Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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SUPPLEMENTARY APPENDIX

Table of Contents

Glossary of Abbreviations

ABBREVIATION	DEFINITION
Ad26	Adenovirus serotype 26 vector
Ad26.COV2.S	Replication incompetent adenovirus serotype 26 vector and a stabilized SARS-CoV-2 spike protein
AE	Adverse event
ATC	Anatomic Therapeutic Chemical
BMI	Body mass index
CI	Confidence interval
CMC	Carboxymethyl cellulose
COVID-19	Coronavirus disease 2019
CRF	Case report form
CSF	Cerebrospinal fluid
CTP	Clinical Trial Protocol
DD	Daily dose
DMC	Data monitoring committee
DMSO	Dimethyl sulfoxide
DPS	Data Presentation Specification Documents
DRC	Data Review Committee
ED	Emergency department
ELISA	Enzyme-linked immunosorbent assay
ELISpot	Enzyme-linked immunospot
FAS	Full analysis set
FDA	Food and Drug Administration
FHCRC	Fred Hutchinson Cancer Research Center
FU	Follow-up
GCP	Good Clinical Practice
GMC	Geometric mean concentration
GMT	Geometric mean titers
IC50	50% neutralization antibody titer
IC90	Samples with defined 90% inhibitory concentration
ICS	Intracellular cytokine staining
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IFN-γ	Interferon gamma

IL	Interleukin
LOD	Limit of detection
LLOQ	Lower limit of quantification
PBMC	Peripheral blood mononuclear cells
PFU	Plaque-forming units
PHE	Public Health England
PL	Placebo
PPE	Per protocol efficacy
PPI	Per protocol immunogenicity
PRO	Patient-reported outcome
psVNA	Pseudovirion neutralization assay
PT	Preferred terms
RNA	Ribonucleic acid
S	Spike protein
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SARS-COV-2	Severe acute respiratory syndrome coronavirus 2
SD	Standard deviation
SDTM	Study data tabulation model
SEB	Staphylococcal enterotoxin B
SIC	Symptoms of infection with coronavirus-19
SoA	Schedule of activities
TNFα	Tumor necrosis factor alpha
ULOQ	Upper limit if quantification
VE	Vaccine efficacy
VNA	Virus neutralization assay
Vp	Viral particles
WBC	White blood cells
WHO	World Health Organization
wtVNA	Wild-type virus neutralization assay

Supplementary Materials and Methods

Description of cohort 2

Cohort 2 will be initiated after the interim or primary analyses of Cohort 1a. In Cohort 2a, approximately 120 participants will receive Ad26.COV2.S at a dose level of 5×10^{10} vp and approximately 15 participants will receive a placebo in a single-dose primary regimen. In Cohort 2b, approximately 120 participants will receive Ad26.COV2.S at a dose level of 5×10^{10} vp and approximately 15 participants will receive a placebo in a 2-dose primary regimen.

To gain preliminary insight into the safety and immunogenicity of a single booster vaccination, designated participants in Cohort 2 who received Ad26.COV2.S for the single-dose (Cohort 2a) or 2 dose (Cohort 2b) primary regimen will receive a single booster vaccination of Ad26.COV2.S at 6 months, 12 months, or 24 months after completion of the primary regimen, and will receive placebo at other applicable time points. As a control, a subgroup of participants who received Ad26.COV2.S for the primary regimen will receive placebo at 6 months, 12 months, and 24 months after completion of the primary regimen. In addition, participants who received placebo for the primary regimen will receive placebo at 6 months, 12 months, and 24 months after completion of the primary regimen. See the below tables for further details. An Ad26.COV2.S dose level of 5×1010 vp will be used for the booster vaccination in Cohorts 2a and 2b.

SARS-CoV-2 wild-type virus neutralization assays

Neutralizing antibodies capable of inhibiting wild type virus infections were quantified using the wild type virus microneutralization assay (MNA) that was developed and qualified by Public Health England (PHE). The virus stocks used were derived from the Victoria/1/2020 strain.

In brief, 6 two-fold serial dilutions of the heat-inactivated human serum samples were prepared in 96-well transfer plate(s). The SARS-CoV-2 wild-type virus was subsequently added to the serum dilutions at a target working concentration (approximately 100 plaque-forming units [PFU]/well) and incubated at 37°C for 60 to 90 minutes. The serum-virus mixture was then transferred onto assay plates, previously seeded overnight with Vero E6 African green monkey kidney cells and incubated at 37°C and 5% CO_2 for 60 to 90 minutes before the addition of carboxymethyl cellulose (CMC) overlay medium and further incubation for 24 hours. Following this incubation, the cells were fixed and stained using an antibody pair specific for the SARS-CoV-2 RBD S protein and

immunoplaques were visualized using TrueBlue™ substrate. Immunoplaques were counted using the Immunospot Analyzer from CTL. The immunoplaque counts were exported to SoftMax Pro (Molecular Devices) and the neutralizing titer of a serum sample was calculated as the reciprocal serum dilution corresponding to the 50% neutralization antibody titer (IC_{50}) for that sample.

Spike enzyme-linked immunosorbent assay (ELISA)

SARS-CoV-2 Pre-Spike-specific binding antibody concentrations were determined using the human SARS-CoV-2 Pre-Spike IgG ELISA, an indirect ELISA which is based on the antibody/antigen interactions. The SARS-CoV-2 antigen used was a stabilized pre-fusion spike protein ((2P), Δfurin, T4 foldon, His-Tag), derived from the first clinical isolate of the Wuhan strain (Wuhan, 2019, whole genome sequence NC_045512), produced in ES-293 cells. The ELISA was developed and qualified for human serum at Nexelis, Laval, Canada.

In brief, purified SARS-CoV-2 Pre-Spike Antigen was adsorbed to the wells of a microplate and diluted serum samples (test samples, standard, and quality controls) were added. Unbound sample was washed away, and enzyme-conjugated anti-human IgG added. After washing excess conjugate away, 3,3′,5,5′-Tetramethylbenzidine (TMB) colorimetric substrate was added. After the established time period, the reaction was stopped. A reference standard on each test plate was used to quantify the amount of antibodies against SARS-CoV-2 Pre-Spike in the sample according to the unit assigned by the standard (ELISA Laboratory Unit per milliliter: EU/mL)

COVID-19 human convalescent serum (HCS) panels

For humoral assays, two HCS panels were used. One panel (N=32) was provided by Nexelis, Canada, and was used in the ELISA and wtVNA. Patients included in this HCS were mostly affected by severe COVOD-19 disease A second panel (N=31) was provided by BioIVT, Belgium, and was used in the ELISA only. This serum panel includes patients with asymptomatic or not defined disease severity. Donor characteristics (age, gender, blood collection from diagnosis) are available in table S4 and S5.

Ad26 neutralizing antibody assay

Ad26 neutralizing antibodies were measured essentially as previously described[1]. The Ad26 neutralization assay detects the amount of Ad26 neutralizing antibodies present in serum samples, which can inhibit the infection of A549 cells by Ad26. To this end, human serum samples were titrated 11 times in 2-fold steps from 1:4 to 1:4096, after which $5x10^6$ Ad26 virus particles encoding a luciferase reporter gene were added. After 30 to 90 minutes incubation, cells were added to the neutralization mix and further incubated for 24 hours (37 C° + 10% CO2). Subsequently, Luc substrate (NeoLite, Perkin Elmer) was added which lyses the cells, and functional expression of the reporter gene by Ad26 was measured. As a control, serum samples with defined 90% inhibitory concentration (IC90) values were included in each run. IC90 was calculated from a 4-parameter logistical regression curve and reported as the neutralization titer. The lower limit of qualification (LLOQ) for this assay was an IC90 of 17.

