Supplemental Online Content

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

eMethods. Supplemental Methods

Pseudovirus neutralizing antibody assay

The SARS-CoV-2 pseudoviruses expressing a luciferase reporter gene were used to measure pseudovirus neutralizing antibodies[1,2]. In brief, the packaging construct psPAX2 (AIDS Resource and Reagent Program), luciferase reporter plasmid pLenti-CMV Puro-Luc (Addgene) and spike protein expressing pcDNA3.1-SARS-CoV-2 SΔCT were co-transfected into HEK293T cells (ATCC CRL_3216) with lipofectamine 2000 (ThermoFisher Scientific). Pseudoviruses of SARS-CoV-2 variants were generated by using WA1/2020 strain (Wuhan/WIV04/2019, GISAID accession ID: EPI_ISL_402124), Beta B.1.351 variant (GISAID accession ID: EPI_ISL_712096), Delta B.1.617.2 (GISAID accession ID: EPI_ISL_2020950), Omicron B.1.1.529 BA.1 (GISAID ID: EPI_ISL_7358094.2), or BA.2 (GSAID ID: EPI_ISL_6795834.2). The supernatants containing the pseudotype viruses were collected 48h after transfection; pseudotype viruses were purified by filtration with 0.45-μm filter. To determine the neutralization activity of human serum, HEK293T-hACE2 cells were seeded in 96-well tissue culture plates at a density of 2×10^4 cells per well overnight. Three-fold serial dilutions of heat-inactivated serum samples were prepared and mixed with 50 μl of pseudovirus. The mixture was incubated at 37 °C for 1 h before adding to HEK293T-hACE2 cells. After 48 h, cells were lysed in Steady-Glo Luciferase Assay (Promega) according to the manufacturer's instructions. SARS-CoV-2 neutralization titers were defined as the sample dilution at which a 50% reduction (NT50) in relative light units was observed relative to the average of the virus control wells.

Live virus neutralizing antibody assay

Full-length SARS-CoV-2 D614G, Delta and Omicron viruses were designed to express nanoluciferase (nLuc) and were recovered via reverse genetics[3]. One day before the assay, Vero C1008 cells were plated at 20,000 cells per well in black bottomed 96 well plates. Cells were inspected to ensure confluency on the day of assay. Serum samples were tested at a starting dilution of 1:20 and were serially diluted 5-fold up to 8 dilution spots. Serially diluted serum samples were mixed in equal volume with diluted virus. Antibody–virus, virus-only mixtures and appropriate controls were then incubated at 37 °C with 5% CO_2 for 1 h. After incubation, the serum-virus mixtures were added in duplicate to the cells at 800 plaque-forming units per well at 37 °C with 5% CO_2. Twenty-four hours later, the cells were lysed, and luciferase activity was measured via Nano-Glo Luciferase Assay System (Promega) according to the manufacturer specifications. Luminescence was measured by a Promega Glomax. Virus neutralization titers were defined as the sample dilution at which a 50% reduction in RLU was observed relative to the average of the virus control wells.

Enzyme-linked immunosorbent assay (ELISA)

SARS-CoV-2 spike receptor-binding domain (RBD)-specific binding antibodies in serum were assessed by ELISA. 96-well plates were coated with 0.5 μg/mL of SARS-CoV-2 WA1/2020, B.1.617.2 (Delta), B.1.351 (Beta), B.1.1.529 (Omicron BA.1), or B.1.1.529 (Omicron BA.2) RBD protein in 1× Dulbecco phosphate-buffered saline (DPBS) and incubated at 4 °C overnight. After incubation, plates were washed once with wash buffer (0.05% Tween 20 in 1× DPBS) and blocked with 350 μL of casein block solution per well for 2 to 3 hours at room temperature. Following incubation, block solution was discarded and plates were blotted dry. Serial dilutions of heat-inactivated serum diluted in Casein block were added to wells, and plates

were incubated for 1 hour at room temperature, prior to 3 more washes and a 1-hour incubation with a 1:4000 dilution of anti–human IgG horseradish peroxidase (HRP) (Invitrogen, ThermoFisher Scientific) at room temperature in the dark. Plates were washed 3 times, and 100 μL of SeraCare KPL TMB SureBlue Start solution was added to each well; plate development was halted by adding 100 μL of SeraCare KPL TMB Stop solution per well. The absorbance at 450 nm, with a reference at 650 nm, was recorded with a VersaMax microplate reader (Molecular Devices). For each sample, the ELISA end point titer was calculated using a 4-parameter logistic curve fit to calculate the reciprocal serum dilution that yields a corrected absorbance value (450 nm-650 nm) of 0.2. Interpolated end point titers were reported.

