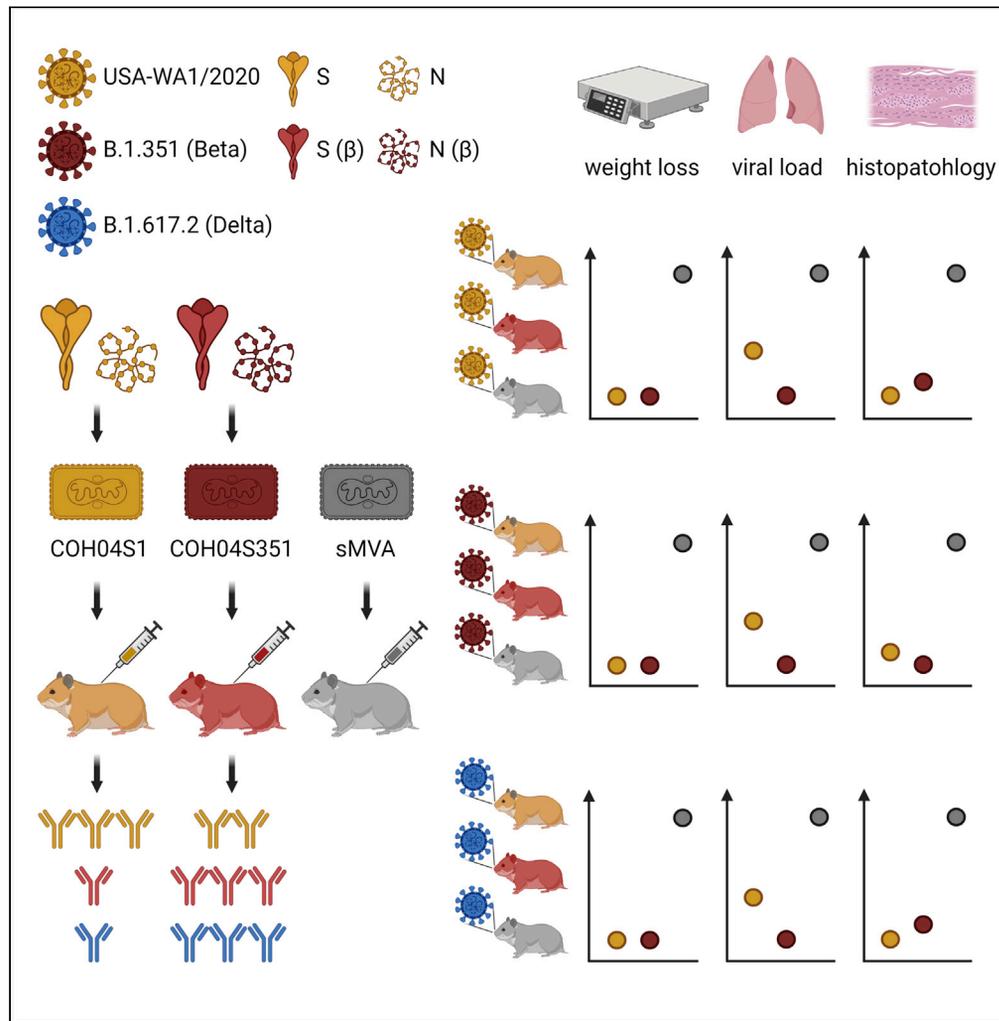


Article

# COH04S1 and beta sequence-modified vaccine protect hamsters from SARS-CoV-2 variants



Felix Wussow,  
Mindy Kha,  
Katelyn Faircloth,  
..., Mark G. Lewis,  
Flavia Chiuppesi,  
Don J. Diamond

fchiuppesi@coh.org (F.C.)  
ddiamond@coh.org (D.J.D.)

**Highlights**  
sMVA-vectored COVID-19 vaccines based on SARS-CoV-2 ancestral virus or Beta variant

Vaccinated hamsters develop antibodies to ancestral virus and variants of concern

Vaccines protect hamsters against ancestral SARS-CoV-2 and Beta and Delta variants

sMVA vectors induce potent cross-protective immunity to SARS-CoV-2 and its variants

Wussow et al., iScience 25, 104457  
June 17, 2022 © 2022 The Author(s).  
<https://doi.org/10.1016/j.isci.2022.104457>



## Article

## COH04S1 and beta sequence-modified vaccine protect hamsters from SARS-CoV-2 variants

Felix Wussow,<sup>1</sup> Mindy Kha,<sup>1</sup> Katelyn Faircloth,<sup>1</sup> Vu H. Nguyen,<sup>1</sup> Angelina Iniguez,<sup>1</sup> Joy Martinez,<sup>1</sup> Yoonsuh Park,<sup>1</sup> Jenny Nguyen,<sup>1</sup> Swagata Kar,<sup>2</sup> Hanne Andersen,<sup>2</sup> Mark G. Lewis,<sup>2</sup> Flavia Chiappesi,<sup>1,3,\*</sup> and Don J. Diamond<sup>1,3,4,\*</sup>

## SUMMARY

**COVID-19 vaccine efficacy is threatened by emerging SARS-CoV-2 variants of concern (VOC) with the capacity to evade protective neutralizing antibody responses. We recently developed clinical vaccine candidate COH04S1, a synthetic modified vaccinia Ankara vector (sMVA) co-expressing spike and nucleocapsid antigens based on the Wuhan-Hu-1 reference strain that showed potent efficacy to protect against ancestral SARS-CoV-2 in Syrian hamsters and non-human primates and was safe and immunogenic in healthy volunteers. Here, we demonstrate that intramuscular immunization of Syrian hamsters with COH04S1 and an analogous Beta variant-adapted vaccine candidate (COH04S351) elicits potent cross-reactive antibody responses and protects against weight loss, lower respiratory tract infection, and lung pathology following challenge with major SARS-CoV-2 VOC, including Beta and the highly contagious Delta variant. These results demonstrate efficacy of COH04S1 and a variant-adapted vaccine analog to confer cross-protective immunity against SARS-CoV-2 and its emerging VOC, supporting clinical investigation of these sMVA-based COVID-19 vaccine candidates.**

## INTRODUCTION

Immediately after the outbreak of the COVID-19 pandemic (Zhou et al., 2020; Zhu et al., 2020), several severe acute respiratory syndrome virus 2 (SARS-CoV-2) variants of concern (VOC) have emerged with the capacity to spread more effectively and to evade neutralizing antibodies (NAb) stimulated through natural infection or vaccination (Tegally et al., 2021; Garcia-Beltran et al., 2021; Zhou et al., 2021; Faria et al., 2021; Dejnirattisai et al., 2021; Moore and Ofit, 2021; Planas et al., 2021). This includes the Beta variant (B.1.351) first identified in South Africa as well as the Delta variant (B.1.617) first identified in India, which has rapidly spread worldwide and globally surfaced as a dominant SARS-CoV-2 VOC (Tegally et al., 2021; Garcia-Beltran et al., 2021; Zhou et al., 2021; Planas et al., 2021), prior to the recent predominance by the Omicron variant (B.1.1.529) (Carreno et al., 2021). The ongoing emergence of SARS-CoV-2 VOC with mutant spike (S) proteins poses imminent challenges to establish protective immunity by approved first-generation COVID-19 vaccines (Polack et al., 2020; Baden et al., 2021; Voysey et al., 2021; Sadoff et al., 2021), which were antigenically designed based on the Wuhan-Hu-1 reference strain initially discovered in China. Recent reports suggest that two doses of approved COVID-19 vaccines confer reduced clinical effectiveness against SARS-CoV-2 Beta and Delta variants as well as SARS-CoV-2 Omicron (Madhi et al., 2021; Shinde et al., 2021; Lopez Bernal et al., 2021; Collie et al., 2021; Tang et al., 2021; Andrews et al., 2022; Sheikh et al., 2021). These findings highlight the need to develop next-generation COVID-19 vaccines that confer improved efficacy against SARS-CoV-2 and its emerging VOC.

We previously developed COH04S1, a multi-antigenic COVID-19 vaccine candidate based on a synthetic version of the well-characterized and clinically proven modified vaccinia Ankara (sMVA) vector (Chiappesi et al., 2020), which is widely used to develop poxvirus vaccines for infectious disease and cancer (Gilbert, 2013; Sutter and Moss, 1992; Volz and Sutter, 2017). We have used MVA to develop vaccine candidates for preclinical testing in animal models of congenital cytomegalovirus disease while demonstrating vaccine efficacy in several clinical trials in solid tumor and stem cell transplant patients (Wussow et al., 2013, 2014; La Rosa et al., 2017; Aldoss et al., 2020; Chiappesi et al., 2018; Yuan et al., 2017). COH04S1 was designed to co-express full-length, unmodified S and nucleocapsid (N) antigens based on the Wuhan-Hu-1 reference strain

<sup>1</sup>Department of Hematology and Transplant Center, City of Hope National Medical Center, Duarte, CA 91010, USA

<sup>2</sup>Bioqual, Rockville, MD, USA

<sup>3</sup>These authors contributed equally

<sup>4</sup>Lead contact

\*Correspondence: fchiappesi@coh.org (F.C.), ddiamond@coh.org (D.J.D.)  
<https://doi.org/10.1016/j.isci.2022.104457>



and demonstrated potent efficacy to protect against respiratory infection by ancestral SARS-CoV-2 in Syrian hamster and non-human primate models through different immunization routes and dose regimens (Chiuppesi et al., 2020). COH04S1 has demonstrated to be safe and immunogenic in a Phase 1 clinical trial in healthy adults (Chiuppesi et al., 2022b), and is currently being tested in Phase 2 clinical trials as a primary series vaccination in hematology and transplant patients (NCT04977024) and as a booster dose to authorized SARS-CoV-2 vaccines in healthy adults (NCT04639466).

In this report, we demonstrate that COH04S1 and an analogous Beta variant-adapted vaccine construct, termed COH04S351, protect Syrian hamsters from respiratory infection by major SARS-CoV-2 VOC, including SARS-CoV-2 Beta and Delta variants. These results demonstrate efficacy of COH04S1 and COH04S351 to provide cross-protective immunity against SARS-CoV-2 and its emerging VOC, supporting clinical evaluation of these COVID-19 vaccine candidates.

