A single-dose of the deactivated rabies virus vectored COVID-19 vaccine, CORAVAX, is highly efficacious and alleviates lung inflammation in the hamster model.

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Abstract:

Without sufficient herd immunity through either vaccination or natural infection, the Coronavirus Disease 2019 pandemic is unlikely to be controlled. Waning immunity with the currently approved vaccines suggests the need to evaluate vaccines causing the induction of long-term responses. Here we report the immunogenicity and efficacy of our adjuvanted single-dose Rabies vectored SARS CoV-2 S1 vaccine, CORAVAX, in hamsters. CORAVAX induces high SARS CoV-2 S1 specific and virus-neutralizing antibodies (VNA) that prevent weight loss, viral loads, disease, lung inflammation, and the cytokine storm in hamsters. We also observed high Rabies VNA titers. In summary, CORAVAX is a promising dual antigen vaccine candidate for clinical evaluation against SARS CoV-2 and Rabies virus.

Introduction

Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) originated in Wuhan, China, in December 2019 and accounts for over 264 million infections and 5.22 million deaths at the time of this report¹. SARS Cov-2 infection in humans causes coronavirus-19 disease (COVID-19), resulting in mild or severe clinical manifestations that mainly affect the respiratory system.

SARS-CoV-2 is an enveloped virus containing the spike (S) glycoprotein on its surface required for viral entry into cells². The S protein is the main target for current vaccine development because antibodies directed against this protein can neutralize the virus. Fast-tracked research efforts by academic institutions and Biotech companies led to the development and approval of several SARS CoV-2 S protein based vaccines that induce neutralizing antibodies². Recent studies suggest that neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection³. Whereas the previous SARS CoV was controlled using social isolation and screening, control of the spread of SARS CoV-2 has proven very challenging. Several countries began administering vaccines by December 2020 and into early January 2021. Despite these efforts, controlling the pandemic has been difficult due to several factors: 1) high transmissibility, 2) transmission by asymptomatic individuals, 3) unequal distribution of vaccines worldwide, 4) emergence of variants of higher transmissibility, 5) vaccine hesitancy.

The first mRNA-based COVID-19 vaccines were approved around December 2020 (Pfizer: BNT162b1, mRNA-1273: Moderna). These vaccines use a lipid-based delivery platform and an almost identical antigen, a pre-fusion stabilized SARS-CoV-2 spike protein-encoding mRNA. The vaccines differ in their proprietary lipid delivery platform and the amount of antigen used (30 µg

for Pfizer and 100 µg for Moderna). Both vaccines provided exceptionally high protection, preventing about 95% of symptomatic COVID-19 disease and 100% severe COVID-19 disease^{4,5}. However, the induced immune responses are not long-lasting, and a booster is required after six months.

Single-dose vaccines have a significant advantage as they allow for greater vaccination coverage and better patient compliance. The Johnson & Johnson (J&J), Ad26.COV2.S vaccine is the only single-dose vaccine that has been evaluated and received FDA approval in the United States. The J&J vaccine comprises a recombinant, replication-incompetent human adenovirus type 26 (Ad26) vector encoding a full-length, membrane-bound prefusion-stabilized S protein. Although phase 3 clinical trial data reported a vaccine efficacy of 66.9% against moderate to severe-critical COVID-19, the vaccine immunity wanes after two months, requiring a booster dose^{6,7} and limiting the utility of this vaccine candidate. Interestingly, a preclinical study with a single intranasal dose of a live-attenuated parainfluenza virus-vectored SARS-CoV-2 vaccine was protective in hamsters⁸. However, long-term responses are yet to be determined for this vector.

The high transmissibility of SARS CoV-2 in the absence of sufficient vaccine coverage has led to the emergence of several variants. The World Health Organization (WHO) has recently classified two variants of concern, Delta and Omicron. Follow-up studies conducted during the emergence of the Delta variant have shown waning immunity for all approved vaccines, including those from Pfizer and Moderna, with the most extreme decline for the J&J vaccine⁹. Due to the prevalence of the Delta variant, the CDC recommends booster doses for all three approved vaccines. Scientists have also recommended differential dosing for infected versus naïve individuals, as a single-dose

of an mRNA vaccine in previously infected individuals provided antibody titers similar to two doses in a naïve person.¹⁰ These results indicate great success for vaccines in preventing COVID-19 but also suggest that new, cost-effective vaccines inducing long-lasting immunity are crucial.

Our previous studies demonstrated the efficacy of two doses of our inactivated COVID-19 vaccine, CORAVAX, in hamsters. CORAVAX is an adjuvanted inactivated vaccine generated using the SAD-B19 Rabies vaccine strain encoding the S1 subunit of SARS CoV-2. This study evaluates the efficacy of a single-dose of CORAVAX in a hamster model of severe disease. A single-dose of adjuvanted CORAVAX induced high S1 and RBD IgG responses along with high virus-neutralizing antibodies against SARS CoV-2. The single-dose vaccinated animals were protected from weight loss, lung inflammation, reduced lung viral loads and alleviated the lung cytokine storm.

Materials and Methods

Ethics statement

The studies were carried out according to the recommendations described in the Guide for the Care and Use of Laboratory Animals of the National Research Council. Thomas Jefferson University (TJU) is an AAALAC-accredited institution, and the IACUC Committee approved all animal work of TJU. All efforts were made to minimize animal suffering, and all procedures involving potential pain were performed with the appropriate anesthetic or analgesic. The number of hamsters used was scientifically justified based on statistical analyses of virological and immunological outcomes. Six to eight-week-old golden Syrian female hamsters (Envigo) were anesthetized with 5% isoflurane before immunization, blood collection, and the SARS-CoV-2 challenge. Animals were monitored daily for weight loss and signs of disease. Five of the animals in each group were euthanized by an overdose of CO2 inhalation on day 3 (day 42), day 7 (day 46), and day 14(53) p.c. for viral load determination and analysis.

Viruses

The SARS-CoV-2 strain used in this study is the first U.S. isolate SARS-CoV-2 USA_WA1/2020 from the Washington State patient identified on January 22, 2020. This virus was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH (SARS-Related Coronavirus 2, Isolate USA-WA1/2020, NR-52281). Virus stocks were obtained at passage 4 (GenBank: MN985325.1) and propagated in Vero E6 cells. The stock used in this study is passage 5.

Cells

Vero (ATCC, CCL81), Vero E6 (ATCC, CRL-1586), BSR (a clone of Baby hamster kidney cells), HEK-293T (ATCC, CRL 3216), Effector cells (murine FcγRIV ADCC Bioassay Effector Cells, Promega-M1201)

Vaccine production and purification

Recombinant RABV were recovered, purified, inactivated, and tittered as described previously¹¹. Briefly, X-tremeGENE 9 transfection reagent (Millipore Sigma, Cat# 6365809001) was used to co-transfect the full-length viral cDNA clone encoding CORAVAX and the plasmids encoding RABV N, P, and L proteins and the T7 RNA polymerase into BSR cells in 6-well plates (RABV). At eight days post-transfection, the supernatant was collected, filtered through a 0.22 μm membrane filter (Millipore), and titrated on Vero cells. The presence of recombinant virus was verified by immunostaining with a monoclonal antibody against the RABV nucleoprotein (FujiRebio, Cat# 800–092) and polyclonal antiserum against the SARS CoV/CoV-2 S1 domain (Thermo Fisher, Cat# PA581798). The filtered virus was then used to inoculate Vero cells seeded in Cellstack Culture Chambers (Corning) and propagated in VP-SFM medium (Thermo Fisher Scientific) over 18 days. Supernatant collected on day ten post-infection was filtered through 0.45 μm PES membrane filters (Nalgene) and layered onto 20% sucrose in DPBS. Virions were sedimented by ultracentrifugation in an SW32 rotor for 1.5 hrs at 25,000 rpm. Viral particles were resuspended in phosphate-buffered saline (PBS) and inactivated with 50 μl per mg of particles of a 1:100 dilution of β-propiolactone (BPL, Millipore Sigma, Cat# P5648) in cold water. The absence of infectious particles was verified by inoculating BSR cells with 10 μg of BPL-inactivated viruses over three passages.

