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## T cell responses to SARS-CoV-2 spike cross-recognize Omicron

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**The SARS-CoV-2 Omicron variant has multiple Spike (S) protein mutations<sup>1,2</sup> that contribute to escape from antibody neutralization<sup>3-6</sup> and reduce vaccine protection from infection<sup>7,8</sup>. The extent to which other components of the adaptive response such as T cells may still target Omicron and contribute to protection from severe outcomes is unknown. We assessed the ability of T cells to react with Omicron spike in participants**

who were vaccinated with Ad26.CoV2.S, BNT162b2, or unvaccinated convalescent COVID-19 patients (n=70). We found that 70-80% of the CD4+ and CD8+ T cell response to spike was maintained across study groups. Moreover, the magnitude of Omicron cross-reactive T cells was similar to Beta and Delta variants, despite Omicron harboring considerably more mutations. In Omicron-infected hospitalized patients (n=19), there were comparable T cell responses to ancestral spike, nucleocapsid and membrane proteins to those patients hospitalized in previous waves dominated by the ancestral, Beta or Delta variants (n=49). Thus, despite Omicron's extensive mutations and reduced susceptibility to neutralizing antibodies, the majority of T cell responses, induced by vaccination or infection, cross-recognize the variant. It remains to be determined whether well-preserved T cell immunity to Omicron contributes to protection from severe COVID-19, and is linked to early clinical observations from South Africa and elsewhere<sup>9-12</sup>.

The newest SARS-CoV-2 variant of concern, designated Omicron<sup>1</sup>, was described on 26 November 2021 from sequences from Botswana, Hong Kong and South Africa<sup>2</sup>. Omicron is responsible for the current surge of infections in South Africa, and is becoming globally dominant. With over 30 mutations in the spike protein, a substantial ability to evade the neutralizing antibody response has been described<sup>3-6</sup>. This associates with greater capacity for reinfection<sup>13</sup>, as well as lower early estimates of vaccine effectiveness against symptomatic disease<sup>7,8</sup>. SARS-CoV-2-specific T cells play a role in modulating COVID-19 severity. A study of acute COVID-19 suggested, through combined measurement of CD4+ T cells, CD8+ T cells, and neutralizing antibodies in COVID-19, that co-ordination of these three arms of the adaptive response leads to lower disease severity<sup>14</sup>. A greater CD8+ T cell response in blood and highly clonally expanded CD8+ T cells in bronchoalveolar lavage were observed in convalescent patients who experienced mild or moderate disease compared to severe disease<sup>15-16</sup>, and CD8+ T cells provided partial protective immunity in the context of suboptimal antibody titers in macaques<sup>17</sup>. A total of 138 participants were included in this study, grouped according to their vaccination and COVID-19 status, in order to 1) determine whether T cells generated in response to vaccination or previous SARS-CoV-2 infection could cross-recognize Omicron, and 2) define the profile of T cell responses in Omicron-infected patients compared to those infected with other variants of concern.

### **T cell cross-reactivity to Omicron**

We examined T cell responses in participants who had received one or two doses of the Ad26.COVS vaccine (Johnson and Johnson/Janssen, n = 20/group), two doses of the BNT162b2 mRNA vaccine (Pfizer–BioNTech, n = 15), or who had recovered from infection (n = 15) (**Fig 1a, Extended Data Table 1a and 1b**). Convalescent donors were examined a median of 1.4 months (interquartile range [IQR]: 1.3-6 months) after mild or asymptomatic infection. More than 85% of vaccinees generated a T cell response to vaccination, measured 22-32 days after the last dose (**Fig. 1b**). Both vaccination and infection induced spike-specific CD4+ T cell responses, while a CD8 response was less consistently detected (**Fig. 1c**). We measured cytokine production (IFN- $\gamma$ , IL-2 and TNF- $\alpha$ ) by intracellular cytokine staining in response to peptide pools covering the full Wuhan-1 spike protein (ancestral) and the Omicron spike (**Fig. 1d and Extended Data Fig. 1a**).

CD4+ T cell frequencies to Omicron spike were consistently and significantly lower than ancestral spike in all groups tested (**Fig. 1e**). This translated to a median decrease of 14-30% of the CD4 response to Omicron, as demonstrated by fold-change (**Fig. 1f**). Similar results were observed for the CD8+ T cell response (**Fig. 1g-h**), where vaccinees who had received two doses of Ad26.COVS and convalescent donors demonstrated a significant decrease in the magnitude of Omicron spike-specific CD8+ T cells, although the other groups did not. There was a median reduction of 17-25% of the CD8 response to Omicron compared to the ancestral virus. Of note, a fraction of responders (5/32; 15%) exhibited a loss of CD8+ T cell recognition of Omicron (**Figure 1g and Extended Data Fig. 1b**), likely reflecting specific HLA molecules being adversely affected by mutations in particular CD8 epitopes<sup>18</sup>.

In parallel, we also measured the neutralizing activity against ancestral and Omicron spike from the plasma of the same participants who received BNT162b2 (n=10) or 2 doses of Ad26.COVS (n=19) (**Extended data Fig. 2**). As previously described<sup>3,5,6</sup>, Omicron escapes

the SARS-CoV-2 neutralizing antibodies generated after BNT162b2 vaccination. Here we present neutralizing responses to Omicron after two doses of Ad26.COVS (Extended data Fig. 2b), demonstrating diminished neutralization capacity compared to D614G ancestral virus and the Beta variant. Comparison of the fold change in T cell responses and neutralizing antibodies targeting ancestral or Omicron spike further emphasizes the preservation of the T cell response, even when neutralization is severely reduced.

Mutations in variant epitopes have the potential to affect the functional capacity of cells<sup>19</sup>. Thus, we compared the polyfunctional profiles of T cells in vaccinees and convalescent individuals and demonstrate similar capacities for cytokine co-expression across all groups for both ancestral and Omicron-specific T cells (Extended Data Fig. 3a-b and 4a-b). Notably, there were also no differences in the polyfunctional profiles between ancestral and Omicron spike for either CD4+ or CD8+ T cells (Extended Data Fig. 3c and 4c), indicating the absence of a functional deficit in cross-reactive Omicron T cell responses. We also compared Omicron spike responses to other variants of concern in Ad26.CoV2.S vaccinees, by testing spike peptide pools corresponding to the viral sequences of the Beta and Delta strains (Extended Data Fig. 5a). There were no significant differences in cross-reactive CD4+ and CD8+ T cell responses between Beta, Delta and Omicron (Extended Data Fig. 5b), with the exception of a greater decrease in the Omicron CD4 response compared to Beta in recipients of two doses of Ad26.COVS. Of note, while prior SARS-CoV-2 infection in vaccinees associated with a higher frequency of spike-specific T cells (Extended Data Fig. 6a), it had no impact on Omicron cross-reactivity (Extended Data Fig. 6b). Overall, these results show that CD4+ and CD8+ T cell recognition of Omicron spike is largely preserved compared to the ancestral strain, and is similar to other variants of concern carrying three times fewer mutations.

### T cell response to different variants

The SARS-CoV-2 epidemic in South Africa has been characterized by four virologically distinct infection waves (Fig. 2b). This enabled us to compare T cell responses in patients from the current fourth epidemic wave, dominated by Omicron, with those infected in prior waves dominated by ancestral (Wave 1, n = 17), Beta (Wave 2, n = 16) and Delta (Wave 3, n = 16) variants (Fig. 2a). In addition to extensive mutations in spike, Omicron has 20 additional mutations in other proteins which could also result in T cell escape. Therefore, we measured the frequency of CD4+ and CD8+ T cells to ancestral spike (S), nucleocapsid (N) and membrane (M) proteins, all major targets of the T cell response<sup>20</sup>. We studied SARS-CoV-2-infected patients who were hospitalized with COVID-19 (Fig. 2a). These recently hospitalized patients, recruited between December 1<sup>st</sup> and 15<sup>th</sup>, 2021 (n = 19), had no history of prior COVID-19 and were unvaccinated. Omicron infection was inferred by S-gene target failure (SGTF)<sup>21</sup> in nine of these patients. Although swabs were unavailable for the remainder, with Omicron accounting for >90% of sequences from South Africa at the time of recruitment and 98% in Tshwane from where the samples originated (Fig. 2b), there was a high probability of Omicron infection in all patients.

Despite differences in age, disease severity and co-morbidities across the infection waves (Fig. 2a and Extended Data Table 1c), T cell responses directed at S, N and M in wave 4 patients were of similar magnitude as those in patients infected with other SARS-CoV-2 variants in previous waves (Fig. 2c and d). The frequency of responders also did not

differ markedly across the waves. Of note, we did not find any association between the absence of detectable CD4<sup>+</sup> T cell responses and the time post COVID-19 diagnosis or disease severity. Furthermore, the magnitude of Omicron spike-specific CD4 responses mounted by Wave 4 patients was highly comparable to ancestral spike (**Fig. 2e**), suggesting that most patients target conserved epitopes in spike. Using data from the Immune Epitope Database ([www.iedb.org](http://www.iedb.org)), we assessed the frequency of T cell recognition of experimentally-confirmed epitopes spanning the entire spike protein. Data show that Omicron spike mutations occur in regions poorly targeted by CD4<sup>+</sup> T cells, but are more common in regions frequently targeted by CD8<sup>+</sup> T cells (**Extended Data Fig. 7**).

To gain deeper insight into the recognition of variable spike epitopes by CD8<sup>+</sup> T cells, we also performed *in silico* analysis to define predicted HLA Class I restriction for Omicron variable epitopes (**Extended Data Table 2**). Six confirmed spike epitopes containing Omicron mutations (A67V/del 69-70, G142D/143-145 del, S373P, S375F, D614G, P681H and N764K) would be detrimentally affected for binding to specific class I alleles, four of which were located at positions that recorded a frequency of recognition greater than 10%. However, we also found another seven confirmed epitopes that contained Omicron mutations (T95I, S371L/S373P/S375F, K417N, G446S, Q493R, N764K, L981F) but had no impact on class I binding compared to the ancestral sequence, five of which were located at positions with a frequency of recognition greater than 10%. Overall, this suggests that while some Omicron mutations may mediate escape from specific HLA-restricted CD8<sup>+</sup> T cells, not all mutations appear to have an impact on class I binding.

## Discussion

In this study, we measured the ability of individuals to cross-recognize Omicron spike, following vaccination, prior infection or both. We also studied unvaccinated individuals with no history of previous infection, whose first encounter with spike was the Omicron version. We demonstrate that vaccination and infection induce robust CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses that largely cross-react with Omicron, consistent with recent work from our laboratory and others on limited T cell escape by Beta, Delta and other variants<sup>22-24</sup>. Despite extensive neutralization escape against Omicron<sup>5</sup>, 70-80% of the T cell response is cross-reactive. In contrast to neutralizing antibody epitopes, T cell epitopes are abundant and located across the entire spike protein<sup>20</sup>, suggesting that the majority of SARS-CoV-2 spike-specific T cell responses are directed against conserved epitopes and SARS-CoV-2 viral evasion from T cells may be limited.

Of note, Omicron mutations appear to abolish CD8<sup>+</sup> T cell recognition in 5 out of 32 participants (15%), in agreement with a recent report<sup>25</sup>. This loss of cross-reactive CD8<sup>+</sup> T cell responses could have pathological consequences for some individuals. Further analyses are required to define specific HLA class I profiles and epitopes linked to loss of T cell responses.

T cells are crucial components of the antiviral immune response. Whilst they do not prevent infection, CD4<sup>+</sup> T cells are indispensable for the generation of protective antibody responses and supporting the maturation of CD8<sup>+</sup> T cells. Hence, given the ability of variants of concern to escape neutralization, the generation and maintenance of robust SARS-CoV-2-specific T cell responses could contribute to long-term vaccine efficacy against severe

disease. Several studies have reported a waning of the neutralizing response after vaccination or infection<sup>26-28</sup>. However, humoral responses can be enhanced upon booster vaccination, improving Omicron neutralization<sup>3,6,29,30</sup>. Vaccine- and infection-induced T cell responses also decay after antigen clearance<sup>31,32</sup>, but SARS-CoV-2-specific CD8+ T cells exhibit the hallmarks of long-lived cells<sup>33</sup>, and T cell responses to SARS-CoV-1 infection were detectable 17 years later<sup>34</sup>. The longer-term durability of SARS-CoV-2-specific T cells remains to be determined, and whether vaccine boosters can further enhance cellular immunity.

Despite the sharp increase in cases in South Africa in the current surge<sup>35</sup>, this has not translated into the expected increase in hospitalization or deaths, compared to previous waves<sup>12</sup>. This uncoupling of caseloads and severe outcomes could be attributed to population immunity, including maintenance of cross-reactive T cell responses observed in our study, or intrinsic differences in Omicron severity, or both. South Africa has high levels of SARS-CoV-2 seropositivity, driven mainly by prior infection (estimated at >60%) and a modest proportion of vaccinated people (40%)<sup>36</sup>. Emerging data hint at reduced intrinsic severity of Omicron, including reduced infection of lower airway cells<sup>37,38</sup>. The relative contribution of high levels of immunity and potential changes in intrinsic virulence on clinical outcomes are difficult to disentangle. Moreover, it remains to be determined whether the apparently milder outcomes at a population level will be observed in other contexts with different exposure histories and vaccination coverage, or whether the higher transmissibility of Omicron and the expected massive increase in cases in a short period will offset any gains. To date, immune correlates of protection from disease are not clearly defined and large-scale prospective studies would be necessary to evaluate correlates of protection and define the role of T cell responses in disease.

