

Omicron-specific mRNA vaccination alone and as a heterologous booster against SARS-CoV-2

Authors

Zhenhao Fang^{1,2,3,*}, Lei Peng^{1,2,3,*}, Renata Filler^{4,5},
Kazushi Suzuki^{1,2,3}, Andrew McNamara^{4,5}, Qianqian Lin^{1,2,3},
Paul A. Renauer^{1,2,3,6}, Luojia Yang^{1,2,3,6}, Bridget Menasche^{4,5,7},
Angie Sanchez^{1,2,3,8}, Ping Ren^{1,2,3}, Qiancheng Xiong^{9,10,11},
Madison Strine^{4,5,7}, Paul Clark^{1,2,3},
Chenxiang Lin^{9,10,11}, Albert I. Ko^{12,13}, Nathan D. Grubaugh^{12,14},
Craig B. Wilen^{4,5,7,@} and Sidi Chen^{1,2,3,6,7,15,16,17,#}

Affiliations

1. Department of Genetics, Yale University School of Medicine, New Haven, CT, USA
2. System Biology Institute, Yale University, West Haven, CT, USA
3. Center for Cancer Systems Biology, Yale University, West Haven, CT, USA
4. Department of Laboratory Medicine, Yale University, New Haven, CT, USA
5. Department of Immunobiology, Yale University, New Haven, CT, USA
6. Molecular Cell Biology, Genetics, and Development Program, Yale University, New Haven, CT, USA
7. Immunobiology Program, Yale University, New Haven, CT, USA
8. Yale College, New Haven, CT, USA
9. Department of Cell Biology, Yale University, New Haven, CT, USA
10. Department of Biomedical Engineering, Yale University, New Haven, CT, USA
11. Nanobiology Institute, Yale University, New Haven, CT, USA
12. Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT, USA
13. Department of Medicine, Section of Infectious Diseases, Yale University School of Medicine, New Haven, CT, USA
14. Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, USA
15. Comprehensive Cancer Center, Yale University School of Medicine, New Haven, CT, USA
16. Stem Cell Center, Yale University School of Medicine, New Haven, CT, USA
17. Center for Biomedical Data Science, Yale University School of Medicine, New Haven, CT, USA

* Co-first authors.

@ Correspondence:

SC (sidi.chen@yale.edu)

CBW (craig.wilen@yale.edu)

Lead contact:

SC (sidi.chen@yale.edu)

+1-203-737-3825 (office)

+1-203-737-4952 (lab)

45 **Abstract**

46 The Omicron variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has high
47 transmissibility and recently swept the globe. Due to the extensive number of mutations, this
48 variant has high level of immune evasion, which drastically reduced the efficacy of existing
49 antibodies and vaccines. Thus, it is important to test an Omicron-specific vaccine, evaluate its
50 immune response against Omicron and other variants, and compare its immunogenicity as boosters
51 with existing vaccine designed against the reference wildtype virus (WT). Here, we generated an
52 Omicron-specific lipid nanoparticle (LNP) mRNA vaccine candidate, and tested its activity in
53 animals, both alone and as a heterologous booster to existing WT mRNA vaccine. Our Omicron-
54 specific LNP-mRNA vaccine elicited strong and specific antibody response in vaccination-naïve
55 mice. Mice that received two-dose WT LNP-mRNA, the one mimicking the commonly used
56 Pfizer/Moderna mRNA vaccine, showed a >40-fold reduction in neutralization potency against
57 Omicron variant than that against WT two weeks post second dose, which further reduced to
58 background level >3 months post second dose. As a booster shot for two-dose WT mRNA
59 vaccinated mice, a single dose of either a homologous booster with WT LNP-mRNA or a
60 heterologous booster with Omicron LNP-mRNA restored the waning antibody response against
61 Omicron, with over 40-fold increase at two weeks post injection as compared to right before
62 booster. Interestingly, the heterologous Omicron LNP-mRNA booster elicited neutralizing titers
63 10-20 fold higher than the homologous WT booster against the Omicron variant, with comparable
64 titers against the Delta variant. All three types of vaccination, including Omicron mRNA alone,
65 WT mRNA homologous booster, and Omicron heterologous booster, elicited broad binding
66 antibody responses against SARS-CoV-2 WA-1, Beta, and Delta variants, as well as other
67 *Betacoronavirus* species such as SARS-CoV, but not Middle East respiratory syndrome
68 coronavirus (MERS-CoV). These data provided direct proof-of-concept assessments of an
69 Omicron-specific mRNA vaccination *in vivo*, both alone and as a heterologous booster to the
70 existing widely-used WT mRNA vaccine form.

71

72 **Key words**

73 Omicron variant, mRNA vaccine, Omicron-specific mRNA vaccine, COVID-19, SARS-CoV-2,
74 lipid nanoparticle (LNP), antibody response, neutralizing antibody, waning immunity, Omicron
75 booster shot, homologous booster, heterologous booster

76 Introduction

77 Since its first identification in specimen collected in November 2021¹, the Omicron variant
78 (lineage B.1.1.529) of SARS-CoV-2 has rapidly spread across the globe²⁻⁵. The Omicron variant
79 was associated with increased risk of reinfection according to a population-level evidence in South
80 Africa⁶. Two days after its initial report to World Health Organization (WHO), Omicron was
81 designated as a variant of concern (VoC) by WHO on November 26⁷. Population-level data
82 indicated that Omicron has become the dominant variant in South Africa in mid-November⁸, only
83 one week after the first traceable case. Similarly in January 2022, Omicron variant has dominated
84 newly diagnosed cases in many states of the US⁹, Canada¹⁰ and UK⁴. Omicron variant drove the
85 fourth “wave” of Coronavirus Disease 2019 (COVID-19) in South Africa¹¹ and around the world¹².
86 Its case doubling time, every 3-4 days, is faster than previous waves¹², hinting its increased
87 intrinsic transmissibility and/or immune evasion. A number of urgent questions on Omicron have
88 quickly become central concerns, which include whether and when Omicron-specific vaccines or
89 therapeutic antibodies will be effective against Omicron variant.

90
91 Mounting clinical and laboratory evidence have shown that most therapeutic and natural antibodies
92 for COVID-19 failed to retain potency against Omicron¹³⁻¹⁸. The Omicron variant has 60 mutations
93 compared to the ancestral variant’s reference sequence (also referred to as prototypic virus / variant,
94 reference, wildtype (WT), Wuhan-Hu-1, or Wuhan-1, in lineage A)⁵. There are 50 nonsynonymous,
95 8 synonymous, and 2 non-coding mutations in Omicron, of which many are not observed in any
96 other variants. There are a total of 32 mutations in the spike gene, which encodes the main antigen
97 target of therapeutic antibodies and of many widely administered vaccines. This results in 30
98 amino acid changes, three small deletions, and one small insertion, of which 15 are within the
99 receptor-binding domain (RBD)¹⁹. Due to its extensive number of mutations, this variant has high
100 level of immune evasion, which drastically reduced the efficacy of existing antibodies and
101 vaccines. Omicron spike mutations are concerning as they cluster on known neutralizing antibody
102 epitopes¹⁵ and some of them have well-characterized consequences such as immune evasion and
103 higher infectivity. In fact, recent reports showed that the majority of the existing monoclonal
104 antibodies developed against SARS-CoV-2 have dramatic reduction in effectiveness against the
105 Omicron variant¹³⁻¹⁸, leading to recall or exclusion of recommended use of certain therapeutic
106 antibodies under emergency authorization²⁰.

107

108 The mRNA vaccines have achieved immense success in curbing the viral spread and reducing the
109 risk of hospitalization and death of COVID-19^{21,22}. However, a significant drop of mRNA
110 vaccine's effectiveness against Omicron has been reported from clinical⁸ and laboratory studies of
111 samples of vaccinated individuals^{23,24}. In light of the heavily altered antigen landscape of Omicron
112 spike, assessing the efficacy of Omicron-specific mRNA vaccine is urgently needed. A number of
113 critical questions regarding Omicron-specific mRNA vaccine need to be addressed. For examples:
114 What is the immunogenicity of Omicron spike used in a vaccine form? Whether potent antibody
115 immunity can be induced by an Omicron-specific mRNA vaccine, and how well does that
116 neutralize the Omicron variant? If, and how, does the immune response induced by the Omicron-
117 specific react to other variants, such as WA-1 (lineage A with a spike gene identical to WT or
118 Wuhan-1) or Delta (lineage B.1.617.2)? Because a large share of world population received
119 authorized Pfizer/BioNTech or Moderna mRNA vaccines that encode the reference (WT) spike
120 antigen, it is important to know whether an Omicron mRNA vaccine can boost the waning
121 immunity of existing vaccinated population. Clinical data showed that heterologous boosting with
122 different types of COVID-19 vaccines elicited neutralizing titers similar to or greater than
123 homologous boosting^{25,26}. It is critical to compare the immunogenicity and efficacy of a
124 heterologous Omicron booster with a homologous WT booster. Last but not least, as the antibody
125 epitopes are closely related to their cross reactivity and susceptibility to variant mutations, it is
126 crucial to know if and to what extent WT or Omicron mRNA vaccine can elicit plasma antibodies
127 possessing broad antibody responses to SARS-CoV-2 variants and *Betacoronavirus* species.

128

129 To answer some of these questions, we directly generated an Omicron-specific lipid nanoparticle
130 (LNP) mRNA vaccine candidate that encodes an engineered full-length Omicron spike with
131 HexaPro mutations, and evaluated its effect alone, and compared its immunogenicity with WT
132 LNP-mRNA as booster shots after SARS-CoV-2 WT mRNA vaccination in animal models.

133

134

135 Results

136 Design, generation and physical characterization of an Omicron-specific LNP-mRNA
137 vaccine candidate

138 We designed an Omicron-specific LNP-mRNA vaccine candidate based on the full-length spike
139 sequence of the Omicron variant (lineage B.1.1.529/BA.1) from two North America patients
140 identified on Nov23rd, 2021 (GISAID EpiCoV: EPI_ISL_6826713 and EPI_ISL_6826714). The
141 spike coding sequence of Wuhan-Hu-1 (WT) and Omicron variant were flanked by 5' UTR, 3'
142 UTR and 3' PolyA tail (Figure 1A). We introduced six Proline mutations (HexaPro) to the spike
143 gene sequence, as they were reported to improve spike protein stability and prefusion state²⁷. The
144 furin cleave site (RRAR) in spike was replaced with GSAS stretch to keep integrity of S1 and S2
145 units. We then encapsulated the transcribed spike mRNA into lipid nanoparticles to produce WT
146 and Omicron LNP-mRNAs, and characterized the quality and biophysical properties by
147 downstream assays including dynamic light scattering, transmission electron microscope and
148 receptor binding assay.

149

150 The dynamic light scattering and transmission electron microscope were applied to evaluate the
151 size distribution and shape of Omicron LNP-mRNA, which showed a monodispersed sphere shape
152 with an average radius of 52 nm and polydispersity index of 0.17 (Figure 1C-1E). To evaluate the
153 effectiveness of LNP-mRNA mediated Omicron spike expression in cells as well as the receptor
154 binding ability of the designed Omicron HexaPro spike, Omicron LNP-mRNA was directly added
155 to HEK293T cells 16 hours before subjecting cells to flow cytometry. Evident surface expression
156 of functional Omicron HexaPro spike capable of binding to human angiotensin-converting
157 enzyme-2 (hACE2) was observed by staining cells with hACE2-Fc fusion protein and PE anti-Fc
158 secondary antibody (Figure 1F). These data showed that the Omicron spike sequence was
159 successfully encoded into an mRNA, encapsulated into the LNP, can be introduced into
160 mammalian cells efficiently without additional manipulation, and express functional spike protein
161 that binds to hACE2.

162

163 **Specific binding and neutralizing antibody response elicited by Omicron LNP-mRNA** 164 **against the Omicron variant**

165 After ensuring functional spike expression mediated by Omicron LNP-mRNA, we proceeded to
166 characterizing the immunogenicity of Omicron LNP-mRNA *in vivo*. In order to test rapid immune
167 elicitation against Omicron variant, we performed the following vaccination and testing schedule.
168 Two doses of 10 µg Omicron LNP-mRNA, as prime and boost two weeks apart were

169 intramuscularly injected into ten C57BL/6Ncr (B6) mice (**Figure 1G; Figure S1A**). Retro-orbital
170 blood was collected prior to immunization on day 0, 13 and 21, i.e. two weeks post prime (one day
171 before boost), and one week post boost. We then isolated plasma from blood, which was used in
172 enzyme-linked immunosorbent assay (ELISA) and neutralization assay to quantify binding and
173 neutralizing antibody titers. A significant increase in antibody titers against Omicron spike RBD
174 was observed in ELISA and neutralization assays from plasma samples post prime and boost
175 (**Figure 1H-1I; Figure S1A-B**). We performed neutralization with infectious virus (also
176 commonly referred to as authentic virus or live virus) using a local SARS-CoV-2 Omicron isolate
177 in a biosafety level 3 (BSL3) setting (Methods), and validated that the plasma samples from mice
178 vaccinated with Omicron-specific LNP-mRNA showed potent neutralization activity against
179 infectious Omicron virus, with significant prime / boost effect (**Figure 1J-K**). These data showed
180 that the Omicron LNP-mRNA induced strong and specific antibody responses in vaccinated mice.
181

182 **Waning immunity of WT LNP-mRNA immunized mice**

183 In light of the wide coverage of the ancestral **WT**-based LNP-mRNA vaccine (to model those
184 widely administered in the current general population), we sought to test: (i) the effect of **WT** LNP-
185 mRNA vaccination against Omicron variant, (ii) the decay of immunity induced by **WT** LNP-
186 mRNA over time, and (iii) whether a homologous **WT** LNP-mRNA booster or a heterologous
187 Omicron LNP-mRNA booster could enhance the waning immunity against Omicron variant, WA-
188 1 and/or Delta variant, and if there is a difference between homologous and heterologous boost.
189 To gain initial answers to these questions in animal models, we sequentially vaccinated two cohorts
190 of B6 mice with two doses of **WT** and one dose of **WT** or Omicron LNP-mRNA booster in two
191 independent experiments (Batch 1 in **Figure S2** and batch 2 in **Figure S3**). Over 100-day interval
192 between 2nd dose of **WT** and **WT**/Omicron booster was ensured in order to observe the waning
193 immunity in **WT**-vaccinated mice (the combined and individual datasets from the two independent
194 experiments were presented in **Figures 2** and **S2-S3** respectively). We collected blood samples of
195 these animals in a rational time series, including day 35 (2 weeks post 2nd dose of **WT** LNP-
196 mRNA), >3.5 months post 2nd doses of **WT** LNP-mRNA (day 127 in batch 1 or day 166 in batch
197 2, immediately before **WT**/Omicron booster), ~2 weeks post **WT**/Omicron LNP-mRNA booster
198 (day 140, one day before the second Omicron booster in batch 1 or day 180 in batch 2), and day
199 148 (1 week post two doses of Omicron LNP-mRNA vaccination in batch 1).

200
201 Plasma samples were isolated from blood samples and analyzed in ELISA and neutralization
202 assays against SARS-CoV-2 Omicron, Delta or WA-1. Comparing to the titers against WA-1 and
203 Delta RBD, the binding antibody titers against Omicron RBD elicited by WT mRNA-LNP were
204 significantly weaker in samples from both day 35 and >3.5 months (Figures 2B, S4 and S5). The
205 group average Omicron reactivity is 15-fold (day 35) and 21-fold (>3.5 months) lower than that
206 of WT RBD (fold change = ratio -1), and 11-fold (day 35) and 14-fold (>3.5 months) lower than
207 Delta (Figure S5). A steep (orders of magnitude) drop of antibody titers from mice immunized
208 with WT LNP-mRNA was observed after three months (day 35 vs. >3.5 months) from all three
209 RBD datasets. It is worth noting that the antibody titers >3.5 months post WT boost decreased to
210 a level that is near-baseline (Phosphate buffered saline, PBS controls, Figure 2), particularly for
211 titers against Omicron RBD.

212
213 **Heterologous booster with Omicron LNP-mRNA as compared to homologous booster with**
214 **WT LNP-mRNA in mice that previously received a two-dose WT LNP-mRNA vaccination**

215 A single dose booster shot, either a homologous booster with WT LNP-mRNA, or a heterologous
216 booster with Omicron LNP-mRNA, drastically increased the antibody titers against Omicron RBD,
217 by over 100-fold as compared to the sample right before booster shot (Figure 2B), reaching a level
218 comparable to the post-boost titer by Omicron LNP-mRNA alone (Figure 1H). The mice that
219 received the Omicron LNP-mRNA booster showed a trend of higher binding antibody titer against
220 Omicron RBD than those administered with WT booster. Interestingly, the Omicron LNP-mRNA
221 shot boosted not only titers against Omicron RBD, but also titers against Delta and WA-1 RBD,
222 of which levels were comparable with those elicited by WT LNP-mRNA booster (Figure 2B). For
223 both WT and Omicron boosters, the extent of titer increase was more drastic in the Omicron RBD
224 dataset than other RBD datasets, signifying the extra benefit of booster shots against Omicron
225 variant (Figure 2B). The antibody titers did not increase one week after a second booster of
226 Omicron LNP-mRNA (Figure S2B).

227
228 Because pseudovirus neutralization is a relatively safer and widely-used assay that strongly
229 correlates with infectious virus results and has been regarded as a standard proxy by the field^{24,28-}
230³⁰, we set out to first use pseudovirus neutralization assay to measure the neutralizing antibody

231 responses induced by Omicron LNP-mRNA booster in these animals. We first generated human
232 immunodeficiency virus-1 (HIV-1) based Omicron pseudovirus system, which contains identical
233 Omicron mutations in vaccine antigen, but lacks the HexaPro or furin site modifications.
234 Interestingly, we found that under exactly the same virus production and assay conditions, the
235 Omicron pseudovirus has higher infectivity than both WA-1 (8x increase) and Delta (4x)
236 pseudoviruses (**Figure S6A-C**), which was also observed by another group²⁴, in concordance with
237 the Omicron - hACE2 interactions from biophysical and structural studies^{31,32}, and correlated with
238 higher transmissibility reported previously^{24,33,34}.

239
240 We then normalized the pseudoviruses by functional titers (number of infected cells / volume),
241 and used this system to perform pseudovirus neutralization assays on all of plasma samples
242 collected (**Figure S6D-E**). The neutralization results showed a consistent overall pattern as ELISA
243 results, with a stronger contrast among titers against Omicron pseudovirus (**Figure 2C**). On day
244 35 and > 3.5 months post WT boost, the mice showed significantly lower neutralizing antibody
245 titers against Omicron variant than titers against Delta variant or WA-1 (**Figure S7A-B**). For the
246 samples two weeks post boost (day 35), the group average Omicron neutralization reactivity is 40-
247 fold lower than that of WA-1 RBD, and 10-fold lower than Delta (**Figure S7B**). When comparing
248 samples collected on day 35 and >3.5 months post WT boost, around two orders of magnitude
249 (10s~100s of fold change) time-dependent titer reduction was unequivocally observed in all three
250 pseudovirus neutralization data (**Figure S2C**). The Omicron-neutralization activity of WT
251 vaccinated mice >3.5 months post boost was as low as PBS background (**Figure 2C**). These data
252 suggested that there was waning antibody immunity in the standard two-dose WT vaccinated
253 animals, which lost neutralization ability against the Omicron variant pseudovirus.

254
255 A single booster shot of WT or Omicron LNP-mRNA vaccine enhanced the antibody titers against
256 Omicron variant two weeks after the injection by >40-fold (**Figure 2C**). The heterologous
257 Omicron LNP-mRNA booster induced significantly higher neutralizing titer against Omicron
258 pseudovirus than the homologous WT LNP-mRNA booster (**Figure 2C**). The neutralizing titer
259 after this surge by Omicron vaccine numerically surpassed the titer two weeks post WT vaccine
260 boost (day 35, **Figure S2C**). Interestingly, the Omicron mRNA vaccine also rescued the antibody
261 titers against Delta and WA-1 pseudoviruses, with two orders of magnitude increase in both ELISA

262 titers and neutralization activity (**Figure 2B-2C**). The neutralization titers of Delta pseudovirus
263 were found similar between WT and Omicron booster groups (**Figure 2C**). A second booster shot
264 two weeks after the first of Omicron mRNA vaccine yielded little increase in neutralization activity
265 against Omicron, WA-1 or Delta variants at the time measured (day 148, 1 week after the second
266 dose) (**Figure S2C**).

267
268 We went on to further evaluate the effects of WT and Omicron LNP-mRNA boosters in infectious
269 virus neutralization assay, which closely correlated with pseudovirus neutralization results. The
270 Omicron LNP-mRNA booster led to over 200-fold increase in neutralizing titers of infectious
271 Omicron virus (**Figure 2D**), while WT booster induced a moderate increase (10-fold) in titers
272 against Omicron live virus (**Figure 2D**). A significant boost of infectious Delta virus neutralizing
273 titers was observed in mice receiving WT (12-fold) and Omicron (19-fold) LNP-mRNA boosters.
274 A 20-fold difference in post-booster (day 180) neutralizing titers against infectious Omicron virus
275 was observed between WT and Omicron booster groups (**Figure 2D**). Together, these data suggest
276 that while both WT LNP-mRNA and Omicron LNP-mRNA boosters can strengthen the waning
277 immunity; however, the heterologous booster with Omicron-specific mRNA vaccination (WT x2
278 + Omicron x1) has an effect significantly stronger than the homologous booster (WT x 3) against
279 the live virus of Omicron variant, with comparable activity against the Delta variant.

