher and more uniform levels ( $\sim 10^7$   
282 copies of  $N$  per mg) of viral RNA were detected after challenge with all SARS-CoV-2 strains  
283 (**Fig 5D**). The brains of control RNA-vaccinated K18-hACE2 mice showed some variability, as  
284 seen previously (Winkler et al., 2020), with many but not all animals showing substantial  
285 infection ( $10^8$  copies of  $N$  per mL) (**Fig S4A**). The high 5  $\mu$ g dose of mRNA-1273 or mRNA-  
286 1273.351 vaccines protected against infection in nasal washes, lung, and brain, with virtually no  
287 viral breakthrough regardless of the challenge strain. After the 0.25  $\mu$ g dose immunization with  
288 mRNA-1273, a loss of protection against infection in the nasal washes (3 of 7 mice) and lungs  
289 (4 of 4 mice) was observed after challenge with B.1.351 and in the lungs only after challenge  
290 with B.1.1.7/E484K (6 of 7 mice) or B.1.617.2 (8 of 8 mice) viruses. After the 0.25  $\mu$ g dose  
291 immunization with mRNA-1273.351, incomplete protection against infection in the nasal  
292 washes, lungs, and brain also was observed after challenge with WA1/2020 D614G (6, 7, and 4  
293 of 8 mice, respectively), WA1/2020 D614G/N501Y (8, 4, and 6 of 8 mice, respectively),  
294 B.1.1.7/E484K (8, 6, and 3 of 8 mice, respectively), and B.1.617.2 (7, 8, and 5 of 8 mice,  
295 respectively). The 0.25  $\mu$ g dose of the mRNA-1273.351 vaccine protected better against lung  
296 and brain infection by the homologous B.1.351 virus than against other strains.

297 We explored whether vaccine-induced neutralizing antibody titers correlated with  
298 protection after challenge with WA1/2020 D614G, WA1/2020 N501Y/D614G, B.1.1.7/E484K,  
299 B.1.351, or B.1.617.2 viruses. In general, serum levels of neutralizing antibody inversely  
300 correlated with viral RNA levels in the lung (**Fig 5E**) for all viruses, with more infection occurring  
301 in animals with lower neutralization titers. However, for WA1/2020 D614G, WA1/2020  
302 N501Y/D614G, B.1.1.7/E484K, and B.1.351, some of the animals with low neutralization titers

303 still were protected against infection in the lung. The correlation was most linear for B.1.617.2-  
304 challenged animals, with a minimum neutralizing titer of approximately 2,000 required to  
305 completely prevent infection at 6 dpi. Most of the breakthrough B.1.617.2 infections occurred  
306 with the 0.25 µg dose of mRNA vaccines. The threshold for complete protection in the lung after  
307 virus challenge varied somewhat with lower levels required for WA1/2020 D614G and  
308 WA1/2020 N501Y/D614G. When we compared body weight change in K18-hACE2 mice at 6  
309 dpi with neutralizing titers, a linear relationship was observed with all challenge viruses except  
310 B.1.351 (**Fig S4B**). The best correlation was seen after B.1.617.2 challenge, with greater weight  
311 loss in mice immunized with the 0.25 µg vaccine dose and having lower serum neutralizing  
312 antibody titers.

313 Because a pro-inflammatory host response to SARS-CoV-2 infection can contribute to  
314 pulmonary pathology and severe COVID-19, we assessed the ability of the mRNA vaccines to  
315 suppress cytokine and chemokine levels in the lung after virus challenge (**Fig S5**). For these  
316 studies, K18-hACE2 mice were immunized and boosted with 5 or 0.25 µg of control, mRNA-  
317 1273 or mRNA-1273.351 vaccines and then challenged with WA1/2020 N501Y/D614G,  
318 B.1.351, or B.1.617.2. SARS-CoV-2 infection of control mRNA vaccinated K18-hACE2 mice  
319 resulted in high levels of expression in lung homogenates of several pro-inflammatory cytokines  
320 and chemokines including G-CSF, IFN $\gamma$ , IL-1 $\beta$ , IL-6, CXCL1, CXCL5, CXCL9, CXCL10, CCL2,  
321 and CCL4. Pro-inflammatory cytokine and chemokines in the lung at 6 dpi generally were  
322 decreased in all mice vaccinated with 5 µg doses of mRNA-1273 or mRNA-1273.351 regardless  
323 of the challenge virus (**Fig S5A and B**). While this pattern also trended for the 0.25 µg dose of  
324 both mRNA vaccines, some cytokines and chemokines (e.g., IL-1 $\beta$ , IL-6, CXCL9, and CXCL10)  
325 remained elevated especially after challenge with B.1.617.2 (**Fig S5C and D**).

326 We evaluated the ability of the mRNA-1273 and mRNA-1273.351 vaccines to prevent  
327 disease in K18-hACE2 mice by performing histological analysis of lung tissues from immunized

328 animals that were challenged with WA1/2020 D614G, WA1/2020 N501Y/D614G,  
329 B.1.1.7/E484K, B.1.351, or B.1.617.2. As expected, lung sections obtained at 6 dpi from mice  
330 immunized with the control mRNA vaccine and challenged with any of the SARS-CoV-2 strains  
331 showed severe pneumonia characterized by immune cell infiltration, alveolar space  
332 consolidation, vascular congestion, and interstitial edema (**Fig 6 and 7**). In comparison, mice  
333 immunized with the high 5  $\mu$ g dose of mRNA-1273 or mRNA-1273.351 did not develop lung  
334 pathology, with histological findings similar to uninfected K18-hACE2 mice (**Fig 6**). Mice  
335 immunized with the low 0.25  $\mu$ g dose of the mRNA vaccines however, showed different results  
336 (**Fig 7**): (a) mice vaccinated with mRNA-1273 showed few, if any, pathological changes after  
337 WA1/2020 D614G, WA1/2020 N501Y/D614G, or B.1.1.7/E484K challenge. Nonetheless, some  
338 mRNA-1273 vaccinated mice challenged with B.1.351 showed pulmonary vascular congestion  
339 and mild lung inflammation; (b) mice vaccinated with mRNA-1273.351 showed almost complete  
340 protection after WA1/2020 D614G, B.1.1.7/E484K, or B.1.351 challenge, whereas scattered  
341 inflammation and alveolar septal thickening was apparent in sections from some WA1/2020  
342 N501Y/D614G challenged mice; (c) of note, lung sections from mice vaccinated with the lower  
343 0.25  $\mu$ g dose mRNA-1273 or mRNA-1273.351 and challenged with B.1.617.2 showed evidence  
344 of viral pneumonia with prominent foci of immune cells inflammation and airspace consolidation.  
345 Thus, low doses immunization of original or modified mRNA vaccines do not fully protect K18-  
346 hACE2 mice from challenge with B.1.617.2 and result in mild to moderate infection and lung  
347 pathology.

348

349 **DISCUSSION**

350 Robust vaccine-induced immune responses and sustained protective activity against  
351 emerging SARS-CoV-2 variants are needed to limit human disease and curtail the COVID-19  
352 global pandemic. A concern in the field is whether immunity generated by vaccines will wane  
353 sufficiently to lose activity against VOC with mutations or deletions in regions of the spike  
354 protein recognized by neutralizing antibodies. In the current study, we evaluated the  
355 immunogenicity and protective activity of high- and low-dose formulations of Moderna mRNA  
356 vaccines targeting historical (mRNA-1273) or variant (mRNA-1273.351) strains. The low-dose  
357 formulation study arm was designed to model waning immunity and assess for possible strain-  
358 specific breakthrough infections. Indeed, the lower neutralizing antibody and T cell responses  
359 measured with the 0.25 µg dose of mRNA-1273 or mRNA-1273.351 parallel those seen for  
360 human antibody and T cell responses six months after a primary vaccination series with mRNA-  
361 1273 (Mateus et al., 2021; Pegu et al., 2021).

362 Immunization of 129S2 or K18-hACE2 transgenic mice with mRNA-1273, mRNA-  
363 1273.351, or the mRNA-1273.211 mixture induced neutralizing antibodies against spike in  
364 serum against historical WA1/2020 and several key VOC. Challenge studies performed  
365 approximately one month after the second vaccine dose showed robust protection against  
366 weight loss and lung pathology with all high-dose vaccine formulations and infecting SARS-  
367 CoV-2 strains. Nonetheless, the low-dose vaccine formulations showed evidence of viral  
368 infection breakthrough and lung pathological changes consistent with pneumonia especially with  
369 the B.1.617.2 strain, which correlated with lower strain-specific neutralizing antibody levels. In  
370 general, variant-specific vaccine designs appeared to induce greater antibody responses and  
371 confer more protection against homologous virus strains.

372 Our experiments expand upon a preliminary immunogenicity study, which showed that  
373 vaccination of H-2<sup>d</sup> BALB/c mice with mRNA-1273.351 resulted in high serum neutralizing  
374 antibody titers against the B.1.351 lineage, whereas the mRNA-1273.211 vaccine induced

375 broad cross-variant neutralization (Wu et al., 2021). We performed experiments with two H-2<sup>b</sup>  
376 strains, 129S2 and K18-hACE2 C57BL/6, and observed some similarities and differences. In  
377 K18-hACE2 mice, the mRNA-1273 vaccine, which encodes for the Wuhan-1 pre-fusion  
378 stabilized spike, induced higher neutralizing titers against WA1/2020 strains but lower  
379 responses against viruses containing E484K mutations in spike (B.1.1.7/E484K and B.1.351),  
380 which agrees with recent immunization studies in NHPs (Corbett et al., 2021). Reciprocally, the  
381 mRNA-1273.351 vaccine, which encodes for the B.1.351 pre-fusion stabilized spike, induced  
382 higher neutralizing titers against B.1.1.7/E484K and B.1.351. In 129S2 mice, only the mRNA-  
383 1273.351 vaccine induced a lower neutralizing response against WA1/2020 D614G, as the  
384 remainder of the neutralizing antibody responses were largely equivalent between vaccines.  
385 However, in both K18-hACE2 and 129S2 mice, the mRNA-1273 and mRNA-1273.351 vaccines  
386 induced antibody responses that neutralized B.1.617.2 less efficiently than the other SARS-  
387 CoV-2 strains. Analysis of serum antibodies and B cell repertoire against SARS-CoV-2 VOC  
388 from ongoing human clinical trials comparing mRNA-1273 and mRNA-1273.351 vaccines will be  
389 needed to corroborate our results obtained in small animal models. Indeed, the differences in  
390 neutralizing antibody titers induced by mRNA-1273 against WA1/2020 D614G and B.1.351 in  
391 mice were smaller in magnitude than that seen in humans one month after boosting, but similar  
392 to that observed six months after boosting (Pegu et al., 2021).

