BNT162b2-induced memory T cells respond to the Omicron variant with preserved polyfunctionality

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The Omicron variant (B.1.1.529) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) extensively escapes neutralizing antibodies elicited by SARS-CoV-2 infection or vaccination. In the present study, we investigated whether BNT162b2 messenger RNA vaccine-induced memory T cells functionally respond to the Omicron spike protein. Experiments were performed using samples from healthcare workers who were immunized with two or three doses of the BNT162b2 mRNA vaccine and individuals with prior SARS-CoV-2 infection who were immunized with two doses of the BNT162b2 vaccine. Vaccine-induced memory T cells exhibited substantial responses to the Omicron spike protein, with no difference between healthcare workers with two versus three vaccine doses. In individuals with prior infection, two-dose vaccination robustly boosted memory T cells that responded to the Omicron spike protein and the SARS-CoV-2 wild-type (lineage B) spike protein. Importantly, polyfunctionality was preserved in vaccine-induced memory T cells responding to the Omicron spike protein. The present findings indicate that BNT162b2-induced memory T cells substantially respond to the Omicron variant with preserved polyfunctionality.

Although coronavirus disease 2019 (COVID-19) vaccines have been developed at an unprecedented pace and administered to large populations on a global scale, the pandemic continues due to the rapid decay of neutralizing antibodies (nAbs) and the emergence of variants[1]. Since the COVID-19 pandemic began, several variants of concern (VOCs) have emerged, including the Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and Omicron (B.1.1.529) severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants[2]. The Omicron variant carries over 30 mutations in its spike protein and infects host cells more efficiently than other variants[3,4]; thus, it has rapidly replaced other variants and is the dominant SARS-CoV-2 strain worldwide[3].

The high number of mutations in the Omicron spike protein prompted concerns that this variant might escape from the immunity elicited by COVID-19 vaccination or natural infection[5]. In fact, recent studies demonstrate that the Omicron variant extensively escapes the neutralizing activities of antibodies present in COVID-19 convalescent or vaccinated individuals[6–11]. Although a third-dose booster vaccination significantly increases neutralizing activities against the Omicron variant[7,10,12–16], this variant frequently causes breakthrough infections among individuals immunized with COVID-19 vaccines[17,18].

In the present study, we investigated whether COVID-19 vaccine-induced memory T cells functionally respond to the Omicron spike protein by analysing samples from BNT162b2-vaccinated healthcare workers (HCWs) and individuals with prior SARS-CoV-2 infection. We report that BNT162b2-induced memory T cells substantially respond to the Omicron variant with preserved polyfunctionality.

Results

BNT162b2-induced memory T cell responses against the Omicron spike protein. The present study enrolled HCWs without previous SARS-CoV-2 infection (seronegative for SARS-CoV-2 nucleocapsid) who were immunized with 2 ($n = 20$) or 3 ($n = 20$) doses of the BNT162b2 messenger RNA vaccine. We also recruited and longitudinally investigated individuals with prior SARS-CoV-2 infection who were immunized with 2 doses of BNT162b2 vaccine ($n = 20$).

We performed intracellular cytokine staining (ICS) assays for interferon-γ (IFN-γ), interleukin-2 (IL-2) and tumour necrosis factor (TNF) in peripheral blood mononuclear cells (PBMCs) that were stimulated using overlapping peptide (OLP) pools for the spike protein of the SARS-CoV-2 wild-type (WT) (lineage B) strain and the Omicron variant (Fig. 1a). We compared the frequency of IFN-γ-producing CD4+ T cells against the WT spike protein versus the Omicron spike protein and found that the average frequency against the Omicron spike protein was 22% ($P < 0.001$) and 10% ($P < 0.05$) lower in PBMCs from HCWs with 2 and 3 doses of the vaccine, respectively (Fig. 1b, left). Nevertheless, BNT162b2-induced memory CD4+ T cells maintained substantial responses to the Omicron spike protein. Notably, the two- and three-dose vaccinated groups did not exhibit significantly different responses, although a third vaccination dose is known to boost the neutralizing activities of nAbs against the Omicron variant[7,12]. The frequency of TNF-producing CD4+ T cells against the Omicron spike protein, compared to the WT spike protein, was decreased by 14% ($P < 0.005$) and 6% (non-significant) in the two- and three-dose vaccinated groups, respectively (Fig. 1b, middle). Similarly, the frequency of IL-2-producing CD4+ T cells against the Omicron spike protein, compared to the WT spike protein, was decreased by 21% ($P < 0.001$) in the two-dose vaccinated group and by 3% (non-significant) in the three-dose vaccinated group (Fig. 1b, right).

