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

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Article

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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.

Introduction At the end of 2019, the causative agent of COVID-19, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first detected in Wuhan, China[1,2]. As of February 2nd 2022, SARS-CoV-2 has infected an estimated 380 million people, causing more than 5 million deaths[3]. Its emergence prompted the rapid development of vaccines based on the viral receptor binding protein, spike (S)[4–6]. Several vaccines demonstrated efficacy through clinical trials in less than a year[7–11] and were approved for emergency use by different regulatory bodies worldwide. Over 4.4 billion people are estimated to have received at least one dose of COVID-19 vaccination[3]. One of those vaccines is AZD1222 (ChAdOx1 nCoV-19), developed by Oxford University and produced by AstraZeneca. AZD1222 is a replication-deficient simian adenovirus–vectored vaccine encoding the non-stabilized S protein of Wuhan-1, one of the first published full-length SARS-CoV-2 sequences[12]. AZD1222 was shown to be highly effective in clinical trials, demonstrating 74% vaccine efficacy against symptomatic disease[7]. A two dose primary series of AZD1222 is approved for usage in more than 170 countries, and more than two billion doses of vaccine have been distributed worldwide[13].

Despite the development and administration of these vaccines, a large portion of the world's population is still unvaccinated, particularly in low-income countries[3]. Furthermore, although COVID-19 vaccination is protective against severe disease, it is not fully protective against infection with SARS-CoV-2, and breakthrough infections regularly occur[14]. High levels of circulating virus, asymptomatic infections, low vaccine coverage and break-through infection together means SARS-CoV-2 continues to circulate in the population. As a consequence, several variants of concern (VoCs) have been detected. A variant is termed a VoC if it is associated with an increase in transmission or virulence, or a decrease in the effectiveness of public health and social measures, such as diagnostics, vaccines, or therapeutics[15]. Since COVID-19 vaccines were developed early in the pandemic, they are based on the ancestral S protein, and substitutions in S may result in a reduced vaccine efficacy against VoCs. Several VoCs have substitutions in the receptor binding domain of S which are associated with a reduction in neutralizing virus titers[16–20], which is a strong predictor of vaccine efficacy[21], but the current vaccines clinically available are largely 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 x 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 x 10^8 IU/animal, intramuscular

67   injection), or with two injections of ChAdOx1 GFP (control group, day -56 and -28, 2.5 x 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 $TCID_{50}$ 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, Kruskall 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 x 10^9 and 1.6 x 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 Kruskall 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 Kruskall 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 TCID$_{50}$ 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 x 10^9 and 5.0 x 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 reported[25], 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 l/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 infection[26]. 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 gRNA[27]. 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-2[28]. 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   VoC[29], whereas this was limited in rhesus macaques that were challenged with ancestral virus[30]. 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 booster[32]. 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

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

A significant correlation was found between sgRNA load in oropharyngeal swabs and neutralizing antibody titers in animals, whereas binding antibodies titers were also predictors for sgRNA load in swabs in animals challenged with the Delta VoC. This finding confirms previous reports of a correlation between binding and neutralizing antibodies and viral load in both hamsters[35] and non-human primates[29,36].

Our study confirms that AZD2816 is immunogenic in the hamster model and protects against infection of the lower respiratory tract against the Omicron, Beta, and Delta VoC. Likewise, a single dose of AZD1222 protects against the Omicron VoC. Furthermore, initial immunization with AZD1222 followed by immunization with AZD2816 results in full protection against the Beta and Delta VoCs, and we predict it will also protect against Omicron. This confirms previous reports that a full antigenic match 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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Materials and Methods

Ethics Statement

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

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

Cells and virus

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

Animal Experiments

ChAdOx1 nCoV-19 was formulated as previously described[37]. 4-6-week-old Syrian hamsters (Envigo Indianapolis) were vaccinated with 2.5×10^8 infectious units of AZD1222, AZD2816, or ChAdOx1-GFP delivered intramuscularly in two 100 µL doses into the posterior thighs 56 or 28 days prior to challenge. Prior to challenge, a serum sample was collected via the retro-orbital plexus under isoflurane anesthesia. All animals were challenged intranasally with 40 µl containing 10^4 $TCID_{50}$/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 μL 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 $TCID_{50}$ of SARS-CoV-2 was added to the diluted sera. After a

335 60 min incubation at 37°C and 5% CO_2, 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 described[40]. 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 sequence[41], with the additional substitutions D614G

349   (except for clade A) and K1255*STOP (aka the Δ19 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 µg/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 µL 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 x 10^4 in 100 µL

363   and incubated at 37 °C, 5% CO_2 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 (2µg/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 µL of Blocker A

385 solution at room temperature with shaking for 30 minutes, then washed 3 times with 150 µL/well of MSD