280
281 Overall, the ELISA titers, pseudovirus and infectious virus neutralization activity were
282 significantly correlated with each other across all groups and animals tested (**Figure S9**). These
283 data suggested that a single dose of Omicron LNP-mRNA heterologous booster not only induced
284 more potent anti-Omicron antibody response than WT booster, but also elicited broad activity
285 against the WA-1 and Delta variant, in mouse models at the timepoints measured.

286
287 **Cross reactivity and epitope characterization of plasma antibodies from homologous**
288 **Omicron mRNA, WT mRNA or heterologous WT + Omicron mRNA vaccination schemes**

289 In light of the broad activity elicited by heterologous vaccination of WT and Omicron LNP-mRNA,
290 we ask if these vaccination schemes can induce antibody responses against other SARS-CoV-2
291 variants and other pathogenic *Betacoronavirus* species. We sought to answer these questions by
292 characterizing and comparing the anti-coronavirus cross reactivity conferred by Omicron mRNA

293 vaccination alone, WT mRNA vaccination alone (homologous booster), or their uses in
294 combination (Omicron mRNA vaccination as a heterologous booster on top of WT mRNA
295 vaccination). The cross reactivity was evaluated using six spike RBDs, including SARS-CoV-2
296 WA-1, Beta (lineage B.1.351) variant, Delta variant, Omicron variant, SARS-CoV spike RBD
297 (SARS RBD) and MERS-CoV spike RBD (MERS RBD). Two doses of Omicron LNP-mRNA
298 induced high titers of antibodies that cross reacted with all spike RBDs tested except for MERS
299 RBD, which shared low sequence identity (<40%) to SARS or SARS-CoV-2 spikes (**Figure 3A**).
300 The antibody titer against SARS RBD was significantly lower than those against SARS-CoV-2
301 WA-1 or variants (**Figure S10A**). Among the SARS-CoV-2 variants characterized, the antibody
302 response to Delta variant by Omicron LNP-mRNA was slightly weaker than others. Both WT and
303 Omicron boosters after WT LNP-mRNA prime and boost led to potent antibody response to
304 SARS-CoV and SARS-CoV-2 Beta variant (**Figure 3B**), while the response to MERS RBD was
305 negligible and similar to PBS control. Within each ELISA antigen except for MERS RBD and
306 Omicron RBD, the antibody response post WT or Omicron boosters (3 shots total) was numerically
307 higher than that of plasma samples post a two-dose Omicron vaccine (Omicron x 2) (**Figure S10C**).
308
309 A number of studies have shown that antibodies whose epitopes overlap with hACE2-binding
310 motif were largely escaped by RBD mutations in variants of concerns, while antibodies whose
311 epitopes fall outside the hACE2-binding motif were rarer and often exhibit broad neutralizing
312 activity to SARS-like *Betacoronaviruses* (*Sarbecoviruses*)³⁵⁻³⁸. Because of such correlation
313 between antibody epitope and cross reactivity, we performed competition ELISA using hACE2 or
314 antibodies with known epitopes as competing agents to evaluate the epitopes, population and
315 affinity of plasma antibodies elicited by Omicron or WT LNP-mRNA. The epitopes of RBD can
316 be categorized into several major classes based on cluster analysis of available neutralizing
317 antibody-RBD complex structures³⁸⁻⁴². We displayed representative antibodies in each major
318 epitope classes by aligning them with the recently solved Omicron RBD:hACE2 complex
319 structure^{31,32} (**Figure 3C**). We then performed hACE2 and antibody competition ELISA using
320 hACE2, Clone 13A, S309 and CR3022 as competing reagents to see if and to what extent group
321 A-D³⁵ (class I-III³⁶, epitopes overlapped with hACE2) and group E-F (class IV, S309 and CR3022)
322 antibodies were induced by these immunization schemes. Low-density Omicron RBD was coated
323 in ELISA plate to ensure adequate competition between plasma antibodies and competing hACE2

324 or antibodies. In two independent experiments (hACE2 and antibody competition assays), the
325 baseline titer of heterologous Omicron booster treated mice (WT x 2 + Omicron) in the absence
326 of competing reagents was significantly higher than those of homologous WT booster treated mice
327 (WT x 3), or mice receiving Omicron vaccination alone (Omicron x 2) (**Figure 3D**). Addition of
328 high concentration hACE2 (Methods) resulted in a significant reduction of plasma antibody titers
329 in mice vaccinated with Omicron (Omicron x2), WT (WT x 3) or WT + Omicron (WT x2 +
330 Omicron) LNP-mRNA (**Figure 3E**). In the antibody competition assay, we used three antibodies
331 with known RBD epitopes. Two of them (CR3022 and S309) are well-characterized representative
332 antibodies from non-hACE2 competing classes. The Clone 13A is a humanized neutralizing
333 antibody developed in our lab previously⁴³ and has an epitope that overlaps with the hACE2
334 binding motif. All three antibodies led to a significant decrease of plasma titers from Omicron
335 vaccinated mice (Omicron x 2), while only CR3022 and S309 mediated a titer reduction in WT
336 booster group (WT x 3) (**Figure 3F**). The WT + Omicron heterologous vaccination group showed
337 minimal titer changes to all three antibodies (**Figure 3F**). These data suggested that a significant
338 percentage of the pool of antibodies elicited by Omicron- or WT- vaccination shared binding
339 epitopes with hACE2. In addition, antibody competition ELISA showed that both Omicron LNP-
340 mRNA and WT LNP-mRNA vaccinated animals contained plasma antibodies targeting rare
341 epitopes in class IV (or group E/F), which often exhibit broad activity against *Sarbecoviruses*.

342

343

344 Discussion

345 The rapid spread of Omicron around the world, especially in countries with wide coverage of
346 vaccines designed based on the ancestral antigen (e.g. WT mRNA vaccine), is particularly
347 concerning. The extensive mutations in **the Omicron spike gene** mark a dramatic alteration in its
348 antigenicity¹⁵. Omicron has high transmissibility and high level of immune evasion from WT
349 mRNA vaccine induced immunity, which was reported from various emerging literature^{6,14-17}.
350 Omicron's strong association with reinfection⁶ or breakthrough infection^{8,12} and its heavily altered
351 antigenicity prompted the idea of developing Omicron-specific mRNA vaccine.

352

353 To date (as of Feb 20, 2022), 4.35 billion people, i.e. 56% of the global population, received
354 COVID-19 vaccination (Our World in Data⁴⁴). Almost all those vaccines were designed based on

355 the antigen from the ancestral virus, including the two approved mRNA vaccine BNT162b2²¹ and
356 mRNA-1273²². Individuals receiving existing COVID-19 vaccines have waning immunity
357 overtime⁴⁵⁻⁴⁸. Consistent with past reports, we observed a dramatic time-dependent decrease
358 (around 40-fold) of antibody titers against Omicron, Delta variants and WA-1 strains 3 months
359 after the second dose of WT mRNA vaccine in mice. This observed waning immunity is
360 particularly concerning in the scenario of rapid spreading of Omicron variant, which largely
361 escapes the humoral immune response elicited by **WT** mRNA vaccines as evident in published
362 studies^{14-16,18} as well as in our current data. **A recent report showed waning immunity in vaccinated**
363 **individuals²⁴ and that a booster shot using the WT based mRNA vaccine helps recover partial**
364 **immunity. Our data showed that the neutralizing antibody titers after the boost with a WT based**
365 **vaccine were still lower against Omicron than against WA-1 and other variants, urging for**
366 **development and testing of an Omicron-specific vaccine. Vaccinee receiving heterologous**
367 **vaccination of WT and Omicron LNP-mRNA have been exposed to both antigens and may have**
368 **robust antibody response against cognate strains and other VoCs. Thus, it is important to evaluate**
369 **and compare the immunogenicity of Omicron-specific vaccine candidate with WT vaccine as**
370 **booster shots on top of two doses of WT mRNA vaccine. In fact, very recently, both Pfizer and**
371 **Moderna have started their clinical trials to evaluate the efficacy of Omicron-specific mRNA**
372 **vaccine in either homologous or heterologous vaccination settings⁴⁹⁻⁵¹. Moderna has released an**
373 **updated Phase 2/3 clinical trial for their Omicron-specific mRNA vaccine (mRNA-1273.529)**
374 **along with the WT vaccine mRNA-1273 against COVID-19 Omicron variant (NCT05249829).**
375 **The scale and swiftness of initiating these clinical trials exemplify the clinical importance and**
376 **urgent need of curbing the Omicron surge and evaluating the Omicron-specific mRNA vaccine.**
377
378 **In this study, we generated a HexaPro-version full-length Omicron spike LNP-mRNA vaccine**
379 **candidate. In mouse models, we found that it can induce potent Omicron-specific and broad anti-**
380 **Sarbecovirus antibody response. With this vaccine candidate, we compared its boosting effect with**
381 **WT counterpart on animals that previously received two-dose WT mRNA vaccine. An observation**
382 **is that a single dose of WT or Omicron boosters significantly strengthened the waning immunity**
383 **against Omicron and Delta variants. A number of recent preprints generated and tested Omicron-**
384 **specific vaccine candidates, which had different vaccine antigen designs, compositions, and**
385 **showed varying results of antibody responses alone or as boosters⁵²⁻⁵⁷ (briefly summarized in**

386 **Table S1**). Three of them focused on evaluation of Omicron RBD mRNA vaccine alone in mice
387 through neutralization assay and reported antibody response against Omicron but not other
388 variants^{52,55,56}. Two studies characterized the Omicron full-length spike mRNA stabilized by two
389 proline mutations (S-2P) and compared their boosting efficacy with WT vaccine in mice⁵³ and
390 macaque⁵⁴. Preprints Gagne et. al. and Ying et. al have shown that both WT and Omicron full-
391 length spike mRNA boosters provided equivalent protection from Omicron challenge in non-
392 human primates (NHPs) ⁵⁴ or mice⁵³. These results shared some commonalities, i.e. the
393 effectiveness of an Omicron-specific vaccine; however, they diverged in the specific titers, as well
394 as in the difference between WT- and Omicron-specific vaccines, potentially due to differences in
395 vaccine antigen designs, compositions, modifications, experimental settings, animal models, or a
396 combination of factors. Our study evaluated the potency of an Omicron-specific full-length spike
397 mRNA vaccine with HexaPro mutations²⁷, which were shown to stabilize the spike in prefusion
398 state. Through well-correlated data from ELISA, pseudovirus and infection virus neutralization
399 assays, we showed that both WT and Omicron boosters significantly restored waning immunity
400 against Omicron and Delta variants. Interestingly, without sacrificing potency against Delta,
401 heterologous Omicron booster achieved significantly higher neutralizing titers against Omicron
402 than homologous WT booster. This observation is in line with findings from heterologous booster
403 vaccination of different COVID-19 vaccines in clinical trials^{25,26}. The broad anti-coronavirus
404 activity after homologous or heterologous boosting was likely associated with plasma antibodies
405 in rarer epitope classes, as observed in competition ELISA.

406
407 It has been shown that the neutralizing antibody level is highly predictive of immune protection
408 from SARS-CoV-2 infection and the initial neutralization level is associated with decay of vaccine
409 efficacy over time⁵⁸. Compared to WT booster, we found that Omicron booster group consistently
410 showed 10-20 fold higher titers against Omicron variant in ELISA, pseudovirus and infectious
411 virus neutralization assays. Within the WT vaccinated group, the titer contrast against Omicron vs.
412 Delta variants persisted over time. Omicron-booster group have been exposed to both WT and
413 Omicron antigens and showed equally potent titers against Omicron and Delta. While our study is
414 in animals, the antibody responses to vaccination are conserved between mouse and human,
415 highlighted by the fact that mice are the main preclinical model used by vaccine developers ^{59,60}.

416

417 The titer against Omicron by single dose Omicron LNP-mRNA was similar to that observed 2
418 weeks post boost of WT LNP-mRNA (log₁₀ AUC or log₁₀ IC₅₀ around 3), although it is still
419 unclear whether the potency of the Omicron mRNA vaccine is associated with the high number of
420 Omicron mutations. As various extent of cross reactivity was observed among WT and/or Omicron
421 vaccinated animals, we sought to understand their cross-reactive immunity by characterizing
422 vaccine-elicited antibody epitopes and population through competition ELISA. In the Omicron
423 RBD competition ELISA, the baseline titer of Omicron LNP-mRNA booster group (WT x2 +
424 Omicron) was significantly higher than WT booster (WT x 3) or Omicron LNP-mRNA (Omicron
425 x 2), which may explain its lower susceptibility to the block of competing antibodies. All three
426 vaccination groups showed significant titer reduction in presence of hACE2, suggestive of
427 abundant plasma antibody population sharing hACE2 binding epitopes, which are often associated
428 with immune escape by variants mutations. The plasma from mice vaccinated with two doses of
429 Omicron LNP-mRNA (Omicron x 2) or three doses of WT LNP-mRNA (WT x 3) exhibited
430 comparable baseline titers and significant titer decrease when co-incubated with CR3022 or S309
431 blocking antibodies, indicating the existence of plasma antibody population sharing group E/F³⁵
432 or class IV³⁶ epitopes. Because of their similar baseline titers, the greater titer reduction in WT
433 booster group may stem from larger population of group E/F antibodies, which was associated
434 higher cross-reactive response against SARS RBD (Figure S10C). Albeit insignificant, the titer
435 change of Omicron booster group (WT x 2 + Omicron) by S309 antibody was greatest among three
436 competing antibodies, hinting a role of epitope IV antibodies in the cross immunity elicited by
437 heterologous vaccination of WT and Omicron LNP-mRNA.

438
439 In summary, this study generated an Omicron-specific HexaPro spike LNP-mRNA vaccine
440 candidate, studied its immunogenicity, and compared it with the WT counterpart in the context of
441 previously WT vaccinated animals. Our results showed that a single dose of either a homologous
442 booster with WT LNP-mRNA or a heterologous booster with Omicron LNP-mRNA restored the
443 waning antibody response, with over 200-fold titer increase by Omicron boosters. Interestingly,
444 the heterologous Omicron LNP-mRNA booster elicited Omicron neutralizing titers higher than the
445 homologous WT booster. The heterologous Omicron booster shot provided strong neutralizing
446 antibody response against Omicron variant and comparable humoral antibody against WA-1 and
447 Delta variants. All three types of vaccination, including Omicron mRNA alone, WT mRNA alone,

Omicron vs WT vaccine boosters

448 and Omicron as a heterologous booster on top of WT mRNA, elicited broad antibody responses,
449 including activities against SARS-CoV-2 VoCs, as well as other *Betacoronavirus* species such as
450 SARS-CoV, but not MERS-CoV. Together, these data provided direct proof-of-concept
451 assessments of Omicron-specific mRNA vaccination *in vivo*, both alone and as a heterologous
452 booster to the existing widely-used mRNA vaccine form.

453

454

455 **Acknowledgments**

456 We thank various members from our labs for discussions and support. We thank staffs from various
457 Yale core facilities (Keck, YCGA, HPC, YARC, CBDS and others) for technical support. We
458 thank Drs. Tsemperouli, Karatekin, and others for providing equipment and related support. **We**
459 **thank Drs. Lucas and Iwasaki for sharing the Omicron virus isolate.** We thank various support
460 from Department of Genetics; Institutes of Systems Biology and Cancer Biology; Dean's Office
461 of Yale School of Medicine and the Office of Vice Provost for Research.

462

463 **Funding**

464 This work is supported by DoD PRMRP IIAR (W81XWH-21-1-0019) and discretionary funds to
465 SC; **and Ludwig Foundation, Mathers Foundation, Burroughs Wellcome Fund to CBW.** The TEM
466 core is supported by NIH grant GM132114 to CL.

467

468 **Institutional Approval**

469 This study has received institutional regulatory approval. All recombinant DNA (rDNA) and
470 biosafety work were performed under the guidelines of Yale Environment, Health and Safety (EHS)
471 Committee with approved protocols (Chen 18-45, 20-18, 20-26). All animal work was performed
472 under the guidelines of Yale University Institutional Animal Care and Use Committee (IACUC)
473 with approved protocols (Chen-2020-20358; Chen 2021-20068; **Wilen 2021-20198**).

474

475 **Figure legends**

476 **Figure 1. Omicron-specific LNP-mRNA vaccine elicited neutralizing antibodies against**
477 **SARS-CoV-2 Omicron variant.**

478 **A**, Illustration of mRNA vaccine construct expressing SARS-CoV-2 **WT** and Omicron spike **genes**.
479 The spike open reading frame were flanked by 5' untranslated region (UTR), 3' UTR and polyA
480 tail. The Omicron mutations (red) and HexaPro mutations (black) were numbered based on WA-
481 1 spike residue number.

482 **B**, Distribution of Omicron spike mutations (magenta) were displayed in one protomer of spike
483 trimer of which NTD, RBD, hinge region and S2 were colored in purple, blue, green and orange
484 respectively (PDB: 7SBL). The HexaPro mutations in S2 were colored in cyan.

485 **C**, Schematics illustrating the formulation and biophysical characterization of LNP-mRNA.

486 **D**, Dynamic light scattering derived histogram depicting the particle radius distribution of Omicron
487 spike LNP-mRNA.

488 **E**, Omicron LNP-mRNA image collected on transmission electron microscope.

489 **F**, human ACE2 receptor binding of LNP-mRNA encoding Omicron spike expressed in 293T cells
490 as detected by human ACE2-Fc fusion protein and PE-anti-human Fc antibody on Flow cytometry.

491 **G**, Immunization and sample collection schedule. Retro-orbital blood were collected prior
492 Omicron LNP-mRNA vaccination on day 0, day 13 and day 21. Ten mice (n=10) were
493 intramuscularly injected with 10 µg Omicron LNP-mRNA on day 0 (prime, Omicron x 1) and day
494 14 (boost, Omicron x 2). The plasma and peripheral blood mononuclear cells (PBMCs) were
495 separated from blood for downstream assays. The slight offset of the labels reflects the fact that
496 each of the blood collections were perform prior to the vaccination injections. **Data were collected**
497 **from two independent experiments and each experiment has five mice.**

498 **H**, Binding antibody titers of plasma from mice vaccinated with Omicron LNP-mRNA against
499 Omicron spike RBD as quantified by area under curve of log₁₀-transformed titration curve (Log₁₀
500 AUC) in Figure S1. Each dot in bar graphs represents value from one mouse **(n = 10 mice)**.

501 **I**, Neutralization of Omicron pseudovirus by plasma from Omicron LNP-mRNA vaccinated mice.

502 **J**, Omicron live virus titration curves over serial dilution points of plasma from mice before and
503 after immunization with Omicron LNP-mRNA at defined time points. Data of each sample were
504 collected from three replicates (n = 10 mice).

505 **K, Neutralization of Omicron infectious virus by plasma from Omicron LNP-mRNA vaccinated**
506 **mice (n = 10 mice).**

507 Data on dot-bar plots are shown as mean \pm s.e.m. with individual data points in plots. One-way
508 ANOVA with Tukey's multiple comparisons test was used to assess statistical significance.
509 Statistical significance labels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Non-
510 significant comparisons are not shown, unless otherwise noted as n.s., not significant.