## RESULTS

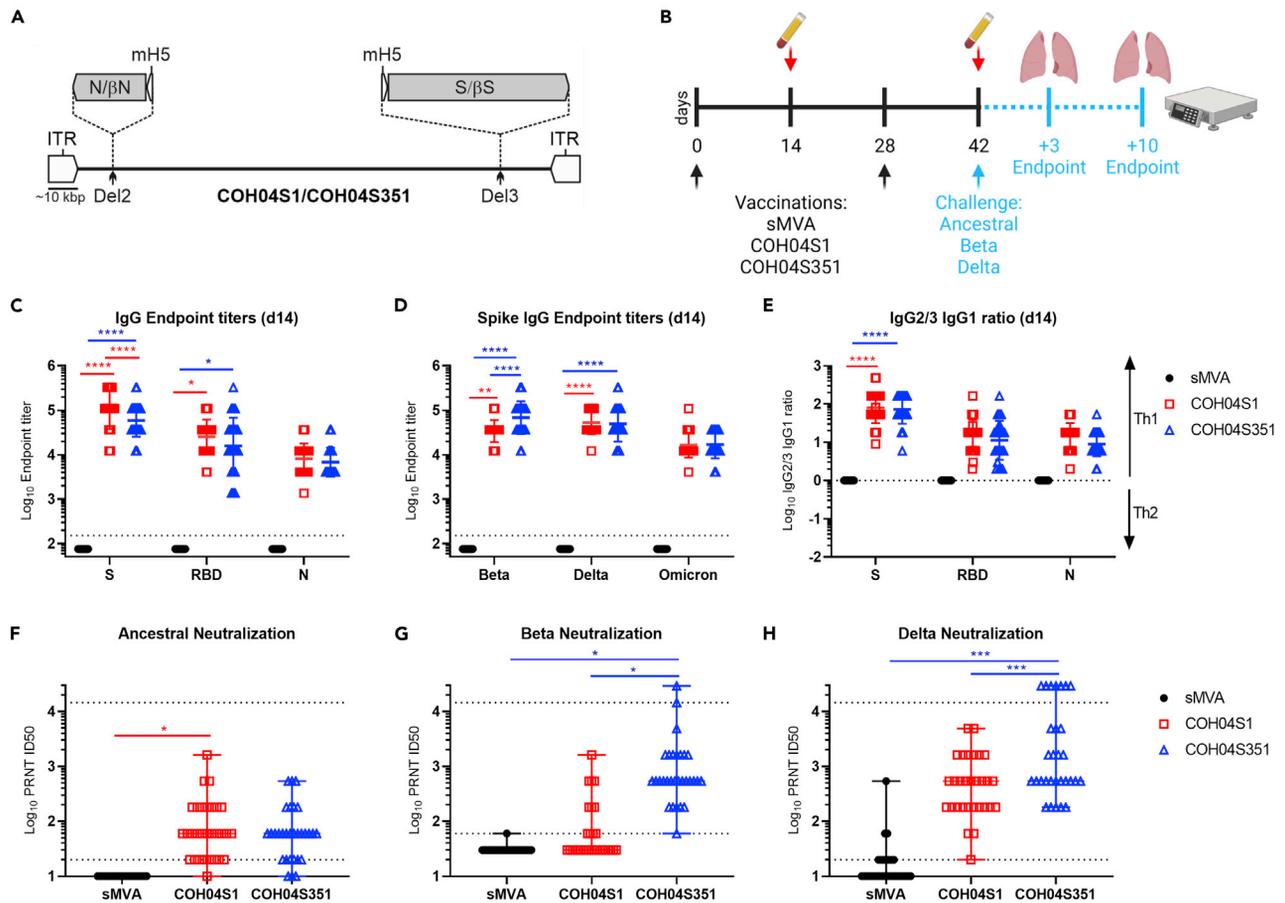
### COH04S1 and COH04S351 elicit cross-reactive antibody responses against SARS-CoV-2 ancestral virus and Beta and Delta variants

Using the Syrian hamster model (Tostanoski et al., 2020; Sanchez-Felipe et al., 2021; Yahalom-Ronen et al., 2020; Chan et al., 2020; Sia et al., 2020; Imai et al., 2020), we evaluated COH04S1 and COH04S351 vaccine efficacy against viral challenge by ancestral SARS-CoV-2 (USA-WA1/2020) or SARS-CoV-2 Beta and Delta variants. The sMVA-derived COH04S1 vaccine contains Wuhan-Hu-1-based N and S antigen sequences (Chiuppesi et al., 2020, 2022a), while the analogously constructed COH04S351 contains modified N and S antigen sequences based on the SARS-CoV-2 Beta strain (Figure 1A and Table S1). Hamsters were vaccinated twice in a four week interval with  $1 \times 10^8$  plaques forming units (PFU) of vaccine or empty sMVA control vector by intramuscular route (Figure 1B). Both COH04S1- and COH04S351-vaccinated animals developed robust binding antibody responses to ancestral-specific S, receptor-binding domain (RBD), and N antigens as well as S antigens based on Beta, Delta, and the currently dominating Omicron variant (Figures 1C and 1D). These ancestral- and VOC-specific responses measured after the first immunization were overall similar between COH04S1- and COH04S351-vaccinated animals, although ancestral-specific S antibody titers tended to be significantly higher in COH04S1-vaccinated hamsters than in COH04S351-vaccinated animals, and Beta-specific S antibody titers tended to be significantly higher in COH04S351-vaccinated animals than in COH04S1-vaccinated animals. In addition, ancestral-specific S, RBD, and N antibody responses elicited in COH04S1- and COH04S351-vaccinated hamsters were predominantly of IgG2/3 isotype and only to a minor proportion of IgG1 isotype (Figure 1E), indicating stimulation of Th1-biased immunity.

NAb measurements by plaque reduction neutralization titer (PRNT) assay after the booster vaccination revealed strain-specific NAb responses in COH04S1- and COH04S351-vaccinated animals (Figures 1F–1H). NAb responses against the ancestral virus were measured in both COH04S1- and COH04S351-vaccinated animals, with no significant differences between the two vaccine groups, although ancestral-specific NAb titers in COH04S351-vaccinated animals tended to be slightly lower than those in COH04S1-vaccinated animals. While ancestral-specific NAb titers were significantly elevated in COH04S1-vaccinated animals compared to controls, there was no statistical significance of ancestral-specific NAb titers in COH04S351-vaccinated animals compared to controls, indicating reduced neutralizing activity against the ancestral virus in COH04S351-vaccinated animals compared to COH04S1-vaccinated animals. In contrast, neutralizing titers measured against the Beta and Delta variants were significantly higher in COH04S351-vaccinated animals than in COH04S1-vaccinated animals. Both Beta- and Delta-specific neutralizing responses were significantly elevated in COH04S351-vaccinated animals compared to controls, whereas there was no statistical significance in Beta- and Delta-specific neutralizing titers when comparing COH04S1-vaccinated animals to controls, indicating reduced or relatively low levels of Beta- and Delta-specific NAb responses in COH04S1-vaccinated animals. In total, these results demonstrate that COH04S1 stimulated robust humoral immunity against the ancestral virus but reduced antibody levels against the Beta and Delta variants, whereas COH04S351 elicited significantly elevated humoral responses against the Beta and Delta variant but reduced antibody responses against the ancestral virus.

### COH04S1 and COH04S351 protect hamsters from severe weight loss following challenge with SARS-CoV-2 ancestral virus and Beta and Delta variants

At six weeks post immunization, vaccinated hamsters and control animals were challenged intranasally with  $8.0 \times 10^6$  TCID<sub>50</sub> (median tissue culture infectious dose) of ancestral SARS-CoV-2 (USA-WA1/2020) or



**Figure 1. COH04S1 and COH04S351 elicit cross-reactive antibody responses against SARS-CoV-2 ancestral virus and Beta and Delta variants**

(A) Vaccine constructs. COH04S1 and COH04S351 are sMVA-based vaccine vectors designed to co-express N and S antigen sequences based on ancestral Wuhan-Hu-1 SARS-CoV-2 or SARS-CoV-2 Beta variant ( $\beta$ ), respectively. The antigen sequences were inserted into the MVA deletion sites 2 (Del2) and 3 (Del3) as indicated.

(B) Study design. Hamsters were vaccinated twice with COH04S1 ( $n = 30$ ) or COH04S351 ( $n = 30$ ) by intramuscular route as indicated (black arrows). Hamsters vaccinated with empty sMVA vector ( $n = 30$ ) were used as controls. Blood samples were collected at day 14 and 42 (red arrows) after the first and second vaccination. Hamsters were challenged intranasally at day 42 with ancestral virus or Beta or Delta variants ( $n = 10$ /group) and body weight changes were recorded daily for 10 days. Nasal turbinates and lung tissue for viral load measurements and lung histopathology were collected at day 3 and 10 post challenge ( $n = 5$ /group/timepoint).

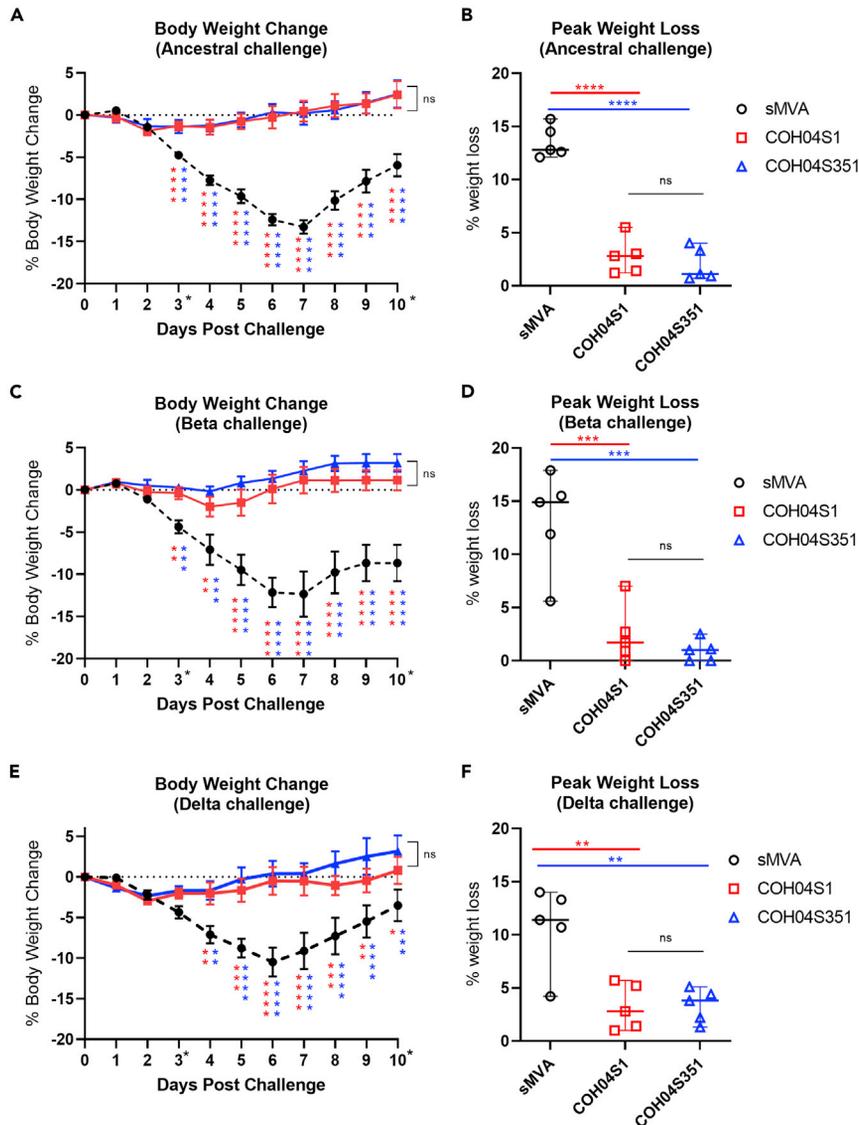
(C) IgG endpoint titers. S, RBD, and N antigen-specific binding antibody titers to ancestral SARS-CoV-2 were measured in serum samples of vaccine and control groups at day 14 (d14) post prime vaccination via ELISA. Dotted lines indicate lower limit of detection.