Our study had several limitations. We studied Omicron cross-reactivity of vaccine responses approximately one month after vaccination. Since T cell responses decline over time, the detection of continued cross-reactivity with variants over time will be related to the durability of the T cell response. Recall memory responses *in vivo* are likely to expand rapidly upon viral infection and contribute to limiting viral replication. We also focused on Th1 cytokine production to quantify CD4+ and CD8+ T cell responses. Additional approaches such as the activation-induced marker (AIM) assay may capture the cellular immune response in a more comprehensive manner<sup>39</sup>. The use of 15mer peptides will have underestimated SARS-CoV-2 specific CD8+ T cells as 9-10mer peptides are optimal for HLA class I binding and it has been estimated that 15mer peptides capture 77% of the frequency of CD8+ T cells when compared to shorter peptides<sup>40</sup>. Moreover, the saturating concentration of peptides used in these studies may underestimate the impact of mutations on T cells. In addition, the use of peptides does not permit us to define the potential effect of mutations on antigen processing and presentation, thus underestimating the impact of Omicron mutations on T cell cross-recognition. Finally, confirmation of our results from cohorts in other geographical areas and exposure to other vaccines would offer further reassurance of the maintenance of T cell responses against Omicron. Indeed, emerging data suggest this to be the case<sup>25,41-45</sup>.

Overall, our data show that unlike neutralizing antibodies, the SARS-CoV-2 T cell response generated upon vaccination or prior infection are highly cross-reactive with Omicron. Early reports emerging from South Africa, England and Scotland have reported a

lower risk of hospitalization and severe disease compared to the previous Delta wave<sup>9-12</sup>. It remains to be defined whether cell-mediated immunity provides protection from severe disease and contributes to the apparent milder outcomes for Omicron. Moreover, the resilience of the T cell response demonstrated here also bodes well in the event that more highly mutated variants emerge in the future.

**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 [Article DOI].

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## METHODS

### Human Participants

At total of 138 participants were included in this study and grouped according to their vaccination and COVID-19 status. Participants were selected based on PBMC availability and clinical data were recorded by trained clinicians using RedCap (v9.5.36). The study was approved by the University of Cape Town Human Research Ethics Committee (ref: HREC 190/2020, 207/2020 and 209/2020) and the University of the Witwatersrand Human Research Ethics Committee (Medical) (ref. M210429 and M210752), the Biomedical Research Ethics Committee at the University of KwaZulu–Natal (ref. BREC/00001275/2020) and the University of Pretoria Health Sciences Research Ethics Committee (ref. 247/2020). Written informed consent was obtained from all participants.

#### 1- Participants vaccinated with Ad26.COV2.S (one or two doses) or BNT162b2 (two doses)

PBMC samples from 40 participants (20 who received one dose of Ad26.COV2.S vaccine and 20 who received two doses) were included in this study. These participants are enrolled in the Sisonke Phase 3b trial, an implementation trial of Ad26.COV2.S in healthcare workers. Recruitment took place at Groote Schuur Hospital (Cape Town, Western Cape, South Africa) between July 2020 and December 2021. Prior COVID-19 infection was recorded in 13 out of the 20 participants who had received one dose of the Ad26.COV2.S vaccine and in 14 out of 20 participants who had received two doses. Additionally, we also included samples from 15 participants vaccinated with two doses of BNT162b2 (Pfizer), enrolled in a prospective cohort study in KwaZulu Natal (South Africa). Prior COVID-19 infection was recorded for 6 out of 15 participants. The demographic and clinical characteristics of vaccinated participants are summarized in **Extended Data Table 1a**, with individual participant details presented in **Extended Data Table 4**.

#### 2- Convalescent COVID-19 participants

COVID-19 convalescent volunteers (n = 15) were recruited from Groote Schuur Hospital in Cape Town (Western Cape, South Africa). Based on the reported date of infection, seven were likely infected with ancestral SARS-CoV-2 (prior to August 2020), while for other 8, the infection date occurred in December 2020, suggesting an infection with the Beta variant. Samples were obtained between January 19<sup>th</sup> and February 15<sup>th</sup>, 2021 prior to SARS-CoV-2 vaccination becoming available in South Africa. All had a documented positive SARS-CoV-2 PCR swab result or a positive SARS-CoV-2 nucleocapsid-specific antibody result (Roche Elecsys assay, Roche Diagnostics, Basel, Switzerland). The median time post positive test was 1.4 months, ranging from 1 to 7 months. The demographic and clinical characteristics of convalescent volunteers are summarized in **Extended Data Table 1b**, with individual participant details presented in **Extended Data Table 3**.

#### 3- Hospitalized COVID-19 patients

Sixty-eight hospitalized COVID-19 patients were included in this study. These participants were grouped according to the time of their hospitalization, reflecting four distinct infection waves in South Africa, each dominated by a different SARS-CoV-2 strain (**Fig. 2b**). Wave 1, 2 and 3 participants were recruited from Groote Schuur Hospital in Cape Town (Western Cape, South Africa) and wave 4 patients were recruited from Groote Schuur Hospital and Tshwane District Hospital in Tshwane (Gauteng, South Africa). Wave 1 patients (n = 17) were enrolled between June 11<sup>th</sup> and July 24<sup>th</sup>, 2020, at a time when ancestral (Wuhan-1 D614G)-related SARS-CoV-2 strains were circulating. No viral sequences are available for these patients, but we assumed that all were infected with a virus closely related to the ancestral virus, as sampling occurred almost three months before the emergence of the Beta variant in South Africa. Wave 2 patients (n = 16) were recruited between December 31<sup>st</sup>, 2020, and January 15<sup>th</sup>, 2021, when the Beta variant dominated. Viral sequences were available for six second wave participants, all of which were confirmed Beta infection (GISAID accession numbers: EPI\_ISL\_1040693, 1040658, 1040661, 1040685, 1040657, 1040663). Wave 3 patients (n = 16) were recruited between July 14<sup>th</sup> and 21<sup>st</sup>, 2021. Wave 3 was dominated by the Delta variant. Viral sequences were available for seven third wave participants, all of which were confirmed to be Delta infection (GISAID accession numbers: EPI\_ISL\_3506484, 3506367, 3957813, 3506504, 3506512, 3506518). Wave 4 patients (n = 19) were recruited between December 1<sup>st</sup> and 15<sup>th</sup>, 2021. The SARS-CoV-2 Omicron variant was dominant during this current wave. Amongst those patients, nine had a Taqpath PCR test performed (ThermoFisher, Waltham, Massachusetts, USA), all of which were characterized by S gene target failure, highly suggestive of an Omicron infection. Whilst we did not have confirmation of Omicron for the remaining samples, they were recruited at a time when Wave 4 was driven by Omicron infection (**Fig. 2b**; there was no concomitant Delta wave in South Africa as has occurred elsewhere), with the prevalence of Omicron in South Africa at the time of recruitment >90% by whole genome sequencing. Moreover, in Tshwane from where the remainder of the samples originated, Omicron was responsible for 98% of infections sequenced at the time of sampling (61/62 samples sequenced).

All hospitalized patients from wave 1, 2 and 4 were unvaccinated at the time of sampling. Third wave participants with known vaccination status were all unvaccinated (n=8), and the remainder (n=8) had unknown vaccination status. Moreover, all hospitalized patients from wave 1, 2 and 4 had no clinical record of a previous symptomatic COVID-19 episode, apart from one Wave 4 participant with an unknown history. The majority of wave 3 patients had an unknown history of prior COVID-19. The demographic and clinical characteristics of hospitalized COVID-19 participants are summarized in **Extended Data Table 1c**, and individual patient clinical data are presented in **Extended Data Table 3**.

### **SARS-CoV-2 spike WGS and phylogenetic analysis**

Whole genome sequencing (WGS) of SARS-CoV-2 was performed from nasopharyngeal swabs. Sequencing was performed as previously published<sup>2</sup>. Briefly, RNA was extracted on an automated Chemagic 360 instrument, using the CMG-1049 kit (Perkin Elmer, Hamburg, Germany). Libraries for whole genome sequencing were prepared using either the Oxford Nanopore Midnight protocol with Rapid Barcoding or the Illumina COVIDseq Assay. The quality control checks on raw sequence data and the genome assembly were performed using Genome Detective 1.133 (<https://www.genomedetective.com>) which was updated for the

accurate assembly and variant calling of tiled primer amplicon Illumina or Oxford Nanopore reads, and the Coronavirus Typing Tool. Phylogenetic classification of the genomes was done using the widespread dynamic lineage classification method from the ‘Phylogenetic Assignment of Named Global Outbreak Lineages’ (PANGOLIN) software suite (v1.2.106) (<https://github.com/hCoV-2019/pangolin>).

### **Isolation of PBMC**

Blood was collected in heparin tubes and processed within 4 hours of collection. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient sedimentation using Ficoll-Paque (Amersham Biosciences, Little Chalfont, UK) as per the manufacturer’s instructions and cryopreserved in freezing media consisting of heat-inactivated fetal bovine serum (FBS, Thermofisher Scientific) containing 10% DMSO and stored in liquid nitrogen until use.

### **SARS-CoV-2 antigens**

For T cell assays on hospitalized patients, we used commercially available peptide pools (15mer sequences with a 11 amino acids overlap) covering the full length of the Wuhan-1 SARS-CoV-2 nucleocapsid, membrane and near full-length spike proteins (PepTivator®, Miltenyi Biotech, Bergisch Gladbach, Germany). For spike, we combined i) a pool of peptides (15-mer sequences with 11 amino acids (aa) overlap) covering the ancestral N-terminal S1 domain of SARS-CoV-2 (GenBank MN908947.3, Protein QHD43416.1) from aa 1 to 692 and ii) a pool of peptides (15-mer sequences with 11 aa overlap) covering the immunodominant sequence domains of the ancestral C-terminal S2 domain of SARS-CoV-2 (GenBank MN908947.3, Protein QHD43416.1) including the sequence domains aa 683-707, aa 741-770, aa 785-802, and aa 885-1273. Pools were resuspended in distilled water at a concentration of 50 µg/mL and used at a final concentration of 1 µg/mL. To determine T cell responses to SARS-CoV-2 variants in vaccinated and convalescent volunteers, we used custom mega pools of peptides. These peptides (15-mers overlapping by 10 amino acids) spanned the entire spike protein corresponding to the ancestral Wuhan sequence (GenBank: MN908947), Beta (B.1.351; GISAID: EPI\_ISL\_736932), Delta SARS-CoV-2 variants (B.1.617.2; GISAID: EPI\_ISL\_2020950) or Omicron (B.1.1.529), carrying in the spike sequence all the 38 currently described mutations (A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, S152W, N211del, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F). Briefly, peptides were synthesized as crude material (TC Peptide Lab, San Diego, CA). All individual peptides included in each mega pool are listed in **Supplementary Table 1**. All peptides were individually resuspended in dimethyl sulfoxide (DMSO) at a concentration of 10-20 mg/mL. Megapools for each antigen were created by pooling aliquots of these individual peptides in the respective SARS-CoV-2 spike sequences, followed by sequential lyophilization steps, and resuspension in DMSO at 1 mg/mL. There were 253 peptides in the Ancestral, Beta and Delta variant pool, and 254 peptides in the Omicron pool. Pools were used at a final concentration of 1 µg/mL with an equimolar DMSO concentration in the non-stimulated control.

### **Cell stimulation and flow cytometry staining**

Cryopreserved PBMC were thawed, washed and rested in RPMI 1640 containing 10% heat-inactivated FCS for 4 hours prior to stimulation. PBMC were seeded in a 96-well V-bottom plate at  $\sim 2 \times 10^6$  PBMC per well and stimulated with either the commercial ancestral SARS-CoV-2 spike (S), Nucleocapsid (N) or membrane protein (M) peptide pools (1  $\mu\text{g}/\text{mL}$ ) obtained from Miltenyi or custom spike mega pools corresponding to the ancestral (Wuhan-1), Beta, Delta or Omicron variants (1  $\mu\text{g}/\text{mL}$ ). All stimulations were performed in the presence of Brefeldin A (10  $\mu\text{g}/\text{mL}$ , Sigma-Aldrich, St Louis, MO, USA) and co-stimulatory antibodies against CD28 (clone 28.2) and CD49d (clone L25) (1  $\mu\text{g}/\text{mL}$  each; BD Biosciences, San Jose, CA, USA). As a negative control, PBMC were incubated with co-stimulatory antibodies, Brefeldin A and an equimolar amount of DMSO. After 16 hours of stimulation, cells were washed, stained with LIVE/DEAD™ Fixable VIVID Stain (1/2500, Invitrogen, Carlsbad, CA, USA) and subsequently surface stained with the following antibodies: CD14 Pac Blue (1/100, TuK4, Invitrogen Thermofisher Scientific), CD19 Pac Blue (1/100, SJ25-C1, Invitrogen Thermofisher Scientific), CD4 PERCP-Cy5.5 (1/100, L200, BD Biosciences, San Jose, CA, USA), CD8 BV510 (1/100, RPA-8, Biolegend, San Diego, CA, USA). Cells were then fixed and permeabilized using a Cytotfix/Cyto perm buffer (BD Biosciences) and stained with CD3 BV650 (1/100, OKT3) IFN- $\gamma$  Alexa 700 (1/250, B27), TNF- $\alpha$  BV786 (1/100, Mab11) and IL-2 APC (1/100, MQ1-17H12) from Biolegend. Finally, cells were washed and fixed in CellFIX (BD Biosciences). Samples were acquired on a BD Fortessa flow cytometer and analyzed using FlowJo (v10.8, FlowJo LLC, Ashland, OR, USA) and Pestle and Spice v6.1 (<https://niaid.github.io/spice>). A gating strategy is provided in **Extended Data Fig. 1**. Results are expressed as the frequency of CD4<sup>+</sup> or CD8<sup>+</sup> T cells expressing IFN- $\gamma$ , TNF- $\alpha$  or IL-2. Due to high TNF- $\alpha$  backgrounds, cells producing TNF- $\alpha$  alone were excluded from the analysis. All data are presented after background subtraction.

