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Article

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The Ad26.COV2.S vaccine[1–3] has demonstrated clinical efficacy against symptomatic COVID-19, including against the B.1.351 variant that is partially resistant to neutralizing antibodies[1]. However, the immunogenicity of this vaccine in humans against SARS-CoV-2 variants of concern remains unclear. Here we report humoral and cellular immune responses from 20 Ad26.COV2.S vaccinated individuals from the COV1001 phase 1/2 clinical trial[2] against the original SARS-CoV-2 strain WA1/2020 as well as against the B.1.1.7, CAL.20C, P.1., and B.1.351 variants of concern. Ad26.COV2.S induced median pseudovirus neutralizing antibody titers that were 5.0- and 3.3-fold lower against the B.1.351 and P.1 variants, respectively, as compared with WA1/2020 on day 71 following vaccination. Median binding antibody titers were 2.9- and 2.7-fold lower against the B.1.351 and P.1 variants, respectively, as compared with WA1/2020. Antibody-dependent cellular phagocytosis, complement deposition, and NK cell activation responses were largely preserved against the B.1.351 variant. CD8 and CD4 T cell responses, including central and effector memory responses, were comparable among the WA1/2020, B.1.1.7, B.1.351, P.1, and CAL.20C variants. These data show that neutralizing antibody responses induced by Ad26.COV2.S were reduced against the B.1.351 and P.1 variants, but functional non-neutralizing antibody responses and T cell responses were largely preserved against SARS-CoV-2 variants. These findings have implications for vaccine protection against SARS-CoV-2 variants of concern.

SARS-CoV-2 variants that partially escape from neutralizing antibodies to the WA1/2020 strain have emerged, including the B.1.351 variant first identified in South Africa[4,5]. Such variants of concern may reduce the efficacy of current vaccines and natural immunity from SARS-COV-2 strains that were prevalent at the beginning of the pandemic. The mRNA-1273 and BNT162b2 vaccines have been reported to induce lower neutralizing antibody titers against the B.1.351 variant than against the original WA1/2020 strain[4,6,7]. Additional SARS-CoV-2 variants include the B.1.1.7 variant first identified in the United Kingdom[8], the P.1 and P.2 variants first identified in Brazil[9], and the CAL.20C variant first identified in California[10].

Ad26.COV2.S is a replication-incompetent human adenovirus type 26 (Ad26) vector[11] expressing a prefusion stabilized SARS-CoV-2 spike[12] from the Wuhan 2019 strain, which is identical to the spike in the WA1/2020 strain. Ad26.COV2.S demonstrated protective efficacy against SARS-CoV-2 challenges in hamsters and nonhuman primates[3,13] and showed safety and immunogenicity in humans[14,15]. In the phase 3 ENSEMBLE trial, a single dose of 5x10^{10} viral particles (vp) Ad26.COV2.S

resulted in 72%, 68%, and 64% protection against moderate to severe COVID-19 and 86%, 88%, and 82% protection against severe/critical COVID-19 in the United States, Brazil, and South Africa, respectively, by day 28 following vaccination[1]. In this trial, 69% of sequenced viruses from confirmed COVID-19 cases in Brazil were the P.2 variant, and 95% of sequenced viruses from confirmed COVID-19 cases in South Africa were the B.1.351 variant, demonstrating that Ad26.COV2.S maintained protective efficacy against these SARS-CoV-2 variants.

COV1001 is a multicenter, randomized, double-blind, placebo-controlled Phase 1/2a trial to evaluate safety, reactogenicity and immunogenicity of Ad26.COV2.S at 5x10^{10} or 1x10^{11} vp administered intramuscularly as single-shot or two-shot vaccine schedules, 56 days apart, in healthy adults (NCT04436276)[14]. Cohort 1b enrolled 25 adults 18-55 years of age from July 29, 2020 to August 7, 2020 at a single site at Beth Israel Deaconess Medical Center (BIDMC), Boston, MA for exploratory immunogenicity studies[15]. The study was approved by the BIDMC institutional review board, and all participants provided written informed consent. Participants were randomly allocated to one

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of five experimental groups (N=5/group): (1) 5×10^{10} vp Ad26.COV2.S on day 1 and day 57 (low-dose/low-dose); (2) 5×10^{10} vp Ad26.COV2.S on day 1 and placebo on day 57 as a single-shot vaccine (low-dose/placebo); (3) 1×10^{11} vp Ad26.COV2.S on day 1 and day 57 (high-dose/high-dose); (4) 1×10^{11} vp Ad26.COV2.S on day 1 and placebo on day 57 as a single-shot vaccine (high-dose/placebo); or (5) placebo on day 1 and day 57 (placebo/placebo).

Antibody Responses to Variants

Antibody responses were assessed against the SARS-CoV-2 WA1/2020 strain as well as against B.1.351 and other variants of concern. Using a luciferase-based pseudovirus neutralizing antibody (psVNA) assay[3,16,17], the median psVNA titers were 169, 142, 102, 80, 60, and 51 against the WA1/2020, D614G, B.1.1.7, CAL.20C, P.1, and B.1.351 strains, respectively, on day 57 (Fig. 1a). The median psVNA titers were 340, 190, 121, 133, 102, and 67, respectively, against these variants on day 71. These data show a 3.3-fold reduction of psVNA titers against P.1 and a 5.0-fold reduction of psVNA titers against B.1.351 as compared with WA1/2020 on day 71. No psVNA titers were observed in placebo recipients. Live virus neutralizing antibody assays[18] showed a greater 10.6-fold reduction in antibody titers against B.1.351 as compared with WA1/2020 on day 71 (Extended Data Fig. 1). This study was not powered to compare responses for the different vaccine doses or regimens.

On day 57, median receptor binding domain (RBD)-specific binding antibody ELISA titers were 1772, 1364, 486, and 392 against the WA1/2020, B.1.1.7, P.1, and B.1.351 variants, respectively (Fig. 1b). On day 71, median ELISA titers were 1962, 1682, 714, and 683, respectively, against these variants. These data show a 1.2-, 2.7-, and 2.9-fold reduction of ELISA titers against B.1.1.7, P.1, and B.1.351 RBD, respectively, as compared with WA1/2020 RBD on day 71. Minimal ELISA titers were observed in placebo recipients.

An electrochemiluminescence assay (ECLA)[19] was also used to evaluate Spike (S)- and RBD-specific binding antibody responses to WA1/2020, B.1.1.7, P.1, and B.1.351 (Fig. 1c). Similar to the ELISA titers, median RBD-specific ECLA responses against B.1.1.7, P.1, and B.1.351 were reduced 1.3-, 1.8-, and 2.9-fold, and median S-specific ECLA responses were reduced 1.6-, 1.8-, and 2.6-fold, respectively, as compared with WA1/2020 on day 71.

Antibody Fc effector function was assessed on day 71 by systems serology[20], including antibody-dependent cellular phagocytosis (ADCP), antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent complement deposition (ADCD), and antibody-dependent NK cell activation (ADNKA). S-specific ADCP, ADNP, ADCD, and ADNKA responses against the B.1.351 variant were 1.5-, 2.9-, 1.6-, and 1.1-fold reduced, respectively, compared with the WA1/2020 strain with the D614G mutation (Fig. 2a). Comparable IgG, IgM, and IgA subclasses and Fc-receptor binding were observed across the variants, with only a slight loss in FcγR2b binding compared to the WA1/2020 strain (Fig. 2b). RBD-specific ADCP, ADNP, and ADCD responses were comparable against the WA1/2020, B.1.1.7, and B.1.351 variants (Extended Data Fig. 2). These data show robust S- and RBD-specific Fc-effector functions against these SARS-CoV-2 variants.

Cellular Immune Responses to Variants

S-specific cellular immune responses were assessed by pooled peptide ELISPOT assays in peripheral blood mononuclear cells (PBMC) on days 57 and 85. IFN-γ ELISPOT responses were comparable to WA1/2020, B.1.351, B.1.1.7, P.1, and CAL.20C at both timepoints, with no evidence of decreased responses against the variants (Fig. 3a). No S-specific ELISPOT responses were observed in placebo recipients. S-specific CD8+ and CD4+ T cell responses were evaluated by multiparameter intracellular cytokine staining (ICS) assays on days 57 and 85 (Extended

Data Fig. 3). IFN-γ CD8+ and CD4+ T cell responses were comparable to WA1/2020, B.1.351, B.1.1.7, P.1, and CAL.20C variants (Fig. 3b). The median ratios of B.1.351, B.1.1.7, and P.1 to WA1/2020 IFN-γ CD8+ T cell responses were 0.98, 0.98, and 0.98, respectively, on day 57 and 0.92, 0.94, and 1.26, respectively, on day 85 (Extended Data Fig. 4). Central memory CD27+CD45RA- and effector memory CD27-CD45RA- CD4+ and CD8+ T cell responses were also comparable across these variants (Extended Data Figs. 5-6). These data show that S-specific cellular immune responses were not detectably impacted by SARS-CoV-2 variants. Polyfunctional analyses showed that CD8+ T cells were primarily IFN-γ, TNF-α, and IFN-γ/TNF-α responses, whereas CD4+ T cells were primarily TNF-α, IFN-γ/TNF-α, IL-2/TNF-α, and IFN-γ/IL-2/TNF-α responses (Extended Data Fig. 7).

To evaluate the specificity and breadth of individual T-cell receptors (TCRs) following vaccination, TCRβ sequencing[21] was performed to define the repertoires of 8 convalescent individuals and 19 vaccine and 5 placebo participants on day 63 (Extended Data Table 1). To identify SARS-CoV-2 specific TCRs, the observed TCRs were compared to a TCR dataset that had previously been determined to be SARS-CoV-2-specific and enriched in subjects with natural infection relative to placebos[22]. The breadth (unique rearrangements) and depth (frequency of TCRs) of TCRs specific to either Spike (S) or non-Spike SARS-CoV-2 proteins were determined, although these analyses may have underestimated total T cell responses. Higher breadth of S-specific TCRs was observed in vaccine recipients compared with placebos (P=0.0014, Wilcoxon rank-sum test; Fig. 4a; Extended Data Figs. 8-9). In contrast, the breadth of non-Spike TCRs was comparable in vaccine recipients and controls, as expected since the vaccine did not contain any non-Spike immunogens. Substantial breadth of CD8+ and CD4+ T cell responses was also observed (Fig. 4b).