(D) Variant-specific IgG endpoint titers. Serum IgG binding antibody titers to Beta, Delta, and Omicron-specific S antigens were measured in serum samples of vaccine and control groups at day 14 (d14) post prime vaccination via ELISA. Dotted lines indicate lower limit of detection.

(E) IgG2-3/IgG1 ratios. S, RBD, and N antigen-specific IgG2-3 and IgG1 endpoint titers were measured at day 14 (d14) in serum samples of vaccine and control groups and used to assess IgG2-3/IgG1 ratios. An IgG2-3/IgG1 ratio  $>1$  (dotted line) is indicative of a Th1-biased response.

(F–H) NAb titers. NAb titers were measured in serum samples of vaccine and control groups at day 42 (d42) post second vaccination via PRNT assay against ancestral SARS-CoV-2 (F) and Beta (G) and Delta (H) variants. Dotted lines indicate lower and upper limit of detection (LLOD and ULOD, respectively). Values below the LLOD (ID50 = 20 in F and H, ID50 = 60 in G) are indicated as half the LLOD, values above the ULOD (ID50 = 14,580) are indicated as twice the ULOD. In C–H data are presented as geometric mean values  $\pm$  geometric SD. Two-way ANOVA with Tukey's multiple comparison test was used. \* $0.05 < p < 0.01$ , \*\* $0.01 < p < 0.001$ , \*\*\* $0.001 < p < 0.0001$ , \*\*\*\* $p < 0.0001$ .

SARS-CoV-2 Beta variant or  $1.3 \times 10^7$  TCID50 of SARS-CoV-2 Delta variant (Figure 1B). A higher viral challenge dose was used for the Delta variant compared to the ancestral virus and the Beta variant based on prior dose-titration experiments. Body weight changes were measured daily over a 10 days period (Figure 1B). Control animals challenged with the ancestral virus or the variant viruses showed rapid body weight loss for 6–7 days, reaching maximum body weight loss between 5% and 18%. In contrast to the controls, COH04S1- and COH04S351-vaccinated hamsters challenged with the ancestral virus or the variant viruses

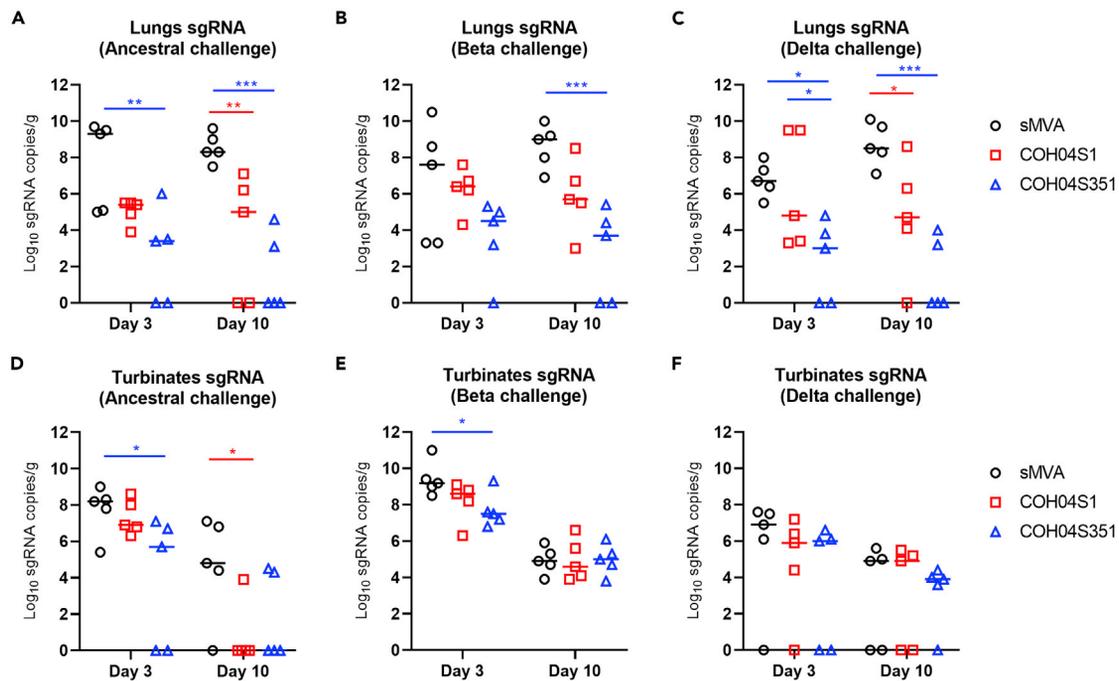


**Figure 2. COH04S1 and COH04S351 protect hamsters from severe weight loss following challenge with SARS-CoV-2 ancestral virus and Beta and Delta variants**

(A, C, and E) Body weight change. Body weight of COH04S1- and COH04S351-vaccinated animals and sMVA-immunized control animals was measured daily for 10 days following challenge with ancestral SARS-CoV-2 (A) or SARS-CoV-2 Beta (C) and Delta (E) variants. Weight loss compared to day 0 is reported as mean  $\pm$  SEM. Two-way ANOVA followed by Tukey's multiple comparison test was used to compare group mean values at each timepoint.

(B, D and F) Maximal weight loss. Percentage of maximal weight loss is shown in single animals of vaccine and control groups challenged with ancestral SARS-CoV-2 (B) or Beta (D) and Delta (F) variants. Lines and bars represent median values and 95% CI, respectively. Peaks of weight loss in each group were compared using one-way ANOVA followed by Tukey's multiple comparison test. Only animals with endpoint at day 10 were included. ns= not significant ( $p > 0.05$ ), \* =  $0.05 < p < 0.01$ , \*\* =  $0.01 < p < 0.001$ , \*\*\* =  $0.001 < p < 0.0001$ , \*\*\*\* =  $p < 0.0001$ .

showed no or only very minor body weight decline, with median peak weight loss below 4% for all groups during the entire 10 days observation period (Figures 2A–2F). COH04S1- and COH04S351-vaccinated animals showed comparable body weight following challenge with the ancestral virus. In contrast, slightly but consistently increased body weight was observed over time for COH04S351-vaccinated animals compared to COH04S1-vaccinated animals following challenge with the variant viruses, suggesting marginally improved efficacy of COH04S351 over COH04S1 to protect against weight loss caused by SARS-CoV-2



**Figure 3. COH04S1 and COH04S351 protect hamsters from lower respiratory tract infection following challenge with SARS-CoV-2 ancestral virus and Beta and Delta variants**

SARS-CoV-2 subgenomic RNA (sgRNA) copies were quantified by qPCR in lung tissue (A–C) and nasal turbinates (D–F) of COH04S1 and COH04S351 vaccine and control groups at days 3 and 10 following challenge with ancestral SARS-CoV-2 (A, D) or SARS-CoV-2 Beta (B, E) and Delta (C, F) variants. Lines indicate median sgRNA copies. One-way ANOVA followed by Tukey’s multiple comparison test was used. \* = 0.05 < p < 0.01, \*\* = 0.01 < p < 0.001, \*\*\* = 0.001 < p < 0.0001.

Beta and Delta variants. These results demonstrate that both COH04S1 and COH04S351 protect Syrian hamsters from severe weight loss following challenge with ancestral SARS-CoV-2 and SARS-CoV-2 Delta and Beta variants.

### COH04S1 and COH04S351 protect hamsters from lower respiratory tract infection following challenge with SARS-CoV-2 ancestral virus and Beta and Delta variants

Viral loads were measured at day 3 and 10 post challenge in lung tissue and nasal turbinates by quantification of SARS-CoV-2 subgenomic RNA (sgRNA) to gauge the amount of replicating virus at lower and upper respiratory tracts. Viral sgRNA loads measured in lung tissue of COH04S1- and COH04S351-vaccinated animals at day 3 and 10 following challenge with the ancestral virus or the variant viruses were consistently lower than those of control animals, indicating efficacy of both vaccines to control lower respiratory infection by ancestral SARS-CoV-2 and SARS-CoV-2 Beta and Delta variants at early and late stages after viral challenge (Figures 3A–3C). Notably, lungs viral loads of COH04S351-vaccinated animals were consistently lower than those of COH04S1-vaccinated animals following challenge with either the ancestral virus or the two variant viruses, indicating improved efficacy of COH04S351 over COH04S1 to protect hamsters from lower respiratory tract infection by ancestral virus and SARS-CoV-2 VOC. In addition, while sgRNA was undetectable in the lungs of only one or two COH04S1-vaccinated hamsters at day 10 following challenge with the ancestral virus or the Delta variant, sgRNA was undetectable in the lungs of a proportion of COH04S351-vaccinated hamsters at day 3 and 10 following challenge with the ancestral virus or either of the two variant viruses. Viral loads measured in nasal turbinates of COH04S1- and COH04S351-vaccinated animals at day 3 or 10 following challenge with the ancestral virus or the variant viruses were either similar or only moderately reduced compared to those of controls, suggesting limited efficacy of COH04S1 and COH04S351 to control upper respiratory tract infection by ancestral SARS-CoV-2 or SARS-CoV-2 VOC (Figures 3D–3F). These results show that COH04S1 and COH04S351 protect hamsters from lower respiratory tract infection following challenge with SARS-CoV-2 ancestral virus and Beta and Delta variants.