Vaccination and SARS-CoV-2 challenge

Thirty hamsters were housed individually in microisolator cages. Each animal received 10 µg of vaccine or control mixed with 50µL of Seppic SEPIVAC adjuvant (50:50, by volume) brought up to 100 µL with 1X DPBS. Hamsters were anesthetized using isoflurane for vaccination, bleeding, and challenge procedures. The hamsters were bled on days 0 (pre-dosing), 14, and 28. Hamsters were vaccinated once on day 0 with 50µL of formulated vaccine or control injected intramuscularly into each thigh of the hamster (100µL total dose/animal). On day 39, hamsters were weighed and then challenged intranasally with 100µL of 1×10^4 PFU of SARS COV-2 Washington strain dropwise into each nostril. Post-challenge, the hamsters were weighed daily and assessed for symptoms. On days 3, 7, and 14 post-challenge, five hamsters from each group were weighed and humanely sacrificed under steady CO₂ conditions. Cardiac blood was collected, and cervical dislocation was performed. The nasal turbinates, lungs, and kidneys were harvested for determining qPCR viral RNA titers and infectious viral load. One lung lobe was placed in 4% formaldehyde and placed on ice. The spleen was harvested for RNA isolation in tubes prefilled with ceramic beads and 1000µl of TRIzol tubes.

Recombinant proteins for ELISA

Purification of the HA-tagged S1 protein from the supernatant of transfected cells for ELISA: Subconfluent T175 flasks of 293T cells (human embryonic kidney cell line, ATCC) were transfected with a pDisplay vector encoding amino acids 16 to 682 of SARS-CoV-2 S (S1) fused to a C-terminal hemagglutinin (HA) peptide using X-tremeGENE 9 reagent (Millipore Sigma, Cat# 6365809001). The supernatant was collected six days post-transfection, filtered through 0.22 µm PES membrane filters (Nalgene), and then loaded onto anti-HA agarose (Pierce, Cat# 26182)

column equilibrated in PBS. After washing with ten-bed volumes of PBS, the column was loaded with two column volumes of HA peptide at a 400 µg/ml concentration in PBS and incubated overnight at 4°C. The following day, the protein was eluted with two column volumes of HA peptide followed by two column volumes of PBS. Fractions were collected and analyzed by western blotting with polyclonal antiserum against the S1 domain (Thermo Fisher, Cat# PA581798). Peak fractions were then pooled and dialyzed against PBS in 10,000 molecular weight cutoff (MWCO) dialysis cassettes (Thermo Fisher Scientific) to remove excess HA peptides. After dialysis, the protein was quantitated by UV spectrophotometry and frozen in small aliquots at -80 °C.

Purification of the RBD-His protein for ELISA: The SARS CoV-2 RBD-His tagged plasmid was purchased from Bei Resources (NR-52309). Sub-confluent T175 flasks of 293T cells (human embryonic kidney cell line) were transfected with the RBD-His tagged plasmid using XtremeGENE 9 reagent (Millipore Sigma, Cat# 6365809001). The supernatant was collected six days post-transfection and filtered through 0.22µm PES membrane filters (Nalgene). The 5 mL HisTALON cartridge (Clontech Laboratories, Cat# 635683) column was equilibrated with ten column volumes of Equilibration Buffer (HisTALON Buffer Set, Clontech Laboratories, Cat# 635651). The filtered supernatant was loaded onto the HisTALON cartridge (Clontech Laboratories, Cat# 635683) column at a speed of 1 mL/min. After washing with ten column volumes of Wash Buffer (prepared by mixing 6.6 parts of Elution Buffer with 93.4 parts of Equilibration Buffer of the HisTALON Buffer Set), the sample was eluted (at a flow rate of ~1 ml/min) with approximately 8 column volumes of Elution Buffer, collecting 1 ml fractions. The sample protein concentration was assessed by measuring the absorbance of the eluted fractions at

280 nm (Nanodrop, Thermo Fisher Scientific). Eluted fractions were analyzed by western blotting with a mouse monoclonal RBD-specific antibody (InvivoGen, Cat# srbdmab10). Peak fractions were then pooled and dialyzed against PBS in 10,000 molecular weight cutoff (MWCO) dialysis cassettes (Thermo Fisher Scientific). After dialysis, the protein was quantitated by UV spectrophotometry and frozen in small aliquots at -80°C .

Enzyme-linked immunosorbent assay

To determine antibody responses to the S protein of SARS-CoV-2, an indirect ELISA was developed utilizing purified S1 or receptor-binding domain (RBD) protein. The production of the recombinant proteins is described above. An indirect ELISA measured humoral responses to SARS-CoV-2 S1 and RBD protein. We tested individual hamster sera by enzyme-linked immunosorbent assay (ELISA) for the presence of IgG specific to SARS-CoV-2 S1 or RBD. To test for anti-SARS CoV-2 S1 humoral responses, we produced soluble S1 or RBD as described above. The two recombinant proteins were resuspended in coating buffer (50 mM Na_2CO_3 [pH 9.6]) at a concentration of 0.5 $\mu\text{g/mL}$ of S1 or 1 $\mu\text{g/mL}$ of RBD, and then they were plated in 96-well ELISA MaxiSorp plates (Nunc) at 100 μL in each well. After overnight incubation at 4°C , plates were washed three times with $1\times$ PBST (0.05% Tween 20 in $1\times$ PBS), followed by the addition of 250 μL blocking buffer (5% dry milk powder in $1\times$ PBST) and incubation at room temperature for 1.5 h. The plates were then washed three times with PBST and incubated overnight at 4°C with serial dilutions of sera (three-fold dilutions in triplicate) in $1\times$ PBST containing 0.5% BSA. Plates were washed three times the next day, followed by the addition of HRP-conjugated goat anti-Syrian hamster IgG secondary antibody (Jackson ImmunoResearch, Cat# 107-035-142, 1:8000 in PBST) or mouse anti-hamster-IgG2/3-HRP (Southern Biotech, Cat# 1935-05, 1:8000

in PBST) or mouse anti-hamster-IgG1-HRP (Southern Biotech, Cat# 1940–05, 1:8000 in PBST) for 2 h at RT. After the incubation, plates were washed three times with PBST, and 100 µl of o-phenylenediamine dihydrochloride (OPD) substrate (Thermo Scientific, 34006 and 34062) was added to each well. The reaction was stopped by adding 50 µL of 3 M H₂SO₄ per well. Optical density was determined at 490 nm (OD₄₉₀) and 630 nm (OD₆₃₀) using an ELX800 plate reader (Biotek Instruments, Inc., Winooski, VT). Plates were incubated for 15 min (IgG) or 20 min (IgG2/3 or IgG1) with OPD substrate before stopping the reaction with 3 M H₂SO₄. Data were analyzed with GraphPad Prism (Version 8.0 g) using 4-parameter nonlinear regression to determine the titer at which the curves reach 50% of the top plateau value (50% effective concentration [EC₅₀]).