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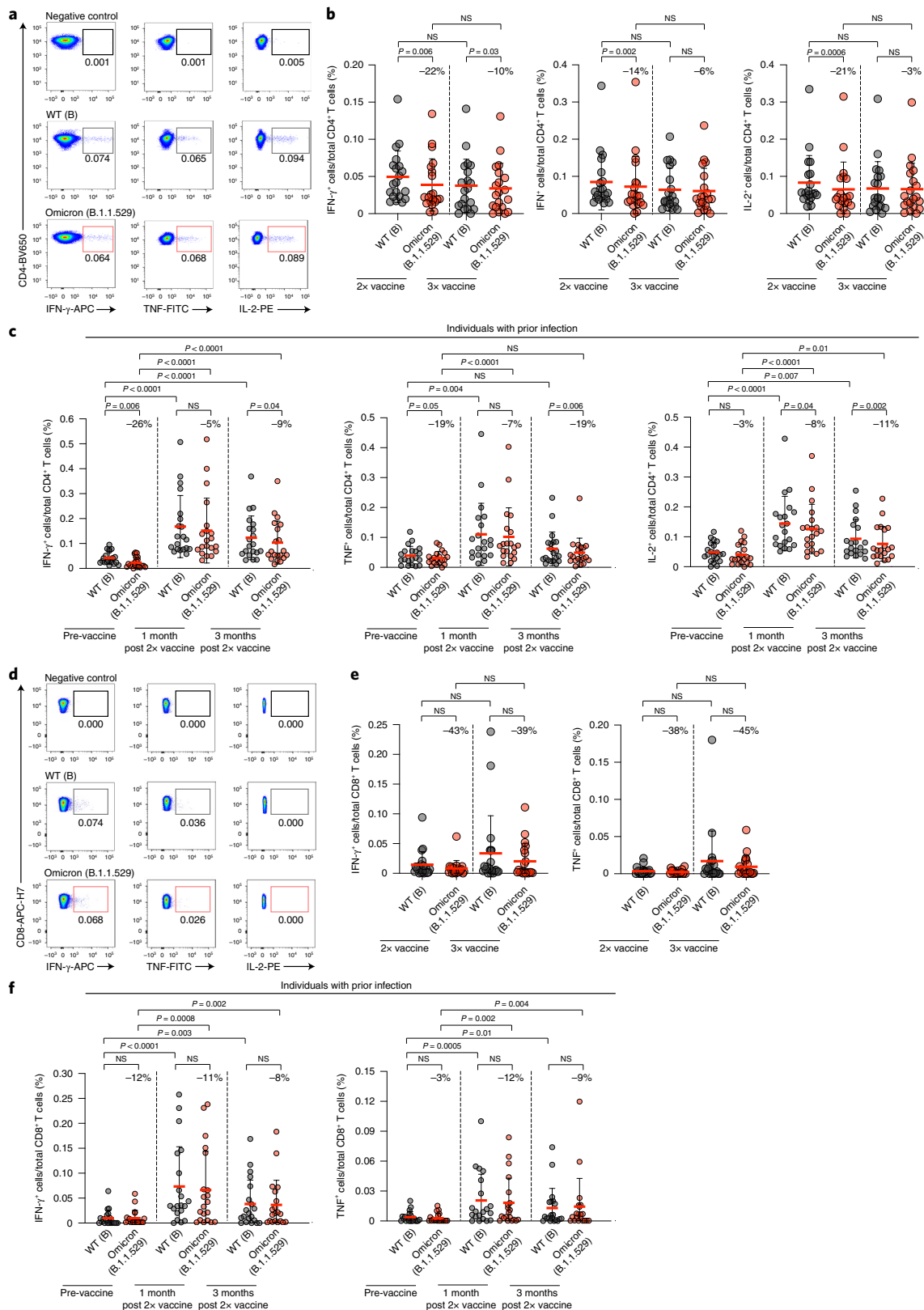
Fig. 1 | Cytokine-secreting functions of BNT162b2-induced memory T cells against the WT and Omicron spike proteins. ICS was performed to examine the frequency of CD4+ or CD8+ T cells responding to the WT and Omicron spike proteins. **a,d**, Representative flow cytometry plots showing cytokine-producing cells among CD4+ (**a**) or CD8+ (**d**) T cells. **b,c,e,f**, The frequency of cytokine-producing CD4+ (**b,c**) or CD8+ (**e,f**) T cells against the WT and Omicron spike proteins in HCWs with 2 (n = 20) or 3 (n = 20) doses of the BNT162b2 vaccine (**b,e**) and in COVID-19-recovered individuals with 2 BNT162b2 vaccine doses (n = 20) (**c,f**). Data are presented as the mean ± s.d. The percentage numbers in each graph indicate the mean decrease in the frequency of cytokine-producing cells against the Omicron spike protein compared to the WT spike protein. P values were calculated with a two-tailed, matched-pairs Wilcoxon signed-rank test (WT versus Omicron) or two-tailed, unpaired Mann–Whitney U-test. NS, not significant.

We also performed a longitudinal analysis among individuals with prior SARS-CoV-2 infection and two-dose BNT162b2 vaccination at three time points: before vaccination (ten months after recovery from mild-to-moderate COVID-19) and at one and three months after the second vaccination. Two-dose BNT162b2 vaccination significantly increased the frequency of IFN-γ-producing CD4^{+} T cells against both the WT and Omicron spike protein (Fig. 1c, left). The frequency of IFN-γ-producing CD4^{+} T cells against the Omicron spike protein, compared to against the WT spike protein, was reduced by 26% ($P < 0.01$) before vaccination, 5% (non-significant) at 1 month after the second vaccination and 9% ($P < 0.05$) at 3 months after the second vaccination. Similarly, against the Omicron spike protein compared to the WT spike protein, the frequency of TNF-producing CD4^{+} T cells decreased by 19% ($P < 0.05$), 7% (non-significant) and 19% ($P < 0.01$) at the 3 time points (Fig. 1c, middle); the frequency of IL-2-producing CD4^{+} T cells decreased by 3% (non-significant), 8% ($P < 0.05$) and 11% ($P < 0.005$) at the 3 time points (Fig. 1c, right). These findings indicated that vaccine-induced memory T cells substantially responded to the Omicron spike protein.

The observed CD8^{+} T cell responses were relatively weak and IL-2-producing cells were not detected among CD8^{+} T cells (Fig. 1d). Therefore, we analysed IFN-γ and TNF production from CD8^{+} T cells and found that memory CD8^{+} T cells exhibited substantial responses to the Omicron spike protein. Both the IFN-γ and TNF responses were non-significantly decreased against the Omicron spike protein compared to the WT spike protein (Fig. 1e,f) and did not significantly differ between the two- and three-dose vaccinated groups (Fig. 1e). Vaccination induced significantly increased responses against both WT and Omicron spike protein among individuals with prior infection (Fig. 1f).

We also analysed the geometric mean fluorescence intensity (MFI) for each cytokine in cytokine^{+} cells. Our results showed that the geometric MFI did not differ between T cells responding to the WT versus Omicron spike protein in the CD4^{+} or CD8^{+} T cell population (Extended Data Fig. 1).

Omicron spike protein-responding memory T cell responses in individual donors. Next, we analysed the percentages of positive responders for each cytokine. A positive response was defined as when the percentage of cytokine^{+} cells after OLP stimulation was at least threefold higher than in the negative controls for each sample[19–21]. Our analysis of CD4^{+} T cells from HCWs revealed an IFN-γ^{+} response against the WT spike protein in 90% (18 of 20) of two-dose vaccinated donors and 60% (12 of 20) of three-dose vaccinated donors (Fig. 2a, left). The corresponding percentages of IFN-γ[+] responses against the Omicron spike protein were reduced to 80% (16 of 20 two-dose vaccinated donors) and 55% (11 of 20 three-dose vaccinated donors); however, these percentages did not notably differ from the percentages of positive responders against the WT spike protein. Similar results were observed in analyses of TNF- or IL-2-producing CD4^{+} T cells (Fig. 2a, middle and right).

In individuals with prior infection, a two-dose BNT162b2 vaccination increased the percentage of IFN-γ^{+} responders to 100% against both WT and Omicron spike protein and these responses were maintained for 3 months (Fig. 2b, left). Similar results were obtained in analyses of TNF- or IL-2-producing CD4^{+} T cells (Fig. 2b, middle and right).

In our analysis of CD8^{+} T cell responses, the percentages of positive responders were relatively low compared to our analysis of CD4^{+} T cell responses. However, the percentages of IFN-γ^{+} or TNF^{+} responders did not notably differ between CD8^{+} T cell responses against the WT versus Omicron spike protein in HCWs (Fig. 2c) or in individuals with prior infection (Fig. 2d).

We also calculated the ratio of the frequency of cytokine-producing T cells against the Omicron spike protein to the frequency of cytokine-producing T cells against the WT spike protein for each cytokine in each individual. In the CD4^{+} T cell population, this ratio ranged from 0.5 to 1.5, irrespective of the cytokines in most cases (Fig. 3a). However, this ratio was <0.1 for IFN-γ in 2 donors and for TNF in a single donor. Within the CD8^{+} T cell population, this ratio was <0.5 for IFN-γ and TNF in several donors (Fig. 3b). These findings indicate that although BNT162b2-induced memory T cells generally exhibited a substantial response to the Omicron spike protein, they could not respond to the Omicron spike protein in certain individuals.