511

512 **Figure 2. Heterologous booster with Omicron LNP-mRNA as compared to homologous**
513 **booster with WT LNP-mRNA in mice that previously received a two-dose WT LNP-mRNA**
514 **vaccination**

515 **A, Schematics showing the immunization and blood sampling schedule of mice administered with**
516 **1 μ g WT LNP-mRNA prime (WT x 1) and boost (WT x 2) as well as 10 μ g WT or Omicron-**
517 **specific LNP-mRNA booster shots. The data was collected and combined from two independent**
518 **experiments shown in Extended Data Figures S2 and S3.**

519 **B, Bar graph comparing binding antibody titers of mice administered with PBS or WT and**
520 **Omicron LNP-mRNA against Omicron, Delta and WA-1 RBD (ELISA antigens). The antibody**
521 **titers were quantified as Log₁₀ AUC based on titration curves in Extended Data Figure 1A. PBS**
522 **sub-groups (n=6 each) collected from different matched time points showed no statistical**
523 **differences between each other, and were combined as one group (n=18).**

524 **C, Pseudovirus neutralizing antibody titers in the form of log₁₀-transformed reciprocal IC50**
525 **calculated from fitting the titration curve with a logistic regression model (n = 12 before booster,**
526 **n=5 in WT x 3, n = 7 in WT x 2 + Omicron).**

527 **D, Infectious virus neutralization titer comparisons between mice before and after vaccination with**
528 **WT or Omicron boosters (n = 9 before booster, n=5 in WT x 3, n = 4 in WT x 2 + Omicron).**

529 **Titer ratios were indicated in each graph and fold change described in manuscript is calculated**
530 **from (ratio - 1). Data on dot-bar plots are shown as mean \pm s.e.m. with individual data points in**
531 **plots. Two-way ANOVA with Tukey's multiple comparisons test was used to assess statistical**
532 **significance. Statistical significance labels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.**
533 **Non-significant comparisons are not shown, unless otherwise noted as n.s., not significant.**

534

535 **Figure 3. Cross reactivity and targeting sites characterization of plasma antibodies elicited**
536 **by Omicron and WT LNP-mRNAs against SARS-CoV-2 VoCs and *Betacoronavirus* species.**
537 **A, cross reactivity of plasma antibody from mice immunized with Omicron LNP mRNA (prime**
538 **and boost) to SARS-CoV-2 VoCs and pathogenic coronavirus species (n = 10).**
539 **B, cross reactivity of plasma antibody from mice immunized with WT (WT x 3) or Omicron**
540 **(WT x 2 + Omicron) boosters to SARS-CoV-2 beta variant and pathogenic coronavirus species**
541 **(n = 6 in PBS, n=5 in WT x 3, n = 7 in WT x 2 + Omicron).**
542 **C, representative antibodies from major classes of RBD epitopes were shown by aligning spike**
543 **RBDs in each of complex structures. The Omicron RBD surface was set to semi-transparent to**
544 **visualize 15 RBD mutations and their relative positions to antibody epitopes.**
545 **D, baseline titers of plasma from mice of different vaccination status (WT x 3, WT x 2 +**
546 **Omicron, Omicron x 2) were shown as log₁₀ AUC determined in hACE2 and antibody**
547 **competition ELISA. Each group sample number is denoted with n (n = 10 in Omicron x 2, n=5**
548 **in WT x 3, n = 7 in WT x 2 + Omicron) in two independent assays (hACE2 and antibody**
549 **competition ELISA).**
550 **E, significant portion of plasma antibody from mice receiving Omicron (Omicron x 2, left panel)**
551 **or WT + Omicron (WT x 3 middle, or WT x 2 + Omicron, right panel) LNP-mRNA competed**
552 **with hACE2 for Omicron RBD binding in ELISA (n = 10 in Omicron x 2, n=5 in WT x 3, n = 7**
553 **in WT x 2 + Omicron).**
554 **F, plasma antibody from mice receiving Omicron (Omicron x 2, n = 10, left panel) or WT +**
555 **Omicron (WT x 3, n = 5, middle or WT x 2 + Omicron, n = 7, right panel) LNP-mRNA showed**
556 **various extent of binding reduction in the presence of blocking antibodies with known epitopes**
557 **on RBD.**
558 **Data on dot-bar plots are shown as mean ± s.e.m. with individual data points in plots. Two-way**
559 **ANOVA with Tukey's multiple comparisons test and one-way ANOVA with Dunnett's multiple**
560 **comparisons were used to assess statistical significance. Statistical significance labels: * p < 0.05;**
561 **** p < 0.01; *** p < 0.001; **** p < 0.0001. Non-significant comparisons are not shown, unless**
562 **otherwise noted as n.s., not significant.**

563

564

565

566 **Extended Data Figures**

567 **Extended Data Figure 1 (Figure S1). ELISA and neutralization titration curves over serial**
568 **dilution of plasma collected at different timepoints from mice administered with PBS or WT**
569 **and/or Omicron LNP-mRNA.**

570 **A, ELISA titration curves over serial \log_{10} -transformed dilution points of plasma collected from**
571 **mice before and after immunization with Omicron LNP-mRNA at defined time points ($n = 10$).**
572 **Average curves, data are shown as mean \pm s.e.m..**

573 **B, Omicron pseudovirus titration curves over serial \log_{10} -transformed dilution points of plasma**
574 **collected from mice before and after immunization with Omicron LNP-mRNA at defined time**
575 **points ($n = 10$). Left panel, average curves, data are shown as mean \pm s.e.m.; Right panel,**
576 **individual curves.**

577

578

579 **Extended Data Figure 2 (Figure S2). Both WT and Omicron specific LNP-mRNA booster**
580 **shots greatly improved waning immunity of mice vaccinated with SARS-CoV-2 WT LNP-**
581 **mRNA against SARS-CoV-2 Delta and Omicron variants (Independent experiment 1 or**
582 **batch 1).**

583 **A, Schematics showing the immunization and blood sampling schedule of mice administered with**
584 **1 μ g WT LNP-mRNA prime (WT x 1) and boost (WT x 2) as well as 10 μ g WT or Omicron-**
585 **specific LNP-mRNA booster shots. The plasma and PBMCs were separate from blood for**
586 **downstream assays.**

587 **B, Bar graph comparing binding antibody titers of mice administered with PBS or WT and**
588 **Omicron LNP-mRNA against Omicron, Delta and WT RBD (ELISA antigens). The antibody titers**
589 **were quantified as \log_{10} AUC based on titrations curves in Extended Data Figure 1A. PBS sub-**
590 **groups ($n=3$ each) collected from different matched time points showed no statistical differences**
591 **between each other, and were combined as one group ($n=9$).**

592 **C, Neutralizing antibody titers in the form of \log_{10} -transformed reciprocal IC_{50} calculated from**
593 **fitting the titration curve with a logistic regression model ($n = 3$).**

594 **D, Correlation of neutralization titers (\log_{10} reciprocal IC_{50} , y axis) and ELISA titers (\log_{10} AUC,**
595 **x axis) from matched vaccination group (left panel) or individual mouse (right panel). PBS samples**
596 **from different timepoints were shown as one group in correlation map and were not included in**

597 linear regression model. Each dot in bar graphs represents value from one group average (left
598 panel), or one individual mouse (right panel).

599 Titer ratios were indicated in each graph and fold change is calculated from (ratio - 1). Data on
600 dot-bar plots are shown as mean \pm s.e.m. with individual data points in plots. Two-way ANOVA
601 with Tukey's multiple comparisons test was used to assess statistical significance. Statistical
602 significance labels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Non-significant
603 comparisons are not shown, unless otherwise noted as n.s., not significant.

604

605 **Extended Data Figure 3 (Figure S3). Omicron specific LNP-mRNA booster shots greatly**
606 **improved waning immunity of mice vaccinated with SARS-CoV-2 WT LNP-mRNA against**
607 **SARS-CoV-2 Delta and Omicron variants (Independent experiment 2 or batch 2).**

608 **A**, Schematics showing the immunization and blood sampling schedule of mice administered with
609 1 μ g WT LNP-mRNA prime (WT x 1) and boost (WT x 2) as well as 10 μ g Omicron-specific
610 LNP-mRNA booster shots. The plasma and PBMCs were separate from blood for downstream
611 assays.

612 **B**, Bar graph comparing binding antibody titers of mice administered with PBS or WT and
613 Omicron LNP-mRNA against Omicron, Delta and WT RBD (ELISA antigens). The antibody titers
614 were quantified as \log_{10} AUC based on titrations curves in Extended Data Figure 1A. PBS sub-
615 groups (n=3 each) collected from different matched time points showed no statistical differences
616 between each other, and were combined as one group (n=6).

617 **C**, Neutralizing antibody titers in the form of \log_{10} -transformed reciprocal IC₅₀ calculated from
618 fitting the titration curve with a logistic regression model (n = 9 before booster, n=5 in WT x 3, n
619 = 4 in WT x 2 + Omicron).

620 **D**, Correlation of neutralization titers (\log_{10} reciprocal IC₅₀, y axis) and ELISA titers (\log_{10} AUC,
621 x axis) from matched vaccination group (left panel) or individual mouse (right panel). PBS samples
622 from different timepoints were shown as one group in correlation map and were not included in
623 linear regression model. Each dot in bar graphs represents value from one group average (left
624 panel), or one individual mouse (right panel).

625 Titer ratios were indicated in each graph and fold change is calculated from (ratio - 1).

626 Data on dot-bar plots are shown as mean \pm s.e.m. with individual data points in plots. Two-way
627 ANOVA with Tukey's multiple comparisons test was used to assess statistical significance.

628 Statistical significance labels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Non-
629 significant comparisons are not shown, unless otherwise noted as n.s., not significant.

630

631 **Extended Data Figure 4 (Figure S4). ELISA titration curves over serial dilution of plasma**
632 **collected at different timepoints from mice administered with PBS or WT and/or Omicron**
633 **LNP-mRNA.**

634 **A.** ELISA titration curves of batch 1 experiment ($n = 3$).

635 **B.** ELISA titration curves of batch 2 experiment ($n = 9$ before booster, $n=5$ in WT x 3, $n = 4$ in
636 WT x 2 + Omicron).

637 The OD450 values were plotted against a series of \log_{10} -transformed dilution points of plasma
638 from mice 35 days post WT prime, >4 months post WT prime (day 127 in batch 1 and day 166 in
639 batch 2) and 2 weeks post booster (day 140 in batch 1 and day 180 in batch 2) of WT or Omicron
640 LNP-mRNA, against spike receptor binding domain (RBD) antigens of Omicron variant (left),
641 Delta (mid) and WT (right) were shown.

642

643 **Extended Data Figure 5 (Figure S5). Binding antibody titers of mice administered with PBS**
644 **or WT and Omicron LNP-mRNA against Omicron, Delta and WT RBD (ELISA antigens),**
645 **were grouped by vaccination timepoints to compare titers against different RBD antigens.**

646 The antibody titers were quantified as area under curve of \log_{10} -transformed titration curve (Log_{10}
647 AUC) in Extended Data Figure 2. The data were derived from independent experiment 1 and 2.

648

649 **Extended Data Figure 6 (Figure S6). Omicron, Delta and WT pseudovirus production,**
650 **characterization and neutralization assay.**

651 **A.** Functional titration curves of Omicron, Delta and WA-1 pseudoviruses in hACE2+ cells.

652 **B.** Representative Flow Cytometry plots of infectivity of Omicron, Delta and WA-1 pseudoviruses
653 in hACE2+ cells.

654 **C.** Quantification of infectivity of Omicron, Delta and WT pseudoviruses in hACE2+ cells.

655 **D.** Neutralization titration curves from batch 1 experiment ($n = 3$).

656 **E.** Neutralization titration curves from batch 2 experiment ($n = 9$ before booster, $n=5$ in WT x 3,
657 $n = 4$ in WT x 2 + Omicron).

658 Percent of pseudovirus infected cells was plotted over serial dilutions of plasma from mice 35 days
659 post WT prime, >4 months post WT prime (day 127 in batch 1 and day 166 in batch 2) and 2 weeks
660 post booster (day 140 in batch 1 and day 180 in batch 2) of WT and Omicron LNP-mRNA against
661 Omicron (left), Delta (mid) and WT (right) pseudovirus. Pseudovirus infection rate was calculated
662 from percent of GFP positive cells and was plotted against plasma dilution (\log_{10} transformed) as
663 titration curve. Top panels, average curves, data are shown as mean \pm s.e.m.; Bottom panels,
664 individual curves.

665

666 **Extended Data Figure 7 (Figure S7). Neutralizing antibody titers in the form of \log_{10} -**
667 **transformed reciprocal IC50 were grouped by vaccination timepoints to compare titers**
668 **against different pseudoviruses.**

669 The neutralization titers from combined datasets (A) or batch 1 (B) were quantified as \log_{10} -
670 transformed reciprocal IC50 values (Log_{10} reciprocal IC50, or Log_{10} IC50) based on titration
671 curves in Extended Data Figure 6.

672 Titer ratios were indicated in each graph and fold change is calculated from (ratio - 1).

673 Data on dot-bar plots are shown as mean \pm s.e.m. with individual data points in plots. Two-way
674 ANOVA with Tukey's multiple comparisons test was used to assess statistical significance.
675 Statistical significance labels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Non-
676 significant comparisons are not shown, unless otherwise noted as n.s., not significant.

677

678 **Extended Data Figure 8 (Figure S8). Live virus neutralization titration curves over serial**
679 **dilution of plasma collected at different timepoints from mice administered with PBS or WT**
680 **and/or Omicron LNP-mRNA.**

681 **A.** Omicron live virus titration curves (n = 9 before booster, n=5 in WT x 3, n = 4 in WT x 2 +
682 Omicron)

683 **B.** Delta live virus titration curves (n = 9 before booster, n=5 in WT x 3, n = 4 in WT x 2 + Omicron)
684 Titration curves were plotted over serial dilution points of plasma collected from mice before and
685 after WT or Omicron LNP-mRNA boosters at defined time points. Data of each sample were
686 collected from two replicates.

687

688 **Extended Data Figure 9 (Figure S9). Correlation analysis of antibody titers determined by**
689 **ELISA, pseudovirus neutralization and live virus neutralization assays.**

690 **A, Correlation between pseudovirus neutralization titers (\log_{10} reciprocal IC₅₀, y axis) and ELISA**
691 **titers (\log_{10} AUC, x axis) from matched vaccination group (left panel) or individual mouse (right**
692 **panel).**

693 **B. Correlation between live virus neutralization titers (\log_{10} IC₅₀, x axis) and ELISA titers (\log_{10}**
694 **AUC, y axis) from matched vaccination group (left panel) or individual mouse (right panel).**

695 **C. Correlation between live virus neutralization titers (\log_{10} IC₅₀, x axis) and pseudovirus**
696 **neutralization titers (\log_{10} AUC, y axis) from matched vaccination group (left panel) or individual**
697 **mouse (right panel).**

698 **PBS samples from different timepoints were shown as one group in correlation map and were not**
699 **included in linear regression model. Each dot in bar graphs represents value from one group**
700 **average (left panel), or one individual mouse (right panel).**

701

702 **Extended Data Figure 10 (Figure S10). Assessment of WT or Omicron LNP-mRNA mediated**
703 **cross reactivity against a panel of SARS-CoV-2 variants and pathogenic coronavirus species**
704 **in ELISA.**

705 **A, binding antibody titers (Log_{10} AUC) of plasma from mice that received Omicron LNP-mRNA**
706 **prime and boost (Omicron x 2, n = 10).**

707 **B, binding antibody titers of plasma from mice that received WT (WT x 3, n = 5) or Omicron (WT**
708 **x 2 + Omicron, n = 7) LNP-mRNA boosters.**

709 **This supplemental figure is a combination of data from the experiment shown in Figures 1 and 3**
710 **for comparison clarity.**

711 **Data on dot-bar plots are shown as mean \pm s.e.m. with individual data points in plots. Two-way**
712 **ANOVA with Tukey's multiple comparisons test was used to assess statistical significance.**

713 **Multiple comparisons between titers against different ELISA antigens were made within same**
714 **vaccination group. All comparisons with MERS RBD were significant and not shown in graph to**

715 **simplify comparisons. Statistical significance labels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ******
716 **$p < 0.0001$. Non-significant comparisons are not shown.**

717

718 **Extended Data Figure 11 (Figure S11). Competition ELISA titration curves and binding**
719 **antibody titers against low-density Omicron RBD from mice vaccinated with WT and/or**
720 **Omicron LNP-mRNA.**

721 **A. hACE competition ELISA titration curves over a series of log₁₀-transformed dilution points of**
722 **plasma from mice vaccinated with Omicron LNP-mRNA (Omicron x 2 plasma, left, n = 10) or**
723 **WT/Omicron LNP-mRNA (WT x 3, middle, n = 5 and WT x 2 + Omicron plasma, right, n = 7).**

724 **B. antibody competition ELISA titration curves over a series of log₁₀-transformed dilution points**
725 **of plasma from mice vaccinated with Omicron LNP-mRNA (Omicron x 2 plasma, left, n = 10) or**
726 **WT/Omicron LNP-mRNA (WT x 3 plasma, middle, n = 5 and WT x 2 + Omicron plasma, right,**
727 **n = 7).**

728 **C. PBS buffer as negative control to show minimal cross reactivity of anti-mouse secondary**
729 **antibody with human IgG blocking antibodies, including Clone 13A, CR3022 and S309. n = 2 and**
730 **each contains 8 mock dilution points.**

731

732 **Extended Data Figure 12 (Figure S12). Representative flow cytometry gating strategy for**
733 **detecting Omicron spike binding to human ACE2 receptor.**

734

735

736 **Methods**

737 **Molecular cloning**

738 The Omicron spike amino acid sequence was derived from two lineage BA.1 Omicron cases
739 identified in Canada on Nov.23rd, 2021 (GISAID EpiCoV, EPI_ISL_6826713 and
740 EPI_ISL_6826714). Omicron spike cDNA were codon optimized, synthesized as gblocks (IDT)
741 and cloned to mRNA vector with 5', 3' untranslated region (UTR) and poly A tail. The furin cleave
742 site (RRAR) was replaced with a GSAS short stretch in the mRNA vector. HexaPro mutations
743 were introduced in the WT sequence (Wuhan-Hu-1, which was used for the current clinical mRNA
744 vaccines) and Omicron variant spike sequence of mRNA vector to improve expression and
745 prefusion state²⁷. The accessory plasmids for pseudovirus assay including pHIVNLGagPol and
746 pCCNanoLuc2AEGFP were from Dr. Bieniasz' lab⁶¹. The C-terminal 19 amino acids were deleted
747 in the SARS-CoV-2 spike sequence for the pseudovirus assay.

748

749 **Cell Culture**

750 HEK293T, HEK293FT (Thermo Fisher) and 293T-hACE2 (gifted from Dr Bieniasz' lab) cell lines
751 were maintained in Dulbecco's modified Eagle's medium (DMEM, Thermo fisher) supplemented
752 with 10% Fetal bovine serum (Hyclone) and 1% penicillin-streptomycin (Gibco, final
753 concentration penicillin 100 unit/ml, streptomycin 100 µg/ml), which is denoted as complete
754 growth medium. Cells were split every 2 days at a split ratio of 1:4 when the confluency reached
755 over 80%. Vero-E6 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 5%
756 heat-inactivated fetal bovine serum (FBS).

757

758 **In vitro mRNA transcription and vaccine formulation**

759 A Hiscribe™ T7 ARCA mRNA Kit (with tailing) (NEB, Cat # E2060S) was used to in vitro
760 transcribe codon-optimized mRNA encoding HexaPro spikes of SARS-CoV-2 WT and Omicron
761 variant with 50% replacement of uridine by N1-methyl-pseudouridine. The DNA template was
762 linearized before mRNA transcription and contained 5' UTR, 3' UTR and 3' polyA tail as flanking
763 sequence of spike open reading frame.

764

765 The purified mRNA was generated by following NEB manufacturer's instructions and kept frozen
766 at -80 °C until further use. The lipid nanoparticles mRNA was assembled using the

767 NanoAssemblr® Ignite™ instrument (Precision Nanosystems) according to manufacturers'
768 guidance. In brief, lipid mixture was mixed with prepared mRNA in 25mM sodium acetate at pH
769 5.2 on Ignite instrument at a molar ratio of 6:1 (LNP: mRNA), similar to previously described^{59,62}.
770 The LNP encapsulated mRNA (LNP-mRNA) was buffer exchanged to PBS using 100kDa Amicon
771 filter (Macrosep Centrifugal Devices 100K, 89131-992). Sucrose was added as a cryoprotectant.
772 The particle size of mRNA-LNP was determined by DLS device (DynaPro NanoStar, Wyatt,
773 WDPN-06) and TEM described below. The encapsulation rate and mRNA concentration were
774 quantified by Quant-iT™ RiboGreen™ RNA Assay (Thermo Fisher).

775

776 **Validation of LNP-mRNA mediated spike expression *in vitro* and receptor binding capability** 777 **of expressed Omicron HexaPro spikes**

778 On day 1, HEK293T cells were seeded at 50% confluence in 24-well plate and mixed with 2 µg
779 Omicron LNP-mRNA. After 16 hours, the cells were collected for flow cytometry. The spike
780 expression on cell surface were detected by staining cells with human ACE2-Fc chimera (Sino
781 Biological, 10108-H02HG) in MACS buffer (D-PBS with 2 mM EDTA and 0.5% BSA) for 20
782 min on ice. Cells were washed twice after the primary stain and incubated with PE-anti-human Fc
783 antibody (Biolegend, 410708) in MACS buffer for 20 min on ice. During secondary antibody
784 staining, live/Dead aqua fixable stain (Invitrogen) was used to assess cell viability. Data was
785 collected on BD FACSAria II Cell Sorter (BD) and analyzed using FlowJo software.

786

787 **Negative-stain TEM**

788 Formvar/carbon-coated copper grid (Electron Microscopy Sciences, catalog number FCF400-Cu-
789 50) was glow-discharged and covered with 6 µl of the sample for 1 min before blotting away the
790 sample. The sample was double-stained with 6 µl of 2% (w/v) uranyl formate (Electron
791 Microscopy Sciences, catalog number 22450) for 5 seconds (first stain) and 1 min (second stain),
792 blotting away after each stain. Images were collected using a JEOL JEM-1400 Plus microscope
793 with an acceleration voltage of 80 kV and a bottom-mount charge-coupled device camera (4k by
794 3k, Advanced Microscopy Technologies).

795

796 **Mouse vaccination**

797 Vaccine immunogenicity study used 6 weeks old female C57BL/6Ncr (B6) mice purchased from
798 Charles River. The mice-housing condition was maintained at regular ambient room temperature
799 (65-75°F, or 18-23°C), 40-60% humidity, and a 14 h:10 h day/night cycle. Each mice cage was
800 individually ventilated with clean food, water, and bedding. Two sets of immunization experiments
801 were performed: vaccination with Omicron LNP-mRNA, and sequential vaccination with **WT**
802 **LNP-mRNA, followed by WT or Omicron LNP mRNA booster**. For the Omicron LNP-mRNA
803 vaccination experiment, five mice were immunized with 10 µg Omicron LNP-mRNA on day 0
804 (prime) and day 14 (boost). Retro-orbital blood was collected prior to vaccine injection on day 0,
805 day 13 and day 21. For **WT** and Omicron LNP-mRNA sequential vaccination experiment, three
806 mice were administered with either 100 µl PBS or two-dose 1 µg WT (21 days apart) and 10 µg
807 Omicron LNP-mRNA (14 days apart). Retro-orbital blood was collected prior to vaccine injection
808 on day 35, day 127, day 140 and day 148.