SARS CoV-2 neutralizing antibody response

Sera collected from animals were tested for neutralizing capabilities against SARS-CoV-2. Briefly, serum samples were heat-inactivated (30 min at 56°C) and then diluted at 1:10. The diluted sera were serially diluted two-fold in a 96 well plate, and 30 µl of each serum dilution was mixed with 30 µl of SARS-CoV-2-WA (100 PFU). The serum/virus mixtures were incubated for 1 h at room temperature; 60 µl of the serum/virus mixtures were then transferred to Vero E6 cell monolayers in flat-bottom 96-well plates and incubated for two days at 37°C. Plates were fixed with 4 % PFA and removed from containment. Plates were blocked and then stained with DAPI at 0.5 µg/mL, and a SARS-CoV-2 specific human antibody CR3022 (produced in-house from plasmids- BEI, NR-53260) conjugated with a Dylight 650 fluorophore at a concentration of 4 µg/mL in 1X DPBS with 5% FBS. Plates were imaged and read on a Cytation5 plate reader (Biotek).

Rabies Virus Neutralization by RFFIT

Rapid fluorescent focus inhibition tests (RFFIT) were performed as previously described¹². In brief, mouse neuroblastoma cells (NA) cultured in RPMI media (with 5 % FBS) were seeded in 96-well plates and incubated for 48 hours. Individual hamster sera from days 0, 28 were serially diluted 3-fold, starting at a 1:20 dilution. A prediluted mixture of Rabies virus (strain CVS-11, previously determined to achieve 90% infection in confluent NA cells) was added to each serum dilution. The US standard Rabies immune globulin (World Health Organization standard [WHO STD]) was used at 2 IU/mL starting dilution. The sera/virus or WHO STD/virus mixture was incubated for 1 hour at 34°C. The medium was then removed from the NA cell plate, replaced with the sera/virus mix, and incubated for 2 hours at 34°C. The sera/virus mixture was aspirated and replaced with a fresh medium post-infection. The plates were then incubated for 22 hours (24 hours' total infection) at 34°C. After incubation, cells were fixed with 80% acetone, dried, and stained with fluorescein isothiocyanate Anti-Rabies N Monoclonal Globulin (Fujirebio) for >4 hours. Wells were assessed for percentage infection using a fluorescent microscope. Fifty percent endpoint titers (EPTs) were calculated using the Reed–Muench method and converted to international units per milliliter by comparing the sample 50% EPTs to that of the US standard Rabies immune globulin.

Tissue processing

Animals were euthanized on days 3, 7, or 14 post-challenge (p.c.), and lungs and nasal turbinates were harvested. One lobe of the right lung, right kidney, and right nasal turbinates was placed in pre-filled tubes containing 1 mL viral transport medium for viral load analysis. The right lung (one lobe), left nasal turbinates, left kidney, and spleen was placed in pre-filled tubes containing 1mL

TRIZol reagent. The left lung lobe was perfused with 4% Formaldehyde, placed in a conical tube filled with 6 mL of 4% Formaldehyde, and put on ice. The lung samples were placed in 4°C for three days before processing for histology and staining.

Viral load determination

Tubes containing organs in viral transport medium were homogenized using the BeadBlaster 24R homogenizer, and tissue homogenates were aliquoted and stored at -80°C. Tissue homogenates were titrated by TCID₅₀ assay on Vero E6 cell monolayers (ATCC) in 96-well plates to determine viral loads. Plates were incubated for 2 days at 37°C before being fixed with 4 % PFA and removed from containment. Plates were blocked and then stained with DAPI at 0.5 µg/mL, and a SARS-CoV-2 specific human antibody CR3022 (produced in-house from plasmids- BEI, NR-53260) conjugated with a Dylight 650 fluorophore at a concentration of 4 µg/mL in 1X DPBS with 5% FBS. Plates were imaged and read on a Cytation5 plate reader (Biotek). The limit of detection was determined by mixing 1000 PFU/mL, 100 PFU/mL, 50 PFU/mL, and 10 PFU/mL of a known titer SARS CoV-2 WA strain virus with lung or tissue homogenates followed by TCID₅₀ assay to calculate the limit of detection.

Viral RNA copies by qRT-PCR

Tubes containing organs in TRIZol were homogenized in Bead Ruptor 12 (Omni). 100uL of tissue homogenates were mixed with 900 uL TRIZol Reagent (Life Technologies). The RNA extraction protocol for biological fluids using TRIZol Reagent was followed until the phase separation. The remaining RNA extraction was done using the PureLink RNA Mini Kit (Ambion). The quantity and quality (260/280 ratios) of RNA extracted was measured using NanoDrop (Thermo Fisher).

SARS-CoV-2 envelope gene subgenomic mRNA cDNA was generated from RNA (Bei Resources, NR-52285) by One-Step RT PCR (SuperScript IV, Thermo Fisher) with primers SARS COV-2 E sgmRNA IVT Fwd (5'- GAATTCTAATACGACTCACTATAGGGGATTAAAGGTT TATACCTTCCC-3') and SARS COV-2 E sgmRNA IVT Rev (5'-GCTAGCTTAGACCAGAAG ATCAGGAACTC-3'). The SARS-CoV-2 E sgmRNA standards were generated by in-vitro transcription of the generated SARS-CoV-2 E sgmRNA cDNA using the MegaScript T7 Transcription kit (Invitrogen), followed by the MEGAclean Transcription Clean-Up Kit. Aliquots of 2×10^{10} copies/ μ L were frozen at -20°C. Five microliters of RNA per sample were run in triplicate, using the primers: SARS COV-2 E sgmRNA Fwd (5'- CGATCTCTTG TAGATCTGTT CTC-3'), SARS COV-2 E sgmRNA Rev (5'- ATATTGCAGCAGTACGCACACA -3') and probe-FAM (5'- ACACTAGCCATCCTTACTGCGCTTCG -3').

Cytokine qPCR

Duplicates of each lung RNA sample were assessed for the presence of several cytokines in a multiplex format. Three 5-Plex assays were generated: 1) γ -actin, IFN- γ , CCL17, CCL3, CCL5, 2) γ -actin, CXCL10, IFN- α , IL10, IL12 p40, 3) γ -actin, IL17, IL21, IL4. One 4-Plex assay was generated to assess γ -actin, IL-6, TGF- β 1, TNF- α . The mRNA assay primer and probes sequences are listed in Table S2. The fold gene expression was calculated by $2^{-\Delta\Delta C_t}$ by calculating the ΔC_t for vaccinated, control, and untreated hamster lungs. Of note, CXCL10 was not detected in any of the animals.