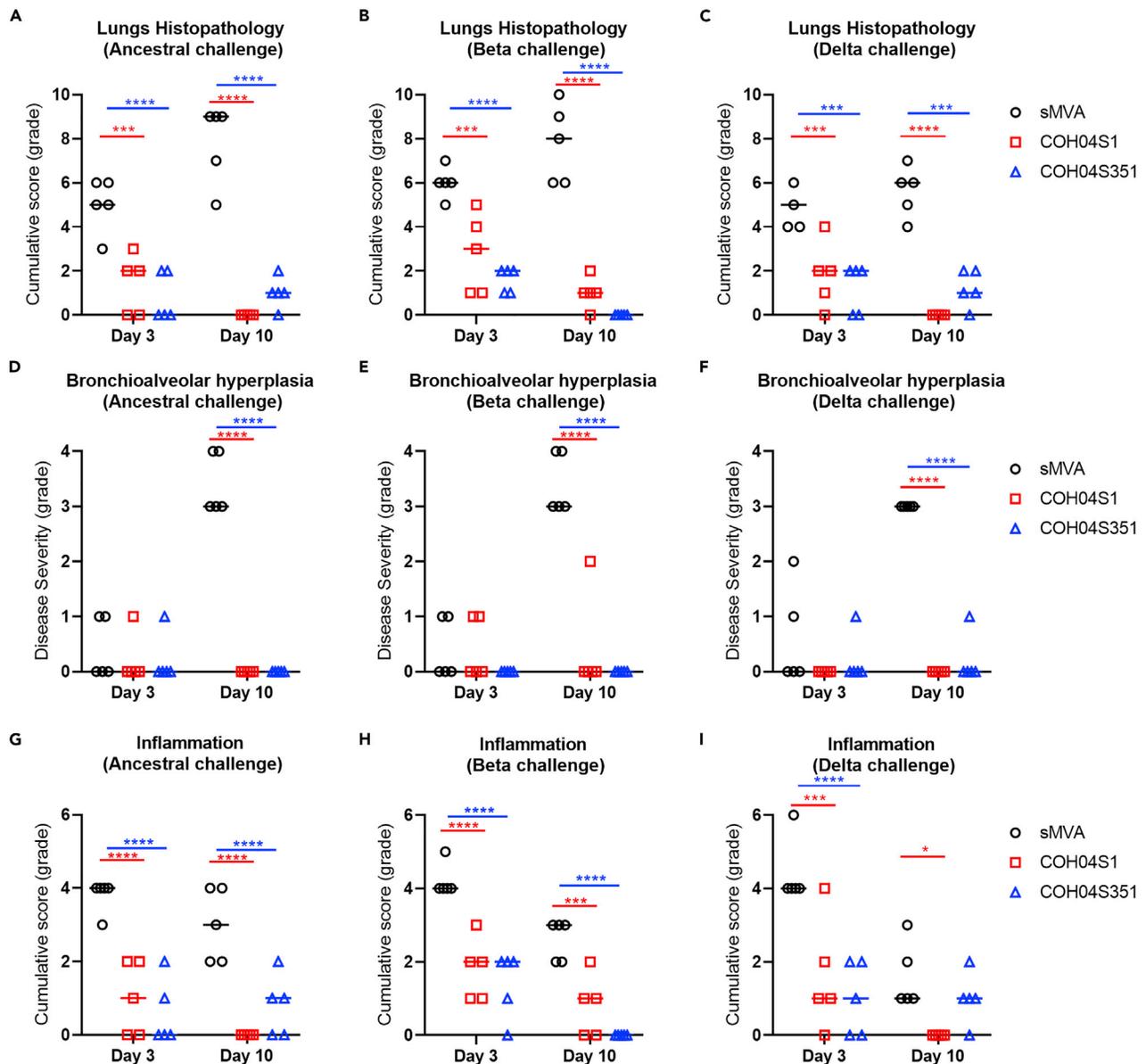
### **COH04S1 and COH04S351 protect hamsters from lung pathology following challenge with SARS-CoV-2 ancestral virus and Beta and Delta variants**

Compared to controls, COH04S1- and COH04S351-vaccinated hamsters showed significantly reduced lung histopathology at day 3 and 10 following challenge with the ancestral virus or the two variant viruses (Figures 3A–3C and S1). Lung pathology in COH04S1- and COH04S351-vaccinated hamsters following challenge with the ancestral virus or the two variant viruses was in most cases either limited or undetectable, with no significant differences between the two vaccine groups. In addition, while all control animals showed high-grade bronchioalveolar hyperplasia (i.e. type II pneumocyte hyperplasia) at day 10 following challenge with the ancestral virus or the two variant viruses, COH04S1- and COH04S351-vaccinated animals showed no bronchioalveolar hyperplasia in almost all cases, regardless of the challenge virus (Figures 4D–4F). Furthermore, lung inflammation assessed at day 3 and 10 following challenge with the ancestral virus or the two variant viruses in COH04S1- and COH04S351-vaccinated hamsters was significantly reduced when compared to control animals, independent of the used challenge strain (Figures 4G–4I and S2). Lung inflammation in COH04S1- and COH04S351-vaccinated hamsters appeared nearly identical, although COH04S1-vaccinated animals did not show lung inflammation at day 10 following challenge with the ancestral virus or the Delta variant, whereas COH04S351-vaccinated animals did not show lung inflammation at day 10 following challenge with Beta variant. Finally, we consistently observed an inverse correlation between vaccine-induced humoral immunity and post-challenge virological and histopathological measurements (Figure S3). When vaccine immunogenicity and protective efficacy were stratified by sex, we observed minor differences between female- and male-vaccinated hamsters (Figure S4). A significant body weight reduction in male versus female animals was observed after five days in control hamsters following challenge with the Beta and Delta VOC, which appears consistent with the known differential susceptibility of female and male hamsters to SARS-CoV-2 (Yuan et al., 2021). In addition, female COH04S351-vaccinated hamsters showed significantly improved body weight than male COH04S351-vaccinated hamsters at late time points following challenge with Delta. Immune responses and protection against lung viral loads and lung pathology appeared generally similar between male and female hamsters, although male COH04S351-vaccinated hamsters showed significant higher Delta-specific NAb responses than female COH04S351-vaccinated hamsters, while lung viral loads in male COH04S1-vaccinated hamsters were significantly lower than those in female COH04S1-vaccinated hamsters, indicating a minor strain-specific vaccine impact depending on sex. These results demonstrate that COH04S1 and COH04S351 afford potent efficacy to protect Syrian hamsters from lung pathology following challenge with ancestral SARS-CoV-2 and SARS-CoV-2 Beta and Delta variants.

### **DISCUSSION**

The ongoing emergence of SARS-CoV-2 VOC jeopardizes the efficacy of approved COVID-19 vaccines that were antigenically designed based on the original Wuhan-Hu-1 reference isolate. It is therefore of critical importance to address whether Wuhan-Hu-1-specific vaccines have the capacity to protect against major SARS-CoV-2 VOC as much as it is important to assess whether COVID-19 vaccine efficacy against SARS-CoV-2 VOC can be improved through variant-adapted vaccine formulations. Several studies indicate that Wuhan-Hu-1-based COVID-19 vaccines have the capacity to protect against SARS-CoV-2 Alpha and Beta variants in different animal models (Yu et al., 2021; Hoffmann et al., 2021; Wuertz et al., 2021), while more recent animal studies have also shown efficacy of approved COVID-19 vaccines against the highly infectious Delta variant (Gagne et al., 2022; Potts et al., 2021). In this study, we have shown that multiantigen sMVA-vectored COVID-19 vaccine COH04S1 based on Wuhan-Hu-1-specific S and N antigens and a Beta variant-adapted vaccine analog (COH04S351) protect against ancestral SARS-CoV-2 and SARS-CoV-2 Beta and Delta variants in Syrian hamsters. These results support the clinical evaluation of COH04S1 or an analogous variant-adapted vaccine candidate based on the sMVA platform. While repeated booster immunizations have been shown to improve the clinical effectiveness of approved COVID-19 vaccines against emerging SARS-CoV-2 VOC (Andrews et al., 2022; Regev-Yochay et al., 2022), COH04S1 or COH04S351 could function as a complementary booster shot to approved COVID-19 vaccines to improve the quality and durability of cross-protective SARS-CoV-2 immunity.

In contrast to most approved COVID-19 vaccines, including mRNA vaccines BNT162b2 and mRNA-1273 and the adenovirus-vectored Ad26.CoV2.S vaccine, COH04S1 and COH04S351 were not designed to utilize S antigens with prefusion-stabilizing 2P modification. Early reports in animal studies for COVID-19 vaccine development using the MVA vector system have indicated that the 2P modification does not significantly improve the stimulation of SARS-CoV-2-specific NAb responses (Liu et al., 2021). The precise reasons for



**Figure 4. COH04S1 and COH04S351 protect hamsters from lung pathology following challenge with SARS-CoV-2 ancestral virus and Beta and Delta variants**

Hematoxylin/eosin-stained lung sections of COH04S1- and COH04S351-vaccinated hamsters and control animals at days 3 and 10 following challenge with ancestral SARS-CoV-2 (A, D, G) or SARS-CoV-2 Beta (B, E, H) and Delta (C, F, I) variants were evaluated by a board-certified pathologist and microscopic findings were graded based on severity on a scale from one to 5 (Table S3).

Panels A–C show the cumulative pathology score of all histopathologic findings in each group.

Panels D–F show grading of bronchioalveolar hyperplasia disease severity in each group.