### **Live virus neutralization assay**

A live neutralization assay was performed on plasma obtained from 10 out of the 15 participants vaccinated with BNT162b2 included in this study. H1299-E3 cells were plated in a 96-well plate (Corning) at 30,000 cells per well 1 day pre-infection. Plasma was separated from EDTA-anticoagulated blood by centrifugation at 500 rcf for 10 min and stored at -80 °C. Aliquots of plasma samples were heat-inactivated at 56 °C for 30 min and clarified by centrifugation at 10,000 rcf for 5 min. Virus stocks were used at approximately 50-100 focus-forming units per microwell and added to diluted plasma. Antibody-virus mixtures were incubated for 1 h at 37 °C, 5% CO<sub>2</sub>. Cells were infected with 100  $\mu\text{L}$  of the virus-antibody mixtures for 1 h, then 100  $\mu\text{L}$  of a 1X RPMI 1640 (Sigma-Aldrich, R6504), 1.5% carboxymethylcellulose (Sigma-Aldrich, C4888) overlay was added without removing the inoculum. Cells were fixed 18 h post-infection using 4% PFA (Sigma-Aldrich) for 20 min. Foci were stained with a rabbit anti-spike monoclonal antibody (BS-R2B12, GenScript A02058) at 0.5  $\mu\text{g}/\text{mL}$  in a permeabilization buffer containing 0.1% saponin (Sigma-Aldrich), 0.1% BSA (Sigma-Aldrich) and 0.05% Tween-20 (Sigma-Aldrich) in PBS. Plates were incubated with primary antibody overnight at 4 °C, then washed with wash buffer containing 0.05% Tween-20 in PBS. Secondary goat anti-rabbit horseradish peroxidase (Abcam ab205718) antibody was added at 1  $\mu\text{g}/\text{mL}$  and incubated for 2 h at room temperature with shaking. TrueBlue peroxidase substrate (SeraCare 5510-0030) was then

added at 50  $\mu$ L per well and incubated for 20 min at room temperature. Plates were imaged in an ELISPOT instrument with built-in image analysis (C.T.L).

### **SARS-CoV-2 pseudovirus-based neutralization assay**

A pseudovirus-based neutralization assay was performed on plasma obtained from all participants vaccinated with two doses of Ad26.COVID.S (n = 20). SARS-CoV-2 pseudotyped lentiviruses were prepared by co-transfecting the HEK 293T cell line with the SARS-CoV-2 614G spike (D614G) or SARS-CoV-2 Beta spike (L18F, D80A, D215G, K417N, E484K, N501Y, A701V, 242-244 del) plasmids with a firefly luciferase encoding lentivirus backbone plasmid. The parental plasmids were provided by Drs Elise Landais and Devin Sok (IAVI). For the neutralization assays, heat-inactivated plasma samples were incubated with SARS-CoV-2 pseudotyped virus for 1 hour at 37°C, 5% CO<sub>2</sub>. Subsequently, 1x10<sup>4</sup> HEK293T cells engineered to overexpress ACE-2, provided by Dr Michael Farzan (Scripps Research Institute), were added and incubated at 37°C, 5% CO<sub>2</sub> for 72 hours, upon which the luminescence of the luciferase gene was measured. CB6 and CA1 monoclonal antibodies were used as controls.

### **Statistical analysis**

Statistical analyses were performed in Prism (v9; GraphPad Software Inc, San Diego, CA, USA). Non-parametric tests were used for all comparisons. The Mann-Whitney, Friedman and Wilcoxon tests were used for unmatched and paired samples, respectively. *P* values less than 0.05 were considered statistically significant. Details of analysis performed for each experiment are described in the figure legends.

### **Reporting summary**

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

### **Data availability**

Datasets (raw data) underlying the figures have been provided as Source Data. Complete genome sequences for the viral isolates were deposited in GISAID.

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**Author contributions** W.A.B, C.R. and R.K. designed the study. C.R. and W.A.B analyzed the data and wrote the manuscript. K.K., S.C., M.B., F.K. and S.V.M. performed neutralization assays and analyzed the data. R.K., M.B.T., A.N., R.B., N.B., and A.Su. performed flow cytometry assays. M.M., S.S., M.A., D.M., O.A., C.S., E.dB., M.A.vdM., Z.dB., T.R.dV. A.B., G.vdB, A.M., A.St., M.V. recruited patients and managed cohorts. A.G., D.W. and A.Se. designed and provided variant peptide pools. T.dO., G.J., S.N., Y.P. and H.T. generated and analyzed sequence data. T.M-G., J.B, P.L.M. and A.Si. supervised the neutralization assay. N.A.B.N., R.J.W., V.U., M.T.B and T.R. led the clinical cohorts. L.G.B and G.G. led the Sisonke Ad26.COVS vaccination trial. All authors reviewed and edited the manuscript.

**Competing interests** A. Sette is a consultant for Gritstone Bio, Flow Pharma, Arcturus Therapeutics, ImmunoScape, CellCarta, Avalia, Moderna, Fortress and Repertoire. All of the other authors declare no competing interests. La Jolla Institute for Immunology has filed a patent for protection for various aspects of vaccine design and identification of specific epitopes.

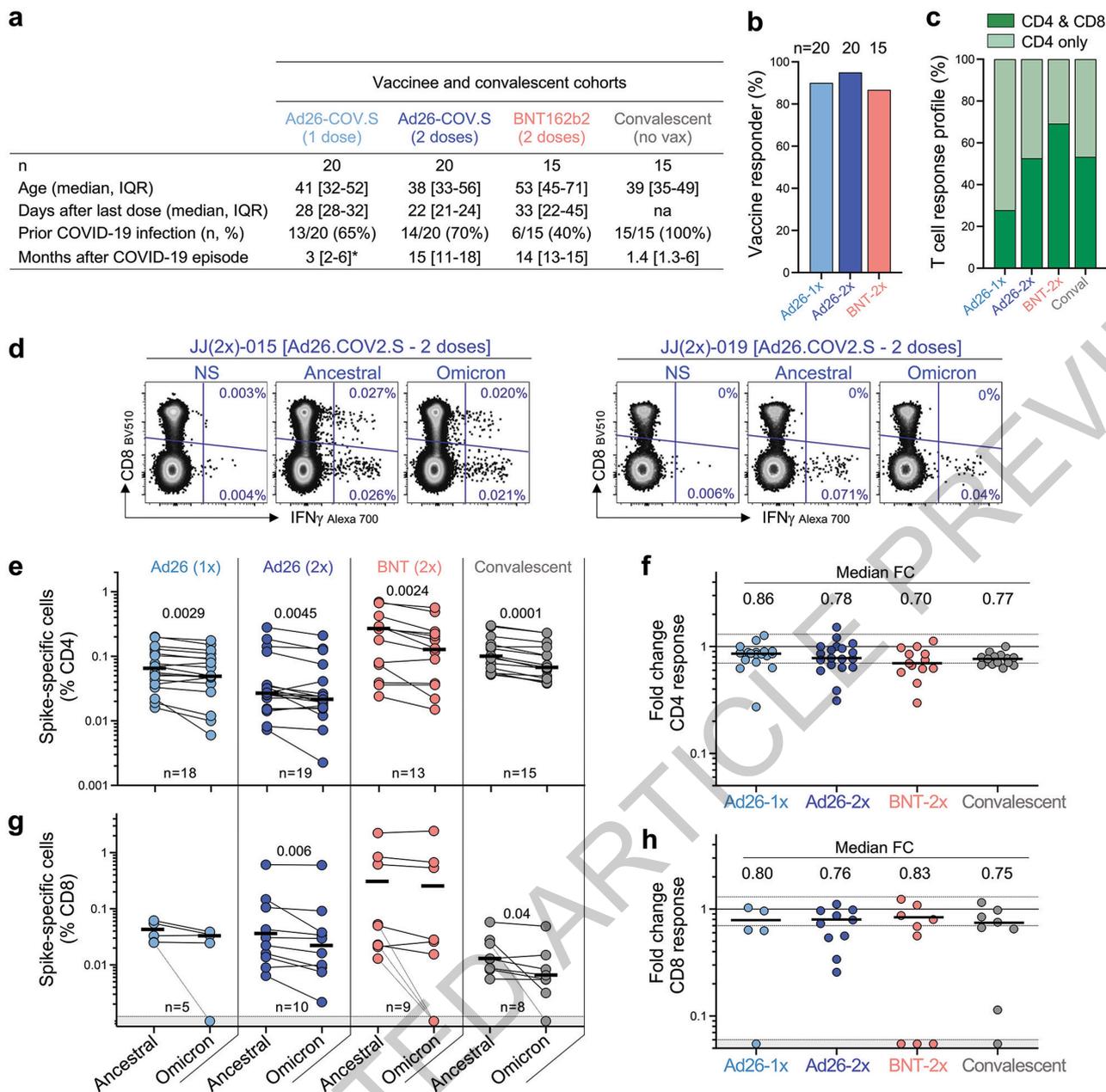
**Additional information**

**Supplementary information** is available for this paper at

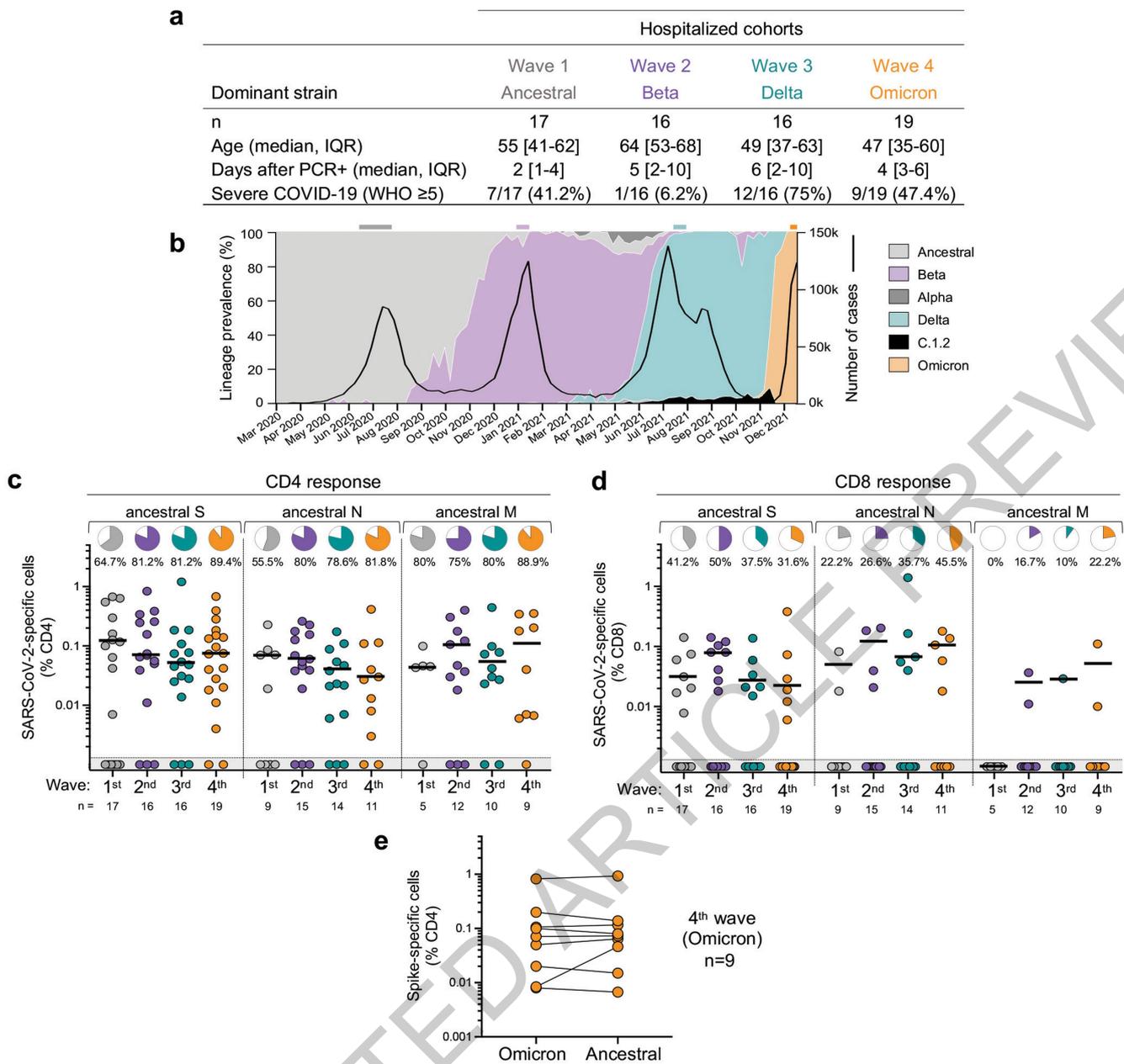
**Correspondence and requests for materials** should be addressed to Wendy A. Burgers or Catherine Riou.