Histology

Hamster lungs were harvested in 4% PFA and stored at 4°C. The tissue was dehydrated, fixed, and infiltrated with paraffin on the Leica Polaris 2 tissue processor. The infiltrated tissue was then embedded and sectioned at 5 microns. Antigen retrieval was performed on the Roche Ventana Discovery ULTRA staining platform using Discovery CC1 (Roche cat#950-500) for a total application time of 64 minutes. The primary antibody, human Anti-SARS COV-2 Nucleocapsid monoclonal (E16C) (Thermo Fisher, MA1-7403, 0.1 mg/mL), was incubated as a 1:100 dilution, at room temperature, for 45 minutes. Secondary immunostaining used a horseradish peroxidase (HRP) multimer cocktail (Roche cat#760-500), and immune complexes were visualized using the ultraView Universal DAB (diaminobenzidine tetrahydrochloride) Detection Kit (Roche cat#760-500). Slides were then washed with a Tris-based-reaction buffer (Roche cat#950-300) and counter-stained with Hematoxylin II (Roche cat #790-2208) for 4 minutes. Scoring was performed as shown in Table2.

Antibody-dependent effector functions

The assay was performed in 96-well, flat, white-bottom plates (Corning) preseeded with 30,000 Vero CCL81 cells and infected after 24 h with Measles vaccine (Edmonston B) vector expressing full-length SARS CoV-2 spike at an MOI of 0.5. After 60 h, the medium was removed, and 25 µl of assay buffer (RPMI 1640 with 4% low-IgG FBS) was added to each well. Sera were pooled from each group and heat-inactivated for analysis. Then, 25 µl sera at a starting dilution of 1:30 were serially diluted 3-fold in assay buffer (in duplicates). The sera were then incubated with the infected cells for 30 min at 37°C. Post-challenge (day 15 post-challenge) hamster sera from the unvaccinated challenged group are used as a control. Genetically modified Jurkat cells expressing mouse FcγR IV with a luciferase reporter gene under the transcriptional control of nuclear-factor-

activated T cell (NFAT) promoter were added at 1.5×10^5 cells in 25 μ l per well, which is approximately a 5:1 ratio of effector cells (Promega) to target cells. Cells were then incubated for another 6 h at 37°C. After the incubation, 75 μ L Bio-Glo Luciferase assay reagent was added, and luminescence was quantified using a microplate reader. Fold induction was measured in relative light units and calculated by subtracting the background signal from wells without effector cells and dividing values for wells with antibodies by values for those with no antibody added. Specifically, fold induction was calculated: $(RLU_{\text{induced}} - RLU_{\text{background}})/(RLU_{\text{uninduced}} - RLU_{\text{background}})$. The mean values and standard errors of the means (SEM) were reported, and a nonlinear regression curve was generated using GraphPad Prism 9.

Statistical analysis

An unpaired two-tailed nonparametric t-test (Mann Whitney) was performed on log-transformed data for each time point for the ELISA, virus-neutralizing antibody, viral load, and qPCR assays. An unpaired nonparametric two-tailed Mann Whitney test was performed on percentage weights for each time point for the weight curves. P values are reported.

Results

Immunogenicity of a single-dose of CORAVAX in Syrian hamsters

We previously described the Rabies virus vectored SARS COV-2 vaccine CORAVAX^{TM11}. Utilizing the SAD-B19 Rabies vaccine strain, the S1 subunit of the SARS-CoV-2 spike protein (S) was incorporated into RABV virions. To promote the incorporation of the S1 domain, we constructed a gene encoding a fusion protein between 682aa of the N-terminal of SARS-CoV-2 S fused to a C-terminal domain of RABV glycoprotein (G)¹¹. Two doses of inactivated CORAVAX were efficacious in hamsters and mice and induced high virus-neutralizing antibodies (VNA)^{11,13}. Here, we evaluated the effectiveness of a single-dose of CORAVAX in the Syrian hamster model. To this end, we vaccinated two groups of fifteen golden Syrian hamsters intramuscularly (i.m.) with either 10 µg of inactivated CORAVAX or the control vaccine FILORAB1 (Rabies vectored Ebola vaccine)¹⁴ adjuvanted with a squalene-based adjuvant (SEPIVAC SWE, Seppic). The animals were inoculated on day 0 and bled on days 0, 14, and 28 (Fig 1). On day 39, vaccinated and control hamsters were challenged intranasally with a dose of 10⁴ PFU of the SARS-CoV-2 isolate USA_WA1/2020. Five animals from each group were necropsied on days 3, 7, and 14 post-challenge.

To analyze the immunogenicity of the CORAVAX vaccine, the sera from both groups of hamsters were assayed for SARS CoV-2 specific antibody responses by ELISA specific for SARS-CoV-2 S1 as well as the receptor-binding site (RBD) (Fig 2). We observed high SARS CoV-2 S1 and RBD-specific IgG responses in the CORAVAX group as early as day 14 post-vaccination. These responses were significantly increased until day 28 (Fig 2A, B), indicating the long-term *in-vivo* stability of the antigen. As previously reported for CORAVAX with another adjuvant, we observed

a strong Th1 biased immune response on day 28 indicated by the induction of S1 IgG2/3 responses (Fig 2C). As expected, no SARS-CoV-2 S1 or RBD-specific immune response was detected before the challenge in the control animals vaccinated with the EBOV vaccine FILORAB1 (Fig 2A, B, C). These results suggested that a single inoculation may provide some level of protection.

We analyzed the sera post-challenge (p.c.). Of note, on day 3 p.c. (day 42), we observed S1 IgG and IgG2/3, and RBD IgG responses only in the CORAVAX vaccinated animals, except one animal in the control group, which had a low level of RBD IgG responses (Fig 2A, B, C). On days 7 and 14 p.c. (day 46 and 52), we observed significantly increased S1 IgG responses in the CORAVAX group, which were significantly higher than the responses seen in the control group (Fig 2A). Moreover, the CORAVAX vaccinated animals induced a strong Th1 biased immune response on time points post-challenge, a feature previously shown to be beneficial in combatting SARS-CoV-2 infection¹⁵. In contrast, control animals immunized with FILORAB1 induced either no S1 IgG2/3 responses or low levels in the case of two animals only on day 14 p.c. (day 53) (Fig 2C). As previously reported, we detected no significant differences in immune responses against the RBD IgG responses on days 7 and 14 p.c. for all groups (Fig 2B).

While the ELISA data is helpful to follow immune responses over time and analyze the quality of such responses, we tested the potency of the sera to neutralize SARS CoV-2 using a virus neutralization antibody (VNA) assay. As shown in figure 3, VNA titers against SARS-CoV-2 in the CORAVAX group followed the trend seen in the S1 IgG ELISA, wherein high VNA titers were detected on day 28, maintained on day 3 p.c., and increased on days 7 and 14 p.c. We did not detect 100% VNA titers on day 14 p.c., which may reflect levels lower than our limit of detection

(dilution 1:20) (Fig 3). However, VNA was present for CORAVAX immunized animals before the challenge (day 28), and no VNA titers were observed in the FILORAB1 group. The FILORAB1 group had no detectable VNA titers at day 3 p.c (similar to S1 IgG responses), but some VNAs were detected on days 3 and 7 after the challenge. Of note, VNA titers of the FILORAB1 group are significantly lower than the CORAVAX group. On day 14 p.c. CORAVAX immunized animals showed VNA response surpassing responses detected for human convalescent sera. No background neutralization was observed in the negative control human sera (Fig 3).