393 While the high-dose vaccination regimen with mRNA-1273 and mRNA-1273.351  
394 induced robust neutralizing antibody and T cell responses that conferred almost complete  
395 protection against all strains, animals immunized with the low-dose scheme showed virological  
396 and pathological breakthrough that varied with the vaccine formulation and challenge strain. The  
397 low-dose vaccination approach we employed as a model for waning immunity resulted in  
398 approximately 10 to 20-fold reduced peak neutralization titers and T cell responses compared to  
399 the high-dose arm, which corresponds to the 90% loss observed 90 days after natural infection  
400 or vaccination in humans (Ibarrondo et al., 2021). The greatest loss in antibody neutralization

401 (both 129S2 and K18-hACE2 mice) and protection (K18-hACE2 mice) consistently occurred  
402 with the B.1.617.2 variant. In contrast, recent longitudinal studies in humans immunized with  
403 mRNA-1273 showed lower levels of serum antibody recognition of B.1.351 than other VOC,  
404 although live virus neutralization assays were not performed with B.1.617.2 (Pegu et al., 2021).  
405 In our experiments with live virus, the loss of neutralizing activity was equivalent if not greater  
406 for B.1.617.2 than B.1.351, as seen by others (Liu et al., 2021a). Based on sequence changes  
407 in the spike protein (B.1.617.2: T19R, 156del, 157del, R158G, L452R, T478K, D614G, P681R,  
408 and D950N; and B.1.351: D80A, D215G, 241del, 242del, 243del, K417N, E484K, N501Y,  
409 D614G, A701V) and known binding sites in the receptor binding motif of neutralizing antibodies  
410 (at residue E484), it is not apparent why neutralizing activity and protection in mice were less  
411 against B.1.617.2 than B.1.351, although there was a direct correlation with levels of  
412 neutralizing antibody and B.1.617.2 burden in the lung. Nonetheless, mutations in the B.1.617.2  
413 alter key antigenic sites and can abrogate recognition by neutralizing antibodies (McCallum et  
414 al., 2021b). Other possible explanations for the loss of potency of antibodies against B.1.617.2  
415 include differential display of B.1.617.2 spike proteins on the surface of infected cells and  
416 engagement of Fc effector functions (Ravetch et al., 2021; Winkler et al., 2021) or differential  
417 ability of antibodies to block cell-to-cell spread in a strain-dependent manner (Kruglova et al.,  
418 2021). Our observation of B.1.617.2 infection and lung disease in low-dose mRNA-vaccinated  
419 K18-hACE2 mice as a model of waning immunity corresponds to descriptions of B.1.617.2  
420 breakthrough infections in vaccinated humans in the United States, Israel, and elsewhere, some  
421 of which have required hospitalization (Brown et al., 2021; Puranik et al., 2021).

422 Our studies in 129S2 and K18-hACE2 mice with parental and modified mRNA vaccines  
423 show robust immunogenicity and protection against multiple SARS-CoV-2 strains when high-  
424 dose immunization schemes are used, although some differences in immunity are seen with  
425 particular vaccines against selected variants. While the lower dose of mRNA vaccines generally  
426 protected against matched virus challenge infection (e.g., mRNA-1273 vaccination and

427 WA1/2020 challenge or mRNA-1273.351 vaccination and B.1.351 challenge), breakthrough  
428 events were seen with some non-matched challenges (e.g., mRNA-1273 vaccination and  
429 B.1.351 challenge or mRNA-1273.351 vaccination and WA1/2020 challenge). As the low dose  
430 of mRNA-1273 and 1273.351 vaccines induced lower neutralizing titers and protected less  
431 against challenge with the B.1.617.2 variant, higher titers will be needed to minimize B.1.617.2  
432 infection, transmission, and disease. Although studies in humans are required, boosting with  
433 historical or variant (e.g., mRNA encoding B.1.617.2 spike genes) vaccines might be required to  
434 prevent breakthrough events as vaccine-induced immunity wanes.

435 **Limitations of study.** We note several limitations in our study. (1) The studies in 129S2  
436 mice precluded challenge with B.1.617.2, as it does not infect murine cells because it lacks an  
437 N501Y mutation. The generation of recombinant SARS-CoV-2 strains with spike genes  
438 encoding B.1.617.2 and an N501Y mutation could overcome this limitation. (2) Female 129S2  
439 and K18-hACE2 mice were used to allow for group caging of the large cohorts required for  
440 these multi-arm vaccination studies. Follow-up experiments in male mice are needed to confirm  
441 results are not sex-biased. (3) We used lower vaccine dosing as a model for waning immunity.  
442 Studies that directly address durability of immune responses and protection are needed for  
443 corroboration. (4) We used historical, variant, or mixed mRNA vaccine formulations with  
444 homologous boosting schemes. Animals studies that test heterologous boosting (mRNA-1273  
445 prime followed by mRNA-1273.351 boost) (Wu et al., 2021) also are needed to support clinical  
446 trials. (5) Our studies focused on immunogenicity and protection in two strains of mice because  
447 of the ability to set up large animal cohorts and the tools available for analysis. These results  
448 require confirmation in other animal models of SARS-CoV-2 infection including hamsters and  
449 non-human primates (Muñoz-Fontela et al., 2020). (6) We did not establish direct immunological  
450 correlates of vaccine protection or failure for all vaccine and challenge strain pairs. While some  
451 relationships were more predictive (e.g., low B.1.617.2 neutralizing titers and viral burden in the  
452 lung), others were not.

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466

467 **AUTHOR CONTRIBUTIONS**

468 L.A.V. and B.Y. performed and analyzed neutralization assays. B.Y., B.W., A.O.H.,  
469 L.A.V., S.S., C.E.K., S.M., N.M.K., R.E.C., and L.B.T. performed mouse experiments. B.W.,  
470 S.S., and C-Y.L. performed viral burden analyses. A.O.H. and B.Y. performed T cell analyses.  
471 J.M.C., G.S., and F.K. performed and analyzed the ELISA experiments. S.H.W and D.J.S.  
472 performed the antigenic cartography analysis. A.C., S.E., and D.E. provided the mRNA vaccines  
473 and helped design experiments. M.S.D. obtained funding. L.B.T. and M.S.D. supervised the  
474 research. M.S.D. and L.B.T. wrote the initial draft, with the other authors providing editorial  
475 comments.

476

477 **COMPETING FINANCIAL INTERESTS**

478 M.S.D. is a consultant for Inbios, Vir Biotechnology, Fortress Biotech, and Carnival  
479 Corporation, and on the Scientific Advisory Boards of Moderna and Immunome. The Diamond  
480 laboratory has received unrelated funding support in sponsored research agreements from Vir  
481 Biotechnology, Kaleido, Moderna, and Emergent BioSolutions. A.C., S.E., and D.K.E. are  
482 employees of and shareholders in Moderna Inc. F.K. is a coinventor on a patent application for  
483 serological assays and SARS-CoV-2 vaccines (international application numbers  
484 PCT/US2021/31110 and 62/994,252).

485 **FIGURE LEGENDS**

486 **Figure 1. Immunogenicity analysis of mRNA vaccines in 129S2 mice.** Seven to  
487 nine-week-old female 129S2 mice were immunized and boosted with 5 or 0.25  $\mu$ g of mRNA  
488 vaccines. **A.** Scheme of immunizations, blood draw, and virus challenge. **B.** Serum anti-spike  
489 IgG responses at three weeks after booster immunization with mRNA vaccines (control (black  
490 symbols), mRNA-1273 (red symbols), mRNA-1273.351 (blue symbols), and mRNA-1273.211  
491 (green symbols) against indicated spike proteins (Wuhan-1, B.1.1.7, B.1.351, or B.1.617.2) ( $n =$   
492 3 (control vaccine) or 8 (spike vaccines), two independent experiments, boxes illustrate mean  
493 values, dotted line shows the limit of detection (LOD); two-way ANOVA with Tukey's post-test: \*,  
494  $P < 0.05$ ). **C-L.** Serum neutralizing antibody responses three weeks after boosting as assessed  
495 by FRNT (half-maximal reduction,  $FRNT_{50}$  values) with WA1/2020 D614G (**C, H**), WA1/2020  
496 D614G/N501Y (**D, I**), B.1.1.7/E484K (**E, J**), B.1.351 (**F, K**), or B.1.617.2 (**G, L**) in mice  
497 immunized with 5 (**C-G**) or 0.25 (**H-L**)  $\mu$ g of control ( $n = 6-10$ ), mRNA-1273, mRNA-1273.351, or  
498 mRNA-1273.211 ( $n = 12-21$ ) vaccines (two independent experiments, boxes illustrate geometric  
499 mean values, dotted line shows LOD; one-way Kruskal-Wallis ANOVA with Dunn's post-test: \*,  
500  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; \*\*\*\*  $P < 0.0001$ ). **M-N.** Antigenic map of sera from 129S2  
501 mice titrated against WA1/2020 D614G, WA1/2020 N501Y/D614G, B.1.1.7/E484K, B.1.351,  
502 and B.1.617.2. The maps show sera from mice that received 5  $\mu$ g (**M**) or 0.25  $\mu$ g (**N**) doses,  
503 respectively. Antigens (viruses) are shown as circles (WA1/2020 D614G: red, bigger circle,  
504 WA1/2020 N501Y/D614G: red, smaller circle, B.1.1.7/E484K: turquoise, B.1.351: blue, and  
505 B.1.617.2: orange) and sera as squares (blue for mRNA-1273.351-induced sera and red for  
506 mRNA-1273-induced sera). The X and Y axes correspond to antigenic distance, with one grid  
507 line corresponding to a two-fold serum dilution in the neutralization assay. The antigens and  
508 sera are arranged on the map such that the distances between them best represent the  
509 distances measured in the neutralization assay.

510 **Figure 2. Protection against SARS-CoV-2 infection after mRNA vaccination in**  
511 **129S2 mice.** Seven to nine-week-old female 129S2 mice were immunized and boosted with 5  
512 or 0.25  $\mu$ g of mRNA vaccines (control (black symbols), mRNA-1273 (red symbols), mRNA-  
513 1273.351 (blue symbols), and mRNA-1273.211 [mixture, green symbols]) as described in  
514 **Figure 1A.** Three weeks after boosting, mice were challenged via intranasal inoculation with  $10^5$   
515 focus-forming units (FFU) of WA1/2020 N501Y/D614G, B.1.1.7/E484K, or B.1.351. **A-B.** Body  
516 weight change over time. Data shown is the mean  $\pm$  SEM ( $n = 6-9$ , two independent  
517 experiments; one-way ANOVA of area under the curve from 2-4 dpi with Dunnett's post-test,  
518 comparison to control immunized group: ns, not significant;  $P > 0.05$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ;  
519 \*\*\*,  $P < 0.001$ ; \*\*\*\*  $P < 0.0001$ ). **C-D.** Viral burden at 4 dpi in the nasal washes (**C**) and lungs (**D**)  
520 as assessed by qRT-PCR of the *N* gene after challenge of immunized mice with the indicated  
521 mRNA vaccines ( $n = 6-8$ , two independent experiments, boxes illustrate median values, dotted  
522 line shows LOD; one-way Kruskal-Wallis ANOVA with Dunn's post-test, comparison among all  
523 immunization groups: ns, not significant;  $P > 0.05$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; \*\*\*\*  $P$   
524  $< 0.0001$ ). **E.** Correlation analyses comparing serum neutralizing antibody concentrations three  
525 weeks after boosting plotted against lung viral titer (4 dpi) in 129S2 mice after challenge with the  
526 indicated SARS-CoV-2 strain (Pearson's correlation  $P$  and  $R^2$  values are indicated as insets;  
527 closed symbols 5  $\mu$ g vaccine dose; open symbols, 0.25  $\mu$ g vaccine dose).