Intracellular Cytokine Staining (ICS)

T-cell responses to the S protein of SARS-CoV-2 were characterized by a validated 27-color ICS assay performed at the Fred Hutchinson Cancer Research Center (FHCRC), Seattle, WA, United States. Cryopreserved peripheral blood mononuclear cells (PBMC) were thawed, cultured overnight, and then stimulated for 6 hours at 37°C with two consensus peptide pools covering the entire length of the SARS-CoV-2 S protein, with dimethyl sulfoxide (DMSO, the peptide diluent) as the negative control, and staphylococcal enterotoxin B (SEB) as the positive control. Brefeldin A was included during the stimulation to prevent cytokine release. Cells were stained first with the viability dye, second with the extracellular antibody cocktail, then fixed and permeabilized and stained with the antibody cocktail for intracellular markers, including Th1 and Th2 cytokines. Cell fluorescence was acquired with 5-laser, 30-parameter Becton-Dickinson FACSymphony cytometers and analyzed using the FlowJo software.

Statistical methods

SARS-CoV-2 Spike (S)-binding antibody titers expressed as ELISA Unit per milliliters (EU/mL), and neutralizing antibody titers in the wtVNA, expressed as the reciprocal serum dilution neutralizing 50% of the test virus dose (50% inhibitory concentration [IC_{50}]), are displayed on a

log_{10} scale and described using geometric mean titers (GMT) and 95% confidence intervals (95%CIs). For both assays, seroconversion was defined as having an antibody titer above the lower limit of quantification (LLOQ) post vaccination if the baseline titer was below the LLOQ, or a 4-fold increase over baseline post vaccination if the baseline titer was above the LLOQ. ICS responses were described as percentage of total CD4+ or CD8+ T cell population. Sample positivity was determined with a one-sided Fisher's exact test comparing non-stimulated versus S peptide stimulated wells.[2] LLOQ was 0.022% and non-quantifiable values were imputed to LLOQ/2. Th1/Th2 ratio was calculated if the Th1 and/or Th2 responses were positive and above 2xLLOQ. If the Th1 or Th2 response from a participant was not fulfilling these criteria, the Th1/Th2 ratio was considered >1 if a Th2 response could not be measured and <1 if a Th1 response could not be measured. More details can be found in the Statistical Analysis Plan (SAP).

Supplementary Results

Length of follow-up for the participants per cohort

For cohort 1a, the median follow-up time is 94 days in all 5 arms. For Cohort, the median follow-up time is 24.5 days in the two active groups and 23 days in the Placebo group. For Cohort, participants were follow-up for 30 days in all 5 arms.

Vaccine safety and reactogenicity of Ad26.COV2.S

There were five SAEs. One related SAE of pyrexia was reported in a participant with no significant past medical history who received blinded study vaccination. Approximately 6 hours after vaccination, the participant reported a fever of 39.6ºC (103.3ºF), felt shortness of breath and had some palpitations. The participant went to the local emergency department (ED) without consulting the study investigators. Physical examination in the ED showed tachycardia, anxious appearance, and body temperature of 39.8ºC (103.6ºF) The participant was admitted to hospital overnight, given 1 g of intravenous paracetamol and became afebrile in less than 2 hours. The participant was discharged the following day. As the fever was considered related to blinded study vaccination by both the investigator and the sponsor, this report met criteria for expedited reporting.

A participant with a past medical history of hypotension with fainting spells since early adulthood and estrogen deficiency received blinded study vaccination. Approximately 8 hours later the participant developed a fever of 40ºC (104ºF) and a fainting episode. The participant took a single Tylenol tablet and the fever resolved but went to the ED because of the fainting spell. In the ED the participant was hypotensive so was admitted for treatment with fluid, pressors, and dexamethasone. The participant was released the next day. As the participant had a past history of hypotensive episodes with fainting, the investigator's and sponsor’s causality assessment was not related to blinded study vaccination. Although the fever was reported as related to blinded study vaccination, it was not considered serious since it was not the reason for hospitalization. This report did not meet criteria for expedited reporting.

A 67-year-old male participant was hospitalized for pneumonia legionella 60 days after receiving the first dose of blinded study vaccination. The presenting symptoms included headache, myalgia, malaise, chills, and fever of 38.8 degrees Celsius. Nasal swabs were negative for COVID-19. A

chest CT scan revealed bilateral pneumonia with pericardial effusion and ruled out COVID-19 pneumonia. Sputum culture was positive for Legionella pneumophila A. Treatment included levofloxacin, amoxicillin/clavulanic acid, paracetamol, and ibuprofen. The participant was discharged from the hospital after 3 days. He was afebrile, doing well with only a mild cough ongoing. The event was considered not related to study vaccination.

A 49-year-old female participant experienced worsening of multiple sclerosis approximately 5 weeks after receiving the first dose of the blinded study vaccination. Approximately 5 weeks prior to vaccination the participant had dizziness and cervicalgia. The symptoms of cervicalgia got better and then worse, however the participant was considered eligible for study vaccination. Subsequently, back pain and shoulder pain developed, as well as tingling and numb feeling in her right lower leg, belly and thorax and numbness in both arms. CSF showed intrathecal IgG-synthesis with 4 or more extra bands in CSF. Per the MRI of the neck and the brain, a neurologist diagnosed the participant with inflammatory high cervical myelin lesion with extensive cerebral demyelinating lesions, probably first manifestation of a longer (8-10 years) existing multiple sclerosis. The subject was admitted to the hospital and started on intravenous methylprednisolone 1g/day for 3 days. She had favorable response to the treatment. She was recovering with all symptoms resolved, apart from slightly reduced sensitivity in fingers and reduced power in both hands. Based on the MRI and neurological findings suggesting old identified lesions and preexisting symptomatology, the event was considered not related to study vaccination. The second dose of the study vaccine was withdrawn.

A 45-year-old male with a relevant medical history of kidney stones started having flank pain approximately 1.5 months after the first dose of the blinded study vaccination and went to the emergency care. He reported that the stone was “too large to pass” and therefore was admitted and consulted by a urologist who ended up placing a left ureteral stent. He was kept overnight and discharged on the next day. He had hematuria which has further improved. The participant was discharged on ciprofloxacin, phenazopyridine, oxybutynin, tamsulosin, and ibuprofen, which was further replaced by acetaminophen, as needed for pain. He was also prescribed oxycodone, as needed for severe pain. A lithotripsy procedure would be further planned. The event was considered not related to study vaccination.