Electrochemiluminescence assay (ECLA)

ECLA plates (MesoScale Discovery SARS-CoV-2 IgG Cat No: K15463U-2; Panel 22) were designed and produced with up to 10 antigen spots in each well[4]. The plates were blocked with 50 uL of Blocker A (1% BSA in distilled water) solution for at least 30 minutes at room temperature shaking at 700 rpm with a digital microplate shaker. During blocking the serum was diluted to 1:5,000 in Diluent 100. The calibrator curve was prepared by diluting the calibrator mixture from MSD 1:9 in Diluent 100 and then preparing a 7-step 4-fold dilution series plus a blank containing only Diluent 100. The plates were then washed 3 times with 150 μL of Wash Buffer (0.5% Tween in 1x PBS), blotted dry, and 50 μL of the diluted samples and calibration curve were added in duplicate to the plates and set to shake at 700 rpm at room temperature for at least 2 h. The plates were again washed 3 times and 50 μL of SULFO-Tagged anti-Human IgG detection antibody diluted to 1x in Diluent 100 was added to each well and incubated shaking at 700 rpm at room temperature for at least 1 h. Plates were then washed 3 times and 150 μL of

MSD GOLD Read Buffer B was added to each well and the plates were read immediately after on a MESO QuickPlex SQ 120 machine. MSD titers for each sample was reported as Relative Light Units (RLU) which were calculated using the calibrator.

Antibody-dependent phagocytosis (ADCP) assay

Antigens were biotinylated using sulfo-NHS LC-LC biotin, coupled to yellow-green fluorescent Neutravidin 1 μm beads (Invitrogen, F8776) for 2 hours at 37˚C and washed two times in 0.1% BSA in PBS. 10 μL/well of coupled beads were added to 96-well plates with 10 μL/well of diluted sample for 2 hours at 37˚C to form immune complexes. After incubation, the immune complexes were spun down and the supernatant was removed. THP-1 cells were added at a concentration of 2.5 x 104 cells/well and incubated for 18 hours at 37˚C. After incubation, the plates were spun down, the supernatant was removed, and cells were fixed with 4% PFA for 10 minutes. Fluorescence was acquired with a Stratedigm 1300EXi cytometer. Phagocytic score was calculated using the following formula: (percentage of FITC+ cells) * (the geometric mean fluorescent intensity (gMFI) of the FITC+ cells)/10,000.

Antibody-dependent neutrophil phagocytosis (ADNP) assay

Antigens were coupled to beads and immune complexes were formed as described for ADCP. Neutrophils were isolated from fresh whole ACD blood using EasySep Direct Human Neutrophil Isolation kit (Stem Cell,19666), resuspended in R10, and added to plates at a concentration of 5x104 cells/well. The plates were incubated for 30min at 37˚C. The neutrophil marker CD66b (Pacific Blue conjugated anti-CD66b; BioLegend, 305112) was used to stain

cells. Cells were fixed for 10 minutes in 4% PFA. Fluorescence was acquired with a Stratedigm 1300EXi cytometer and phagocytic score was calculated as described for ADCP.

Antibody-dependent complement deposition (ADCD) assay

Antigens were coupled to non-fluorescent Neutravidin 1 μm beads (Invitrogen, F8777) as described for ADCP. Immune complexes were formed by incubating 10 μL of coupled beads with 10 μL of diluted sample for 2 hours at 37˚C. Plates were spun down, and immune complexes were washed with PBS. Lyophilized guinea pig complement (Cedarlane, CL4051) was resuspended in cold water, diluted in Gelatin Veronal Buffer, Boston BioProducts, IBB-290X) and added to the immune complexes. The plates were incubated for 50 minutes at 37˚C and the reaction was stopped by washing the plates twice with 15mM EDTA in PBS. To detect complement deposition, plates were incubated with Fluorescein-conjugated goat anti-guinea pig complement C3 (MP Biomedicals, 0855385) for 15 minutes in the dark. Fluorescence was acquired with a Stratedigm 1300EXi cytometer.