386 Wash buffer. 50 µL 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 µL 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 µL 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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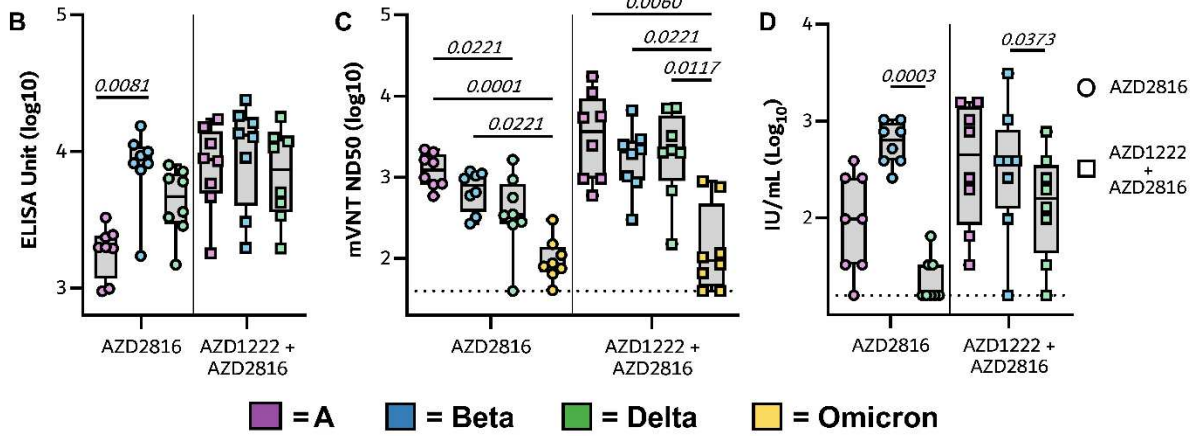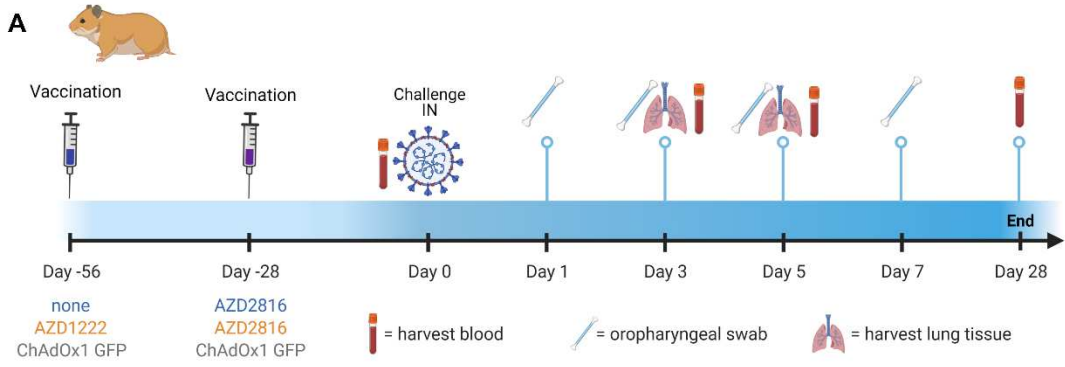
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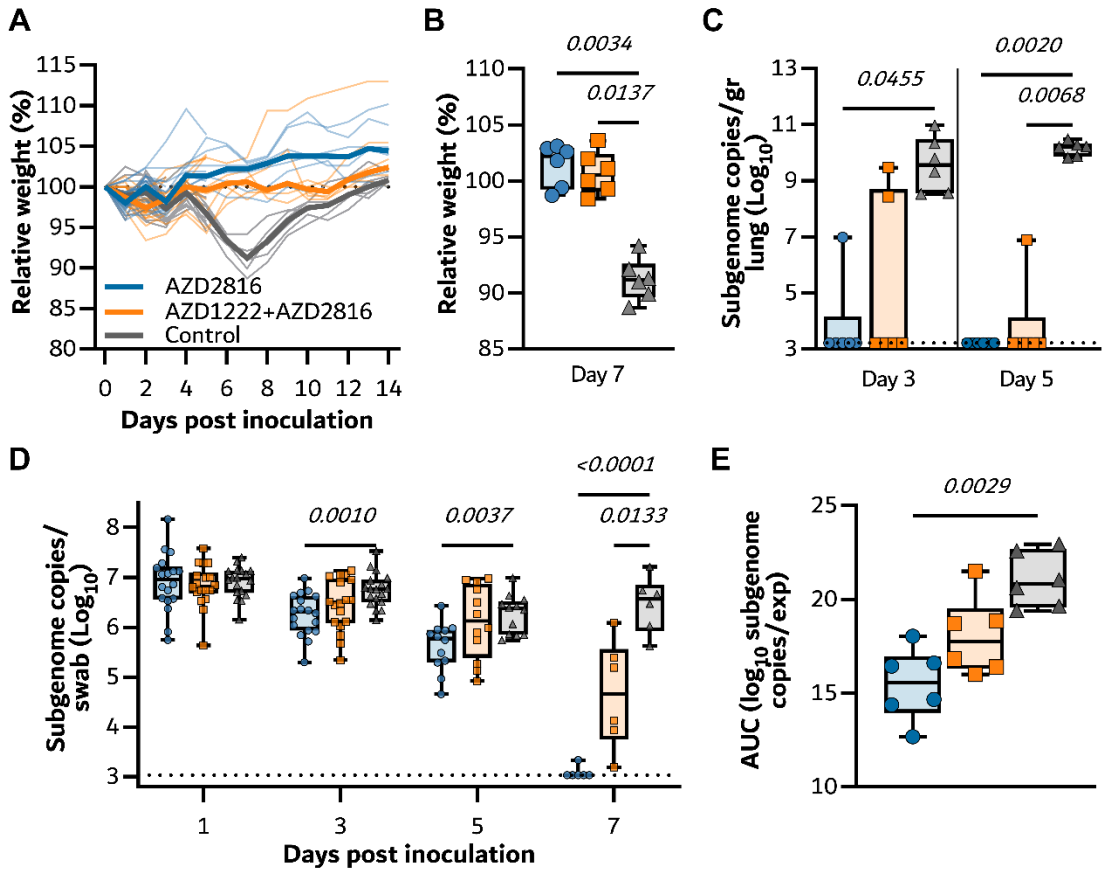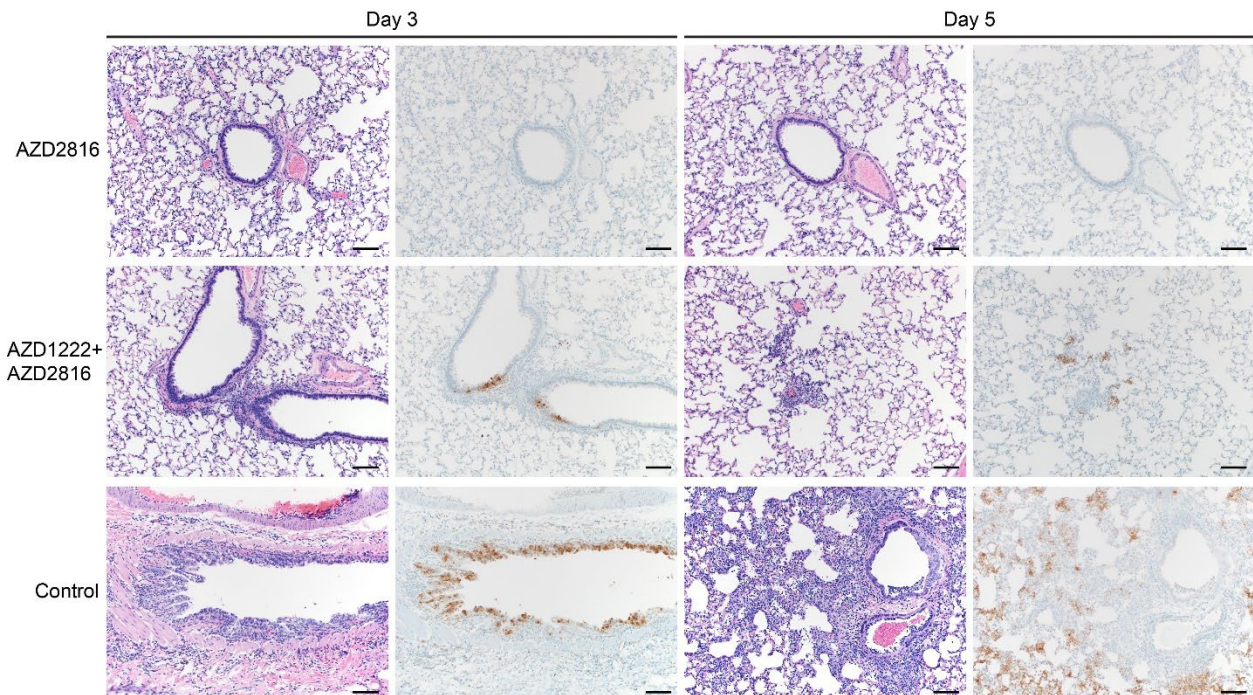
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A