528 **Figure 3. Vaccine-mediated protection against lung pathology in 129S2 mice.**  
529 Seven to nine-week-old female 129S2 mice were immunized, boosted with 0.25 µg of mRNA  
530 vaccines (control, mRNA-1273, mRNA-1273.351, or mRNA-1273.211), and challenged with  
531 B.1.351 as described in **Figure 2**. Hematoxylin and eosin staining of lung sections harvested at  
532 4 dpi or from a mock-infected animal. Images show low- (top; scale bars, 1 mm) and high-power  
533 (bottom; scale bars, 50 µm). Representative images from  $n = 2$  per group.

534 **Figure 4. Immunogenicity analysis of mRNA vaccines in K18-hACE2 transgenic**

535 **mice.** Seven-week-old female K18-hACE2 mice were immunized and boosted with 5 or 0.25  $\mu$ g

536 of mRNA vaccines (control (black symbols), mRNA-1273 (red symbols), or mRNA-1273.351

537 (blue symbols). **A.** Scheme of immunizations, spleen harvest, blood draw, and virus challenge.

538 **B.** Serum anti-spike IgG responses at three weeks after booster immunization with mRNA

539 vaccines (control (black symbols), mRNA-1273 (red symbols), mRNA-1273.351 (blue symbols))

540 against indicated spike proteins (Wuhan-1, B.1.1.7, B.1.351, or B.1.617.2). (n = 3 (control

541 vaccine) or 8 (spike vaccines), two independent experiments, boxes illustrate mean value,

542 dotted line shows the LOD; two-way ANOVA with Tukey's post-test: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ). **C-**

543 **L.** Serum neutralizing antibody responses three weeks after boosting as judged by FRNT (half-

544 maximal reduction, FRNT<sub>50</sub> values) with WA1/2020 D614G (**C, H**), WA1/2020 D614G/N501Y

545 (**D, I**), B.1.1.7/E484K (**E, J**), B.1.351 (**F, K**), or B.1.617.2 (**G, L**) in mice immunized with 5 (**C-G**)

546 or 0.25 (**H-L**)  $\mu$ g of control (n = 5-10), mRNA-1273 (n = 20-24), and mRNA-1273.351 (n = 21-

547 24) vaccines (two independent experiments, boxes illustrate geometric mean values, dotted line

548 shows LOD; one-way Kruskal-Wallis ANOVA with Dunn's post-test: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,

549  $P < 0.001$ ; \*\*\*\*  $P < 0.0001$ ). **M-N.** Antigenic map of sera from K18-hACE2 mice titrated against

550 WA1/2020 D614G, WA1/2020 N501Y/D614G, B.1.1.7/E484K, B.1.351, and B.1.617.2. The

551 maps show sera from immunized mice that received 5  $\mu$ g (**M**) or 0.25  $\mu$ g (**N**) doses, respectively

552 with symbol details described in **Figure 1.** **O-R.** CD8<sup>+</sup> (**O, P**) and CD4<sup>+</sup> (**Q, R**) T cell responses

553 in K18-hACE2 mice at day 35, two weeks after booster immunization with mRNA vaccines (n=

554 10 for each group, two independent experiments, boxes illustrate median values, one-way

555 ANOVA with Tukey's post-test: ns, not significant; \*,  $P < 0.05$ ; \*\*,  $P > 0.01$ ; \*\*\*,  $P < 0.001$ ; \*\*\*\*  $P$

556 < 0.0001).

**Figure 5. Protection against SARS-CoV-2 infection after mRNA vaccination in K18-hACE2 transgenic mice.** Seven-week-old female K18-hACE2 mice were immunized and

559 boosted with 5 or 0.25  $\mu$ g of mRNA vaccines (control (black symbols), mRNA-1273 (red  
560 symbols), or mRNA-1273.351 (blue symbols) as described in **Figure 4A**. Four weeks after  
561 boosting, mice were challenged via intranasal inoculation with  $10^3$  to  $3 \times 10^4$  FFU of WA1/2020  
562 D614G, WA1/2020 N501Y/D614G, B.1.1.7/E484K, B.1.351, or B.1.617.2, depending on the  
563 strain. **A-B**. Body weight change over time. Data shown is the mean +/- SEM ( $n = 4-8$ , two  
564 independent experiments; one-way ANOVA of area under the curve from 2-4 dpi with Dunnett's  
565 post-test, comparison to control immunized group: \*\*\*\*  $P < 0.0001$ ). **C-D**. Viral burden levels at  
566 6 dpi in the nasal washes (**C**) and lungs (**D**) as assessed by qRT-PCR of the *N* gene after  
567 challenge of immunized mice with the indicated mRNA vaccines ( $n = 4-8$ , two independent  
568 experiments, boxes illustrate median values, dotted line shows LOD; one-way Kruskal-Wallis  
569 ANOVA with Dunn's post-test, comparison among all immunization groups: \*,  $P < 0.05$ ; \*\*,  $P <$   
570 0.01; \*\*\*,  $P < 0.001$ ; \*\*\*\*  $P < 0.0001$ ). **E**. Correlation analyses comparing serum neutralizing  
571 antibody concentrations three weeks after boosting plotted against lung viral titer (6 dpi) in K18-  
572 hACE2 mice after challenge with the indicated SARS-CoV-2 strain (Pearson's correlation  $P$  and  
573  $R^2$  values are indicated as insets; closed symbols 5  $\mu$ g vaccine dose; open symbols, 0.25  $\mu$ g  
574 vaccine dose).

575 **Figure 6. High dose mRNA vaccine protection against lung inflammation and**  
576 **pathology in K18-hACE2 transgenic mice.** Seven to nine-week-old female K18-hACE2  
577 transgenic mice were immunized, boosted with 5  $\mu$ g of mRNA vaccines (control, mRNA-1273,  
578 or mRNA-1273.351, and challenged with WA1/2020, WA1/2020 N501Y/D614G, B.1.1.7/E484K,  
579 B.1.351, or B.1.617.2, as described in **Figure 5**. Hematoxylin and eosin staining of lung sections  
580 harvested at 6 dpi or from an uninfected animal. Images show low- (top; scale bars, 1 mm) and  
581 high-power (bottom; scale bars, 50  $\mu$ m). Representative images of multiple lung sections from  $n$   
582 = 2 per group.

583 **Figure 7. Low dose mRNA vaccine protection against lung inflammation and**  
584 **pathology in K18-hACE2 transgenic mice.** Seven to nine-week-old female K18-hACE2  
585 transgenic mice were immunized, boosted with 0.25 µg of mRNA vaccines (control, mRNA-  
586 1273, or mRNA-1273.351, and challenged with WA1/2020, WA1/2020 N501Y/D614G,  
587 B.1.1.7/E484K, B.1.351, or B.1.617.2. as described in **Figure 5**. Hematoxylin and eosin staining  
588 of lung sections harvested at 6 dpi or from an uninfected animal. Images show low- (top; scale  
589 bars, 1 mm) and high-power (bottom; scale bars, 50 µm). Representative images of multiple  
590 lung sections from n = 2 per group.

591

592 **SUPPLEMENTAL FIGURE LEGENDS**

593 **Figure S1. Analysis of serum neutralization of SARS-CoV-2 strains from 129S2**  
594 **mice immunized with mRNA vaccines, Related to Figure 1.** Comparison of neutralizing  
595 activity of sera against WA1/2020 D614G, WA1/2020 D614G/N501Y, B.1.1.7/E484K, B.1.351,  
596 and B.1.617.2. Sera was obtained three weeks after boosting with 5  $\mu$ g (**A-C**) or 0.25  $\mu$ g (**D-F**)  
597 of mRNA vaccines: mRNA-1273 (**A and D**), mRNA-1273.351 (**B and E**), and mRNA-1273.211  
598 (**C and F**). Results are from experiments performed in **Figure 1C-L**. Geometric mean  
599 neutralization titers (GMT) are shown above each graph, dotted line represents the LOD. Solid  
600 lines connect data points from the same serum sample across strains.

601 **Figure S2. Protection against SARS-CoV-2 infection after mRNA vaccination in**  
602 **129S2 mice, Related to Figure 2.** Seven to nine-week-old female 129S2 mice were immunized  
603 and boosted with 5 or 0.25  $\mu$ g of mRNA vaccines as described in **Figure 1A**. Three weeks after  
604 boosting, mice were challenged via intranasal inoculation with  $10^5$  FFU of WA1/2020  
605 N501Y/D614G, B.1.1.7/E484K, or B.1.351. **A.** Viral burden at 4 dpi in the spleen as assessed  
606 by qRT-PCR of the *N* gene after challenge of immunized mice with the indicated mRNA  
607 vaccines ( $n = 6-8$ , two independent experiments; boxes illustrate median values, dotted line  
608 shows LOD; one-way Kruskal-Wallis ANOVA with Dunn's post-test, comparison among all  
609 immunization groups: ns, not significant;  $P > 0.05$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; \*\*\*\*  $P$   
610  $< 0.0001$ ). **B.** Correlation analyses comparing serum neutralizing antibody concentrations three  
611 weeks after boosting plotted against weight change in 129S2 mice after challenge with the  
612 indicated SARS-CoV-2 strain (Pearson's correlation  $P$  and  $R^2$  values are indicated as insets;  
613 closed symbols, 5  $\mu$ g vaccine dose; open symbols, 0.25  $\mu$ g vaccine dose).

614 **Figure S3. Analysis of serum neutralization of SARS-CoV-2 strains from K18-**  
615 **hACE2 mice immunized with mRNA vaccines, Related to Figure 4.** Comparison of  
616 neutralizing activity of sera against WA1/2020 D614G, WA1/2020 D614G/N501Y,

617 B.1.1.7/E484K, B.1.351, and B.1.617.2. Sera was obtained three weeks after boosting with 5  $\mu$ g  
618 (**A-B**) or 0.25  $\mu$ g (**C-D**) mRNA vaccines: mRNA-1273 (**A and C**) and mRNA-1273.351 (**B and**  
619 **D**), Results are from experiments performed in **Figure 4C-L**. GMTs are shown above each  
620 graph, dotted line represents the LOD. Solid lines connect data points from the same serum  
621 sample across strains.