Panels G–I show the severity of lung inflammatory microscopic findings. Lines indicate median values. Two-way ANOVA followed by Tukey's multiple comparison test was used. \* = 0.05 < p < 0.01, \*\* = 0.01 < p < 0.001, \*\*\* = 0.001 < p < 0.0001, \*\*\*\* = p < 0.0001.

this finding remain unclear, although it may be associated with the robust MVA antigen expression system that potentially results in effective immune exposure of various S antigen forms, including the prefusion intermediate known to promote NAb stimulation. These findings for an MVA-vectored COVID-19 vaccine seems to be different from approved COVID-19 vaccines, such as Ad26.CoV2.S, where the prefusion stabilization has been shown to significantly improve the vaccine immunogenicity and efficacy in animal models (Mercado et al., 2020). Whether 2P-mediated prefusion stabilization of the S antigen could potentially further enhance the efficacy of COH04S1 and COH04S351 remains to be clarified in future studies.

Prior studies in mice and non-human primates and our recently published Phase 1 clinical trial with COH04S1 have shown that this vaccine stimulates potent T cell responses against both the S and N antigens (Chiuppesi et al., 2020, 2022a, 2022b), suggesting that T cells may have partially contributed to the observed vaccine efficacy of COH04S1 and COH04S351 in hamsters. While the precise role of N-specific immune responses for the protection against SARS-CoV-2 remains unclear, the N protein, in addition to the S protein, is well recognized as a dominant target of T cell responses (Grifoni et al., 2020). In addition, recent data suggest that the N protein is a dominant target of non-neutralizing humoral responses that promote potent antibody-dependent cellular cytotoxicity (ADCC) (Fielding et al., 2021; López-Muñoz et al., 2021), and N-specific responses have been shown to provide protection against SARS-CoV-2 in animal models (Trimpert et al., 2021; Dangi et al., 2021, 2022). The inclusion of the N antigen in COH04S1 and COH04S351 may be important to broaden or enhance the stimulation of T cell responses, as well as being important for the stimulation of protective non-neutralizing antibody functions. Furthermore, considering the low degree of variability of the N protein compared to the S protein, immune responses to N may provide broader cross-reactivity against SARS-CoV-2 VOC than S-specific immune responses.

While COH04S1 stimulated robust humoral immunity against the ancestral virus but reduced antibody levels against the Beta and Delta variants, COH04S351 elicited significantly elevated humoral responses against the Beta and Delta variant but reduced antibody responses against the ancestral virus. The precise reasons for the differential strain-specific neutralizing responses induced by the two vaccines remain unclear, although they may reflect differences in the epitope specificity and antigenicity of the S antigens. Despite the strain-specific neutralizing responses elicited by the two vaccines, both vaccines provided potent protection against the ancestral virus and the two variants. This suggests that only relatively low levels of strain-specific NAb responses were required for vaccine protection, which appears consistent with prior observations for COVID-19 vaccines in animal models (McMahan et al., 2021). Whether these strain-specific differences in neutralization by the vaccine-elicited antibodies in hamsters will have clinically relevant consequences is difficult to conclude. NAb titers have been shown to be highly predictive of immune protection against SARS-CoV-2 (Khoury et al., 2021). The observation that COH04S351 stimulated higher neutralizing titers against the two variant viruses than COH04S1 in the hamster model may suggest that the Beta variant-adapted COH04S351 vaccine could provide superior clinical efficacy against emerging SARS-CoV-2 VOC than the original ancestral-specific COH04S1 vaccine.

Both COH04S1 and COH04S351 afforded potent efficacy to protect Syrian hamsters from weight loss, lower respiratory tract infection, and lung pathology following challenge with either the ancestral virus or the two VOC-specific viruses, consistently demonstrating that both vaccines elicited broadly cross-protective SARS-CoV-2 immunity. However, considering the strain-specific neutralizing responses stimulated by the two vaccines, it was surprising that the two vaccines appeared to largely afford similar levels of protection to prevent lung pathology following challenge with the ancestral virus or the two variant viruses, while COH04S351 appeared consistently more effective than COH04S1 to control lower respiratory tract infection by the ancestral virus or the two variant viruses. While the precise reasons for this discrepancy between the strain-specific neutralizing responses and the different levels of protection against lung pathology and lower respiratory tract infection remain unclear, they could be associated with qualitative differences in the vaccine-elicited ancestral and VOC-specific immune responses. The Beta variant-adapted COH04S351 vaccine may be more effective than the ancestral-specific COH04S1 vaccine in eliciting protective NAb responses to conserved epitopes that are shared between the ancestral and VOC-specific viruses, thereby providing improved control of lower respiratory tract infection by the ancestral virus and the variant viruses *in vivo*. Other reasons may also account for the differential efficacy observed with the two vaccines, including differences in non-neutralizing effector functions of S- and N-specific antibody responses or differences in antigen-specific T cell responses elicited by the two vaccines. Regardless of the precise reasons for the improved vaccine impact of COH04S351 compared to COH04S1 on lower respiratory tract infection, these findings may suggest that variant-specific vaccine adaptation can augment the stimulation of cross-protective immune responses against SARS-CoV-2 and its emerging VOC.

### Limitations of the study

A major limitation of the study was that we did not independently address the contribution of the S and N antigens to the protective efficacy observed with COH04S1 and COH04S351, nor did we evaluate T cell responses elicited by the two vaccines in this animal model. Another limitation was that we

used a 3-fold higher challenge dose of the Delta variant compared to the ancestral virus and the Beta variant to compensate for the reduced pathogenicity observed with the Delta strain in a prior dose-titration experiment. Yet, despite using an increased viral challenge dose of the Delta virus, the Delta challenge seemed less aggressive than the challenge with the ancestral virus or the Beta variant. The apparent attenuation of the Delta challenge virus could be seen in the less severe weight loss and lower viral loads measured in control animals challenged with Delta variant compared to control animals challenged with the ancestral virus or the Beta variant. Post-hoc sequencing analysis indicated that the Delta strain used in this study contained a deletion in Orf7a, a known type-I interferon signaling antagonist (Xia et al., 2020), which may have accounted for the reduced pathogenicity and the elevated NAb responses observed with the used Delta strain. However, the mutations within the S and N protein, the two viral proteins targeted by the vaccine-induced immune responses, were identical to those of the native Delta variant. Therefore, we conclude that despite the attenuation, the observed vaccine efficacy against the mutated Delta variant closely resembles the vaccine efficacy against the wild-type Delta variant in this model.

### STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Vaccine vectors
  - Animals, study design and challenge
- METHOD DETAILS
  - Binding antibody detection
  - Neutralization assay
  - Subgenomic RNA quantification
  - Histopathology
- QUANTIFICATION AND STATISTICAL ANALYSIS

### SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2022.104457>.

### ACKNOWLEDGMENTS

We would like to thank Angela Wong and Christina Ulloa for helping with laboratory logistics and secretarial support. We would like to acknowledge the Bioqual team for their technical support. We gratefully acknowledge City of Hope's unique internal funding mechanism of selected scientific discoveries, enabling preclinical and clinical development to speedily advance products toward commercialization, such as COH04S1. The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: hCoV-19/South Africa/KRISP-K005325/2020, NR-54974; hCoV-19/USA/PHC658/2021, NR-55612; Vero E6-hACE2 cells, NR-53726. D.J.D. was partially supported by the following Public Health Service grants: U19 AI128913, CA111412, CA181045, and CA107399. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Graphical abstract and Figure 1B were created using [Biorender.com](https://biorender.com).

### AUTHOR CONTRIBUTIONS

F.W. and F.C. conceptualized and supervised the study, designed the experiments, analyzed the data, and prepared the manuscript. D.J.D. conceptualized the study, designed the experiments, provided supervision, and oversaw final manuscript preparation. M.K., K.F., V.H.N., A.I., J.M., Y.P., and J.N. performed the *in vitro* experiments and contributed to data acquisition and manuscript editing. S.K., H.A., and M.G.L. supervised clinical care of the animals and virological assays. All authors contributed to and approved the final version of this manuscript.

## DECLARATION OF INTERESTS

While unknown whether publication of this report will aid in receiving grants and contracts, it is possible that this publication will be of benefit to City of Hope (COH). COH had no role in the conceptualization, design, data collection, analysis, decision to publish, or preparation of the manuscript. D.J.D. and F.W. are co-inventors on patent application PCT/US2021/016247 which covers the design and construction of the synthetic MVA platform. D.J.D., F.W., and F.C. are co-inventors on PCT/US2021/032821 and 63/244,103 which cover the development of a COVID-19 vaccine. D.J.D. is a consultant for GeoVax Labs Inc. All remaining authors declare no competing interests. GeoVax Labs Inc. has taken a worldwide exclusive license for COH04S1 from COH.

