

Efficacy of ChAdOx1 vaccines against SARS-CoV-2 Variants of Concern Beta, Delta and Omicron in the Syrian hamster model

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1 **ChAdOx1 nCoV-19 (AZD1222) or ChAdOx1 nCoV-19-Beta (AZD2816) protect Syrian hamsters**
2 **against the Beta, Delta, and Omicron variants of concern**

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21 **Abstract** - ChAdOx1 nCoV-19 (AZD1222) is a replication-deficient simian adenovirus–vectored vaccine
22 encoding the spike (S) protein of SARS-CoV-2, based on the first published full-length sequence
23 (Wuhan-1). AZD1222 was shown to have 74% vaccine efficacy (VE) against symptomatic disease in
24 clinical trials and over 2.5 billion doses of vaccine have been released for worldwide use. However,
25 SARS-CoV-2 continues to circulate and consequently, variants of concern (VoCs) have been detected,
26 with substitutions in the S protein that are associated with a reduction in virus neutralizing antibody titer.
27 Updating vaccines to include S proteins of VoCs may be beneficial over boosting with vaccines encoding
28 the ancestral S protein, even though current real-world data is suggesting good efficacy against
29 hospitalization and death following boosting with vaccines encoding the ancestral S protein. Using the
30 Syrian hamster model, we evaluated the effect of a single dose of AZD2816, encoding the S protein of the
31 Beta VoC, and efficacy of AZD1222/AZD2816 as a heterologous primary series against challenge with
32 the Beta or Delta variant. We then investigated the efficacy of a single dose of AZD2816 or AZD1222
33 against the Omicron VoC. As seen previously, minimal to no viral sgRNA could be detected in lungs of
34 vaccinated animals obtained at 5 days post inoculation, in contrast to lungs of control animals. Thus, these
35 vaccination regimens are protective against the Beta, Delta, and Omicron VoCs in the hamster model.

36 **Introduction** At the end of 2019, the causative agent of COVID-19, severe acute respiratory syndrome
37 coronavirus 2 (SARS-CoV-2), was first detected in Wuhan, China^{1,2}. As of February 2nd 2022, SARS-
38 CoV-2 has infected an estimated 380 million people, causing more than 5 million deaths³. Its emergence
39 prompted the rapid development of vaccines based on the viral receptor binding protein, spike (S)⁴⁻⁶.
40 Several vaccines demonstrated efficacy through clinical trials in less than a year⁷⁻¹¹ and were approved
41 for emergency use by different regulatory bodies worldwide. Over 4.4 billion people are estimated to
42 have received at least one dose of COVID-19 vaccination³. One of those vaccines is AZD1222
43 (ChAdOx1 nCoV-19), developed by Oxford University and produced by AstraZeneca. AZD1222 is a
44 replication-deficient simian adenovirus–vectored vaccine encoding the non-stabilized S protein of
45 Wuhan-1, one of the first published full-length SARS-CoV-2 sequences¹². AZD1222 was shown to be
46 highly effective in clinical trials, demonstrating 74% vaccine efficacy against symptomatic disease⁷. A
47 two dose primary series of AZD1222 is approved for usage in more than 170 countries, and more than
48 two billion doses of vaccine have been distributed worldwide¹³.
49 Despite the development and administration of these vaccines, a large portion of the world’s population is
50 still unvaccinated, particularly in low-income countries³. Furthermore, although COVID-19 vaccination is
51 protective against severe disease, it is not fully protective against infection with SARS-CoV-2, and
52 breakthrough infections regularly occur¹⁴. High levels of circulating virus, asymptomatic infections, low
53 vaccine coverage and break-through infection together means SARS-CoV-2 continues to circulate in the
54 population. As a consequence, several variants of concern (VoCs) have been detected. A variant is termed
55 a VoC if it is associated with an increase in transmission or virulence, or a decrease in the effectiveness of
56 public health and social measures, such as diagnostics, vaccines, or therapeutics¹⁵. Since COVID-19
57 vaccines were developed early in the pandemic, they are based on the ancestral S protein, and
58 substitutions in S may result in a reduced vaccine efficacy against VoCs. Several VoCs have substitutions
59 in the receptor binding domain of S which are associated with a reduction in neutralizing virus titers¹⁶⁻²⁰,
60 which is a strong predictor of vaccine efficacy²¹, but the current vaccines clinically available are largely
61 able to protect against severe disease and hospitalization caused by VoCs^{22,23}. Here, we investigate the

62 efficacy of an updated vaccine based on the S protein of the Beta variant (AZD2816)²⁴ against three
63 different VoCs; the Beta, Delta, and Omicron variants, relative to the original AZD1222 vaccine.

64 **Results** We vaccinated Syrian hamsters either with a single dose of AZD2816 (prime only group, day -
65 28, 2.5×10^8 IU/animal, intramuscular injection), or with a prime dose of AZD1222 followed by a boost
66 dose of AZD2816 (prime-boost group, day -56 and -28, both 2.5×10^8 IU/animal, intramuscular
67 injection), or with two injections of ChAdOx1 GFP (control group, day -56 and -28, 2.5×10^8 IU/animal,
68 intramuscular injection) (*Figure 1A*). On day 0, serum was obtained from eight hamsters per group and
69 binding antibody titers against S protein were determined. In the prime-boost group, high binding titers
70 were detected against all three S proteins. In the prime only group, binding antibody titers were
71 significantly higher against Beta S compared to ancestral S (*Figure 1B*). Virus neutralizing (VN) antibody
72 titers were determined using both a lentivirus-based pseudotype and live VN assay. Pseudotype VN titers
73 were significantly lower against Omicron in both vaccine groups. In the prime only group, pseudotype
74 VN titers were significantly lower for Delta than ancestral S (*Figure 1C*). Using the pseudotype VN
75 assay, the influence of single substitutions K417N, N501Y (present in the Beta and Omicron VoC),
76 E484K (present in the Beta VoC) as well as L452R (present in the Delta VoC) compared to ancestral
77 spike were investigated. Significantly higher VN titers were found against the E484K mutant compared to
78 ancestral S in prime only sera (*Extended Data Figure 1A*). Live VN titers were significantly higher
79 against the Beta VoC compared to the Delta VoC in both vaccinated groups (*Figure 1D*). Together, these
80 data show that a single vaccination with AZD2816 induces a robust immune response against S protein in
81 hamsters, specifically against the Beta S or an S with the E484K mutation. However, vaccination with
82 AZD1222 followed by AZD2816 does not result in a significant increase in binding to Beta or E484K S
83 compared to ancestral S.

84 On Day 0, animals were challenged via the intranasal route with 10^4 TCID₅₀ of SARS-CoV-2 Beta VoC.
85 Controls showed weight loss starting at day 5 and full recovery at day 12-14. In contrast, vaccinated
86 hamsters maintained weight during the experiment (*Figure 2A*). Significant differences were observed
87 between relative weights of the control and vaccinated groups on the day of peak weight loss compared to

88 day 0 (Day 7, *Figure 2B*, $p= 0.0034$ and 0.0137 , Kruskal Wallis test). On day 3 and 5, six animals per
89 group were euthanized and lung tissue was collected. In lung tissue of controls, subgenomic viral RNA
90 (sgRNA) was high on both days (12/12 samples positive, median of 3.6×10^9 and 1.6×10^{10} copies/gram,
91 respectively). In contrast, no or limited sgRNA was detected in lung tissue collected from prime only
92 animals (1/6 and 0/6 samples positive on day 3 and 5 respectively, significant by Kruskal Wallis test).
93 Likewise, sgRNA in lung tissue collected on day 5 from prime-boost animals was low (1/6 samples
94 positive, significant by Kruskal Wallis test), whereas 2/6 samples collected on day 3 from the prime-
95 boost group were found to be positive at levels equivalent to the control group (no significance, *Figure*
96 *2C*). Oropharyngeal swabs were collected on day 1, 3, 5, and 7, and the presence of sgRNA was
97 investigated. No reduction of sgRNA load in swabs was detected on day 1 in vaccinated groups compared
98 to the control. However, a significant reduction in sgRNA load was found on day 3 and 5 in prime only
99 animals. On day 7, both vaccinated groups showed a reduction in sgRNA load in oropharyngeal swabs,
100 showing that although vaccination did not prevent infection, it did significantly reduce the window of
101 shedding (*Figure 2D*). Area-under-the-curve analysis, used as a measurement of total amount of sgRNA
102 detected in oropharyngeal swabs throughout the experiment, showed a significant reduction in the prime
103 only group, but not the prime-boost group, compared to controls (*Figure 2E*).

104 Serum was collected from all hamsters on day 5 post challenge, and antibody titers were determined via
105 ELISA as well as pseudovirus and live VN assays as described above (*Extended Data Figure 2*). A
106 significantly higher binding antibody titer against Beta S compared to ancestral and Delta S was detected
107 in both vaccine groups via ELISA. In live VN assays, higher VN titers were found against Beta compared
108 to Delta VoC in the prime only group. In the pseudotype VN assay, titers against Omicron were lower
109 than against ancestral, Beta and Delta. Furthermore, titers against K417N S were higher than ancestral S.
110 (*Extended Data Figure 1B and 2*). We then investigated the correlation between sgRNA load in swabs on
111 the day of necropsy with corresponding antibody levels. A significant correlation could be found between
112 infectious VN titer against ancestral virus, the Beta, and Delta VoC, and sgRNA in swabs, but not

113 between binding antibodies or pseudotype VN titer against any S variant and sgRNA in swabs (*Extended*
114 *Data Figure 3*).