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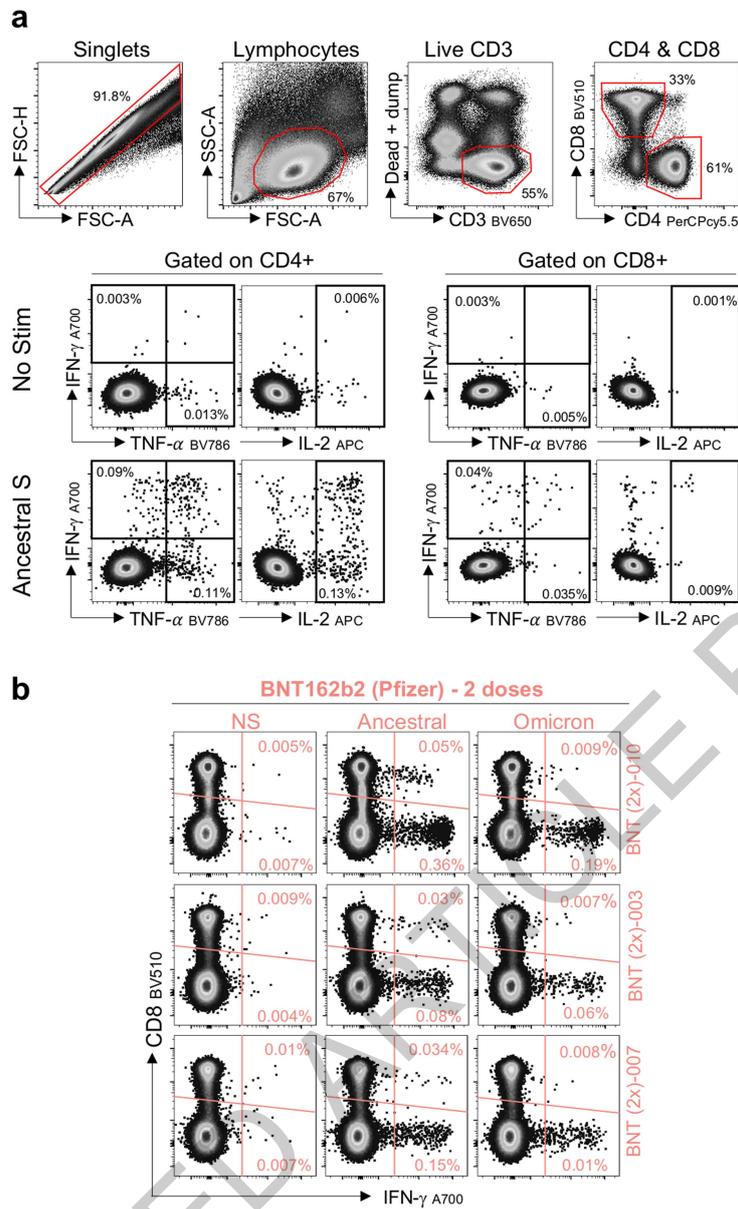


**Fig. 1 | T cell response to the ancestral and Omicron SARS-CoV-2 spike after vaccination and in unvaccinated COVID-19 convalescent patients.** **a**, Clinical characteristics of the study groups. \*: data regarding time post Covid-19 infection were available for only 6 out of the 13 participants who received one dose of Ad26.COV2.S. **b**, Proportion of participants exhibiting an ancestral spike-specific CD4+ T cell response after vaccination with one or two doses of Ad26.COV2.S or two doses of BNT162b2. **c**, Profile of the ancestral spike-specific T cell response in vaccinees and convalescent individuals. **d**, Representative examples of IFN- $\gamma$  production in response to ancestral and Omicron spike in two individuals who received two doses of Ad26.COV2.S. **e,g**, Frequency of spike-specific CD4+ (**e**) and CD8+ T cells (**g**) producing any of the measured cytokines (IFN- $\gamma$ , IL-2 or TNF- $\alpha$ ) in response to ancestral and Omicron spike peptide pools. Bars represent median of responders. Differences between SARS-CoV-2 variants were calculated using a two-tailed Wilcoxon paired test. **f, h**, Fold change in the frequency of spike-specific CD4+ (**f**) and CD8+ T cells (**h**) between ancestral and Omicron spike responses. Bars represent medians. No significant differences were observed between groups using a Kruskal-Wallis test with Dunn's multiple comparisons post-test. The number of participants included in each analysis is indicated on the graphs.

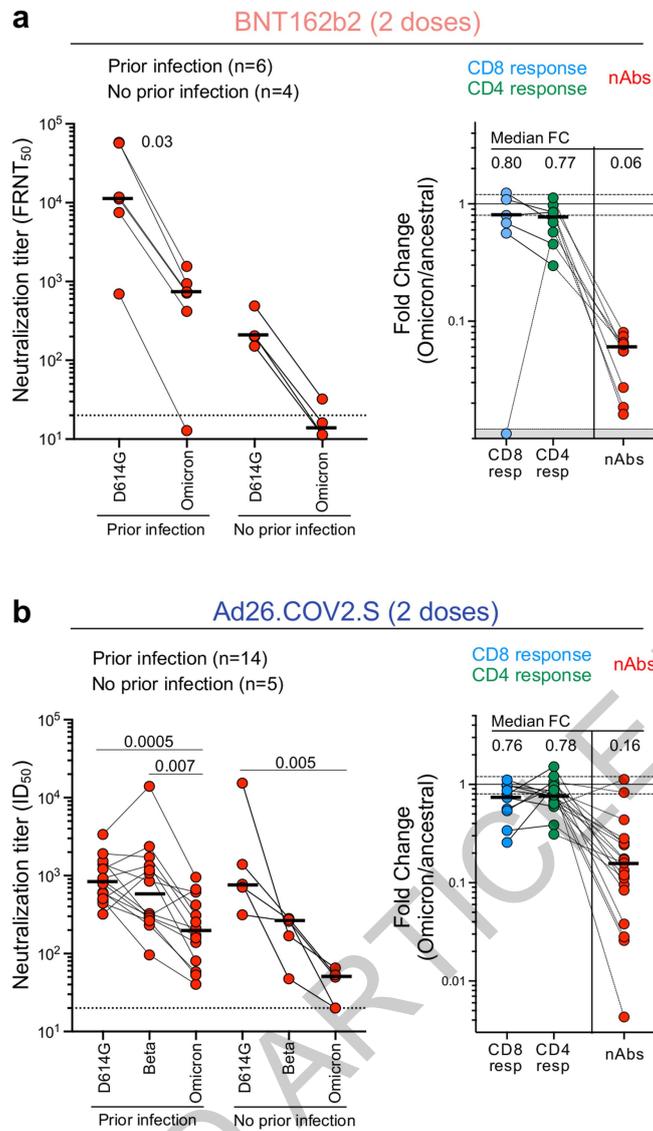


**Fig. 2 | T cell response to ancestral SARS-CoV-2 in unvaccinated hospitalized COVID-19 patients infected with the ancestral, Beta, Delta or Omicron SARS-CoV-2 variants.**

**a**, Clinical characteristics of the study groups. Severe disease was defined based on oxygen therapy requirement according to the WHO ordinal scale scoring system ( $O_2$  via high flow to ECMO). **b**, SARS-CoV-2 epidemiological dynamics in South Africa showing the prevalence of different SARS-CoV-2 strains (based on 24,762 sequences; left axis) and the number of COVID-19 cases (right axis). The bars on top of the graph indicate the periods when samples were collected for each epidemic wave. **c,d**, Frequency of SARS-CoV-2-specific CD4<sup>+</sup> (**c**) and CD8<sup>+</sup> T cells (**d**) producing any of the measured cytokines (IFN- $\gamma$ , IL-2 or TNF- $\alpha$ ) in response to ancestral SARS-CoV-2 spike (S), nucleocapsid (N) and membrane (M) peptide pools. Pies depict the proportion of participants exhibiting a detectable T cell response to each protein. **e**, Comparison of T cell response to ancestral or Omicron spike in Omicron-infected patients. Bars represent medians of responders. No significant differences were observed between antigens amongst responders using a Kruskal-Wallis test with Dunn's multiple comparisons post-test. The number of participants included in each analysis is indicated on the graphs.

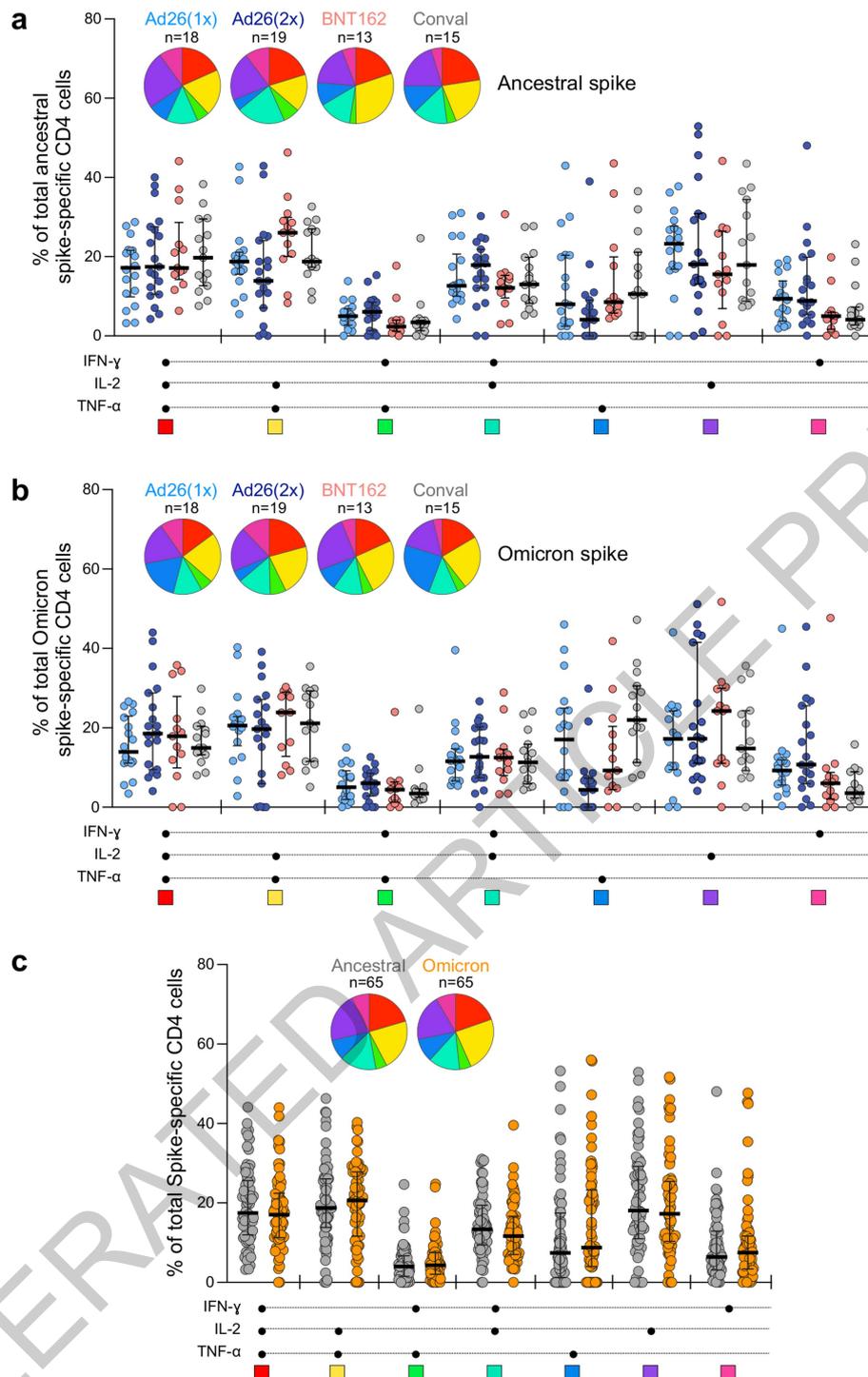


**Extended Data Fig. 1 | Gating strategy and examples of flow cytometry plots. a**, Gating strategy and representative examples of SARS-CoV-2 spike-specific IFN- $\gamma$ , IL-2 and TNF- $\alpha$  production. **b**, Spike-specific expression of IFN- $\gamma$  in the T cell compartment of the three BNT162b2-vaccinated participants where Omicron-specific CD8+ T cells were undetectable.