Appendix 2. Changes to protocol-planned analyses

Appendix 3. Guidance for industry: toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials

Vital Signs *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Fever (°C) ** (°F) *	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute***	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

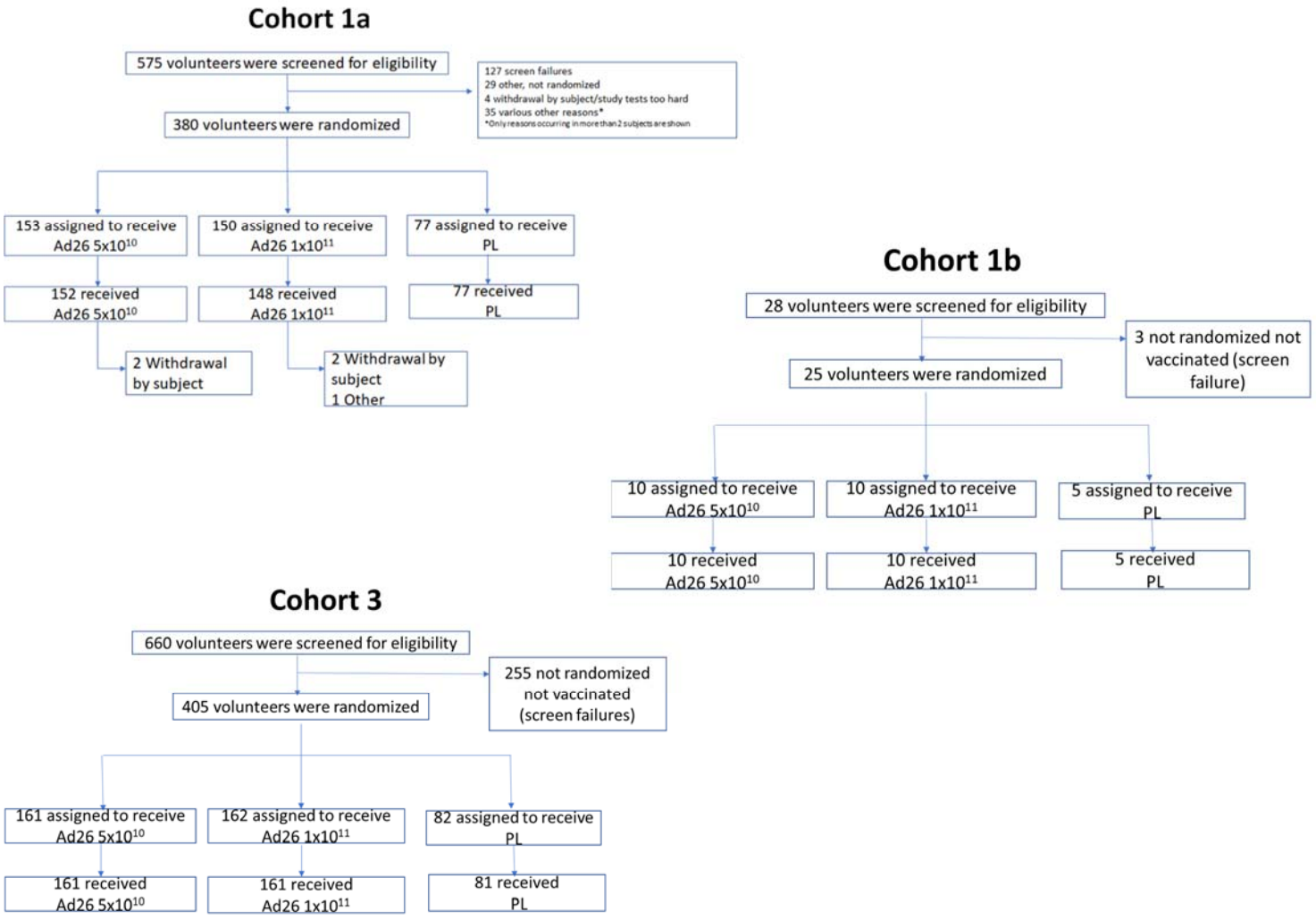
* Participant should be at rest for all vital sign measurements.

** Oral temperature; no recent hot or cold beverages or smoking.

*** When resting heart rate is between 60 - 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy participant populations, for example, conditioned athletes.

Supplementary Figures

Figure S1. Consort flow charts for cohort 1a, cohort 1b and cohort 3



Legend to Figure S1 Participants were enrolled concurrently at Belgian and US sites. Participants were randomized in parallel in a 1:1:1:1:1 ratio to one of five vaccination groups to receive one or two IM injections of Ad26.COV2.S at two dose levels of either $5x10^{10}$ vp or $1x10^{11}$ vp, or placebo. All four on active groups shown will equally receive one or two vaccinations. For cohort 1 and 3, in the absence of clinically significant findings 24 hours after the first vaccination was administered to five sentinel participants (two per dose level and one placebo), another ten participants were vaccinated across all groups. Safety data up to Day 28 were then reviewed by an internal data review committee before the remaining participants were randomized.

Figure S2. Summary of solicited AEs from cohort 1a, post dose 2.