622 **Figure S4. Protection against SARS-CoV-2 infection after mRNA vaccination in**  
623 **K18-hACE2 transgenic mice, Related to Figure 5.** Seven-week-old K18-hACE2 mice were  
624 immunized and boosted with 5 or 0.25  $\mu$ g of mRNA vaccines (control (black symbols), mRNA-  
625 1273 (red symbols), or mRNA-1273.351 (blue symbols) as described in **Figure 4A**. Three to  
626 four weeks after the last boost, mice were challenged via intranasal inoculation with WA1/2020  
627 D614, WA1/2020 N501Y/D614G, B.1.1.7/E484K, B.1.351, or B.1.617.2 as described in **Figure**  
628 **5. (A)** Viral burden levels at 6 dpi in the brain as assessed by qRT-PCR of the *N* gene after  
629 challenge of immunized mice with the indicated mRNA vaccines ( $n = 4-8$ , two independent  
630 experiments; boxes illustrate median values, dotted line shows LOD; one-way Kruskal-Wallis  
631 ANOVA with Dunn's post-test, comparison among all immunization groups: \*,  $P < 0.05$ ; \*\*,  $P <$   
632 0.01; \*\*\*,  $P < 0.001$ ; \*\*\*\*  $P < 0.0001$ ). **B.** Correlation analyses comparing serum neutralizing  
633 antibody concentrations three weeks after boosting plotted against weight change in K18-  
634 hACE2 mice after challenge with the indicated SARS-CoV-2 strain (Pearson's correlation  $P$  and  
635  $R^2$  values are indicated as insets; closed symbols, 5  $\mu$ g vaccine dose; open symbols, 0.25  $\mu$ g  
636 vaccine dose).

637 **Figure S5. Cytokine induction in lungs after mRNA vaccination and SARS-CoV-2**  
638 **challenge, Related to Figure 5.** Cytokine levels from mice immunized with 5  $\mu$ g (**A-B**) or 0.25  
639  $\mu$ g (**C-D**) dose of mRNA vaccines as measured by multiplex platform in lung tissues of SARS-  
640 CoV-2-infected mice at 6 dpi. **A and C.** For each cytokine, fold-change was calculated  
641 compared to mock-inoculated mice and  $\log_2$  (fold-change) was plotted in the corresponding

642 color-coded heat-map. **B and D.** Cytokine levels as measured by multiplex platform in the lungs  
643 of SARS-CoV-2-infected mice after vaccination (n = 7-8 per group, two independent  
644 experiments, mean values +/- SEM is shown).

645

646 **STAR METHODS**

647 **RESOURCE AVAILABILITY**

648 **Lead Contact.** Further information and requests for resources and reagents should be  
649 directed to and will be fulfilled by the Lead Contact, Michael S. Diamond  
650 ([diamond@wusm.wustl.edu](mailto:diamond@wusm.wustl.edu)).

651 **Materials Availability.** All requests for resources and reagents should be directed to  
652 and will be fulfilled by the Lead Contact author. This includes mice, antibodies, viruses,  
653 vaccines, proteins, and peptides. All reagents will be made available on request after  
654 completion of a Materials Transfer Agreement.

655 **Data and code availability.** All data supporting the findings of this study are available  
656 within the paper and are available from the corresponding author upon request.

657

658 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

659 **Cells.** Vero-TMPRSS2 (Zang et al., 2020) and Vero-hACE2-TMPRSS2 (Chen et al.,  
660 2021b) cells were cultured at 37°C in Dulbecco's Modified Eagle medium (DMEM)  
661 supplemented with 10% fetal bovine serum (FBS), 10 mM HEPES pH 7.3, 1 mM sodium  
662 pyruvate, 1× non-essential amino acids, and 100 U/mL of penicillin–streptomycin. Vero-  
663 TMPRSS2 cells were supplemented with 5 µg/mL of blasticidin. Vero-hACE2-TMPRSS2 cells  
664 were supplemented with 10 µg/mL of puromycin. All cells routinely tested negative for  
665 mycoplasma using a PCR-based assay.

666 **Viruses.** The WA1/2020 recombinant strain with substitutions (D614G or  
667 N501Y/D614G) were obtained from an infectious cDNA clone of the 2019n-  
668 CoV/USA\_WA1/2020 strain as described previously (Plante et al., 2020). The B.1.351,  
669 B.1.1.7/E484K, and B.1.617.2 isolates were originally obtained from nasopharyngeal isolates.  
670 All viruses were passaged once in Vero-TMPRSS2 cells and subjected to next-generation

671 sequencing as described previously (Chen et al., 2021b) to confirm the introduction and stability  
672 of substitutions. All virus experiments were performed in an approved biosafety level 3 (BSL-3)  
673 facility.

674 **Mice.** Animal studies were carried out in accordance with the recommendations in the  
675 Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The  
676 protocols were approved by the Institutional Animal Care and Use Committee at the Washington  
677 University School of Medicine (assurance number A3381–01). Virus inoculations were  
678 performed under anesthesia that was induced and maintained with ketamine hydrochloride and  
679 xylazine, and all efforts were made to minimize animal suffering.

680 Heterozygous K18-hACE2 C57BL/6J mice (strain: 2B6.Cg-Tg(K18-ACE2)2PrImn/J) and  
681 129 mice (strain: 129S2/SvPasCrl) were obtained from The Jackson Laboratory and Charles  
682 River Laboratories, respectively. Animals were housed in groups and fed standard chow diets.

683 **Pre-clinical vaccine mRNA and lipid nanoparticle production process.** A sequence-  
684 optimized mRNA encoding prefusion-stabilized Wuhan-Hu-1 (mRNA-1273) or B.1.351-variant  
685 (mRNA-1273.351) SARS-CoV-2 S-2P protein was synthesized *in vitro* using an optimized T7  
686 RNA polymerase-mediated transcription reaction with complete replacement of uridine by N1m-  
687 pseudouridine (Nelson et al., 2020). The reaction included a DNA template containing the  
688 immunogen open-reading frame flanked by 5' untranslated region (UTR) and 3' UTR sequences  
689 and was terminated by an encoded polyA tail. After transcription, the cap-1 structure was added  
690 to the 5' end using the vaccinia virus capping enzyme (New England Biolabs) and vaccinia  
691 virus 2'-O-methyltransferase (New England Biolabs). The mRNA was purified by oligo-dT affinity  
692 purification, buffer exchanged by tangential flow filtration into sodium acetate, pH 5.0, sterile  
693 filtered, and kept frozen at –20°C until further use.

694 The mRNA was encapsulated in a lipid nanoparticle through a modified ethanol-drop  
695 nanoprecipitation process described previously (Hassett et al., 2019). Ionizable, structural,  
696 helper, and polyethylene glycol lipids were briefly mixed with mRNA in an acetate buffer, pH 5.0,

697 at a ratio of 2.5:1 (lipid:mRNA). The mixture was neutralized with Tris-HCl, pH 7.5, sucrose was  
698 added as a cryoprotectant, and the final solution was sterile-filtered. Vials were filled with  
699 formulated lipid nanoparticle and stored frozen at -20°C until further use. The vaccine product  
700 underwent analytical characterization, which included the determination of particle size and  
701 polydispersity, encapsulation, mRNA purity, double-stranded RNA content, osmolality, pH,  
702 endotoxin, and bioburden, and the material was deemed acceptable for *in vivo* study.

703 **Antigens.** Recombinant soluble S proteins from different SARS-CoV-2 strains were  
704 expressed as previously described (Amanat et al., 2021; Stadlbauer et al., 2020). Briefly,  
705 mammalian cell codon-optimized nucleotide sequences coding for the soluble ectodomain of the  
706 S protein of SARS-CoV-2 including a C-terminal thrombin cleavage site, T4 foldon trimerization  
707 domain, and hexahistidine tag were cloned into mammalian expression vector pCAGGS. The  
708 spike protein sequence was modified to remove the polybasic cleavage site (RRAR to A), and  
709 two pre-fusion stabilizing proline mutations were introduced (K986P and V987P, wild type  
710 Wuhan-Hu-1 numbering). Recombinant proteins were produced in Expi293F cells  
711 (ThermoFisher) by transfection with purified DNA using the ExpiFectamine 293 Transfection Kit  
712 (ThermoFisher). Supernatants from transfected cells were harvested 3 days post-transfection,  
713 and recombinant proteins were purified using Ni-NTA agarose (ThermoFisher), then buffer  
714 exchanged into PBS and concentrated using Amicon Ultracel centrifugal filters (EMD Millipore).  
715

## 716 **METHOD DETAILS**

717 **ELISA.** Assays were performed in 96-well microtiter plates (Thermo Fisher) coated with  
718 50 µL of recombinant spike from wild type SARS-CoV-2 or variant viruses B.1.1.7, B.1.351, or  
719 B.1.617.2. Plates were incubated at 4°C overnight and then blocked with 200 µL of 3% non-fat  
720 dry milk (AmericanBio) in PBS containing 0.1% Tween-20 (PBST) for one hour at room  
721 temperature (RT). Sera were serially diluted in 1% non-fat dry milk in PBST and added to the  
722 plates. Plates were incubated for 120 min at room temperature and then washed 3 times with

723 PBST. Goat anti-mouse IgG-HRP (Sigma-Aldrich, 1:9000) was diluted in 1% non-fat dry milk in  
724 PBST before adding to the wells and incubating for 60 min at room temperature. Plates were  
725 washed 3 times with PBST before the addition of peroxidase substrate (SigmaFAST o-  
726 phenylenediamine dihydrochloride, Sigma-Aldrich). Reactions were stopped by the addition of 3  
727 M hydrochloric acid. Optical density (OD) measurements were taken at 490 nm, and endpoint  
728 titers were calculated in excel using a 0.15 OD 490 nm cutoff. Graphs were generated using  
729 Graphpad Prism v9.

730 **Focus reduction neutralization test.** Serial dilutions of sera were incubated with  $10^2$   
731 focus-forming units (FFU) of different strains of SARS-CoV-2 for 1 h at 37°C. Antibody-virus  
732 complexes were added to Vero-TMPRSS2 cell monolayers in 96-well plates and incubated at  
733 37°C for 1 h. Subsequently, cells were overlaid with 1% (w/v) methylcellulose in MEM. Plates  
734 were harvested 30 h later by removing overlays and fixed with 4% PFA in PBS for 20 min at  
735 room temperature. Plates were washed and sequentially incubated with an oligoclonal pool of  
736 SARS2-2, SARS2-11, SARS2-16, SARS2-31, SARS2-38, SARS2-57, and SARS2-71 (Liu et al.,  
737 2021c) anti-S antibodies and HRP-conjugated goat anti-mouse IgG (Sigma, 12-349) in PBS  
738 supplemented with 0.1% saponin and 0.1% bovine serum albumin. SARS-CoV-2-infected cell  
739 foci were visualized using TrueBlue peroxidase substrate (KPL) and quantitated on an  
740 ImmunoSpot microanalyzer (Cellular Technologies).