Polyfunctionality of BNT162b2-induced memory T cells against the Omicron spike protein. We further focused on the polyfunctionality of the memory T cells that responded to the WT or Omicron spike protein. Polyfunctional T cells, which simultaneously exert several effector functions, play a crucial role in host protection during viral infection[22–25]. Representative flow cytometry dot plots showed that a proportion of CD4^{+} T cells simultaneously produced IFN-γ, TNF and IL-2 in response to both WT and Omicron spike protein OLPs (Fig. 4a). Notably, the triple-positive population exhibited significantly higher geometric MFI for each cytokine compared to the double- and single-positive populations and the double-positive population had a significantly higher geometric MFI than the single-positive population (Fig. 4b and Extended Data Fig. 2). We additionally analysed polyfunctional (triple-positive or double-positive) CD4^{+} T cells from two- and three-dose vaccinated HCWs. Against the Omicron spike protein compared to the WT spike, the average percentage of polyfunctional cells decreased by 8% ($P < 0.05$) in two-dose vaccinated donors and 2% (non-significant) in three-dose vaccinated donors (Fig. 4c). In our analysis of polyfunctionality using every possible combination of functions, we observed no significant difference between CD4^{+} T cells responding to the WT versus Omicron spike protein (Fig. 4d).

In individuals with prior infection, two-dose BNT162b2 vaccination significantly increased the percentage of polyfunctional cells against both the WT and Omicron spike protein (Fig. 4e). At all three time points, the percentage of polyfunctional cells did not significantly differ between CD4^{+} T cells responding to the WT versus Omicron spike protein. Polyfunctionality analysis using pie graphs confirmed that two-dose vaccination was associated with a significant increase in the percentage of polyfunctional cells against both the WT and Omicron spike protein (Fig. 4f). In our analysis of polyfunctionality using every possible combination of functions, we found no significant difference between the CD4^{+} T cells responding to the WT versus Omicron spike protein (Fig. 4g).

We also analysed the polyfunctionality of BNT162b2-induced memory CD8^{+} T cells. In the HCW groups with two and three doses of the vaccine, the percentage of polyfunctional cells was not significantly decreased against the Omicron spike protein compared to the WT spike (Fig. 5a). In our analysis of polyfunctionality using every possible combination of functions, we observed no significant difference between CD8^{+} T cells responding to the WT versus Omicron spike protein (Fig. 5b). In individuals with prior infection, at all three time points, the percentage of polyfunctional cells did not significantly differ between CD8^{+} T cells responding to WT versus Omicron spike protein (Fig. 5c). The pie graphs also confirmed that the polyfunctional responses did not significantly differ against WT versus Omicron spike protein (Fig. 5d); when we analysed polyfunctionality using every possible combination of functions, we again found no significant difference between CD8^{+} T cells responding to the WT versus Omicron spike protein (Fig. 5e). Taken together, these results demonstrate that BNT162b2-induced memory CD4^{+} and CD8^{+} T cells exhibit preserved polyfunctionality against the Omicron spike protein.
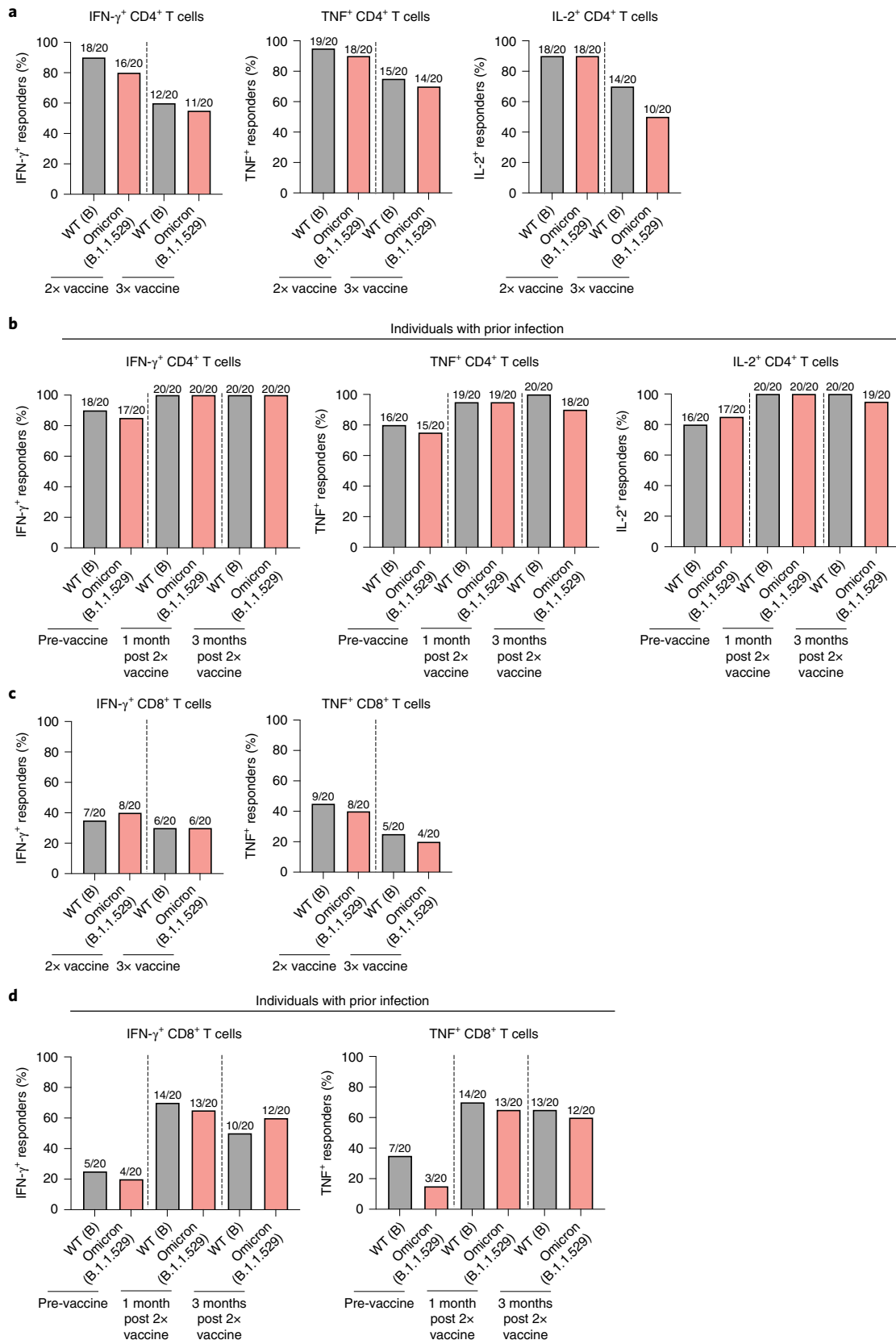
Fig. 2 | Percentage of positive responders against the WT and Omicron spike proteins. a–d, The percentage of positive responders was determined on the basis of the percentage of cells positive for each cytokine in the CD4+ (**a,b**) or CD8+ (**c,d**) T cell population in HCWs with 2 ($n = 20$) or 3 ($n = 20$) doses of the BNT162b2 vaccine (**a,c**) and in COVID-19-recovered individuals with 2 BNT162b2 vaccine doses ($n = 20$) (**b,d**). A positive response was defined as when the percentage of cytokine+ cells after OLP stimulation was more than threefold higher than the percentage of cytokine+ cells in the negative controls for each sample.
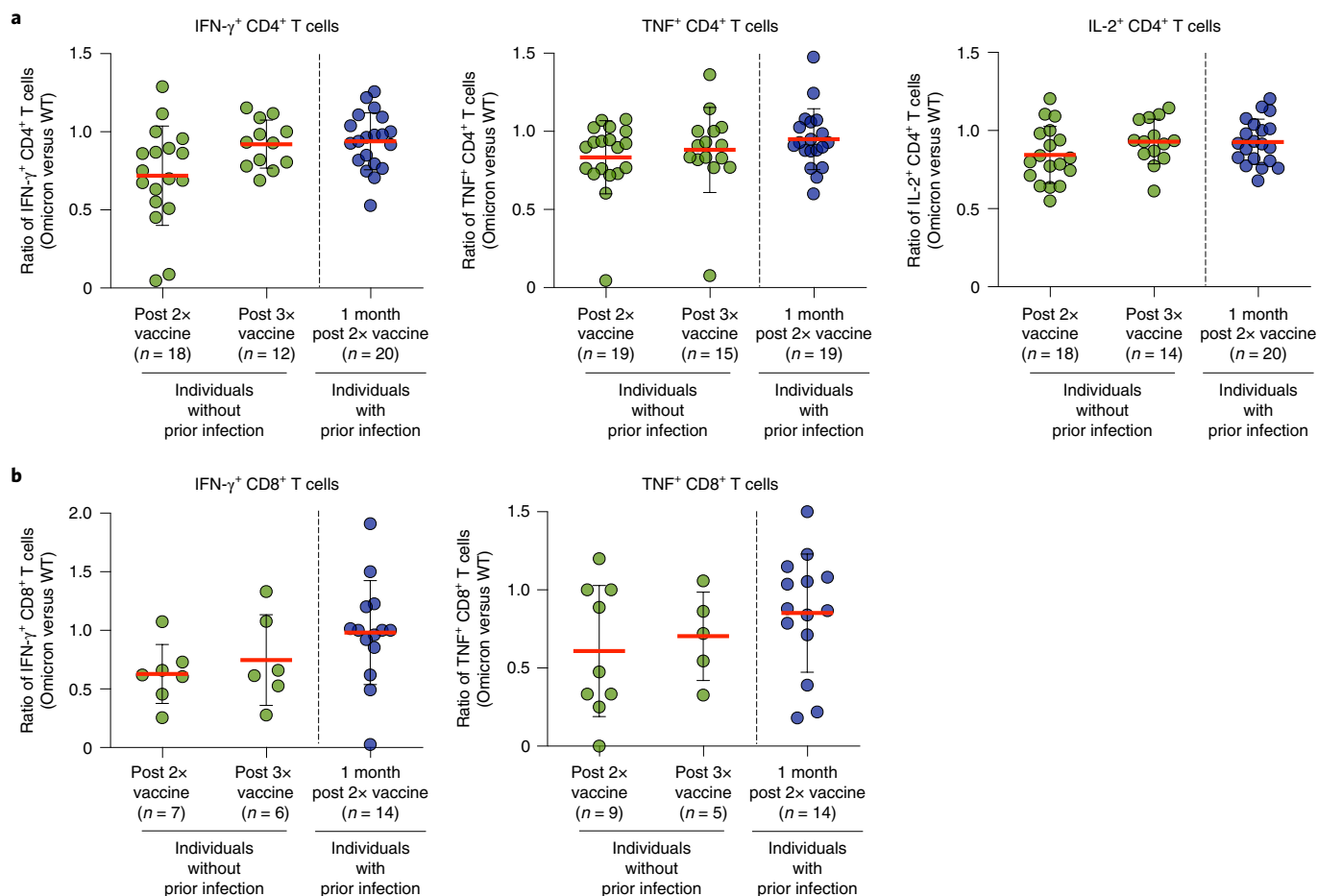
Fig. 3 | The ratio of the response against the Omicron spike protein to the response against the WT spike protein. a,b, The ratio of the frequency of cytokine-producing T cells against the Omicron spike protein to the frequency of cytokine-producing T cells against the WT spike protein was determined for each cytokine in each individual. CD4+ (**a**) and CD8+ (**b**) T cells were analysed in HCWs with two or three doses of the BNT162b2 vaccine and in COVID-19-recovered individuals with two BNT162b2 vaccine doses. Positive responders for each cytokine against the WT spike protein were included in this analysis. Data are presented as mean values ± s.d.