CORAVAX induces potent immune responses against RABV

In addition to the VNA detected for SARS-CoV-2, we also analyzed the VNA induced by the two vaccines against RABV and observed no significant differences between the CORAVAX and the FILORAB1 group (Fig S1). The CORAVAX and the FILORAB1 vaccine induced strong RABV VNA titers exceeding the protective levels against RABV, i.e., 0.5 IU/mL.

A single-dose of CORAVAX protects the hamsters from weight loss and viral burden in the lungs post-SARS CoV-2 challenge.

As shown above, CORAVAX is immunogenic; therefore, the hamsters were challenged intranasally on day 39 with 10^4 PFU of SARS-CoV-2 isolate USA_WA1/2020 and were monitored for up to 14 days. The CORAVAX vaccinated animals showed significantly less weight loss than the FILORAB1 controls, which lost more than 6% weight (Fig 4). On days 3, 7, and 14 p.c., five hamsters in each study group were humanely euthanized, and lungs, nasal turbinates, spleen, and kidneys were harvested. The viral loads were determined by assaying RNA for viral copies of replicating virus for the subgenomic (sg) mRNA by RT-qPCR assay (Fig 5A, B) and infectious

viral loads by TCID₅₀ assay (Fig 5C, D). Significantly lower sgRNA and infectious viral loads were detected in the lungs of the CORAVAX vaccinated animals on day 3 post-challenge (Fig 5A, C). On day 7 post-challenge, significantly lower sgRNA was detected in the lungs of the CORAVAX group, but no detectable infectious virus was seen in either the vaccine or control groups (Fig 5A, C), suggesting RNA stability. On day 14 post-challenge, there was no detectable infectious SARS-CoV-2 in either group, although similarly low levels of sgRNA were detected in both groups (Fig 5A, C). In the nasal turbinates, we only detected infectious virus on day 3 p.c. with no significant differences between the two groups. (Fig 5B,D). We could not detect infectious virus or sgRNA in the kidneys or spleens of animals in either group (data not shown).

A single-dose of CORAVAX can significantly reduce lung pathology in hamsters

Lung sections were collected from control and vaccinated animals at days 3,7, and 14 p.c. and processed for histology (Figs 6,7,8). Sections were scored in a blinded manner (Scoring criteria, Table S1). On day 3 p.c., the control, FILORAB1 specimens showed severe, patchy inflammation involving more than 50% of the examined lung with severe involvement of the airways (bronchitis/bronchiolitis), extending into the alveolar septa (pneumonitis) with alveolar edema and hemorrhage. We also noted severe vasculitis. The immunohistochemical stain for the SARS-CoV-2 Nucleoprotein antigen showed extensive multifocal positive cells involving bronchial epithelium, alveolar epithelium, and macrophages. In contrast, on day 3 p.c., the CORAVAX vaccinated specimens showed only mild to moderate patchy inflammation involving less than 50% of the examined lung with mild to moderate airway inflammation, mild alveolar wall infiltration, congestion, and interstitial edema. We also noted focal mild vasculitis. Immunohistochemical stain for the SARS-CoV-2 Nucleoprotein antigen showed only multifocal positive bronchial cells.

417
 418 On day 7 p.c., the control FILORAB1 specimens still showed significant inflammation involving
 419 around 50% of the examined lung. The disease is multifocal, nodular, and bronchiolocentric with
 420 moderate to severe involvement of the airways and alveolar wall infiltration, edema, congestion,
 421 and focal hemorrhage. We also noted mild to moderate vasculitis. Immunohistochemical stain for
 422 the SARS-CoV-2 Nucleoprotein antigen showed multifocal positive cells involving both bronchial
 423 epithelia and alveolar epithelia but to a much lesser extent than those observed on day 3 p.c. On
 424 day 7, the CORAVAX vaccinated group showed significant improvement with only mild airway
 425 inflammation, mild alveolar edema, and congestion involving less than 30% of the examined lung.
 426 No significant vasculitis was noted. Immunohistochemical stain for the SARS-CoV-2
 427 Nucleoprotein antigen showed no definite positive cells.

428 On day 14 p.c., the control FILORAB1 specimens showed improvement, with inflammation
 429 involving less than 30% of the lung with mild to moderate airway inflammation, mild to moderate
 430 alveolar congestion, edema, and mild vasculitis. Immunohistochemical stain for the SARS-CoV-
 431 2 Nucleoprotein antigen showed no definite positive cells. On day 14 p.c., the CORAVAX
 432 vaccinated group showed no significant airway inflammation, only mild alveolar congestion and
 433 edema, and no significant vasculitis. Immunohistochemical stain for the SARS-CoV-2
 434 Nucleoprotein antigen showed no definite positive cells.

435
 436 *A single-dose of CORAVAX can significantly prevent SARS COv-2 induced cytokine storm in*
 437 *the lungs.*

438 Uncontrolled induction of inflammation is one of the hallmarks of pathology after infection with
 439 SARS-CoV-2¹⁶. Therefore, the lungs of the vaccinated and control animals were assayed for

cytokine (IFN- γ , IFN- α , IL-10, IL-6, IL-4, IL-2, IL-21, IL-17, TGF- β 1, TNF- α) and chemokine (CCL5, CCL3) expression by qRT-PCR (Primer and probe sequences, Table S2), normalized to γ -actin and expressed as fold-changes over the average expression of three uninfected, unchallenged hamsters. On days 3 and/or 7 post-challenge, we observed significantly higher levels of inflammatory cytokines IFN- γ , IFN- α , IL-10, and IL-6 in the control groups than were detected in CORAVAX immunized animals (Fig 9). CCL5 and IL-4 were only detectable in the control groups, suggesting a more substantial Th2 bias in the control group, as seen in the isotype-specific ELISA presented above (Fig 2C). On day 7 p.c., there was a significant upregulation of CCL3, IL-2, IL-21, and TGF- β 1 in the CORAVAX vaccinated animals, which may have contributed to dampening the inflammation and allowing for viral clearance as observed in the lung histology (Fig 6B, C, and 7B, C). We saw no significant difference in the IL-17 and IL-12 p40 expression between the two groups after the challenge (Fig 9 and S2).

A single-dose of CORAVAX induces antibodies with antibody-dependent cellular cytotoxicity activity.

While we previously found high VNA titers in animals immunized with CORAVAX, we wanted to test if CORAVAX also induced non-neutralizing antibody functions. To understand the potential mechanism of S1 antibody-mediated protection, we tested pre-challenge hamster sera (day 28) from vaccinated and controlled animals for antibody-dependent cellular cytotoxicity (ADCC) activity using a recombinant measles virus expressing SARS-CoV-2 S in Vero-CCL81 cells. These reporter cells showed ADCC activation Reporter Bioassay where the Fc portion of the antibody binds to the Fc γ RIIIa (mouse) of the effector cells (Jurkat) and result in a quantifiable luminescence signal from NFAT (nuclear factor of activated T-cells) pathway. We observed the

463 highest ADCC activity in the CORAVAX serum. In contrast, we observed no ADCC activity in
464 the post-challenge hamster sera (15 days p.c.) or the control FILORAB1 groups (Fig 10),
465 suggesting that CORAVAX induced antibodies with neutralizing and non-neutralizing functions.