809

810 **Isolation of plasma and PBMCs from blood**

811 At the defined time points, retro-orbital blood was collected from mice. The isolation of PBMCs
812 and plasma was achieved via centrifugation using SepMate-15 and Lymphoprep gradient medium
813 (StemCell Technologies). 200 µl blood was immediately diluted with 800 µl PBS with 2% FBS.
814 The blood diluent was then added to SepMate-15 tubes with 6ml Lymphoprep (StemCell
815 Technologies). Centrifugation at 1200 g for 20 minutes was used to isolate RBCs, PBMCs and
816 plasma. 250ul diluted plasma was collected from the surface layer. The remaining solution at the
817 top layer was poured to a new tube to isolate PBMCs, which were washed once with PBS + 2%
818 FBS. The separated plasma was used in ELISA and neutralization assay.

819

820 **ELISA**

821 3 µg/ml of spike antigens were coated onto the 384-well ELISA plates (VWR, Cat # 82051-300)
822 overnight at 4 degree. The antigen panel used in the ELISA includes RBDs of **SARS RBD**
823 **(AcroBiosystems, SPD-S52H6), MERS RBD (AcroBiosystems, SPD-M52H6),** 2019-nCoV WA-
824 1 (Sino Biological 40592-V08B), Delta variant B.1.617.2 (Sino Biological 40592-V08H90), **Beta**
825 **variant B.1.351 (Sino Biological 40592-V08H85)** and Omicron variant B.1.1.529 (Sino Biological
826 40592-V08H121). Plates were washed with PBST (PBS plus 0.5% Tween 20) three times in the
827 50TS microplate washer (Fisher Scientific, NC0611021) and blocked with 0.5% BSA in PBST at

828 room temperature for one hour. Plasma was fourfold serially diluted starting at a 1:500 dilution.
829 Diluted plasma samples were added to the plates and incubated at room temperature for one hour,
830 followed by washes with PBST five times. Anti-mouse secondary antibody (Fisher, Cat# A-10677)
831 at 1:2500 dilution in blocking buffer was incubated at room temperature for one hour. Plates were
832 washed five times and developed with tetramethylbenzidine substrate (Biolegend, 421101). The
833 reaction was stopped with 1 M phosphoric acid after 20 min at room temperature, and OD at 450
834 nm was measured by multimode microplate reader (PerkinElmer EnVision 2105). The binding
835 response (OD450) was plotted against the dilution factor in log₁₀ scale as the dilution-dependent
836 response curve. The area under curve of the dilution-dependent response (Log₁₀ AUC) was
837 calculated to quantify the potency of the plasma antibody binding to spike antigens. The fold
838 change of antibody titer was estimated using this equation: ratio = 10[^] (AUC₁ - AUC₂)

839

840 **hACE2 and antibody competition ELISA**

841 The 384-well plate was coated with 0.6 µg/ml Omicron RBD at 4 degree overnight before washed
842 with PBST (0.5% Tween-20) three times and blocked with 2% BSA in PBST for 1 hour at room
843 temperature. In hACE2 and antibody competition ELISA, 15 µg/ml hACE2 (Sino, 10108-H08H)
844 or 10 µg/ml antibodies including Clone 13A (Chen lab), CR3022 (Abcam, Ab273073) and S309
845 (BioVision, A2266) were respectively added to the plate 1 hour prior to subsequent incubation
846 with serially diluted plasma for another hour at room temperature. After coincubation of plasma
847 and hACE2/antibodies, the plate was washed five times with PBST and incubated with anti-mouse
848 secondary antibody with minimal cross reactivity with human IgG (Biolegend, 405306). The plate
849 was washed five times after 1-hour secondary antibody incubation and developed with
850 tetramethylbenzidine substrate (Biolegend, 421101). The reaction was stopped with 1 M
851 phosphoric acid after 20 min at room temperature, and OD at 450 nm was measured by multimode
852 microplate reader (PerkinElmer EnVision 2105). The normalized AUC was calculated by
853 normalizing the value with AUC determined in PBS group.

854

855 **Omicron, **WA-1** and Delta pseudovirus production and characterization**

856 For the neutralization assay, HIV-1 based SARS-CoV-2 WA-1, B.1.617.2 (Delta) variant, and
857 B.1.1.529 (Omicron) variant pseudotyped virions were packaged using a coronavirus spike
858 plasmid, a reporter vector and a HIV-1 structural protein expression plasmid. The reporter vector,

859 pCCNanoLuc2AEGFP, and plasmid expressing HIV-1 structural proteins (pHIVNLGagPol) were
860 gifts from Dr Bieniasz's lab. The spike plasmid for SARS-CoV-2 WA-1 pseudovirus truncated C-
861 terminal 19 amino acids (denoted as SARS-CoV-2- Δ 19) and was from Dr Bieniasz' lab. Spike
862 plasmids expressing C-terminally truncated SARS-CoV-2 B.1.617.2 variant S protein (Delta
863 variant- Δ 19) and SARS-CoV-2 B.1.1.529 variant S protein (Omicron variant- Δ 19) were made
864 based on the pSARS-CoV-2- Δ 19. All pseudoviruses were produced under the same conditions.
865 Briefly, 293FT cells were seeded in 150 mm plates, and transfected with 21 μ g pHIVNLGagPol,
866 21 μ g pCCNanoLuc2AEGFP, and 7.5 μ g of corresponding plasmids, in the presence of 198 μ l
867 PEI (1mg/ml, PEI MAX, Polyscience). At 48 h after transfection, the supernatant was filtered
868 through a 0.45- μ m filter, and frozen in -80°C.

869
870 To characterize the titer of WA-1, Delta, and Omicron pseudoviruses packaged, 1×10^4 293T-
871 hACE2 cells were plated in each well of a 96-well plate. In the next day, different volumes of
872 pseudovirus supplemented with culture medium to a total volume of 100 μ L were added into 96-
873 well plates with 293T-hACE2. Plates were incubated at 37°C for 24 hr. Then cells were washed
874 with MACS buffer once and the percent of GFP-positive cells were counted by Attune NxT
875 Acoustic Focusing Cytometer (Thermo Fisher). To normalize pseudovirus titer, 1×10^4 293T-
876 hACE2 cells were plated in each well of a 96-well plate. In the next day, 50 μ L pseudovirus was
877 mixed with 50 μ L culture medium to 100 μ L. The mixture was incubated for 1 hr in the 37 °C
878 incubator, supplied with 5% CO₂, and added into 96-well plates with 293T-hACE2. Plates were
879 incubated at 37°C for 24 hr. Then cells were washed with MACS buffer once and the percent of
880 GFP-positive cells were counted by Attune NxT Acoustic Focusing Cytometer (Thermo Fisher).
881 Delta pseudovirus and Omicron pseudovirus were diluted accordingly to match the functional titer
882 of WA-1 pseudovirus for neutralization assay of plasma samples.

883

884 **Pseudovirus neutralization assay**

885 The SARS-CoV-2 pseudovirus assays were performed on 293T-hACE2 cells. One day before
886 infection, 1×10^4 293T-hACE2 cells were plated in each well of a 96-well plate. In the next day,
887 plasma collected from mice were serially diluted by 5 fold with complete growth medium at a
888 starting dilution of 1:100. 55 μ L diluted plasma was mixed with the same volume of SARS-CoV-
889 2 WA-1, Delta variant, or Omicron variant pseudovirus and was incubated for 1 hr in the 37 °C

890 incubator, supplied with 5% CO₂. 100 μ L of mixtures were added into 96-well plates with 293T-
891 hACE2. Plates were incubated at 37°C for 24 hr. Then cells were washed with MACS buffer once
892 and the percent of GFP-positive cells were counted by Attune NxT Acoustic Focusing Cytometer
893 (Thermo Fisher). The 50% inhibitory concentration (IC₅₀) was calculated with a four-parameter
894 logistic regression using GraphPad Prism (GraphPad Software Inc.). If the fitting value of IC₅₀ is
895 negative (i.e. negative titer), which suggested undetectable neutralization activity, the value was
896 set to baseline (1, 0 in log scale).

897

898 **Omicron and Delta live virus production and characterization**

899 Full-length SARS-CoV-2 Omicron (BA.1) and Delta (B.1.617.2) isolates were a gift of Carolina
900 Lucas and Akiko Iwasaki, and were isolated as previously described⁶³. To expand viral stocks, 10⁷
901 Vero-E6 cells stably overexpressing ACE2 and TMPRSS2 were infected with SARS-CoV-2 at an
902 MOI of approximately 0.01. The Omicron stock was collected 2 dpi, clarified by centrifugation
903 (450 xg for 10 minutes), filtered through a 0.45-micron filter, and concentrated ten-fold using
904 Amicon Ultra-15 columns. To increase titer, the Delta stock was collected at 1 dpi, clarified,
905 filtered, and used to infect 5 x 10⁷ Vero-E6 cells overexpressing ACE2 and TMPRSS2. At 1 dpi,
906 supernatant was harvested, clarified, filtered and concentrated as above. Viral stocks were titered
907 by plaque assay in Vero-E6 cells as previously described⁶⁴.

908

909 **Infectious virus neutralization assay**

910 The complements and other potential neutralizing agents were heat inactivated in mouse plasma
911 prior to infectious virus neutralization assay. Mouse plasma samples were serially diluted, then
912 incubated with SARS-CoV-2 Omicron live virus for 1 h at 37°C. The Omicron live virus was
913 isolated from nasopharyngeal specimens and sequenced as part of the Yale SARS-CoV-2 Genomic
914 Surveillance Initiative's weekly surveillance Program in Connecticut⁶⁵. After coinubation,
915 plasma/virus mixture was added to Vero-E6 cells overexpressing ACE2/TMPRSS2. Cell viability
916 was measured at 3dpi or 5dpi using CellTiter Glo.

917

918 **Standard statistics**

919 Standard statistical methods were applied to non-high-throughput experimental data. The
920 statistical methods are described in figure legends and/or supplementary Excel tables. The

921 statistical significance was labeled as follows: n.s., not significant; * $p < 0.05$; ** $p < 0.01$; *** p
922 < 0.001 ; **** $p < 0.0001$. Prism (GraphPad Software) and RStudio were used for these analyses.
923 Additional information can be found in the supplemental excel tables.

924

925 **Schematic illustrations**

926 Schematic illustrations were created with Affinity Designer or BioRender.

927

928 **Replication, randomization, blinding and reagent validations**

929 Biological or technical replicate samples were randomized where appropriate. In animal
930 experiments, mice were randomized by littermates.

931 Experiments were not blinded.

932 Commercial antibodies were validated by the vendors, and re-validated in house as appropriate.

933 Custom antibodies were validated by specific antibody - antigen interaction assays, such as ELISA.

934 Isotype controls were used for antibody validations.

935 Cell lines were authenticated by original vendors, and re-validated in lab as appropriate.

936 All cell lines tested negative for mycoplasma.

937

938 **Data availability**

939 All data generated or analyzed during this study are included in this article and its supplementary
940 information files. Specifically, source data and statistics are provided in a supplementary table
941 excel file. **No custom code was used in this study.** Additional information related to this study are
942 available from the corresponding author(s) upon reasonable request.

943

944 **References**

945

946 1 *Science Brief: Omicron (B.1.1.529) Variant*, <[https://www.cdc.gov/coronavirus/2019-](https://www.cdc.gov/coronavirus/2019-ncov/science/science-briefs/scientific-brief-omicron-variant.html#1)
947 [ncov/science/science-briefs/scientific-brief-omicron-variant.html#1](https://www.cdc.gov/coronavirus/2019-ncov/science/science-briefs/scientific-brief-omicron-variant.html#1)> (

948 2 Khare, S. *et al.* GISAID's Role in Pandemic Response. *China CDC Wkly* **3**, 1049-1051,
949 doi:[10.46234/ccdcw2021.255](https://doi.org/10.46234/ccdcw2021.255) (2021).

950 3 *Omicron daily overview: 31 December 2021*,
951 <[https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1044522/20211231_OS_Daily_Omicron_Overview.pdf)
952 [data/file/1044522/20211231_OS_Daily_Omicron_Overview.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1044522/20211231_OS_Daily_Omicron_Overview.pdf)> (

953 4 *Omicron cases tracker by Newsnodes*, <https://newsnodes.com/nu_tracker> (

954 5 *Tracking Omicron variant, GISAID*, <<https://www.gisaid.org/hcov19-variants/>> (

955 6 Pulliam, J., *et al.* Increased risk of SARS-CoV-2 reinfection associated with emergence
956 of the Omicron variant in South Africa. *MedRxiv*,
957 doi:<https://doi.org/10.1101/2021.11.11.21266068> (2021).

958 7 *Classification of Omicron (B.1.1.529): SARS-CoV-2 Variant of Concern*,
959 <[https://www.who.int/news/item/26-11-2021-classification-of-omicron-\(b.1.1.529\)-sars-](https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern)
960 [cov-2-variant-of-concern](https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern)> (

961 8 Collie, S., Champion, J., Moultrie, H., Bekker, L. G. & Gray, G. Effectiveness of
962 BNT162b2 Vaccine against Omicron Variant in South Africa. *N Engl J Med*,
963 doi:[10.1056/NEJMc2119270](https://doi.org/10.1056/NEJMc2119270) (2021).

964 9 *SARS-CoV-2 Omicron variant: statistics*, <[https://en.wikipedia.org/wiki/SARS-CoV-](https://en.wikipedia.org/wiki/SARS-CoV-2_Omicron_variant#Epidemiology)
965 [2_Omicron_variant#Epidemiology](https://en.wikipedia.org/wiki/SARS-CoV-2_Omicron_variant#Epidemiology)> (

966 10 *Tracking variants of the novel coronavirus in Canada*,
967 <[https://www.ctvnews.ca/health/coronavirus/tracking-variants-of-the-novel-coronavirus-](https://www.ctvnews.ca/health/coronavirus/tracking-variants-of-the-novel-coronavirus-in-canada-1.5296141)
968 [in-canada-1.5296141](https://www.ctvnews.ca/health/coronavirus/tracking-variants-of-the-novel-coronavirus-in-canada-1.5296141)> (

969 11 Viana, R., *et al.* Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in
970 southern Africa. *Nature*, doi:<https://www.nature.com/articles/d41586-021-03832-5>.

971 12 Karim, S. S. A. & Karim, Q. A. Omicron SARS-CoV-2 variant: a new chapter in the
972 COVID-19 pandemic. *Lancet* **398**, 2126-2128, doi:[10.1016/S0140-6736\(21\)02758-6](https://doi.org/10.1016/S0140-6736(21)02758-6)
973 (2021).

- 974 13 Kozlov, M. Omicron overpowers key COVID antibody treatments in early tests. *Nature*,
975 doi:10.1038/d41586-021-03829-0 (2021).
- 976 14 Cameroni, E. *et al.* Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron
977 antigenic shift. *bioRxiv*, doi:10.1101/2021.12.12.472269 (2021).
- 978 15 Cao, Y., *et al.* Omicron escapes the majority of existing SARS-CoV-2 neutralizing
979 antibodies. *Nature*, doi:<https://doi.org/10.1038/d41586-021-03796-6> (2021).
- 980 16 Planas, D., *et al.* Considerable escape of SARS-CoV-2 variant Omicron to antibody
981 neutralization. *Nature*, doi:<https://doi.org/10.1038/d41586-021-03827-2> (2021).
- 982 17 Aggarwal, A., *et al.* SARS-CoV-2 Omicron: evasion of potent humoral responses and
983 resistance to clinical immunotherapeutics relative to viral variants of concern. *MedRxiv*,
984 doi:<https://doi.org/10.1101/2021.12.14.21267772> (2021).
- 985 18 Hoffmann, M., *et al.* The Omicron variant is highly resistant against antibody-mediated
986 neutralization – implications for control of the COVID-19 pandemic. *Cell*,
987 doi:<https://doi.org/10.1016/j.cell.2021.12.032> (2021).
- 988 19 *Implications of the emergence and spread of the SARS-CoV-2 B.1.1. 529 variant of*
989 *concern (Omicron) for the EU/EEA*,
990 <[https://www.ecdc.europa.eu/sites/default/files/documents/Implications-emergence-](https://www.ecdc.europa.eu/sites/default/files/documents/Implications-emergence-spread-SARS-CoV-2%20B.1.1.529-variant-concern-Omicron-for-the-EU-EEA-Nov2021.pdf)
991 [spread-SARS-CoV-2%20B.1.1.529-variant-concern-Omicron-for-the-EU-EEA-](https://www.ecdc.europa.eu/sites/default/files/documents/Implications-emergence-spread-SARS-CoV-2%20B.1.1.529-variant-concern-Omicron-for-the-EU-EEA-Nov2021.pdf)
992 [Nov2021.pdf](https://www.ecdc.europa.eu/sites/default/files/documents/Implications-emergence-spread-SARS-CoV-2%20B.1.1.529-variant-concern-Omicron-for-the-EU-EEA-Nov2021.pdf)> (
- 993 20 *U.S. Pauses Distribution Of Monoclonal Antibody Treatments That Proved Ineffective*
994 *Against Omicron*, <[https://www.forbes.com/sites/zacharysmith/2021/12/23/us-pauses-](https://www.forbes.com/sites/zacharysmith/2021/12/23/us-pauses-distribution-of-mono-clonal-antibody-treatments-that-proved-ineffective-against-omicron/?sh=57bf26bf4c62)
995 [distribution-of-mono-clonal-antibody-treatments-that-proved-ineffective-against-](https://www.forbes.com/sites/zacharysmith/2021/12/23/us-pauses-distribution-of-mono-clonal-antibody-treatments-that-proved-ineffective-against-omicron/?sh=57bf26bf4c62)
996 [omicron/?sh=57bf26bf4c62](https://www.forbes.com/sites/zacharysmith/2021/12/23/us-pauses-distribution-of-mono-clonal-antibody-treatments-that-proved-ineffective-against-omicron/?sh=57bf26bf4c62)> (
- 997 21 Polack, F. P. *et al.* Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N*
998 *Engl J Med* **383**, 2603-2615, doi:10.1056/NEJMoa2034577 (2020).
- 999 22 Baden, L. R. *et al.* Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N*
1000 *Engl J Med* **384**, 403-416, doi:10.1056/NEJMoa2035389 (2021).
- 1001 23 Cele, S. *et al.* SARS-CoV-2 Omicron has extensive but incomplete escape of Pfizer
1002 BNT162b2 elicited neutralization and requires ACE2 for infection. *medRxiv*,
1003 doi:10.1101/2021.12.08.21267417 (2021).

- 1004 24 Garcia-Beltran, W. F. *et al.* mRNA-based COVID-19 vaccine boosters induce
1005 neutralizing immunity against SARS-CoV-2 Omicron variant. *Cell*,
1006 doi:10.1016/j.cell.2021.12.033 (2022).
- 1007 25 Atmar, R. L. *et al.* Homologous and Heterologous Covid-19 Booster Vaccinations. *N*
1008 *Engl J Med*, doi:10.1056/NEJMoa2116414 (2022).
- 1009 26 Costa Clemens, S. A. *et al.* Heterologous versus homologous COVID-19 booster
1010 vaccination in previous recipients of two doses of CoronaVac COVID-19 vaccine in
1011 Brazil (RHH-001): a phase 4, non-inferiority, single blind, randomised study. *Lancet* **399**,
1012 521-529, doi:10.1016/S0140-6736(22)00094-0 (2022).
- 1013 27 Hsieh, C. L. *et al.* Structure-based design of prefusion-stabilized SARS-CoV-2 spikes.
1014 *Science* **369**, 1501-1505, doi:10.1126/science.abd0826 (2020).
- 1015 28 Bewley, K. R. *et al.* Quantification of SARS-CoV-2 neutralizing antibody by wild-type
1016 plaque reduction neutralization, microneutralization and pseudotyped virus neutralization
1017 assays. *Nat Protoc* **16**, 3114-3140, doi:10.1038/s41596-021-00536-y (2021).
- 1018 29 Nie, J. *et al.* Quantification of SARS-CoV-2 neutralizing antibody by a pseudotyped
1019 virus-based assay. *Nat Protoc* **15**, 3699-3715, doi:10.1038/s41596-020-0394-5 (2020).
- 1020 30 Liu, L. *et al.* Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2
1021 spike. *Nature* **584**, 450-456, doi:10.1038/s41586-020-2571-7 (2020).
- 1022 31 McCallum, M. *et al.* Structural basis of SARS-CoV-2 Omicron immune evasion and
1023 receptor engagement. *Science*, eabn8652, doi:10.1126/science.abn8652 (2022).
- 1024 32 Mannar, D. *et al.* SARS-CoV-2 Omicron variant: Antibody evasion and cryo-EM
1025 structure of spike protein-ACE2 complex. *Science*, eabn7760,
1026 doi:10.1126/science.abn7760 (2022).
- 1027 33 Luo, C. H., et al. Infection with the SARS-CoV-2 Delta Variant is Associated with
1028 Higher Infectious Virus Loads Compared to the Alpha Variant in both Unvaccinated and
1029 Vaccinated Individuals. *medRxiv*, doi:<https://doi.org/10.1101/2021.08.15.21262077>.
- 1030 34 Liu, Y. & Rocklöv, J. The reproductive number of the Delta variant of SARS-CoV-2 is
1031 far higher compared to the ancestral SARS-CoV-2 virus. *J Travel Med* **28**,
1032 doi:10.1093/jtm/taab124 (2021).
- 1033 35 Cao, Y. *et al.* Omicron escapes the majority of existing SARS-CoV-2 neutralizing
1034 antibodies. *Nature*, doi:10.1038/s41586-021-04385-3 (2021).