Discussion

Since its emergence, the Omicron variant has shown remarkable ability to escape the neutralizing activities of antibodies present in individuals convalescing from COVID-19 or vaccinated individuals[6–8]. However, our current study demonstrates that memory T cells elicited by BNT162b2 vaccination substantially respond to the Omicron variant with polyfunctionality. This finding is in line with recent reports of relatively preserved responses of infection- or vaccine-induced memory T cells against the Omicron variant[26–30]. Notably, it has also been demonstrated that T cell epitopes in SARS-CoV-2 proteins are substantially conserved in the spike and non-spike proteins of the Omicron variant[31].

The other SARS-CoV-2 VOCs, including the Alpha, Beta, Gamma and Delta variants, also rarely escape the memory T cell responses elicited by COVID-19 vaccination or natural infection[32–36], although they can considerably evade the neutralizing activities of antibodies induced by vaccination or infection[1]. In principle, it is difficult for variants to completely escape T cell responses because viral proteins have multiple T cell epitopes depending on each individual's allotype of human leukocyte antigen[36]. These findings indicate that vaccine-induced memory T cells may recognize and respond to a broad range of variants even if nAbs cannot neutralize them.

The host-protective roles of T cells have been demonstrated in patients and animal models with SARS-CoV-2 infection. In a macaque model, depletion of CD8+ T cells in convalescent animals partially abrogated host protection against SARS-CoV-2 rechallenge[37]. Additionally, mice immunized with a vaccine expressing T cell epitopes exhibited reduced viral titres and lung pathology when challenged with SARS-CoV-2, even in the absence of nAbs[38]. Moreover, higher levels of CD8+ T cell immunity are associated with improved patient survival in patients with COVID-19 who have humoral immunodeficiency caused by anti-CD20 therapy[39]. Collectively, these data indicate that T cells contribute to host protection against SARS-CoV-2, particularly when nAb activity is suboptimal and insufficient, for example, because of waning nAbs and emerging variants[36].

Our current study has several technical limitations. The present results may underestimate the impact of mutations on T cell responses because T cells were stimulated using a high concentration of OLPs (1 μg ml⁻¹ for each peptide) for ICS. Our results may also underestimate the impact of mutations outside of epitopes that affect processing or presentation.