Intracellular cytokine staining (ICS) assay

CD4+ and CD8+ T cell responses were quantitated by pooled peptide-stimulated intracellular cytokine staining (ICS) assays[5]. Peptide pools contained 15 amino acid peptides overlapping by 11 amino acids spanning the SARS-CoV-2 WA1/2020 or B.1.1.529 (Omicron BA.1) Spike proteins (21st Century Biochemicals). 10^6 peripheral blood mononuclear cells were re-suspended in 100 μL of R10 media supplemented with CD49d monoclonal antibody (1 μg/mL) and CD28 monoclonal antibody (1 μg/mL). Each sample was assessed with mock (100 μL of R10 plus 0.5% DMSO; background control), peptides (2 μg/mL), and/or 10 pg/mL

phorbol myristate acetate (PMA) and 1 µg/mL ionomycin (Sigma-Aldrich) (100µL; positive control) and incubated at 37°C for 1 h. After incubation, 0.25 µL of GolgiStop and 0.25 µL of GolgiPlug in 50 µL of R10 was added to each well and incubated at 37°C for 8 h and then held at 4°C overnight. The next day, the cells were washed twice with DPBS, stained with aqua live/dead dye for 10 mins and then stained with predetermined titers of monoclonal antibodies against CD279 (clone EH12.1, BB700), CD4 (clone L200, BV711), CD27 (clone M-T271, BUV563), CD8 (clone SK1, BUV805), CD45RA (clone 5H9, APC H7) for 30 min. Cells were then washed twice with 2% FBS/DPBS buffer and incubated for 15 min with 200 µL of BD CytoFix/CytoPerm Fixation/Permeabilization solution. Cells were washed twice with 1X Perm Wash buffer (BD Perm/WashTM Buffer 10X in the CytoFix/CytoPerm Fixation/ Permeabilization kit diluted with MilliQ water and passed through 0.22µm filter) and stained with intracellularly with monoclonal antibodies against Ki67 (clone B56, BB515), IL21 (clone 3A3-N2.1, PE), CD69 (clone TP1.55.3, ECD), IL10 (clone JES3-9D7, PE CY7), IL13 (clone JES10-5A2, BV421), IL4 (clone MP4-25D2, BV605), TNF-α (clone Mab11, BV650), IL17 (clone N49-653, BV750), IFN-γ (clone B27; BUV395), IL2 (clone MQ1-17H12, BUV737), IL6 (clone MQ2-13A5, APC), and CD3 (clone SP34.2, Alexa 700) for 30 min. Cells were washed twice with 1X Perm Wash buffer and fixed with 250µL of freshly prepared 1.5% formaldehyde. Fixed cells were transferred to 96-well round bottom plate and analyzed by BD FACSymphony™ system. Data were analyzed using FlowJo v9.9.

RBD-specific B cell staining

PBMCs were stained[6] with Aqua live/dead dye for 20 min, washed with 2% FBS/DPBS buffer, and cells were suspended in 2% FBS/DPBS buffer with Fc Block (BD) for 10 min,

followed by staining with monoclonal antibodies against CD45 (clone HI30, BV786), CD3 (clone UCHT1, APC-R700), CD16 (clone 3G8, BUV496), CD14 (clone M5E2, BV605), CD19 (clone SJ25C, BUV615), CD20 (clone 2H7, PE-Cy5), IgM (clone G20-127, BUV395), IgD (clone IA6-2, PE), IgG (clone G18-145, BUV737), CD95 (clone DX2, BV711), CD27 (clone M-T271, BUV563), CD21 (clone B-ly4, PE-CF594), CD38 (clone HB7, BUV805), CD71 (clone M-A712, BV750) and staining with SARS-CoV-2 antigens including biotinylated SARS-CoV-2 (WA1/2020) RBD proteins (Sino Biological), SARS-CoV-2 (WA1/2020) RBD proteins (Sino Biological) labeled with FITC, SARS-CoV-2 (B.1.1.529) RBD proteins (Sino Biological) labeled with DyLight 405 and SARS-CoV-2 (B.1.617.2) RBD proteins (Sino Biological) labeled with APC. Staining was done at 4 °C for 30 min. After staining, cells were washed twice with 2% FBS/DPBS buffer, followed by incubation with BV650 streptavidin (BD Pharmingen) for 10 min, then washed twice with 2% FBS/DPBS buffer. After staining, cells were washed and fixed by 2% paraformaldehyde. All data were acquired on a BD FACSymphony flow cytometer. Subsequent analyses were performed using FlowJo software (BD Bioscience, v10.8.1). For analyses, in singlet gate, dead cells were excluded by Aqua dye and CD45 was used as a positive inclusion gate for all leukocytes. Within class-switched B cell population gated as CD19+ IgG+ IgM- IgD- CD3- CD14- CD16-, SARS-CoV-2 (WA1/2020) RBD-specific B cells were identified as double positive for WA1/2020 RBD proteins labeled with different fluorescent probes. From WA1/2020 RBD-specific B cells, B.1.1.529 or B.1.617.2 RBD-specific B cells were defined as cell population positive for B.1.1.529 or B.1.617.2 RBD proteins. The SARS-CoV-2-specific B cells were further distinguished according to CD21/CD27 phenotype distribution.