Discussion

SARS-CoV-2 variants have emerged with multiple mutations in targets of neutralizing antibodies, such as the E484K mutation. Median pseudovirus neutralizing antibody titers induced by Ad26.COV2.S were 5.0-fold lower against the B.1.351 variant and 3.3-fold lower against the P.1 variant as compared with the original WA1/2020 strain, which is a comparable reduction of psVNA titers that has been reported for other vaccines[4,6,7]. In contrast, functional non-neutralizing antibody responses and CD8+ and CD4+ T cell responses were largely preserved against SARS-CoV-2 variants of concern.

In the phase 3 ENSEMBLE trial[1], Ad26.COV2.S was evaluated in the United States, Latin America including Brazil, and South Africa. In South Africa, 95% of sequenced viruses from COVID-19 cases were of the B.1.351 variant, and in Brazil, 69% of sequenced viruses from COVID-19 cases were of the P.2 lineage. Protective efficacy of Ad26.COV2.S against severe/critical disease was similar in all geographic locations by day 28, and protective efficacy against moderate to severe disease was only slightly reduced in South Africa and Brazil compared with the United States. Although the mechanistic correlates of protection for COVID-19 are not yet known, the robust protective efficacy in these regions despite reduced neutralizing antibodies raises the possibility that functional non-neutralizing antibodies and/or CD8+ T cell responses may also contribute to protection. Indeed, TCRβ sequencing revealed substantial breadth of T cell responses in individuals vaccinated with Ad26.COV2.S. Alternatively, it is possible that low levels of neutralizing antibodies are sufficient for protection. In a nonhuman primate model, adoptive transfer of purified IgG was sufficient for protection against SARS-CoV-2 if psVNA titers exceeded a threshold of approximately 50, but CD8+ T cells also contributed to protection if antibody titers were subprotective[23].

In conclusion, neutralizing antibody responses elicited by Ad26.COV2.S were reduced against the B.1.351 and P.1 variants, but other functional antibody responses and T cell responses were largely preserved

against these variants. The relevance of these immune parameters to mechanistic correlates of vaccine efficacy remains to be determined.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-021-03681-2.