741 **Mouse experiments.** Female 129S2 (catalog 287) and K18-hACE2 C57BL/6 (catalog  
742 034860) mice were purchased from the Charles River and The Jackson Laboratory,  
743 respectively. Seven to nine-week-old animals were immunized and boosted three weeks apart  
744 with 0.25 or 5  $\mu$ g of mRNA vaccines (control, mRNA-1273, mRNA-1273.351, or mRNA-  
745 1273.211) in 50  $\mu$ l PBS via intramuscular injection in the hind leg. Animals were bled at  
746 specified time points via the mandibular vein to obtain sera for immunogenicity analysis. Three  
747 to four weeks after boosting, mice were inoculated with  $10^5$  FFU (129S2) or  $10^3$  to  $3 \times 10^4$  FFU  
748 (K18-hACE2) of WA1/2020 D614G ( $10^4$ ), WA1/2020 N501Y/D614G ( $10^3$ ), B.1.1.7/E484K ( $10^3$ ),

749 B.1.351 ( $10^3$ ), or B.1.617.2 ( $3 \times 10^4$ ) of SARS-CoV-2 strains by the intranasal route. Different  
750 doses of viruses were used in K18-hACE2 mice to match weight loss and infection in the nasal  
751 wash and lungs. This approach was necessary as some viruses (WA1/2020 N501Y/D614G,  
752 B.1.1.7/E484K, and B.1.351) encode N501Y mutations that enhance pathogenicity in mice (Gu  
753 et al., 2020; Muruato et al., 2021; Rathnasinghe et al., 2021). Animals were euthanized at 4 or 6  
754 dpi, and tissues were harvested for virological, immunological, and pathological analyses.

755 **Measurement of viral burden.** Tissues were weighed and homogenized with zirconia  
756 beads in a MagNA Lyser instrument (Roche Life Science) in 1000  $\mu$ L of DMEM medium  
757 supplemented with 2% heat-inactivated FBS. Tissue homogenates were clarified by  
758 centrifugation at 10,000 rpm for 5 min and stored at  $-80^{\circ}\text{C}$ . RNA was extracted using the  
759 MagMax mirVana Total RNA isolation kit (Thermo Fisher Scientific) on the Kingfisher Flex  
760 extraction robot (Thermo Fisher Scientific). RNA was reverse transcribed and amplified using  
761 the TaqMan RNA-to-CT 1-Step Kit (Thermo Fisher Scientific). Reverse transcription was carried  
762 out at  $48^{\circ}\text{C}$  for 15 min followed by 2 min at  $95^{\circ}\text{C}$ . Amplification was accomplished over 50  
763 cycles as follows:  $95^{\circ}\text{C}$  for 15 s and  $60^{\circ}\text{C}$  for 1 min. Copies of SARS-CoV-2 *N* gene RNA in  
764 samples were determined using a previously published assay (Case et al., 2020a). Briefly, a  
765 TaqMan assay was designed to target a highly conserved region of the *N* gene (Forward primer:  
766 ATGCTGCAATCGTGCTACAA; Reverse primer: GACTGCCGCCTCTGCTC; Probe: /56-  
767 FAM/TCAAGGAAC/ZEN/AACATTGCCAA/3IABkFQ/). This region was included in an RNA  
768 standard to allow for copy number determination down to 10 copies per reaction. The reaction  
769 mixture contained final concentrations of primers and probe of 500 and 100 nM, respectively.

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

775                   **Lung histology.** Animals were euthanized before harvest and fixation of tissues. Lungs  
776                    were inflated with ~2 mL of 10% neutral buffered formalin using a 3-mL syringe and catheter  
777                    inserted into the trachea and kept in fixative for 7 days. Tissues were embedded in paraffin, and  
778                    sections were stained with hematoxylin and eosin. Images were captured using the  
779                    Nanozoomer (Hamamatsu) at the Alafi Neuroimaging Core at Washington University.

780                   **Peptide restimulation and intracellular cytokine staining.** Two weeks after boosting,  
781                    splenocytes from vaccinated K18-hACE2 mice were stimulated *ex vivo* with an H-2D<sup>b</sup>-restricted  
782                    CD8 or CD4 immunodominant peptide (amino acids 262–270 and 62-76 of the S protein,  
783                    respectively; gift of K. Valentine and S. Shresta, La Jolla Institute for Immunology) for 16 h at  
784                    37°C with brefeldin A (BioLegend, 420601) added for the last 4 h of incubation. Following  
785                    blocking with FcγR antibody (BioLegend, clone 93), cells were stained on ice with CD45  
786                    BUV395 (BD BioSciences clone 30-F11), CD4 PE (BD BioSciences clone GK1.5), CD8 FITC  
787                    (BioLegend clone 53-6.7), and Fixable Aqua Dead Cell Stain (Invitrogen, L34966). Stained cells  
788                    were fixed and permeabilized with the Foxp3/Transcription Factor Staining Buffer Set  
789                    (eBiosciences, 00-5523). Subsequently, intracellular staining was performed with anti-IFN-γ  
790                    Alexa 647 (BioLegend, clone XMG1.2), and anti-TNFα BV605 (BioLegend, clone MP6-XT22).  
791                    Analysis was performed on a BD LSRII Fortessa X-20 cytometer, using FlowJo X 10.0 software.

792                   **Antigenic cartography.** A target distance from an individual serum to each virus was  
793                    derived by calculating the difference between the logarithm (log2) reciprocal neutralization titer  
794                    for that particular virus and the log2 reciprocal maximum titer achieved by that serum (against  
795                    any virus). Thus, the higher the reciprocal titer, the shorter the target distance. As the log2 of the  
796                    reciprocal titer was used, a 2-fold change in titer equates to a fixed change in target distance  
797                    whatever the magnitude of the actual titers. Antigenic cartography (Smith et al., 2004) then was  
798                    used to optimize the positions of the viruses and sera relative to each other on a map,  
799                    minimizing the sum-squared error between map distance and target distance. Each virus is

800 therefore positioned by multiple sera, and the sera themselves also are positioned only by their  
801 distances to the viruses. Hence, sera with different neutralization profiles to the virus panel are  
802 in separate locations on the map but contribute equally to positioning of the viruses.

803

804 **QUANTIFICATION AND STATISTICAL ANALYSIS**

805 Statistical significance was assigned when *P* values were < 0.05 using Prism Version 10  
806 (GraphPad). Tests, number of animals (n), median values, and statistical comparison groups  
807 are indicated in the Figure legends.