Discussion

Several SARS CoV-2 vaccines are now approved and available to the public. Despite the current vaccines, there is a need for better vaccines in terms of the durability of protective responses, limited variant cross-protection, the high production costs, and low-temperature stability of some vaccines, which render them less suitable for countries with less developed infrastructure. Furthermore, none of the current vaccines are approved for children below five years. The immunity provided by prior infection and the current vaccines is high but not complete. The emergence of the Delta and Omicron strains highlights the possibility of reinfection and loss of protection against newly emerging strains, which will require the continued development of modified or new vaccines with broader coverage. While studies have verified waning immunity after six months for the mRNA vaccines (Pfizer and Moderna) and two months after the J&J vaccine, it is still debated if a prior infection can provide long-term protection^{17,18}. Although protection from reinfection decreases with time in previously infected individuals, it is higher than those who received two doses of the Pfizer vaccine¹⁹. Maintaining public health measures to curb transmission as the COVID-19 pandemic continues is critical for preventing morbidity and mortality. Modifying guidelines as per immunity status to improve vaccine coverage may be required. Studies have shown that a single-dose of the vaccine in previously infected individuals is as protective, if not better, than two doses of the mRNA or a single-dose of the AstraZeneca vaccine²⁰⁻²².

Most of the current WHO approved vaccines (mRNA-1273, BNT162b2, and Ad26.COV2.S) utilize the pre-fusion stabilized spike protein as the antigen⁶. For the mRNA vaccines, although the antigen is similar, they differ in their mRNA content (100 µg for mRNA-1273 vs. 30 µg for

BNT162b2), the dosing interval between prime and boost vaccines (4 weeks for mRNA-1273 vs. three weeks for BNT162b2), and the lipid composition of the nanoparticles used for packaging the mRNA content². Initial studies demonstrated similar efficacy for both mRNA vaccines. Among the WHO-approved replication-incompetent adenoviral vaccines, AstraZeneca's ChAdOx1 nCoV-19 vaccine (AZD1222) and the Jansen's Ad26.COV2, the AZD1222, expresses the full-length spike while Ad26.COV2.S expresses a pre-fusion stabilized spike protein. Both vaccines are efficacious (~66-76%) but to a lower extent than the mRNA vaccines²³. Large-scale human adenoviral COVID-19 vaccinations appear to be associated with rare cases of vaccine-induced immune thrombocytopenia (VITT) that may be due to the interaction of the viral vector with platelet factor 4 (PF4)²⁴⁻³⁰. Recently WHO gave emergency use approval to an adjuvanted recombinant nanoparticle vaccine from NOVAVAX, NVX-CoV2373, with an efficacy of 89.7% in phase 3 trials³¹⁻³³.

The emergence of variants in the presence of waning vaccine immunity has raised concerns worldwide. A major current issue is that all vaccines express the ancestral SARS-CoV-2 spike, whereas the currently circulating variants, including Delta and Omicron, have significant mutations in the spike-encoding region, rendering the vaccine less effective giving rise to mutants. Generating and testing new variant vaccines may be required to control the spread of SARS CoV-2. Single-dose variant vaccines that can induce long-term immunity may help to improve vaccine coverage and reduce reinfection rates. Testing vaccines in previously infected animals may become critical as infection rates rise worldwide. In addition, developing standardized assays to determine the virus-neutralizing titer (VNT) thresholds essential for protection may improve vaccine quality³⁴.

512

513 Here we report the immunogenicity, efficacy, reduced lung inflammation, and a potential
514 mechanism of our single-dose S1- based COVID-19 vaccine, CORAVAX, in hamsters. The
515 antigen used is a chimeric protein that contains the S1 domain (1-682aa) fused to the C terminus
516 of RABV-G for tethering to the Rabies virus virion. We previously analyzed the correct folding
517 of our chimeric S1 in CORAVAX by verifying its binding to hACE2 protein¹¹. An S1 based
518 vaccine, when folded correctly, might serve as a good immunogen as it ensures the immune
519 responses against the important neutralizing epitopes identified in human convalescent patients
520 that recognize the RBD, NTD S1, and quaternary epitope that bridges the two RBDs³⁵.

521

522 Our previous study showed that two doses of CORAVAX adjuvanted with MPLA-Addavax, a
523 TRL4 agonist, induced high levels of neutralizing antibodies, generated a strong Th1-biased
524 immune response (indicated by high S1 IgG2/3 responses), and were efficacious in hamsters with
525 viral clearance in the nasal turbinates¹³. In human patients with mild/recovered COVID-19, a Th1
526 biased immune response is beneficial for protection against disease³⁶. The current study used
527 SEPIVAC SWE (developed by VFI and Seppic) adjuvant, a squalene-in-water emulsion, similar
528 to Addavax (Invivogen) tested in our previous study. We observed a significant increase in
529 antibody and VNT's from day 14 to day 28 with a single-dose of adjuvanted inactivated
530 CORAVAX, suggesting antigen stability. Of note, CORAVAX induced high 100% VNT titers.
531 As previously published, CORAVAX induced a strong Th1 biased immune response, which
532 helped significantly reduce inflammation and viral loads in the lungs. The controls induced a
533 stronger inflammatory cytokine and chemokine response in the lungs on day 3 and/ day 7 post-
534 challenge than the vaccinated animals, in addition to the Th2 cytokines only being induced in the

control group. Sera from CORAVAX vaccinated hamsters demonstrate antibody-dependent cytotoxic activity, suggesting a dual function of the antibodies. Therefore, we can conclude that a single-dose of CORAVAX is efficacious and prevents weight loss and the cytokine storm seen in the control lungs.

The histopathologic tissue examination on days 3 and 7 p.c. showed the CORAVAX vaccinated animals had significantly reduced inflammation, as indicated by the accumulation of inflammatory cells compared to the controls. CORAVAX vaccinated animals always had significantly lower average pathology lung scores, indicating excellent protection from lung disease. A significantly higher amount of antigen was detected in the lungs of the controls than in the CORAVAX vaccinated animals on days 3 and 7 p.c. By day 14 p.c., the inflammation was completely cleared in the vaccinated animals and significantly reduced in the control unvaccinated group, and the antigen was absent in the lungs of both groups, which is a common feature of the hamster model. The hamster model is well established for SARS CoV-2 as it mimics the human lung pathology seen in COVID-19 patients¹⁶. This study concludes that SARS CoV-2 challenged CORAVAX vaccinated animals never developed the severe inflammation associated with the disease (compared with the control animals). We also observed a rapid decline and early elimination of the SARS-CoV-2 Nucleoprotein antigen in the vaccinated animals (compared with the unvaccinated counterparts). These findings are a strong indication of protection of the lungs by the CORAVAX vaccine.

This study is the first to show the efficacy of a single-dose RABV vectored COVID-19 vaccine, CORAVAX, in the Syrian hamster model of severe disease. The Rabies vaccine vector has

significant advantages, including 1) an excellent safety profile, 2) approved administration to pregnant women, children, and the immunocompromised, 3) the induction of lifelong immunity, 4) amenable to boosting several times 5) less reactogenicity³⁷. This study showed significant protection with single-dose of CORAVAX, yet it is evident that two doses are required to get complete viral clearance from the nasal turbinates. Studies evaluating the longevity of immune responses after a single-dose and the boosting potential of CORAVAX in previously infected hamsters are yet to be assessed. Production of CORAVAX is facile, as it would follow the existing RABV vaccine manufacturing facilities and technologies. To further evaluate CORAVAX, a Phase I human clinical trial is being conducted in India by Bharat Biotech International Ltd., with plans for a follow-up Phase I study in the US³⁸. To summarize, CORAVAX is a promising COVID-19 vaccine candidate with an excellent safety profile and proven efficacy in several animal studies.