- 1035 36 Wang, e. a. A third dose of inactivated vaccine augments the potency, breadth, and
1036 duration of anamnestic responses against SARS-CoV-2. *MedRxiv* (2021).
- 1037 37 Cameroni, E. *et al.* Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron
1038 antigenic shift. *Nature*, doi:10.1038/s41586-021-04386-2 (2021).
- 1039 38 Hastie, K. M. *et al.* Defining variant-resistant epitopes targeted by SARS-CoV-2
1040 antibodies: A global consortium study. *Science* **374**, 472-478,
1041 doi:10.1126/science.abh2315 (2021).
- 1042 39 Barnes, C. O. *et al.* SARS-CoV-2 neutralizing antibody structures inform therapeutic
1043 strategies. *Nature* **588**, 682-687, doi:10.1038/s41586-020-2852-1 (2020).
- 1044 40 Yuan, M. *et al.* Structural and functional ramifications of antigenic drift in recent SARS-
1045 CoV-2 variants. *Science* **373**, 818-823, doi:10.1126/science.abh1139 (2021).
- 1046 41 Dejnirattisai, W. *et al.* The antigenic anatomy of SARS-CoV-2 receptor binding domain.
1047 *Cell* **184**, 2183-2200 e2122, doi:10.1016/j.cell.2021.02.032 (2021).
- 1048 42 Wang, K. *et al.* Memory B cell repertoire from triple vaccinees against diverse SARS-
1049 CoV-2 variants. *Nature*, doi:10.1038/s41586-022-04466-x (2022).
- 1050 43 Lei, e. a. Monospecific and bispecific monoclonal SARS-CoV-2 neutralizing antibodies
1051 that maintain potency against B.1.617. doi:Monospecific and bispecific monoclonal
1052 SARS-CoV-2 neutralizing antibodies that maintain potency against B.1.617 (2021).
- 1053 44 *Coronavirus (COVID-19) Vaccinations*, <[https://ourworldindata.org/covid-
1054 vaccinations?country=OWID_WRL](https://ourworldindata.org/covid-vaccinations?country=OWID_WRL)> (
- 1055 45 Goldberg, Y. *et al.* Waning Immunity after the BNT162b2 Vaccine in Israel. *N Engl J*
1056 *Med* **385**, e85, doi:10.1056/NEJMoa2114228 (2021).
- 1057 46 Thomas, S. J. *et al.* Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine
1058 through 6 Months. *N Engl J Med* **385**, 1761-1773, doi:10.1056/NEJMoa2110345 (2021).
- 1059 47 Levin, E. G. *et al.* Waning Immune Humoral Response to BNT162b2 Covid-19 Vaccine
1060 over 6 Months. *N Engl J Med* **385**, e84, doi:10.1056/NEJMoa2114583 (2021).
- 1061 48 Doria-Rose, N. *et al.* Antibody Persistence through 6 Months after the Second Dose of
1062 mRNA-1273 Vaccine for Covid-19. *N Engl J Med* **384**, 2259-2261,
1063 doi:10.1056/NEJMc2103916 (2021).

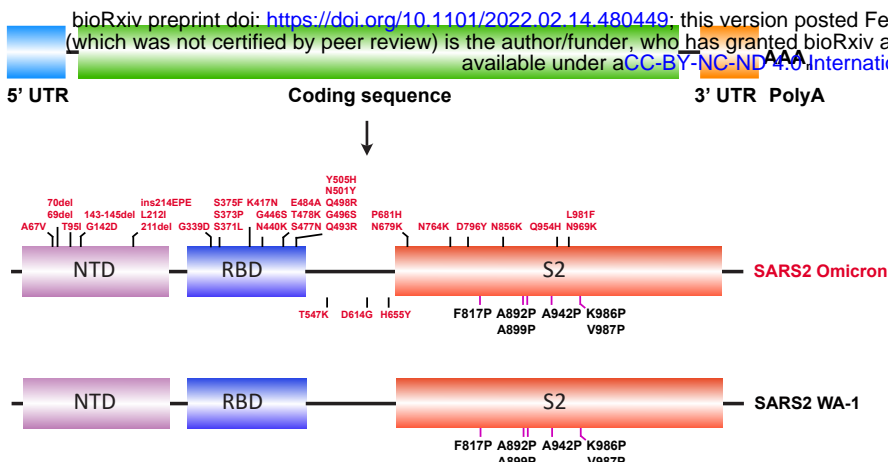
- 1064 49 Moderna starts trial for Omicron-specific booster shot,
1065 <[https://www.reuters.com/business/healthcare-pharmaceuticals/moderna-starts-trial-](https://www.reuters.com/business/healthcare-pharmaceuticals/moderna-starts-trial-testing-omicron-specific-booster-shot-2022-01-26/)
1066 [testing-omicron-specific-booster-shot-2022-01-26/](https://www.reuters.com/business/healthcare-pharmaceuticals/moderna-starts-trial-testing-omicron-specific-booster-shot-2022-01-26/)> (
- 1067 50 Pfizer and BioNTech Initiate Study to Evaluate Omicron-Based COVID-19 Vaccine in
1068 Adults 18 to 55 Years of Age, <[https://www.pfizer.com/news/press-release/press-release-](https://www.pfizer.com/news/press-release/press-release-detail/pfizer-and-biontech-initiate-study-evaluate-omicron-based)
1069 [detail/pfizer-and-biontech-initiate-study-evaluate-omicron-based](https://www.pfizer.com/news/press-release/press-release-detail/pfizer-and-biontech-initiate-study-evaluate-omicron-based)> (2022).
- 1070 51 A Study to Evaluate the Immunogenicity and Safety of mRNA-1273.529 Vaccine for the
1071 COVID-19 Omicron Variant B.1.1.529,
1072 <[https://clinicaltrials.gov/ct2/show/record/NCT05249829?cond=omicron+sars&draw=2](https://clinicaltrials.gov/ct2/show/record/NCT05249829?cond=omicron+sars&draw=2&rank=2)
1073 [&rank=2](https://clinicaltrials.gov/ct2/show/record/NCT05249829?cond=omicron+sars&draw=2&rank=2)> (
- 1074 52 Zhang, N. N. *et al.* Rapid development of an updated mRNA vaccine against the SARS-
1075 CoV-2 Omicron variant. *Cell Res*, doi:10.1038/s41422-022-00626-w (2022).
- 1076 53 Ying, B. *et al.* Boosting with Omicron-matched or historical mRNA vaccines increases
1077 neutralizing antibody responses and protection against B.1.1.529 infection in mice.
1078 *bioRxiv*, doi:10.1101/2022.02.07.479419 (2022).
- 1079 54 Gagne, M., *et al.* mRNA-1273 or mRNA-Omicron boost in vaccinated macaques elicits
1080 comparable B cell expansion, neutralizing antibodies and protection against Omicron.
1081 doi:<https://doi.org/10.1101/2022.02.03.479037>.
- 1082 55 Lee, e. a. Omicron-specific mRNA vaccine induced potent neutralizing antibody against
1083 Omicron but not other SARS-CoV-2 variants.
1084 doi:<https://doi.org/10.1101/2022.01.31.478406> (2022).
- 1085 56 Zang, e. a. An mRNA vaccine candidate for the SARS-CoV-2 Omicron variant. *BioRxiv*,
1086 doi:<https://doi.org/10.1101/2022.02.07.479348>.
- 1087 57 Hawman, e. a. Replicating RNA platform enables rapid response to the SARS-CoV-2
1088 Omicron variant and elicits enhanced protection in naïve hamsters compared to ancestral
1089 vaccine. *BioRxiv*, doi:<https://doi.org/10.1101/2022.01.31.478520> (2022).
- 1090 58 Khoury, D. S. *et al.* Neutralizing antibody levels are highly predictive of immune
1091 protection from symptomatic SARS-CoV-2 infection. *Nat Med* **27**, 1205-1211,
1092 doi:10.1038/s41591-021-01377-8 (2021).
- 1093 59 Corbett, K. S. *et al.* SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen
1094 preparedness. *Nature* **586**, 567-571, doi:10.1038/s41586-020-2622-0 (2020).

Omicron vs WT vaccine boosters

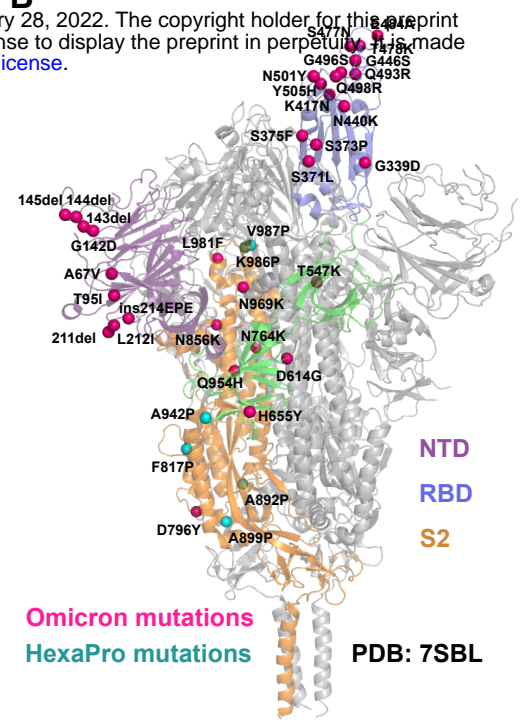
- 1095 60 Vogel, A. B., et al. A prefusion SARS-CoV-2 spike RNA vaccine is highly immunogenic
1096 and prevents lung infection in non-human primates. *bioRxiv*,
1097 doi:<https://doi.org/10.1101/2020.09.08.280818> (2020).
- 1098 61 Schmidt, F. *et al.* Measuring SARS-CoV-2 neutralizing antibody activity using
1099 pseudotyped and chimeric viruses. *J Exp Med* **217**, doi:10.1084/jem.20201181 (2020).
- 1100 62 Hassett, K. J. *et al.* Optimization of Lipid Nanoparticles for Intramuscular Administration
1101 of mRNA Vaccines. *Mol Ther Nucleic Acids* **15**, 1-11, doi:10.1016/j.omtn.2019.01.013
1102 (2019).
- 1103 63 Lucas, C. *et al.* Impact of circulating SARS-CoV-2 variants on mRNA vaccine-induced
1104 immunity. *Nature* **600**, 523-529, doi:10.1038/s41586-021-04085-y (2021).
- 1105 64 Wei, J. *et al.* Genome-wide CRISPR Screens Reveal Host Factors Critical for SARS-
1106 CoV-2 Infection. *Cell* **184**, 76-91 e13, doi:10.1016/j.cell.2020.10.028 (2021).
- 1107 65 Perez-Then, E. *et al.* Neutralizing antibodies against the SARS-CoV-2 Delta and
1108 Omicron variants following heterologous CoronaVac plus BNT162b2 booster
1109 vaccination. *Nat Med*, doi:10.1038/s41591-022-01705-6 (2022).
- 1110

Figure 1

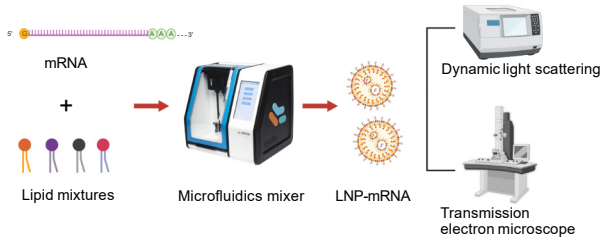
A



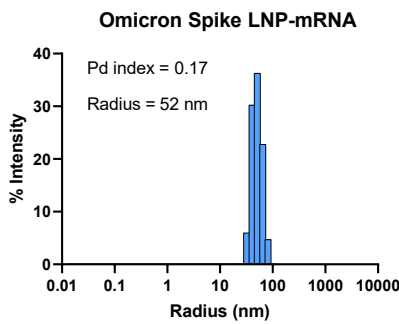
B



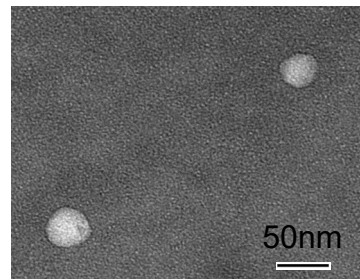
C



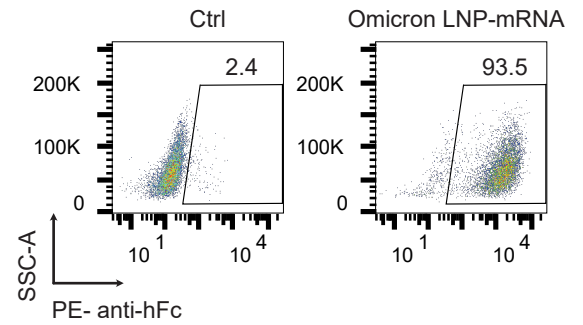
D



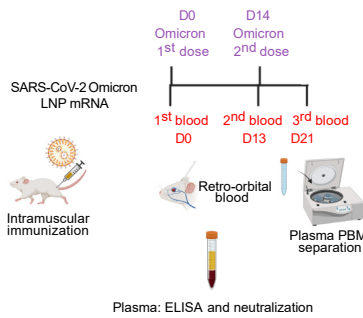
E



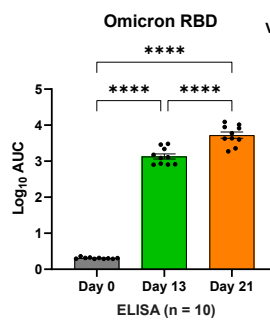
F



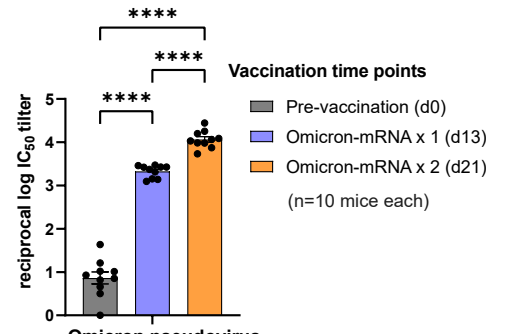
G



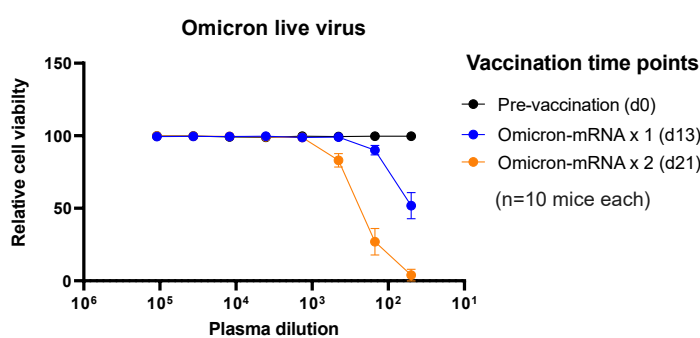
H



I



J



K

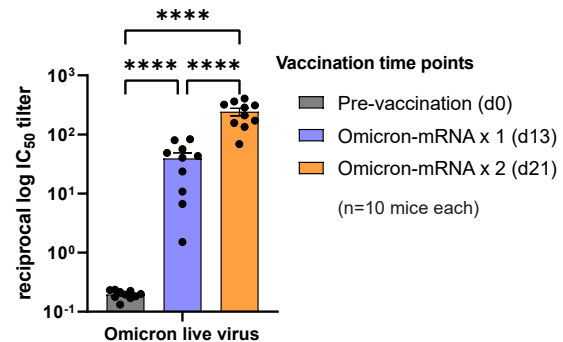


Figure 2

A bioRxiv preprint doi: <https://doi.org/10.1101/2022.02.14.480449>; this version posted February 28, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

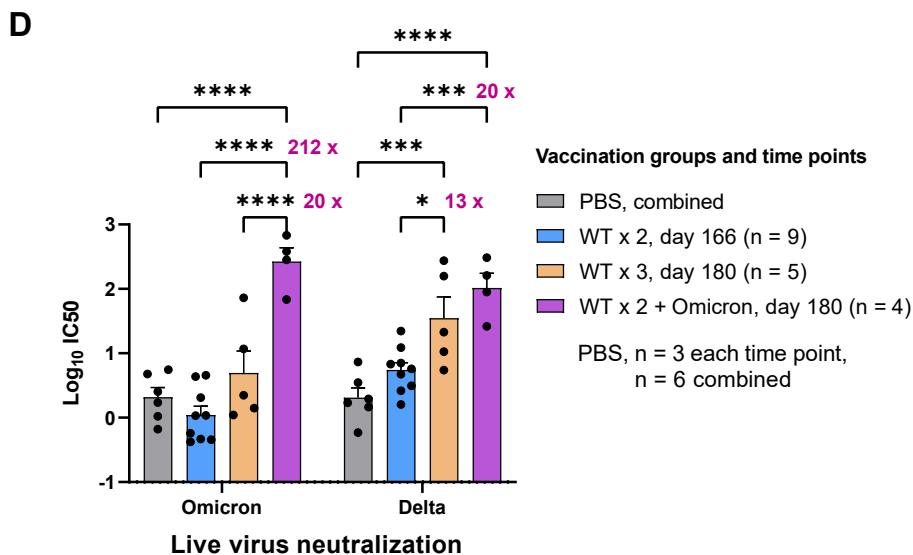
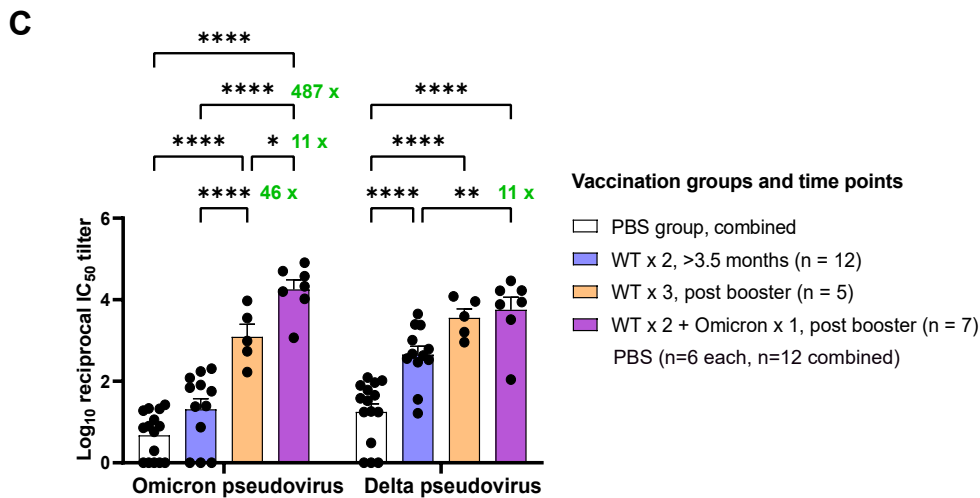
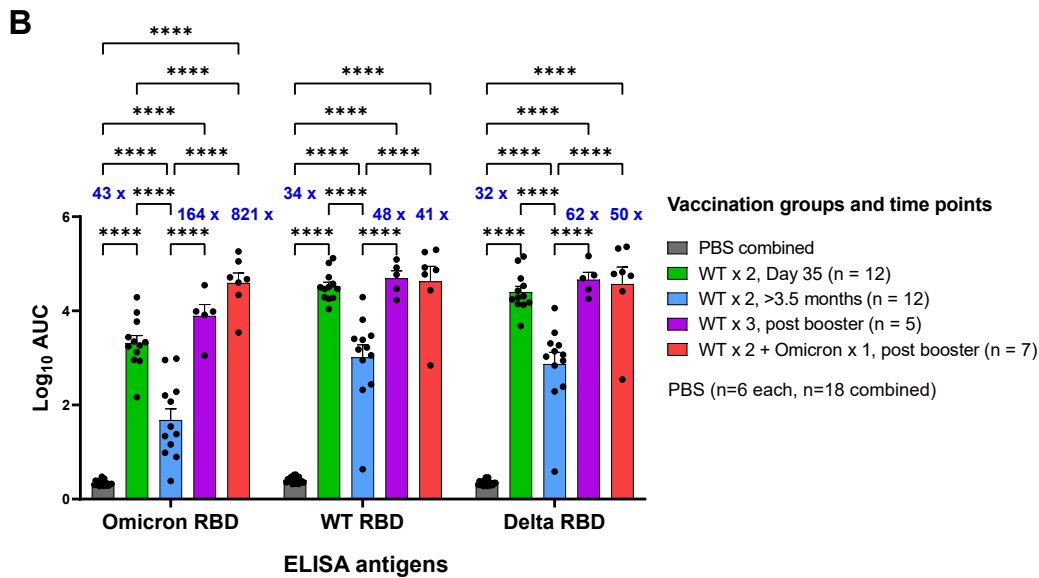
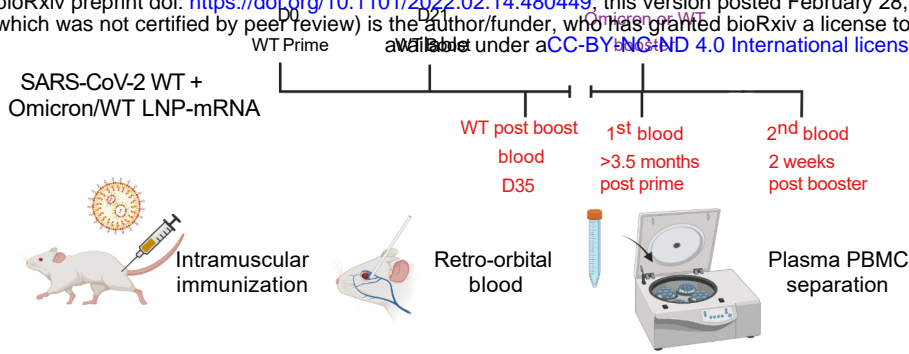
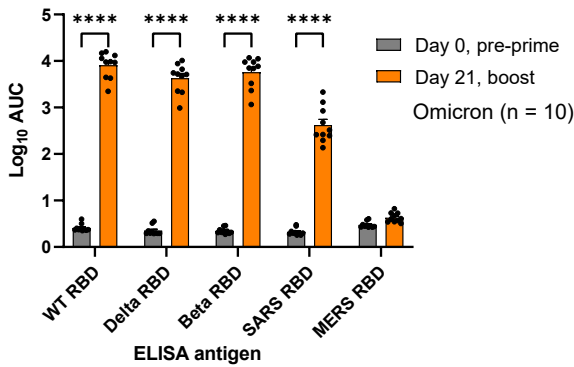