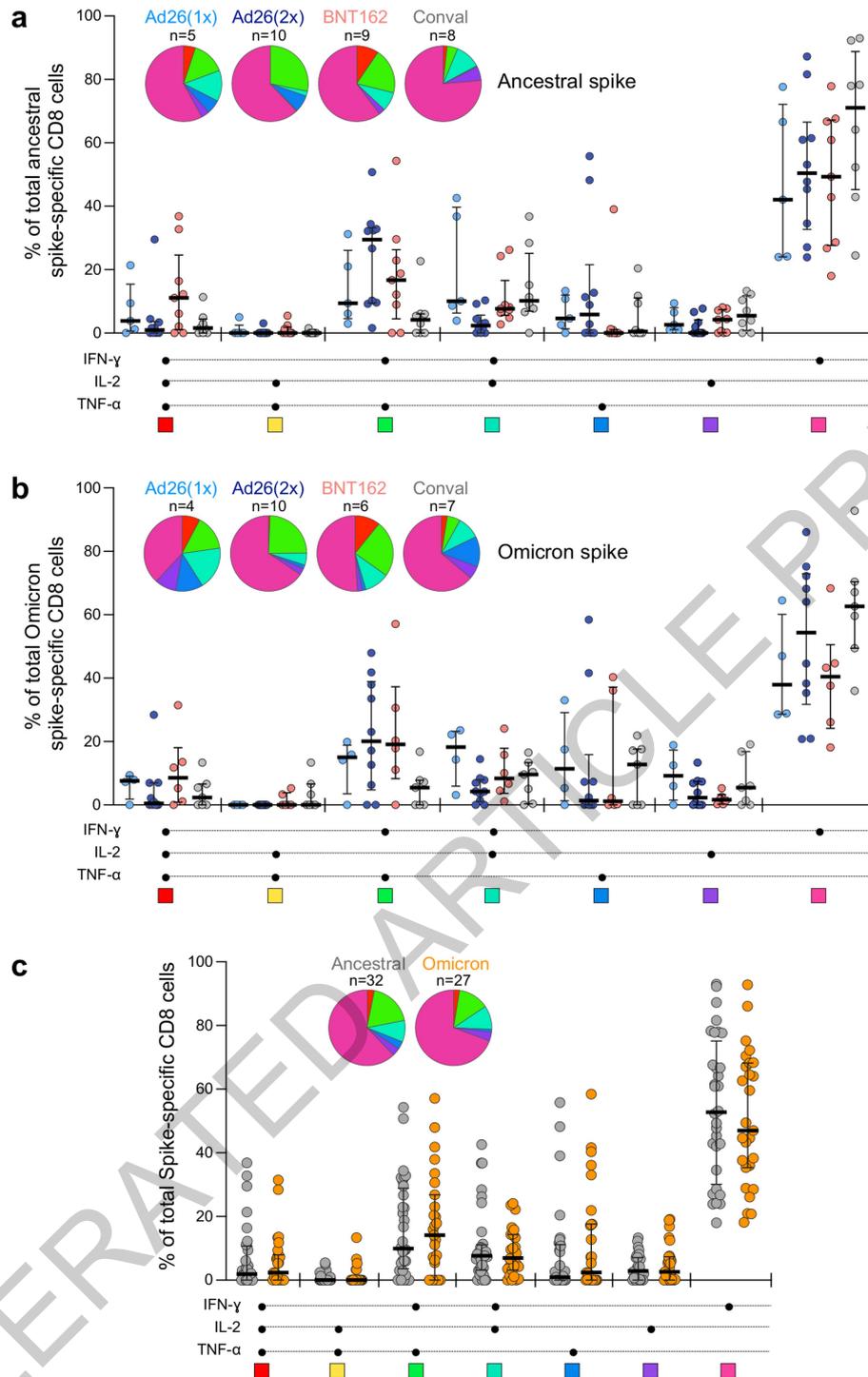
**Extended Data Fig. 2 | Neutralization of Omicron compared to the ancestral SARS-CoV-2 (D614G) by plasma from participants vaccinated with two doses of BNT162b2 or Ad26.COVS.2.S.** **a**, Neutralization by BNT162b2 plasma (n = 10, 6 with prior COVID-19 infection and 4 without) was performed using a live virus neutralization assay. The reciprocal plasma dilution (FRNT<sub>50</sub>) resulting in 50% reduction in the number of infection foci is reported. The threshold of detection was set at a FRNT<sub>50</sub> of 20. A two-tailed paired Wilcoxon test was used to compare ancestral and Omicron titers. Comparison of the fold change in SARS-CoV-2-specific CD8+ and CD4+ T cell responses and neutralization titers (Omicron/ancestral) is depicted in the right panel. Bars represent medians. **b**, Neutralization against ancestral, Beta and Omicron variants by plasma from Ad26.COVS.2.S vaccinees (two doses; n = 19), including 14 with prior COVID-19 infection and 5 without, was performed using a SARS-CoV-2 pseudovirus-based neutralization assay. The threshold of detection was a 50% inhibitory dilution (ID<sub>50</sub>) of 20. A Friedman test with Dunn's multiple comparisons post-test was used to compare the titers of the three variants tested. Comparison of the fold change in SARS-CoV-2-specific CD8+ and CD4+ T cell response and neutralization titers (Omicron/ancestral) is depicted in the right panel. Bars represent medians.

## CD4 response

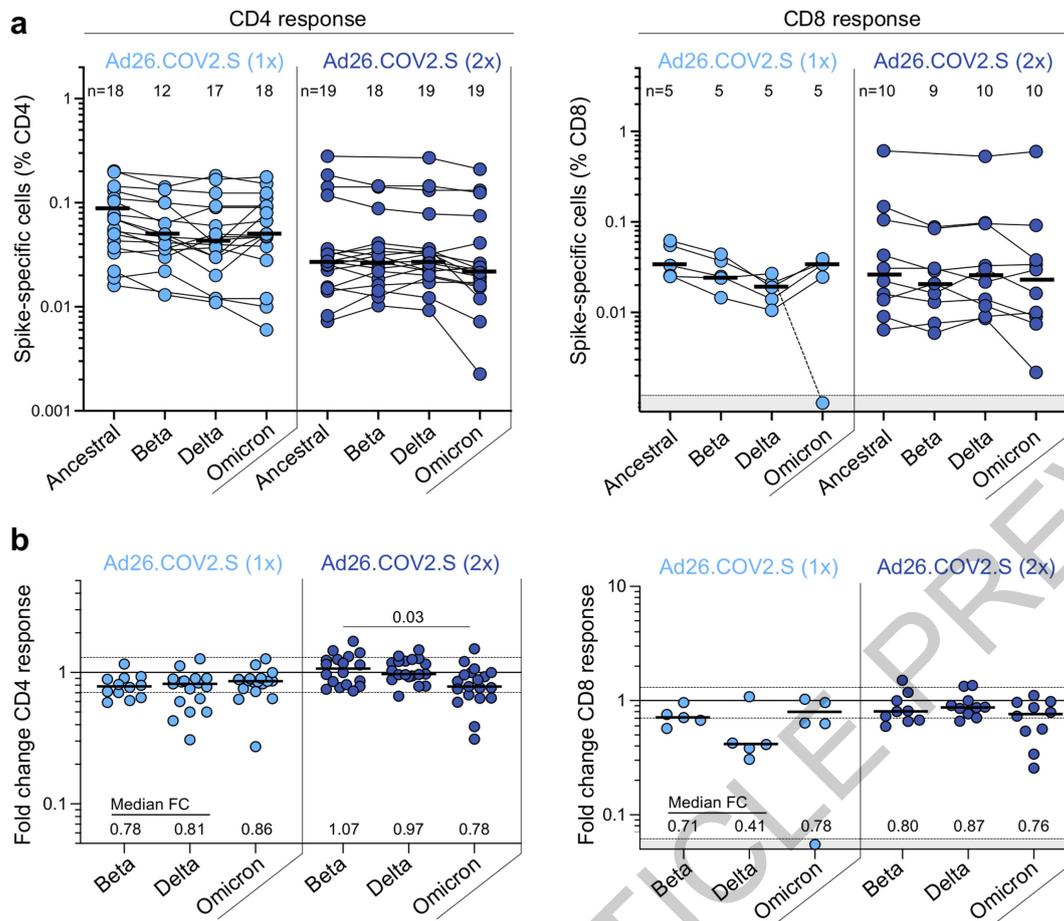


**Extended Data Fig. 3 | Polyfunctional profiles of SARS-CoV-2-specific CD4<sup>+</sup> T cells after vaccination and in unvaccinated convalescent volunteers. a, b,** Comparison of the polyfunctional profile of ancestral (a) and Omicron (b) spike-specific CD4<sup>+</sup> T cells between the four groups (Ad26.COVS-one dose, Ad26.COVS-two doses, BNT162b-two doses and unvaccinated convalescent volunteers). **c,** Comparison of the polyfunctional profile between ancestral and Omicron spike-specific CD4<sup>+</sup> T cells including all CD4<sup>+</sup> T cell responding participants, irrespective of their clinical grouping. The medians and IQR are shown. Each response pattern (i.e., any possible combination of IFN- $\gamma$ , IL-2 or TNF- $\alpha$  expression) is color-coded, and data are summarized in the pie charts. No significant differences were observed between pies using a permutation test. The number of participants included in each analysis is indicated on the graphs.

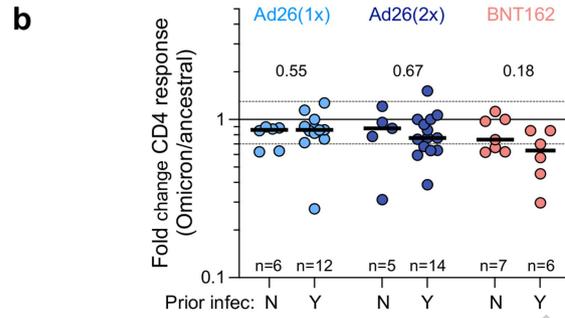
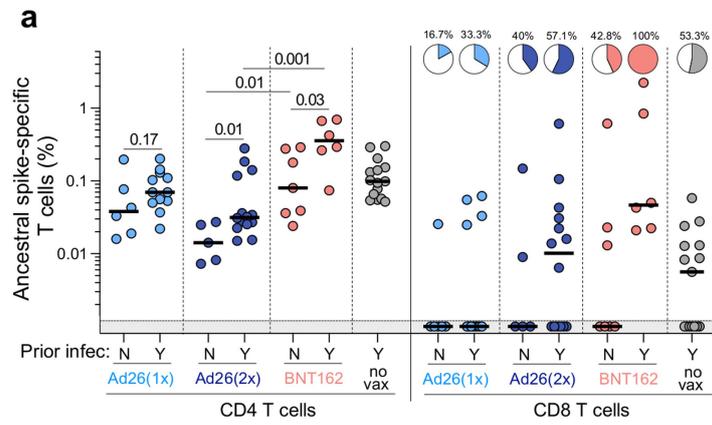
## CD8 response



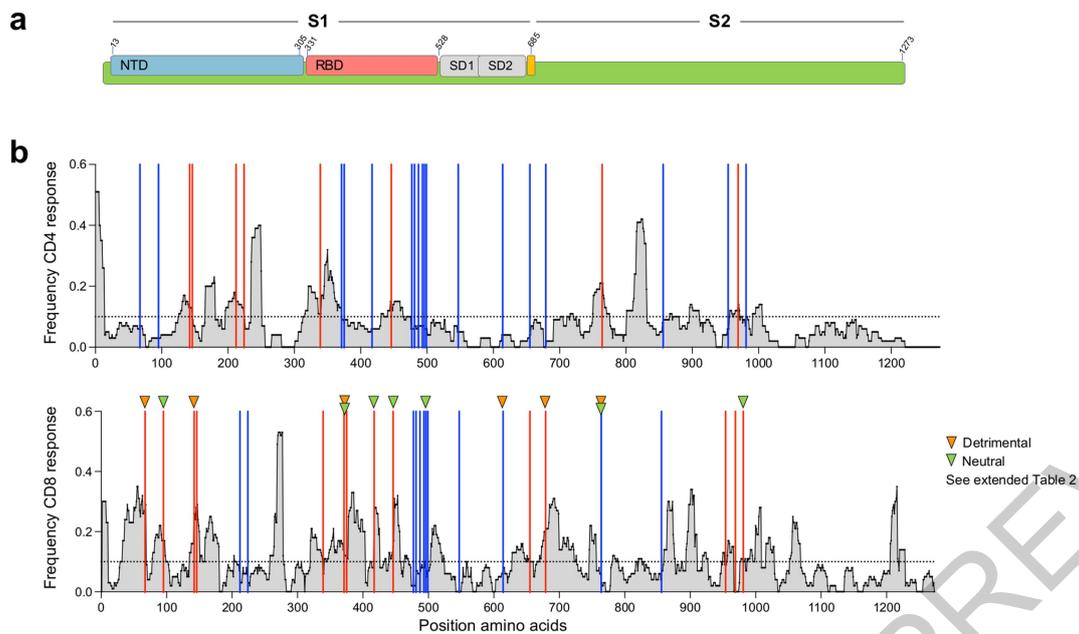
**Extended Data Fig. 4 | Polyfunctional profiles of SARS-CoV-2-specific CD8+ T cells after vaccination and in unvaccinated convalescent volunteers. a, b,** Comparison of the polyfunctional profile of ancestral (a) and Omicron (b) spike-specific CD8+ T cells between the four groups (Ad26.COVID.S-one dose, Ad26.COVID.S-two doses, BNT162b2-two doses and unvaccinated convalescent COVID-19 volunteers). **c,** Comparison of the polyfunctional profile between ancestral spike and Omicron spike-specific CD8+ T cells including all CD8+ T cell responding participants, irrespective of their clinical grouping. The medians and IQR are shown. Each response pattern (i.e., any possible combination of IFN- $\gamma$ , IL-2 or TNF- $\alpha$  expression) is color-coded, and data are summarized in the pie charts. No significant differences were observed between pies using a permutation test. The number of participants included in each analysis is indicated on the graphs.