Figure Legends:

Fig 1. Vaccine constructs and vaccination schedule.

A) Schematic illustration of CORAVAX, the Rabies virus-based SARS-CoV-2 vaccine construct wherein a SARS-CoV-2 S1 RABV G chimeric protein cDNA was inserted between the N and P genes of the SAD-B19-derived RABV virus vaccine vector BNSP333 and control vaccine FILORAB1, the Rabies virus-based EBOLA vaccine used in this study. B) Syrian hamsters were immunized on day 0 with 10 µg chemically inactivated CORAVAX or FILORAB1 with Seppic (SEPIVAC SWE) adjuvant. The animals were challenged on day 39 with a dose of 10⁴ PFU of the SARS-CoV-2 isolate USA_WA1/2020. Serum was collected from each hamster at days 0, 14, 28, and at necropsy (days 42, 46, or 53) for analysis. Animals were necropsied at days 42 (day 3 p.c.), 46 (day 7 p.c.), and 53 (day 14 p.c.). Created with BioRender.com.

Fig 2. SARS CoV-2 immune responses.

Serum samples collected from each hamster were evaluated for SARS-CoV-2 S-specific immune responses by A) ELISA, Anti-SARS CoV-2 S1 IgG responses represented as EC50 titers over time, B) ELISA, Anti-SARS CoV-2 RBD responses, and C), ELISA, Anti-SARS COV-2 S1 IgG2/3 responses. The CORAVAX vaccine group is shown in blue, and the FILORAB1 group in black. For A-C, mean titers ± SD are depicted for each group per time point. P values determined by Mann-Whitney test. Only significant differences are depicted.

Fig 3. SARS CoV-2 virus-neutralizing antibody responses.

Serum samples collected from each hamster were evaluated for SARS-CoV-2 S-specific virus-neutralizing antibody (VNA) responses using 100 PFU of SARS-CoV-2 isolate

USA_WA1/2020.100% VNA titers are depicted for hamster sera at different time points along with convalescent sera (N=7) and negative control sera (N=7). Mean titers $\pm$ SD are depicted for each group per time point. P values determined by Mann-Whitney test. Only significant differences are depicted.

Fig 4. Hamster body weight after SARS CoV-2 infection.

Hamsters were vaccinated at day 0, challenged intranasally with 10^4 PFU SARS-CoV-2 on day 39. Percent change in body weight. CORAVAX vaccine group is shown in blue and FILORAB1 group in black. N = 15 for FILORAB1 group (5 hamsters each euthanized at days 3, 7 and 14p.c.) and N = 15 for CORAVAX group (5 hamsters each euthanized at days 3, 7 and 14 p.c.). Body weight P value determined by Wilcoxon test. P > 0.123 (ns), P < 0.033 (*), P < 0.002 (**), P < 0.001 (***).

Fig 5. SARS-CoV-2 tissue viral load in hamsters.

Hamsters were challenged intranasally with 10^4 PFU SARS-CoV-2, and five of the animals in each group were euthanized on days 3, 7, and 14 p.c. Right lungs (A, C) and nasal turbinates (B, D) from each animal were homogenized in media, and viral loads were determined by qRT-PCR (A, B) or by plaque assays on Vero E6 cells (C, D). The limit of detection for the qRT-PCR assay is 10 copies. The limit of detection for the plaque assay was 50 PFU/mL per lung/nasal turbinate tissue. The CORAVAX vaccine group is shown in blue, and the FILORAB1 group in black. Data represent mean $\pm$ S.D., N = 5 for FILORAB1 group days 3, 7, and 14 time points, and N = 5 for CORAVAX group days 3, 7, and 15 time points. P values determined by Mann-Whitney test. Only significant differences are depicted.

616

617 **Fig 6. SARS-CoV 2 lung pathology.**

618 Representative histological images of SARS-CoV-2 infection in control and vaccinated hamster
619 lungs. (A,C,E): CORAVAX vaccinated on days 3, 7, and 14 p.c. (B,D,F): FILORAB1 control on
620 days 3, 7, and 14 p.c. All images at magnification 40X. The images show significantly less lung
621 inflammation in the vaccinated animals than the control unvaccinated animals. The difference is
622 more prominent at day 3p.c. followed by day 7p.c.

623

624 **Fig 7. Comparative pathology scores for lungs from CORAVAX vaccinated, and control**
625 **hamsters post SARS-CoV-2 challenge.**

626 Overall lung pathology scores at days 3-, 7- and 14-days p.c. The pathology scores (mean) were
627 calculated based on the criteria described in Table S1. The CORAVAX vaccine group is shown in
628 blue, and the control group in black. Data represent mean $\pm$ S.D., N = 5 for FILORAB1 group and
629 CORAVAX group for days 3,7 and 15 time points. P > 0.123 (ns), P < 0.033 (*), P < 0.002 (**),
630 P < 0.001 (***). Only significant differences are depicted.

631

632 **Fig 8. SARS-CoV 2 nucleoprotein antigen lung staining**

633 Representative histological images of SARS-CoV-2 Nucleoprotein antigen staining in the lungs
634 of control and vaccinated hamster. (A,C,E) CORAVAX day 3,7,14 p.c. (B,D,F) FILORAB1 day
635 3,7,14 p.c. The presence of the SARS CoV-2 nucleoprotein is indicated by the brown DAB (3, 3'-
636 diaminobenzidine) stain in the lungs. A significantly high amount of N antigen staining is observed
637 in the FILORAB1 lungs (B) than in CORAVAX lungs (A) on day 3 p.c. On day 7p.c.
638 immunohistochemical stain for the SARS-CoV-2 Nucleoprotein antigen show scattered positive

cells in the control group (D) and no definite positive cell in the vaccinated group (C). N antigen staining is absent in the FILORAB1 (F) and CORAVAX lungs (E) on day 14 p.c.

Fig 9. A single-dose of CORAVAX immunization prevents a cytokine storm in the lungs.

Total RNA was extracted from the lungs of hamsters necropsied on days 3,7, and 14 after challenge with SARS-CoV-2. Hamster (A) IFN- γ , (B) IFN- α , (C) IL-10, (D) IL-6, (E) CCL5, (F) IL-4, (G) CCL3, (H) IL-2, (I) IL-21, (J) TGF- β 1, (K) TNF- α and (L)IL-17. Cytokine/chemokine mRNAs were quantified by real-time RT-PCR. γ -actin mRNA was used as an internal control. Data are shown as fold change in gene expression compared to normal animals (unimmunized and unchallenged) after normalization. Data were analyzed using Mann Whitney test for each time point (*P < 0.05; **P < 0.01; ***P < 0.001).

Fig 10. A single-dose of CORAVAX immunization induces antibody-dependent cellular cytotoxicity activity

Antibody-dependent cellular cytotoxicity (ADCC) activation activity luciferase reporter assay. Measles virus expressing full SARS CoV-2 spike infected Vero cells were incubated with hamster sera and then incubated with effector cells (mouse Fc γ RIV expressing Jurkat cells at a ratio of 5:1 (effector to target)). Heat-inactivated pooled sera were used for the assay. The figure represents one replicate of 3 repeats. Fold induction is depicted for each group.