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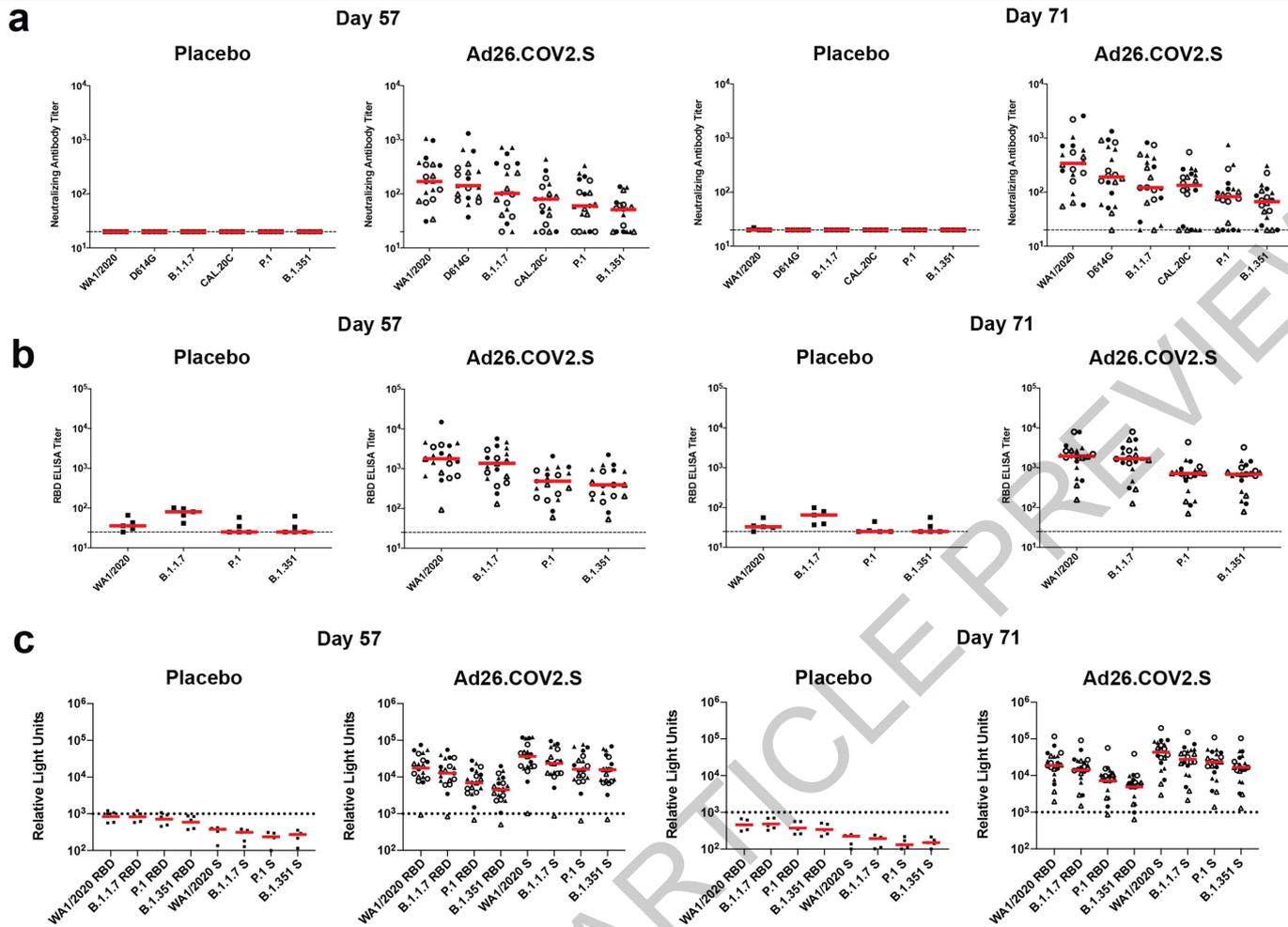
Fig. 1 | Neutralizing and binding antibody responses to SARS-CoV-2 variants. (**a**) SARS-CoV-2 pseudovirus neutralizing antibody (psVNA) responses against WA1/2020, D614G, B.1.1.7, CAL.20C, P.1, and B.1.351, (**b**) receptor binding domain (RBD)-specific binding antibodies by ELISA against WA1/2020, B.1.1.7, P.1, and B.1.351, and (**c**) RBD- and S-specific binding antibodies by electrochemiluminescence assay (ECLA) against WA1/2020, B.1.1.7, P.1, and B.1.351 (Meso Scale Discovery Panel 7) on day 57 and day 71. Red bars reflect median responses. Dotted lines reflect lower limits of quantitation. Filled squares, placebo-placebo; filled circles, high dose-placebo; open circles, high dose-high dose; filled triangles, low dose-placebo; open triangles, low dose-low dose. n=25 independent samples (5 placebo recipients, 20 Ad26. COV2.S vaccine recipients).
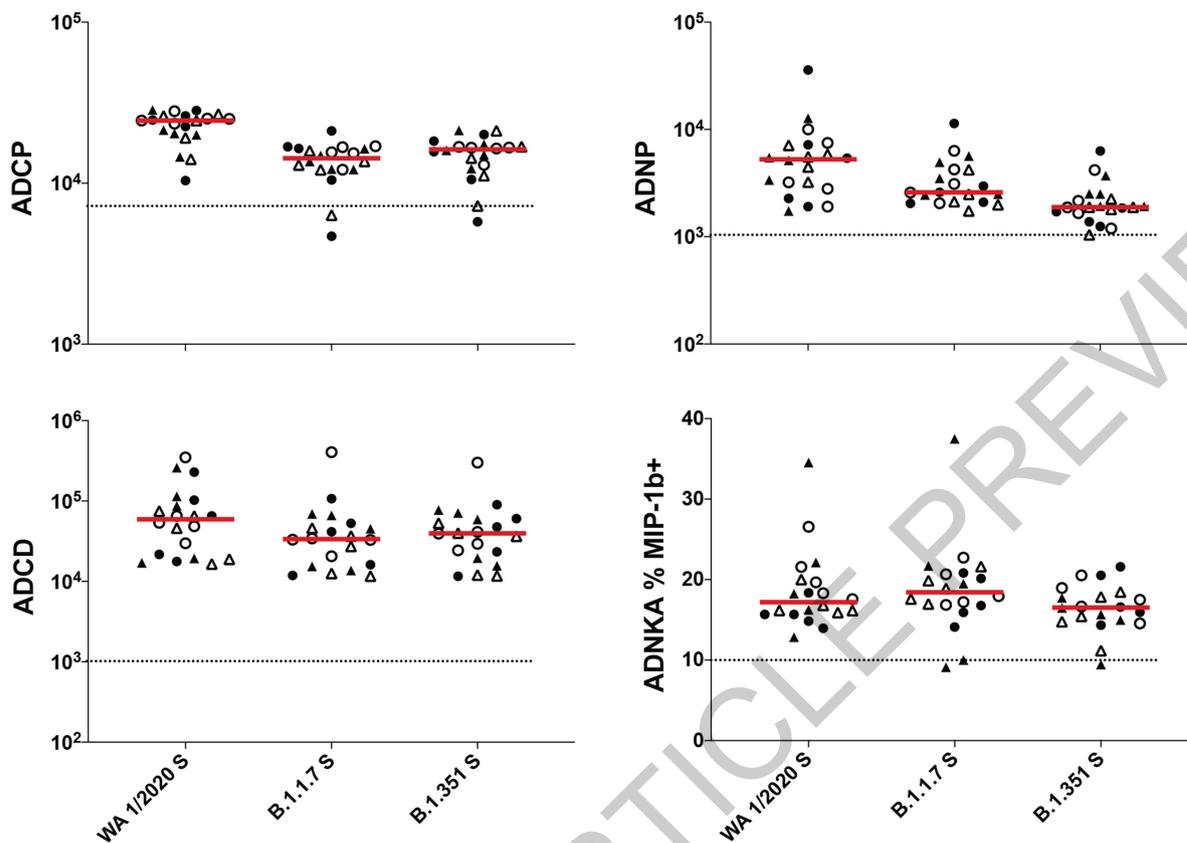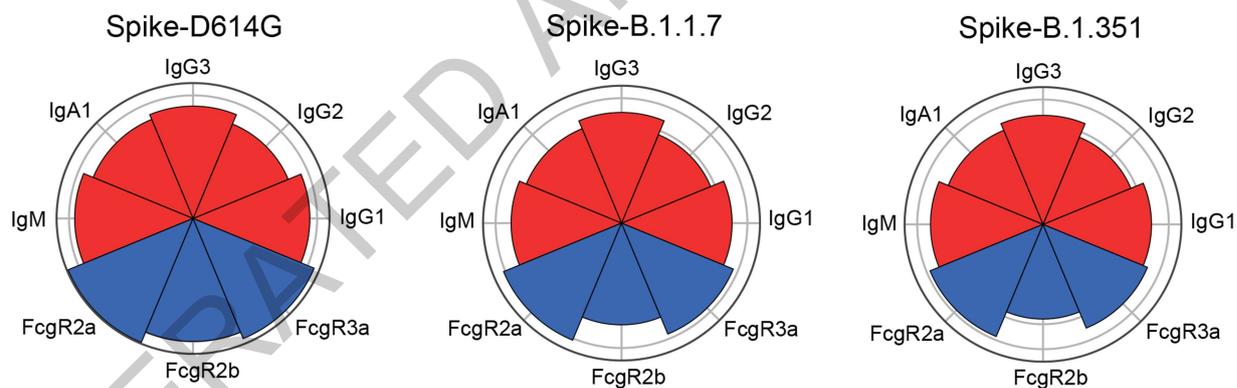
Fig. 2 | Systems serology to SARS-CoV-2 variants. (**a**) S-specific ADCP (antibody-dependent cellular phagocytosis), ADNP (antibody-dependent neutrophil phagocytosis), ADCD (antibody-dependent complement deposition), and ADNKA (antibody-dependent NK cell activation) against WA1/2020 (D614G), B.1.1.7, and B.1.351 on day 71. Red bars reflect median responses. Dotted lines reflect median of placebo recipients. Filled circles, high dose-placebo; open circles, high dose-high dose; filled triangles, low dose-placebo; open triangles, low dose-low dose. (**b**) The nightingale plots show the median levels of WA1/2020 (D614G), B.1.1.7, B.1.351 S-specific isotype (IgM, IgA, IgG1, IgG2, IgG3) (red) and FcγR2a, FcγR2b, FcγR3a (blue) binding. n=20 independent samples from Ad26.COV2.S vaccine recipients.
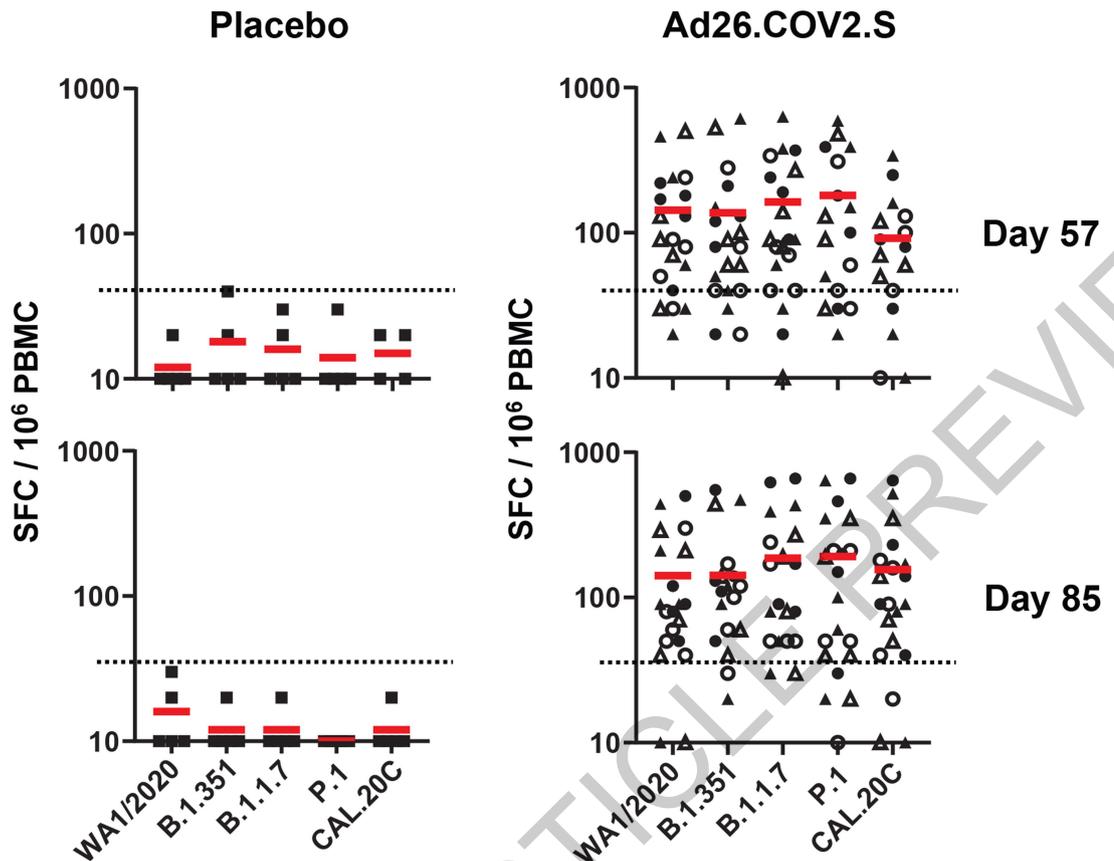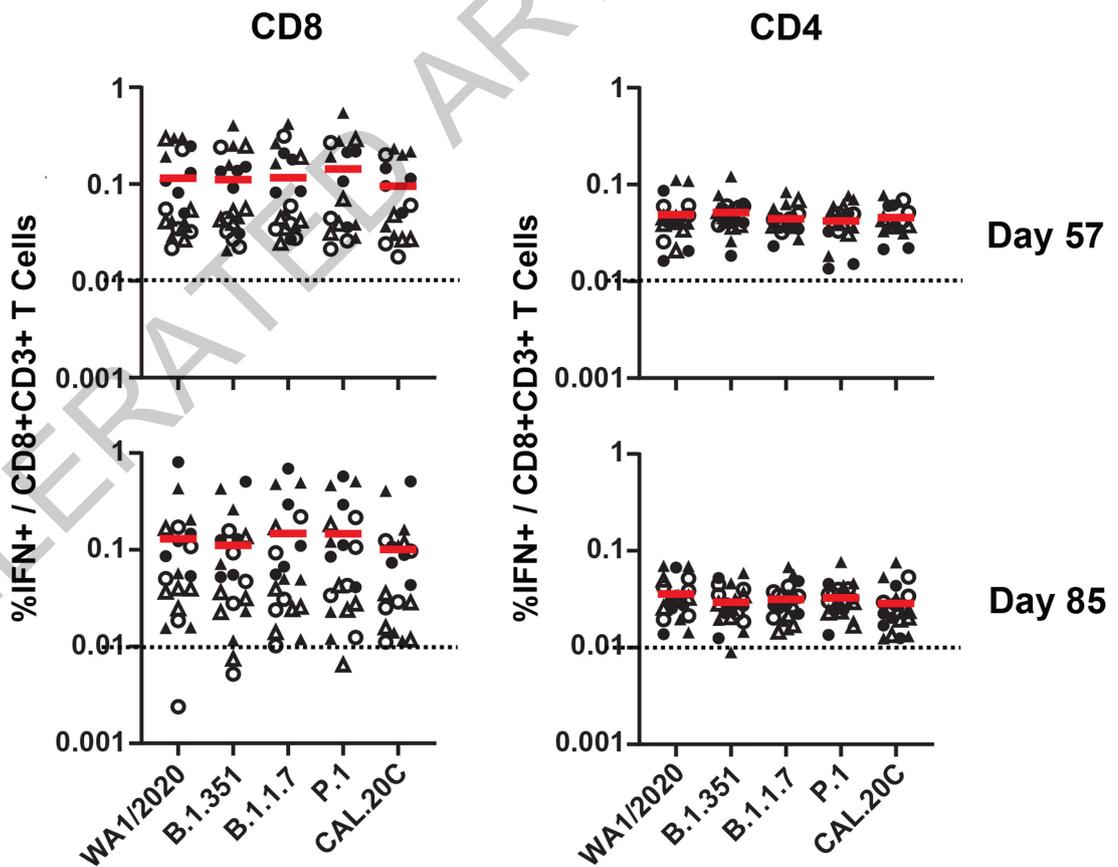
Fig. 3 | See next page for caption.

Fig. 3 | Cellular immune responses to SARS-CoV-2 variants. (**a**) S-specific pooled peptide IFN-γ ELISPOT responses against WA1/2020, B.1.351, B.1.1.7, P.1, and CAL.20C. n=25 independent samples (5 placebo recipients, 20 Ad26. COV2.S vaccine recipients). (**b**) S-specific pooled peptide IFN-γ CD4+ and CD8+ T cell responses by intracellular cytokine staining (ICS) assays against WA1/2020, B.1.351, B.1.1.7, P.1, and CAL.20C. SFC, spot-forming cells.