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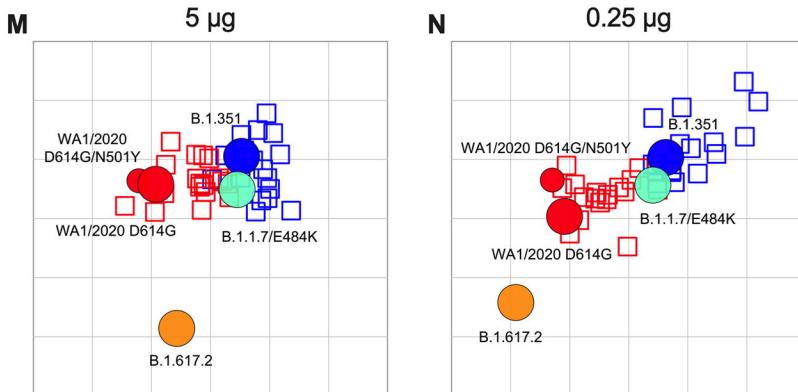
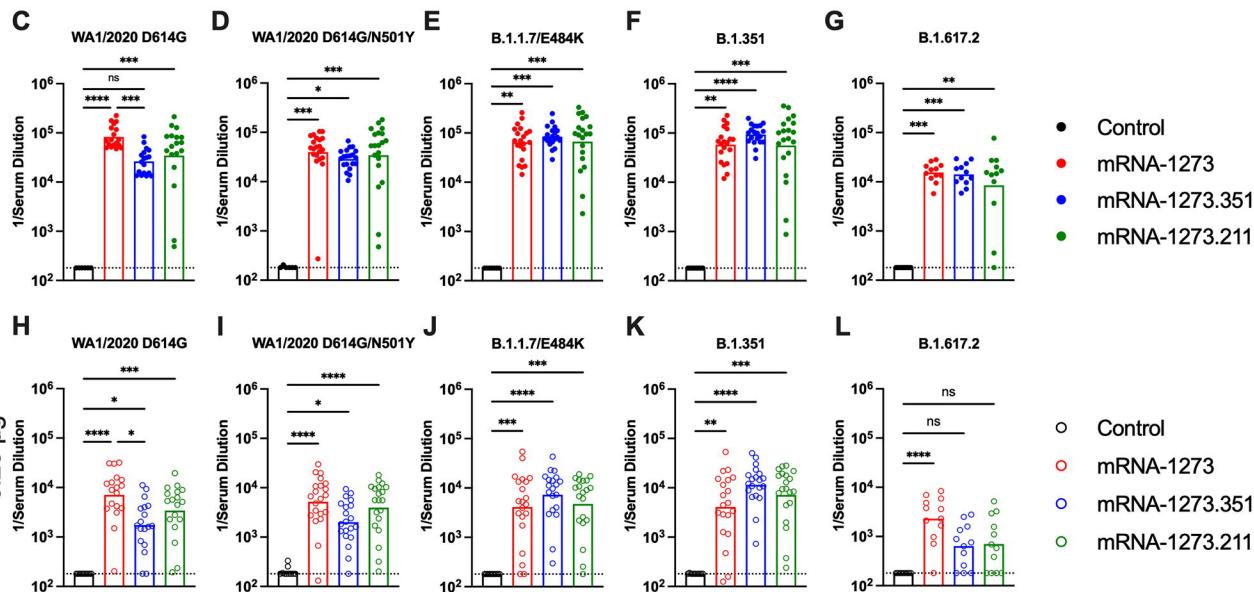
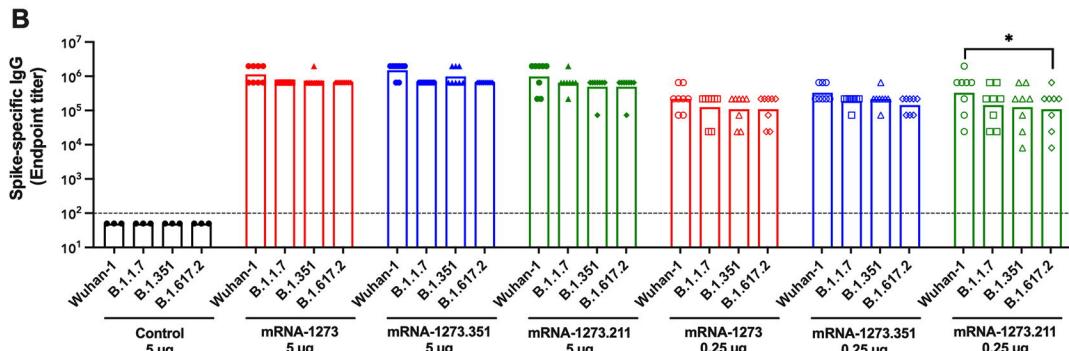
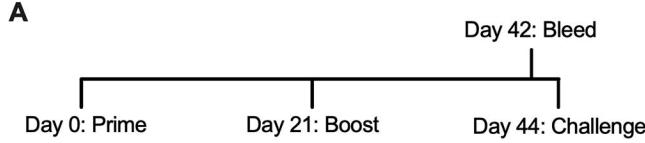
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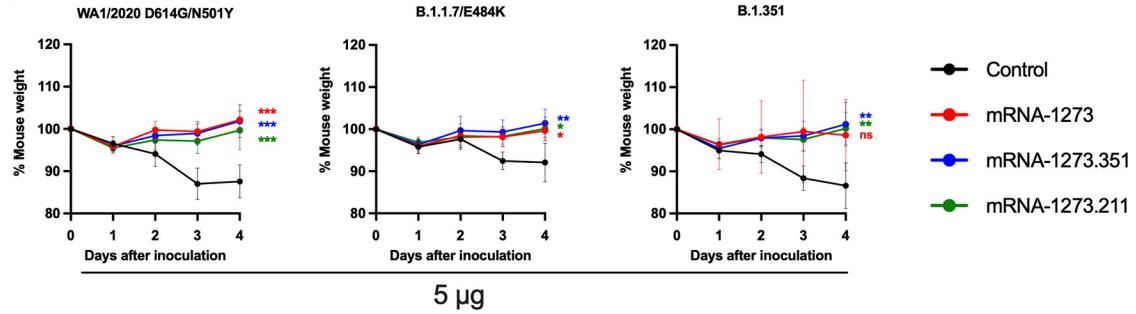
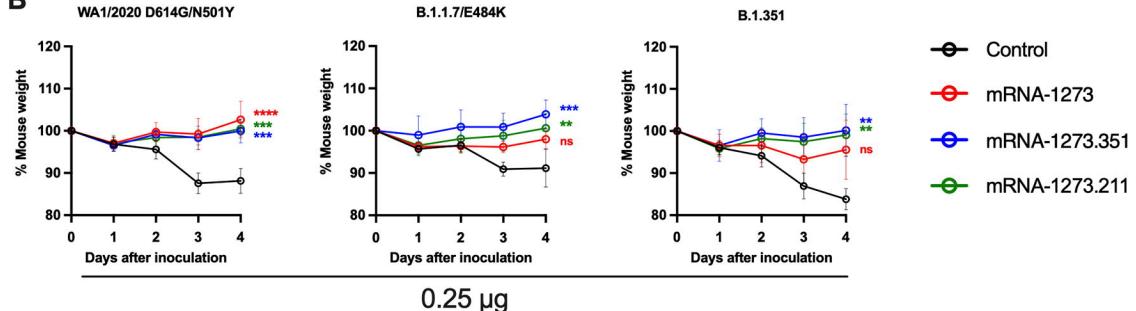
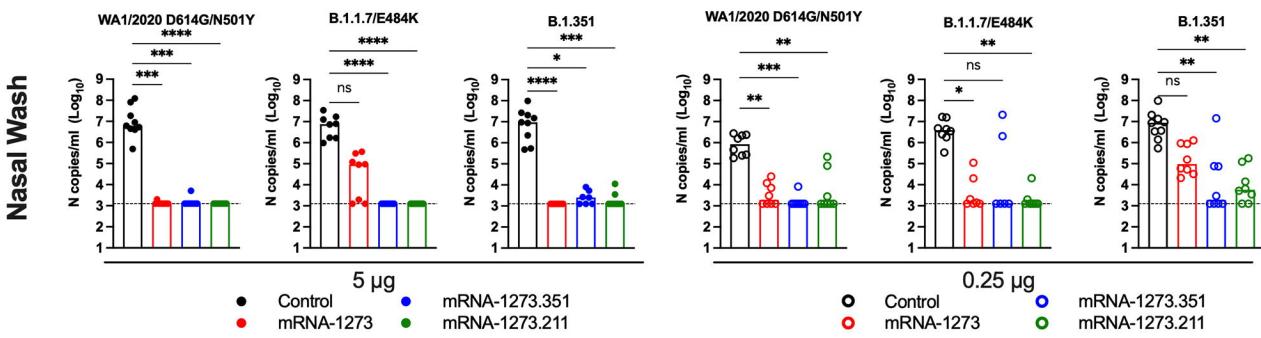
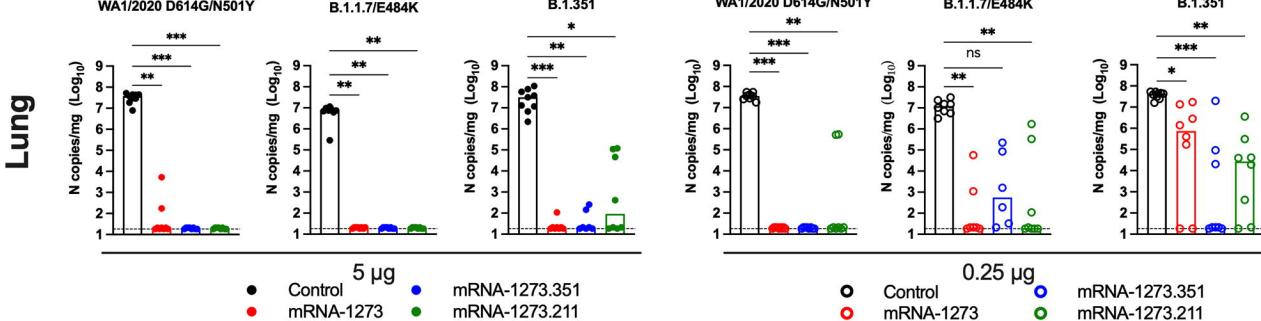
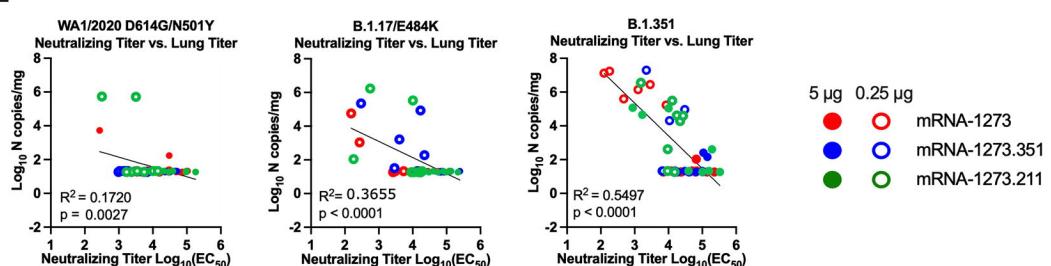
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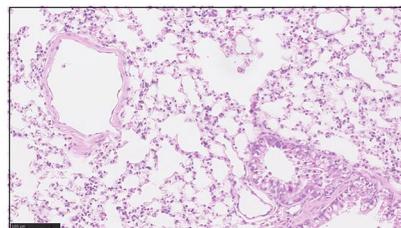
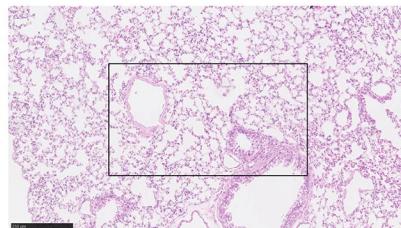
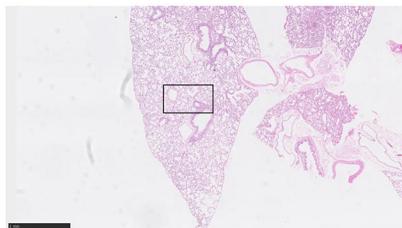
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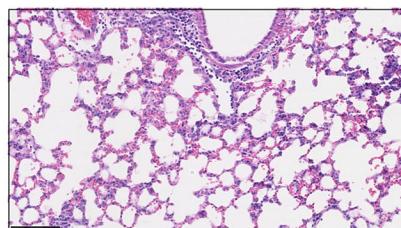
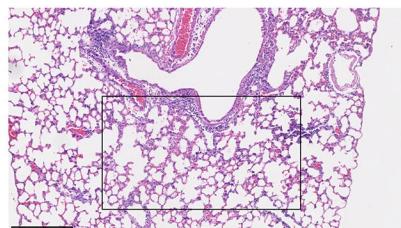
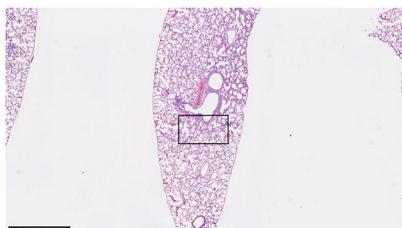
**A****B****C****D****E**