eReferences

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2. Yu J, Li Z, He X, et al. Deletion of the SARS-CoV-2 Spike Cytoplasmic Tail Increases Infectivity in Pseudovirus Neutralization Assays. *J Virol.* 2021.
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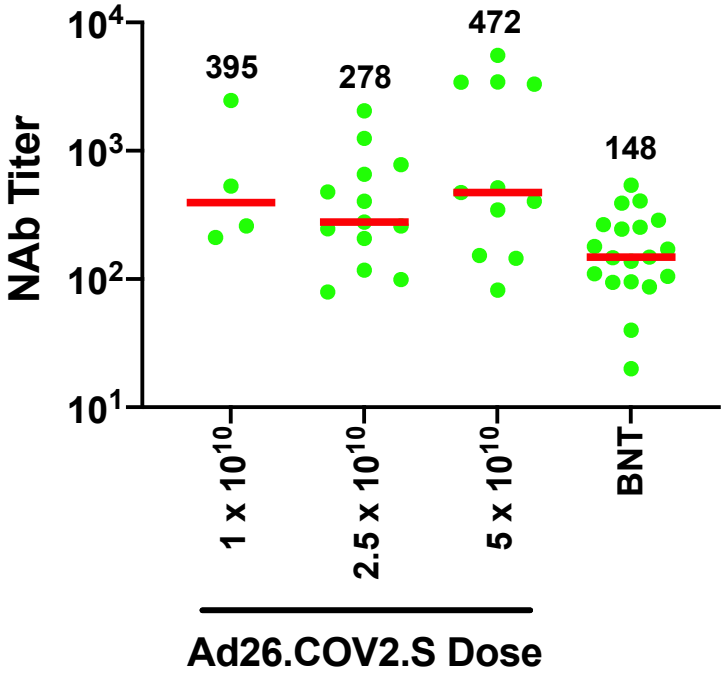
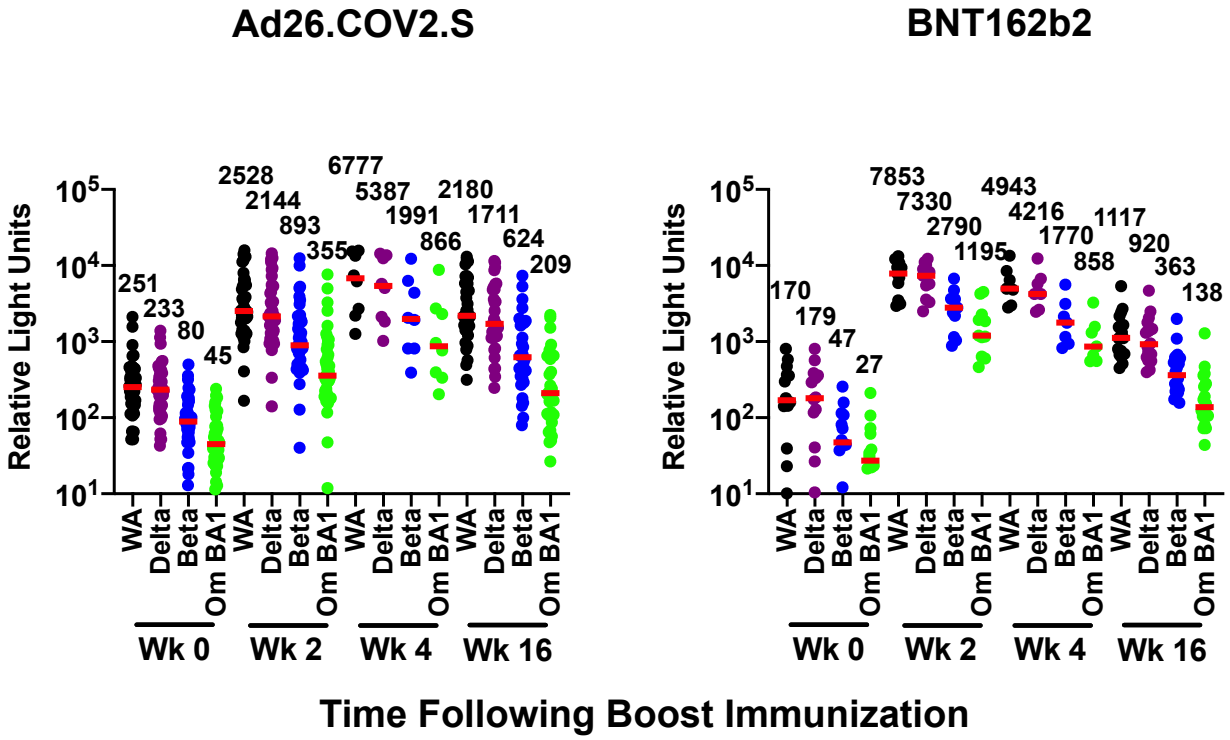
4. Jacob-Dolan C, Feldman J, McMahan K, et al. Coronavirus-Specific Antibody Cross Reactivity in Rhesus Macaques Following SARS-CoV-2 Vaccination and Infection. *J Virol.* 2021.
5. Liu J, Chandrashekar A, Sellers D, et al. Vaccines elicit highly conserved cellular immunity to SARS-CoV-2 Omicron. *Nature.* 2022;603(7901):493-496.
6. He X, Chandrashekar A, Zahn R, et al. Low-dose Ad26.COV2.S protection against SARS-CoV-2 challenge in rhesus macaques. *Cell.* 2021;184(13):3467-3473 e3411.