Responses are shown on day 57 and day 85. Red bars reflect median responses. Dotted lines reflect lower limits of quantitation. Filled squares, placebo-placebo; filled circles, high dose-placebo; open circles, high dose-high dose; filled triangles, low dose-placebo; open triangles, low dose-low dose. n=20 independent samples from Ad26.COV2.S vaccine recipients.
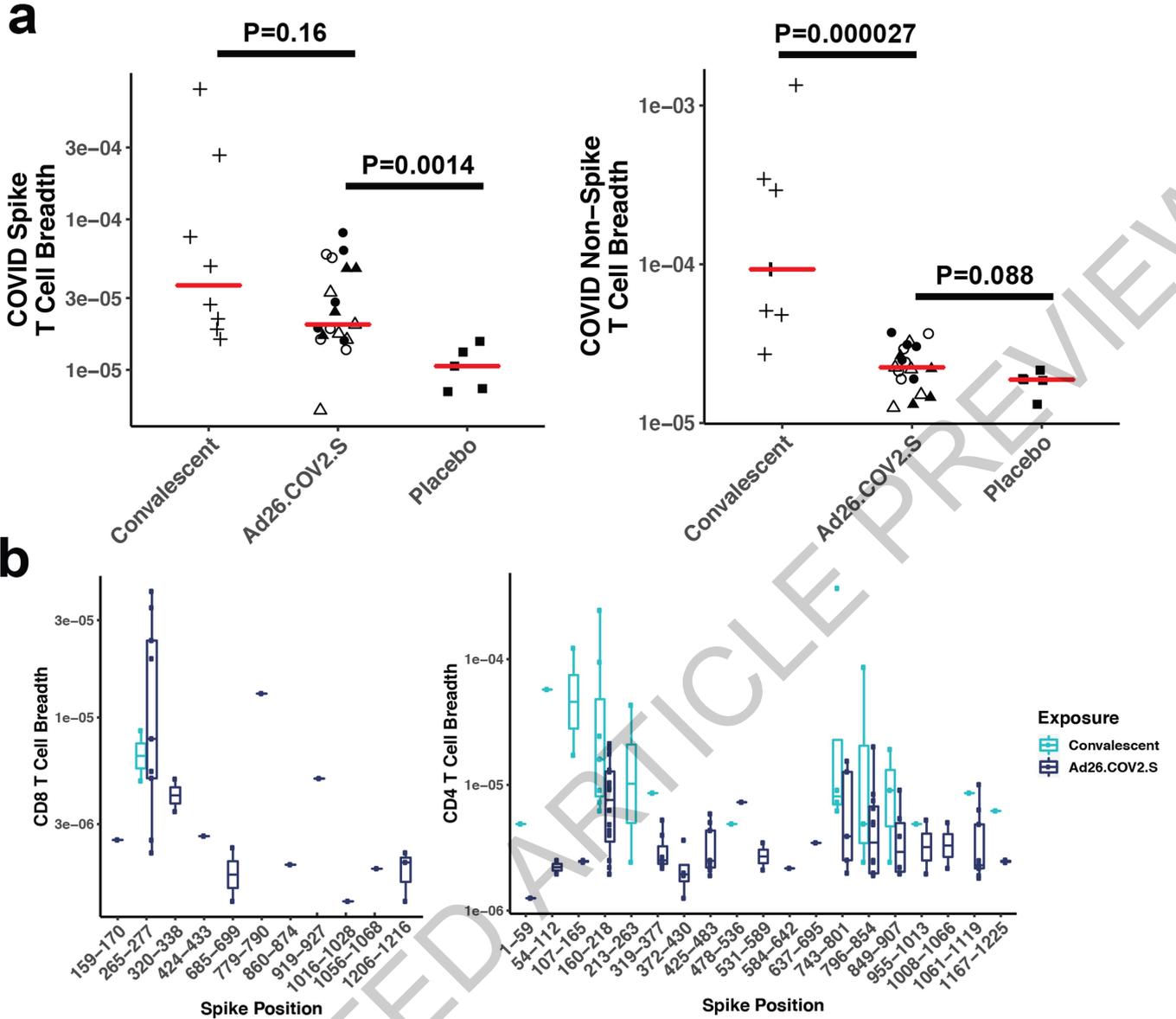
Fig. 4 | TCRβ repertoire analysis. (**a**) Spike and non-Spike T cell breadth by TCRβ sequencing on day 63. P-values represent 2-sided Wilcoxon rank-sum tests. Red bars reflect median responses. (**b**) Breadth of Spike-specific CD8+ and CD4+ T cell responses. Filled squares, placebo-placebo; filled circles, high dose-placebo; open circles, high dose-high dose; filled triangles, low dose-placebo; open triangles, low dose-low dose; plus signs, convalescent samples. In the box-and-whisker plots, the middle line reflects the median, the box reflects the 25-75 percentiles, and the whiskers extend the full range up to 1.5x the interquartile range, with outlier points marked individually. n=32 independent samples (8 SARS-CoV-2 convalescent individuals, 5 placebo recipients, 19 Ad26.COV2.S vaccine recipients).

Methods

Pseudovirus-based neutralization assay

The SARS-CoV-2 pseudoviruses expressing a luciferase reporter gene were generated in an approach similar to as described previously[17,24]. Briefly, the packaging plasmid psPAX2 (AIDS Resource and Reagent Program), luciferase reporter plasmid pLenti-CMV Puro-Luc (Addgene), and spike protein expressing pcDNA3.1-SARS CoV-2 SΔCT of variants were co-transfected into HEK293T cells by lipofectamine 2000 (ThermoFisher). Pseudoviruses of SARS-CoV-2 variants were generated by using WA1/2020 strain (Wuhan/WIV04/2019, GISAID accession ID: EPI_ISL_402124), D614G mutation, B.1.1.7 variant (GISAID accession ID: EPI_ISL_601443), CAL.20C (GISAID accession ID: EPI_ISL_824730), P.1 (GISAID accession ID: EPI_ISL_792683), or B.1.351 variant (GISAID accession ID: EPI_ISL_712096). The supernatants containing the pseudotype viruses were collected 48 h post-transfection, which were purified by centrifugation and filtration with 0.45 μm filter. To determine the neutralization activity of the plasma or serum samples from participants, HEK293T-hACE2 cells were seeded in 96-well tissue culture plates at a density of 1.75×10^4 cells/well overnight. Three-fold serial dilutions of heat inactivated serum or plasma 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. 48 h after infection, 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 in relative light unit (RLU) was observed relative to the average of the virus control wells.

Live virus neutralization assay

Full-length SARS-CoV-2 WA1/2020, B.1.351, and B.1.1.7, viruses were designed to express nanoluciferase (nLuc) and were recovered via reverse genetics[18]. One day prior to the assay, Vero E6 USAMRID cells were plated at 20,000 cells per well in clear bottom black walled 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 3-fold up to nine dilution spots. Serially diluted serum samples were mixed in equal volume with diluted virus. Antibody-virus and virus only mixtures were then incubated at 37 °C with 5% CO2 for one hour. Following incubation, serially diluted sera and virus only controls were added in duplicate to the cells at 75 PFU at 37 °C with 5% CO2. 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 Spectramax M3 plate reader (Molecular Devices, San Jose, CA). 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.

ELISA

WA1/2020, B.1.1.7, and B.1.351 RBD-specific binding antibodies were assessed by ELISA. Briefly, 96-well plates were coated with 2μg/ml RBD proteins (provided by Dr. Florian Krammer) in 1X DPBS and incubated at 4 °C overnight. After incubation, plates were washed once with wash buffer (0.05% Tween 20 in 1 X DPBS) and blocked with 350 μL Casein block/well for 2-3 h at room temperature. After 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 h at room temperature, prior to three further washes and a 1 h incubation with a 1:4000 dilution of anti-human IgG HRP (Invitrogen) at room temperature in the dark. Plates were then washed three times, and 100 μL of SeraCare KPL TMB SureBlue Start solution was added to each well; plate development was halted by the addition of 100 μL SeraCare KPL TMB Stop solution per well. The absorbance at 450nm, with a reference at 650nm, was recorded using a VersaMax microplate reader. For each sample, ELISA endpoint titer was calculated in Graphpad Prism software, using a four-parameter logistic curve fit to calculate the reciprocal serum dilution that yields a corrected absorbance value (450nm-650nm) of 0.2. Log10 endpoint titers are reported.

Electrochemiluminescence assay (ECLA)

ECLA plates (MesoScale Discovery SARS-CoV-2 IgG Cat No: N05CA-1; Panel 7) were designed and produced with up to 9 antigen spots in each well. The antigens included were WA1/2020, B.1.1.7, P.1, and B.1.351 S and RBD. The plates were blocked with 50 uL of Blocker A (1% BSA in MilliQ water) solution for at least 30 m at room temperature shaking at 700 rpm with a digital microplate shaker. During blocking the serum was diluted 1:5,000 in Diluent 100. The plates were then washed 3 times with 150 μL of the MSD kit Wash Buffer, blotted dry, and 50 μL of the diluted samples 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 as Sample RLU minus Blank RLU for each spot for each sample. The limit of detection was defined as 1000 RLU for each assay.

Systems Serology

Both the biophysical and functional quality of polyclonal vaccine induced SARS-CoV-2 antibodies were profiled using systems serology[20]. Biophysical profiling was performed using a custom Luminex based assay where individuals bar-coded beads were coated with Spike (S) or (RBD) variants by carboxy coupling. The D614G, B.1.1.7 and B.1.351 variants (provided by Drs. Erica Ollman Saphire and Florian Krammer) were profiled. The overall levels of IgG1, IgG2, IgG3, IgA, IgM and FcγR2a, FcγR2b, FcγR3a, and FcγR3b binding were assessed. Functional profiling included the assessment of antibody dependent monocyte phagocytosis (ADCP), antibody dependent neutrophil phagocytosis (ADNP), antibody dependent complement deposition (ADCD), and antibody dependent NK cell activation (ADNKA). Briefly, for the ADCP, ADNP, and ADCD assays, fluorescent beads (LifeTechnologies) were coupled via carboxy-coupling, and plasma were added, allowing immune complex formation, excess antibodies were washed away, followed by the addition of THP1 monocytes, primary neutrophils, or guinea pig complement, individually, respectively. The level of phagocytosis and complement deposition was assessed by flow cytometry. For ADNKA, ELISA plates were coated with antigen, followed by the addition of plasma. Excess antibodies were washed away following by the addition of primary NK cells. NK cells were treated with Golgi Stop (BD) and Brefeldin A (Sigma Aldrich) and were stained for the surface markers CD56, CD16 and CD3 and for activity markers CD107a (BD) and MIP-1b (BD). Fluorescence was determined by flow cytometry. NK cells were classified as CD56+/CD16+/CD3-.

Enzyme-linked immunospot (ELISPOT) assay

ELISPOT plates were coated with mouse anti-human IFN-γ monoclonal antibody from MabTech at 1 μg/well and incubated overnight at 4 °C. Plates were washed with DPBS, and blocked with R10 media (RPMI with 10% heat inactivated FBS with 1% of 100x penicillin-streptomycin, 1M HEPES, 100mM Sodium pyruvate, 200mM L-glutamine, and 0.1% of 55mM 2-Mercaptoethanol) for 2-4 h at 37 °C. SARS-CoV-2 pooled S peptides from WA1/2020, B.1.351, B.1.1.7, P.1, and CAL.20C (21st Century Biochemicals) were prepared and plated at a concentration of 2 μg/well, and 100,000 cells/well were added to the plate. The peptides and cells were incubated for 15-20 h at 37 °C. All steps following this incubation were performed at room temperature. The plates were washed with

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ELISPOT wash buffer and incubated for 2-4 h with Biotinylated mouse anti-human IFN-γ monoclonal antibody from MabTech (1 μg/mL). The plates were washed a second time and incubated for 2-3 h with conjugated Goat anti-biotin AP from Rockland, Inc. (1.33 μg/mL). The final wash was followed by the addition of Nitor-blue Tetrazolium Chloride/5-bromo-4-chloro 3 'indolyphosphate p-toludine salt (NBT/BCIP chromagen) substrate solution for 7 min. The chromagen was discarded and the plates were washed with water and dried in a dim place for 24 h. Plates were scanned and counted on a Cellular Technologies Limited Immunospot Analyzer.