Figure 3

bioRxiv preprint doi: <https://doi.org/10.1101/2022.02.14.480449>; this version posted February 28, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

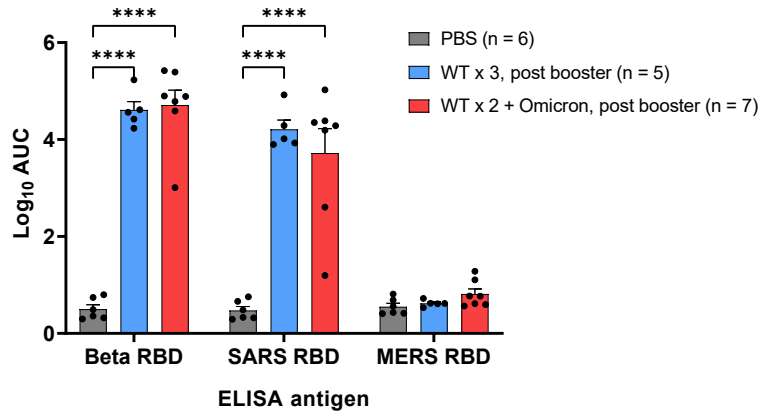
A

Omicron x 2 plasma against RBDs

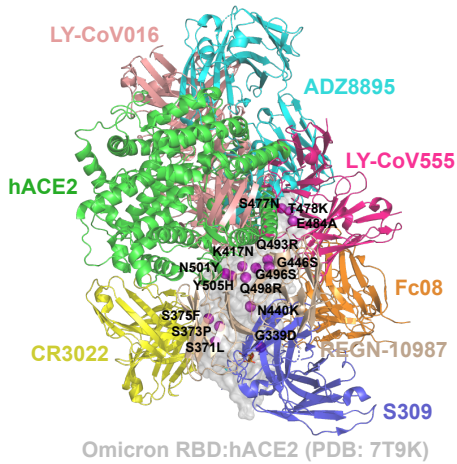


B

WT + Omicron plasma against RBDs

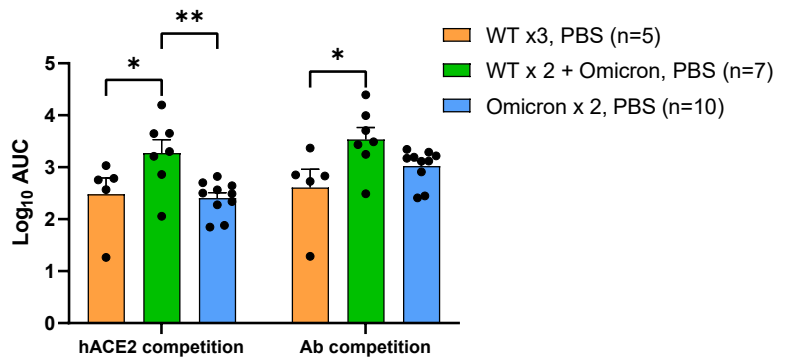


C



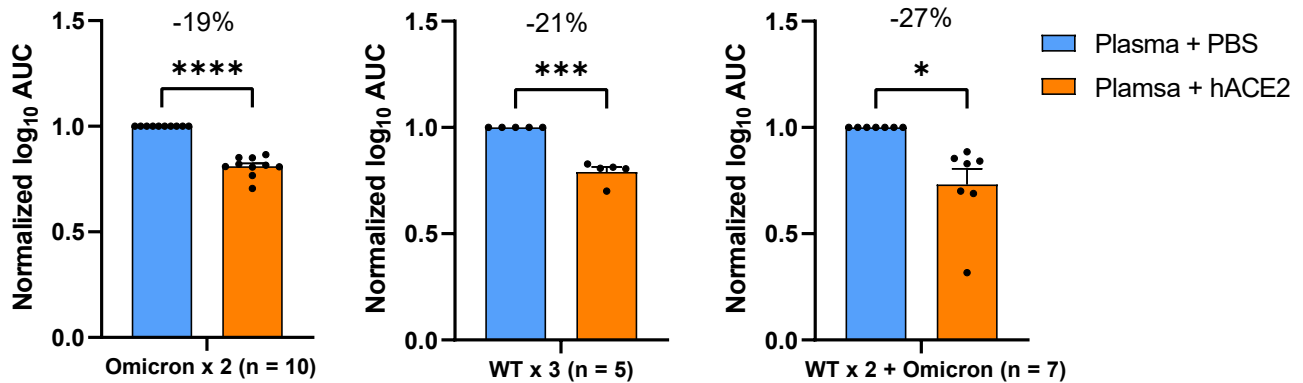
D

Baseline AUC against Omicron RBD



E

Plasma against Omicron RBD in hACE2 competition ELISA



F

Plasma against Omicron RBD in antibody competition ELISA

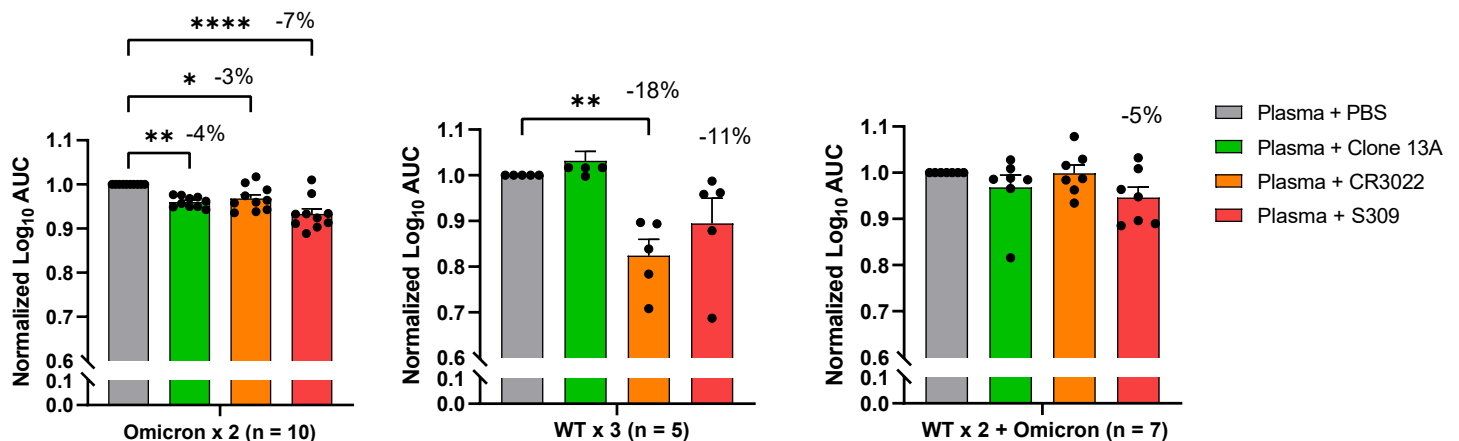
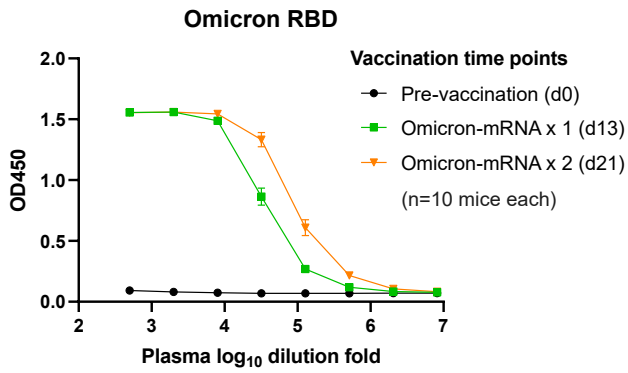


Figure S1

bioRxiv preprint doi: <https://doi.org/10.1101/2022.02.14.480449>; this version posted February 28, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a [CC-BY-NC-ND 4.0 International license](#).

A



B

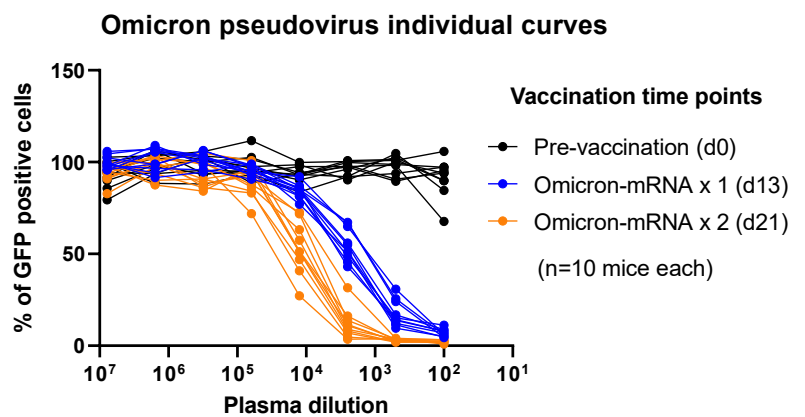
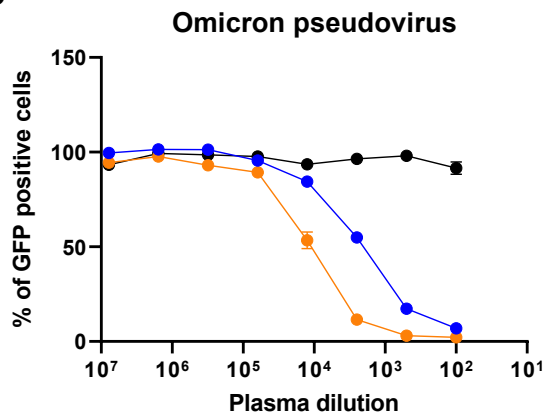


Figure S2

A bioRxiv preprint doi: <https://doi.org/10.1101/2022.02.14.480449>; this version posted February 28, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

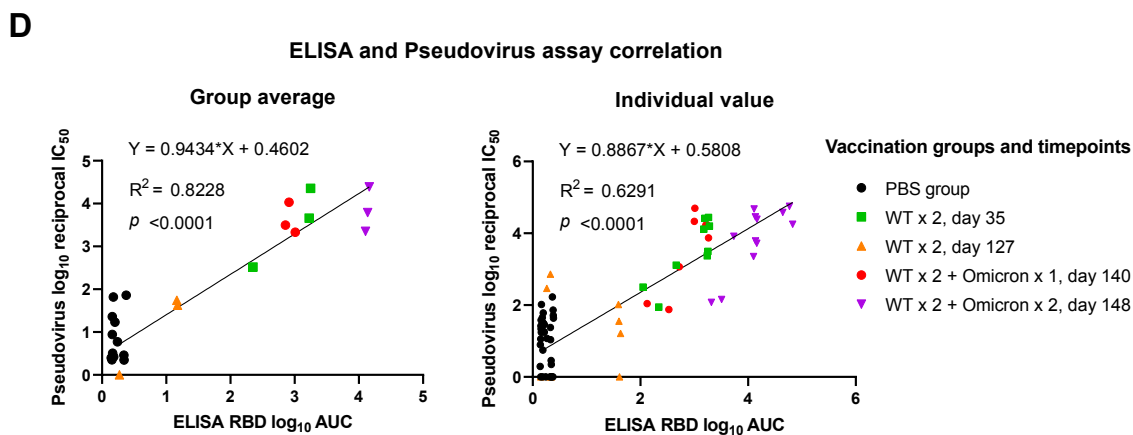
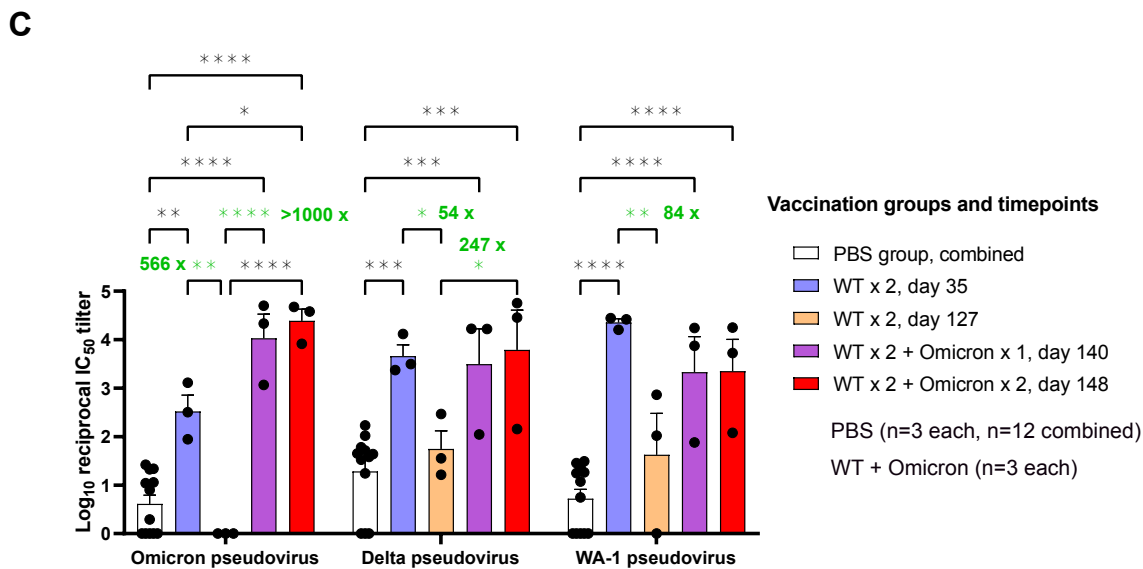
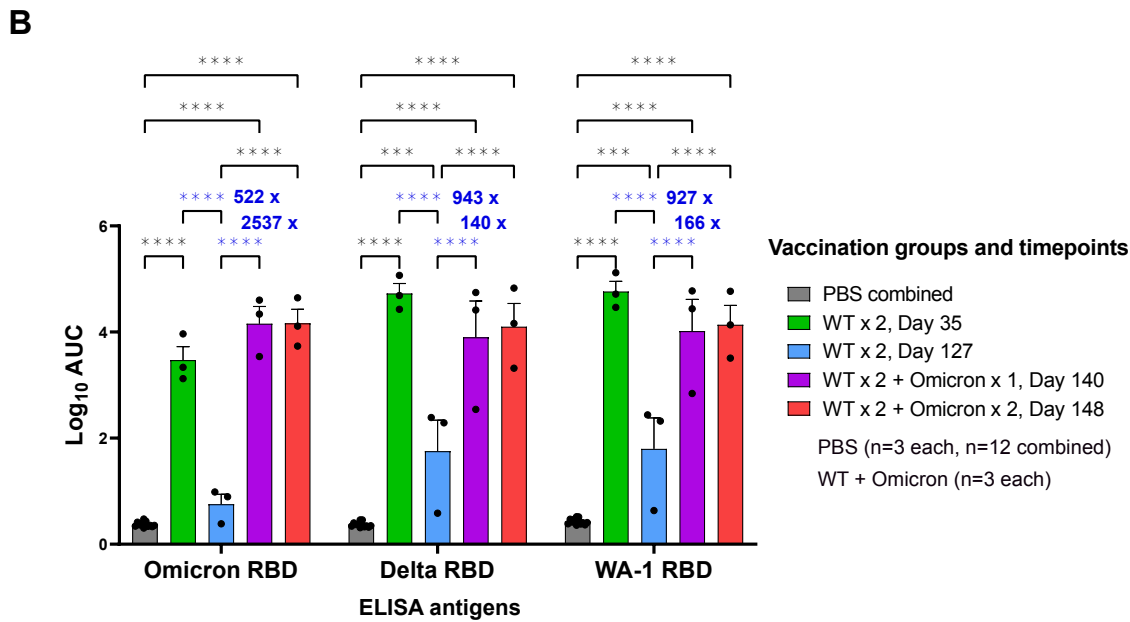
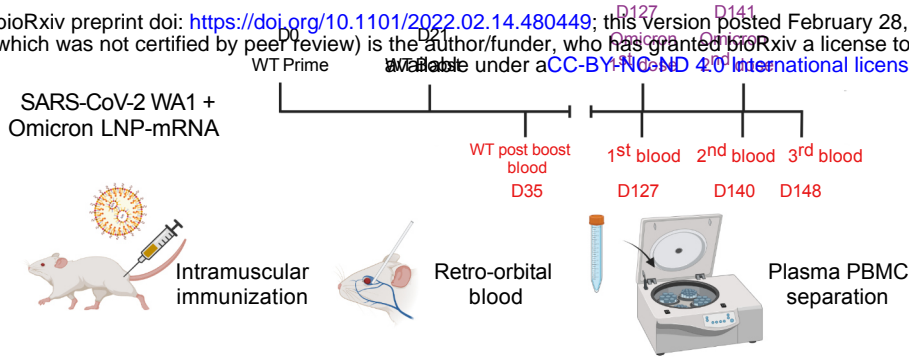
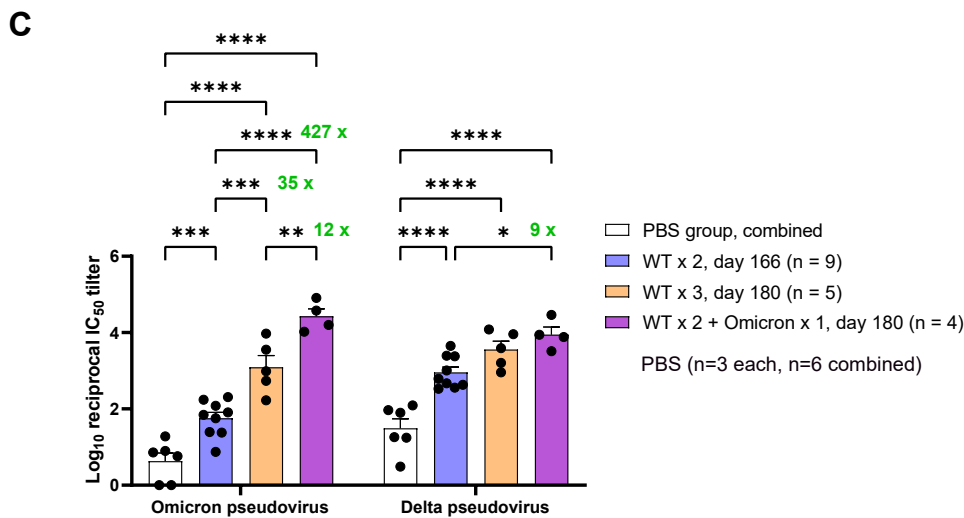
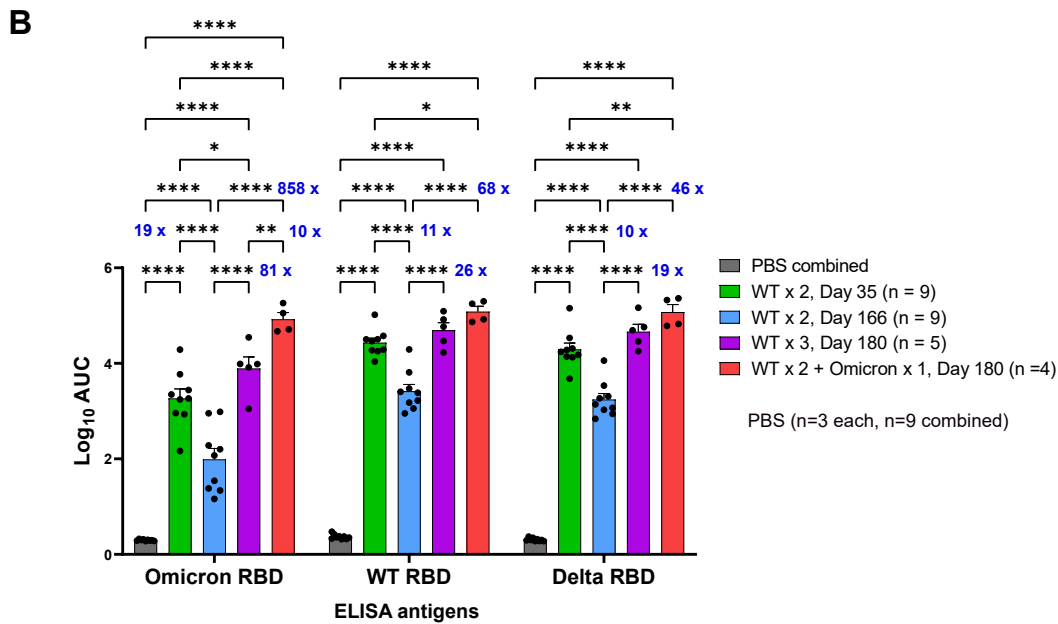
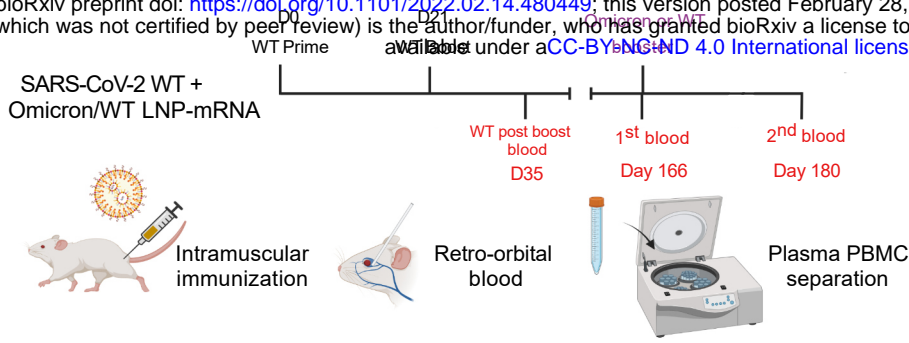


Figure S3

A bioRxiv preprint doi: <https://doi.org/10.1101/2022.02.14.480449>; this version posted February 28, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.