115 Lung pathology was scored by a board-certified veterinary pathologist blinded to study groups (*Figure 3*).
116 SARS-CoV-2-related pathology was observed in all animals of the control group. On day 3 post Beta
117 challenge, minimal-to-moderate acute bronchiolitis was observed affecting less than 1% of the lung.
118 Histological lesions consisted of a moderate subacute broncho-interstitial pneumonia affecting between
119 30-50% of pulmonary tissue. Lesions were characterized by broncho-interstitial pneumonia centered on
120 terminal bronchioles and extending into the adjacent alveoli. Alveolar septa were expanded by edema
121 fluid and leucocytes. SARS-CoV-2 antigen staining was numerous within bronchiolar epithelium on day
122 3, whereas this had mostly moved to type I and II pneumocytes on day 5. In contrast, antigen staining in
123 the vaccinated groups was relatively low; samples obtained from the prime only group were mostly
124 negative, whereas antigen staining in samples obtained from the prime-boost group was between none to
125 moderate (*Figure 3, Extended Data Figure 4*).

126 To investigate the efficacy of a vaccine optimized for the Beta S against the Delta variant, new groups of
127 hamsters vaccinated as described above were challenged with 10^4 TCID₅₀ of the Delta variant via the
128 intranasal route. As observed upon challenge with the Beta variant, vaccinated animals did not show any
129 weight loss throughout the experiment, whereas control animals did lose weight (*Figure 4A*). Indeed,
130 differences in weight loss between the control and vaccinated groups were significant on day 7 (*Figure*
131 *4B*). High levels of sgRNA could be detected on both day 3 and 5 in lung tissue of control animals (12/12
132 samples positive, median of 5.6×10^9 and 5.0×10^9 copies/gram, respectively). In contrast, the majority of
133 lung tissue obtained from vaccinated animals was negative for sgRNA, viral sgRNA was detected in 1/6
134 samples on each day for the prime boost group, versus 2/6 on day 3 and 0/6 on day 5 for the prime only
135 group (*Figure 4C*). Significant differences in sgRNA detected in oropharyngeal swabs were limited to
136 day 3 (both vaccinated groups) and day 5 (prime only group) compared to controls (*Figure 4D*). In
137 contrast to what we observed in animals inoculated with the Beta variant, we did not see a decrease in the
138 window of shedding of vaccinated animals compared to control animals. Area-under-the-curve analysis

139 (as a measurement of total amount of sgRNA detected in oropharyngeal swabs throughout the
140 experiment) showed that animals that received a prime only vaccination shed significantly less than
141 control animals (*Figure 4E*).

142 In sera collected on day 5 post challenge, a significantly higher binding antibody titer against Delta S
143 compared to ancestral S was detected in the prime only group (*Extended Data Figure 2*). In the live VN
144 assay, higher VN titers were found against the Beta VoC compared to ancestral virus in the prime only
145 group (*Extended Data Figure 2*). Significant differences in the pseudotype VN assay were found between
146 Omicron and Beta as well as Delta VoCs (*Extended Data Figure 2*), as well as between ancestral and
147 E484K S in the prime only group, and ancestral and K417N S in the prime boost group. (*Extended Data*
148 *Figure 1C*). A linear correlation was found between sgRNA in swabs and correlating binding antibodies
149 against ancestral, Beta and Delta S. For live VN titers, a significant correlation was found with the Beta
150 and Delta, but not ancestral, VoCs (*Extended Data Figure 5*).

151 SARS-CoV-2 -related pathology post Delta challenge was observed in all animals of the control group
152 and did not differ from animals challenged with the Beta VoC (*Figure 5, Extended Data Figure 4*). In the
153 prime only group, a minimal-to-moderate bronchiolitis was observed in some animals on day 3. In the
154 prime boost group, bronchiolitis was either absent or minimal. This was combined with reduced antigen
155 staining in the bronchiolar epithelium in both groups compared to controls. No pathology or antigen
156 staining was observed on day 5, except for one animal in the prime boost group, which is the only animal
157 that was positive for sgRNA at this time point.

158 We then investigated the efficacy of AZD1222 and AZD2816 against the Omicron VoC. Here, groups of
159 hamsters were vaccinated with a single dose of AZD1222, AZD2816, or ChAdOx1 GFP and serum was
160 collected 14 days post-vaccination (*Figure 6A*). Binding antibodies against different S proteins were
161 detected using the Mesoscale V-PLEX SARS-CoV-2 panel 23, in-house optimized for hamster sera.

162 Upon vaccination with AZD1222, binding antibodies were highest for ancestral and Alpha S and lowest
163 for Omicron. Upon vaccination with AZD2816, antibody levels were similar for ancestral, Alpha, Beta,
164 and Gamma S, but dropped for Delta and Omicron S (*Figure 6B*). Live VN titers against the Omicron

165 VoC were significantly lower compared to the ancestral variant (*Figure 6C*). 28 days after vaccination,
166 animals were challenged with the ancestral variant or Omicron VoC. As previously reported²⁵, we did not
167 see weight loss in control hamsters challenged with the Omicron VoC, whereas this weight loss was
168 present in control hamsters challenged with ancestral virus (*Figure 6D*). Four animals per group were
169 euthanized on day 3 and day 5. No significant differences in lung:body weight ratio were observed,
170 although lung:body weight ratio was relatively high on day 5 for control hamsters inoculated with
171 ancestral virus (*Figure 6E*). As expected, AZD1222 vaccination resulted in significantly reduced viral
172 genome copies in lung tissue (*Figure 6F*). However, replication of the Omicron VoC in hamster lung
173 tissue was low and although a reduction in genome copies in lung tissue of vaccinated hamsters was
174 observed, particularly in the animals which received AZD2816, no significance was reached.

175 Oropharyngeal swabs were obtained on day 1-5 and analyzed for sgRNA. Shedding of the control animals
176 infected with the Omicron VoC was similar to control and vaccinated animals that were infected with the
177 ancestral variant, albeit lower on day 1. However, vaccinated animals inoculated with the Omicron VoC
178 had significantly lower shedding on day 3 (both groups), day 4, and day 5 (AZD2816 vaccinated group
179 only) compared to controls (*Figure 6G*). Area-under-the-curve analysis was performed on the four
180 animals per group that were euthanized on day 5 as a measure of total amount of virus shed throughout
181 the experiment. Significantly lower shedding was detected in the vaccinated animals infected with the
182 Omicron VoC, but not in those infected with the ancestral virus (*Figure 6H*).

183 Lung pathology was scored by a board-certified veterinary pathologist blinded to study groups (*Figure 7*,
184 *Extended Data Figure 6*). SARS-CoV-2-related pathology differed from what was observed in the
185 animals inoculated with the Beta or Delta VoC. On day 3 and 5 post Omicron challenge, no evidence of
186 pulmonary pathology was noted in the lower airway in any of the control animals, however minimal
187 subacute inflammation and multifocal necrosis was noted in the trachea of 2/4 and 1/4 animals on day 3
188 and 5, respectively. SARS-CoV-2 antigen staining was observed in 3/4 tracheal tissues on day 3. On day
189 5, rare staining was observed in bronchial and bronchiolar epithelium in 2/4 animals, in type I and II
190 pneumocytes in 1/4 animals, and in tracheal epithelium in 1/4 animals. In animals vaccinated with

191 AZD1222, 1/4 animals had a minimal subacute inflammation and multifocal necrosis of the tracheal
192 epithelium. Rare SARS-CoV-2 antigen staining was observed in tracheal epithelium of 3/4 animals, and
193 scattered SARS-CoV-2 antigen staining was observed in bronchial and bronchiolar epithelium of 1/4
194 animals. On day 5, no SARS-CoV-2 antigen staining is observed, and minimal interstitial pneumonia was
195 seen in 1/4 animals. In animals vaccinated with AZD2816, no pathology was observed on day 3, and
196 limited to minimal interstitial pneumonia in 1/4 animals on day 5. Antigen staining was limited to rare
197 tracheal epithelium staining on day 3, with no staining observed on day 5 (*Figure 7, Extended Data*
198 *Figure 6*).

199 **Discussion** We have previously shown that despite reduced neutralizing antibody titers in sera obtained
200 from hamsters vaccinated with AZD1222 against the Beta VoC, hamsters were fully protected against
201 lower respiratory tract infection²⁶. Other vaccines have performed differently in animal models. ,
202 Tostanoski *et al.* show that although vaccination with Ad26.COV2.S (an adenovirus-vectored vaccine
203 encoding ancestral S protein) reduced the viral load detected in lung tissue of hamsters challenged with
204 the Beta VoC at 14 days post challenge, this difference was not significant for gRNA²⁷. Likewise, rhesus
205 macaques vaccinated with the same vaccine showed higher viral loads in bronchoalveolar lavage and
206 nasal swabs when challenged with the Beta VoC compared to ancestral SARS-CoV-2²⁸. Finally, Corbett
207 *et al.* show that sgRNA was detected in bronchoalveolar lavage and nasal swabs from rhesus macaques
208 vaccinated with the Moderna vaccine mRNA-1273 (encoding ancestral S) and challenged with the Beta
209 VoC²⁹, whereas this was limited in rhesus macaques that were challenged with ancestral virus³⁰. These
210 data suggest that while vaccines which encode the ancestral spike can protect against hospitalization and
211 death caused by VoC, a variant-specific vaccine may result in increased protection against disease and
212 onward transmission.

213 Thus, we investigated the protective efficacy of the vaccine AZD2816, which encodes the S protein of the
214 Beta VoC, in the hamster model. In contrast to control animals, upon challenge with either the Beta,
215 Delta, or Omicron VoC, little-to-no viral RNA was found at 5 days post challenge in the lower respiratory

216 tract of the vaccinated hamsters. Thus, the vaccine regimens utilized in the current study, including single
217 dose AZD2816, are protective against all three VoCs in the hamster model.

218 Vaccine and variant-specific differences were observed in the different experiments. In the Beta VoC
219 study, lung tissue from 2/6 prime boost vaccinated animals were positive for sgRNA at day 3, combined
220 with higher antigen staining in this group compared to the prime only group. Furthermore, whereas total
221 shedding was reduced in the prime only group compared to controls, this was not the case for the prime
222 boost group. This suggests that initial priming with one VoC S may shape the immune response to
223 subsequent vaccinations. Indeed, our humoral immune response analysis showed higher titers for the Beta
224 S and E484K mutation compared to ancestral or Delta S in the prime only group, but not the prime boost
225 group. In contrast, in the Delta VoC study, the prime boost group appeared to be slightly better protected
226 than the prime only group, mostly evident in pathology scoring. This may be due to the higher quantity of
227 antibodies in the prime boost group compared to the prime only group.