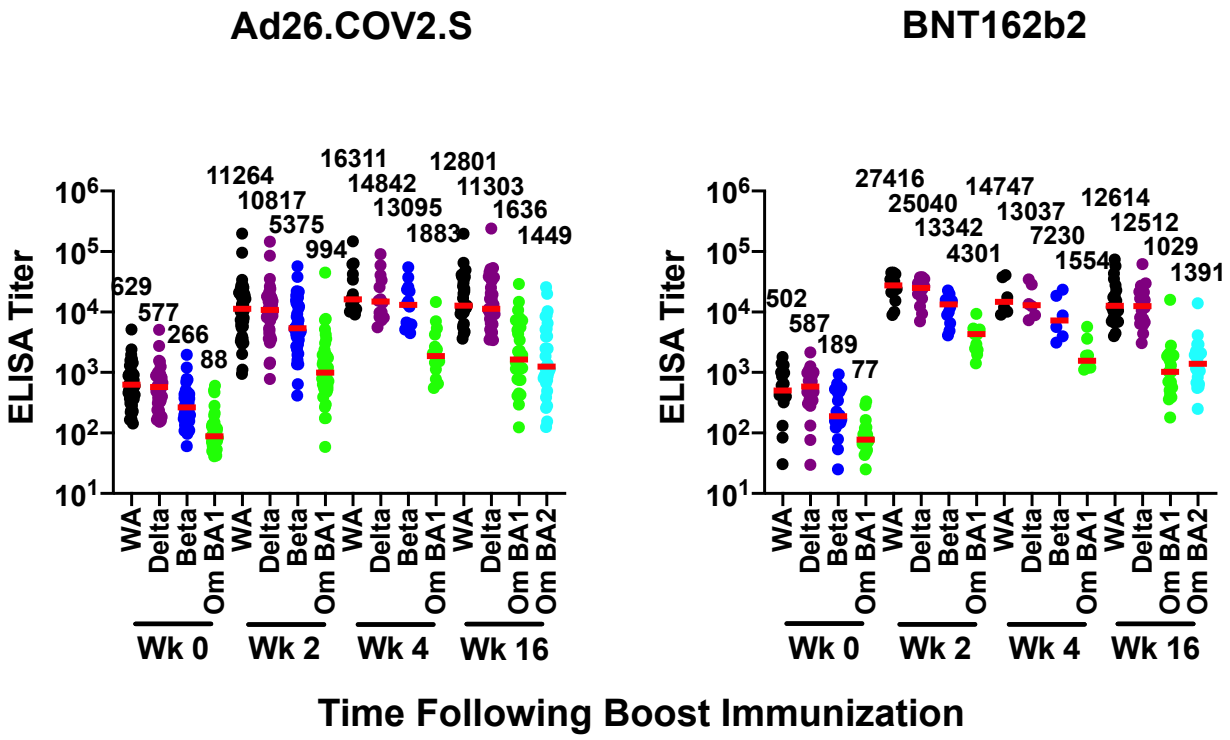
eFigure 1. Pseudovirus Neutralizing Antibody Responses Following Ad26.COV2.S by Dose or BNT162b2 Boosting. Pseudovirus neutralizing antibody (NAb) titers to B.1.1.529 (Omicron BA.1) at week 16 following boosting of BNT162b2 vaccinated individuals with $1x10^{10}$, $2.5x10^{10}$, or $5x10^{10}$ viral particles (vp) Ad26.COV2.S or BNT162b2. Medians (red bars) are depicted and numerically shown.