Our present results demonstrate that BNT162b2-induced memory T cells substantially respond to the Omicron variant with preserved polyfunctionality. These findings suggest that memory T cells exert effector functions against the Omicron variant during breakthrough infections in individuals immunized with COVID-19 vaccines.
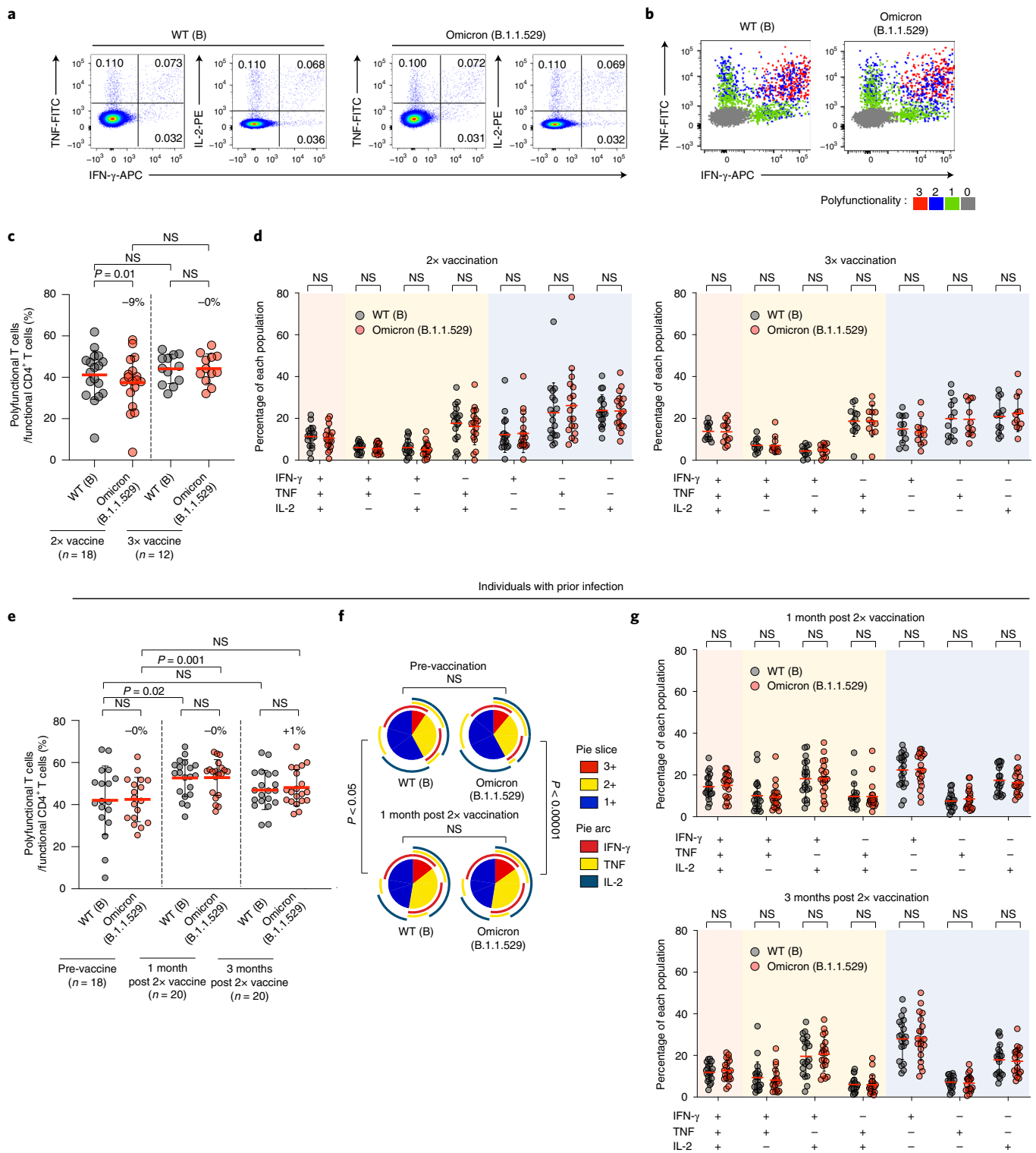
Fig. 4 | Polyfunctionality of BNT162b2-induced memory CD4+ T cells. a, Representative flow cytometry plots showing polyfunctional cells among CD4+ T cells. **b,** Representative polychromatic dot plots for IFN-γ and TNF. T cells positive for a given number of functions are marked in different colours to show their distribution in the dot plots for IFN-γ and TNF. **c,** Percentage of polyfunctional T cells among any cytokine-producing CD4+ T cells from HCWs with 2 (n = 18) or 3 (n = 12) doses of the BNT162b2 vaccine. **d,** Detailed analyses of polyfunctionality are presented with every possible combination of functions (n = 18 for 2× vaccination; n = 12 for 3× vaccination). **e,** Percentage of polyfunctional T cells among any cytokine-producing CD4+ T cells from COVID-19-recovered individuals with 2 doses of the BNT162b2 vaccine (n = 18 for pre-vaccine; n = 20 for 1 month post 2× vaccine; n = 20 for 1 month post 2× vaccine). **f,** Pie graphs representing the fraction of cells positive for a given number of functions among CD4+ T cells with any type of function before vaccination and one month after the second vaccination. Permutation test = 10,000 permutations. **g,** Detailed analyses of polyfunctionality are presented with every possible combination of functions (n = 20). CD4+ T cell IFN-γ responders against the WT spike protein were included in this analysis. **c–e,g,** Data are presented as mean values ± s.d. P values were calculated with a two-tailed, matched-pairs Wilcoxon signed-rank test (WT versus Omicron) or two-tailed, unpaired Mann–Whitney U-test.

Fig. 5 | Polyfunctionality of BNT162b2-induced memory CD8+ T cells. a, Percentage of polyfunctional T cells among any cytokine-producing CD8+ T cells from HCWs with two or three doses of the BNT162b2 vaccine. **b**, Detailed analyses of polyfunctionality are presented with every possible combination of functions. **c**, Percentage of polyfunctional T cells among any cytokine-producing CD8+ T cells from COVID-19-recovered individuals with two doses of the BNT162b2 vaccine. **d**, Pie graphs representing the fraction of cells positive for a given number of functions among CD8+ T cells with any type of functions before vaccination and one month after the second vaccination. Permutation test = 10,000 permutations. **e**, Detailed analyses of polyfunctionality are presented with every possible combination of functions. CD8+ T cell IFN-γ responders against the WT spike protein were included in this analysis. **a–c,e**, Data are presented as mean values ± s.d. P values were calculated with a two-tailed, matched-pairs Wilcoxon signed-rank test (WT versus Omicron) or two-tailed, unpaired Mann–Whitney U-test.