228 Similar to what has been reported by other groups, replication of the Omicron VoC was limited in the
229 lower respiratory tract^{25,31}. We were unable to detect any sgRNA, and gRNA was low compared to
230 ancestral virus. Despite the limited replication in the lower respiratory tract, the upper respiratory tract
231 displayed much higher viral replication, comparable to ancestral virus. Both the AZD1222 and AZD2816
232 vaccine were able to reduce shedding within the Omicron-challenged groups suggesting that both
233 vaccines are effective. This was recently confirmed in a preprint by Gagne *et al.*, in which they show
234 protection of NHPs against the Omicron VoC, both with a regimen of mRNA-1273 against ancestral S,
235 3x, or mRNA-1273 2x and mRNA-Omicron as a booster³². Further research is required to determine to
236 the extent in which the hamster model recapitulates human disease and infection kinetics with the
237 Omicron VoC. For example, a higher inoculum dose as well as a different inoculation route such as
238 intratracheal may increase virus replication in the lower respiratory tract.

239 Interestingly, whereas we previously reported on the lack of reduction in virus detected in oropharyngeal
240 swabs when vaccines were given via the intramuscular route^{33,34}, vaccinated hamsters inoculated with the
241 Omicron VoC displayed significantly reduced shedding compared to controls, even though shedding of

242 control animals was at levels equal to animals inoculated with the ancestral virus. This difference was
243 particularly evident in the AZD2816 group. Omicron has an E484A mutation in the S protein, whereas the
244 Beta VoC has an E484K mutation. In our pseudovirus VN assays, we show a higher neutralization of
245 pseudotypes with the E484K mutation compared to ancestral S in serum obtained from hamsters that only
246 received the AZD2816 vaccination. It is possible that this also translates to the E484A mutation.
247 However, we did not see an increase neutralization in live virus assays against the Omicron VoC in serum
248 obtained from hamsters vaccinated with AZD2816 compared to those vaccinated with AZD1222. Further
249 research is needed to determine whether the small difference observed between the two vaccines against
250 the Omicron VoC is relevant, and why shedding is reduced.

251 A significant correlation was found between sgRNA load in oropharyngeal swabs and neutralizing
252 antibody titers in animals, whereas binding antibodies titers were also predictors for sgRNA load in swabs
253 in animals challenged with the Delta VoC. This finding confirms previous reports of a correlation
254 between binding and neutralizing antibodies and viral load in both hamsters³⁵ and non-human
255 primates^{29,36}.

256 Our study confirms that AZD2816 is immunogenic in the hamster model and protects against infection of
257 the lower respiratory tract against the Omicron, Beta, and Delta VoC. Likewise, a single dose of
258 AZD1222 protects against the Omicron VoC. Furthermore, initial immunization with AZD1222 followed
259 by immunization with AZD2816 results in full protection against the Beta and Delta VoCs, and we
260 predict it will also protect against Omicron. This confirms previous reports that a full antigenic match
261 between the vaccine and the challenged virus is not required for protection of the lower respiratory tract.

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277 **Author contributions**

278 N.v.D. and V.J.M. designed the studies, S.C.G. and T.L. provided the vaccine, N.v.D., J.S., D.R.A.,
279 T.A.S., R.J.F., C.K.Y., N.T., J.N., M.U., S.B.R., G.S., A.S., D.B. and V.J.M. performed the experiments,
280 N.v.D., A.S., and D.B. analyzed results, N.v.D. and V.J.M. wrote the manuscript, all co-authors reviewed
281 the manuscript.

282 **Competing interests**

283 S.C.G. is a co-founder and stock-holder of Vaccitech and named as an inventor on a patent covering the
284 use of ChAdOx1-vector-based vaccines and a patent application covering a SARS-CoV-2 (nCoV-19)
285 vaccine (UK patent application no. 2003670.3). T.L. is named as an inventor on a patent application
286 covering a SARS-CoV-2 (nCoV-19) vaccine (UK patent application no. 2003670.3). The University of
287 Oxford and Vaccitech, having joint rights in the vaccine, entered into a partnership with AstraZeneca in
288 April 2020 for further development, large-scale manufacture and global supply of the vaccine. Equitable
289 access to the vaccine is a key component of the partnership. Neither Oxford University nor Vaccitech will
290 receive any royalties during the pandemic period or from any sales of the vaccine in developing countries.
291 All other authors declare no competing interests.

292 **Materials and Correspondence**

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295 **Materials and Methods**

296 *Ethics Statement*

297 Animal experiments were conducted in an AAALAC International-accredited facility and were approved
298 by the Rocky Mountain Laboratories Institutional Care and Use Committee following the guidelines put
299 forth in the Guide for the Care and Use of Laboratory Animals 8th edition, the Animal Welfare Act,
300 United States Department of Agriculture and the United States Public Health Service Policy on the
301 Humane Care and Use of Laboratory Animals.

302 The Institutional Biosafety Committee (IBC) approved work with infectious SARS-CoV-2 virus strains
303 under BSL3 conditions. Virus inactivation of all samples was performed according to IBC-approved
304 standard operating procedures for the removal of specimens from high containment areas.

305 *Cells and virus*

306 SARS-CoV-2 variant B.1.351 (USA/MD-HP01542/2021, EPI_ISL_890360) was obtained from Andrew
307 Pekosz at John Hopkins Bloomberg School of Public Health. SARS-CoV-2 variant B.1.617.2 (hCoV-
308 19/USA/KY-CDC-2-4242084/2021) was obtained from BEI resources. SARS-CoV-2 variant B.1.1.529
309 (hCoV-19/USA/GA-EHC-2811C/2021, EPI_ISL_7171744) was obtained from Mehul Suthar, Emory
310 University. All virus stocks were sequenced, and no SNPs compared to the patient sample sequence were
311 detected. Virus propagation was performed in VeroE6 cells in DMEM supplemented with 2% fetal
312 bovine serum, 1 mM L-glutamine, 50 U/ml penicillin and 50 µg/ml streptomycin (DMEM2). VeroE6
313 cells were maintained in DMEM supplemented with 10% fetal bovine serum, 1 mM L-glutamine, 50
314 U/ml penicillin, and 50 µg/ml streptomycin. No mycoplasma was detected in cells or virus stocks.

315 *Animal Experiments*

316 ChAdOx1 nCoV-19 was formulated as previously described³⁷. 4-6-week-old Syrian hamsters (Envigo
317 Indianapolis) were vaccinated with 2.5×10^8 infectious units of AZD1222, AZD2816, or ChAdOx1-GFP
318 delivered intramuscularly in two 100 µL doses into the posterior thighs 56 or 28 days prior to challenge.
319 Prior to challenge, a serum sample was collected via the retro-orbital plexus under isoflurane anesthesia.
320 All animals were challenged intranasally with 40 µl containing 10^4 TCID₅₀/mL virus in sterile DMEM.

321 Body weights were recorded daily. Oropharyngeal swabs were collected in 1 mL of DMEM2. On day 3
322 and 5, 4-6 animals from each group were euthanized and lung samples were taken for qRT-PCR analysis,
323 virus titrations and histopathology. The remaining six animals in each group were monitored daily until
324 day 21.

325 *RNA extraction and quantitative reverse-transcription polymerase chain reaction*

326 RNA was extracted from DMEM2 containing oropharyngeal swabs using the QiaAmp Viral RNA kit
327 (Qiagen), and lung samples were homogenized and extracted using the RNeasy kit (Qiagen) according to
328 the manufacturer's instructions and following high-containment laboratory protocols. Five μ L of
329 extracted RNA was tested with the Quantstudio 3 system (Thermofisher) according to the manufacturer's
330 instructions using viral RNA specific assays^{38,39}. A standard curve was generated during each run using
331 SARS-CoV-2 standards containing a known number of genome copies.

332 *Virus neutralization*

333 Sera were heat-inactivated (30 min, 56 °C). After an initial 1:10 dilution of the sera, two-fold serial
334 dilutions were prepared in DMEM2. 100 TCID₅₀ of SARS-CoV-2 was added to the diluted sera. After a
335 60 min incubation at 37°C and 5% CO₂, the virus-serum mixture was added to VeroE6 cells and cells
336 were further incubated for 6 days before assessment of CPE. The virus neutralization titer was expressed
337 as the reciprocal value of the highest dilution of the serum that still inhibited virus replication. Three
338 different positive serum controls were done next to NIBSC sera sample 20/130 by three different
339 technicians, to determine IU/mL equivalent. NIBSC sera readout was 640-1066, compared to reported
340 value at 1300 (1.5x higher). All serum samples were subsequently accompanied by positive controls on
341 the plate. Assays were only approved if positive controls fell within the range previously determined by
342 three technicians. Values were then multiplied by 1.5 to determine IU/mL.

343 *Generating lentiviral based pseudotypes bearing the SARS-CoV-2 S protein*

344 Lentiviral-based SARS-CoV-2 pseudotyped viruses were generated in HEK293T cells incubated at 37°C,
345 5% CO₂ as previously described⁴⁰. Mutant SARS-CoV-2 expression plasmids (Clade A, Beta, Delta,
346 Omicron, N501Y, E484K, K417N, L452R) were generated by site-directed mutagenesis or using the

347 QuikChange Lightning Multi Site-Directed Mutagenesis Kit (Agilent). All SARS-CoV-2 spike expression
348 plasmids were based on the Wuhan-hu-1 reference sequence⁴¹, with the additional substitutions D614G
349 (except for clade A) and K1255*STOP (aka the Δ 19 mutation or cytoplasmic tail truncation). Briefly,
350 HEK293T cells were transfected with SARS-CoV-2 spike, along with the lentiviral plasmids p8.91
351 (encoding for HIV-1 gag-pol) and CSFLW (lentivirus backbone expressing a firefly luciferase reporter
352 gene) with PEI (1 μ g/mL) transfection reagent. Supernatants containing pseudotyped SARS-CoV-2 were
353 harvested and pooled at 48 and 72 hours post transfection, centrifuged at 1,300 x g for 10 minutes at 4 °C
354 to remove cellular debris and stored at -80 °C. SARS-CoV-2 pseudoparticles were titrated on HEK293T
355 cells stably expressing human ACE2 and infectivity assessed by measuring luciferase luminescence after
356 the addition of Bright-Glo luciferase reagent (Promega) and read on a GloMax Multi+ Detection System
357 (Promega).