Intracellular cytokine staining (ICS) assay

10^6 PBMCs/well 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), pooled S peptides from WA1/2020, B.1.351, B.1.1.7, P.1, and CAL.20C (21st Century Biochemicals) (2 μg/mL), 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 mAbs 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 200μL of BD CytoFix/CytoPerm Fixation/Permeabilization solution. Cells were washed twice with 1X Perm Wash buffer (BD Perm/Wash™ Buffer 10X in the CytoFix/CytoPerm Fixation/Permeabilization kit diluted with MilliQ water and passed through 0.22μm filter) and stained with intracellularly with mAbs 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), 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 with FlowJo v9.9.

T-cell receptor variable beta chain sequencing

Immunosequencing of the CDR3 regions of human TCRβ chains was performed using the immunoSEQ® Assay (Adaptive Biotechnologies, Seattle, WA). Extracted genomic DNA was amplified in a bias-controlled multiplex PCR, followed by high-throughput sequencing. Sequences were collapsed and filtered in order to identify and quantitate the absolute abundance of each unique TCRβ CDR3 region for further analysis as previously described[21]. The fraction of T cells was calculated by normalizing TCRβ template counts to the total amount of DNA usable for TCR sequencing, where the amount of usable DNA was determined by PCR-amplification and sequencing of several reference genes that are expected to be present in all nucleated cells. TCR sequences from repertoires were mapped against a set of TCR sequences that are known to react to SARS-CoV-2 by matching on V gene, amino acid sequence and J gene. Briefly, these sequences were first identified by Multiplex Identification of T-cell Receptor Antigen Specificity (MIRA)[22]. TCRs that react were further screened for enrichment in COVID-19 positive repertoires collected as part of ImmuneCODE compared to COVID-19 negative repertoires to remove TCRs that may be highly public or cross-reactive to common antigens. Individual response could be quantified by the number and/or frequency of SARS-CoV-2 TCRs seen post-vaccine. TCRs were further analyzed at the level specific ORF or position within ORF based on the MIRA antigens. The breadth summary metric is calculated as the number of unique annotated rearrangements out of the total number of unique productive rearrangements, while depth summary metric corresponds to the sum frequency of those rearrangements in the repertoire. Sequences of known variants were obtained from GISAID (www.gisaid.org) and aligned to known MIRA antigen locations.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

All data are available in the manuscript and supplementary information.

24. Yu, J. *et al.* Deletion of the SARS-CoV-2 Spike Cytoplasmic Tail Increases Infectivity in Pseudovirus Neutralization Assays. *Journal of Virology, JVI*.00044-00021, https://doi.org/10.1128/jvi.00044-21 (2021).

Author contributions J.Y., L.H.T., K.M., C.J.D., A.C., T.A., and J.N. led the binding and pseudovirus neutralizing antibody assays. D.R.M. and R.S.B. led the live virus neutralization assays. G.A., C.A., and S.S. performed the systems serology. F.A. and F.K. produced the purified proteins. J.L., A.C., E.N.B., M.L. performed the cellular immune assays. P.F., I.K., H.R. led the TCR sequencing. K.E.S., M.L.G., J.S., A.M.G., D.H., F.S., M.D., J.V.H., and H.S. led the clinical study. D.H.B. coordinated the study and wrote the paper with all co-authors.

Placebo

Ad26.COV2.S

Day 71

Extended Data Fig. 1 | Live virus neutralizing antibody responses to SARS-CoV-2 variants. SARS-CoV-2 live virus neutralizing antibody responses against WA1/2020, B.1.1.7, and B.1.351. Red bars reflect median responses. Dotted lines reflect lower limits of quantitation. Filled squares, placebo-placebo; filled circles, high dose-placebo; open circles, high dose-high dose; filled triangles, low dose-placebo; open triangles, low dose-low dose. n=25 independent samples (5 placebo recipients, 20 Ad26.COV2.S vaccine recipients).
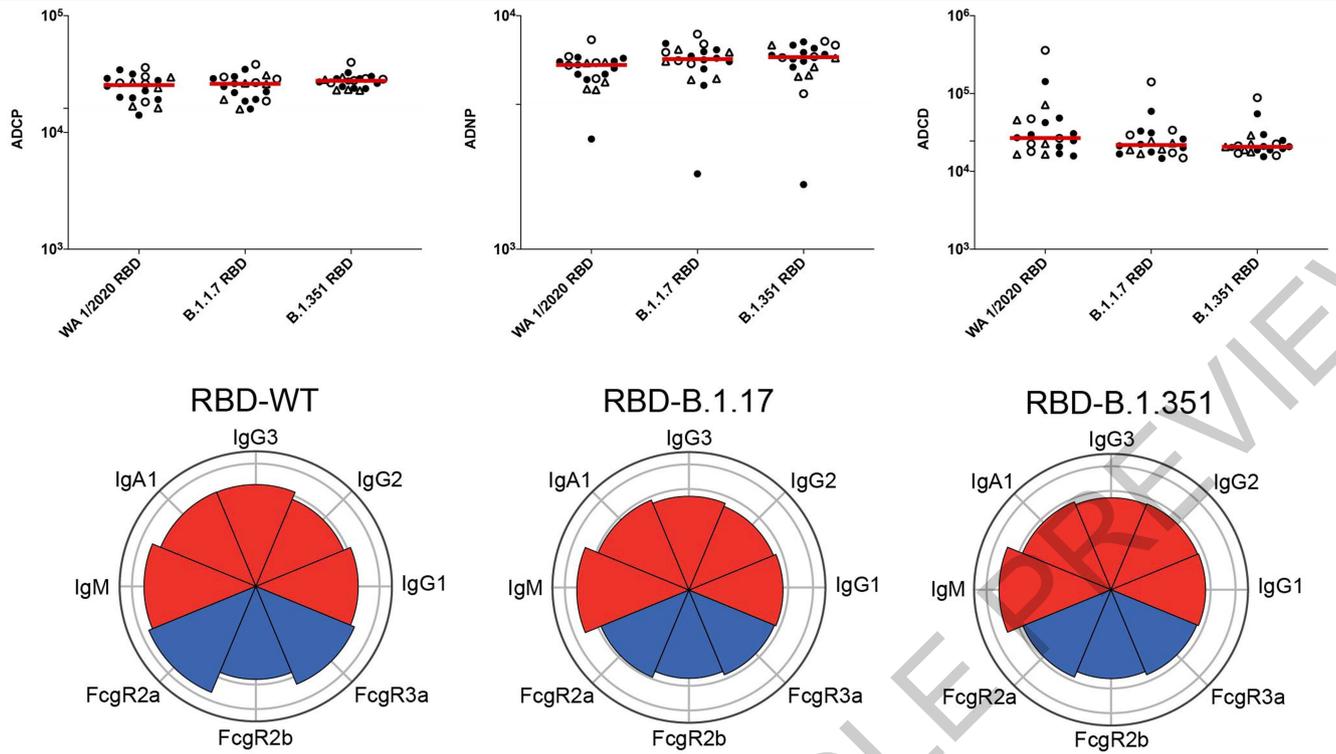
Extended Data Fig. 2 | RBD-specific functional antibody responses to SARS-CoV-2 variants. Top, RBD-specific ADCP (antibody-dependent cellular phagocytosis), ADNP (antibody-dependent neutrophil phagocytosis), and ADCD (antibody-dependent complement deposition) against WA1/2020 (D614G), B.1.1.7, and B.1.351. LD, low dose; HD, high dose; PL, placebo on day 71. Filled circles, high dose-placebo; open circles, high dose-high dose; filled triangles, low dose-placebo; open triangles, low dose-low dose. Bottom, RBD-specific isotype (IgG1, IgG3, IgA, IgM) (red) and FcγR2a, FcγR2b, FcγR3a (blue) binding against WA1/2020 (D614G), B.1.1.7, B.1.351 on day 71. n=20 independent samples from Ad26.COV2.S vaccine recipients.
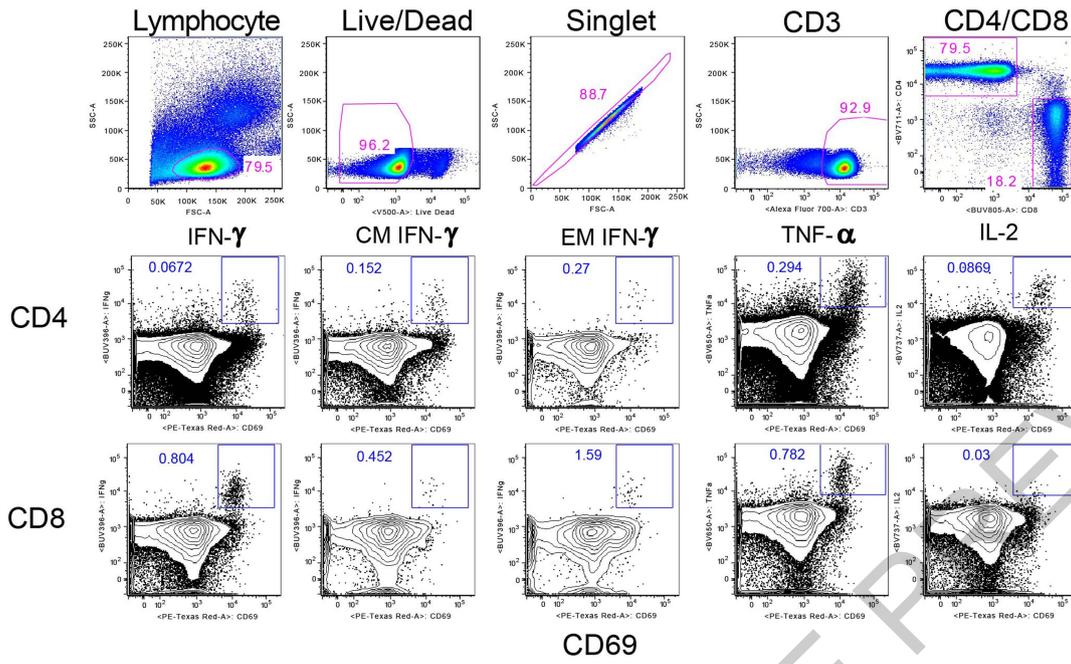
Extended Data Fig. 3 | Representative gating for intracellular cytokine staining (ICS) assays.
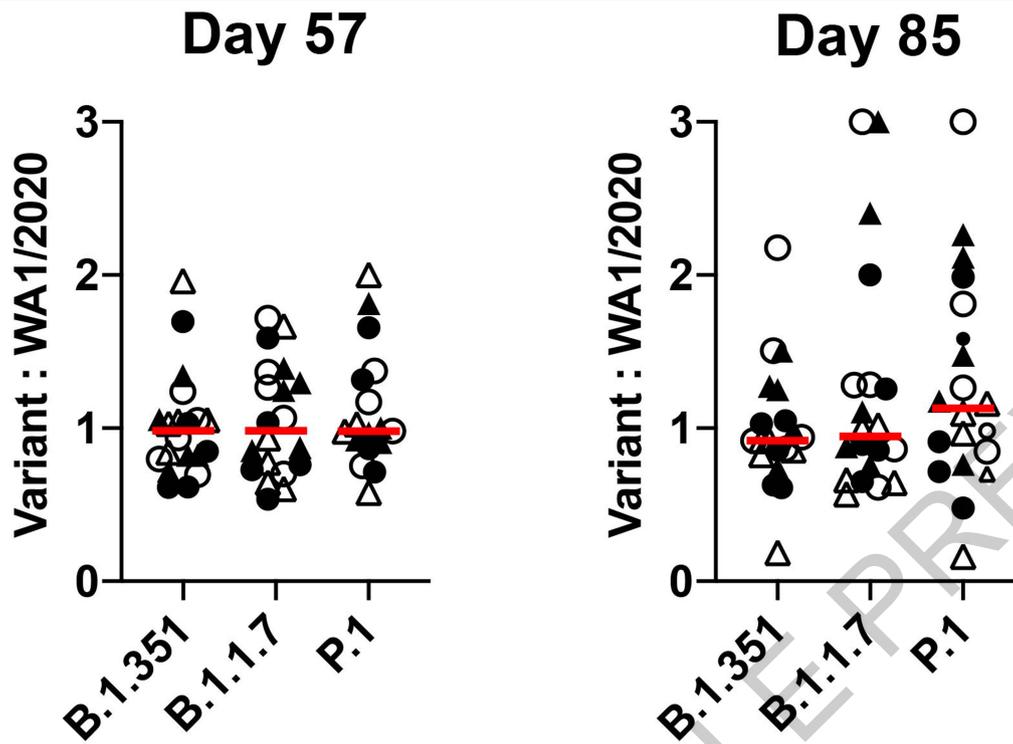
Extended Data Fig. 4 | Ratio of variants vs. WA1/2020 CD8+ T cell responses. Ratio of S-specific pooled peptide IFN-γ CD8+ T cell responses by intracellular cytokine staining (ICS) assays against B.1.351, B.1.1.7, and P.1 vs. WA1/2020 on day 57 and day 85. Red bars reflect median responses. Filled circles, high dose-placebo; open circles, high dose-high dose; filled triangles, low dose-placebo; open triangles, low dose-low dose. n=20 independent samples from Ad26.COV2.S vaccine recipients.