Methods

Patients and specimens. For this study, we enrolled HCWs who had been immunized with 2 (n = 20; male:female = 12:8; mean age = 41.7, range = 27–55) or 3 (n = 20; male:female = 13:7; mean age = 39.35, range = 25–53) doses of the BNT162b2 mRNA vaccine from Chungbuk National University Hospital. All enrolled HCWs were seronegative for SARS-CoV-2 nucleocapsid. Peripheral blood was obtained three months after the second vaccination in the two-dose group and one month after the third vaccination in the three-dose group. We also recruited individuals with prior SARS-CoV-2 infection who were immunized with 2 doses of the BNT162b2 vaccine (n = 20; male:female = 0:20; mean age = 49.3, range = 38–57) from Korea University Guro Hospital. In this group, peripheral blood was obtained

at 3 time points: 6–7 months before vaccination (10 months after recovery from mild-to-moderate COVID-19) and 1 and 3 months after the second vaccination. PBMCs were isolated by density gradient centrifugation using lymphocyte separation medium (Corning). After isolation, cells were cryopreserved in fetal bovine serum (Corning) with 10% dimethyl sulfoxide (DMSO) (Sigma-Aldrich) until use. This study was reviewed and approved by the institutional review boards of Chungbuk National University Hospital (no. 2021-02-010) and Korea University Guro Hospital (nos. 2020GR0130 and 2021GR0099). Informed consent was obtained from all participants and compensation was provided to participants. This study was conducted according to the principles of the Declaration of Helsinki (seventh version).

ICS assay. Cryopreserved PBMCs were thawed, rested overnight at 37 °C and cultured in the presence of OLP pools (1 μg ml^{-1} for each peptide; Peptides & Elephants GmbH) for the spike protein of the WT strain (NM908947) or Omicron variant (hCoV-19/South Africa/CERI-KRISP-K032284/2021; EPI_ISL_6699770 in GISAID) and anti-human CD28 and CD49d monoclonal antibodies (mAbs) (1 μg ml^{-1} for each; BD Biosciences) for 6 h at 37 °C. Negative controls were cultured in the presence of DMSO and anti-CD28 and anti-CD49d antibodies. Brefeldin A (GolgiPlug; BD Biosciences) and monensin (GolgiStop; BD Biosciences) were added 1 h after the initial stimulation. Cells were stained with fluorochrome-conjugated antibodies for specific surface markers for 10 min at 4 °C. Dead cells were excluded using the LIVE/DEAD dye (Invitrogen). For intracellular staining, cells were fixed and permeabilized using the FoxP3 Staining Buffer Kit (Invitrogen) and stained for cytokines for 30 min at 4 °C. The following monoclonal antibodies were used for multicolour flow cytometry: anti-CD3 BV786 (1:100 dilution, clone UCHT1, catalogue no. 565491); anti-CD4 BV650 (1:100 dilution, clone SK3, catalogue no. 563875); anti-CD8 APC-H7 (1:100 dilution, clone SK1, catalogue no. 560179); anti-CD14 BV510 (1:100 dilution, clone MφP9, catalogue no. 563079); anti-CD19 BV510 (1:100 dilution, clone SJ25C1, catalogue no. 562947); and anti-IFN-γ APC (1:100 dilution, clone B27, catalogue no. 554702) all from BD Biosciences. Anti-IL-2 PE (1:100 dilution, clone MQ1-17H12, catalogue no. 12-7029-42) and anti-TNF FITC (1:100 dilution, clone MAb11, catalogue no. 11-7349-82) were obtained from Thermo Fisher Scientific. Multicolour flow cytometry was performed using an LSR II instrument with FACSDiva software (v8.0.1, BD Biosciences). Data were analysed with the FlowJo software (v10.8.1, FlowJo LLC). We calculated the percentage of positive responders based on the percentage of cells positive for each cytokine in the CD4$^+$ or CD8$^+$ T cell population. A positive response was defined as when the percentage of cytokine$^+$ cells after OLP stimulation was at least threefold higher than in the negative controls for each sample.

Statistical analysis. Differences between groups were analysed by non-parametric (matched-pairs Wilcoxon signed-rank test or Mann–Whitney *U*-test). The statistical analyses were performed with Prism v.8.2 (GraphPad Software). To determine the differences in the pie charts, permutation tests in the SPICE software (v6.1) were used (10,000 permutations). Two-sided *P* values were determined in all analyses. *P* < 0.05 was considered statistically significant.

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

Data availability
All data are available within the article or its supplementary material. Source data are provided with this paper.

Code availability
No custom code was generated or used in this study.