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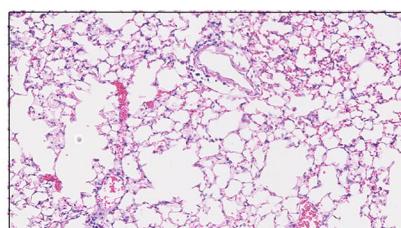
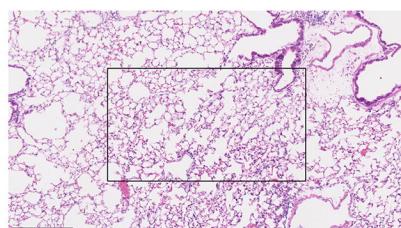
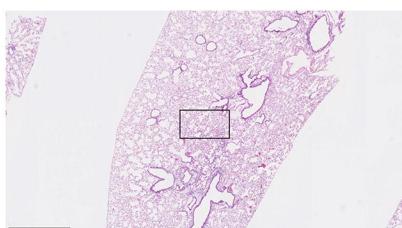
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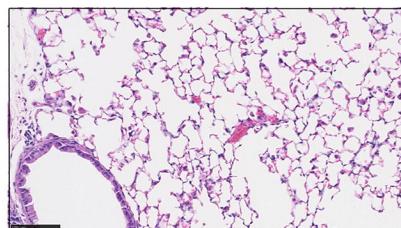
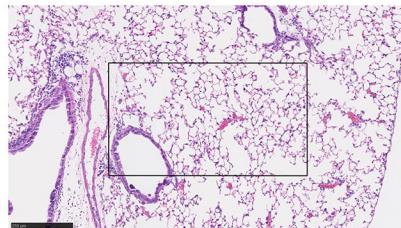
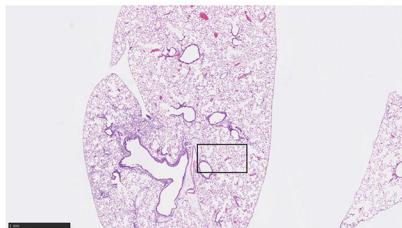
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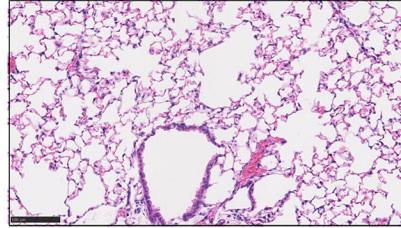
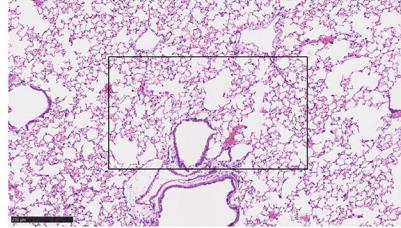
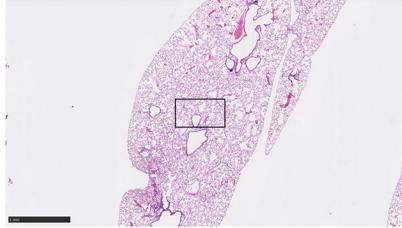
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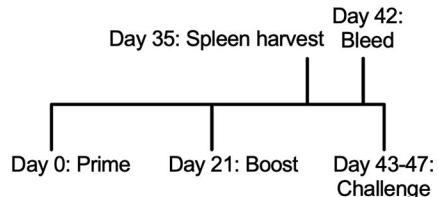
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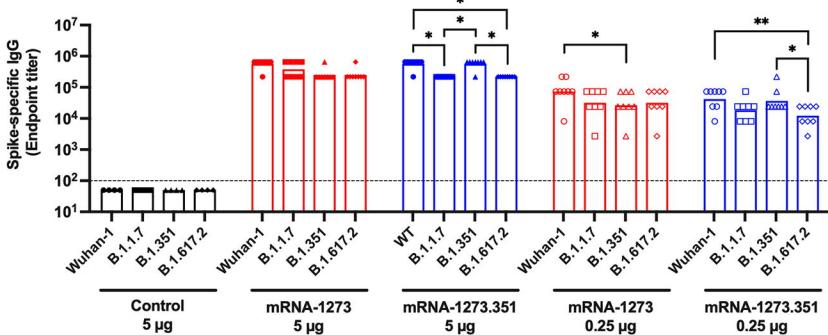
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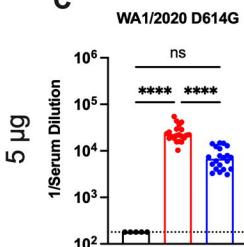
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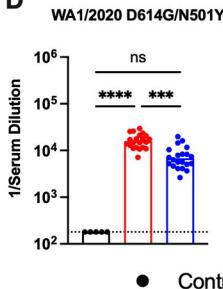
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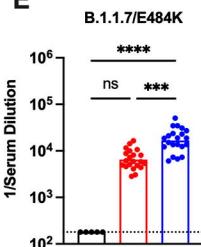
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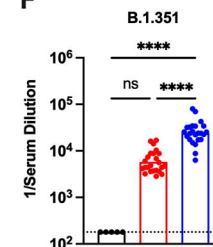
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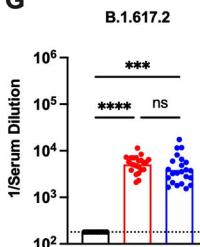
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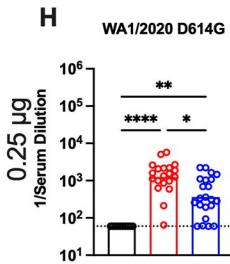
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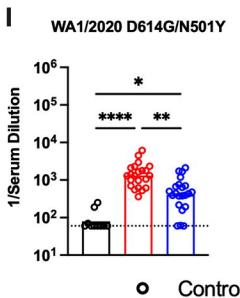
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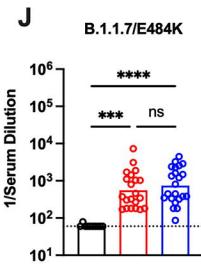
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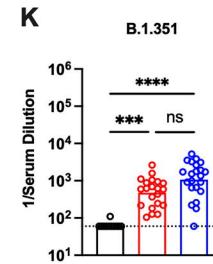
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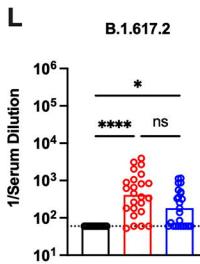
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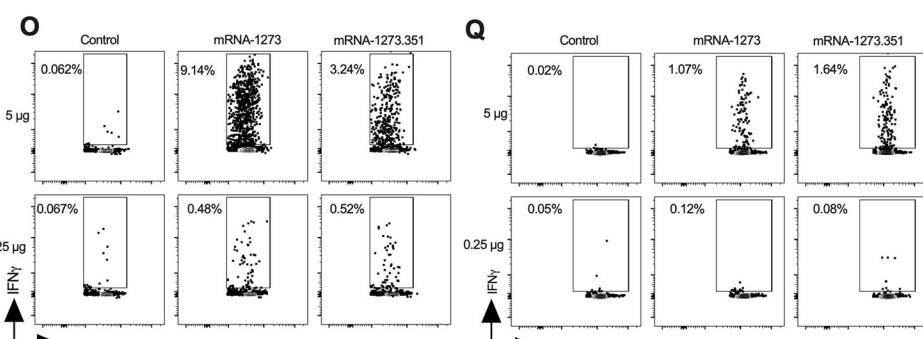
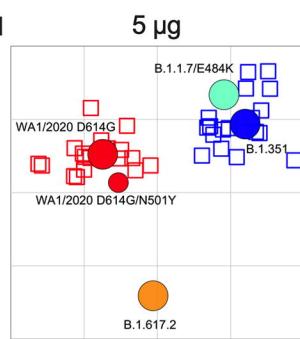
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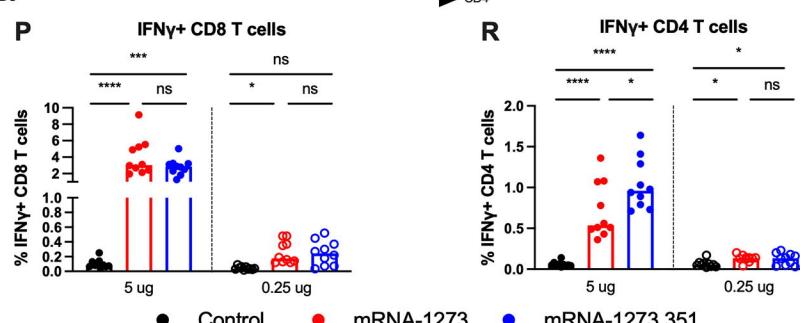
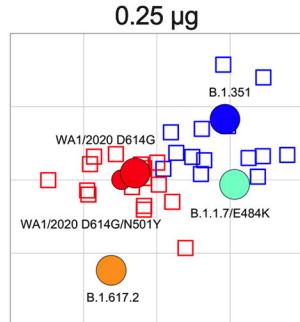
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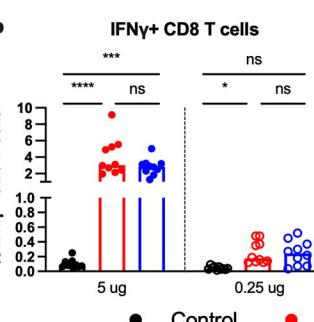
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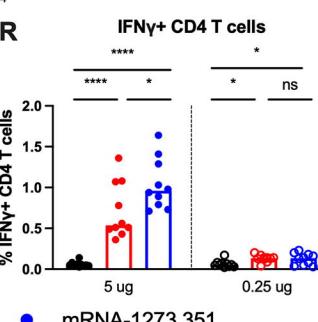
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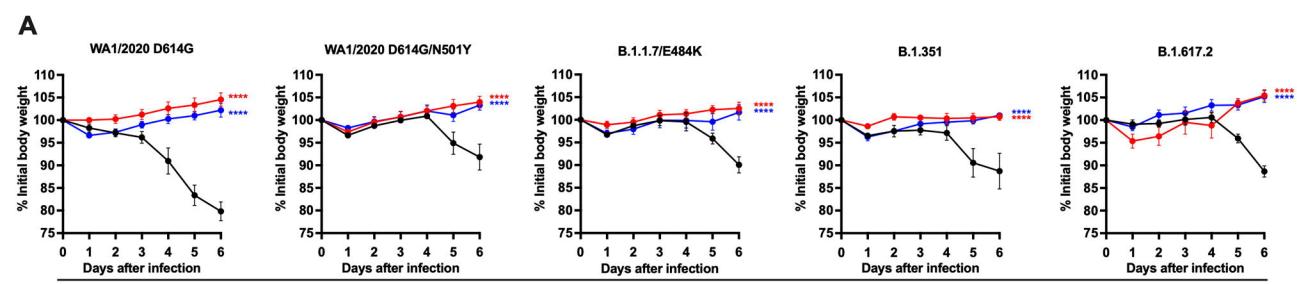


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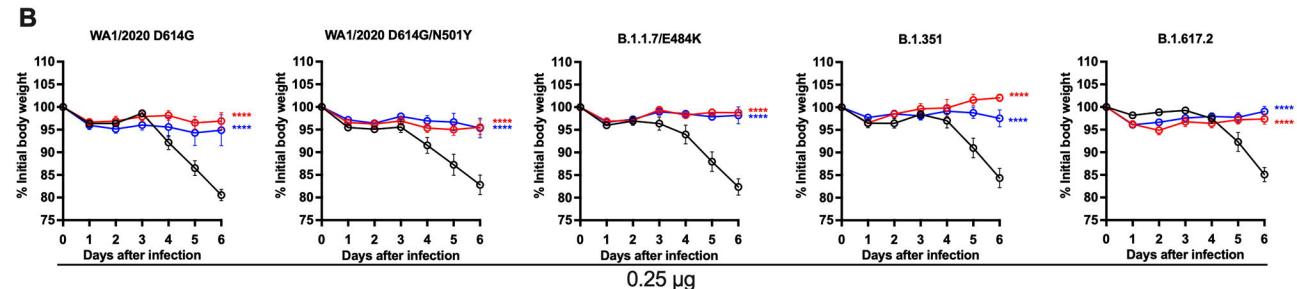
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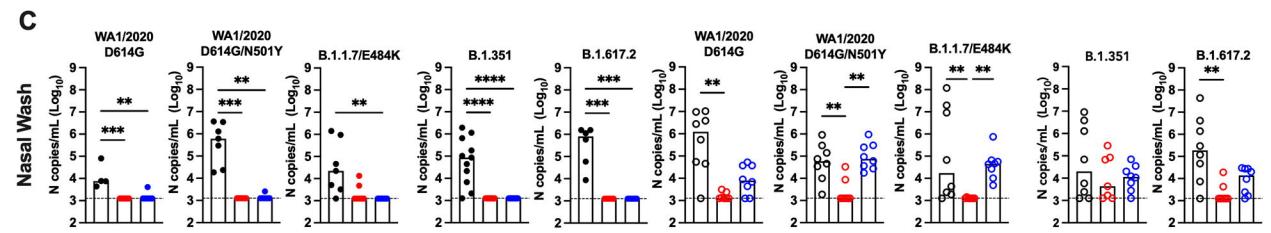
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- Control
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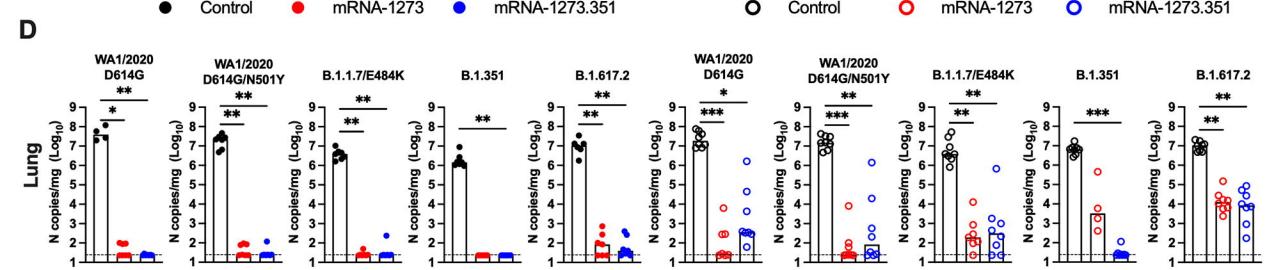