Day 57

Day 85
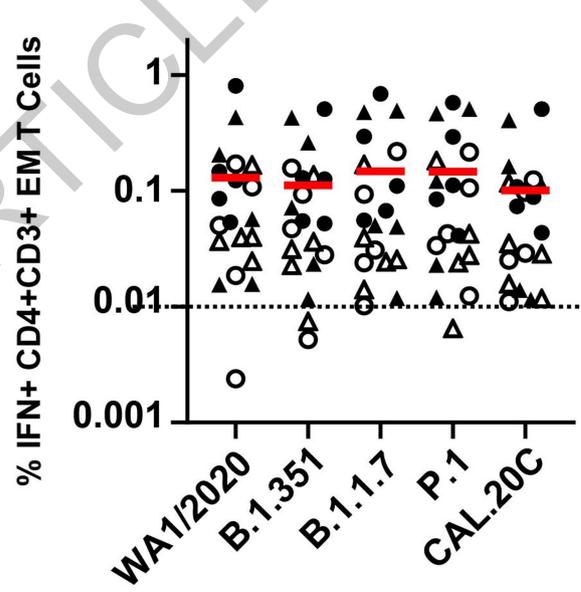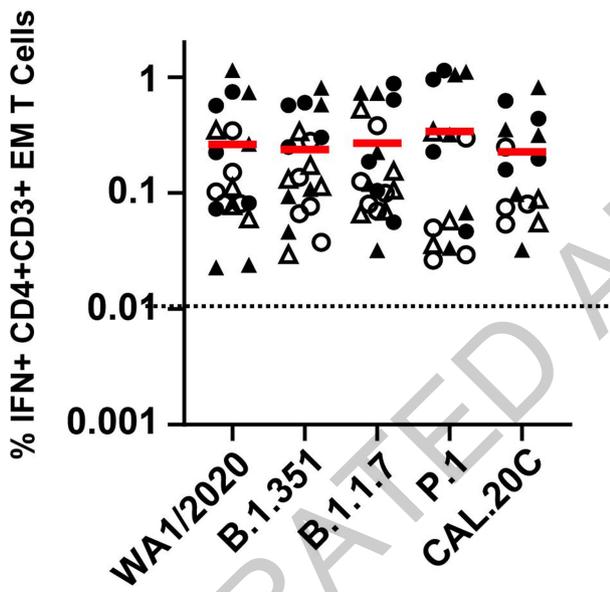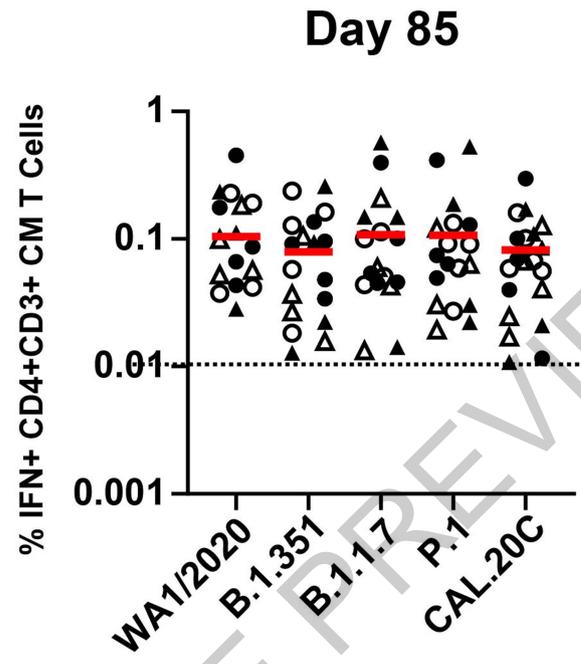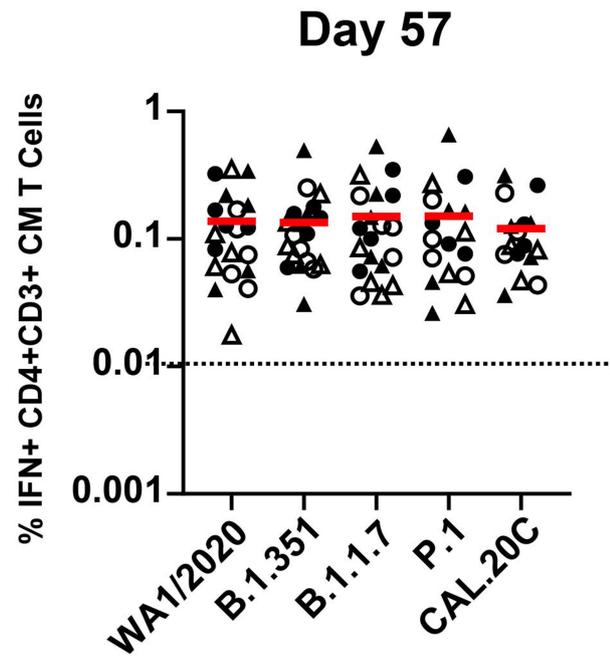
Extended Data Fig. 5 | Central and effector memory CD8+ T cell responses to SARS-CoV-2 variants. S-specific pooled peptide IFN-γ central memory CD27+CD45RA- and effector memory CD27-CD45RA- CD8+ T cell responses by intracellular cytokine staining (ICS) assays against WA1/2020, B.1.351, B.1.1.7, P.1, and CAL.20C on day 57 and day 85. Red bars reflect median responses.

Dotted lines reflect lower limits of quantitation. Filled circles, high dose-placebo; open circles, high dose-high dose; filled triangles, low dose-placebo; open triangles, low dose-low dose. n=20 independent samples from Ad26.COV2.S vaccine recipients.