References

1. Noh, J. Y., Jeong, H. W. & Shin, E.-C. SARS-CoV-2 mutations, vaccines, and immunity: implication of variants of concern. *Signal Transduct. Target. Ther.* **6**, 203 (2021).
2. Koelle, K., Martin, M. A., Antia, R., Lopman, B. & Dean, N. E. The changing epidemiology of SARS-CoV-2. *Science* **375**, 1116–1121 (2022).
3. Hui, K. P. Y. et al. SARS-CoV-2 Omicron variant replication in human bronchus and lung ex vivo. *Nature* **603**, 715–720 (2022).
4. Meng, B. et al. Altered TMPRSS2 usage by SARS-CoV-2 Omicron impacts infectivity and fusogenicity. *Nature* **603**, 706–714 (2022).
5. Callaway, E. Heavily mutated Omicron variant puts scientists on alert. *Nature* **600**, 21 (2021).
6. Liu, L. et al. Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. *Nature* **602**, 676–681 (2022).
7. Planas, D. et al. Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. *Nature* **602**, 671–675 (2022).
8. Cele, S. et al. Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. *Nature* **602**, 654–656 (2022).
9. Hoffmann, M. et al. The Omicron variant is highly resistant against antibody-mediated neutralization: implications for control of the COVID-19 pandemic. *Cell* **185**, 447–456.e11 (2022).
10. Garcia-Beltran, W. F. et al. mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant. *Cell* **185**, 457–466.e4 (2022).
11. Edara, V.-V. et al. mRNA-1273 and BNT162b2 mRNA vaccines have reduced neutralizing activity against the SARS-CoV-2 omicron variant. *Cell Rep. Med.* **3**, 100529 (2022).
12. Carreno, J. M. et al. Activity of convalescent and vaccine serum against SARS-CoV-2 Omicron. *Nature* **602**, 682–688 (2022).
13. Haveri, A. et al. Neutralizing antibodies to SARS-CoV-2 Omicron variant after third mRNA vaccination in health care workers and elderly subjects. *Eur. J. Immunol.* https://doi.org/10.1002/eji.202149785 (2022).
14. Daniel, J. S. et al. Neutralisation sensitivity of the SARS-CoV-2 omicron (B.1.1.529) variant: a cross-sectional study. *Lancet Infect. Dis.* https://doi.org/10.1016/S1473-3099(22)00129-3 (2022).
15. Accorsi, E. K. et al. Association between 3 doses of mRNA COVID-19 vaccine and symptomatic infection caused by the SARS-CoV-2 Omicron and Delta variants. *JAMA* **327**, 639–651 (2022).
16. Gruell, H. et al. mRNA booster immunization elicits potent neutralizing serum activity against the SARS-CoV-2 Omicron variant. *Nat. Med.* **28**, 477–480 (2022).
17. Nyberg, T. et al. Comparative analysis of the risks of hospitalisation and death associated with SARS-CoV-2 omicron (B.1.1.529) and delta (B.1.617.2) variants in England: a cohort study. *Lancet* **399**, 1303–1312 (2022).
18. Kuhlmann, C. et al. Breakthrough infections with SARS-CoV-2 omicron despite mRNA vaccine booster dose. *Lancet* **399**, 625–626 (2022).
19. Nelde, A. et al. SARS-CoV-2-derived peptides define heterologous and COVID-19-induced T cell recognition. *Nat. Immunol.* **22**, 74–85 (2021).
20. Heide, J. et al. Broadly directed SARS-CoV-2-specific CD4$^+$ T cell response includes frequently detected peptide specificities within the membrane and nucleoprotein in patients with acute and resolved COVID-19. *PLoS Pathog.* **17**, e1009842 (2021).
21. Grifoni, A. et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* **181**, 1489–1501.e15 (2020).
22. Betts, M. R. et al. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8$^+$ T cells. *Blood* **107**, 4781–4789 (2006).
23. Park, S.-H. et al. Successful vaccination induces multifunctional memory T-cell precursors associated with early control of hepatitis C virus. *Gastroenterology* **143**, 1048–1060.e4 (2012).
24. Precopio, M. L. et al. Immunization with vaccinia virus induces polyfunctional and phenotypically distinctive CD8$^+$ T cell responses. *J. Exp. Med.* **204**, 1405–1416 (2007).
25. Seder, R. A., Darrah, P. A. & Roederer, M. T-cell quality in memory and protection: implications for vaccine design. *Nat. Rev. Immunol.* **8**, 247–258 (2008).
26. Gao, Y. et al. Ancestral SARS-CoV-2-specific T cells cross-recognize the Omicron variant. *Nat. Med.* **28**, 472–476 (2022).
27. Naranbhai, V. et al. T cell reactivity to the SARS-CoV-2 Omicron variant is preserved in most but not all individuals. *Cell* **185**, 1259 (2022).
28. Tarke, A. et al. SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron. *Cell* **185**, 847–859.e11 (2022).
29. Keeton, R. et al. T cell responses to SARS-CoV-2 spike cross-recognize Omicron. *Nature* **603**, 488–492 (2022).
30. Liu, J. et al. Vaccines elicit highly conserved cellular immunity to SARS-CoV-2 Omicron. *Nature* **603**, 493–496 (2022).
31. Choi, S. J. et al. T cell epitopes in SARS-CoV-2 proteins are substantially conserved in the Omicron variant. *Cell. Mol. Immunol.* **19**, 447–448 (2022).
32. Geers, D. et al. SARS-CoV-2 variants of concern partially escape humoral but not T-cell responses in COVID-19 convalescent donors and vaccinees. *Sci. Immunol.* **6**, eabj1750 (2021).
33. Woldemeskel, B. A., Garliss, C. C. & Blankson, J. N. SARS-CoV-2 mRNA vaccines induce broad CD4$^+$ T cell responses that recognize SARS-CoV-2 variants and HCoV-NL63. *J. Clin. Invest.* **131**, e149335 (2021).
34. Tarke, A. et al. Impact of SARS-CoV-2 variants on the total CD4$^+$ and CD8$^+$ T cell reactivity in infected or vaccinated individuals. *Cell Rep. Med.* **2**, 100355 (2021).
35. Jordan, S. C. et al. T cell immune responses to SARS-CoV-2 and variants of concern (Alpha and Delta) in infected and vaccinated individuals. *Cell. Mol. Immunol.* **18**, 2554–2556 (2021).
36. Noh, J. Y., Jeong, H. W., Kim, J. H. & Shin, E.-C. T cell-oriented strategies for controlling the COVID-19 pandemic. *Nat. Rev. Immunol.* **21**, 687–688 (2021).
37. McMahan, K. et al. Correlates of protection against SARS- CoV-2 in rhesus macaques. *Nature* **590**, 630–634 (2021).
38. Zhuang, Z. et al. Mapping and role of T cell response in SARS-CoV-2-infected mice. *J. Exp. Med.* **218**, e20202187 (2021).
39. Bange, E. M. et al. CD8$^+$ T cells contribute to survival in patients with COVID-19 and hematologic cancer. *Nat. Med.* **27**, 1280–1289 (2021).

Acknowledgements

Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | The geometric mean fluorescence intensity for each cytokine among cytokine-producing cells. The geometric mean fluorescence intensity (gMFI) was determined for each cytokine among cytokine-producing cells. CD4 + (a–b) and CD8 + (c–d) T cells were analyzed among healthcare workers (HCWs) with two or three doses of the BNT162b2 vaccine (a and c), and among COVID-19-recovered individuals with two BNT162b2 doses (b and d). Positive responders for each cytokine against the ancestral spike were included in this analysis. P values are calculated with two-tailed Wilcoxon matched-pairs signed rank test.
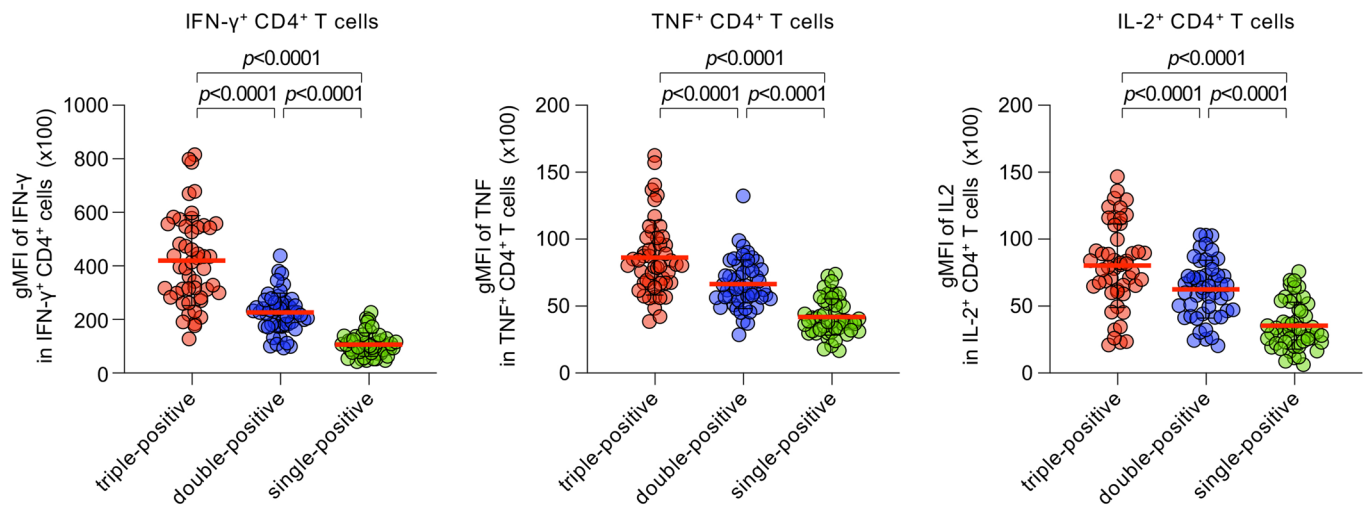
Extended Data Fig. 2 | The geometric mean fluorescence intensity for each cytokine among polyfunctional CD4 + T cells. The geometric mean fluorescence intensity (gMFI) for each cytokine was determined in single-, double-, and triple-positive CD4 + T cell populations. IFN-γ responders against the ancestral spike from healthcare workers (HCWs) with two (n = 18) or three (n = 12) doses of the BNT162b2 vaccine and COVID-19-recovered individuals with two BNT162b2 doses (n = 20; at one month after the second vaccination) were analyzed together. P values are calculated with two-tailed Wilcoxon matched-pairs signed rank test.