D ELISA and Pseudovirus assay correlation

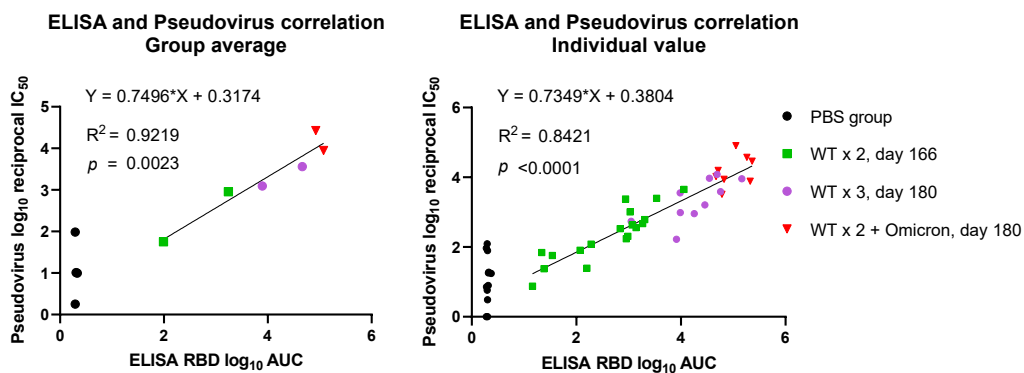
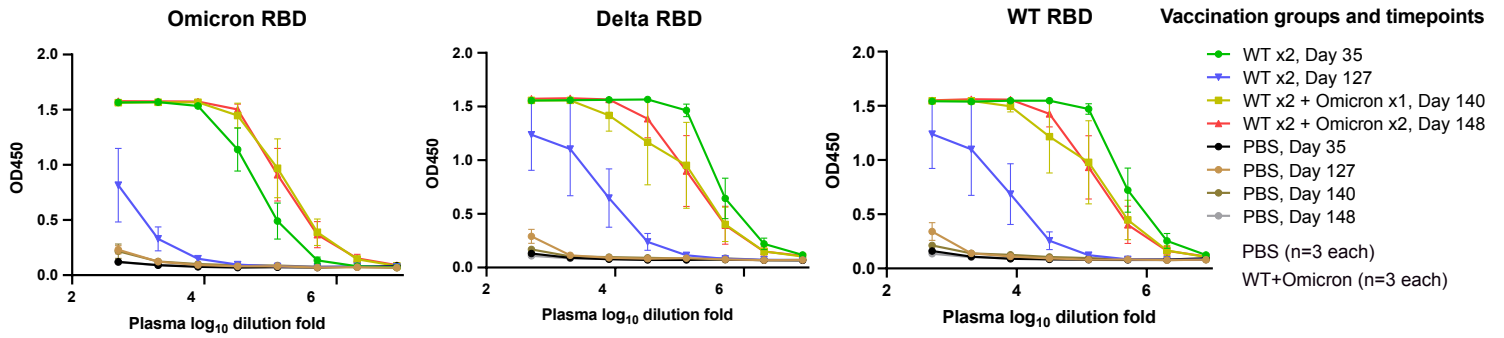


Figure S4

A

bioRxiv preprint doi: <https://doi.org/10.1101/2022.02.14.480449>; this version posted February 28, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.



B

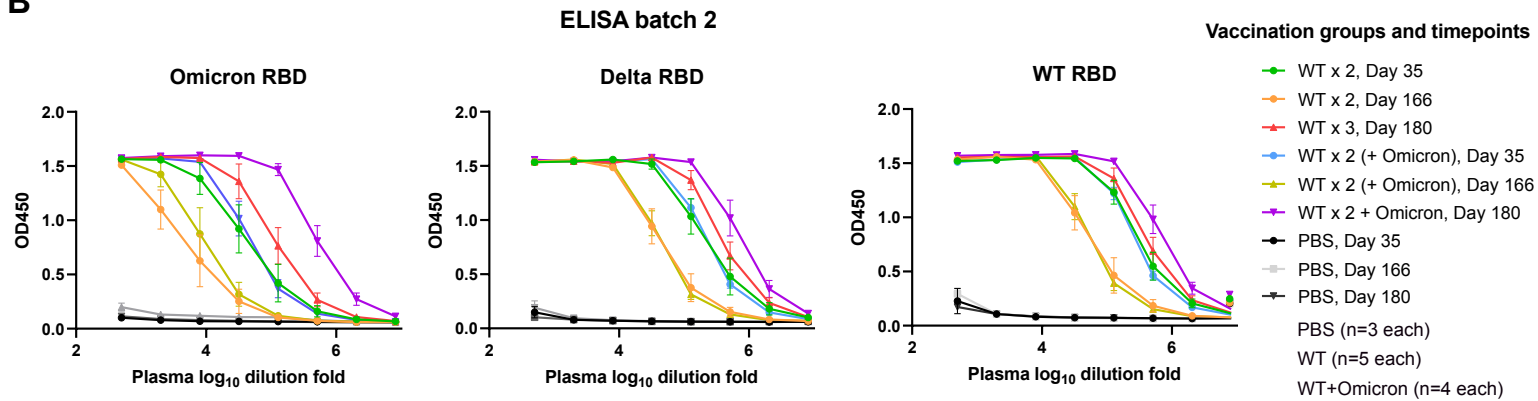


Figure S5

bioRxiv preprint doi: <https://doi.org/10.1101/2022.02.14.480449>; this version posted February 28, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a [CC-BY-NC-ND 4.0 International license](https://creativecommons.org/licenses/by-nc-nd/4.0/).

ELISA batch 1 and 2

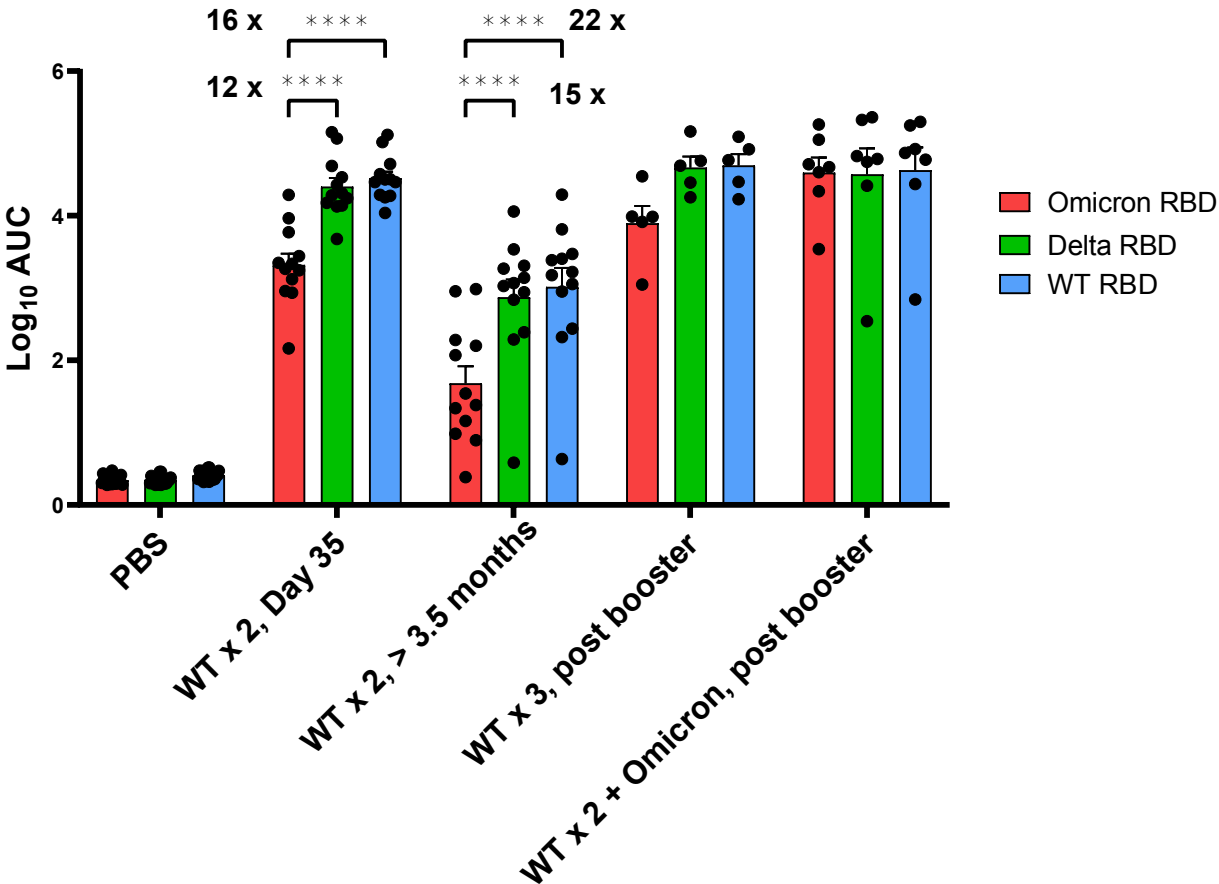
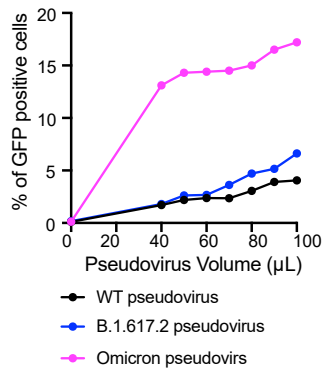


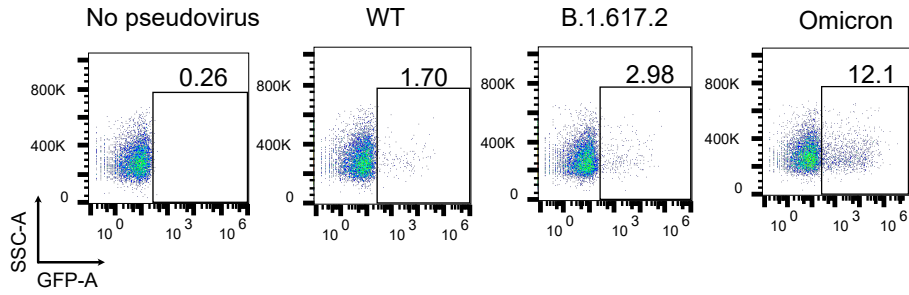
Figure S6

bioRxiv preprint doi: <https://doi.org/10.1101/2022.02.14.480449>; this version posted February 28, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a [CC-BY-NC-ND 4.0 International license](https://creativecommons.org/licenses/by-nc-nd/4.0/).

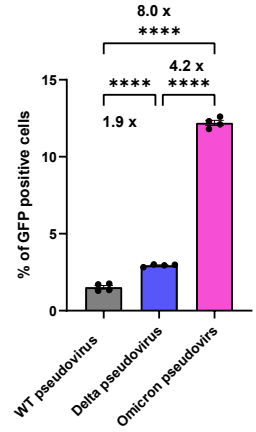
A



B

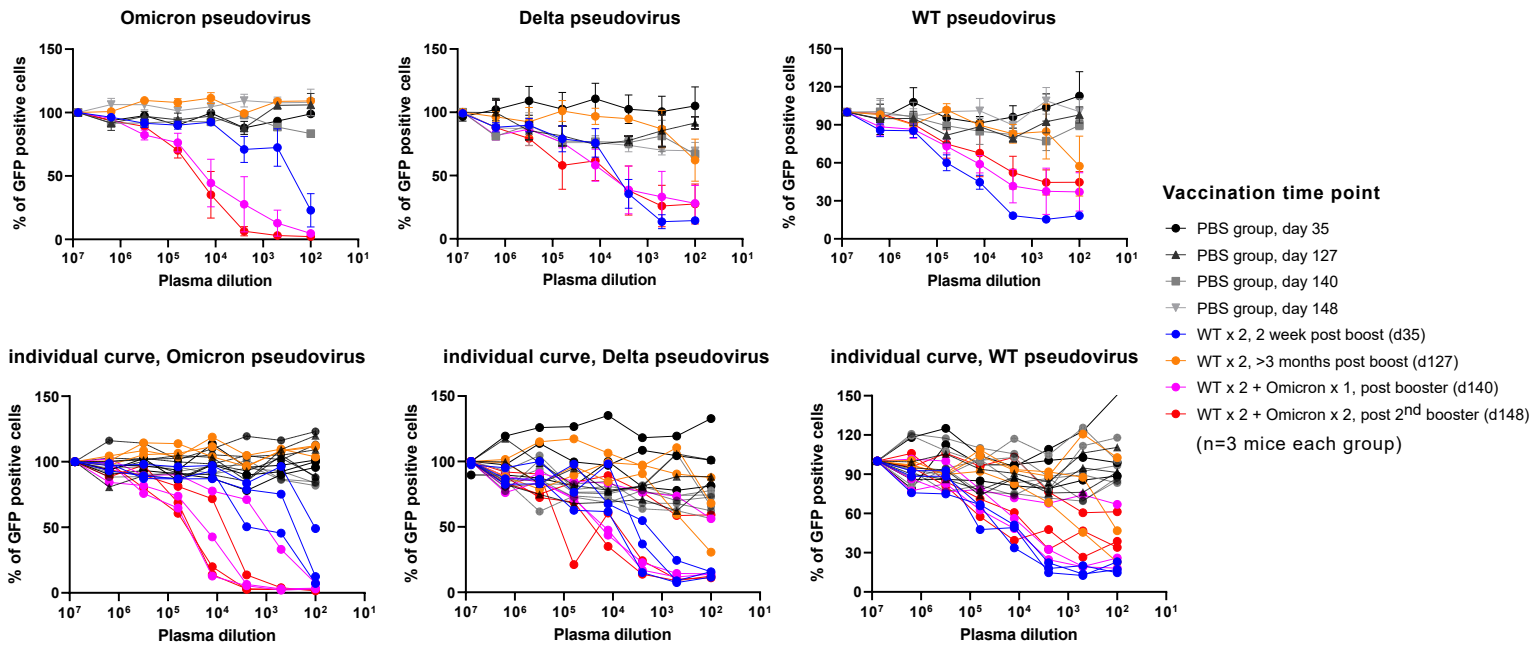


C



D

Pseudovirus neutralization batch 1



E

Pseudovirus neutralization batch 2

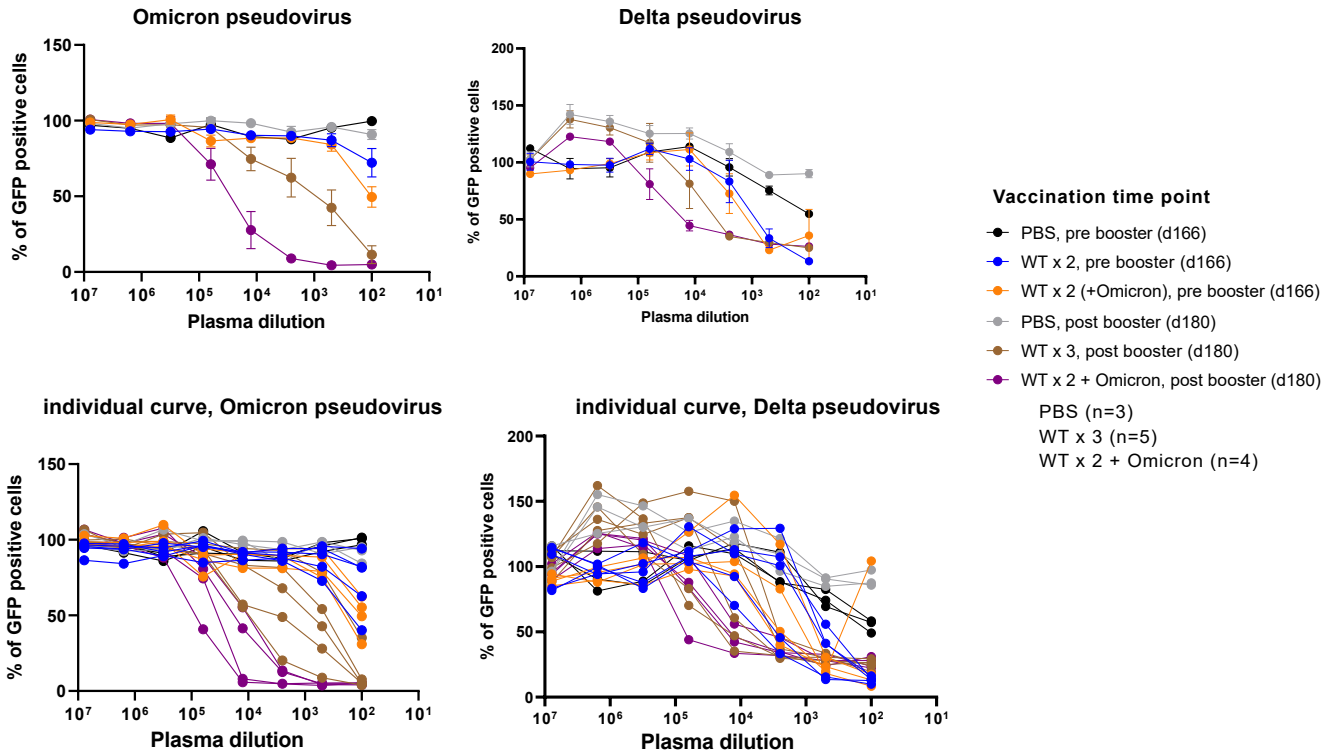
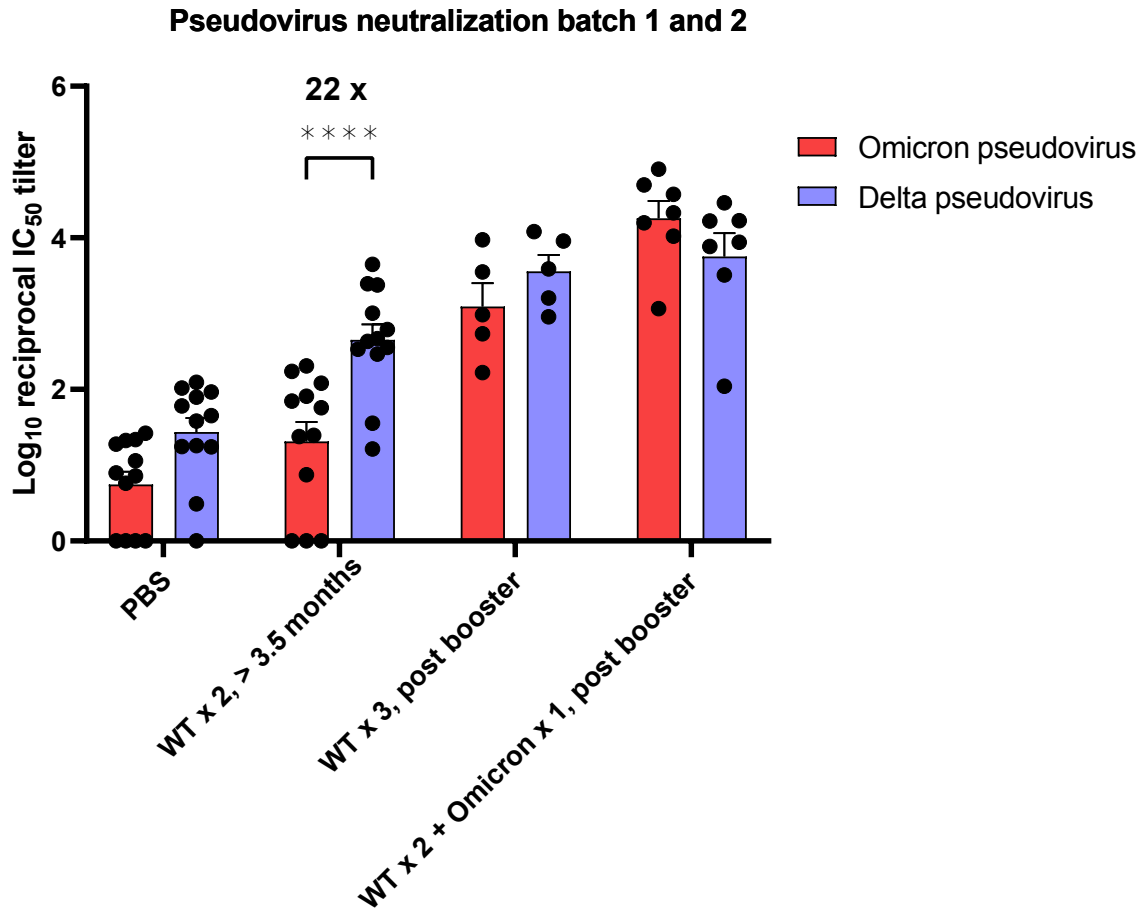


Figure S7

bioRxiv preprint doi: <https://doi.org/10.1101/2022.02.14.480449>; this version posted February 28, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a [CC-BY-NC-ND 4.0 International license](https://creativecommons.org/licenses/by-nc-nd/4.0/).