Day 57

Day 85
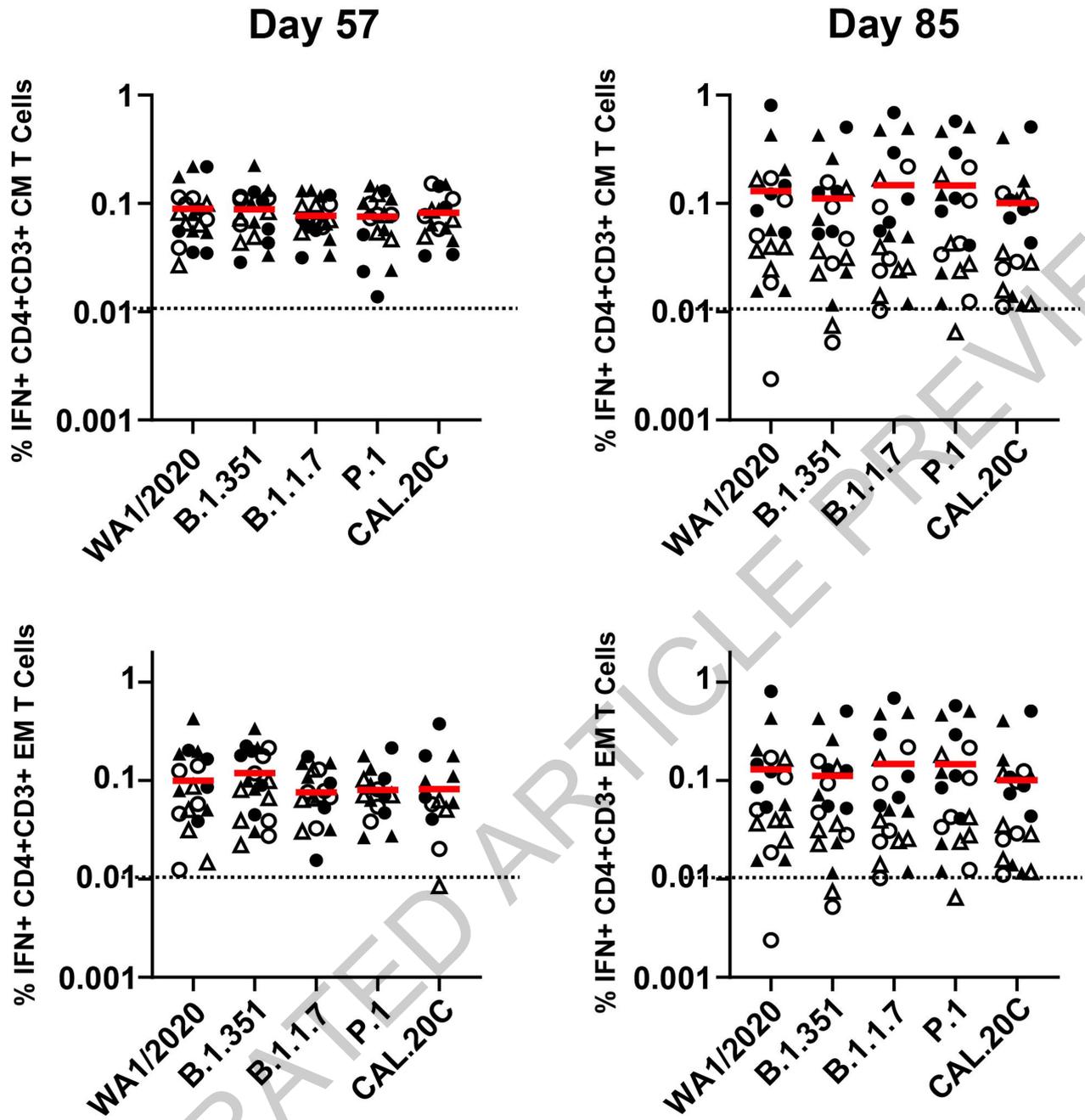
Extended Data Fig. 6 | Central and effector memory CD4+ T cell responses to SARS-CoV-2 variants. S-specific pooled peptide IFN-γ central memory CD27+CD45RA- and effector memory CD27-CD45RA- CD4+ T cell responses by intracellular cytokine staining (ICS) assays against WA1/2020, B.1.351, B.1.1.7, P.1, and CAL.20C on day 57 and day 85. Red bars reflect median responses.

Dotted lines reflect lower limits of quantitation. Filled circles, high dose-placebo; open circles, high dose-high dose; filled triangles, low dose-placebo; open triangles, low dose-low dose. n=20 independent samples from Ad26.COV2.S vaccine recipients.
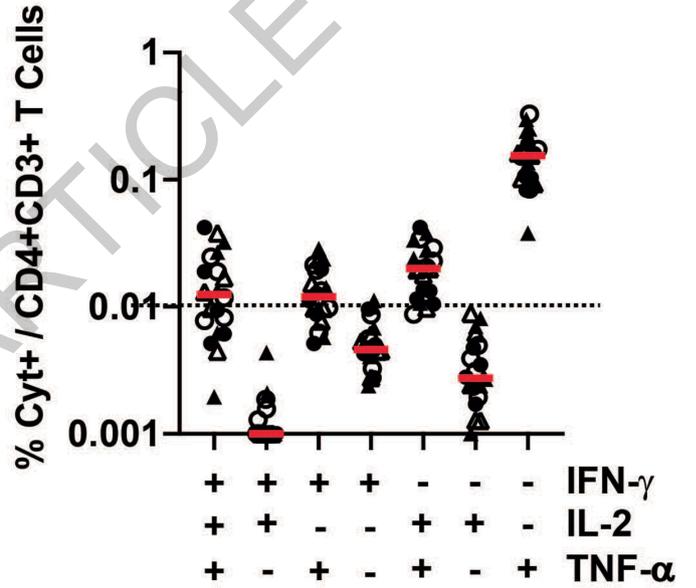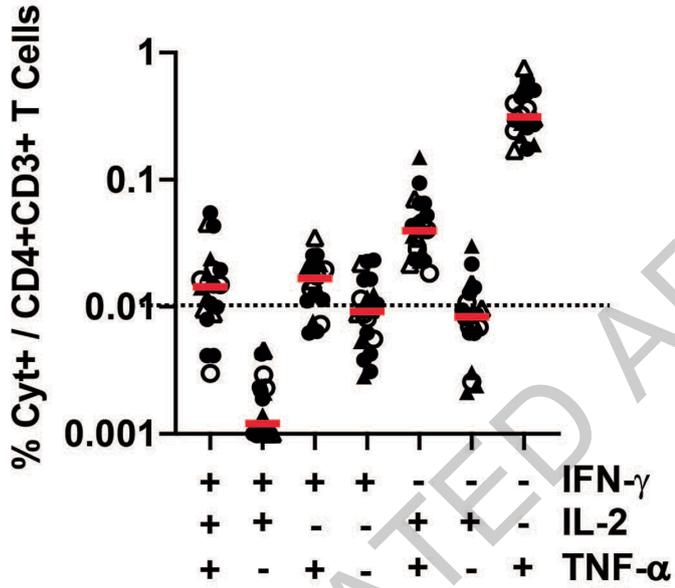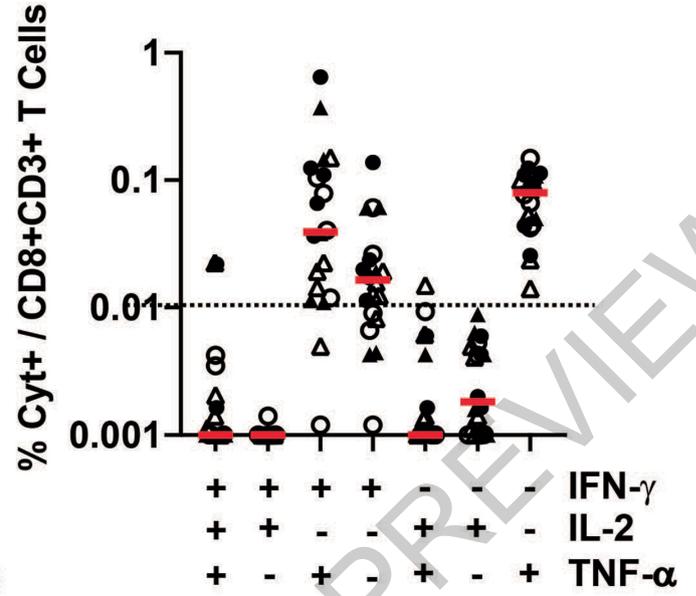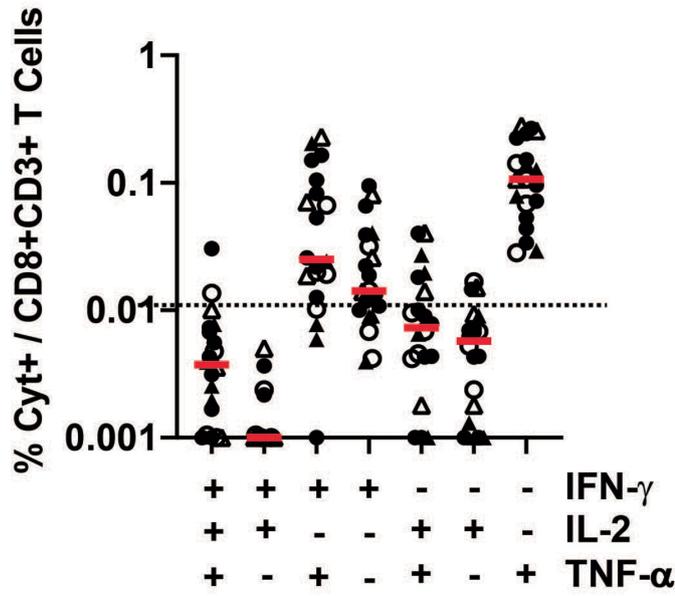
Extended Data Fig. 7 | Polyfunctional CD8+ and CD4+ T cell responses.
WA1/2020 S-specific pooled peptide monofunctional and multifunctional
IFN-γ, IL-2, and TNF-α CD8+ and CD4+ T cell responses by intracellular cytokine
staining (ICS) assays on day 57 and day 85. Red bars reflect median responses.