358 *Micro neutralization test (mVNT) using SARS-CoV-2 pseudoparticles*

359 Sera was diluted 1:20 in serum-free media in a 96-well plate in triplicate and titrated 3-fold. A fixed
360 volume of SARS-CoV-2 pseudoparticles were added at a dilution equivalent to 10^5 signal luciferase units
361 in 50 μ L DMEM-10% and incubated with sera for 1 hour at 37 °C, 5% CO₂ (giving a final sera dilution
362 of 1:40). Target cells stably expressing human ACE2 were then added at a density of 2×10^4 in 100 μ L
363 and incubated at 37 °C, 5% CO₂ for 48 hours. Firefly luciferase activity was then measured after the
364 addition of Bright-Glo luciferase reagent on a Glomax-Multi+ Detection System (Promega, Southampton,
365 UK). CSV files were exported for analysis. Pseudotyped virus neutralization titers were calculated by
366 interpolating the dilution at which a 50% reduction in reduction in luciferase activity was observed,
367 relative to untreated controls, neutralization dose 50% (ND50).

368 *Enzyme-linked immunosorbent assay*

369 MaxiSorp plates (Nunc) were coated with 100ng (2 μ g/ml) whole spike protein diluted in PBS for
370 overnight adsorption at 4°C. Plates were washed in PBS/Tween (0.05% v/v) and wells blocked using
371 casein (ThermoFisher Scientific) for at least 1 hr at RT. Standard positive sera (pool of hamster serum
372 from AZD1222-AZD2816 vaccinated animals with high endpoint titer against original spike protein),

373 individual hamster serum, negative and internal control samples were added to plates and incubated for at
374 least 2 hours at RT. Following washing, bound antibodies were detected by addition of Alkaline
375 Phosphatase-conjugated goat anti-hamster IgG (Sigma-Aldrich, SAB3700455) (1:1000 dilution) for 1hr at
376 RT and addition of p-Nitrophenyl Phosphate, Disodium Salt substrate (Sigma-Aldrich) and optimal
377 density reading at 405nm. An arbitrary number of ELISA units (EU) were assigned to the reference pool
378 and optical density values of each dilution were fitted to a 4-parameter logistic curve using SOFTmax
379 PRO software. ELISA units were calculated for each sample using the optical density values of the
380 sample and the parameters of the standard curve. All data was log-transformed for presentation and
381 statistical analyses.

382 *Binding antibody titers against different spike proteins on the Meso Quickplex*

383 The V-PLEX SARS-CoV-2 Panel 13 (IgG) kit (MSD, K15463U) was used to run the hamster samples on
384 the Meso Quickplex (MSD, K15203D). The 96-well plate was incubated with 150 μ L of Blocker A
385 solution at room temperature with shaking for 30 minutes, then washed 3 times with 150 μ L/well of MSD
386 Wash buffer. 50 μ L of the standard curve and hamster samples were transferred to the plate in duplicates.
387 Vaccinated hamster serum samples were diluted 10,000x, and ChAdOx1 GFP-vaccinated hamster serum
388 samples were diluted 1,000x. The plate was sealed with shaking at room temperature for 2 hours,
389 followed by 3 washes with 1X MSD Wash buffer. An in-house MSD GOLD SULFO-TAG NHS-Ester
390 (MSD, R31AA-2) conjugated goat anti-hamster IgG secondary antibody (Thermo Fischer, SA5-10284)
391 was diluted 10,000x in diluent 100 and 50 μ L was applied to each well of the plate. The plate was sealed
392 with shaking at room temperature for 1 hour. After incubation, the plate was washed with 1X MSD Wash
393 buffer as before, and 150 μ L of MSD Gold Read Buffer B was added per well. The plate was read
394 immediately by the MSD instrument. Arbitrary units (AU) were assigned to the standard curve of pooled
395 SARS-CoV-2-positive hamster sera, which was used on each plate. AU/mL were calculated using the
396 MSD Workbench 4.0 software.

397 *Histology and immunohistochemistry*

398 Lungs were perfused with 10% neutral-buffered formalin and fixed for at least 8 days. Tissue was
399 embedded in paraffin, processed using a VIP 6 Tissue-Tek (Sakura Finetek) tissue processor, and then
400 embedded in Ultraffin paraffin polymer (Cancer Diagnostics). Sections of 5 µm were deparaffinized in
401 xylene, passed through 100% ethanol, and rehydrated in tap water. Sections were stained with Harris
402 hematoxylin (Cancer Diagnostics, no. SH3777), decolorized with 0.125% HCl/70% ethanol, blued in
403 Pureview PH Blue (Cancer Diagnostics, no. 167020), counterstained with eosin 615 (Cancer Diagnostics,
404 no. 16601), dehydrated, and mounted in Micromount (Leica, no. 3801731). An in-house-generated
405 SARS-CoV-2 nucleocapsid protein rabbit antibody (GenScript) at a 1:1000 dilution was used to detect
406 specific anti-SARS-CoV-2 immunoreactivity, carried out on a Discovery ULTRA automated staining
407 instrument (Roche Tissue Diagnostics) with a Discovery ChromoMap DAB (Ventana Medical Systems)
408 kit. The tissue slides were examined by a board-certified veterinary anatomic pathologist blinded to study
409 group allocations. Scoring was done as follows. H&E; no lesions = 0; less than 1% = 0.5; minimal (1-
410 10%) = 1; mild (11-25%) = 2; moderate (26-50%) = 3; marked (51-75%) = 4; severe (76-100%) = 5. IHC
411 attachment; none = 0; less than 1% = 0.5; rare/few (1-10%) = 1; scattered (11-25%) = 2; moderate (26-
412 50%) = 3; numerous (51-75%) = 4; diffuse (76-100%) = 5.