Vaccination — Day -56
none
AZD1222
ChAdOx1 GFP

Vaccination — Day -28
AZD2816
AZD2816
ChAdOx1 GFP

Challenge IN — Day 0

Day 1   Day 3   Day 5   Day 7   Day 28   End

= harvest blood   = oropharyngeal swab   = harvest lung tissue

B

ELISA Unit (log10)

0.0081

AZD2816   AZD1222 + AZD2816

C

mVNT ND50 (log10)

0.0060
0.0221
0.0221
0.0001
0.0117
0.0221

AZD2816   AZD1222 + AZD2816

D

IU/mL (Log$_{10}$)

0.0373
0.0003

AZD2816   AZD1222 + AZD2816

○ AZD2816

☐ AZD1222 + AZD2816

☐ = A   ☐ = Beta   ☐ = Delta   ☐ = Omicron

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Figure 3. Pulmonary effects of intranasal challenge with the Beta VoC in vaccinated and control hamsters at day 3 and 5 post challenge. H&E staining (1st and 3rd column) and IHC staining against N protein (brown, 2nd and 4th column), 100x, scale bar = 100 μm. No pathology nor antigen staining observed in animals which received an AZD2816 vaccination. No pathology observed in animals which received an AZD1222 + AZD2816 vaccination. Compared to control, limited staining of bronchiolar epithelium observed