Received: February 16, 2022

Revised: April 12, 2022

Accepted: May 13, 2022

Published: June 17, 2022

## REFERENCES

- Aldoss, I., La Rosa, C., Baden, L.R., Longmate, J., Ariza-Heredia, E.J., Rida, W.N., Lingaraju, C.R., Zhou, Q., Martinez, J., Kaltcheva, T., et al. (2020). Poxvirus vectored cytomegalovirus vaccine to prevent cytomegalovirus viremia in transplant recipients: a phase 2, randomized clinical trial. *Ann. Intern. Med.* 172, 306–316. <https://doi.org/10.7326/m19-2511>.
- Andrews, N., Stowe, J., Kirsebom, F., Toffa, S., Rickeard, T., Gallagher, E., Gower, C., Kall, M., Groves, N., O'connell, A.M., et al. (2022). Covid-19 vaccine effectiveness against the omicron (B.1.1.529) Variant. *N. Engl. J. Med.* 386, 1532–1546. <https://doi.org/10.1056/NEJMoa2119451>.
- Baden, L.R., El Sahly, H.M., Essink, B., Kotloff, K., Frey, S., Novak, R., Diemert, D., Spector, S.A., Roupheal, N., Creech, C.B., et al. (2021). Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N. Engl. J. Med.* 384, 403–416. <https://doi.org/10.1056/NEJMoa2035389>.
- Carreno, J.M., Alshammary, H., Tcheou, J., Singh, G., Raskin, A., Kawabata, H., Sominsky, L., Clark, J., Adelsberg, D.C., Bielak, D., et al. (2021). Activity of convalescent and vaccine serum against SARS-CoV-2 omicron. *Nature* 602, 682–688. <https://doi.org/10.1038/d41586-021-03846-z>.
- Chan, J.F.W., Zhang, A.J., Yuan, S., Poon, V.K.M., Chan, C.C.S., Lee, A.C.Y., Chan, W.M., Fan, Z., Tsoi, H.W., Wen, L., et al. (2020). Simulation of the clinical and pathological manifestations of coronavirus disease 2019 (COVID-19) in a golden syrian hamster model: implications for disease pathogenesis and transmissibility. *Clin. Infect. Dis.* 71, 2428–2446. <https://doi.org/10.1093/cid/ciaa325>.
- Chiuppesi, F., Nguyen, J., Park, S., Contreras, H., Kha, M., Meng, Z., Kaltcheva, T., Iniguez, A., Martinez, J., La Rosa, C., et al. (2018). Multiantigenic modified vaccinia virus ankara vaccine vectors to elicit potent humoral and cellular immune responses against human cytomegalovirus in mice. *J. Virol.* 92, e01012-18. <https://doi.org/10.1128/jvi.01012-18>.
- Chiuppesi, F., Nguyen, V.H., Park, Y., Contreras, H., Karpinski, V., Faircloth, K., Nguyen, J., Kha, M., Johnson, D., Martinez, J., et al. (2021). Synthetic multiantigen MVA vaccine COH04S1 protects against SARS-CoV-2 in Syrian hamsters and non-human primates. Preprint at bioRxiv. <https://doi.org/10.1101/2021.09.15.460487>.
- Chiuppesi, F., Nguyen, V.H., Park, Y., Contreras, H., Karpinski, V., Faircloth, K., Nguyen, J., Kha, M., Johnson, D., Martinez, J., et al. (2022a). Synthetic multiantigen MVA vaccine COH04S1 protects against SARS-CoV-2 in Syrian hamsters and non-human primates. *NPJ Vaccines* 7, 7. <https://doi.org/10.1038/s41541-022-00436-6>.
- Chiuppesi, F., Salazar, M.D., Contreras, H., Nguyen, V.H., Martinez, J., Park, Y., Nguyen, J., Kha, M., Iniguez, A., Zhou, Q., et al. (2020). Development of a multi-antigenic SARS-CoV-2 vaccine candidate using a synthetic poxvirus platform. *Nat. Commun.* 11, 6121. <https://doi.org/10.1038/s41467-020-19819-1>.
- Chiuppesi, F., Zaia, J.A., Frankel, P.H., Stan, R., Drake, J., Williams, B., Acosta, A.M., Francis, K., Taplitz, R.A., Dickter, J.K., et al. (2022b). Safety and immunogenicity of a synthetic multiantigen modified vaccinia virus Ankara-based COVID-19 vaccine (COH04S1): an open-label and randomized, phase 1 trial. *Lancet Microbe* 3, e252–e264. [https://doi.org/10.1016/s2666-5247\(22\)00027-1](https://doi.org/10.1016/s2666-5247(22)00027-1).
- Collie, S., Champion, J., Moultrie, H., Bekker, L.G., and Gray, G. (2021). Effectiveness of BNT162b2 vaccine against omicron variant in South Africa. *N. Engl. J. Med.* 386, 494–496.
- Dangi, T., Class, J., Palacio, N., Richner, J.M., and Penaloza Macmaster, P. (2021). Combining spike and nucleocapsid-based vaccines improves distal control of SARS-CoV-2. *Cell Rep.* 36, 109664.
- Dangi, T., Sanchez, S., Park, M., Class, J., Richner, M., Richner, J.M., and Penaloza-Macmaster, P. (2022). Nucleocapsid-specific humoral responses improve the control of SARS-CoV-2. Preprint at bioRxiv. <https://doi.org/10.1101/2022.03.09.483635>.
- Dejnirattisai, W., Zhou, D., Supasa, P., Liu, C., Mentzer, A.J., Ginn, H.M., Zhao, Y., Duyvesteyn, H.M.E., Tuekprakhon, A., Nutalai, R., et al. (2021). Antibody evasion by the P.1 strain of SARS-CoV-2. *Cell* 184, 2939–2954.e9. <https://doi.org/10.1016/j.cell.2021.03.055>.
- Faria, N.R., Mellan, T.A., Whittaker, C., Claro, I.M., Candido, D.D.S., Mishra, S., Crispim, M.A.E., Sales, F.C.S., Hawryluk, I., Mccrone, J.T., et al. (2021). Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science* 372, 815–821.
- Fielding, C.A., Sabberwal, P., Williamson, J.C., Greenwood, E.J.D., Crozier, T.W.M., Zelek, W., Seow, J., Graham, C., Huettner, I., Edgeworth, J.D., et al. (2021). ADNKA overcomes SARS-CoV2-mediated NK cell inhibition through non-spike antibodies. Preprint at bioRxiv. <https://doi.org/10.1101/2021.04.06.438630>.
- Gagne, M., Corbett, K.S., Flynn, B.J., Foulds, K.E., Wagner, D.A., Andrew, S.F., Todd, J.P.M., Honeycutt, C.C., McCormick, L., Nurmukhambetova, S.T., et al. (2022). Protection from SARS-CoV-2 Delta one year after mRNA-1273 vaccination in rhesus macaques coincides with anamnestic antibody response in the lung. *Cell* 185, 113–130.e15. <https://doi.org/10.1016/j.cell.2021.12.002>.
- Garcia-Beltran, W.F., Lam, E.C., St Denis, K., Nitido, A.D., Garcia, Z.H., Hauser, B.M., Feldman, J., Pavlovic, M.N., Gregory, D.J., Poznansky, M.C., et al. (2021). Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. *Cell* 184, 2523. <https://doi.org/10.1016/j.cell.2021.04.006>.
- Gilbert, S.C. (2013). Clinical development of modified vaccinia virus ankara vaccines. *Vaccine* 31, 4241–4246. <https://doi.org/10.1016/j.vaccine.2013.03.020>.
- Grifoni, A., Weiskopf, D., Ramirez, S.I., Mateus, J., Dan, J.M., Moderbacher, C.R., Rawlings, S.A., Sutherland, A., Premkumar, L., Jardi, R.S., et al. (2020). Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* 181, 1489–1501. <https://doi.org/10.1016/j.cell.2020.05.015>.
- Hoffmann, D., Corleis, B., Rauch, S., Roth, N., Muhe, J., Halwe, N.J., Ulrich, L., Fricke, C., Schon, J., Kraft, A., et al. (2021). CVnCoV and CV2CoV protect human ACE2 transgenic mice from ancestral B.1.1.7 and emerging B.1.351 SARS-CoV-2. *Nat. Commun.* 12, 4048. <https://doi.org/10.1038/s41467-021-24339-7>.
- Imai, M., Iwatsuki-Horimoto, K., Hatta, M., Loeber, S., Halfmann, P.J., Nakajima, N., Watanabe, T., Ujie, M., Takahashi, K., Ito, M., et al.