Supplementary Figure Legends and Tables

Fig S1. Rabies virus neutralizing antibodies

Hamster Day 28 sera were assessed for RABV VNA. The dotted line represents 0.5 I.U/mL.

Only significant differences are depicted.

Fig S2. IL-12p40 mRNA levels by qPCR

Total RNA was extracted from the lungs of hamsters necropsied on days 3,7, and 14 post-challenge with SARS-CoV-2 and were assayed for the presence of IL-12p40 mRNA levels. RNAs were quantified by real-time RT-PCR. γ -actin mRNA was used as an internal control. Data are shown as fold change in gene expression compared to normal animals (unimmunized and unchallenged) after normalization. Data were analyzed using the Mann Whitney test for each time point (*P < 0.05; **P < 0.01; ***P < 0.001).

Table S1. Semi-quantitative histopathological scoring criteria.

Table S2: List of cytokine/chemokine qPCR primers and probes sequences.

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- CORAVAX inact. + Seppic
- ▲ FILORAB1 inact. + Seppic

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Fig S1

Fig S2

Table S1. Semi-quantitative histopathological scoring criteria.

Categories	Score criteria	Score (Total score=27 per lung) ^a
General evaluation (G)	Assessment percentage of lung damage area under 4x objective lens	0 none 1 focal lung inflammation within one lobe or <30% of total lung cutting area ^b 2 diffuse lung inflammation within one lobe or involving 30-70% total lung cutting area 3 diffuse lung inflammation involving more than one lobe or 70% total lung cutting area
Bronchioles (B)	Assessing appearance and severity of peribronchiolar infiltration, intrabronchiolar wall infiltration and bronchiolar epithelial cell death/desquamation	0 none 1 peribronchiolar infiltration only 2 peribronchiolar and epithelial infiltration (mild to moderate) 3 severe bronchiolar wall infiltration and epithelium desquamation
Alveoli (A)	Assessing the appearance, scope involved, and severity of alveolar septal infiltration, alveolar space infiltration, alveolar space exudation and hemorrhage	0 none 1 only alveolar wall thickening (interstitial congestion and edema), mild interstitial pneumonitis (<30%) 2 alveolar wall infiltration (interstitial pneumonitis), exudation or hemorrhage involving 30-70% of lung cutting area 3 diffuse alveolar infiltration (interstitial pneumonitis), exudation or hemorrhage involving >70% of lung cutting area
Vasculature (V)	Severity of vasculature inflammation	0 none, 1 only perivascular edema and or perivascular infiltration 2 mild infiltration within the vessel wall without endothelium infiltration 3 severe vasculitis

^a Total score = the scores of general assessment × the scores accumulated from individual category assessment ^b More than one lung lobes should be examined for histological assessment, if only one lobe were studied, percentage of the total lung cutting area would be used for scoring. Adapted from Lee et al. 2020 ¹.

1. Lee AC, *et al.* Oral SARS-CoV-2 Inoculation Establishes Subclinical Respiratory Infection with Virus Shedding in Golden Syrian Hamsters. *Cell Rep Med* **1**, 100121 (2020).

Table S2. List of cytokine/chemokine qPCR primers and probes sequences.

Type of Multiplex	Cytokine/Chemokine	Reporter Dye		FW Primer 5' to 3'	REV Primer 5' to 3'	Probe 5' to 3'
5-Plex	γ -actin	VIC	MGB	ACA GAG AGA AGA TGA CGC AGA TAA TG	GCC TGA ATG GCC ACG TAC A	TTG AAA CCT TCA ACA CCC CAG CC
	IFN- γ	FAM	MGB	TGT TGC TCT GCC TCA CTC AGG	AAG ACG AGG TCC CCT CCA TTC	TGG CTG CTA CTG CCA GGG CAC ACT C
	CCL17	ABY	QSY	CGA GTG CTG CCT GGA GAT C	TGA TGG CCT TCT TCA CAT GC	TGTGACTGTCCAGGGCAGGTCCA
	CCL3	JUN	QSY	GGT CCA AGA GTA CGT CGC TG	GAG TTG TGG AGG TGG CAA GG	JIRA-55614; Thermo Fisher generated sequence
	CCL5	ALEXA680	QSY	TCA GCT TGG TTT GGG AGC AA	TGA AGT GCT GGT TTC TTG GGT	JIRA-55614;Thermo Fisher generated sequence
5-Plex	γ -actin	VIC	MGB	ACA GAG AGA AGA TGA CGC AGA TAA TG	GCC TGA ATG GCC ACG TAC A	TTG AAA CCT TCA ACA CCC CAG CC
	CXCL10	FAM	MGB	TAC GTC GGC CTA TGG CTA CT	TTG GGG ACT CTT GTC ACT GG	APH6E47; Thermo Fisher generated sequence
	IFN- α	ABY	QSY	TCG TTG CTG CTC CCG TAG TC	ATG GAT CCC GCT GCA ATT C	TGGGTGCTGTGGGCTTCACTGG
	IL-10	JUN	QSY	AGC TGG ACA ACA TAC TAC TCA CTG A	GGT TTG GCA ACC CAA GTA ACC	CTCCTTACTGCAGGACTTTAAG
	IL-12-p40	ALEXA680	QSY	AAT GCG AGG CAG CAA ATT ACT C	CTG CTC TTG ACG TTG AAC TTC AAG	CCT GCT GGT GGC TGA CTG CAA TCA
5-Plex	γ -actin	VIC	MGB	ACA GAG AGA AGA TGA CGC AGA TAA TG	GCC TGA ATG GCC ACG TAC A	TTG AAA CCT TCA ACA CCC CAG CC
	IL-17	FAM	MGB	AGGAACCTTGGAGAGATGGT	AGACAGGCTTCTCTGTTGG	GCCCAGCTGAGGAACAGCTCTGAGCCAAGAG
	IL-2	ABY	QSY	GTG CAC CCA CTT CAA GCT CTA A	AAG CTC CTG TAA GTC CAG CAG TAA C	AGGAAACCCAGCAGCACCTCGAGC
	IL-21	JUN	QSY	GGA CAG TGG CCC ATA AAA CAA G	TTC AAC ACT GTC TAT AAG ATG ACG AAG TC	CAA GGG CCA GAT CGC CTC CTG ATT
	IL-4	ALEXA680	QSY	ACA GAA AAA GGG ACA CCA TGC A	GAA GCC CTG CAG ATG AGG TCT	AGA CGC CCT TTC AGC AAG GAA GAA CTC C
4-Plex	γ -actin	VIC	MGB	ACA GAG AGA AGA TGA CGC AGA TAA TG	GCC TGA ATG GCC ACG TAC A	TTG AAA CCT TCA ACA CCC CAG CC
	IL-6	FAM	MGB	CCT GAA AGC ACT TGA AGA ATT CC	GGT ATG CTA AGG CAC AGC ACA CT	AGAAGTCACCATGAGGTCTACTCGCAAAA
	TGF- β 1	ABY	QSY	GGC TAC CAC GCC AAC TTC TG	GAG GGC AAG GAC CTT ACT GTA CTG	CCC TGT CCC TAC ATT TGG AGC CTG GA
	TNF- α	JUN	QSY	TGA GCC ATC GTG CCA ATG	AGC CCG TCT GCT GGT ATC AC	CGG CAT GTC TCT CAA AGA CAA CCA G