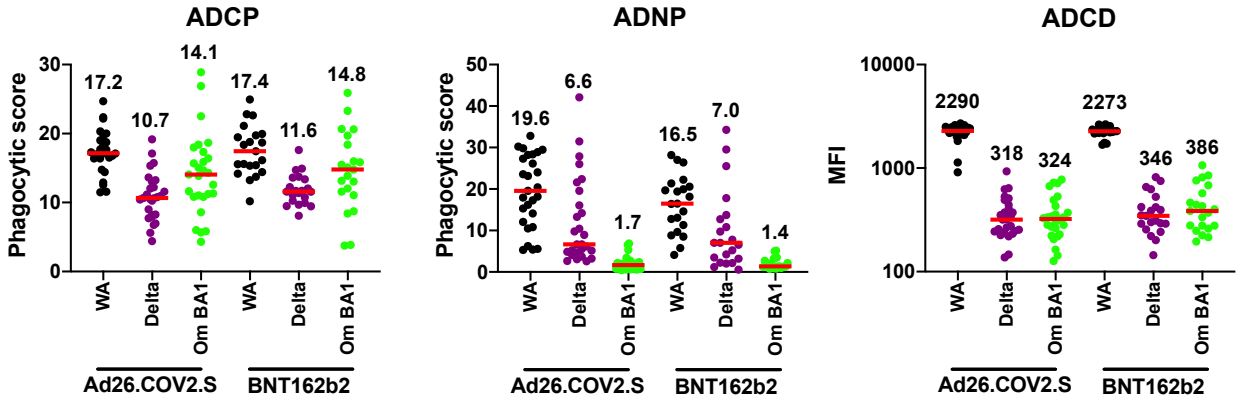
eFigure 2. ECLA Responses Following Ad26.COV2.S or BNT162b2 Boosting. RBD-specific binding antibody titers by ECLA at weeks 0, 2, 4, and 16 following boosting of BNT162b2 vaccinated individuals with Ad26.COV2.S or BNT162b2. ECLA titers to SARS-CoV-2 W1/2020 (WA), B.1.617.2 (Delta), B.1.351 (Beta), and B.1.1.529 (Omicron BA.1; Om BA1). Medians (red bars) are depicted and numerically shown.