- (2020). Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. *Proc. Natl. Acad. Sci. U. S. A.* 117, 16587–16595. <https://doi.org/10.1073/pnas.2009799117>.
- Khoury, D.S., Cromer, D., Reynaldi, A., Schlub, T.E., Wheatley, A.K., Juno, J.A., Subbarao, K., Kent, S.J., Triccas, J.A., and Davenport, M.P. (2021). Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat. Med.* 27, 1205–1211. <https://doi.org/10.1038/s41591-021-01377-8>.
- La Rosa, C., Longmate, J., Martinez, J., Zhou, Q., Kaltcheva, T.I., Tsai, W., Drake, J., Carroll, M., Wussow, F., Chiuppesi, F., et al. (2017). MVA vaccine encoding CMV antigens safely induces durable expansion of CMV-specific T cells in healthy adults. *Blood* 129, 114–125. <https://doi.org/10.1182/blood-2016-07-729756>.
- Liu, R., Americo, J.L., Cotter, C.A., Earl, P.L., Erez, N., Peng, C., and Moss, B. (2021). One or two injections of MVA-vectored vaccine shields hACE2 transgenic mice from SARS-CoV-2 upper and lower respiratory tract infection. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2026785118. <https://doi.org/10.1073/pnas.2026785118>.
- Lopez Bernal, J., Andrews, N., Gower, C., Gallagher, E., Simmons, R., Thelwall, S., Stowe, J., Tessier, E., Groves, N., Dabrera, G., et al. (2021). Effectiveness of Covid-19 vaccines against the B.1.617.2 (delta) variant. *N. Engl. J. Med.* 385, 585–594. <https://doi.org/10.1056/NEJMoa2108891>.
- López-Muñoz, A.D., Lopez-Munoz, A., Kosik, I., Yewdell, J.W., and Holly, J. (2021). Cell surface SARS-CoV-2 nucleocapsid protein modulates innate and adaptive immunity. Preprint at bioRxiv. <https://doi.org/10.1101/2021.12.10.472169>.
- Madhi, S.A., Baillie, V., Cutland, C.L., Voysey, M., Koen, A.L., Fairlie, L., Padayachee, S.D., Dheda, K., Barnabas, S.L., Bhorat, Q.E., et al. (2021). Efficacy of the ChAdOx1 nCoV-19 vaccine against the B.1.351 variant. *N. Engl. J. Med.* 384, 1885–1898. <https://doi.org/10.1056/nejm-moa2102214>.
- Mcmahan, K., Yu, J., Mercado, N.B., Loos, C., Tostanoski, L.H., Chandrashekar, A., Liu, J., Peter, L., Atyeo, C., Zhu, A., et al. (2021). Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nature* 590, 630–634. <https://doi.org/10.1038/s41586-020-03041-6>.
- Mercado, N.B., Zahn, R., Wegmann, F., Loos, C., Chandrashekar, A., Yu, J., Liu, J., Peter, L., Mcmahan, K., Tostanoski, L.H., et al. (2020). Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. *Nature* 586, 583–588. <https://doi.org/10.1038/s41586-020-2607-z>.
- Moore, J.P., and Offit, P.A. (2021). SARS-CoV-2 vaccines and the growing threat of viral variants. *JAMA* 325, 821–822. <https://doi.org/10.1001/jama.2021.1114>.
- Planas, D., Veyer, D., Baidaliuk, A., Staropoli, I., Guivel-Benhassine, F., Rajah, M.M., Planchais, C., Porrot, F., Robillard, N., Puech, J., et al. (2021). Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. *Nature* 596, 276–280. <https://doi.org/10.1038/s41586-021-03777-9>.
- Polack, F.P., Thomas, S.J., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Perez, J.L., Perez Marc, G., Moreira, E.D., Zerbini, C., et al. (2020). Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N. Engl. J. Med.* 383, 2603–2615. <https://doi.org/10.1056/NEJMoa2034577>.
- Potts, K.G., Noyce, R.S., Gafuik, C., John, C.M., Todesco, H.M., De Heuvel, E., Favis, N., Kelly, M.M., Evans, D.H., and Mahoney, D.J. (2021). Booster vaccines protect hamsters with waning immunity from Delta VOC infection, disease, and transmission. Preprint at bioRxiv. <https://doi.org/10.1101/2021.12.27.474282>.
- Regev-Yochay, G., Gonen, T., Gilboa, M., Mandelboim, M., Indenbaum, V., Amit, S., Meltzer, L., Asraf, K., Cohen, C., Fluss, R., et al. (2022). Efficacy of a fourth dose of Covid-19 mRNA vaccine against omicron. *N. Engl. J. Med.* 386, 1377–1380.
- Sadoff, J., Gray, G., Vandebosch, A., Cardenas, V., Shukarev, G., Grinsztejn, B., Goepfert, P.A., Truysers, C., Fennema, H., Spiessens, B., et al. (2021). Safety and efficacy of single-dose Ad26.COV2.S vaccine against Covid-19. *N. Engl. J. Med.* 384, 2187–2201. <https://doi.org/10.1056/nejm-moa2101544>.
- Sanchez-Felipe, L., Vercruyse, T., Sharma, S., Ma, J., Lemmens, V., Van Looveren, D., Arkalagud Javarappa, M.P., Boudewijns, R., Malengier-Devlies, B., Liesenborghs, L., et al. (2021). A single-dose live-attenuated YF17D-vectored SARS-CoV-2 vaccine candidate. *Nature* 590, 320–325. <https://doi.org/10.1038/s41586-020-3035-9>.
- Sheikh, A., Robertson, C., and Taylor, B. (2021). BNT162b2 and ChAdOx1 nCoV-19 vaccine effectiveness against death from the delta variant. *N. Engl. J. Med.* 385, 2195–2197. <https://doi.org/10.1056/nejm-c2113864>.
- Shinde, V., Bhikha, S., Hoosain, Z., Archary, M., Bhorat, Q., Fairlie, L., Laloo, U., Masilela, M.S.L., Moodley, D., Hanley, S., et al. (2021). Efficacy of NVX-CoV2373 Covid-19 vaccine against the B.1.351 variant. *N. Engl. J. Med.* 384, 1899–1909. <https://doi.org/10.1056/nejm-moa2103055>.
- Sia, S.F., Yan, L.M., Chin, A.W.H., Fung, K., Choy, K.T., Wong, A.Y.L., Kaewpreedee, P., Perera, R.A.P.M., Poon, L.L.M., Nicholls, J.M., et al. (2020). Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. *Nature* 583, 834–838. <https://doi.org/10.1038/s41586-020-2342-5>.
- Sutter, G., and Moss, B. (1992). Nonreplicating vaccinia vector efficiently expresses recombinant genes. *Proc. Natl. Acad. Sci. U. S. A.* 89, 10847–10851. <https://doi.org/10.1073/pnas.89.22.10847>.
- Tang, P., Hasan, M.R., Chemaitelly, H., Yassine, H.M., Benslimane, F.M., Al Khatib, H.A., Almkudad, S., Coyle, P., Ayoub, H.H., Al Kanaani, Z., et al. (2021). BNT162b2 and mRNA-1273 COVID-19 vaccine effectiveness against the SARS-CoV-2 Delta variant in Qatar. *Nat. Med.* 27, 2136–2143. <https://doi.org/10.1038/s41591-021-01583-4>.
- Tegally, H., Wilkinson, E., Giovanetti, M., Iranzadeh, A., Fonseca, V., Giandhari, J., Doolabh, D., Pillay, S., San, E.J., Msomi, N., et al. (2021). Detection of a SARS-CoV-2 variant of concern in South Africa. *Nature* 592, 438–443. <https://doi.org/10.1038/s41586-021-03402-9>.
- Tostanoski, L.H., Wegmann, F., Martinot, A.J., Loos, C., Mcmahan, K., Mercado, N.B., Yu, J., Chan, C.N., Bondoc, S., Starke, C.E., et al. (2020). Ad26 vaccine protects against SARS-CoV-2 severe clinical disease in hamsters. *Nat. Med.* 26, 1694–1700. <https://doi.org/10.1038/s41591-020-1070-6>.
- Trimpert, J., Herwig, S., Stein, J., Vladimirova, D., Adler, J.M., Abdelgawad, A., Firsching, T.C., Thoma, T., Sehoul, J., Osterrieder, K., et al. (2021). Deciphering the role of humoral and cellular immune responses in different COVID-19 vaccines—a comparison of vaccine candidate genes in roborovski dwarf hamsters. *Viruses* 13, 2290. <https://doi.org/10.3390/v13112290>.
- Volz, A., and Sutter, G. (2017). Modified vaccinia virus ankara: history, value in basic research, and current perspectives for vaccine development. *Adv. Virus Res.* 97, 187–243. <https://doi.org/10.1016/bs.avirv.2016.07.001>.
- Voysey, M., Clemens, S.A.C., Madhi, S.A., Weckx, L.Y., Folegatti, P.M., Aley, P.K., Angus, B., Baillie, V.L., Barnabas, S.L., Bhorat, Q.E., et al. (2021). Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* 397, 99–111. [https://doi.org/10.1016/S0140-6736\(20\)32661-1](https://doi.org/10.1016/S0140-6736(20)32661-1).
- Wuertz, K.M., Barkei, E.K., Chen, W.H., Martinez, E.J., Lakhali-Naouar, I., Jagodzinski, L.L., Paquin-Proulx, D., Gromowski, G.D., Swafford, I., Ganesh, A., et al. (2021). A SARS-CoV-2 spike ferritin nanoparticle vaccine protects hamsters against Alpha and Beta virus variant challenge. *NPJ Vaccines* 6, 129. <https://doi.org/10.1038/s41541-021-00392-7>.
- Wussow, F., Chiuppesi, F., Martinez, J., Campo, J., Johnson, E., Flechsig, C., Newell, M., Tran, E., Ortiz, J., La Rosa, C., et al. (2014). Human cytomegalovirus vaccine based on the envelope gH/gL pentamer complex. *PLoS Pathog.* 10, e1004524. <https://doi.org/10.1371/journal.ppat.1004524>.
- Wussow, F., Yue, Y., Martinez, J., Deere, J.D., Longmate, J., Herrmann, A., Barry, P.A., and Diamond, D.J. (2013). A vaccine based on the rhesus cytomegalovirus UL128 complex induces broadly neutralizing antibodies in rhesus macaques. *J. Virol.* 87, 1322–1332. <https://doi.org/10.1128/jvi.01669-12>.
- Xia, H., Cao, Z., Xie, X., Zhang, X., Chen, J.Y.C., Wang, H., Menachery, V.D., Rajsbaum, R., and Shi, P.Y. (2020). Evasion of type I interferon by SARS-CoV-2. *Cell Rep.* 33, 108234. <https://doi.org/10.1016/j.celrep.2020.108234>.
- Yahalom-Ronen, Y., Tamir, H., Melamed, S., Politi, B., Shifman, O., Achdout, H., Vitner, E.B., Israeli, O., Milrot, E., Stein, D., et al. (2020). A single dose of recombinant VSV-G-spike vaccine provides protection against SARS-CoV-2 challenge. *Nat. Commun.* 11, 6402. <https://doi.org/10.1038/s41467-020-20228-7>.
- Yu, J., Tostanoski, L.H., Mercado, N.B., Mcmahan, K., Liu, J., Jacob-Dolan, C., Chandrashekar, A., Atyeo, C., Martinez, D.R., Anioke, T., et al. (2021). Protective efficacy of Ad26.COV2.S against SARS-CoV-2

B.1.351 in macaques. *Nature* 596, 423–427. <https://doi.org/10.1038/s41586-021-03732-8>.

Yuan, L., Zhu, H., Zhou, M., Ma, J., Chen, R., Chen, Y., Chen, L., Wu, K., Cai, M., Hong, J., et al. (2021). Gender associates with both susceptibility to infection and pathogenesis of SARS-CoV-2 in Syrian hamster. *Signal Transduct. Targeted Ther.* 6, 136. <https://doi.org/10.1038/s41392-021-00552-0>.

Yuan, Y., Kos, F.J., He, T.F., Yin, H.H., Li, M., Hardwick, N., Zurcher, K., Schmolze, D., Lee, P., Pillai, R.K., et al. (2017). Complete regression of

cutaneous metastases with systemic immune response in a patient with triple negative breast cancer receiving p53MVA vaccine with pembrolizumab. *Oncolmmunology* 6, e1363138. <https://doi.org/10.1080/2162402x.2017.1363138>.

Zhou, D., Dejnirattisai, W., Supasa, P., Liu, C., Mentzer, A.J., Ginn, H.M., Zhao, Y., Duyvesteyn, H.M.E., Tuekprakhon, A., Nutalai, R., et al. (2021). Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. *Cell* 184, 2348–2361.e6. <https://doi.org/10.1016/j.cell.2021.02.037>.

Zhou, P., Yang, X.L., Wang, X.G., Hu, B., Zhang, L., Zhang, W., Si, H.R., Zhu, Y., Li, B., Huang, C.L., et al. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579, 270–273. <https://doi.org/10.1038/s41586-020-2012-7>.