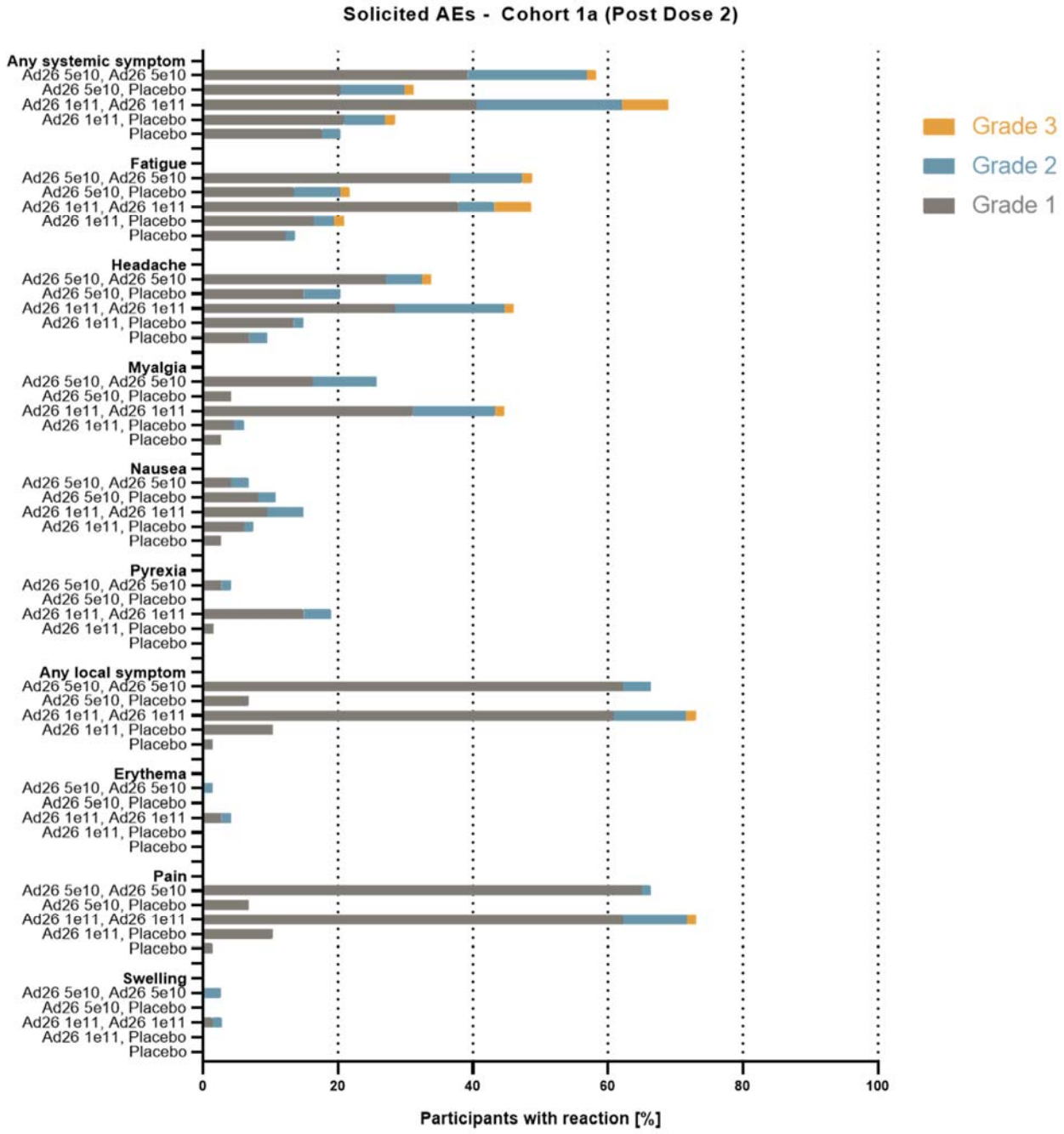
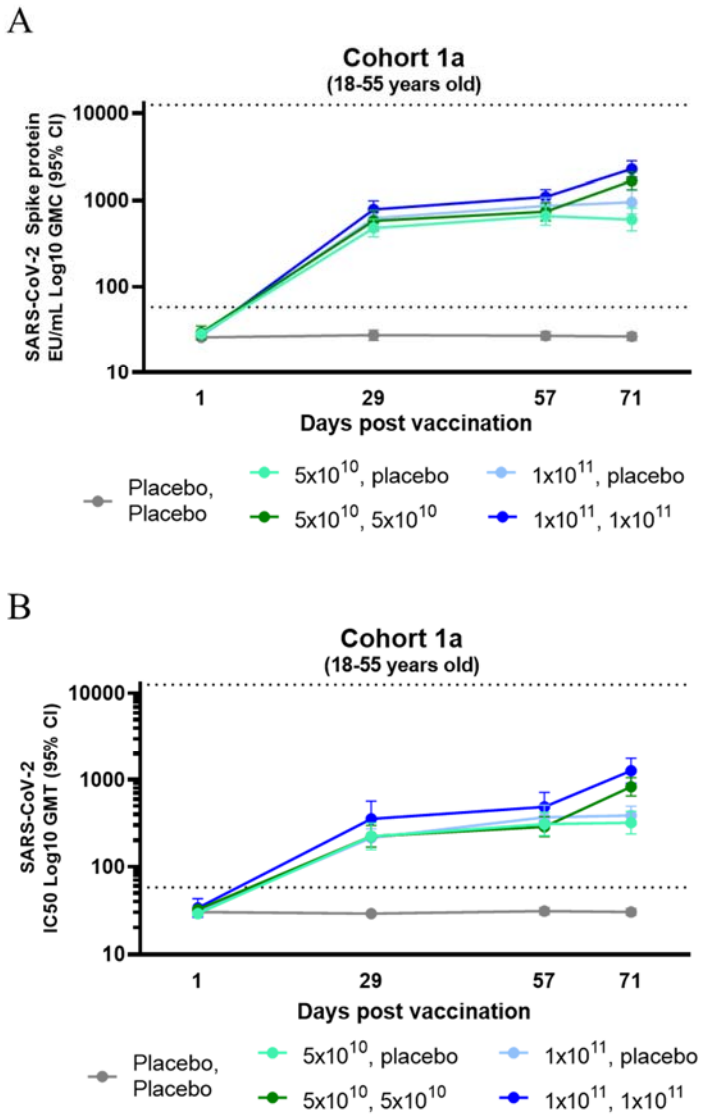
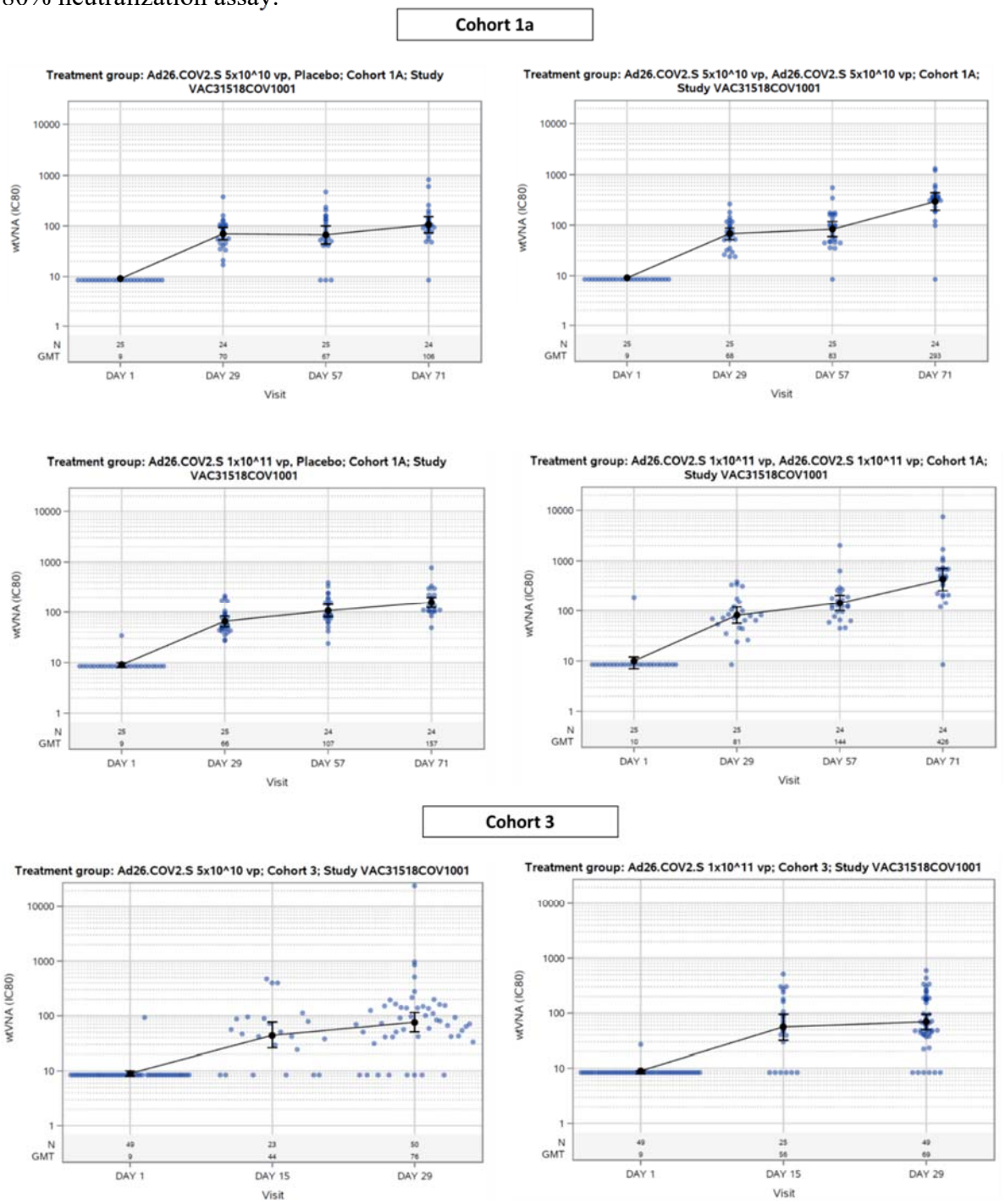


Figure S3. Humoral immunogenicity in cohort 1a.