413 *Data availability statement*

414 Data will be deposited in Figshare.

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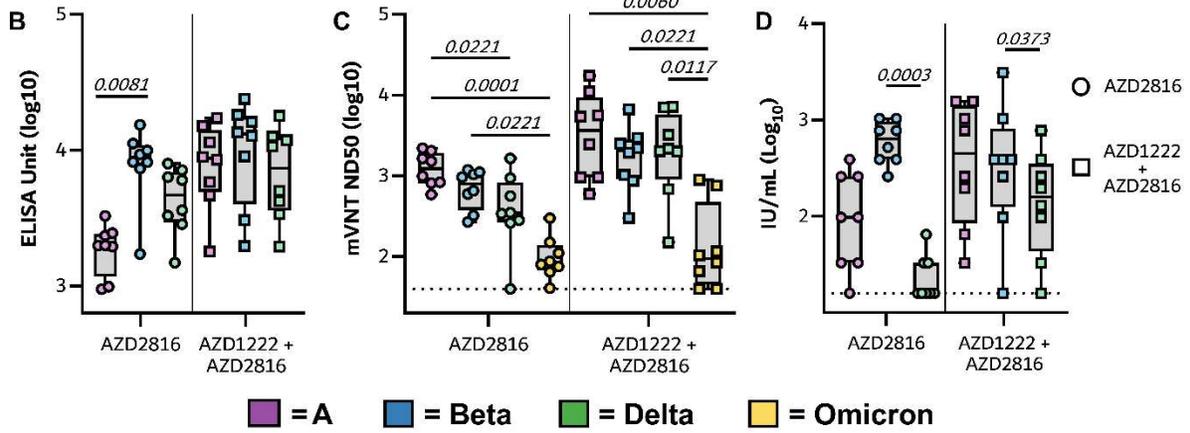
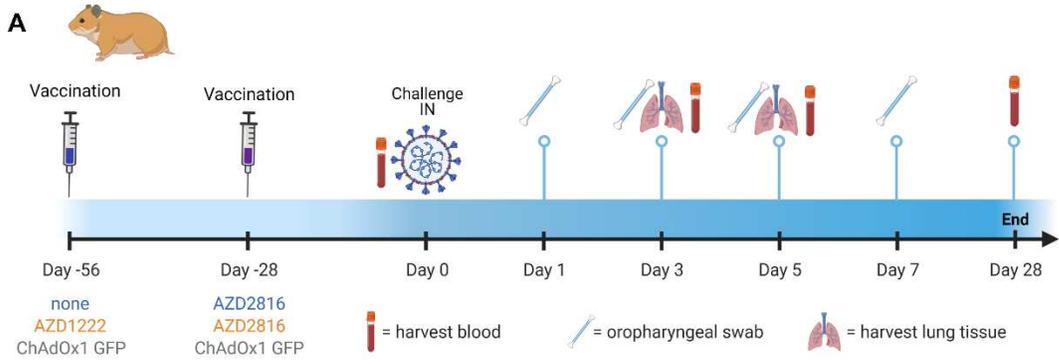
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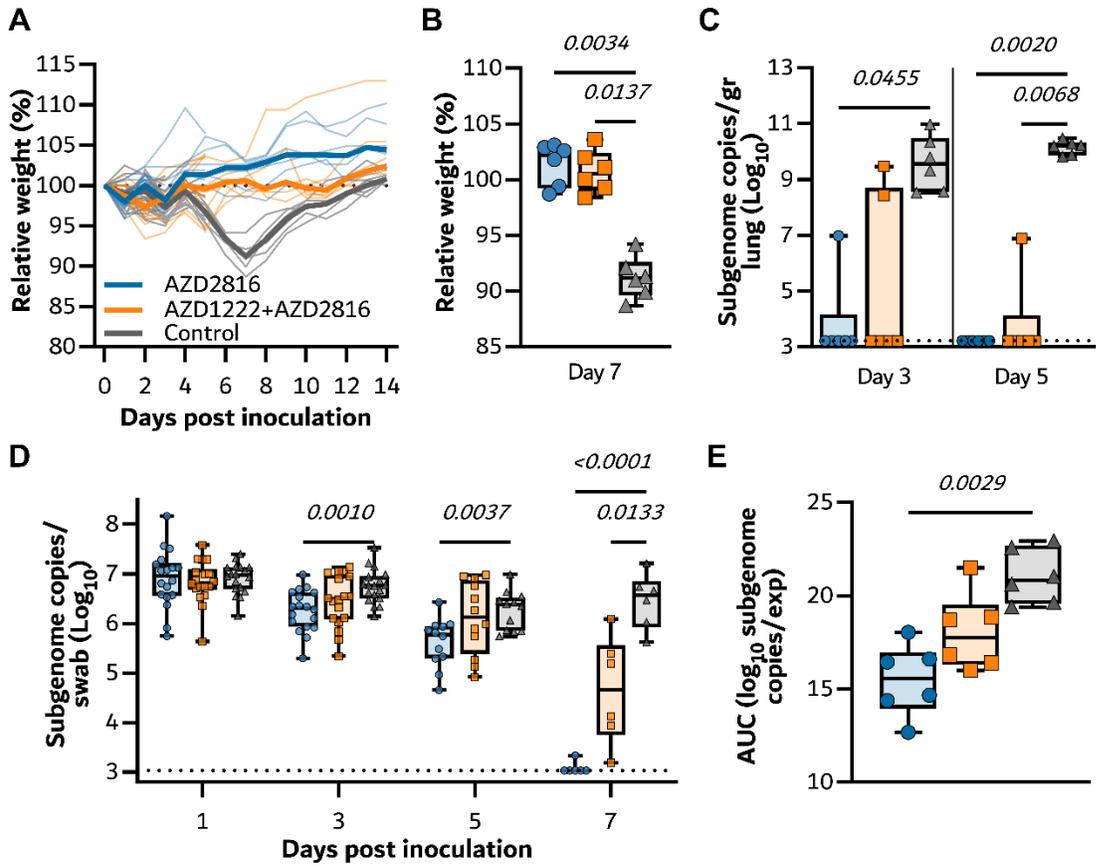
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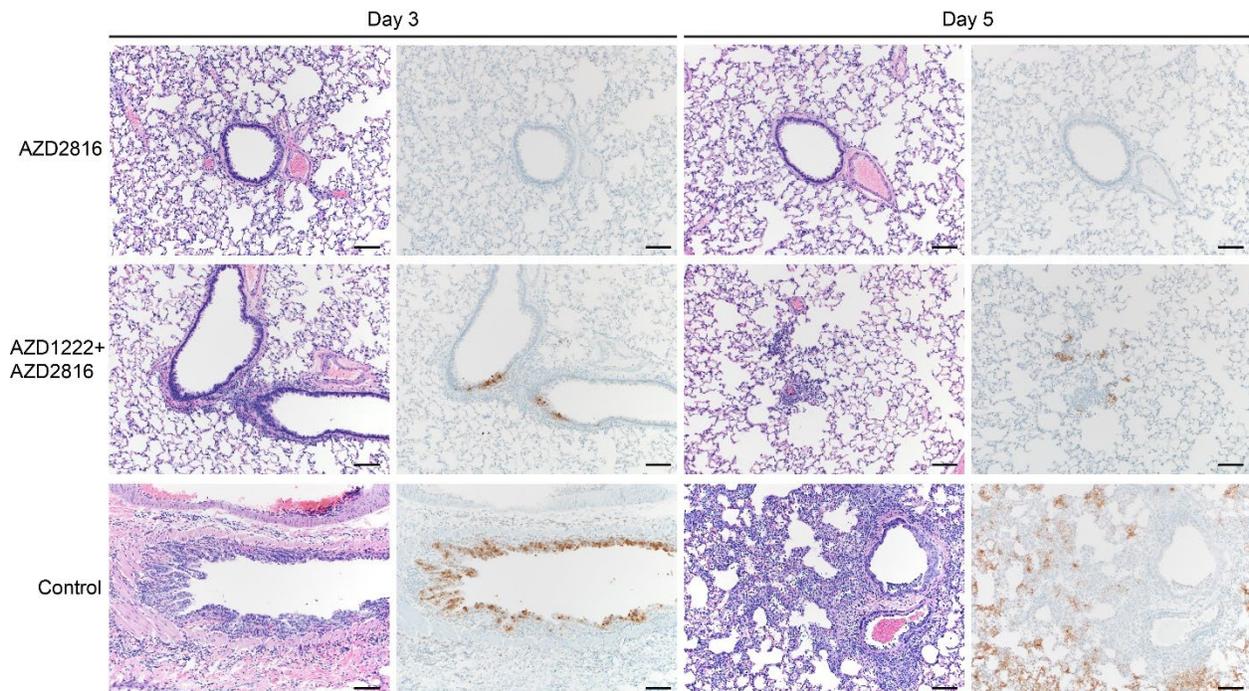
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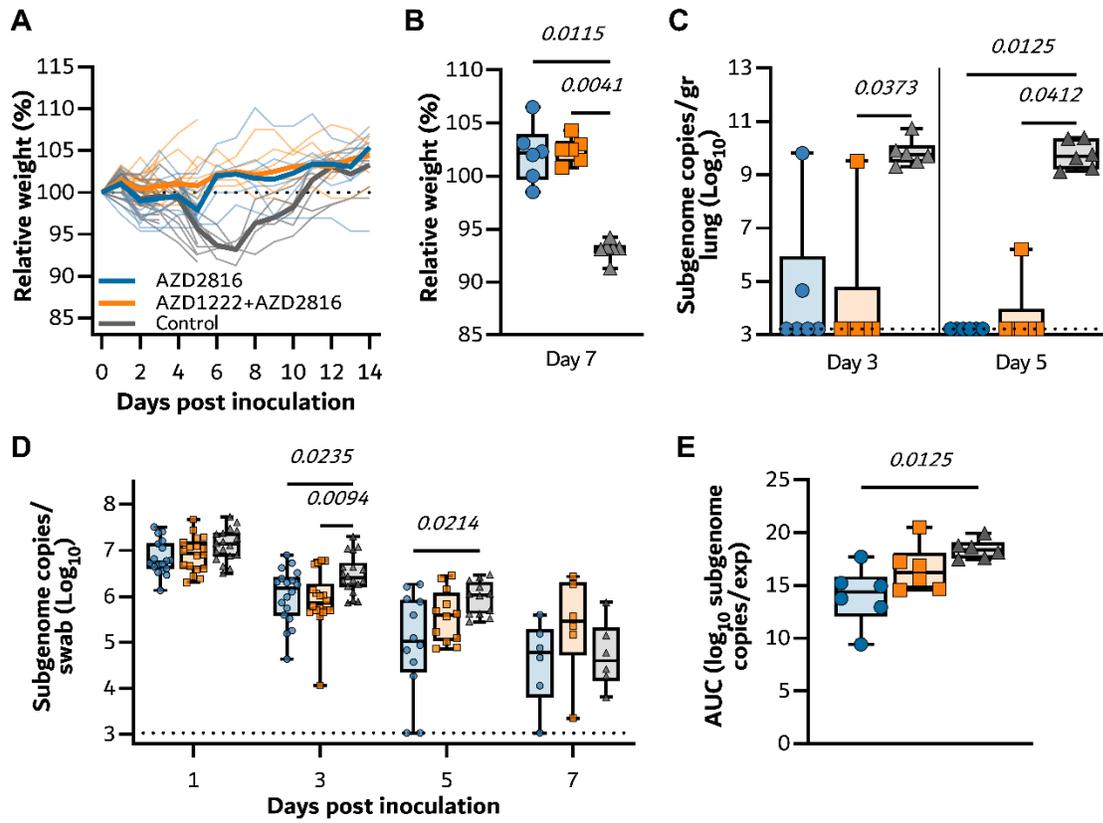
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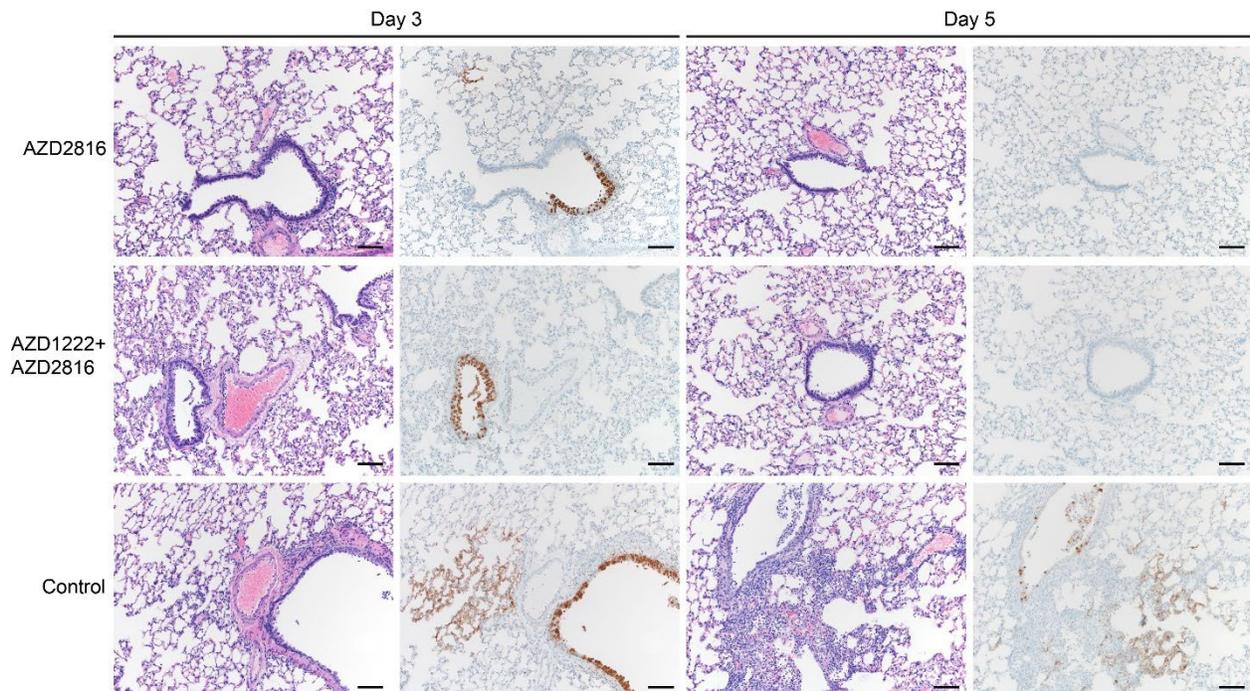
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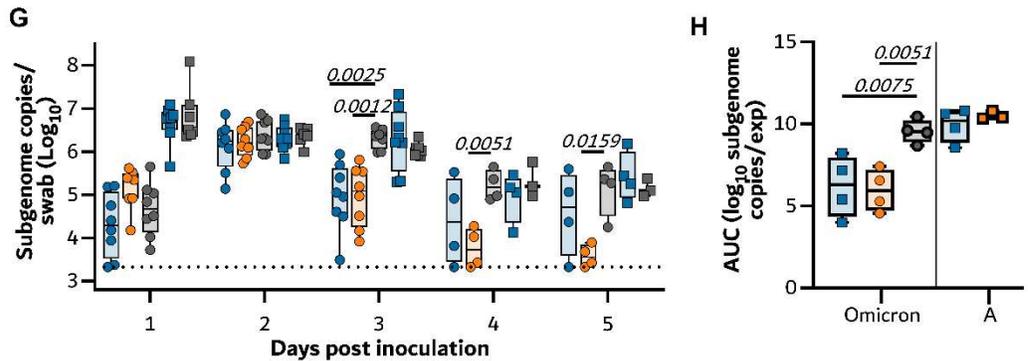
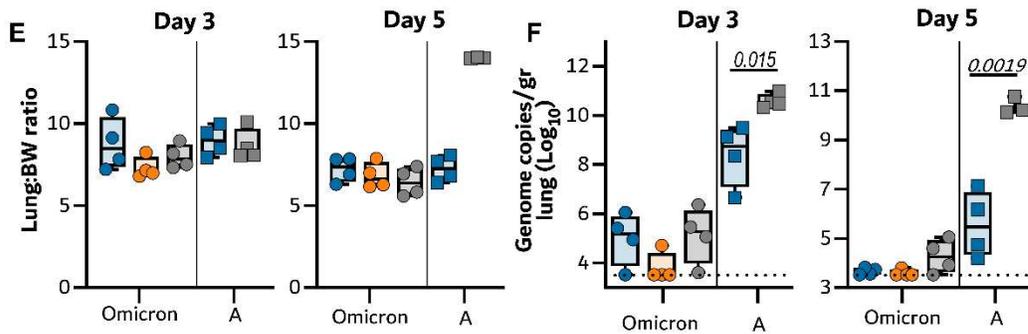
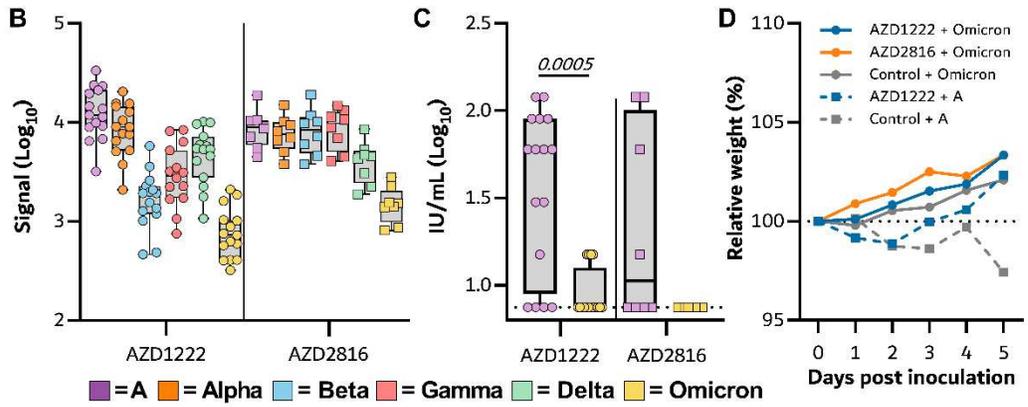
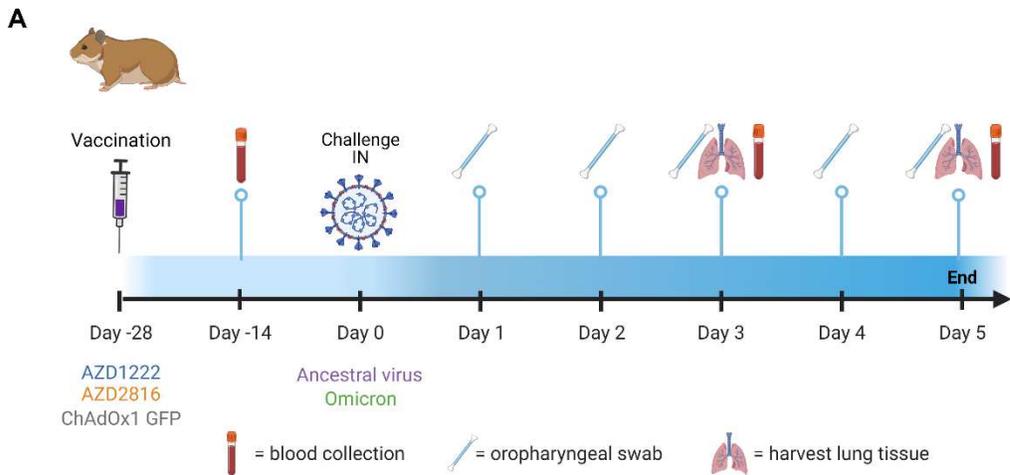
544 **Figure 3. Pulmonary effects of intranasal challenge with the Beta VoC in vaccinated and control**
545 **hamsters at day 3 and 5 post challenge.** H&E staining (1st and 3rd column) and IHC staining against N
546 protein (brown, 2nd and 4th column), 100x, scale bar = 100 μ m. No pathology nor antigen staining
547 observed in animals which received an AZD2816 vaccination. No pathology observed in animals which
548 received an AZD1222 + AZD2816 vaccination. Compared to control, limited staining of bronchiolar
549 epithelium observed on day 3 and 5. Control animals show progression from bronchiolitis on day 3 to
550 bronchointerstitial pneumonia on day 5, at which point alveolar septa are expanded by edema fluid and
551 leucocytes. Staining of bronchiolar epithelial cells, type I&II pneumocytes, and rare macrophages. Images
552 are representative of observations within 100% of a complete lung section containing all lobes.