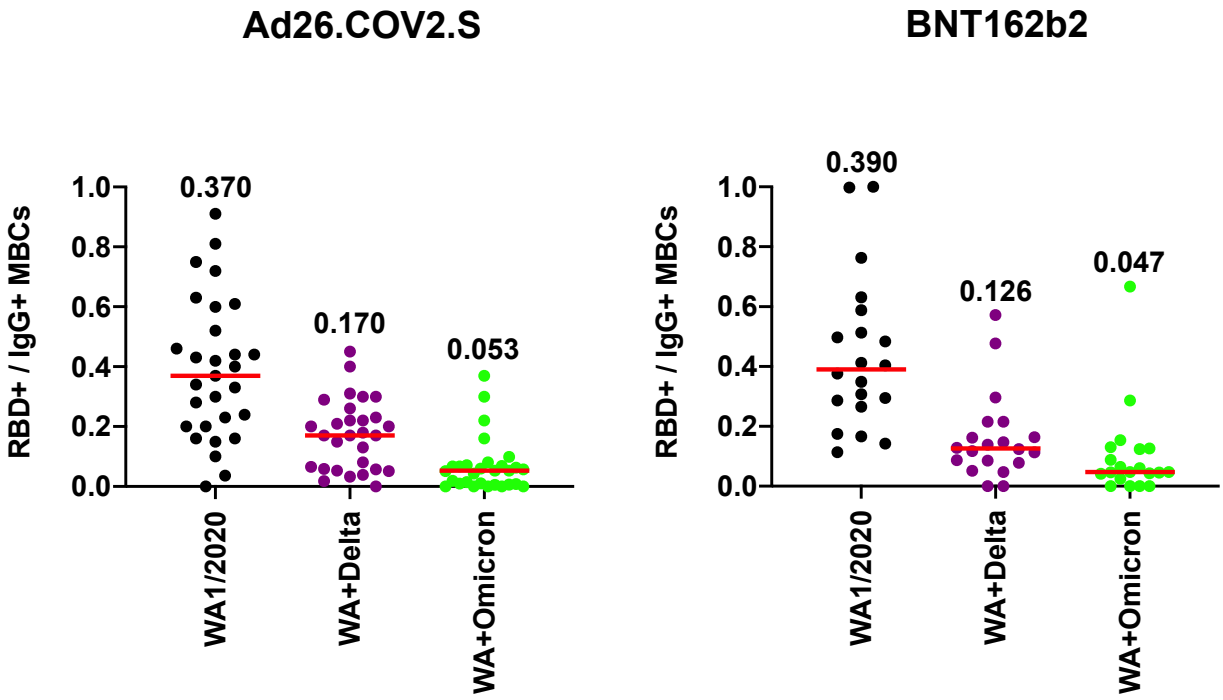
eFigure 3. ELISA Responses Following Ad26.COV2.S or BNT162b2 Boosting. RBD-specific binding antibody titers by ELISA at weeks 0, 2, 4, and 16 following boosting of BNT162b2 vaccinated individuals with Ad26.COV2.S or BNT162b2. ELISA titers to SARS-CoV-2 W1/2020 (WA), B.1.617.2 (Delta), B.1.351 (Beta), B.1.1.529 (Omicron BA.1; Om BA1), and B.1.1.529 (Omicron BA.2; Om BA2). Medians (red bars) are depicted and numerically shown.



eFigure 4. Fc Functional Antibody Responses Following Ad26.COV2.S or BNT162b2 Boosting. Spike-specific antibody-dependent cellular phagocytosis (ADCP), antibody-dependent neutrophil phagocytosis (ADNP), and antibody-dependent complement deposition (ADCD) at week 16 following boosting of BNT162b2 vaccinated individuals with Ad26.COV2.S or BNT162b2. Phagocytic score (ADCP, ADNP) or mean fluorescence intensity (MFI) (ADCD) to SARS-CoV-2 W1/2020 (WA), B.1.617.2 (Delta), and B.1.1.529 (Omicron BA.1; Om BA1). Medians (red bars) are depicted and numerically shown.



eFigure 5. RBD-Specific Memory B Cell Responses Following Ad26.COV2.S or BNT162b2 Boosting. RBD-specific memory B cell (MBC) responses by flow cytometry at week 16 following boosting of BNT162b2 vaccinated individuals with Ad26.COV2.S or BNT162b2. Total WA1/2020 MBC responses, as well as cross-reactive WA1/2020 + Delta and WA1/2020 + Omicron MBC responses are shown. Medians (red bars) are depicted and numerically shown.



eFigure 6. Phenotype of RBD-Specific Memory B Cells Following Ad26.COV2.S Boosting. Phenotype of RBD- specific MBCs at weeks 0, 2, 4, and 16 following boosting BNT162b2 vaccinated individuals with Ad26.COV2.S. Red, activated memory (AM) B cells. Orange, resting memory (RM) B cells.