A



B

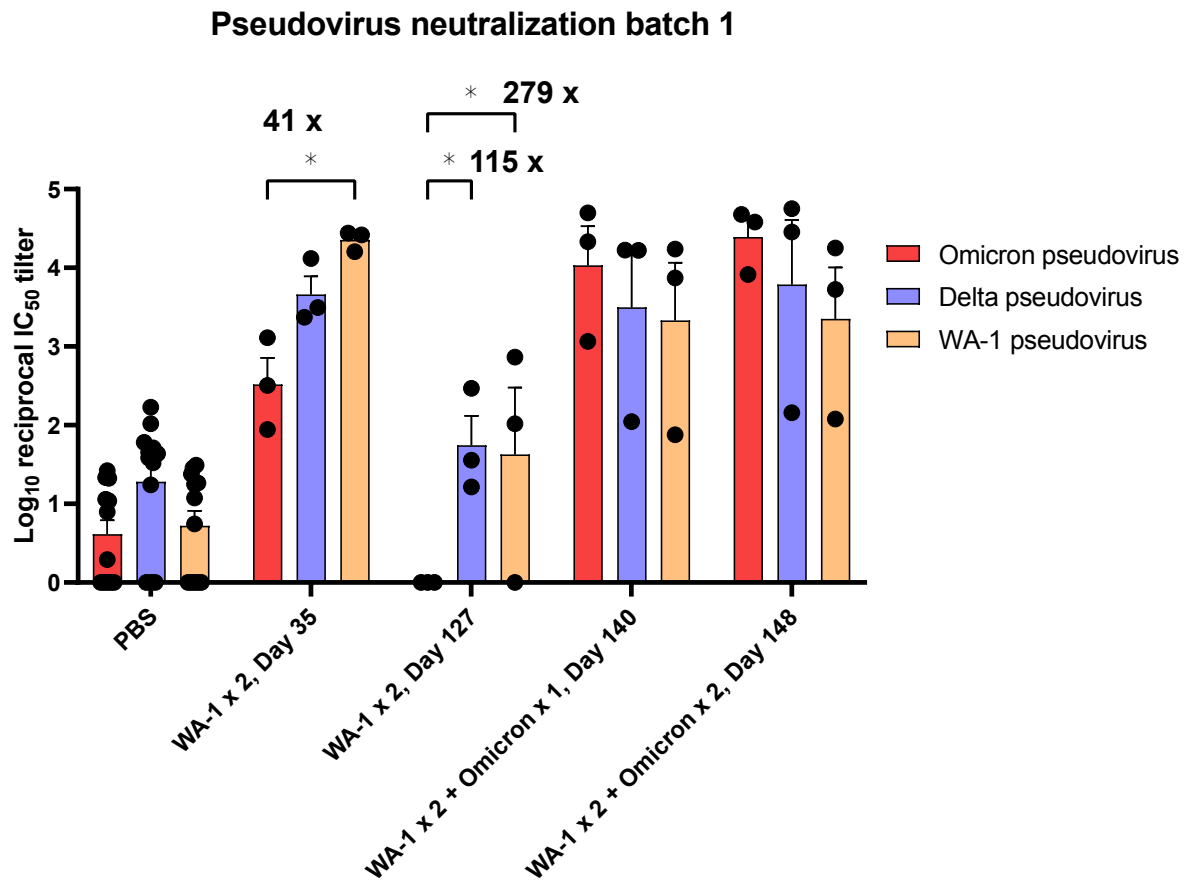


Figure S8

bioRxiv preprint doi: <https://doi.org/10.1101/2022.02.14.480449>; this version posted February 28, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a [CC-BY-NC-ND 4.0 International license](#).

A

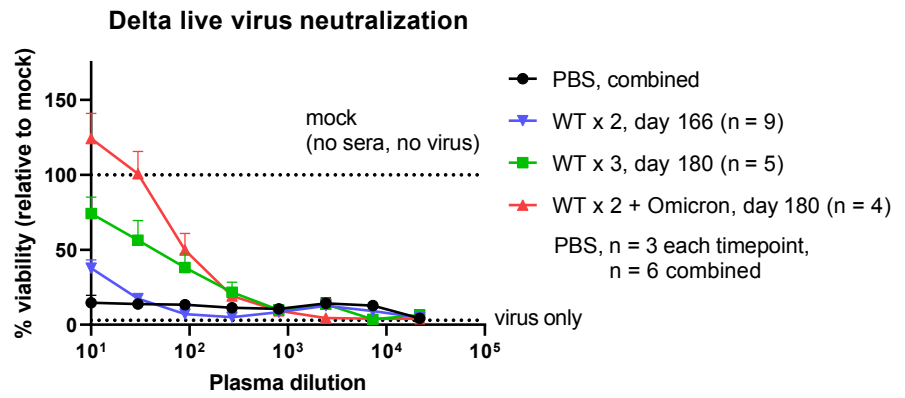
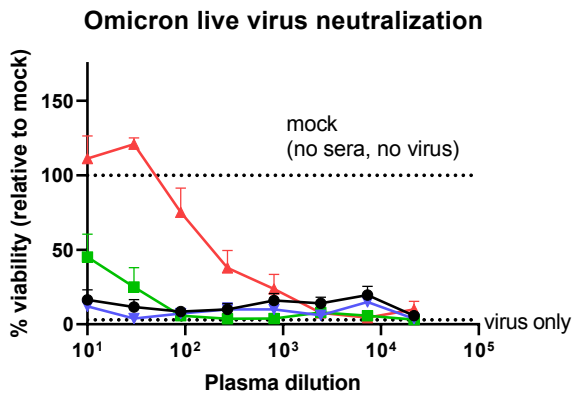
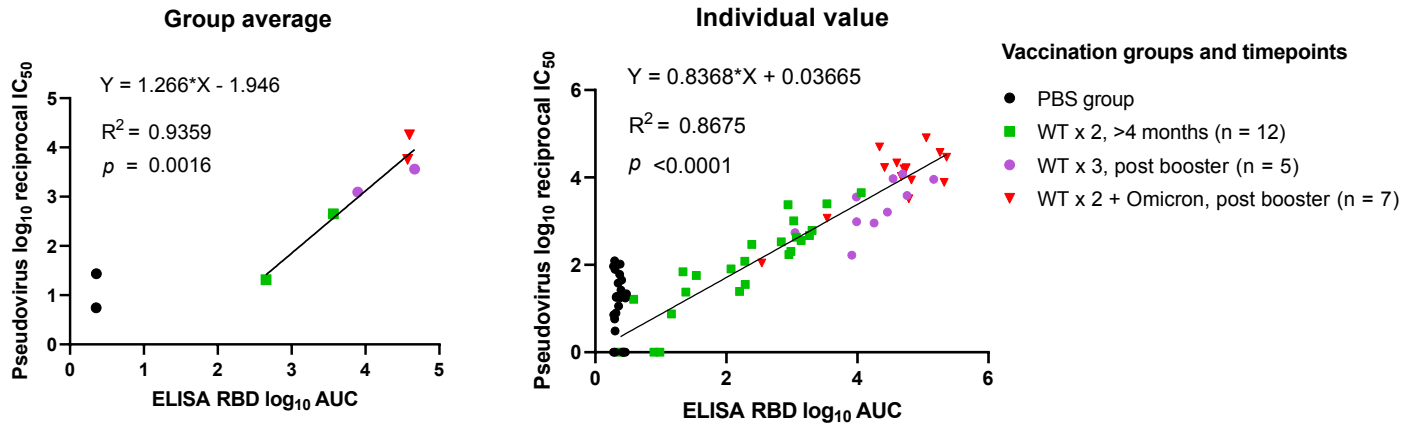


Figure S9

bioRxiv preprint doi: <https://doi.org/10.1101/2022.02.14.480449>; this version posted February 28, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a [CC-BY-NC-ND 4.0 International license](#).

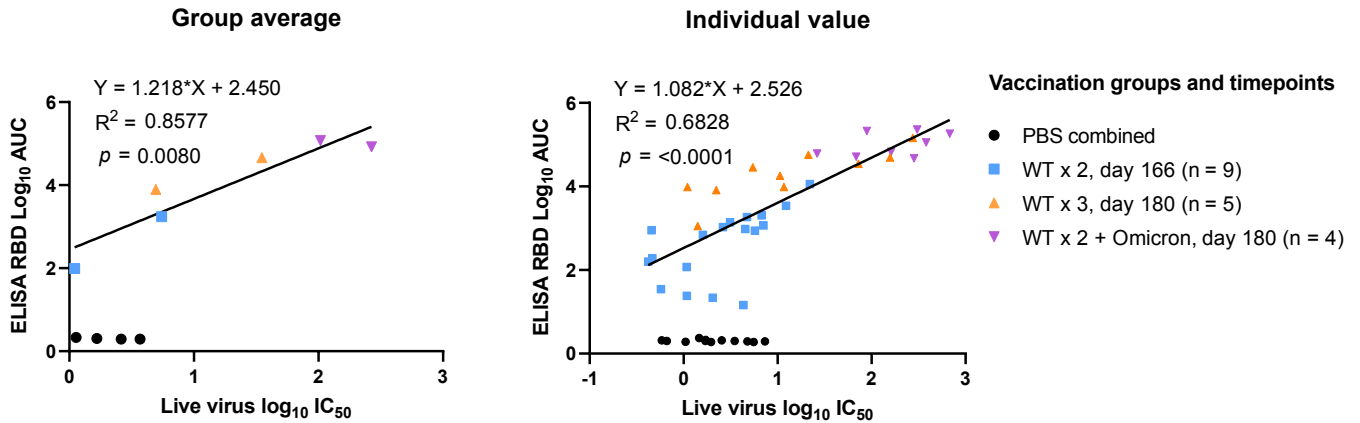
A

ELISA and pseudovirus neutralization titer correlation



B

ELISA and live virus neutralization titer correlation



C

Live virus and pseudovirus neutralization titer correlation

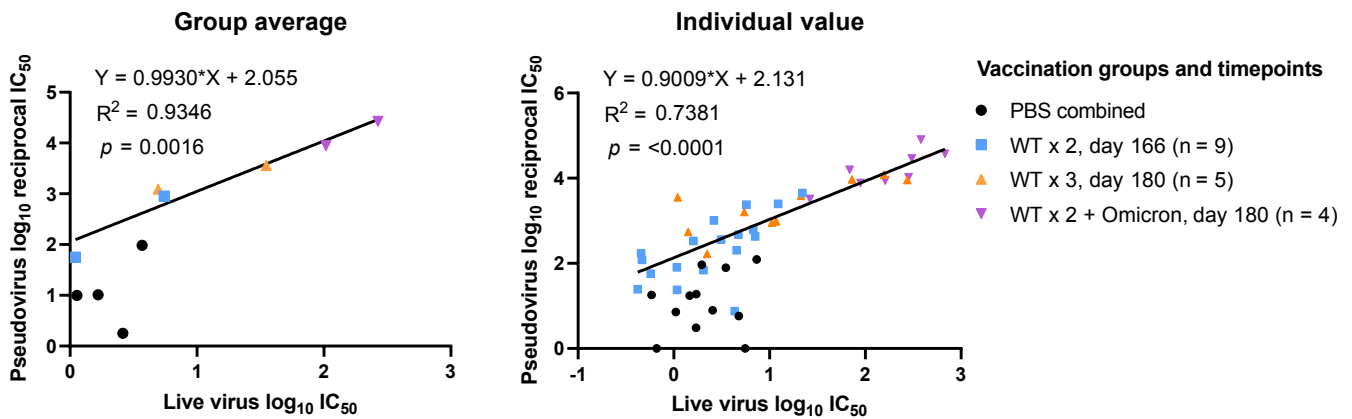
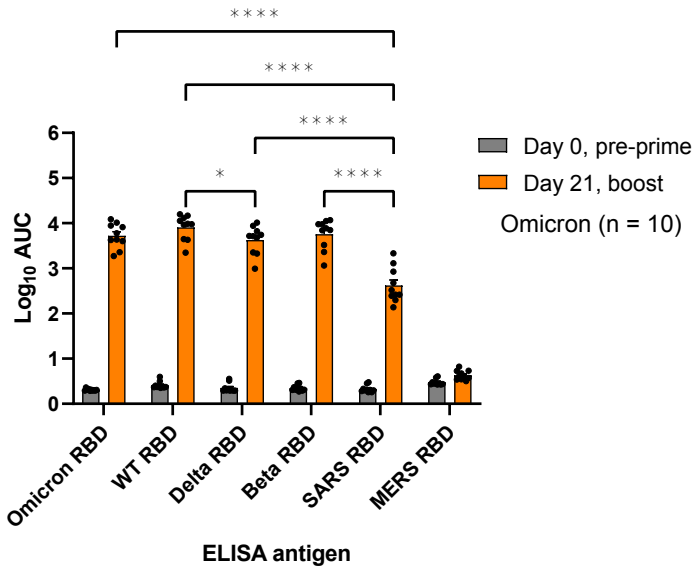


Figure S10

bioRxiv preprint doi: <https://doi.org/10.1101/2022.02.14.480449>; this version posted February 28, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a [CC-BY-NC-ND 4.0 International license](https://creativecommons.org/licenses/by-nc-nd/4.0/).

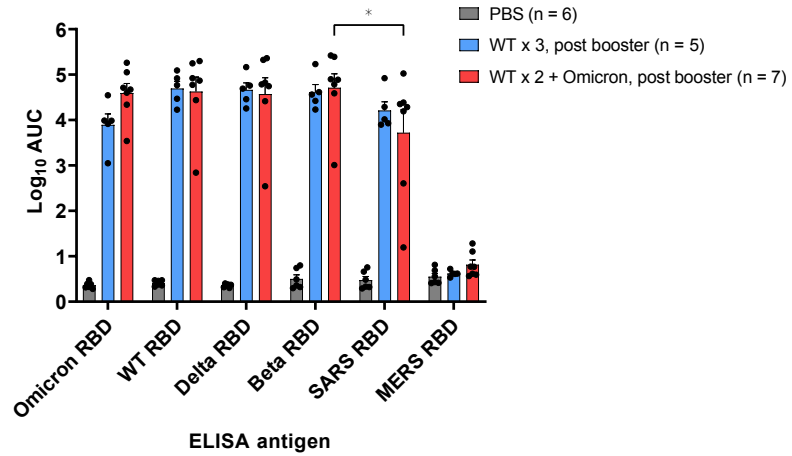
A

Omicron x 2 plasma against RBDs



B

WT + Omicron plasma against RBDs



C

WT + Omicron plasma against RBDs

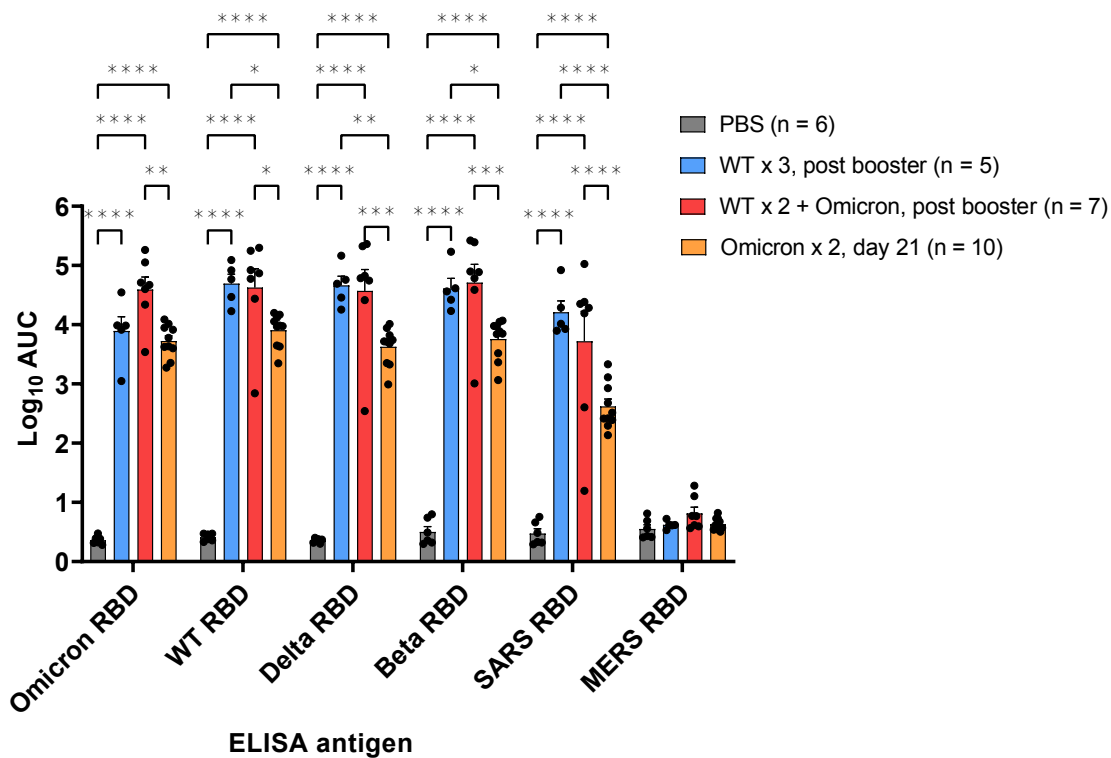
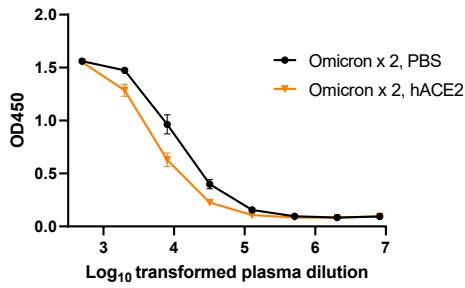


Figure S11

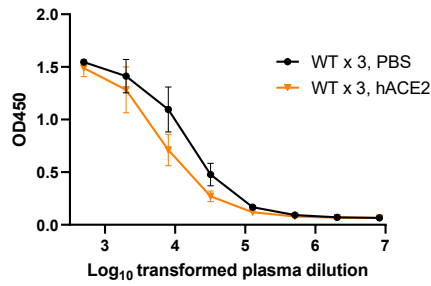
bioRxiv preprint doi: <https://doi.org/10.1101/2022.02.14.480449>; this version posted February 28, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a [CC-BY-NC-ND 4.0 International license](https://creativecommons.org/licenses/by-nc-nd/4.0/).

A

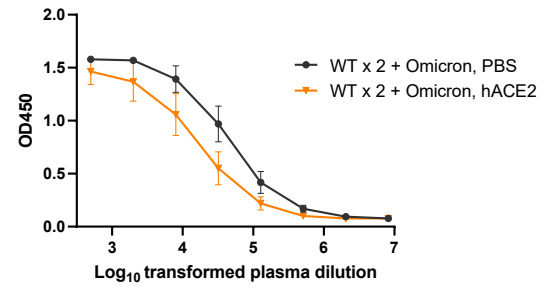
Omicron x 2 plasma against Omicron RBD



WT x 3 plasma against Omicron RBD

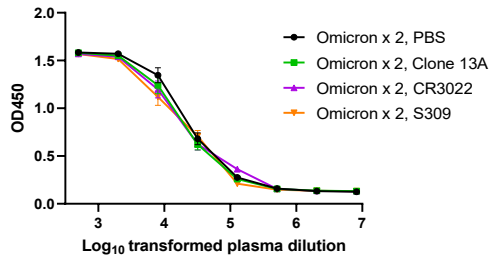


WT x 2 + Omicron plasma against Omicron RBD

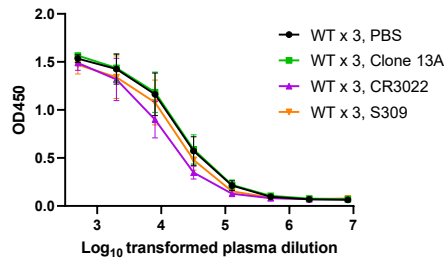


B

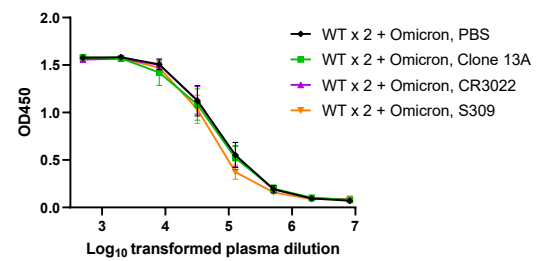
Omicron x 2 plasma against Omicron RBD



WT x 3 plasma against Omicron RBD



WT x 2 + Omicron, plasma against Omicron RBD



C

PBS against Omicron RBD

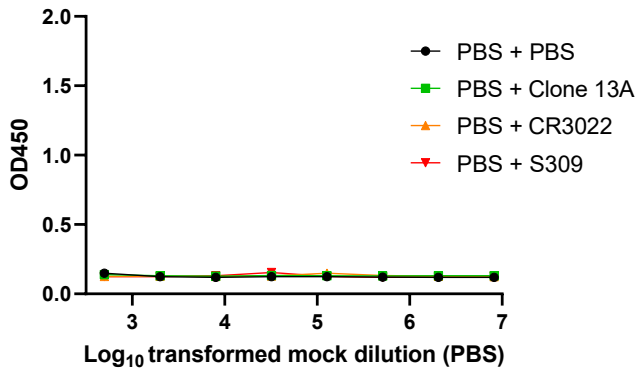


Figure S12

bioRxiv preprint doi: <https://doi.org/10.1101/2022.02.14.480449>; this version posted February 28, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a [CC-BY-NC-ND 4.0 International license](#).

Gating strategy