Dotted lines reflect lower limits of quantitation. Filled circles, high
dose-placebo; open circles, high dose-high dose; filled triangles, low
dose-placebo; open triangles, low dose-low dose. n=20 independent samples
from Ad26.COV2.S vaccine recipients.
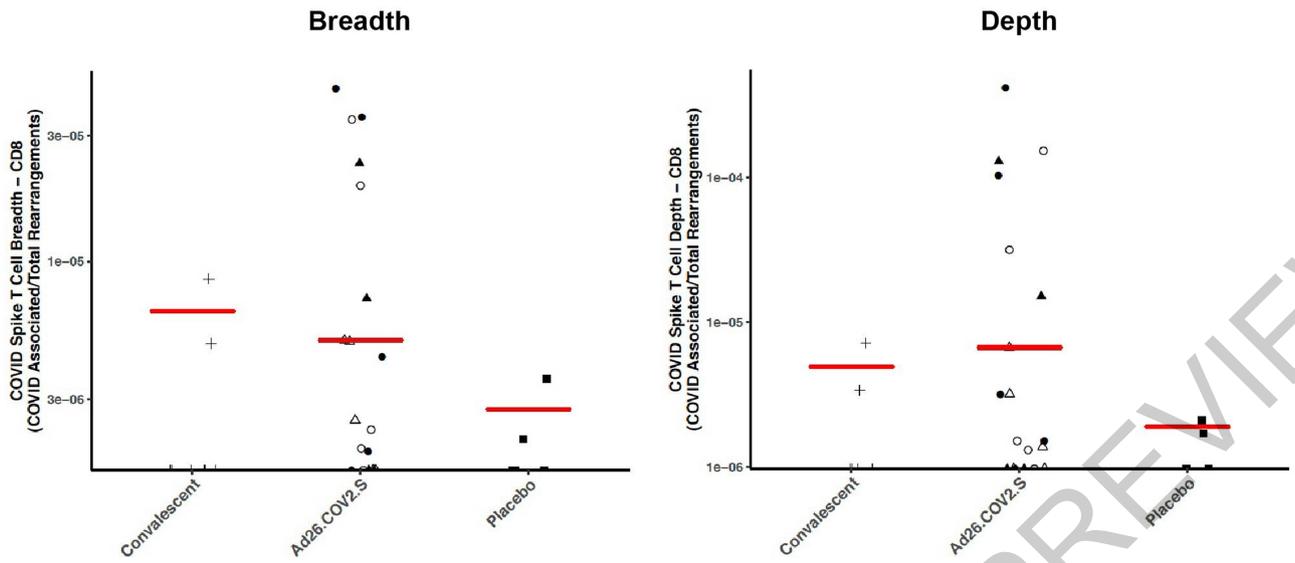
Extended Data Fig. 8 | CD8+ TCRβ repertoire analysis. CD8+ T cell breadth and depth by TCRβ sequencing on day 57. Red bars reflect median responses. Filled squares, placebo-placebo; filled circles, high dose-placebo; open circles, high dose-high dose; filled triangles, low dose-placebo; open triangles, low dose-low dose; plus signs, convalescent samples. n=32 independent samples (8 SARS-CoV-2 convalescent individuals, 5 placebo recipients, 19 Ad26.COV2.S vaccine recipients).
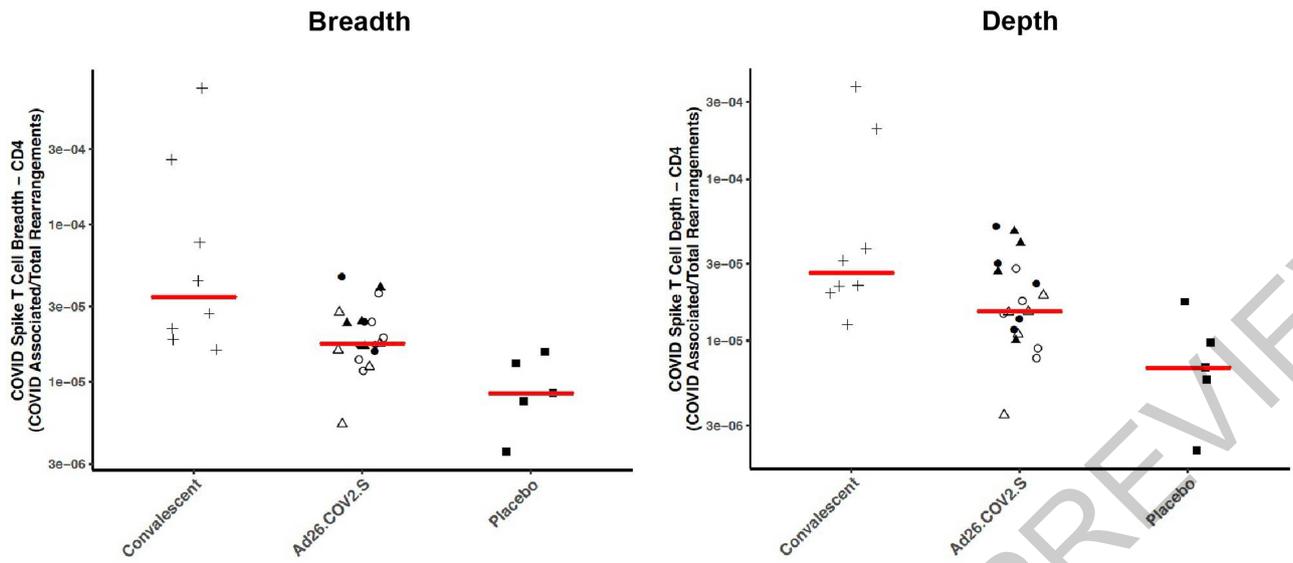
Breadth

Depth

Extended Data Fig. 9 | CD4+ TCRβ repertoire analysis. CD4+ T cell breadth and depth by TCRβ sequencing on day 57. Red bars reflect median responses. Filled squares, placebo-placebo; filled circles, high dose-placebo; open circles, high dose-high dose; filled triangles, low dose-placebo; open triangles, low dose-low dose; plus signs, convalescent samples. n=32 independent samples (8 SARS-CoV-2 convalescent individuals, 5 placebo recipients, 19 Ad26.COV2.S vaccine recipients).

Article

Extended Data Table 1 | TCRβ repertoire analysis

	N	Nucleated Cells	Total T Cells	Total Unique T Cells	T-Cell Fraction	Simpson Clonality	Max Productive Freq	Input DNA (ng)
Convalescent	8	1,052,265 (123,509-1,619,294)	270,534 (16,173-508,294)	172,274 (8,190-412,422)	0.243 (0.121-0.462)	0.08 (0.01-0.231)	0.062 (0.005-0.228)	6,834 (953-10,290)
Placebo	5	1,059,472 (950,153-1,232,508)	525,991 (412,034-695,568)	382,357 (278,871-532,822)	0.449 (0.35-0.548)	0.024 (0.009-0.046)	0.019) (0.006-0.034)	6,979 (6,348-8,102)
Ad26.COV2.S	19	1,221,985 (861,351-1,583,207)	668,538 (426,690-1,043,467)	456,583 (289,281-797,233)	0.492 (0.383-0.628)	0.023 (0.006-0.067)	0.017 (0.003-0.059)	8,124 (5,639-10,281)

Cell parameters analyzed for TCRβ sequencing. n=32 independent samples (8 SARS-CoV-2 convalescent individuals, 5 placebo recipients, 19 Ad26.COV2.S vaccine recipients).

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Last updated by author(s): May 22, 2021

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	None
Data analysis	Analysis of data was performed using FlowJo v9.9 and GraphPad Prism 8.4.2 (GraphPad Software).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data availability statement included. All data are available in the manuscript and supplementary material.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size is 25 indivduals (5 individuals/group reflecting 20 vaccine recipients and 5 placebo recipients). This sample size can differentiate large differences in immunogenicity; details provided in the primary clinical trial manuscript (Stephenson et al. JAMA 2021; 325:1535-1544).
Data exclusions	No data were excluded.
Replication	Virologic and immunologic measures were generally performed in duplicate. Technical replicates were minimally different. All attempts at replication were successful.
Randomization	Participants were randomly allocated to groups.
Blinding	The study was double blinded. All immunologic and virologic assays were also performed blinded.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	For ELISA and ELISPOT assays anti-macaque IgG HRP (NIH NHP Reagent Program), rabbit polyclonal anti-human IFN-γ (U-Cytech); for ICS assays mAbs from BD against CD279 (clone EH12.1, BB700), CD38 (clone OKT10, PE), CD28 (clone 28.2, PE CY5), CD4 (clone L200, BV510), CD45 (clone D058-1283, BUV615), CD95 (clone DX2, BUV737), CD8 (clone SK1, BUV805), Ki67 (clone B56, FITC), CD69 (clone TP1.55.3, ECD), IL10 (clone JES3-9D7, PE CY7), IL13 (clone JES10-5A2, BV421), TNF-α (clone Mab11, BV650), IL4 (clone MP4-25D2, BV711), IFN-γ (clone B27; BUV395), IL2 (clone MQ1-17H12, APC), CD3 (clone SP34.2, Alexa 700) (BD); for 800CW-conjugated goat-anti-human secondary antibody (Li-COR); anti-rhesus IgG1, IgG2, IgG3, IgA, IgM (NIH NHP Reagent Program); tertiary goat anti-mouse IgG-PE antibody (Southern Biotech), anti-CD107a (PE-Cy7, BD), anti-CD56 (PE-Cy7, BD), anti-MIP-1β (PE, BD), mouse anti-human IFN-γ monoclonal antibody (BD), Streptavidin-alkaline phosphatase antibody (Southern Biotech), anti-human IgG HRP (Invitrogen). Antibodies were used at manufacturer's concentrations and were titrated prior to use.
Validation	mAbs used according to manufacturer's instructions and previously published methods; mAbs were titrated with human PBMC and assessed for specificity prior to use

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293T from ATCC
Authentication	Commerically purchased (ATCC) and evaluated in control experiments prior to use
Mycoplasma contamination	Negative for mycoplasma

Commonly misidentified lines (See [ICLAC](#) register)	None were utilized

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	Healthy adults 18-55, mixed gender; details provided in the primary clinical trial manuscript (Stephenson et al. JAMA 2021; 325:1535-1544)
Recruitment	Participants were recruited by education and outreach programs; details provided in the primary clinical trial manuscript (Stephenson et al. JAMA 2021; 325:1535-1544)
Ethics oversight	BIDMC institutional review board; details provided in the primary clinical trial manuscript (Stephenson et al. JAMA 2021; 325:1535-1544)

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	NCT04436276
Study protocol	Primary clinical trial and protocol published (Stephenson et al. JAMA 2021; 325:1535-1544)
Data collection	25 participants were enrolled from July 29, 2020 to August 7, 2020, and the follow-up for this day 71 interim analysis was completed on October 3, 2020. This study was conducted at a single clinical site in Boston, MA as part of a randomized, double-blinded, placebo-controlled phase 1/2a clinical trial (COV1001) of Ad26.COV2.S.
Outcomes	Variant immunogenicity data are reported in this manuscript. Safety and immunogenicity are reported in the primary clinical trial manuscript (Stephenson et al. JAMA 2021; 325:1535-1544)

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	10^6 PBMCs/well were re-suspended in 100 μL of R10 media
Instrument	BD FACSymphony
Software	FlowJo v10
Cell population abundance	See Extended Data Fig. 3
Gating strategy	See Extended Data Fig. 3; FSC/SSC gating of the starting cell population, live/dead staining, gating on CD3+CD4+ or CD3+CD8+ cell populations

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.