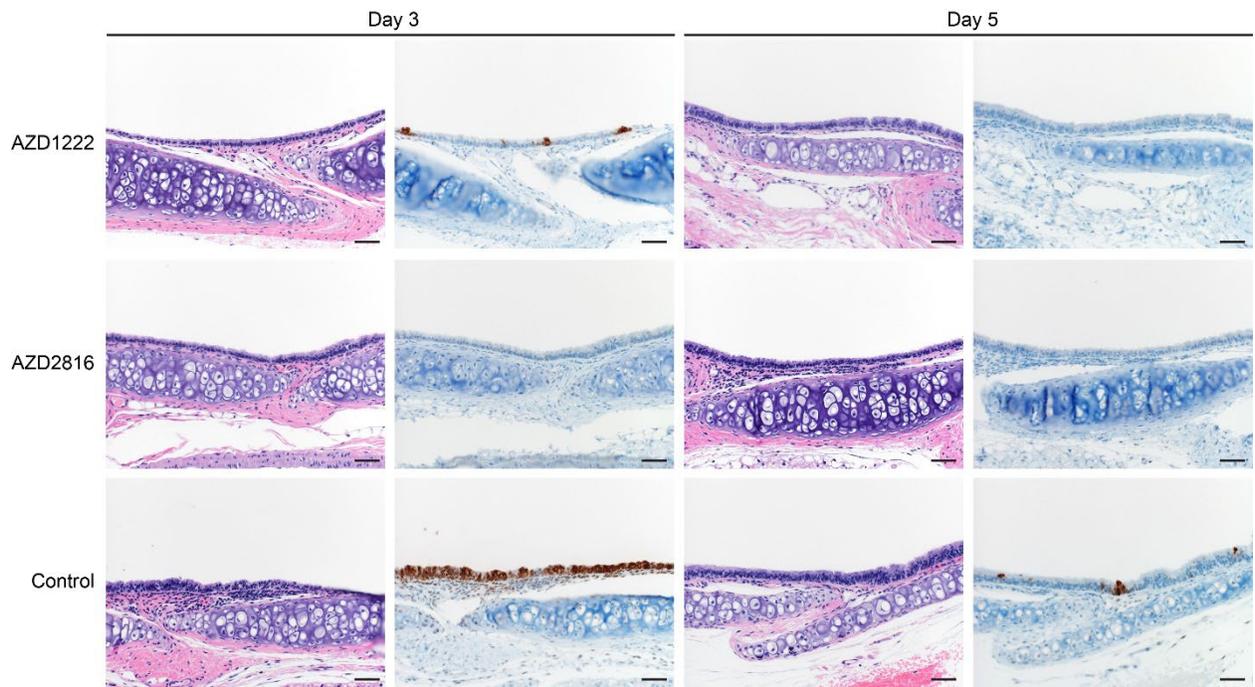


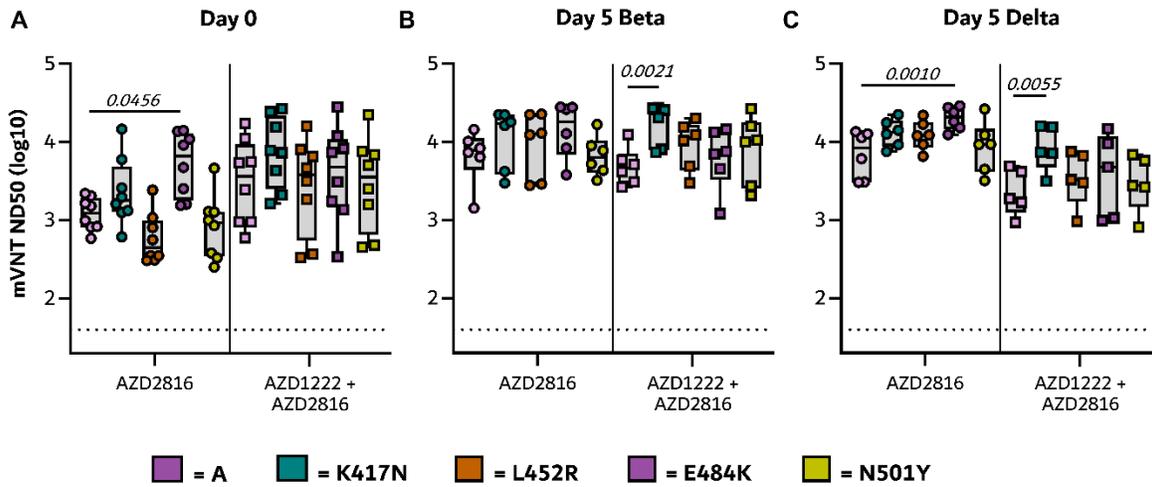
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573 **Figure 5. Pulmonary effects of intranasal challenge with the Delta VoC in vaccinated and control**
574 **hamsters at day 3 and 5 post challenge.** H&E staining (1st and 3rd column) and IHC staining against N
575 protein (brown, 2nd and 4th column), 100x, scale bar = 100 μm; Limited bronchiolitis with epithelial cell
576 necrosis observed on day 3, which was resolved on day 5, in animals that received an AZD2816
577 vaccination. No pathology observed in animals which received an AZD1222 + AZD2816 vaccination.
578 Compared to controls, limited staining of bronchiolar epithelium observed on day 3, which was resolved
579 on day 5 in both vaccine groups. Control animals show progression from bronchiolitis on day 3 to
580 bronchiointerstitial pneumonia on day 5, at which point alveolar septa are expanded by edema fluid and
581 leucocytes. Staining of bronchiolar epithelial cells, type I&II pneumocytes, and rare macrophages on both
582 days. Images are representative of observations within 100% of a complete lung section containing all
583 lobes.



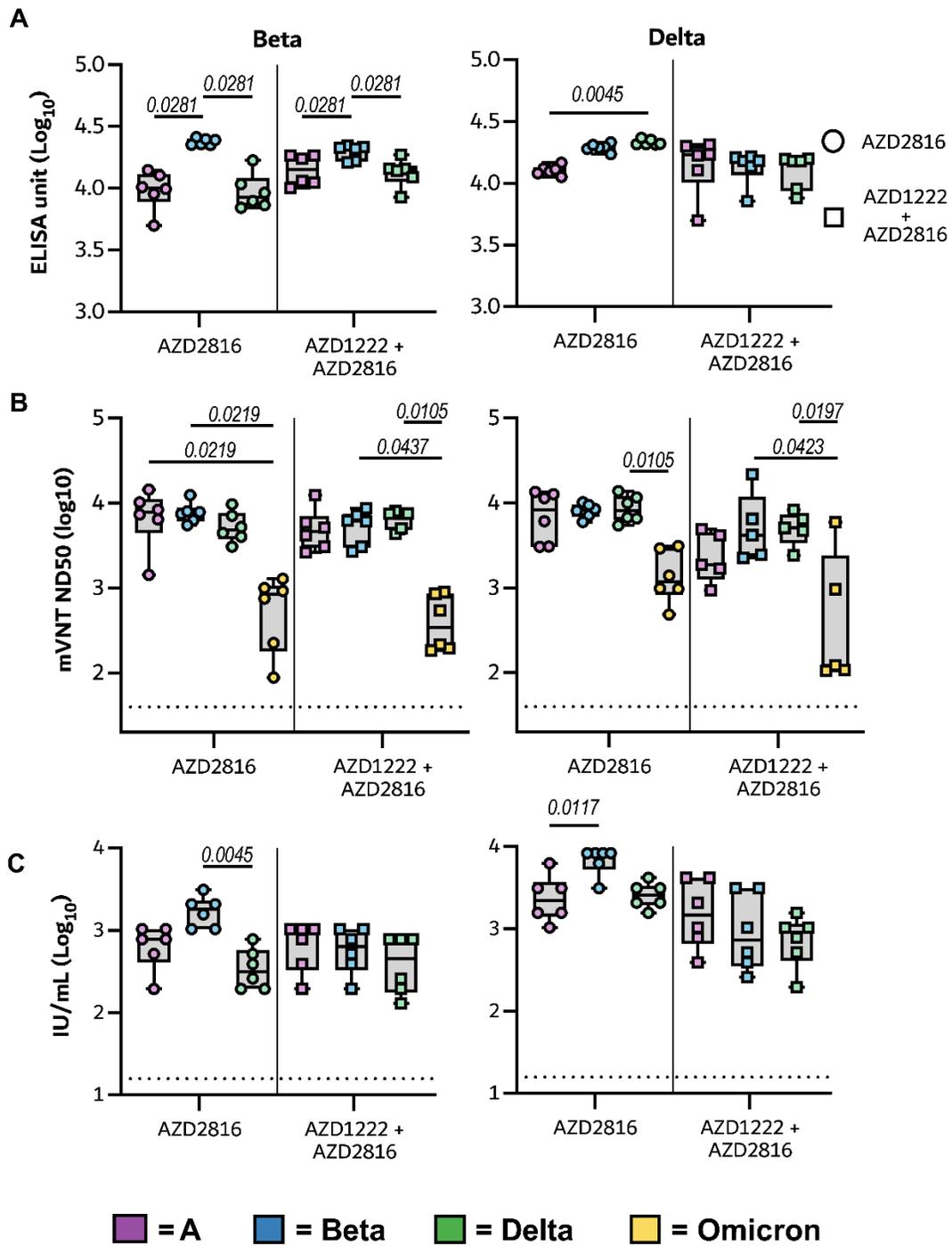
585 **Figure 6. Vaccination of Syrian hamsters with AZD2816 or AZD1222 reduces shedding by the**
586 **Omicron VoC.** A) Schematic overview of experiment. Hamsters were vaccinated with AZD1222,
587 AZD2816, or ChAdOx1 GFP on day -28. Twenty-eight days post final vaccination, hamsters were
588 challenged with 10^3 TCID₅₀ of the Omicron or ancestral variant, via the intranasal route. B) Boxplot
589 (minimum to maximum) of binding IgG antibody signal in hamster sera obtained on day -14 against
590 different SARS-CoV-2 S proteins obtained using the V-PLEX SARS-CoV-2 panel 23 by Meso Scale
591 Discovery. Circles = hamsters vaccinated with AZD2816, squares = hamsters vaccinated with AZD2816.
592 C) Boxplot (minimum to maximum) of virus neutralizing antibody titers in hamster sera obtained on day -
593 14 against different ancestral virus or Omicron VoC. VN titers were normalized against NIBSC standard.
594 Statistical significance was determined via a Wilcoxon test. Circles = hamsters vaccinated with
595 AZD2816, squares = hamsters vaccinated with AZD2816. D) Relative weight in comparison to day 0.
596 Dotted line = 100% relative weight. N=8 (Day 1-3) or 4 (Day 4-5). Circles = hamsters challenged with
597 Omicron, squares = hamsters challenged with ancestral variant. E) Boxplot (minimum to maximum) of
598 lung:body weight ratio. Circles = hamsters challenged with Omicron, squares = hamsters challenged with
599 ancestral variant. F) Boxplot (minimum to maximum) of gRNA in lung tissue harvested on day 3 and 5
600 (N=4). Statistical significance was determined via a Kruskal Wallis test followed by Dunn's multiple
601 comparisons test. Dotted line = limit of detection. Circles = hamsters challenged with Omicron, squares =
602 hamsters challenged with ancestral variant. G) Boxplot (minimum to maximum) of sgRNA in
603 oropharyngeal swabs taken on day 1-3 (N=8), and 4-5 (N=4). Statistical significance was determined via
604 a mixed-effects analysis followed by Dunnett's multiple comparisons test, comparing vaccinated groups
605 against control group. Dotted line = limit of detection. Circles = hamsters challenged with Omicron,
606 squares = hamsters challenged with ancestral variant. H) Boxplot (minimum to maximum) of the AUC
607 analysis of shedding as measured by sgRNA analysis in swabs collected on 1-5 days post inoculation.
608 Circles = hamsters challenged with Omicron, squares = hamsters challenged with ancestral variant.
609 Statistical significance was determined via one-way ANOVA. N=5.