**Extended Data Fig. 5 | T cell responses to the ancestral, Beta, Delta and Omicron SARS-CoV-2 spike in participants who received Ad26.COVS.2.S (one or two doses).** **a**, Frequency of spike-specific CD4+ (left panel) and CD8+ T cells (right panel) producing any of the measured cytokines (IFN- $\gamma$ , IL-2 or TNF- $\alpha$ ) in response to ancestral, Beta, Delta and Omicron spike peptide pools. Bars represent median of responders. No significant differences were observed between variants using a Kruskal-Wallis test with Dunn's multiple comparisons post-test. **b**, Fold change in the frequency of spike-specific CD4+ (left panel) and CD8+ T cells (right panel) between ancestral and Omicron spike responses. Bars represent medians. Differences between SARS-CoV-2 variants were calculated using a Kruskal-Wallis test with Dunn's multiple comparisons post-test. Median fold changes are indicated at the bottom of each graph. The number of participants included in each analysis is indicated on the graphs.



**Extended Data Fig. 6 | Impact of prior COVID-19 infection on T cell responses to the ancestral and Omicron SARS-CoV-2 spike in vaccinated participants. a,** Comparison of the frequency of ancestral spike-specific T cell responses in vaccinated participants who had (Y) or did not have (N) prior SARS-CoV-2 infection. Pies depict the proportion of participants exhibiting a detectable CD8<sup>+</sup> T cell response. Bars represent medians. Statistical differences were calculated using a two-tailed Mann-Whitney test. **b,** Fold change in the frequency of spike-specific CD4<sup>+</sup> T cells between ancestral and Omicron spike responses in the three vaccine groups. Bars represent medians. Statistical differences were calculated using a two-tailed Mann-Whitney test. The number of participants included in each analysis is indicated on the graphs.



**Extended Data Fig. 7 | Distribution of spike SARS-CoV-2 epitopes targeted by CD4+ and CD8+ T cells.** **a**, Schematic of SARS-CoV-2 spike protein primary structure colored by domain. NTD: N-terminal domain, RBD: receptor binding domain, SD1: Sub-domain 1, SD2: Sub-domain 2. **b**, Distribution and frequency of recognition of confirmed CD4+ (top) and CD8+ T cell epitopes (bottom) across the entire spike protein. Data represent experimentally confirmed epitopes from the Immune Epitope Database and Analysis Resource ([www.iedb.org](http://www.iedb.org)). Red lines depict the position of Omicron mutations that recorded a frequency of recognition > 10% and blue lines <10%. The position of variable epitopes associated with specific HLA-class I (see Extended Table 2) is indicated by a triangle. Mutations with a detrimental or neutral impact for HLA binding are depicted in orange and green, respectively.

**Table 1a:**

Vaccinee cohorts	Vaccinees (all)	Without prior infection	With prior infection
<b>Ad26.COVS (Janssen) - 1 dose</b>			
Number	20	7	13
Age (y) (median, IQR)	41 [32-52]	44 [32-54]	41 [32-48]
Gender (% female)	75%	71.4%	76.9%
With co-morbidities (n, %)	6/20 (30%)	2/7 (28.6%)	4/13 (30.8%)
Days since last vaccine dose (median, IQR)	28 [28-32]	31 [28-34]	28 [27-29]
Months since COVID episode (median, IQR)	-	na	3 [2-6]*
<b>Ad26.COVS (Janssen) - 2 doses</b>			
Number	20	6	14
Age (y) (median, IQR)	38 [33-56]	58 [36-62]	36 [32-44]
Gender (% female)	75%	83.3%	71.4%
With co-morbidities (n, %)	9/20 (45%)	4/6 (66.7%)	5/14 (35.7%)
Days since last vaccine dose (median, IQR)	22 [21-24]	23 [21-25]	22 [21-23]
Months since COVID episode (median, IQR)	-	na	15 [11-18]
<b>BNT162b2 (Pfizer) - 2 doses</b>			
Number	15	9	6
Age (y) (median, IQR)	53 [45-71]	53 [41-74]	57 [44-66]
Gender (% female)	53.3%	44.4%	66.7%
With co-morbidities (n, %)	9/15 (60%)	4/9 (44.4%)	5/6 (83.3%)
Days since last vaccine dose (median, IQR)	33 [22-48]	46 [23-65]	27 [20-38]
Months since COVID episode (median, IQR)	-	na	14 [13-15]

**Table 1b:**

Convalescent cohort (unvaccinated)	
Number	15
Age (y) (median, IQR)	39 [35-49]
Gender (% female)	12%
With co-morbidities (n, %)	6/15 (40%)
Months since COVID episode (median, IQR)	1.4 [1.3-6]

**Table 1c:**

Hospitalized COVID-19 cohort	Total	Wave 1 (Ancestral)	Wave 2 (Beta)	Wave 3 (Delta)	Wave 4 (Omicron)
Number	68	17	16	16	19
Age (y) (median, range)	53 [39-64]	55 [41-62]	64 [53-68]	49 [37-63]	47 [35-60]
Gender (% female)	47%	29.4%	56.2%	56.2%	47.4%
With co-morbidities (n, %)	53/68 (77.9%)	11/17 (64.7%)	15/16 (93.7%)	13/16 (81.2%)	14/19 (73.7%)
Day since PCR+ (median, range)	4 [2-8]	2 [1-3.5]	5 [2-10]	6 [2-10]	4 [3-6]
Severe COVID-19 (WHO ≥5)	29/68 (42.6%)	7/17 (41.2%)	1/16 (6.25%)	12/16 (75%)	9/19 (47.4%)

### Extended Data Table 1 Clinical characteristics of vaccinated, convalescent, and hospitalized COVID-19 participants.

**Table 1a:** Clinical characteristics of vaccinee cohorts. Co-morbidities include: asthma, hypertension, obesity or diabetes mellitus.\*: data regarding time post Covid-19 infection were available for only 6 out of the 13 participants who received 1 dose of Ad26-COV.S. **Table 1b:** Clinical characteristics of convalescent COVID-19 patients. Co-morbidities include: asthma, hypertension, obesity or diabetes mellitus. **Table 1c:** Clinical characteristics of hospitalized COVID-19 patient cohort. Co-morbidities include: asthma, hypertension, obesity or diabetes mellitus. Severe COVID-19 was defined based on oxygen therapy requirement according to the WHO ordinal scale scoring system (O<sub>2</sub> via high flow to extracorporeal membrane oxygenation).

	peptide (WT)	aa start	aa stop	Mutation	Peptide (Omicron)	MHC class-I allele	P rank (WT)	P rank (Omi)	IC <sub>50</sub> (WT)	IC <sub>50</sub> (Omi)	Previously described
Detrimental	VTWFHAIHV	62	70	A67V, del 69-70	VTWFHVISG	HLA-A*02:11	0.64	18	18.91	8421.29	Deng, 2021
						HLA-A*68:02	0.26	9.3	45.32	8237.36	
	GVYYHKNNK	142	150	G142D, 143-145 del	DHKNNKSWM	HLA-A*03:01	0.07	89	27.86	42735.87	Tarke, 2021
	ASFSTFKCY	372	380	S373P, S375F	APFFTFKCY	HLA-A*30:02	0.06	2.8	43.85	2759.86	
						HLA-B*15:03	0.26	2.5	25.16	495.99	
	YQDVNCTEV	612	620	D614G	YQGVNCTEV	HLA-A*02:131	0.53	1.3	49.38	149.98	Tarke, 2021
						HLA-A*02:11	0.95	2	31.58	93.34	
SPRRARSA	680	688	P681H	SHRRARSA	HLA-B*07:02	0.02	2.9	4.65	4721.01	Tarke, 2021	
					HLA-B*07:05	0.03	3	4.51	2591.37		
					HLA-B*07:06	0.03	3	4.51	2591.37		
					HLA-B*42:02	0.03	3.6	38.64	8401.27		
QLNRALTGI	762	770	N764K	QLKRALTGI	HLA-A*02:11	0.84	4	27.13	440.69		
Neutral	GVYFASTEK	89	97	T95I	GVYFASIEK	HLA-A*11:01	0.03	0.02	9.66	6.98	Tarke, 2021
						HLA-A*03:01	0.05	0.04	19.15	17.74	
	NSASFSTFK	370	379	S371L, S373P, S375F	NLAPFFTFK	HLA-A*68:01	0.02	0.06	4.4	8.51	Tarke, 2021
						HLA-A*34:02	0.02	0.01	16.17	11.26	
	YNSASFSTF	369	378	S371L, S373P, S375F	YNLAPFFTF	HLA-A*11:01	0.03	0.03	9.13	10.02	
						HLA-B*15:03	0.05	0.18	6.63	18.49	
	KIADYNYKL	417	425	K417N	NIADYNYKL	HLA-A*02:02	0.07	0.17	6.58	11.64	Shomuradova, 2021
						HLA-A*02:05	0.09	0.16	13.49	27.89	
	VGGNYNYLY	445	453	G446S	VSGNYNYLY	HLA-A*30:02	0.06	0.02	45.32	20.21	Tarke, 2021
	SKVGGNYNY	443	451	G446S	SKVSGNYNY	HLA-B*15:03	0.26	0.17	25.38	17.47	Tarke, 2021
YFPLQSYGF	489	498	Q493R	YFPLRSYSF	HLA-A*23:01	0.12	0.06	49.01	24.54	Zhang, 2021	
GSFCTQLNR	757	765	N764K	GSFCTQLKR	HLA-A*11:01	0.18	0.26	37.18	53.31		
QLNRALTGI	762	770	N764K	QLKRALTGI	HLA-A*02:03	0.25	0.47	15.69	27.68		
VLNDILSRL	976	985	L981F	VLNDIFSRL	HLA-A*02:02	0.04	0.05	4.16	4.36	Tarke, 2021	
					HLA-A*02:03	0.06	0.08	5.19	6.38		
					HLA-A*02:11	0.09	0.09	3.4	3.5		
					HLA-A*02:05	0.09	0.09	14.04	14.07		

## Extended Data Table 2 In silico analysis of the impact of Omicron mutations on epitope recognition by MHC Class I.

Putative HLA class I restrictions were inferred using the Immune Epitope Database (IEDB) analysis resource (<http://tools.iedb.org/tepitool/>), NetMHCpan prediction method). Selected ancestral peptides with predicted a percentile rank (P rank)  $\leq 1$  and a IC<sub>50</sub> < 50 nM are shown, and the binding predictions for the corresponding Omicron mutated epitope. References of previously described immunoreactive peptides (WT) are provided in the last column. Deng et al. (doi.org/10.1002/JLB.4MA0621-020R); Tarke et al. (doi: 10.1016/j.xcrm.2021.100204); Shomuradova et al. (doi.org/10.1016/j.immuni.2020.11.004), Zhang et al. (doi.org/10.1016/j.celrep.2021.109708).