on day 3 and 5. Control animals show progression from bronchiolitis on day 3 to bronchointerstitial pneumonia on day 5, at which point alveolar septa are expanded by edema fluid and leucocytes. Staining of bronchiolar epithelial cells, type I&II pneumocytes, and rare macrophages. Images are representative of observations within 100% of a complete lung section containing all lobes.
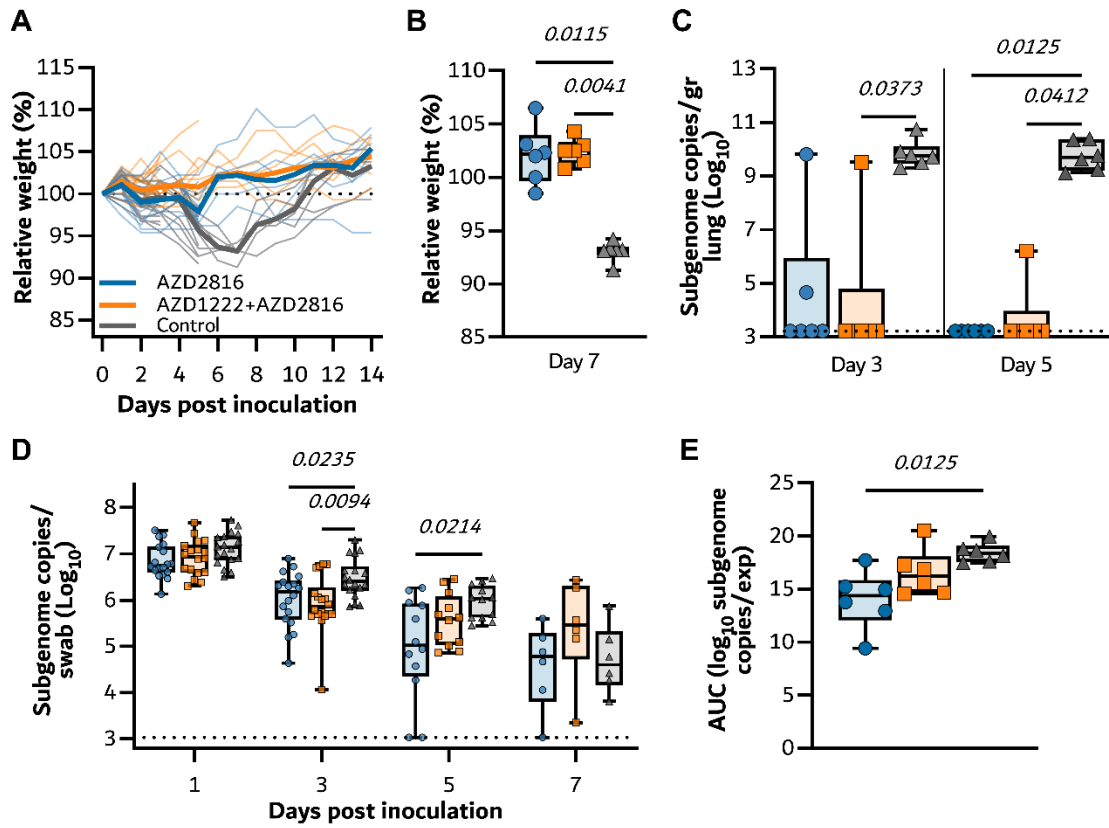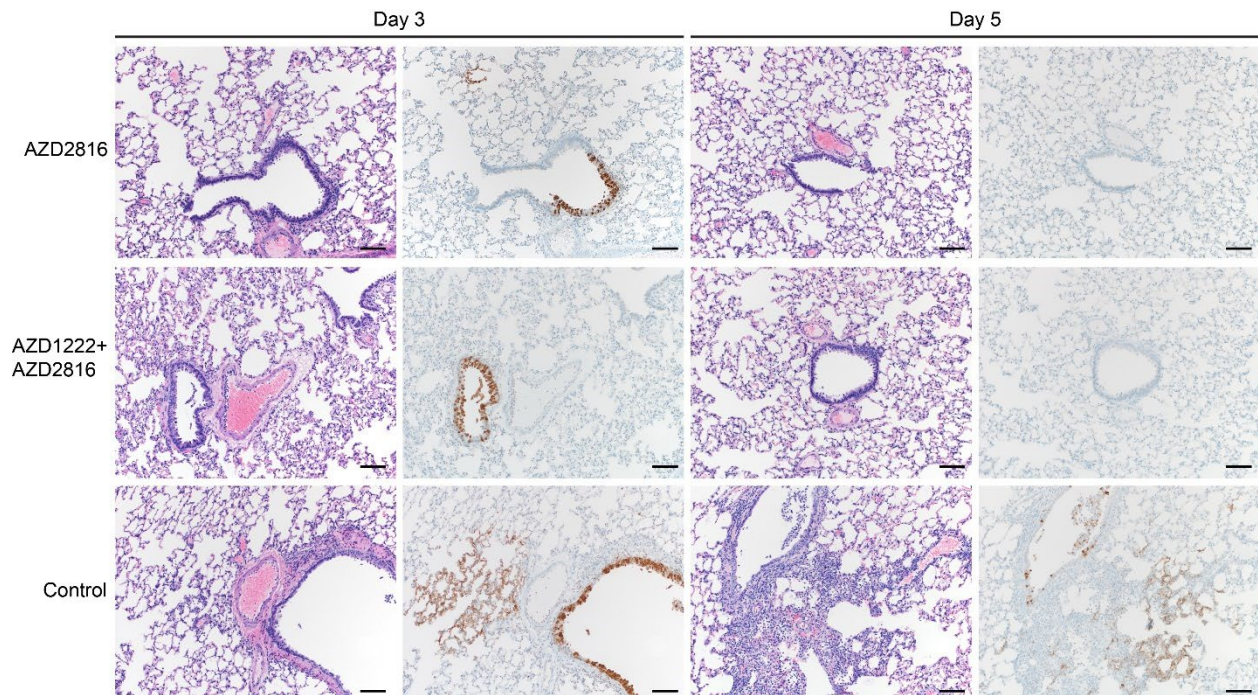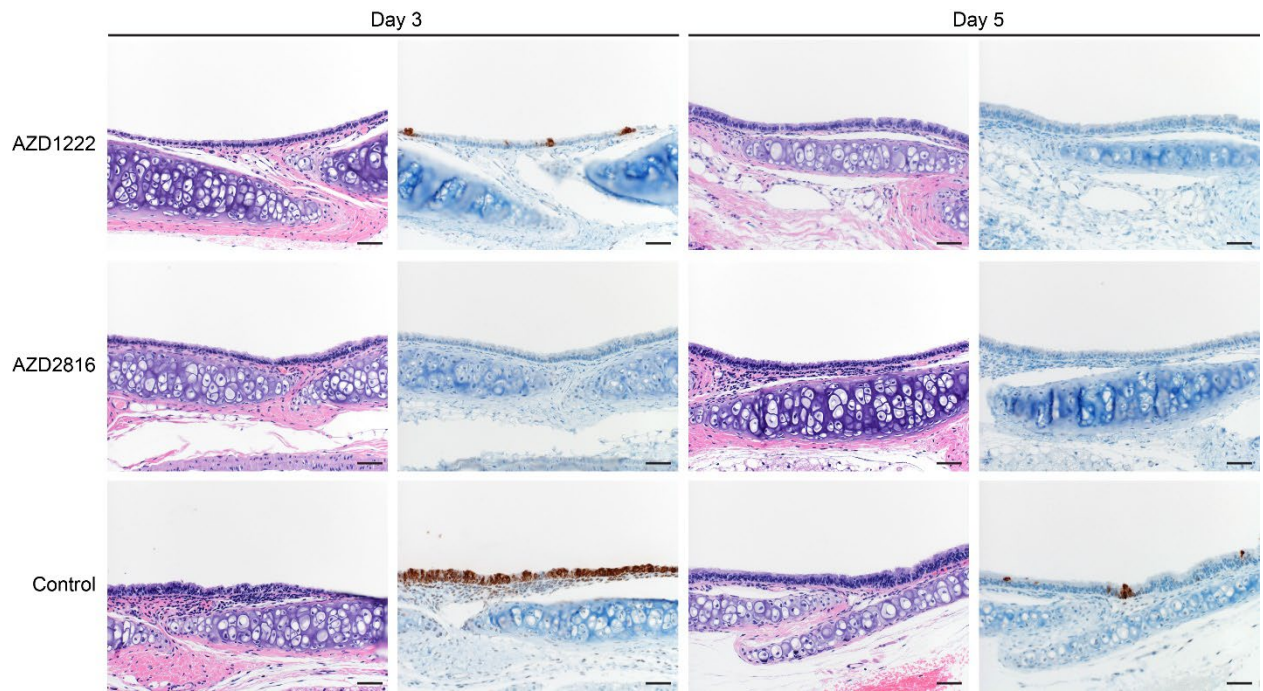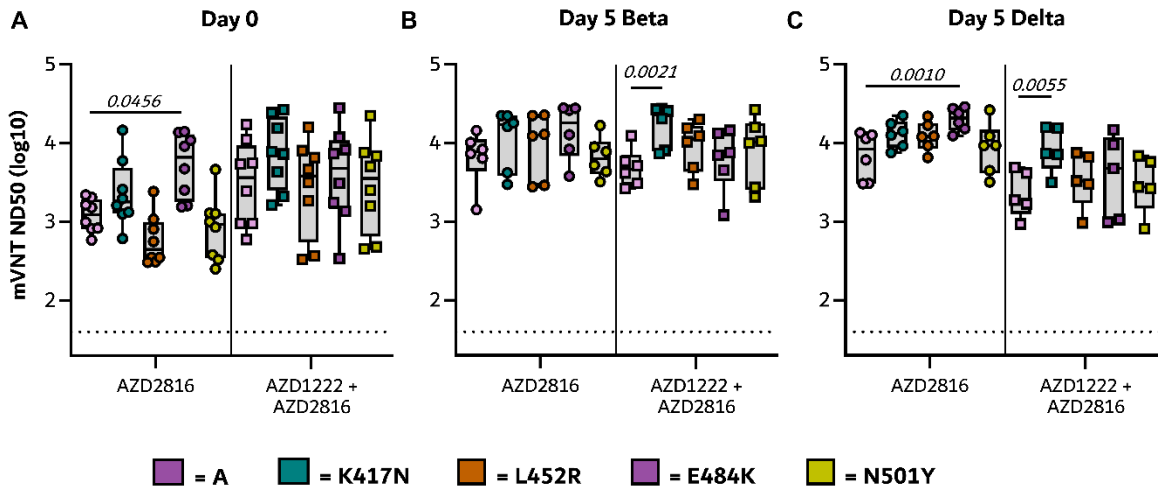
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Figure 5. Pulmonary effects of intranasal challenge with the Delta VoC in vaccinated and control hamsters at day 3 and 5 post challenge. H&E staining (1st and 3rd column) and IHC staining against N protein (brown, 2nd and 4th column), 100x, scale bar = 100 μm; Limited bronchiolitis with epithelial cell necrosis observed on day 3, which was resolved on day 5, in animals that received an AZD2816 vaccination. No pathology observed in animals which received an AZD1222 + AZD2816 vaccination. Compared