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., et al. (2020). A novel coronavirus from patients with pneumonia in China, 2019. *N. Engl. J. Med.* 382, 727–733. <https://doi.org/10.1056/NEJMoa2001017>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
anti-Hamster IgG(H+L) HRP	Southern Biotech	Cat# 6061-05, RRID:AB_2796135
anti-Hamster IgG1 HRP	Southern Biotech	Cat# 1940-05, RRID:AB_2795558
anti-Hamster IgG2/3 HRP	Southern Biotech	Cat# 1935-05, RRID:AB_2795553
<b>Bacterial and virus strains</b>		
SARS-CoV-2 USA-WA1/2020	BEI Resources	NR-53780
isolate hCoV-19/South Africa/KRISP-K005325/2020	BEI Resources	NR-54974
isolate hCoV-19/USA/PHC658/2021	BEI Resources	NR-55612
COH04S1 (sMVA encoding Wuhan-Wu-1 S and N)	This paper	N/A
COH04S351 (sMVA encoding B.1.351 S and N)	This paper	N/A
sMVA	This paper	N/A
<b>Biological samples</b>		
Golden Syrian Hamsters lung tissue	This paper	N/A
Golden Syrian Hamsters turbinates	This paper	N/A
<b>Chemicals, peptides, and recombinant proteins</b>		
S1+S2	SinoBiological	40589-V08B1
N	SinoBiological	40588-V08B
RBD	SinoBiological	40592-V08H
Beta-specific S	Acro Biosystems	SPN-C52Hk
Delta-specific S	Acro Biosystems	SPN-C52He
Omicron-specific S	Acro Biosystems	SPN-C52Hz
<b>Experimental models: Cell lines</b>		
Vero E6-hACE2 cells	BEI Resources	NR-53726
Calu-3 cells	ATCC	HTB-55
Vero E6 TMPRSS2 cells	Dr. A. Creanga [VRC]	N/A
Vero-E6	ATCC	CRL-1586
<b>Experimental models: Organisms/strains</b>		
Male and female golden Syrian hamsters	Envigo	HsdHan:AURA
<b>Oligonucleotides</b>		
N sgRNA forward: 5'-CGA TCT CTT GTA GAT CTG TTC TC-3'	This paper	N/A
N sgRNA reverse: 5'-GGT GAA CCA AGA CGC AGT AT-3'	This paper	N/A
N sgRNA probe: 5'-FAM- TAA CCA GAA TGG AGA ACG CAG TGG G-BHQ-3'	This paper	N/A
<b>Software and algorithms</b>		
Prism 8 v8.3.0	Graph Pad	<a href="https://www.graphpad.com/">https://www.graphpad.com/</a>
SoftMax Pro 7	Molecular devices	<a href="https://www.moleculardevices.com/products/microplate-readers/acquisition-and-analysis-software/softmax-pro-software#ref">https://www.moleculardevices.com/products/microplate-readers/acquisition-and-analysis-software/softmax-pro-software#ref</a>
7500 Real-Time PCR System Software	Applied Biosystems	<a href="https://www.thermofisher.com/us/en/home/technical-resources/software-downloads/applied-biosystems-7500-fast-real-time-pcr-system.html">https://www.thermofisher.com/us/en/home/technical-resources/software-downloads/applied-biosystems-7500-fast-real-time-pcr-system.html</a>

## RESOURCE AVAILABILITY

### Lead contact

Further information and requests for resources and reagents should be directed to the lead contact, Don J. Diamond ([ddiamond@coh.org](mailto:ddiamond@coh.org)).

### Materials availability

All requests for resources and reagents should be directed to the [lead contact](#) author. All reagents will be made available on request after completion of a Materials Transfer Agreement.

### Data and code availability

All data supporting the findings of this study are available within the paper and are available from the corresponding author upon request. This paper does not include original code. Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

## EXPERIMENTAL MODEL AND SUBJECT DETAILS

### Vaccine vectors

COH04S1 is a double-plaque purified virus isolate derived from the previously described sMVA-N/S vector (GenBank: MW036243) with N and S antigen sequences inserted into the MVA deletion sites 2 and 3, respectively ([Chiuppesi et al., 2020, 2022a](#)). COH04S1 co-expresses full-length, unmodified S and N antigen sequences based on the Wuhan-Hu-1 reference strain (GenBank: NC\_045512). COH04S351 is a double-plaque purified virus isolate with analogous vaccine construction compared to the original COH04S1 vector and co-expresses modified S and N antigen sequences based on the B.1.351 Beta variant ([Table S1](#)) ([Zhou et al., 2021](#); [Garcia-Beltran et al., 2021](#)). COH04S1 and COH04S351 were generated using the sMVA platform as previously described ([Chiuppesi et al., 2020](#)). Virus stocks of the vaccine vectors and sMVA control vector were produced using chicken embryo fibroblasts (CEF) and prepared by 36% sucrose cushion ultracentrifugation and virus resuspension in 1 mM Tris-HCl (pH 9). Virus stocks were stored at  $-80^{\circ}\text{C}$  and titrated on CEF by plaque immunostaining as described ([Chiuppesi et al., 2020](#)). Viral stocks were validated for antigen insertion and expression by PCR, sequencing, and Immunoblot.

### Animals, study design and challenge

In life portion of the hamster studies were carried out at Bioqual Inc. (Rockville, MD). Ninety 6–8 weeks old Syrian hamsters were randomly assigned to the groups, with 5 females and 5 males in each challenge group. Hamsters were intramuscularly vaccinated four weeks apart with  $1 \times 10^8$  PFU of COH04S1, COH04S351 or sMVA virus stocks diluted in phosphate-buffered saline (PBS). Two weeks after the second vaccine dose animals were challenged intranasally (50  $\mu\text{L}$ /nare) with  $8.0 \times 10^6$  Median Tissue Culture Infectious Dose, [TCID50] of SARS-CoV-2 USA-WA1/2020 (produced at Bioqual using Calu-3),  $8.0 \times 10^6$  TCID50 of SARS-CoV-2 Beta variant (isolate hCoV-19/South Africa/KRISP-K005325/2020, lot no. 03062021-750, expanded from NR-54974 lot no. 70041987, BEI Resources), or  $1.3 \times 10^7$  TCID50 of SARS-CoV-2 Delta variant (isolate hCoV-19/USA/PHC658/2021, BEI Resources, NR-55612 lot no. 70045240). The USA-WA1/2020 and Beta variant stocks were produced by infecting Calu-3 cells (HTB-55, ATCC) and titrated using TCID50 assay on Vero E6 TMPRSS2 cells (Supplied by Dr. Adrian Creanga [VRC]). Sequence identity verification of the virus stocks by next generation sequencing confirmed a Orf7a deletion (aa-44-100) in the Delta isolate. Body weight was recorded daily for 10 days. Hamsters were humanely euthanized for lung and turbinate samples collection at day 3 ( $n = 5$ /group) and day 10 ( $n = 5$ /group) post-challenge. All animal studies were conducted in compliance with local, state, and federal regulations and were approved by Bioqual and City of Hope Institutional Animal Care and Use Committees (IACUC).

## METHOD DETAILS

### Binding antibody detection

SARS-CoV-2-specific binding antibodies in hamster serum samples were detected by indirect ELISA using purified ancestral-specific S, RBD, and N proteins (Sino Biological 40589-V08B1, 40592-V08H, 40588-V08B), or Beta-, Delta-, and Omicron-specific S proteins (Acro Biosystems SPN-C52Hk, SPN-C52He, and SPN-C52Hz). 96-well plates were coated with 100  $\mu\text{L}$ /well of protein at a concentration of 1  $\mu\text{g}/\text{mL}$  in PBS and incubated overnight at  $4^{\circ}\text{C}$ . Plates were washed 5X with wash buffer (0.05% Tween-20/PBS), then blocked with 250  $\mu\text{L}$ /well of blocking buffer (0.5% casein/154 mM NaCl/10 mM Tris-HCl [pH 7.6]) for 2 hours

at room temperature. After washing, 3-fold diluted heat-inactivated serum in blocking buffer was added to the plates and incubated 2 hours at room temperature. After washing, anti-Hamster IgG HRP secondary antibodies measuring total IgG(H+L), IgG<sub>1</sub>, or IgG<sub>2</sub>/IgG<sub>3</sub> (Southern Biotech 6061-05, 1940-05, 1935-05) were diluted 1:1,000 in blocking buffer and added to the plates. After 1 hour incubation, plates were washed and developed with 1 Step TMB-Ultra (Thermo Fisher 34029). The reaction was stopped with 1M H<sub>2</sub>SO<sub>4</sub> and plates were immediately read on FilterMax F3 (Molecular Devices). Endpoint titers were calculated as the highest dilution to have an absorbance >0.100nm.

### Neutralization assay

NAb were measured by plaque reduction neutralization assay (PRNT) assay using ancestral SARS-CoV-2 (USA-WA1/2020 strain), or Beta (isolate hCoV-19/South Africa/KRISP-K005325/2020-p4) and Delta (isolate hCoV-19/USA/PHC658/2021) variants. Ancestral SARS-CoV-2 stock was generated using Vero-E6 cells infected with seed stock virus obtained from Kenneth Plante at UTMB (lot # TVP 23156). Vero E6 cells (ATCC, CRL-1586) were seeded in 24-well plates at 175,000 cells/well in DMEM/10% FBS/Gentamicin. Serial 3-fold serum dilutions were incubated in 96-well plates with 30 PFU of SARS-CoV-2 ancestral, beta or delta strains for 1 hour at 37°C. The serum/virus mixture was transferred to Vero-E6 cells and incubated for 1 hour at 37°C. After that, 1 mL of 0.5% methylcellulose media was added to each well and plates were incubated at 37°C for three days. Plates were washed, and cells were fixed with methanol. Crystal violet staining was performed, and plaques were recorded. IC50 titers were calculated as the serum dilution that gave a 50% reduction in viral plaques in comparison to control wells.

### Subgenomic RNA quantification

SARS-CoV-2 sgRNA copies were assessed through quantification of N gene mRNA by qRT-PCR as previously described (Chiuppesi et al., 2021). Briefly, SARS-CoV-2 RNA was extracted from tissues using TRIzol, precipitated and resuspended in RNase-free water. For quantification, the standard curve of a plasmid containing a cDNA copy of the N gene mRNA target was used. Applied Biosystems 7500 Real-Time PCR System was used for amplification with the following program: 48°C for 30 minutes, 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds, and 1 minute at 55°C. The number of copies of RNA per mL was calculated by extrapolation from the standard curve and multiplying by the reciprocal of 0.2 mL extraction volume. Primer/probe sequences: 5'-CGA TCT CTT GTA GAT CTG TTC TC-3'; 5'-GGT GAA CCA AGA CGC AGT AT-3'; 5'-FAM- TAA CCA GAA TGG AGA ACG CAG TGG G-BHQ-3'.

### Histopathology

Histopathological evaluation of hamster lung sections was performed by Experimental Pathology Laboratories, Inc. (Sterling, VA). At necropsy organs were collected and placed in 10% neutral buffered formalin for histopathologic analysis. Tissues were processed through to paraffin blocks, sectioned once at approximately 5 microns thickness, and stained with hematoxylin/eosin. Board certified pathologists were blinded to the vaccine groups and mock controls were used as a comparator.

### QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses were performed using Prism 8 (GraphPad, v8.3.0). One-way ANOVA and two-way ANOVA with Tukey's multiple comparison test and Spearman correlation analysis were used for statistical evaluation. The significance level for each test is indicated in the figure legends. Prism 8 was used to derive correlation matrices.