Participant ID	Sex	Age	Date of blood sampling	Time since COVID-19 diagnosis (days)	Vaccination status	Evidence of prior symptomatic COVID-19	WHO score at sampling	Hyper-tension	Diabetes	Obesity	Asthma	Outcome
Hosp-Wave 4-001	Male	36	2021/12/07	9	unvaccinated	No	5	Yes	Yes	Yes	No	Discharged
Hosp-Wave 4-002	Female	26	2021/12/01	5	unvaccinated	No	4	No	Yes	No	No	Still admitted
Hosp-Wave 4-003	Female	17	2021/12/06	3	unvaccinated	No	4	No	Yes	No	No	Discharged
Hosp-Wave 4-004	Female	24	2021/12/07	4	unvaccinated	No	4	No	Yes	No	No	Discharged
Hosp-Wave 4-005	Female	63	2021/12/08	3	unvaccinated	No	4	No	No	No	No	Discharged
Hosp-Wave 4-006	Male	59	2021/12/14	4	unvaccinated	No	6	Yes	Yes	No	No	Still admitted
Hosp-Wave 4-007	Female	62	2021/12/07	8	unvaccinated	No	5	Yes	No	Yes	No	Discharged
Hosp-Wave 4-008	Male	58	2021/12/13	10	unvaccinated	No	5	Yes	Yes	No	No	Discharged
Hosp-Wave 4-009	Male	49	2021/12/13	8	unvaccinated	No	4	No	No	No	No	Discharged
Hosp-Wave 4-010	Female	71	2021/12/07	4	unvaccinated	No	5	Yes	Yes	No	No	Discharged
Hosp-Wave 4-011	Male	60	2021/12/07	1	unvaccinated	No	4	Yes	Yes	No	No	Discharged
Hosp-Wave 4-012	Male	62	2021/12/13	3	unvaccinated	No	5	No	No	No	No	Discharged
Hosp-Wave 4-013	Female	38	2021/12/13	5	unvaccinated	No	5	No	No	No	No	Still admitted
Hosp-Wave 4-014	Male	49	2021/12/13	1	unvaccinated	No	4	Yes	No	No	No	Still admitted
Hosp-Wave 4-015	Male	47	2021/12/13	4	unvaccinated	No	4	No	No	No	No	Discharged
Hosp-Wave 4-016	Female	35	2021/12/14	5	unvaccinated	No	5	No	No	No	No	Discharged
Hosp-Wave 4-017	Female	21	2021/12/14	4	unvaccinated	No	5	No	No	No	No	Discharged
Hosp-Wave 4-018	Male	38	2021/12/15	6	unvaccinated	Unk	3	Yes	No	No	No	Discharged
Hosp-Wave 4-019	Male	37	2021/12/15	6	unvaccinated	No	3	No	No	No	No	Discharged
Hosp-Wave 3-001	Female	33	2021/07/14	9	unvaccinated	No	5	Yes	Yes	No	No	Discharged
Hosp-Wave 3-002	Female	76	2021/07/14	6	Unk	No	5	Yes	No	Yes	No	Discharged
Hosp-Wave 3-003	Male	36	2021/07/15	6	unvaccinated	No	3	No	Yes	No	No	Discharged
Hosp-Wave 3-004	Female	43	2021/07/15	6	unvaccinated	Unk	5	Yes	No	No	No	Discharged
Hosp-Wave 3-005	Male	59	2021/07/15	2	Unk	Unk	5	Yes	Yes	No	No	Discharged
Hosp-Wave 3-006	Female	64	2021/07/15	4	Unk	Unk	6	No	No	Yes	No	Demised
Hosp-Wave 3-007	Male	64	2021/07/16	2	Unk	Unk	3	No	No	No	No	Discharged
Hosp-Wave 3-008	Male	47	2021/07/19	2	unvaccinated	Unk	3	Yes	Yes	No	No	Discharged
Hosp-Wave 3-009	Male	29	2021/07/19	11	unvaccinated	Unk	6	No	Yes	Yes	No	Discharged
Hosp-Wave 3-010	Female	33	2021/07/19	6	unvaccinated	Unk	5	Yes	No	No	No	Discharged
Hosp-Wave 3-011	Female	42	2021/07/19	12	unvaccinated	Unk	5	No	No	Yes	No	Discharged
Hosp-Wave 3-012	Female	52	2021/07/19	26	Unk	Unk	6	Yes	Yes	Yes	No	Demised
Hosp-Wave 3-013	Female	44	2021/07/20	2	unvaccinated	Unk	5	Yes	Yes	No	No	Discharged
Hosp-Wave 3-014	Female	72	2021/07/20	2	Unk	Unk	6	Yes	Yes	Yes	No	Demised
Hosp-Wave 3-015	Male	53	2021/07/20	3	Unk	Unk	5	No	No	No	No	Discharged
Hosp-Wave 3-016	Male	51	2021/07/21	12	Unk	Unk	4	No	No	No	No	Discharged
Hosp-Wave 2-001	Male	64	2020/12/31	3	unvaccinated	No	3	No	Yes	No	No	Discharged
Hosp-Wave 2-002	Male	68	2021/01/06	20	unvaccinated	No	4	Yes	Yes	No	No	Discharged
Hosp-Wave 2-003	Female	61	2021/01/06	2	unvaccinated	No	4	Yes	Yes	No	No	Discharged
Hosp-Wave 2-004	Female	65	2021/01/06	14	unvaccinated	No	4	No	No	No	No	Discharged
Hosp-Wave 2-005	Female	53	2021/01/08	8	unvaccinated	No	4	Yes	Yes	No	No	Discharged
Hosp-Wave 2-006	Female	68	2021/01/08	6	unvaccinated	No	4	No	Yes	No	No	Discharged
Hosp-Wave 2-007	Male	64	2021/01/12	2	unvaccinated	No	4	Yes	Yes	No	No	Discharged
Hosp-Wave 2-008	Male	45	2021/01/12	5	unvaccinated	No	4	Yes	Yes	No	No	Discharged
Hosp-Wave 2-009	Male	83	2021/01/13	0	unvaccinated	No	4	Yes	No	No	No	Discharged
Hosp-Wave 2-010	Male	33	2021/01/13	5	unvaccinated	No	3	Yes	No	No	No	Discharged
Hosp-Wave 2-011	Female	73	2021/01/13	8	unvaccinated	No	3	Yes	No	No	No	Discharged
Hosp-Wave 2-012	Female	53	2021/01/13	4	unvaccinated	No	4	Yes	No	Yes	No	Discharged
Hosp-Wave 2-013	Female	64	2021/01/13	2	unvaccinated	No	4	Yes	Yes	No	No	Discharged
Hosp-Wave 2-014	Female	67	2021/01/13	14	unvaccinated	No	4	Yes	Yes	Yes	No	Discharged
Hosp-Wave 2-015	Female	68	2021/01/15	11	unvaccinated	No	5	Yes	Yes	No	No	Discharged
Hosp-Wave 2-016	Male	49	2021/01/15	4	unvaccinated	No	3	No	Yes	No	No	Discharged
Hosp-Wave 1-001	Male	68.1	2020/06/11	2	unvaccinated	No	3	Yes	Yes	No	No	Discharged
Hosp-Wave 1-002	Male	36.1	2020/06/12	11	unvaccinated	No	4	Yes	Yes	Yes	No	Discharged
Hosp-Wave 1-003	Female	21.2	2020/06/18	3	unvaccinated	No	3	No	No	No	No	Discharged
Hosp-Wave 1-004	Male	56.4	2020/06/22	2	unvaccinated	No	4	No	No	No	No	Discharged
Hosp-Wave 1-005	Male	49.3	2020/06/22	2	unvaccinated	No	3	No	Yes	No	No	Discharged
Hosp-Wave 1-006	Female	55.5	2020/06/25	4	unvaccinated	No	5	No	No	No	No	Discharged
Hosp-Wave 1-007	Male	41.2	2020/06/25	1	unvaccinated	No	4	Yes	No	No	No	Discharged
Hosp-Wave 1-008	Female	63.5	2020/06/25	1	unvaccinated	No	4	Yes	Yes	Yes	No	Discharged
Hosp-Wave 1-009	Female	37.9	2020/06/25	1	unvaccinated	No	4	No	No	Yes	No	Discharged
Hosp-Wave 1-010	Male	58.0	2020/06/29	1	unvaccinated	No	4	Yes	Yes	Yes	No	Discharged
Hosp-Wave 1-011	Male	49.6	2020/07/02	1	unvaccinated	No	5	Yes	No	No	No	Discharged
Hosp-Wave 1-012	Male	63.7	2020/07/02	1	unvaccinated	No	5	No	No	No	No	Discharged
Hosp-Wave 1-013	Male	55.0	2020/07/03	3	unvaccinated	No	5	No	No	No	No	Discharged
Hosp-Wave 1-014	Male	41.6	2020/07/10	14	unvaccinated	No	5	No	No	No	No	Discharged
Hosp-Wave 1-015	Male	60.4	2020/07/16	1	unvaccinated	No	4	Yes	Yes	No	No	Discharged
Hosp-Wave 1-016	Male	67.7	2020/07/20	8	unvaccinated	No	5	Yes	Yes	No	No	Discharged
Hosp-Wave 1-017	Female	56.4	2020/07/24	2	unvaccinated	No	5	Yes	No	Yes	No	Discharged
Convalescent-001	Female	40	2021/01/19	213	unvaccinated	No	na	Yes	No	No	No	na
Convalescent-002	Female	45	2021/02/12	178	unvaccinated	No	na	Yes	No	No	No	na
Convalescent-003	Female	35	2021/01/19	183	unvaccinated	No	na	No	No	No	No	na
Convalescent-004	Female	35	2021/01/19	148	unvaccinated	No	na	No	No	No	No	na
Convalescent-005	Male	31	2021/01/19	213	unvaccinated	No	na	No	No	No	No	na
Convalescent-006	Female	52	2021/02/15	243	unvaccinated	No	na	Yes	No	Yes	No	na
Convalescent-007	Female	35	2021/01/26	38	unvaccinated	No	na	Yes	No	No	No	na
Convalescent-008	Female	55	2021/01/19	179	unvaccinated	No	na	No	No	No	No	na
Convalescent-009	Female	49	2021/01/21	30	unvaccinated	No	na	Yes	No	No	No	na
Convalescent-010	Male	36	2021/01/20	35	unvaccinated	No	na	No	No	No	No	na
Convalescent-011	Female	48	2021/01/21	40	unvaccinated	No	na	No	No	No	No	na
Convalescent-012	Male	39	2021/02/03	42	unvaccinated	No	na	No	No	No	No	na
Convalescent-013	Female	29	2021/02/01	38	unvaccinated	No	na	No	No	No	No	na
Convalescent-014	Female	32	2021/02/03	38	unvaccinated	No	na	No	No	No	No	na
Convalescent-015	Female	57	2021/02/03	38	unvaccinated	No	na	Yes	No	No	No	na