to controls, limited staining of bronchiolar epithelium observed on day 3, which was resolved on day 5 in both vaccine groups. Control animals show progression from bronchiolitis on day 3 to bronchointerstitial pneumonia on day 5, at which point alveolar septa are expanded by edema fluid and leucocytes. Staining of bronchiolar epithelial cells, type I&II pneumocytes, and rare macrophages on both days. Images are representative of observations within 100% of a complete lung section containing all lobes.
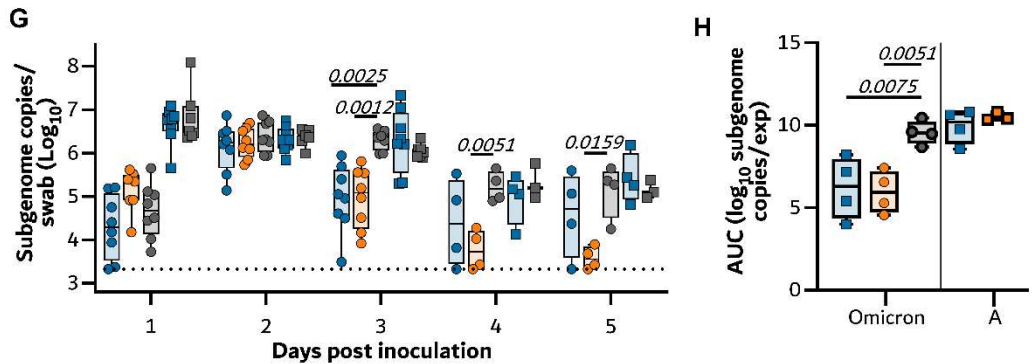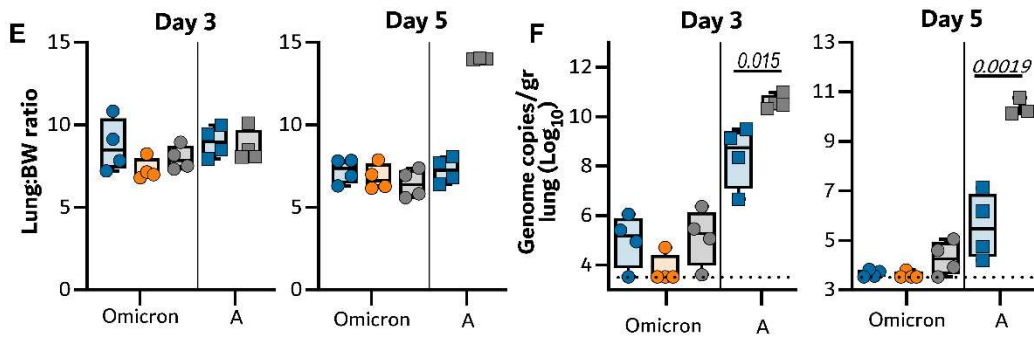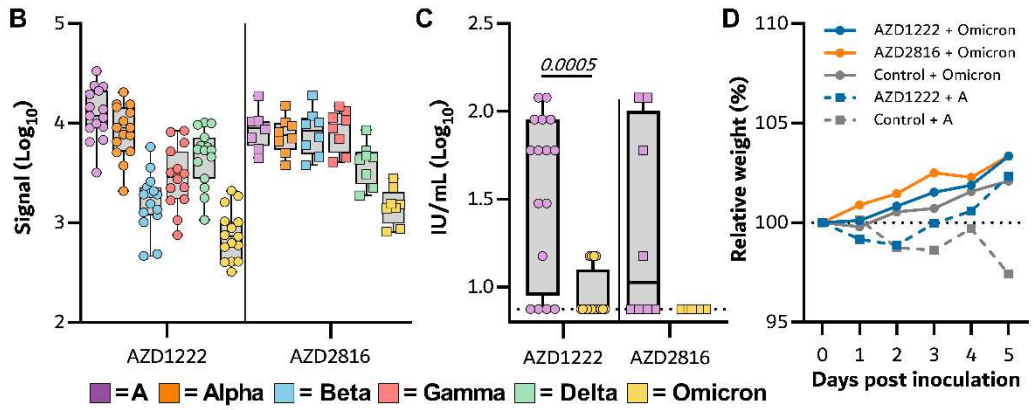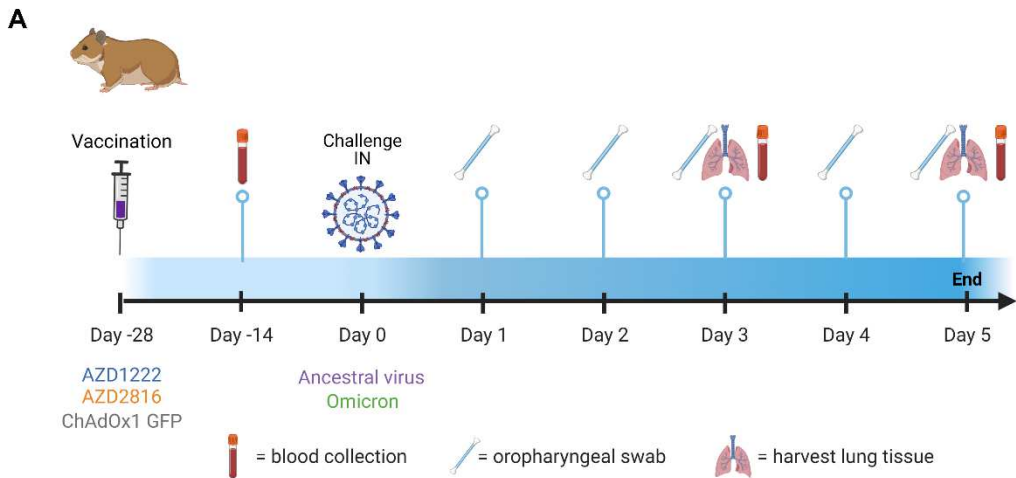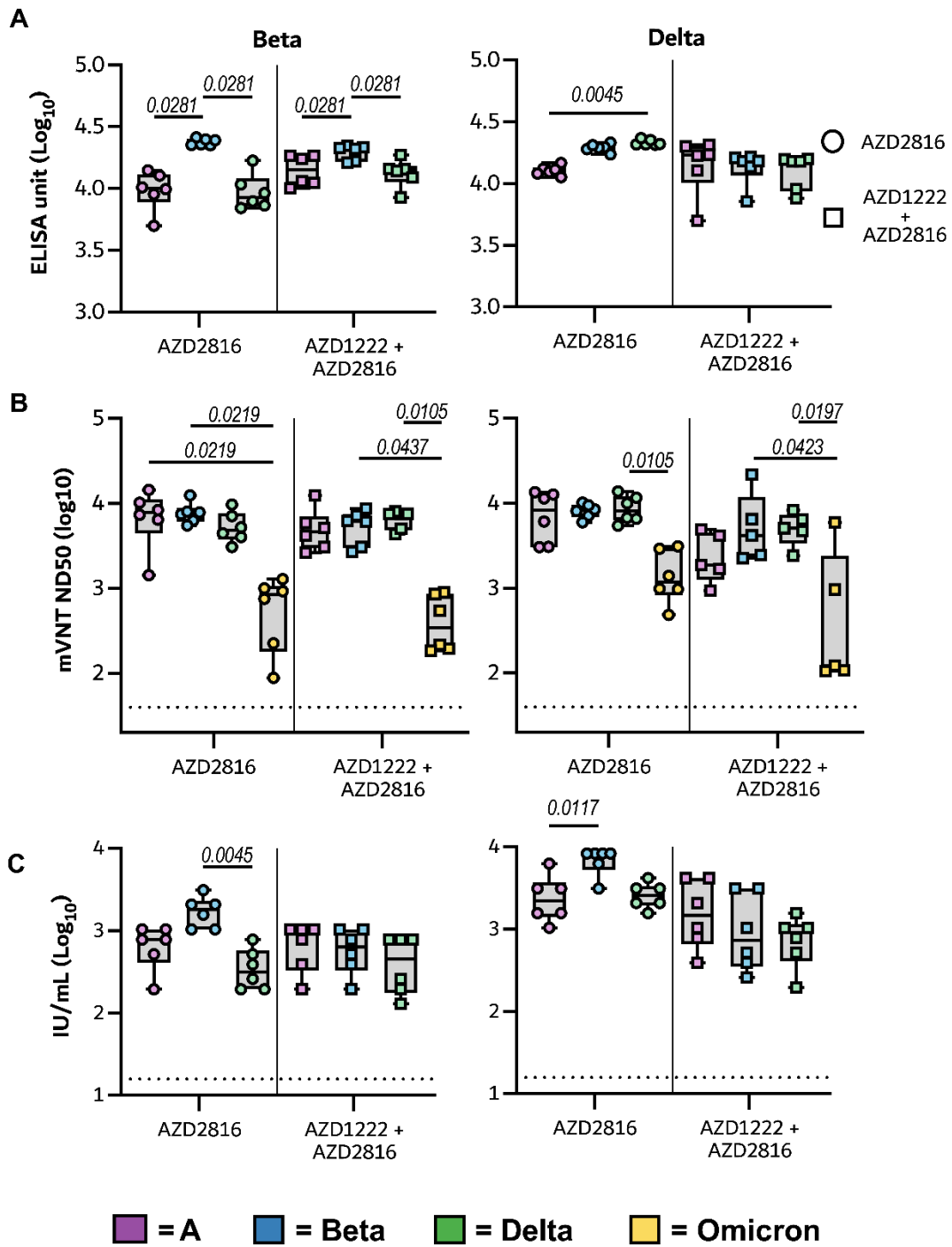
Figure 6. Vaccination of Syrian hamsters with AZD2816 or AZD1222 reduces shedding by the Omicron VoC. A) Schematic overview of experiment. Hamsters were vaccinated with AZD1222, AZD2816, or ChAdOx1 GFP on day –28. Twenty-eight days post final vaccination, hamsters were challenged with 10^3 TCID$_{50}$ of the Omicron or ancestral variant, via the intranasal route. B) Boxplot (minimum to maximum) of binding IgG antibody signal in hamster sera obtained on day -14 against different SARS-CoV-2 S proteins obtained using the V-PLEX SARS-CoV-2 panel 23 by Meso Scale Discovery. Circles = hamsters vaccinated with AZD2816, squares = hamsters vaccinated with AZD2816. C) Boxplot (minimum to maximum) of virus neutralizing antibody titers in hamster sera obtained on day -14 against different ancestral virus or Omicron VoC. VN titers were normalized against NIBSC standard. Statistical significance was determined via a Wilcoxon test. Circles = hamsters vaccinated with AZD2816, squares = hamsters vaccinated with AZD2816. D) Relative weight in comparison to day 0. Dotted line = 100% relative weight. N=8 (Day 1-3) or 4 (Day 4-5). Circles = hamsters challenged with Omicron, squares = hamsters challenged with ancestral variant. E) Boxplot (minimum to maximum) of lung:body weight ratio. Circles = hamsters challenged with Omicron, squares = hamsters challenged with ancestral variant. F) Boxplot (minimum to maximum) of gRNA in lung tissue harvested on day 3 and 5 (N=4). Statistical significance was determined via a Kruskall Wallis test followed by Dunn's multiple comparisons test. Dotted line = limit of detection. Circles = hamsters challenged with Omicron, squares = hamsters challenged with ancestral variant. G) Boxplot (minimum to maximum) of sgRNA in oropharyngeal swabs taken on day 1-3 (N=8), and 4-5 (N=4). Statistical significance was determined via a mixed-effects analysis followed by Dunnett's multiple comparisons test, comparing vaccinated groups against control group. Dotted line = limit of detection. Circles = hamsters challenged with Omicron, squares = hamsters challenged with ancestral variant. H) Boxplot (minimum to maximum) of the AUC analysis of shedding as measured by sgRNA analysis in swabs collected on 1–5 days post inoculation. Circles = hamsters challenged with Omicron, squares = hamsters challenged with ancestral variant. Statistical significance was determined via one-way ANOVA. N=5.
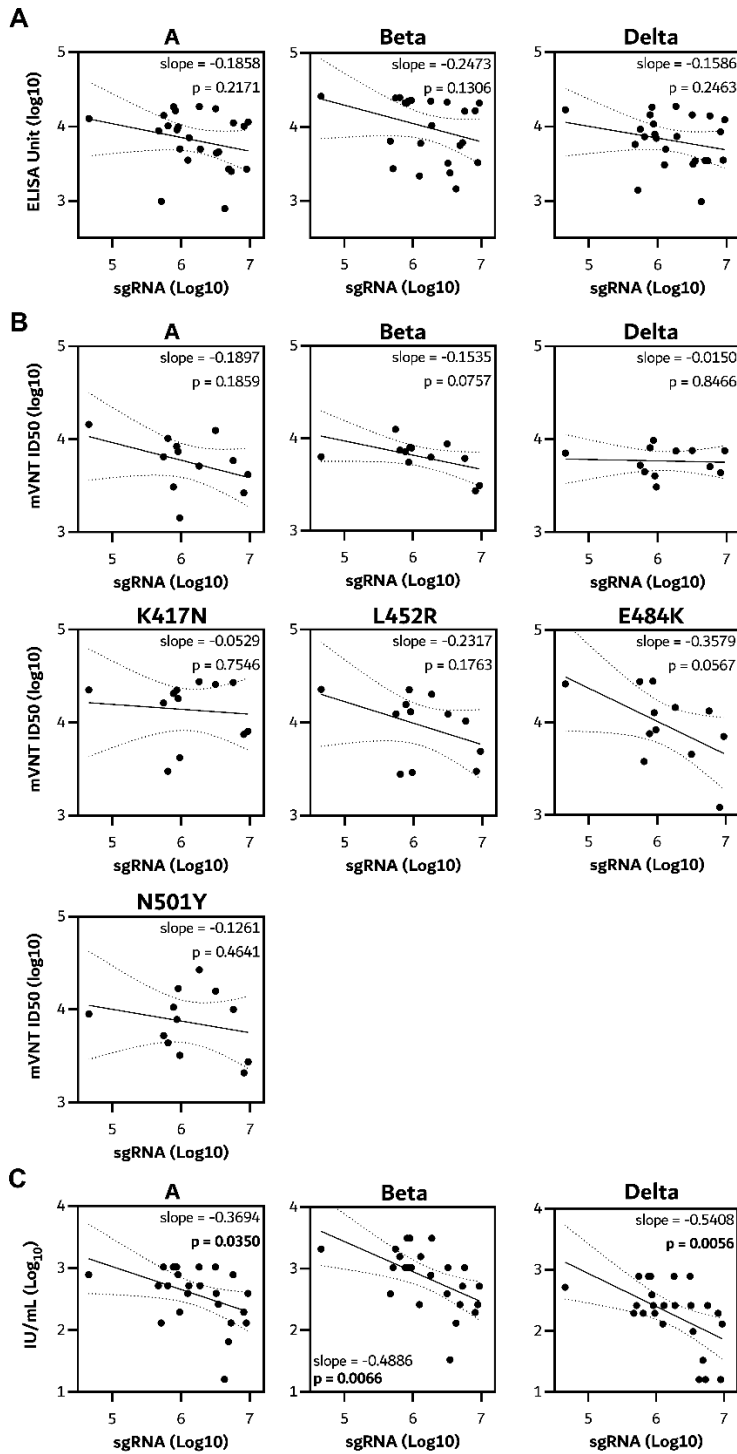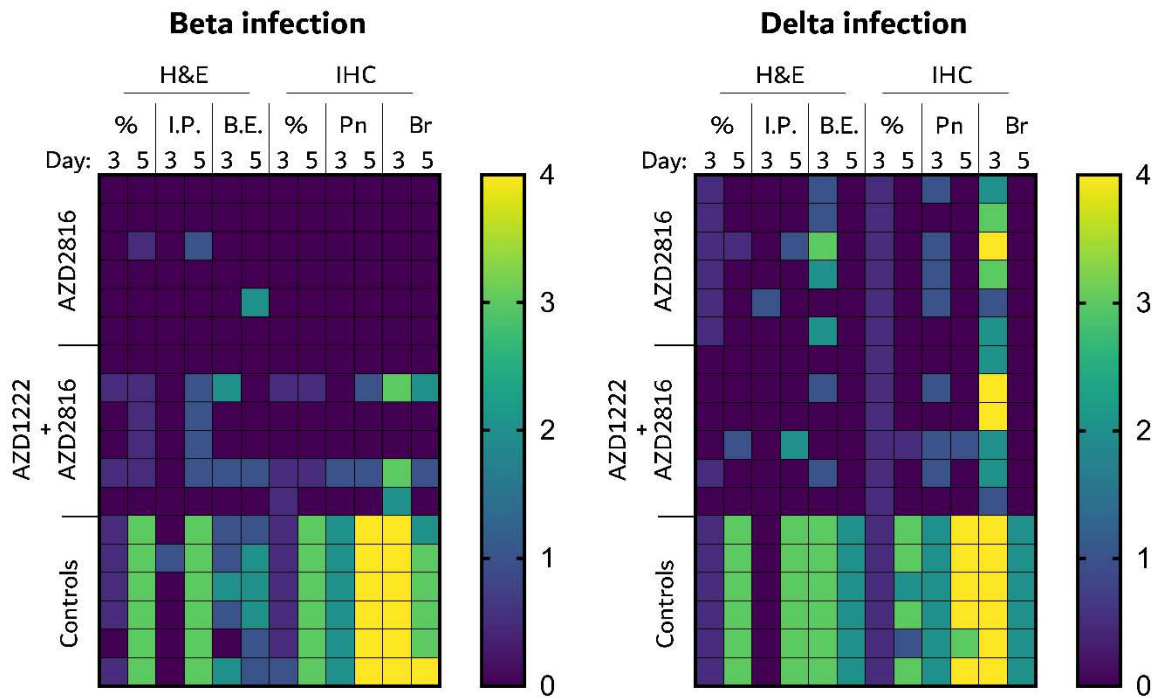
	Day 3	Day 5
AZD1222		
AZD2816		
Control		