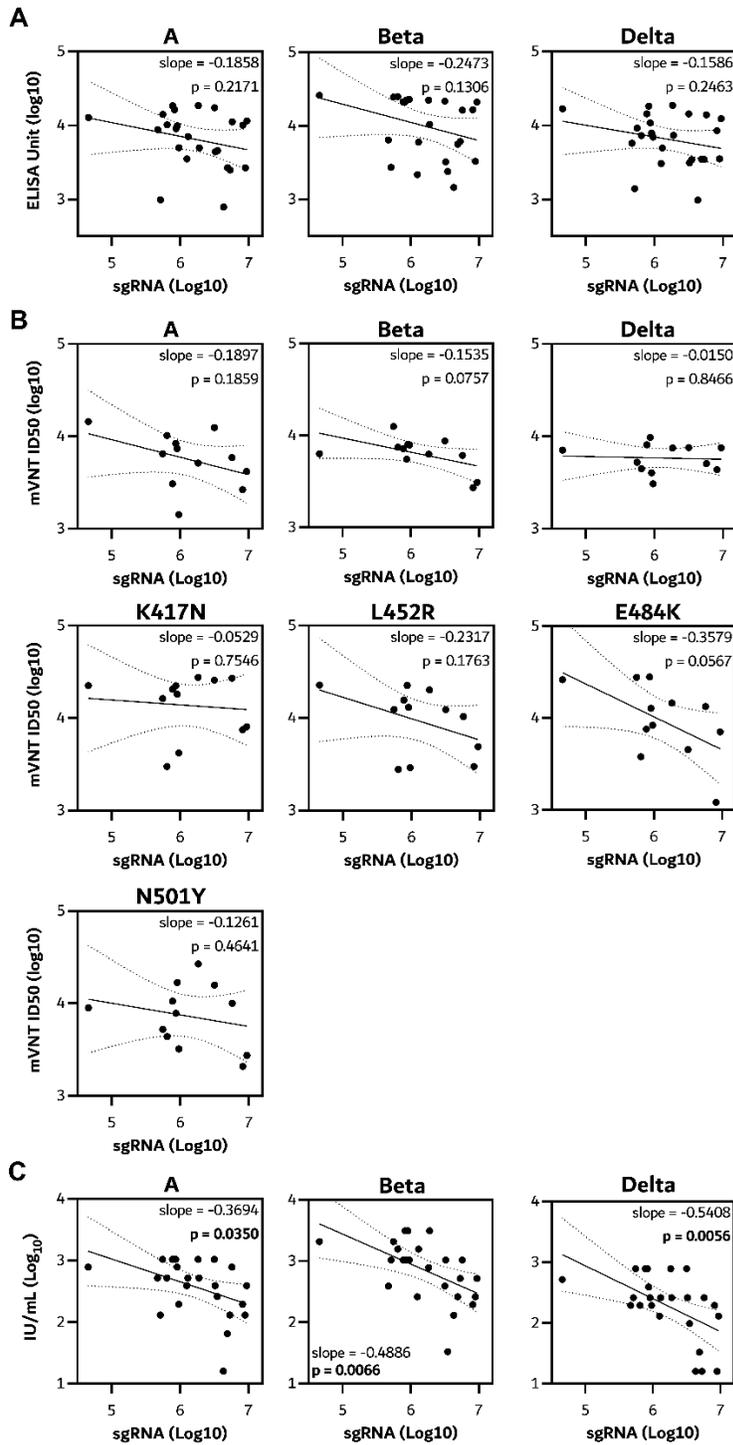


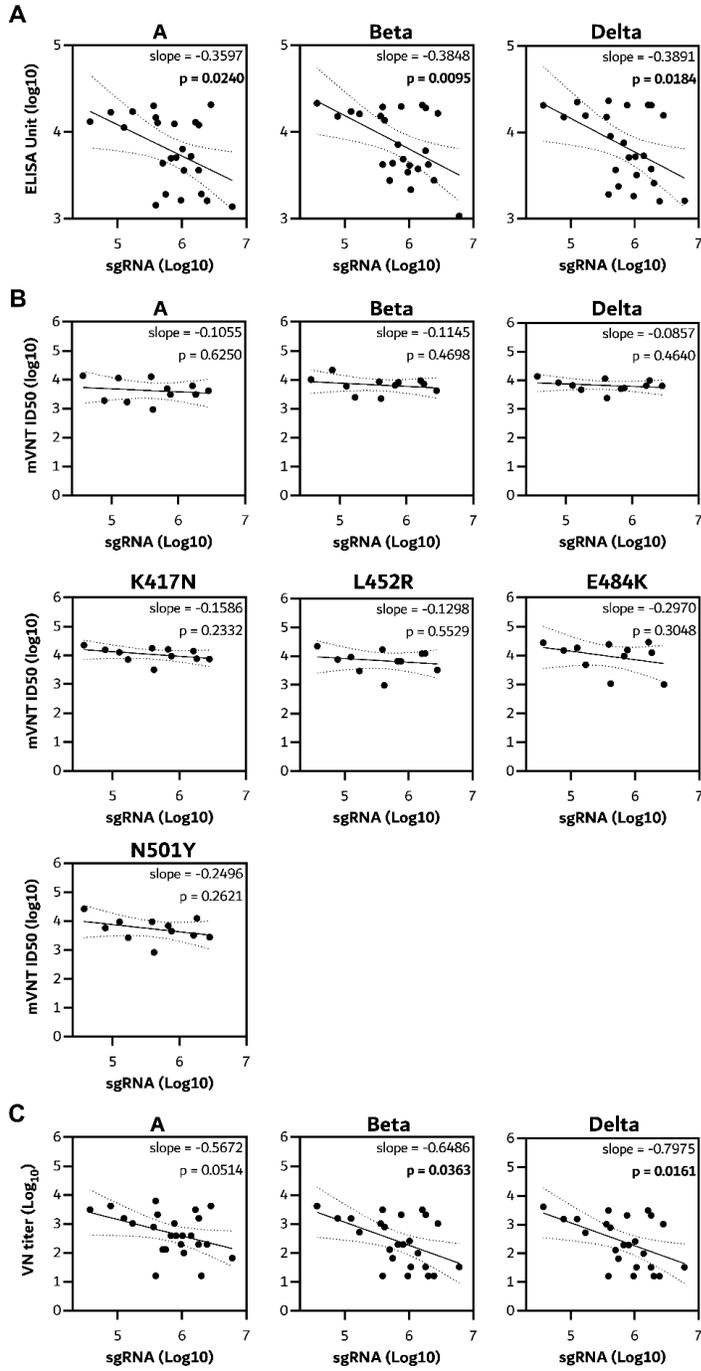


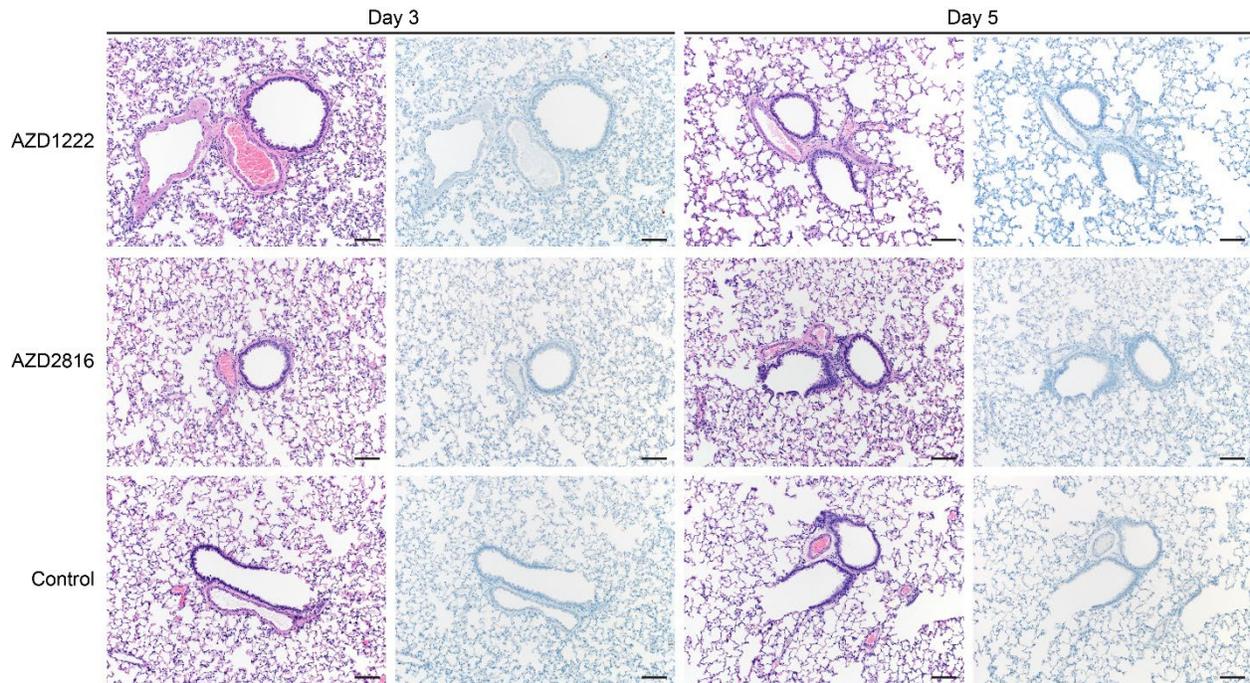
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621 **Extended Data Figure 1. Humoral response of vaccinated hamsters against single mutant**
 622 **pseudotypes.** Boxplots (minimum to maximum) of binding antibody titers as measured by pseudovirus
 623 VN titers in hamster sera obtained on day 0 (left panel), day 5 after Beta VoC challenge (middle panel),
 624 and day 5 after Delta VoC challenge (right panel). Statistical significance was determined via a Friedman
 625 test followed by Dunn's multiple comparisons test comparing ancestral against mutant, p-values in italic
 626 when significant. N=6 per group, day 5 Delta prime boost group N=5.









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653 **Extended Data Figure 6. Pulmonary effects of intranasal challenge with the Omicron VoC in**
 654 **vaccinated and control hamsters at day 3 and 5.** H&E staining (1st and 3rd column) and IHC staining
 655 against N protein (brown, 2nd and 4th column), 100x, scale bar = 100 μ m; Most vaccinated and control
 656 animals showed no pathology in the lower respiratory tract, except for minimal interstitial pneumonia on
 657 day 5 in 1/4 animals in each vaccine group. Antigen staining was limited to bronchial and bronchiolar
 658 epithelium in 2/4 control animals on day 5 and 1/4 AZD1222 vaccinated animals on day 3, as well as in
 659 type I and II pneumocytes in 1/4 animals in control animals on day 5.