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☒	☐	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	For flow cytometry, data were collected with BD FACSDIVA v.9.0.
Data analysis	Flow cytometry Data from flow cytometry were analyzed with FlowJo v.10.7.1 software. Dara regarding polyfunctionality of T cells were analyzed with SPICE v.6.1. Statistical analysis Data were analyzed with GraphPad software v.9.0.

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 data supporting the findings of this study are available within the main manuscript and the supplementary files or provided upon reasonable request.
Raw data corresponding to all the main and extended data figures are included in the Source Data files.

Field-specific reporting

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Life sciences study design

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

Sample size	The sample size was not statistically pre-determined. Upon the emergence of the Omicron variant, we included available PBMC samples in the current study as follows: PBMC samples from healthcare workers without previous SARS-CoV-2 infection (seronegative for SARS-CoV-2 nucleocapsid) who were immunized with two (n = 20) or three (n = 20) doses of the BNT162b2 mRNA vaccine and individuals with prior SARS-CoV-2 infection who were immunized with two doses of BNT162b2 (n = 20). Consequently, this sample size was enough to draw a conclusion in the current study.
Data exclusions	No data were excluded in this study.
Replication	Experiments did not include replicates as all participants and their data are unique.
Randomization	For the comparison of T-cell responses against the Wuhan-Hu-1 versus the Omicron spike, the current study enrolled BNT162b2-immunized individuals only without placebo controls. Therefore, randomization was not applicable to this study.
Blinding	For the comparison of T-cell responses against the Wuhan-Hu-1 versus the Omicron spike, the current study enrolled BNT162b2-immunized individuals only without placebo controls. Therefore, blinding was not applicable to this study.

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	BD Biosciences Anti-human CD3-BV786, UCHT1, 565491, 1084725, 1:100 Anti-human CD4-BV650, SK3, 563875, 1188950, 1:100 Anti-human CD8-APC-H7, SK1, 560179, 1207195, 1:100 Anti-human CD14-BV510, MφP9, 563079, 1180028, 1:100 Anti-human CD19-BV510, SJ25C1, 562947, 1084958, 1:100 Anti-human IFN-γ-APC, B27, 554702, 1047222, 1:100 Purified anti-human CD28/49d, L293/L25, 347690, 1277116, 1:100 ThermoFisher Anti-human TNF-FITC, MAb11, 11-7349-82, 2298674, 1:100 Anti-human IL-2-PE, MQ1-17H12, 12-7029-42, 2363853, 1:100
Validation	Antibody validation is available at each manufacturer's website below. BD Biosciences Anti-human CD3 - BV786 - https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv786-mouse-anti-human-cd3.565491

Anti-human CD4 - BV650 - https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv650-mouse-anti-human-cd4.563875
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Anti-human IFN-γ - APC - https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-mouse-anti-human-ifn.554702
Purified anti-human CD28/49d - https://www.bdbiosciences.com/en-nz/products/reagents/flow-cytometry-reagents/clinical-discovery-research/multicolor-cocktails-and-kits-ruo-gmp/co-stimulatory-antibodies-cd28-cd49d.347690

Thermofisher
Anti-human TNF - FITC - https://www.thermofisher.com/antibody/product/TNF-alpha-Antibody-clone-MAb11-Monoclonal/11-7349-82
Anti-human IL-2 - PE - https://www.thermofisher.com/antibody/product/IL-2-Antibody-clone-MQ1-17H12-Monoclonal/12-7029-42

Human research participants

Policy information about studies involving human research participants

Population characteristics	This study enrolled healthcare workers (HCWs) without previous SARS-CoV-2 infection (seronegative for SARS-CoV-2 nucleocapsid) who were immunized with two (n = 20; male:female = 12:8; age, mean 41.7, and range 27–55) or three (n = 20; male:female = 13:7; age, mean 39.35, and range 25–53) doses of the BNT162b2 mRNA vaccine. Additionally, individuals with prior infection who were immunized with two doses of BNT162b2 (n = 20; male:female = 0:20; age, mean 49.3, and range 38–57) were recruited and longitudinally examined (pre-vaccination, 3 months post 2x vaccination and 2 month post 3x vaccination.
Recruitment	In 2021 when the nationwide COVID-19 vaccination was performed in South Korea, we enrolled healthcare workers without previous SARS-CoV-2 infection and individuals with prior SARS-CoV-2 infection who were vaccinated with BNT162b2 with informed consent according to the study protocol. We performed T-cell analysis without excluding any collected PBMC sample, thus minimizing a possible bias. For this study, we enrolled healthcare workers (HCWs) who had been immunized with two (n = 20) or three (n = 20) doses of the BNT162b2 mRNA vaccine. All enrolled HCWs were seronegative for SARS-CoV-2 nucleocapsid. Peripheral blood was obtained at 3 months after the second vaccination in the two-dose group, and at 1 month after the third vaccination in the three-dose group. We also recruited individuals with prior SARS-CoV-2 infection who were immunized with two doses of BNT162b2 (n = 20). In this group, peripheral blood was obtained at three time-points: before vaccination (10 months after recovery from mild-to-moderate COVID-19), and at one and three months after the second vaccination.
Ethics oversight	This study was reviewed and approved by institutional review boards of the Chungbuk National University Hospital (2021-02-010) and the Korea University Guro Hospital (2020GR0130 and 2021GR0099) and conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all donors.

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

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).

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Methodology

Sample preparation	Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Lymphocyte Separation Medium (Corning). After isolation, the cells were cryopreserved in fetal bovine serum (Corning) with 10% dimethyl sulfoxide (Sigma-Aldrich) until use.
Instrument	Data were collected using BD FACS LSR II.
Software	Data from flow cytometry were analyzed with FlowJo v.10.7.1 software. And, data from flow cytometry were analyzed with FlosJo v.10.7.1 software and SPICE v.6.1.
Cell population abundance	Data analyses were performed using at least >2 million PBMCs per each sample.
Gating strategy	For all the experiments, single cells were gated on a forward scatter height vs. forward scatter area plot. Lymphocytes were

Gating strategy
gated on forward vs. side scatter plot based on their size and granularity. To exclude dead cells and non-T cells, LIVE/DEAD Fixable Dead Cell reactive dye+, CD14+, and CD19+ cells were gated out. T cells were gated with CD3, CD4, and CD8 gating. For ICS assays, IFN-gamma+, IL-2+, and TNF+ cells were gated accroding to the corresponding DMSO controls.

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