**Extended Data Table 3 Clinical characteristics of each hospitalized and convalescent COVID-19 participant.**

Unk: unknown. Na: not applicable

Participant ID	Sex	Age	Date of blood sampling	Vaccine regimen	Date of 1st vaccine dose	Date of 2nd vaccine dose	Time between vaccination and sampling (days)	COVID-19 history prior to sampling	Date of prior infection	Time between prior infection and sampling (months)	Hypertension	Diabetes	Obesity	Asthma
JJ (1x)-001	F	44	2021/03/18	1 dose Ad26.COV2.S	2021/02/18	na	28	No prior Infection	na	na	No	No	No	No
JJ (1x)-002	M	58	2021/03/23	1 dose Ad26.COV2.S	2021/02/17	na	34	No prior Infection	na	na	No	No	No	No
JJ (1x)-003	F	53	2021/04/08	1 dose Ad26.COV2.S	2021/02/24	na	43	No prior Infection	na	na	No	No	No	No
JJ (1x)-004	F	32	2021/03/23	1 dose Ad26.COV2.S	2021/02/24	na	27	No prior Infection	na	na	No	No	No	Yes
JJ (1x)-005	F	54	2021/03/18	1 dose Ad26.COV2.S	2021/02/18	na	28	No prior Infection	na	na	No	No	No	No
JJ (1x)-006	M	30	2021/03/24	1 dose Ad26.COV2.S	2021/02/21	na	31	No prior Infection	na	na	No	No	No	No
JJ (1x)-007	F	33	2021/03/23	1 dose Ad26.COV2.S	2021/02/19	na	32	No prior Infection	na	na	No	No	No	Yes
JJ (1x)-008	F	34	2021/03/18	1 dose Ad26.COV2.S	2021/02/18	na	28	Prior Infection	2021/01/04	2.40	No	No	No	No
JJ (1x)-009	M	30	2021/03/23	1 dose Ad26.COV2.S	2021/02/22	na	29	Prior Infection	2020/12/18	3.13	No	No	No	No
JJ (1x)-010	M	42	2021/03/18	1 dose Ad26.COV2.S	2021/02/18	na	28	Prior Infection	2020/12/19	2.93	No	No	No	Yes
JJ (1x)-011	F	38	2021/03/17	1 dose Ad26.COV2.S	2021/02/18	na	27	Prior Infection	2020/10/07	5.30	No	No	No	No
JJ (1x)-012	M	58	2021/03/24	1 dose Ad26.COV2.S	2021/02/24	na	28	Prior Infection	Unk	Unk	No	No	No	No
JJ (1x)-013	F	43	2021/03/23	1 dose Ad26.COV2.S	2021/02/19	na	32	Prior Infection	Unk	Unk	Yes	No	No	No
JJ (1x)-014	F	36	2021/03/25	1 dose Ad26.COV2.S	2021/02/25	na	28	Prior Infection	Unk	Unk	No	No	No	No
JJ (1x)-015	F	27	2021/04/22	1 dose Ad26.COV2.S	2021/03/24	na	29	Prior Infection	2020/07/08	9.47	No	No	No	No
JJ (1x)-016	F	28	2021/03/26	1 dose Ad26.COV2.S	2021/02/26	na	28	Prior Infection	2021/02/16	1.25	No	No	No	No
JJ (1x)-017	F	53	2021/03/18	1 dose Ad26.COV2.S	2021/02/18	na	28	Prior Infection	Unk	Unk	No	No	Yes	No
JJ (1x)-018	F	46	2021/03/31	1 dose Ad26.COV2.S	2021/03/09	na	22	Prior Infection	Unk	Unk	No	No	Yes	No
JJ (1x)-019	F	51	2021/04/01	1 dose Ad26.COV2.S	2021/03/09	na	23	Prior Infection	Unk	Unk	No	No	No	No
JJ (1x)-020	F	41	2021/04/07	1 dose Ad26.COV2.S	2021/02/24	na	42	Prior Infection	Unk	Unk	No	No	No	No
JJ (2x)-001	F	59	2021/12/06	2 doses Ad26.COV2.S	2021/02/19	2021/11/12	24	No prior Infection	na	na	Yes	No	No	No
JJ (2x)-002	F	64	2021/12/07	2 doses Ad26.COV2.S	2021/02/27	2021/11/11	26	No prior Infection	na	na	No	No	No	Yes
JJ (2x)-003	F	36	2021/12/13	2 doses Ad26.COV2.S	2021/02/26	2021/11/19	24	No prior Infection	na	na	No	No	Yes	No
JJ (2x)-004	F	58	2021/12/13	2 doses Ad26.COV2.S	2021/02/22	2021/11/22	21	No prior Infection	na	na	No	No	No	No
JJ (2x)-005	M	36	2021/12/06	2 doses Ad26.COV2.S	2021/02/19	2021/11/15	21	No prior Infection	na	na	No	No	No	Yes
JJ (2x)-006	F	62	2021/12/07	2 doses Ad26.COV2.S	2021/02/18	2021/11/15	22	No prior Infection	na	na	No	No	No	No
JJ (2x)-007	M	33	2021/12/08	2 doses Ad26.COV2.S	2021/02/18	2021/11/17	21	Prior Infection	2020/10/21	13.58	No	No	No	No
JJ (2x)-008	F	39	2021/12/06	2 doses Ad26.COV2.S	2021/02/17	2021/11/11	25	Prior Infection	2020/07/31	16.21	No	No	No	No
JJ (2x)-009	M	32	2021/12/09	2 doses Ad26.COV2.S	2021/02/24	2021/11/18	21	Prior Infection	2020/05/31	18.31	No	No	No	No
JJ (2x)-010	F	37	2021/12/06	2 doses Ad26.COV2.S	2021/02/18	2021/11/12	24	Prior Infection	2021/01/07	10.95	No	No	No	No
JJ (2x)-011	F	27	2021/12/08	2 doses Ad26.COV2.S	2021/02/23	2021/11/17	21	Prior Infection	2020/08/14	15.81	No	No	No	No
JJ (2x)-012	F	59	2021/12/06	2 doses Ad26.COV2.S	2021/02/19	2021/11/15	21	Prior Infection	2021/01/07	10.95	No	No	No	No
JJ (2x)-013	M	25	2021/12/06	2 doses Ad26.COV2.S	2021/02/23	2021/11/12	24	Prior Infection	2021/01/04	11.05	No	No	No	Yes
JJ (2x)-014	M	52	2021/12/08	2 doses Ad26.COV2.S	2021/02/19	2021/11/16	22	Prior Infection	2020/06/02	18.21	No	No	No	No
JJ (2x)-015	F	34	2021/12/08	2 doses Ad26.COV2.S	2021/02/24	2021/11/15	23	Prior Infection	2020/06/01	18.24	No	Yes	No	No
JJ (2x)-016	F	35	2021/12/09	2 doses Ad26.COV2.S	2021/03/16	2021/11/17	22	Prior Infection	2020/06/18	17.72	No	No	No	Yes
JJ (2x)-017	F	43	2021/12/13	2 doses Ad26.COV2.S	2021/02/26	2021/11/24	19	Prior Infection	2020/12/24	11.64	Yes	No	No	No
JJ (2x)-018	F	31	2021/12/13	2 doses Ad26.COV2.S	2021/02/23	2021/11/22	21	Prior Infection	2020/12/21	11.74	No	No	No	No
JJ (2x)-019	F	48	2021/12/07	2 doses Ad26.COV2.S	2021/02/19	2021/11/15	22	Prior Infection	2020/04/28	19.33	Yes	No	No	No
JJ (2x)-020	F	43	2021/12/06	2 doses Ad26.COV2.S	2021/02/22	2021/11/17	19	Prior Infection	2021/01/07	10.95	No	No	No	No
BNT (2x)-001	F	74	2021/07/30	2 doses BNT162b2	2021/06/22	2021/07/20	10	No prior Infection	na	na	No	No	No	No
BNT (2x)-002	F	36	2021/08/03	2 doses BNT162b2	2021/06/03	2021/07/01	33	No prior Infection	na	na	No	No	Yes	No
BNT (2x)-003	M	53	2021/08/11	2 doses BNT162b2	2021/04/23	2021/05/21	82	No prior Infection	na	na	No	No	No	Yes
BNT (2x)-004	F	46	2021/07/26	2 doses BNT162b2	Unk	2021/05/05	82	No prior Infection	na	na	No	No	Yes	No
BNT (2x)-005	M	74	2021/09/06	2 doses BNT162b2	Unk	2021/07/21	47	No prior Infection	na	na	No	No	No	No
BNT (2x)-006	F	74	2021/09/06	2 doses BNT162b2	Unk	2021/07/20	48	No prior Infection	na	na	No	No	No	No
BNT (2x)-007	M	53	2021/07/06	2 doses BNT162b2	Unk	2021/05/21	46	No prior Infection	na	na	No	No	No	Yes
BNT (2x)-008	M	35	2021/08/02	2 doses BNT162b2	Unk	2021/07/19	14	No prior Infection	na	na	No	No	Yes	No
BNT (2x)-009	M	71	2021/09/02	2 doses BNT162b2	Unk	2021/07/19	45	No prior Infection	na	na	No	No	No	No
BNT (2x)-010	F	66	2021/10/12	2 doses BNT162b2	Unk	2021/08/10	63	Prior Infection	2020/07/01	15.39	Yes	Yes	Yes	Yes
BNT (2x)-011	F	43	2021/09/02	2 doses BNT162b2	Unk	2021/08/11	22	Prior Infection	2020/06/03	14.99	No	Yes	No	No
BNT (2x)-012	M	51	2021/10/06	2 doses BNT162b2	Unk	2021/09/06	30	Prior Infection	2020/10/01	12.16	No	No	Yes	No
BNT (2x)-013	F	67	2021/08/16	2 doses BNT162b2	Unk	2021/07/19	28	Prior Infection	2020/08/03	12.43	Yes	Yes	Yes	No
BNT (2x)-014	F	63	2021/08/18	2 doses BNT162b2	Unk	2021/07/23	26	Prior Infection	2020/08/04	12.46	No	No	No	No
BNT (2x)-015	F	45	2021/10/05	2 doses BNT162b2	Unk	2021/09/21	14	Prior Infection	2020/07/04	15.06	Yes	No	No	No

Extended Data Table 4 Clinical characteristics of each vaccinated participant.

Unk: unknown. Na: not applicable.

## 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 Clinical data were recorded by trained clinicians using RedCap (version 9.5.36)

Data analysis Statistical analysis were performed using GraphPad Prism version 9.3 Flow. Cytometry data were analysed with FlowJo Software (10.8, FlowJo LLC, BD Life Sciences) and Pestle and Spice v6.1 (<https://niaid.github.io/spice>). HLA prediction was performed using TepiTool from IEDB Analysis Resource (<http://tools.iedb.org>). The quality control checks on raw sequence data and the genome assembly were performed using Genome Detective 1.133 (<https://www.genomedetective.com>). Phylogenetic classification of the genomes was done using the PANGOLIN software suite (v1.2.106) (<https://github.com/hCoV-2019/pangolin>).

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 Portfolio [guidelines for submitting code & software](#) for further information.

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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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Datasets (raw data) underlying the figures have been provided as Source Data. Complete genome sequences for the viral isolates were deposited in GISAID.

## 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](https://www.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 was based on available samples rather than on a pre-defined samples size calculation
Data exclusions	Sample (PBMC) with low viability (<60%) or low cell number (CD4+ T cells < 20,000 cells) were excluded from the analysis.
Replication	Samples for each patient were analysed once due to limited availability
Randomization	As this is a observational study, randomization is not applicable.
Blinding	For flow cytometry assay, samples were stained and acquired in 7 consecutive runs over 2 weeks. While performing the experiments, investigators were blinded to patient groups.

## 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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input type="checkbox"/>	<input checked="" type="checkbox"/> Human research participants
<input type="checkbox"/>	<input checked="" type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

### Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

## Antibodies

### Antibodies used

Antibodies  
 purified NA/LE mouse anti-human CD28 (clone 28.2) BD Pharmingen Cat# 555725; RRID:AB\_2130052, dilution 1/1000  
 purified NA/LE mouse anti-human CD49d (clone L25) BD Pharmingen Cat# 555501; RRID:AB\_396068, dilution 1/1000  
 LIVE/DEAD™ Fixable VIVID Stain Invitrogen Cat# L34955, dilution 1/2500  
 CD14 Pac Blue (clone TuK4) Invitrogen Thermofisher Scientific Cat# MHCD1428; RRID:AB\_10373537, dilution 1/100  
 CD19 Pac Blue (clone SJ25-C1) Invitrogen Thermofisher Scientific Cat# MHCD1928; RRID:AB\_10373689, dilution 1/100  
 CD4 PERCP-Cy5.5 (clone L200) BD Biosciences Cat# 552838; RRID:AB\_394488, dilution 1/100  
 CD8 BV510 (clone RPA-8) Biolegend Cat# 301048; RRID:AB\_2561942, dilution 1/100  
 CD3 BV650 (clone OKT3) Biolegend Cat# 317324; RRID:AB\_2563352, dilution 1/100  
 IFN-g Alexa 700 (clone B27) BD Biosciences Cat# 557995; RRID:AB\_396977, dilution 1/250  
 TNF BV786 (clone Mab11) Biolegend Cat# 502948; RRID:AB\_2565858, dilution 1/100  
 IL-2 APC (clone MQ1-17H12) Biolegend Cat# 500310; RRID:AB\_315097, dilution 1/100

### Validation

All antibodies used in this study are commercially available. All antibodies were validated by their manufacturers and were titrated to define the optimal titer for positive and negative separation.

## Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	HEK293T-ACE2 cells were a gift from Dr Michael Farzan, Scripps, USA; H1299-E3 cell line was derived from H1299 (CRL-5803). H1299 cells were a gift from M. Oren, Weizmann Institute of Science.
Authentication	Cell lines were not authenticated
Mycoplasma contamination	Cell lines were tested for mycoplasma contamination and were mycoplasma negative.
Commonly misidentified lines (See <a href="#">ICLAC</a> register)	None

## Human research participants

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

Population characteristics	This study includes participants vaccinated with Ad26.COV2.S or Pfizer BNT162b2, convalescent COVID-19 patients and hospitalized COVID-19 patients. Samples were selected based on PBMC availability. Demographic characteristics are presented in Extended Data Table 1. Clinical characteristics for each participants included in the study are presented in Extended Data Table 3 and 4.
Recruitment	Study participants vaccinated with Ad26.COV2.S. and convalescent donors were included in a prospective cohort study conducted at Groote Schuur Hospital (Western Cape). Pfizer BNT162b2 vaccinees were recruited from KwaZulu-Natal. Hospitalized COVID-19 patients were recruited from Groote Schuur and Tshwane hospitals. All participants were older than 18 years old, and all participants gave written informed consent. One bias that may be present is the ethnic background, since ethnic background differs substantially between the different South African provinces. This could have implications for the prevalence of HLA class I and II molecules between patients recruited from different provinces.
Ethics oversight	The study was approved by the University of Cape Town Human Research Ethics Committee (ref: HREC 190/2020, 207/2020 and 209/2020) and the University of the Witwatersrand Human Research Ethics Committee (Medical) (ref. M210429 and M210752), the Biomedical Research Ethics Committee at the University of KwaZulu-Natal (ref. BREC/00001275/2020) and the University of Pretoria Health Sciences Research Ethics Committee (ref. 247/2020).

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	<i>Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.</i>
Study protocol	<i>Note where the full trial protocol can be accessed OR if not available, explain why.</i>
Data collection	<i>Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.</i>
Outcomes	<i>Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.</i>

## 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	Blood was collected in heparin tubes and processed within 4 hours of collection. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient sedimentation using Ficoll-Paque (Amersham Biosciences, Little Chalfont, UK) as per the manufacturer's instructions and cryopreserved in freezing media consisting of heat-inactivated fetal bovine serum (FBS, Thermofisher Scientific) containing 10% DMSO and stored in liquid nitrogen until use.
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Instrument	BD Fortessa
Software	Flowjo V10.8.1, Pestle and Spice V6.1
Cell population abundance	No cell sorting was performed
Gating strategy	SARS-Cov-2-specific T cells were identified via the following gating strategy: Viable lymphocytes were identified by successive gating in singlets (FSC-A/FSC-H), time gate, live CD3 (SSC-A/ DUMP channel- dead cells, CD14, CD19,). Then, from live CD3+ T cells, CD4+ and CD8+ T cells were gated in CD4/CD8 plots. Next, SARS-CoV-2 -specific T cells were gated by plotting IFN-g, IL-2 and TNF-a

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