610

A Day 0   **B** Day 5 Beta   **C** Day 5 Delta

Legend: ■ = A   ■ = K417N   ■ = L452R   ■ = E484K   ■ = N501Y

619

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

A

Beta

Delta

ELISA unit (Log$_{10}$)

○ AZD2816

□ AZD1222 + AZD2816

B

mVNT ND50 (log10)

C

IU/mL (Log$_{10}$)

■ = A   ■ = Beta   ■ = Delta   ■ = Omicron

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639

Extended Data Figure 4. Heatmap of scores of pathological features in lung tissue of hamsters infected with the Beta or Delta variant. Each square represents an individual score, each column represents a pathological feature at either day 3 or day 5. All features were scored 0 to 5. H&E = hematoxylin and eosin stain. IHC = Immunohistochemistry for SARS2 antigen. % = percentage affected. I.P. = interstitial pneumonia. B.E. = Bronchiolitis with epithelial cell necrosis. Pn = Staining of type I and type II pneumocytes. Br = Staining of bronchiolar epithelium.

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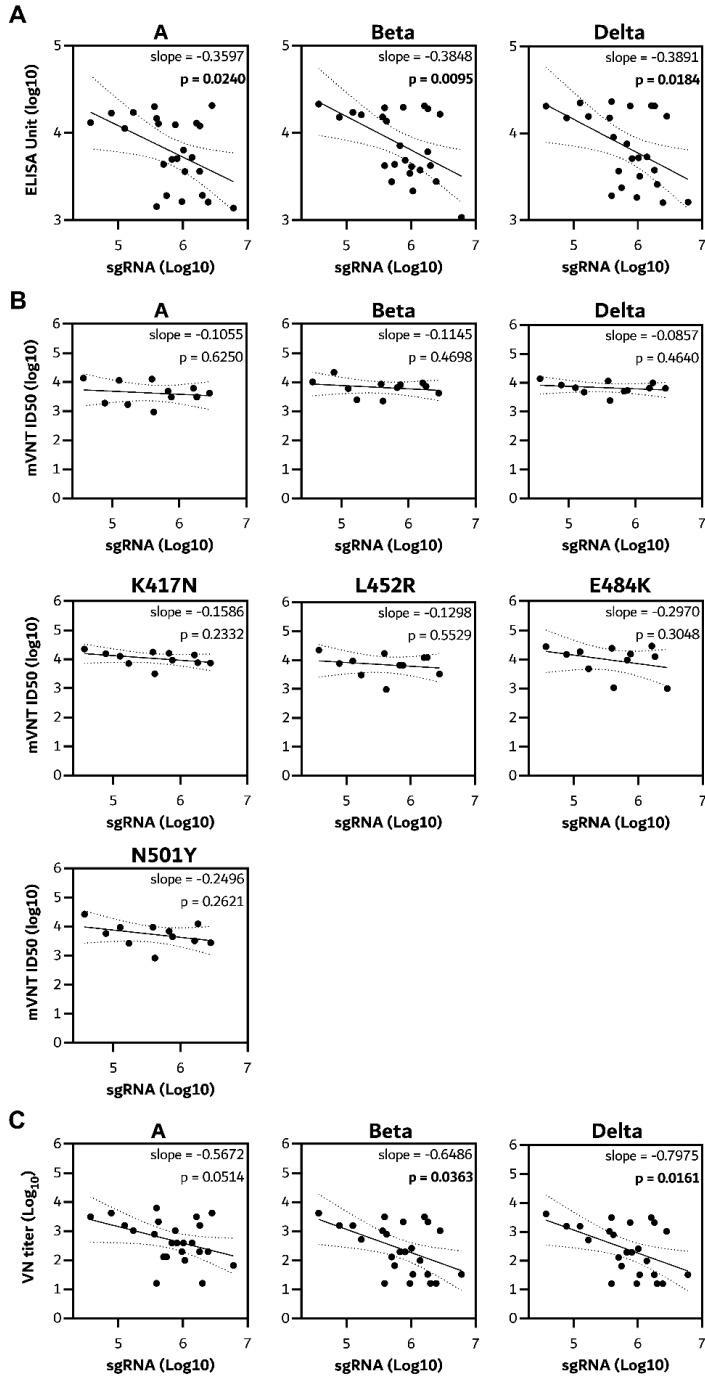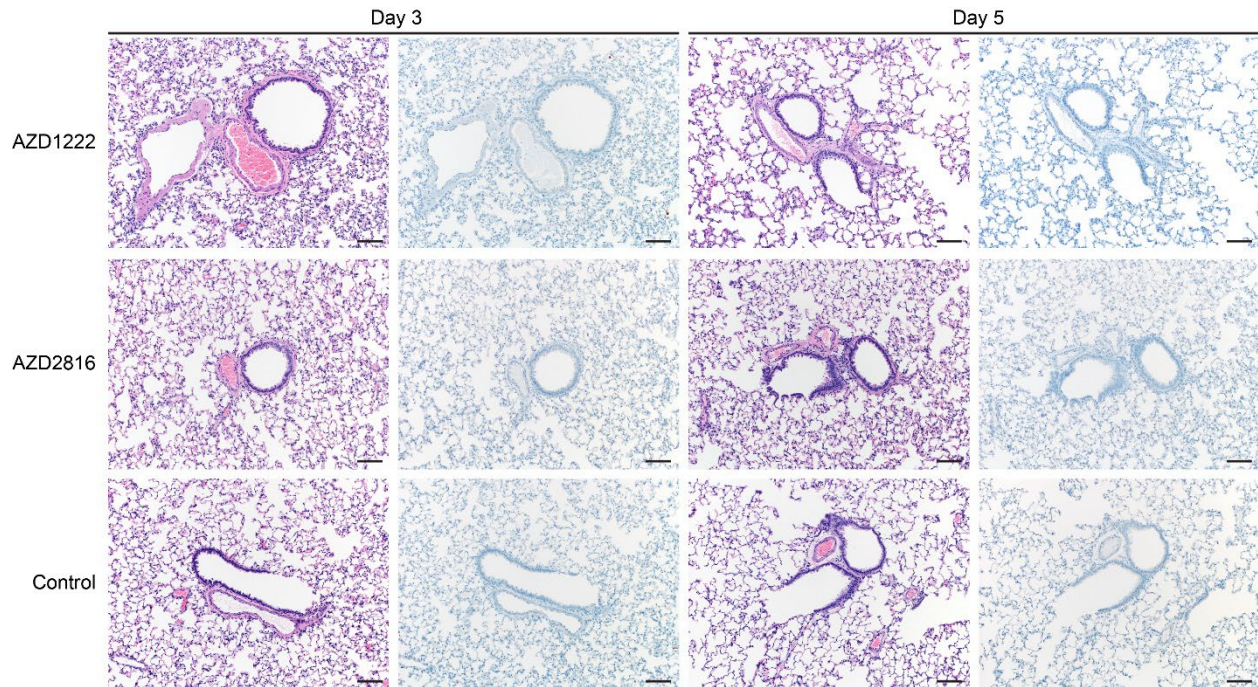
Extended Data Figure 6. Pulmonary effects of intranasal challenge with the Omicron VoC in vaccinated and control hamsters at day 3 and 5. H&E staining (1st and 3rd column) and IHC staining against N protein (brown, 2nd and 4th column), 100x, scale bar = 100 μm; Most vaccinated and control animals showed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