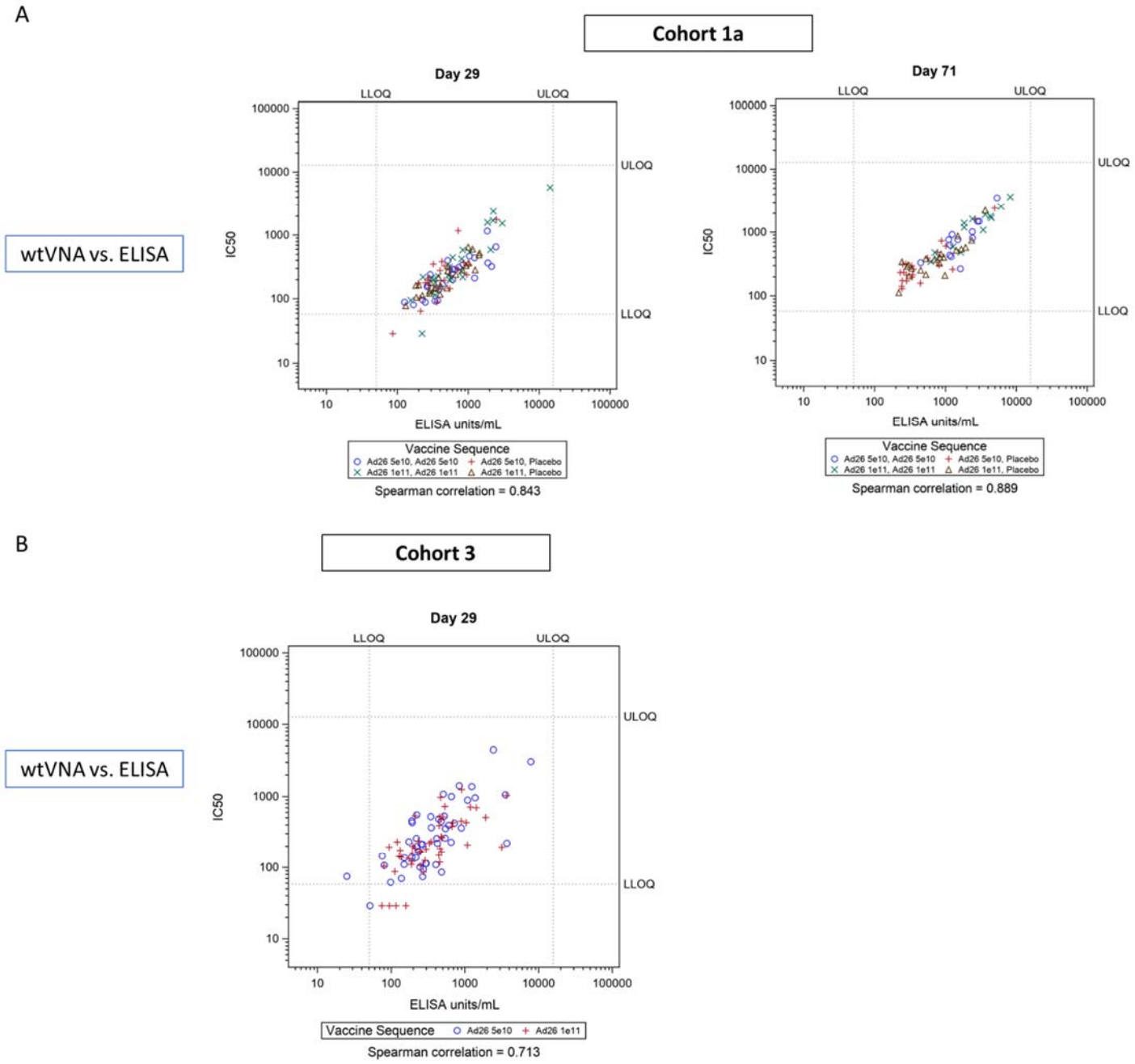
Shown are measures of humoral immunogenicity in serum samples obtained from the participants in cohort 1a, according to the receipt of the low or high dose of Ad26.COV2.S or placebo. Participants received two injections of high-dose or low-dose vaccine or placebo, as indicated with slashes (e.g., placebo/placebo if they received two injections of placebo). The samples were measured on enzyme-linked immunosorbent assay (ELISA) in ELISA units (EU) per milliliter (Panel A) and on wild-type virus neutralization assay, with seropositivity defined as a half maximal inhibitory concentration (IC50) titer of more than 58 at the lower limit of quantitation (Panel B). The values were measured at baseline and at day 29, day 57 and day 71. The two horizontal dotted lines in each panel indicate the lower and upper limits of quantification of the respective assay; I bars indicate 95% confidence intervals. HCS denotes human convalescent serum. For values below the lower limit of quantification, the values were divided by 2 and then plotted.

Figure S4. Log10 GMTs of serum SARS-CoV-2 neutralizing antibodies (wtVNA), measured by 80% neutralization assay.



IC80 Log10 GMT, as illustrated by the black dots and the numbers below each timepoint, at baseline and at Day 29, 57 and 71 post vaccination, among a subset of participants, according to schedule, cohort 1a (18-55 years old) and at day 1, 15 and 29 post vaccination cohort 3 (>65 years old).

Figure S5. Correlation between neutralizing antibody titers by wtVNA (IC50 log10) and binding antibody titers by ELISA (EU/mL log10).



(A) 29- and 71-days post vaccination 1 and 2, respectively in cohort 1a. (B) Correlation between neutralizing antibody titers by wtVNA and binding antibody titers by ELISA 29-days post vaccination 1 in cohort 3.

Figure S6. Correlation plot between Ad26 (IC90 log10) and SARS-CoV-2 (IC50 log10) neutralizing antibody titers: baseline Ad26 VNA baseline vs. wtVNA day 29, and Ad26 VNA at day 57 vs. wtVNA at day 71.