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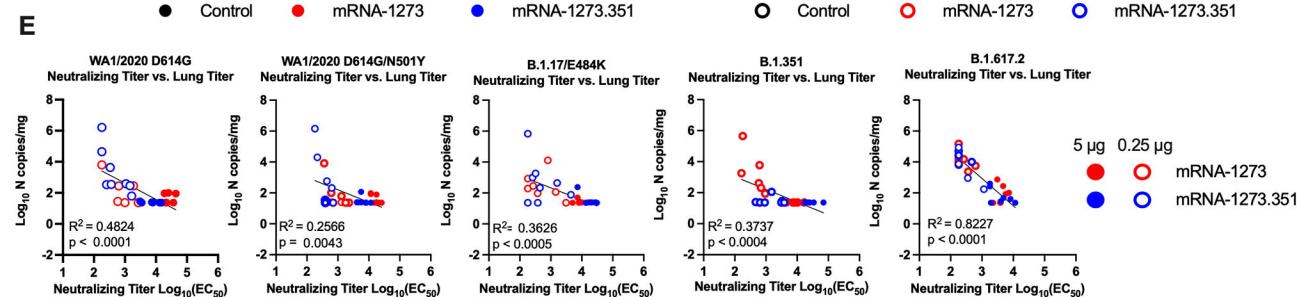
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● Control   ● mRNA-1273   ● mRNA-1273.351



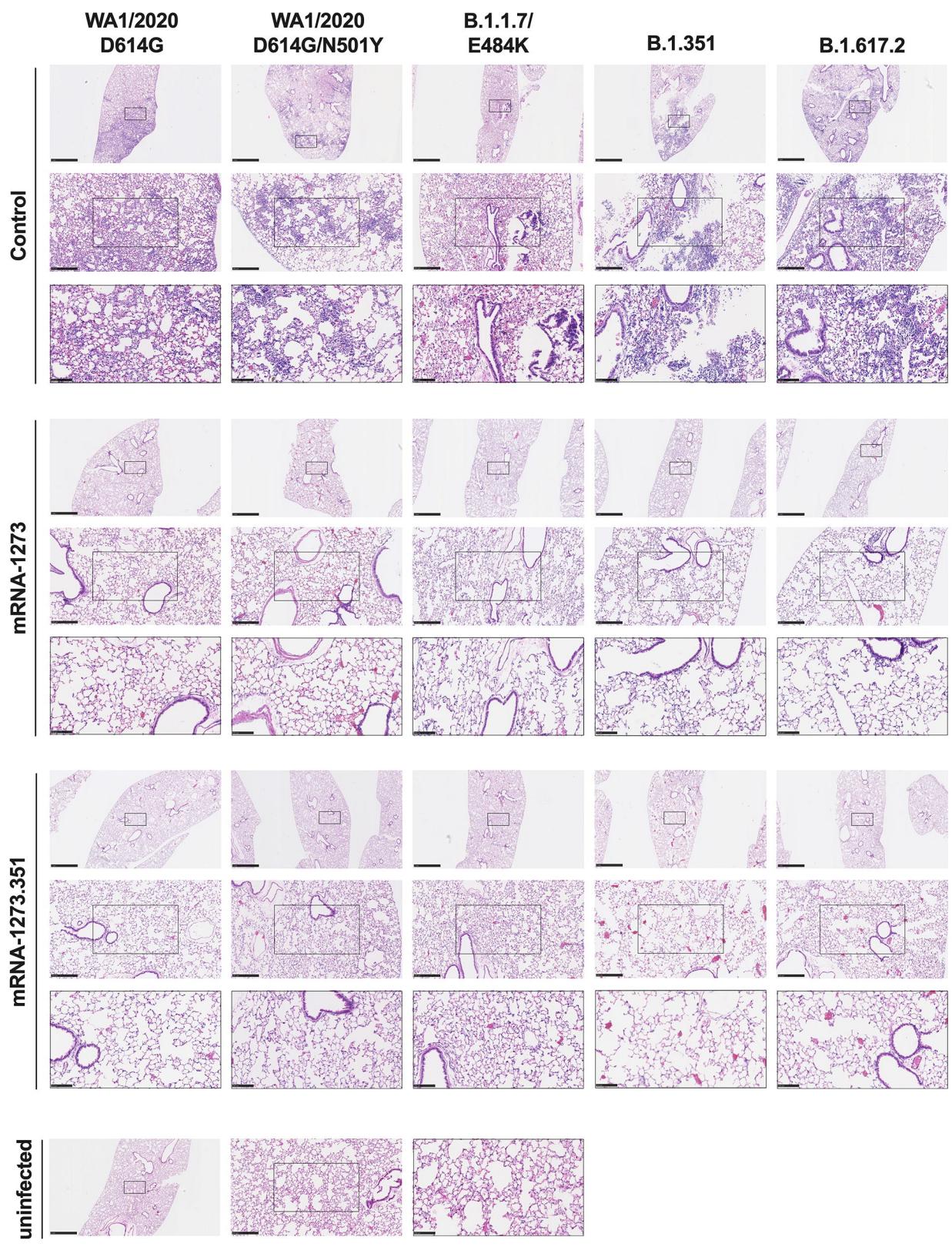
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5  $\mu$ g   0.25  $\mu$ g

● mRNA-1273   ● mRNA-1273.351

5  $\mu$ g



0.25  $\mu$ g

WA1/2020  
D614G

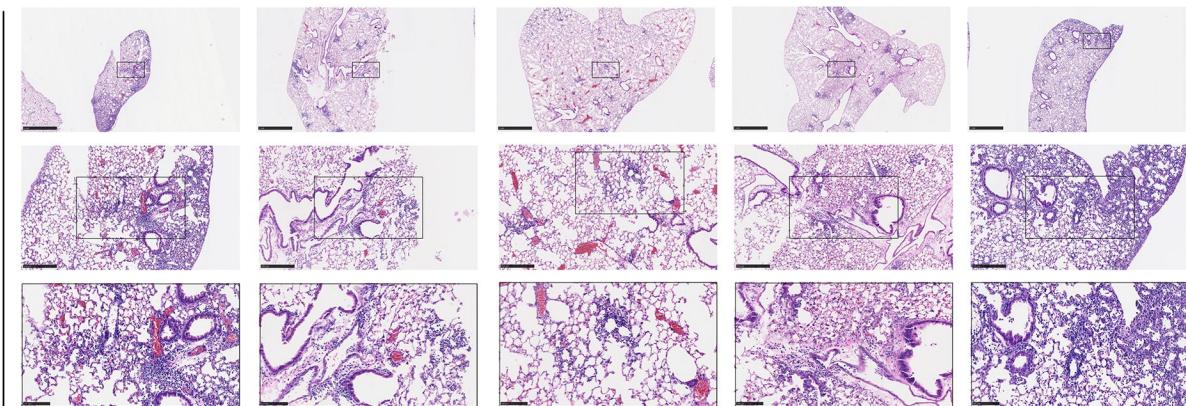
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B.1.1.7/  
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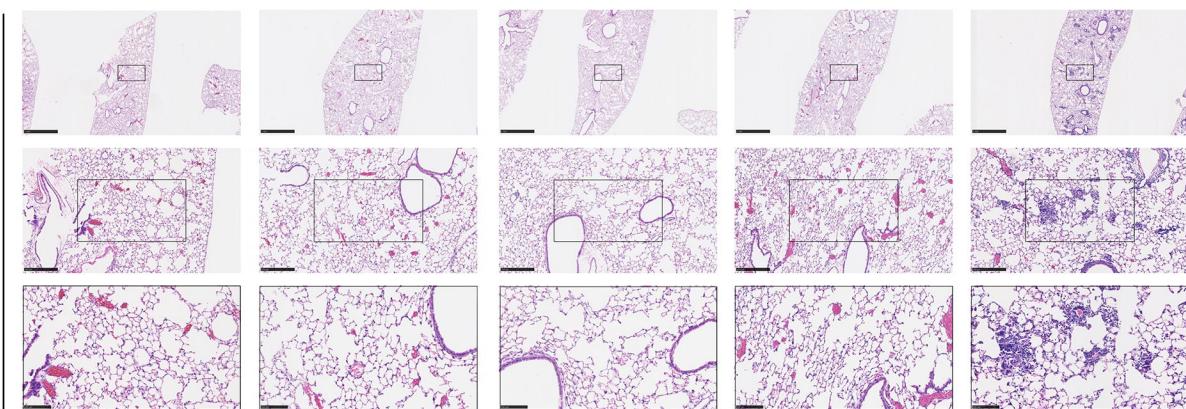
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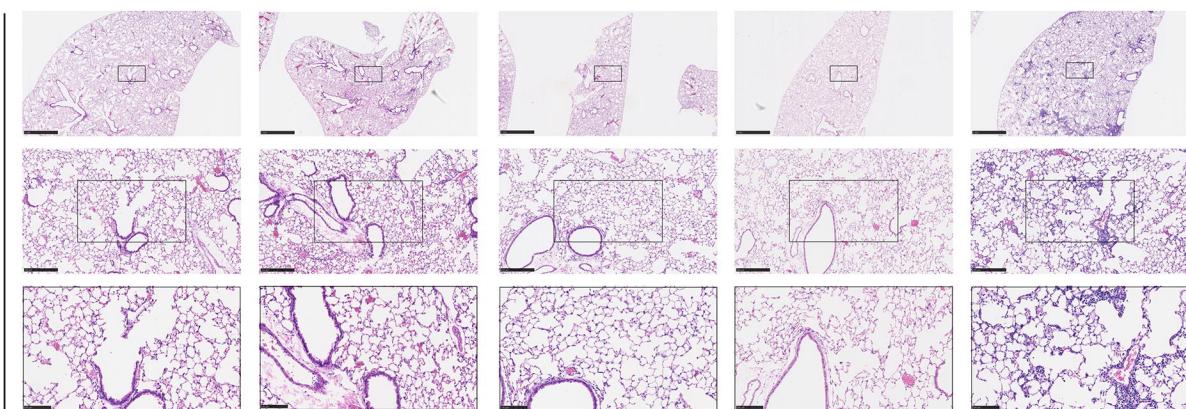
Control



mRNA-1273



mRNA-1273.351



uninfected