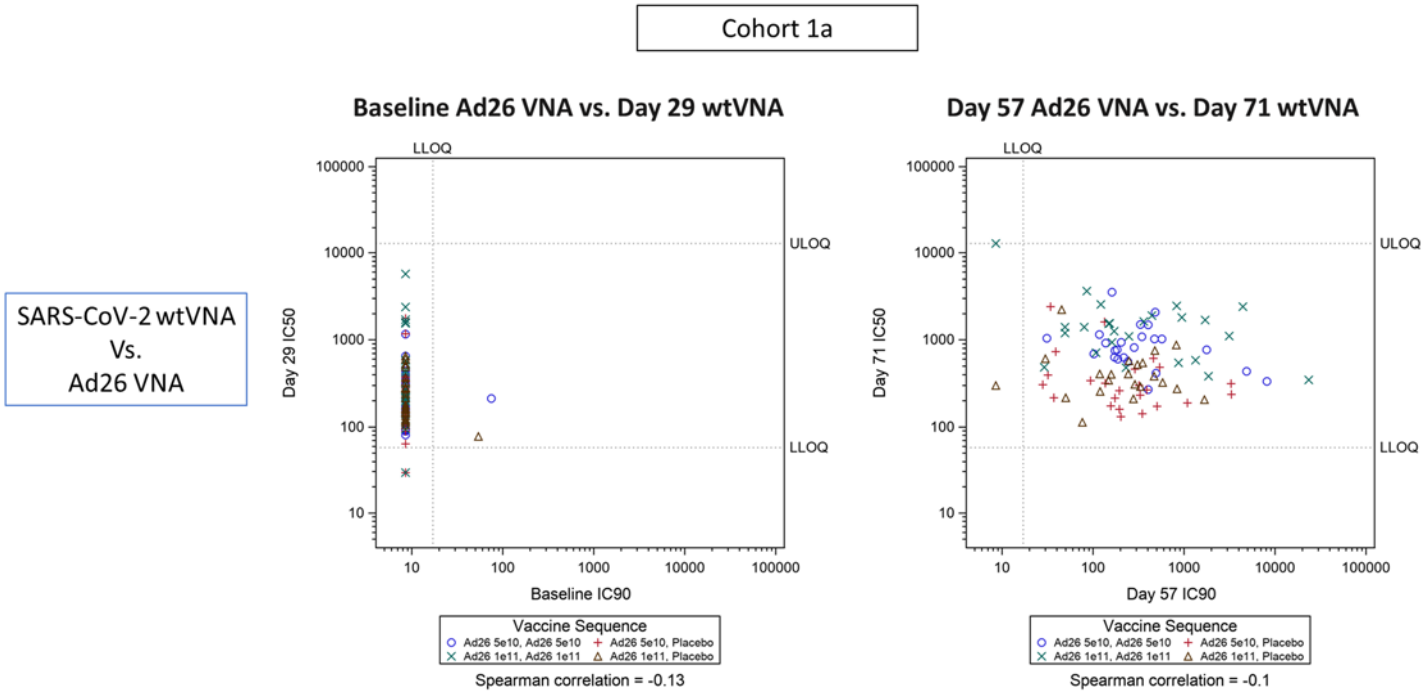
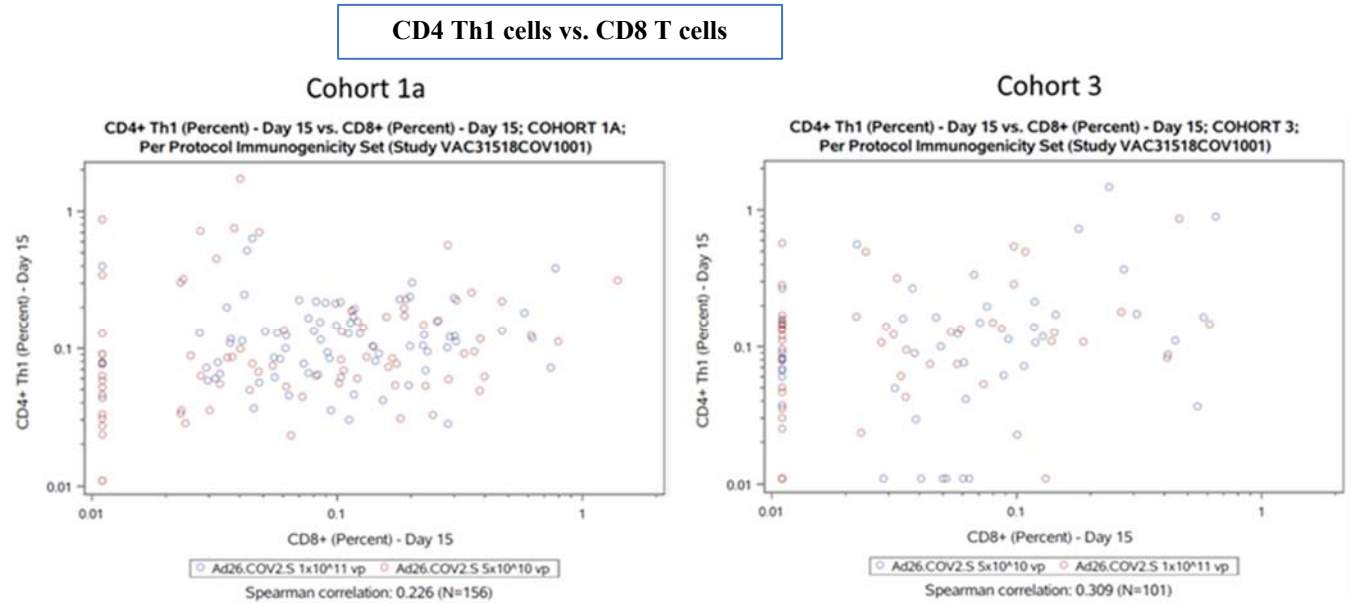


Figure S7. Correlation plot between the percent (log10) of CD4+ Th1 and CD8+T cell responses 15 days post vaccination in cohort 1a and 3.



Supplementary Tables

Table S1. COV1001 study design

Cohort 1a (Adults ≥18 to ≤55 years)			
Group	**N**	**Day 1 (Vaccination 1)**	**Day 57 (Vaccination 2)**
1	75	Ad26.COV2.S 5×10^{10} vp	Ad26.COV2.S 5×10^{10} vp
2	75	Ad26.COV2.S 5×10^{10} vp	Placebo
3	75	Ad26.COV2.S 1×10^{11} vp	Ad26.COV2.S 1×10^{11} vp
4	75	Ad26.COV2.S 1×10^{11} vp	Placebo
5	75	Placebo	Placebo
Cohort 1b (Adults ≥18 to ≤55 years)			
Group	**N**	**Day 1 (Vaccination 1)**	**Day 57 (Vaccination 2)**
1	5	Ad26.COV2.S 5×10^{10} vp	Ad26.COV2.S 5×10^{10} vp
2	5	Ad26.COV2.S 5×10^{10} vp	Placebo
3	5	Ad26.COV2.S 1×10^{11} vp	Ad26.COV2.S 1×10^{11} vp
4	5	Ad26.COV2.S 1×10^{11} vp	Placebo
5	5	Placebo	Placebo
Cohort 2a (Adults ≥18 to ≤55 years)			
Group	**N**	**Day 1 (Vaccination 1)**	**Day 57**
1-4	120	Ad26.COV2.S 5×10^{10} vp	No vaccination
5	15	Placebo	No vaccination
Cohort 2b (Adults ≥18 to ≤55 years)			
Group	**N**	**Day 1 (Vaccination 1)**	**Day 57 (Vaccination 2)**
1-4	120	Ad26.COV2.S 5×10^{10} vp	Ad26.COV2.S 5×10^{10} vp
5	15	Placebo	Placebo
Cohort 3 (Adults ≥65 years)			
Group	**N**	**Day 1 (Vaccination 1)**	**Day 57 (Vaccination 2)**
1	75	Ad26.COV2.S 5×10^{10} vp	Ad26.COV2.S 5×10^{10} vp
2	75	Ad26.COV2.S 5×10^{10} vp	Placebo
3	75	Ad26.COV2.S 1×10^{11} vp	Ad26.COV2.S 1×10^{11} vp
4	75	Ad26.COV2.S 1×10^{11} vp	Placebo
5	75	Placebo	Placebo
Total	**1,045**		

Details on cohort 2 design:

Cohort 2a Vaccination Schedule – Primary Regimen and Single Booster Vaccination

		Primary Regimen	Booster Vaccination		
Group	N	Day 1 [0] (Vac 1)	6 months [0]	12 months [0]	24 months [0]
1	30	Ad26.COV2.S 5×10^{10} vp	Placebo	Placebo	Placebo
2	30	Ad26.COV2.S 5×10^{10} vp	Ad26.COV2.S 5×10^{10} vp	Placebo	Placebo
3	30	Ad26.COV2.S 5×10^{10} vp	Placebo	Ad26.COV2.S 5×10^{10} vp	Placebo
4	30	Ad26.COV2.S 5×10^{10} vp	Placebo	Placebo	Ad26.COV2.S 5×10^{10} vp
5	15	Placebo	Placebo	Placebo	Placebo
Total	**135**				

[a] Study vaccine will be administered as a single-dose primary regimen.
[b] Study vaccine (Ad26.COV2.S or a placebo) will be administered at 6 months, 12 months, and 24 months after completion of the single-dose primary regimen.
N = number of participants; vac = vaccination; vp = virus particles.

Cohort 2b Vaccination Schedule – Primary Regimen and Single Booster Vaccination

		Primary Regimen		Booster Vaccination		
Group	N	Day 1[0] (Vac 1)	Day 57[0] (Vac 2)	8 months[0]	14 months[0]	26 months[0]
1	30	Ad26.COV2.S 5×10^{10} vp	Ad26.COV2.S 5×10^{10} vp	Placebo	Placebo	Placebo
2	30	Ad26.COV2.S 5×10^{10} vp	Ad26.COV2.S 5×10^{10} vp	Ad26.COV2.S 5×10^{10} vp	Placebo	Placebo
3	30	Ad26.COV2.S 5×10^{10} vp	Ad26.COV2.S 5×10^{10} vp	Placebo	Ad26.COV2.S 5×10^{10} vp	Placebo
4	30	Ad26.COV2.S 5×10^{10} vp	Ad26.COV2.S 5×10^{10} vp	Placebo	Placebo	Ad26.COV2.S 5×10^{10} vp
5	15	Placebo	Placebo	Placebo	Placebo	Placebo
Total	**135**					

[a] Study vaccine will be administered as a 2-dose (Day 1 and Day 57) primary regimen.
[b] Study vaccine (Ad26.COV2.S or a placebo) will be administered at 6 months, 12 months, and 24 months after completion of the 2-dose primary regimen. If the second vaccination at Day 57 is not provided, then participants will follow the same SoA as cohort 2a (i.e. booster vaccination at 6, 12, and 24 months after completion of the single-dose primary regimen).
N = number of participants; SoA = schedule of activities; vac = vaccination; vp = virus particles.

Table S2. Age distribution in cohort 3.

Count of Subject Number																				
Row Labels	**66**	**67**	**68**	**69**	**70**	**71**	**72**	**73**	**74**	**75**	**76**	**77**	**78**	**79**	**80**	**81**	**83**	**84**	**88**	**Grand Total**
Randomized	**50**	**44**	**44**	**35**	**23**	**33**	**23**	**26**	**19**	**24**	**12**	**7**	**4**	**3**	**1**	**1**	**2**	**1**	**2**	**403**
Belgium	30	25	19	18	11	16	12	13	9	9										183
United States of America	20	19	25	17	12	17	11	13	10	15	12	7	4	3	1	1	2	1	2	220
Grand Total	**50**	**44**	**44**	**35**	**23**	**33**	**23**	**26**	**19**	**24**	**12**	**7**	**4**	**3**	**1**	**1**	**2**	**1**	**2**	**403**

Table S3. Summary of solicited AEs in all cohorts

OVERALL SUMMARY OF SOLICITED ADVERSE EVENTS; COHORTS 1A, 1B AND 3; FULL ANALYSIS SET (COV1001 STUDY)

	Ad26 5e10	Ad26 1e11	Placebo
Cohort 1a			
Full Analysis Set	152	148	77
Participants with 1 or more:			
Solicited AE	115 (75.7%)	133 (89.9%)	21 (27.3%)
Solicited AE with worst grade of 3 or higher	14 (9.2%)	27 (18.2%)	0
Solicited local AE	98 (64.5%)	116 (78.4%)	8 (10.4%)
Solicited local AE with worst grade of 3 or higher	0	3 (2.0%)	0
Solicited systemic AE	99 (65.1%)	124 (83.8%)	17 (22.1%)
Solicited systemic AE with worst grade of 3 or higher	14 (9.2%)	27 (18.2%)	0
Solicited systemic AEs considered to be related to study vaccine	99 (65.1%)	123 (83.1%)	16 (20.8%)
Solicited systemic AEs of grade 3 or higher considered to be related to study vaccine	14 (9.2%)	27 (18.2%)	0
Cohort 1b			
Full Analysis Set	10	10	5
Participants with 1 or more:			
Solicited AE	7 (70.0%)	8 (80.0%)	4 (80.0%)
Solicited AE with worst grade of 3 or higher	0	5 (50.0%)	0
Solicited local AE	6 (60.0%)	7 (70.0%)	0
Solicited local AE with worst grade of 3 or higher	0	0	0
Solicited systemic AE	6 (60.0%)	8 (80.0%)	4 (80.0%)
Solicited systemic AE with worst grade of 3 or higher	0	5 (50.0%)	0
Solicited systemic AEs considered to be related to study vaccine	6 (60.0%)	8 (80.0%)	4 (80.0%)

OVERALL SUMMARY OF SOLICITED ADVERSE EVENTS; COHORTS 1A, 1B AND 3; FULL ANALYSIS SET (COV1001 STUDY)

	Ad26 5e10	Ad26 1e11	Placebo
Solicited systemic AEs of grade 3 or higher considered to be related to study vaccine	0	5 (50.0%)	0
Analysis set: Full	161	161	81
Cohort 3			
Full Analysis Set	161	161	81
Participants with 1 or more:			
Solicited AE	98 (60.9%)	109 (67.7%)	23 (28.4%)
Solicited AE with worst grade of 3 or higher	1 (0.6%)	4 (2.5%)	0
Solicited local AE	66 (41.0%)	68 (42.2%)	11 (13.6%)
Solicited local AE with worst grade of 3 or higher	0	1 (0.6%)	0
Solicited systemic AE	74 (46.0%)	88 (54.7%)	19 (23.5%)
Solicited systemic AE with worst grade of 3 or higher	1 (0.6%)	4 (2.5%)	0
Solicited systemic AEs considered to be related to study vaccine	72 (44.7%)	86 (53.4%)	16 (19.8%)
Solicited systemic AEs of grade 3 or higher considered to be related to study vaccine	1 (0.6%)	4 (2.5%)	0

Table S4. Grade 3 unsolicited AEs in Cohort 1a and cohort 3

Cohort	ID*	Dose 1	Age Range	Sex	Preferred Term	Reported Term for the Adverse Event	Study Day of Start of Adverse Event	Study Day of End of Adverse Event	Serious Event	Causality	Action Taken with Study Treatment	Outcome of Adverse Event	Concomitant or Additional Tx Given
COHORT 1A	1	Ad26.COV2.S 1x10^11 vp	46-55 years	F	Blood pressure decreased	DECREASED BLOOD PRESSURE	1	4	Y	NOT RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	Y
COHORT 1A	2	Placebo	46-55 years	M	White blood cell count increased	WBC INCREASE	8	12	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	N
COHORT 1A	3	Ad26.COV2.S 1x10^11 vp	18-30 years	F	Malaise	MALAISE	1	2	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	N
COHORT 1A	4	Ad26.COV2.S 5x10^10 vp	18-30 years	F	Back pain	BACKPAIN	2	2	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	Y
COHORT 1A	5	Ad26.COV2.S 5x10^10 vp	31-45 years	F	Hypotensive crisis	HYPOTENSIVE CRISIS	1	1	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	Y
COHORT 1A	6	Ad26.COV2.S 1x10^11 vp	31-45 years	M	Insomnia	INSOMNIA	2	2	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	N
COHORT 1A	7	Placebo	18-30 years	M	Contusion	CONTUSION RIGHT ANKLE	5	43	N	NOT RELATED	DOSE NOT CHANGED	RECOVERING/RESOLVING	Y
COHORT 1A	8	Ad26.COV2.S 1x10^11 vp	31-45 years	M	Pyrexia	FEVER	1	2	Y	RELATED	DRUG WITHDRAWN	RECOVERED/RESOLVED	Y
COHORT 1A	9	Ad26.COV2.S 1x10^11 vp	18-30 years	F	Back pain	BACKPAIN	1	2	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	Y
COHORT 1A	10	Ad26.COV2.S 1x10^11 vp	18-30 years	M	Dizziness	LIGHTHEADEDNESS	2	2	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	N
COHORT 1A	11	Ad26.COV2.S 5x10^10 vp	18-30 years	F	Heat stroke	SUNSTROKE	13	13	N	NOT RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	Y
COHORT 1A	12	Ad26.COV2.S 1x10^11 vp	31-45 years	F	Neck pain	NECK PAIN	1	16	N	NOT RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	Y
COHORT 3	13	Ad26.COV2.S 5x10^10 vp	65-75 years	F	Vomiting	VOMITING	6	7	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	N
COHORT 3	13	Ad26.COV2.S 5x10^10 vp	65-75 years	F	Dizziness	DIZZINESS	6	7	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	N
COHORT 3	14	Ad26.COV2.S 5x10^10 vp	>75 years	M	Systolic hypertension	GRADE 3 SYSTOLIC HYPERTENSION	15	18	N	NOT RELATED	DOSE NOT CHANGED	RECOVERING/RESOLVING	N

Cohort	ID*	Dose 1	Age Range	Sex	Preferred Term	Reported Term for the Adverse Event	Study Day of Start of Adverse Event	Study Day of End of Adverse Event	Serious Event	Causality	Action Taken with Study Treatment	Outcome of Adverse Event	Concomitant or Additional Tx Given
COHORT 3	15	Ad26.COV2.S 5x10^10 vp	65-75 years	M	Hypertension	HYPERTENSION WORSENING	8	17	N	RELATED	DOSE NOT CHANGED	RECOVERING/RESOLVING	N
COHORT 3	16	Ad26.COV2.S 5x10^10 vp	65-75 years	F	Systolic hypertension	SYSTOLIC HYPERTENSION (GRADE 3)	1	1	N	RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	N
COHORT 3	16	Ad26.COV2.S 5x10^10 vp	65-75 years	F	Bradycardia	WORSENING OF BRADYCARDIA	1	1	N	NOT RELATED	DOSE NOT CHANGED	RECOVERED/RESOLVED	N

* ID: scrambled identifier, not tied to the actual participant ID in the clinical database. This scrambled ID allows to determine which participants reported multiple grade 3 unsolicited AEs.

Table S5. Human convalescent serum panel used in the ELISA and wtVNA

Patient #	Date of Visit (Screening)	Age (Screening)	Sex	Disease severity
1	21May2020	49	M	Severe
2	21May2020	38	F	Severe
3	21May2020	74	M	Severe
4	22May2020	41	F	Severe
5	26May2020	65	M	Mild
6	27May2020	23	F	Severe
7	27May2020	27	M	Severe
8	28May2020	50	F	Severe
9	28May2020	60	M	Moderate
10	28May2020	57	F	Severe
11	28May2020	48	F	Severe
12	29May2020	53	F	Severe
13	01Jun2020	32	M	Severe
14	01Jun2020	47	M	N/A
15	01Jun2020	30	F	Severe
16	01Jun2020	32	F	Severe
17	01Jun2020	52	F	Moderate
18	03Jun2020	54	F	Moderate
19	05Jun2020	31	F	Severe
20	05Jun2020	54	F	Severe
21	08Jun2020	49	F	Severe
22	10Jun2020	N/A	F	Severe
23	10Jun2020	79	F	Severe
24	11Jun2020	58	F	Mild
25	11Jun2020	38	F	Severe
26	11Jun2020	30	F	Severe

27	15Jun2020	27	F	Mild
28	15Jun2020	71	F	Mild
29	17Jun2020	36	F	Moderate
30	19Jun2020	44	F	Severe
31	25Jun2020	24	M	Moderate
32	02Jul2020	74	M	Moderate
33	02Jul2020	69	F	Severe

Table S6. Human convalescent serum panel used in the ELISA only.

Gender:	**Age (Years):**	**Race:**	**Severity:**
Male	38	White	N/A
Female	49	White	N/A
Female	25	Caucasian	N/A
Male	53	White	N/A
Male	61	White	N/A
Male	34	White	N/A
Female	34	White	N/A
Female	44	White	N/A
Male	60	White	N/A
Male	31	White	N/A
Male	38	African American	N/A
Male	48	White	N/A
Female	28	White	N/A
Male	41	White	N/A
Female	52	White	N/A
Female	37	White	N/A
Male	35	White	Mild
Male	35	White	Mild
Male	28	White	Asymptomatic
Male	34	White	Mild
Female	30	White	Asymptomatic
Male	37	African American	Asymptomatic
Female	35	White	Asymptomatic
Male	27	White	Asymptomatic
Male	33	White	Asymptomatic
Male	57	Caucasian	Asymptomatic
Female	56	Caucasian	Asymptomatic

Female	60	White	Asymptomatic
Female	61	White	Asymptomatic
Female	35	White	Asymptomatic

References

1. Sprangers MC, Lakhai W, Koudstaal W, Verhoeven M, Koel BF, Vogels R, et al. Quantifying adenovirus-neutralizing antibodies by luciferase transgene detection: addressing preexisting immunity to vaccine and gene therapy vectors. Journal of clinical microbiology. 2003;41(11):5046-52. PubMed PMID: 14605137; PubMed Central PMCID: PMC262545.

2. Horton H, Thomas EP, Stucky JA, et al. Optimization and validation of an 8-color intracellular cytokine staining (ICS) assay to quantify antigen-specific T cells induced by vaccination. J Immunol Methods 2007;323:39–54.